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TRITICEAE IV

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Triticeae IV

Edited by
P. Hernández, M.T. Moreno, J.I. Cubero and A. Martín

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PREFACE

The 4th International Triticeae Symposium, which was held at Cordoba, Spain, in September, 2001, was jointly organized by 'CIFA de Córdoba' (Junta de Andalucía), 'Instituto de Agricultura Sostenible' (CSIC), and 'Universidad de Córdoba' and also sponsored by The Triticeae Consortium and the International Plant Genetic Resources Institute (IPGRI).

Conservation and use of biodiversity are the foundations of a sustainable agriculture. Taxonomic and evolutionary studies are essential to interpreting the biodiversity of the Triticeae, and devising appropriate strategies for conservation of its biodiversity, whether resulting from natural forces or human cultivation. The application of molecular technologies for the analysis and use of this biodiversity was reflected in the high percentage of contributions that made use of these technologies.

The purpose of the Symposium, to foster interdisciplinary discussion and exchange among all those working with the Triticeae, was reflected in the broad spectrum of topics covered by its five sessions and in the many lively discussions that took place outside of the formal sessions. By focusing on a single tribe rather than a discipline, the Symposium brought together people with diverse backgrounds and interests, people who would not encounter each other at 'standard' systematics, breeding, and pathology meetings.

By the end of the Symposium, several new collaborations had been initiated, some involving individuals who met for the first time in Cordoba, and many pre-existing collaborations renewed. The Fifth International Triticeae Symposium, to be held in Prague, Czech Republic, in 2005, will offer an opportunity to present the results of such collaborations and forge new ones. Such links between scientists working on biodiversity, plant breeding, stress and disease resistance, quality and utilization and biotechnology scientists are essential if the problems and challenges of Agriculture in the 21st century are to be successfully addressed.

For various reasons, some of the contributors scheduled to give an oral presentation had to cancel their presentation after the program had been published. Their manuscripts are included in these Proceedings. Conversely, to our regret, there are no manuscripts available for some of the oral presentations. Longer versions of some of the contributions will be published in full paper version during 2002 in a special issue of Hereditas.

The Organizing Committee

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SESSION A:

EVOLUTIONARY AND SYSTEMATIC RELATIONSHIPS IN THE TRITICEAE.

GENOME ORGANIZATION AND GENOME EVOLUTION IN TRITICEAE

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ABSTRACT: We are studying the molecular organization of selected regions in the diploid genomes of *Triticum monococcum* (A^m-genome), barley, *Aegilops tauschii* and the A-genome of the hexaploid wheat *Triticum aestivum*. These studies at the DNA sequence level are using a newly established bacterial artificial chromosome (BAC)-library of *T. monococcum* and a cosmid library of hexaploid wheat. The analysis revealed the existence of gene-rich regions at the *Lrk/Tak* loci in wheat and barley with gene densities similar to *Arabidopsis thaliana* as well as regions with a complex organization of repetitive sequences. A large number of different repetitive sequences was found, among them a variety of retrotransposons, non-LTR retrotransposons, transposons, MITEs as well as a number of new elements not characterized previously. In addition to duplications and nested retrotransposon insertions, evidence for a number of molecularly different DNA deletions was found. These deletions might partially counteract the expansion of genome size due to transposon activity. To better understand the molecular evolution of the wheat genomes we are currently extending our comparative studies at the molecular level using a newly available BAC library of the tetraploid wheat *Triticum turgidum*.

1. GENOME ORGANIZATION

The *Triticeae* species have large and complex genomes, clearly distinguishing them from the model genomes of rice and Arabidopsis [1]. The molecular characterization of these genomes as well as the application of this knowledge for the isolation of agronomically important genes from *Triticeae* has remained very difficult. In order to improve the tools for gene isolation, we are studying gene organization and genome structure and evolution in the *Triticeae*. A BAC library was made from the diploid wheat *Triticum monococcum* [2] and a very good colinearity was found with the A-genome of hexaploid wheat in two regions analysed in detail on chromosome group 1S. These regions are under detailed investigation because in *Triticum aestivum* they contain the *Lr10* leaf rust disease resistance gene and the *Pm3* powdery mildew resistance gene, respectively [3]. We have completely sequenced a region of 211 kb from the *Lr10* containing region. This represents the largest continuous segment of a *Triticeae* genome sequenced so far [4]. Five putative genes were identified, two of which show similarity to disease resistance genes and therefore represent good candidates of orthologous genes for *Lr10*. Three of the five genes are clustered in a 31 kb gene enriched island while the two others are separated from the cluster and from each other by large stretches of repetitive DNA. About 70% of the 211 kb contig is comprised of transposable elements. Ten different types of retrotransposons were identified, most of them forming a pattern of nested insertions. In addition, non-LTR retrotransposons, MITEs as well as new types of repetitive elements were detected. Evidence was found for major deletion, insertion and duplication events within the analysed region, suggesting multiple mechanisms of genome evolution in addition to retrotransposon amplification. Seven types of foldback transposons, an element class previously not described for *Triticeae* genomes, were characterised. One such element was found to be closely associated with genes in several *Triticeae* species and may therefore be of use for the identification of gene-rich regions in these species and the development of molecular markers.

2. GENOME EVOLUTION

Hexaploid wheat is a young polyploid species and represents a good model to study mechanisms of gene and genome evolution after polyploidization. Recent studies at the scale of the whole genome have suggested rapid genomic changes after polyploidization but so far the rearrangements that have occurred in terms of gene content and organization have not been analyzed at the microlevel in wheat. We have isolated members of a receptor-like kinase (*Lrk*) gene family in hexaploid and diploid wheat, *A. tauschii* and barley. We have studied the structure of this gene-rich region in more detail [5,6]. The receptor-like kinase multigene families are located in gene-dense clusters, with gene densities close to the values of Arabidopsis (1 gene per 5 kb). The comparative genetics of these gene families as well as phylogenetic analysis has allowed to establish evolutionary relationships (orthology vs. paralogy) between the different members of the gene family in wheat as well as with *Lrk* genes from barley and *Aegilops tauschii*. It also demonstrated that the sequences of the homoeologous *Lrk* genes evolved independently after polyploidization. In addition, we found evidence for gene loss during the evolution of wheat and barley. Analysis of large genomic fragments isolated from non orthologous *Lrk* loci showed a high conservation of the gene content and gene organization at these loci on the homoeologous group 1 chromosomes of wheat and barley. Finally, sequence comparison of two paralogous fragments containing *Lrk* sequences on chromosome 1B showed a large number of local events (sequence duplications, deletions and insertions) which reveal rearrangements and mechanisms for genome enlargement at the microlevel. We conclude that paralogous genes are ideally suited for the analysis of local genome evolution and can reveal the molecular details of the shaping of plant genomes.

REFERENCES

[1] Keller, B. and Feuillet, C. 2000. Colinearity and gene density in grass genomes. *Trends Plant Sci.* **5**, 246-251.

[2] Lijavetzky, D., Muzzi, G., Wicker, T., Keller, B., Wing, R. and Dubcovsky J. 1999. Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. *Genome* **42**,1176-1182.

[3] Stein, N., Feuillet, C., Wicker, T., Schlagenhauf, E. and Keller, B. 2000. Subgenome chromosome walking in wheat: a 450 kb physical contig in *Triticum monococcum* L. spans the *Lr10* resistance locus in hexaploid wheat (*T. aestivum* L.). Proc. Natl. Acad. Sci. USA **97**, 13436-13441.

[4] Wicker, T., Stein, N., Albar, L., Feuillet, C., Schlagenhauf, E. and Keller, B. 2001. Analysis of a contigous 211 kb sequence in diploid wheat (*Triticum monococcum L.*) reveals multiple mechanisms of genome evolution. *Plant J.* **26**: 307-316.

[5] Feuillet, C. and Keller, B. 1999. High gene density is conserved at syntenic loci of small and large grass genomes. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 8265-8270.

[6] Feuillet, C., Penger, A., Gellner, K., Mast, A. and Keller, B. 2001. Molecular evolution of receptor-like kinase genes in hexaploid wheat: independent evolution of orthologs after polyploidization and mechanisms of local rearrangements at paralogous loci. *Plant Physiol.* **125**: 1304-1313.

MOLECULAR PHYLOGENETIC INVESTIGATION OF ALLOPOLYPLOID ELYMUS IN NORTH AMERICA

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ABSTRACT: Under the genomic system of classification, *Elymus* is defined as one of several allopolyploid combinations that include the **St** genome of *Pseudoroegneria*. The present paper focuses on 1) native North American allotetraploid species of *Elymus*, all of which are presumed to combine the **St** genome with the **H** genome of *Hordeum* in an **StStHH** allotetraploid configuration, and 2) the introduced, weedy *E. repens*, an allohexaploid whose genomic constitution is not well understood, but which may include the **St** and **H** genomes. The genomic complements of these species are examined in a phylogenetic context, using three data sets from the chloroplast genome and one from a nuclear gene encoding granule-bound starch synthase. The data from the native *Elymus* species are consistent with cytogenetic data, and suggest that *Hordeum* and *Pseudoroegneria* are involved in the origin of the native North American allotetraploid species of *Elymus*. The more preliminary results from *Elymus repens* are complex and indicate the involvement of at least four genome donors, including *Taeniatherum* and an unknown donor in addition to *Hordeum* and *Pseudoroegneria*.

INTRODUCTION

Because the Triticeae represent such an economically important group, represented by wheat, barley, rye, and several important forage grasses, it is not surprising that the classification of the tribe has received a disproportionately high amount of attention. The resulting collection of disparate classification systems (reviewed in Barkworth 2000) has served not to enlighten but to confuse, and has hampered communication among disciplines. In North America, the native members of the tribe are usually described under one of two main classification systems (although there are variants of both). The morphology-based classification of Hitchcock (1951) is favored by many people because the genera are easily distinguished using clear, convenient characters. The genomic system (Dewey 1982, 1984; Barkworth and Dewey 1985), on the other hand, is based on an explicit attempt to develop a classification system that reflects the groups's evolutionary history. Such a system is potentially far more useful to workers in all disciplines, but it has not been universally adopted, possibly because the genera in this system are more difficult to distinguish on morphological grounds. Further, some workers have expressed doubt over the ability of genome-pairing data, on which the genomic system is based, to uncover phylogenetic relationships (reviewed in Seberg and Petersen 1998).

In North America, the circumscription of *Elymus* is dramatically different in the two classification systems (see Mason-Gamer in press for a summary). This paper uses the genomic definition, in which *Elymus* is defined to include all allopolyploid species that contain the **St** genome from *Pseudoroegneria*. The ploidal level and the identity of the other genome(s) can differ among species, such that *Elymus* under the genomic definition is explicitly polyphyletic and evolutionarily complex. I focus on only some of the many distinct genome combinations found within *Elymus*. The analyses presented include representatives of the native North American species, nearly all of which are thought to combine the genomes of *Pseudoroegneria* (**St**) and *Hordeum* (**H**) in an **StStHH** allotetraploid configuration (Dewey 1982, 1984, and references therein). I also present more preliminary results from *E. repens*, an allohexaploid that has been introduced to North America and is now widespread. Given its impact, it is surprising that its genomic constitution is so

much less well-known than those of the native *Elymus* species. Early genome-pairing data from *E. repens* X *Hordeum secalinum* hybrids indicated the presence of an **H** genome from *Hordeum* (Cauderon and Saigne 1961), but Dewey later questioned the presence of an **H** genome on the basis of morphology (Dewey 1984). Chromosome pairing in *E. repens* X *Pseudoroegneria spicata* hybrids (Dewey 1976) suggested that the other two sets of genomes are **St**-like, and proposed an **StStStStXX** genomic complement (where **X** represents an unknown donor). Recently, Ørgaard and Anamthawat-Jónsson (2001), in a genomic in situ hybridization study, found that *Hordeum brachyantherum* probes hybridize strongly to one of the genome sets of *E. repens*, indicating the presence of the **H** genome, but *Pseudoroegneria spicata* probes hybridize only very weakly to the other two genome sets, leaving the presence of the **St** genome in doubt. Ørgaard and Anamthawat-Jónsson (2001) thus reported a genomic complement of **XXXXHH** for *E. repens*.

The main objective of the present report is to illustrate the use of molecular phylogenetic techniques to assess, complement, and expand upon the existing genome-pairing data for native North American allotetraploid *Elymus* and for the introduced allohexaploid *E. repens*.

MATERIALS AND METHODS

Because polyploid taxa are interpreted here within the context of the entire tribe, the analyses include representatives of nearly all of the monogenomic genera. The analysis of native North American *Elymus* species includes *E. canadensis*, *E. glaucus*, *E. trachycaulus*, *E. hystrix*, *E. elymoides*, *E. wawawaiensis*, *E. virginicus*, *E. riparius*, and *E. lanceolatus*. (One Asian species, *E. ciliaris*, was sampled in the chloroplast DNA analysis; it is presumed to have an **SSYY** genomic content.) For more details about sampled taxa, see Mason-Gamer (in press). The sample of *E. repens* includes six individuals, representing the states of Maine, Connecticut, Wisconsin, and Idaho.

Chloroplast DNA (cpDNA) restriction site data were described in Mason-Gamer and Kellogg (1996), and represent the monogenomic Triticeae along with *E. ciliaris*, *E. lanceolatus*, and *E. repens*. The chloroplast *rpo*A gene was amplified and sequenced using primers designed by Petersen and Seberg (1997). The chloroplast genes for trnT, trnF-3', trnF-5', and trnL, and their intervening spacers were amplified and sequenced using primers designed by Taberlet et al. (1991). Data from the *rpo*A and tRNA genes were collected for the monogenomic Triticeae, from the ten tetraploid *Elymus* species listed above, and from *E. repens*. Analyses of combined cpDNA data were carried out in PAUP* 4.0 (Swofford 2000) using cladistic parsimony with all characters equally-weighted. Because of space considerations here, more detailed descriptions of the cpDNA data, analyses, and results have been submitted elsewhere.

The starch synthase gene was amplified, cloned, and sequenced as described in Mason-Gamer et al. (1998). Because the taxa in question are allopolyploid, up to eight clones from each tetraploid individual and up to twenty from each hexaploid (*E. repens*) individual were screened in order to reveal most of the intra-individual variation. Data and analyses for the monogenomic Triticeae and North American *Elymus* are presented in detail in Mason-Gamer (in press), and final results for *E. repens* will be presented in detail elsewhere.

RESULTS

Because the chloroplast genome is maternally inherited in most angiosperms, analysis of the combined cpDNA data (Fig. 1) can be used to identify the maternal genome donor. In the case of *Elymus*, there appear to be three possible chloroplast genome donors: *Pseudoroegneria*, *Thinopyrum*, or *Dasypyrum*. The result is complicated by the fact that *Pseudoroegneria* forms a paraphyletic group from within which *Thinopyrum* and *Dasypyrum* arise. Although all three genera are possible donors according to this tree, *Pseudoroegneria* is the possibility most consistent with both genome-pairing data (Dewey 1982, 1984, and references therein) and with the starch synthase sequence data (below).

Phylogenetic analyses of starch synthase gene sequences (Fig. 2) illustrate the reticulate nature of *Elymus*, and confirm the presence of both the **St** (*Pseudoroegneria*) and **H** (*Hordeum*) genomes. The monophyly of the **St** clade, which includes *Elymus* and

Pseudoroegneria, is not strongly confirmed, however; on some of the shortest trees, the **St**1 and **St**2 clades do not together form a monophyletic group (Mason-Gamer in press).

The starch synthase data, on the other hand, yield more complex and unexpected results (Table I). First, although the chloroplast genome appears to have been derived from *Pseudoroegneria*, an apparent *Pseudoroegneria* starch synthase gene has been found in only one of the six individuals. Three of the six individuals have a *Hordeum*-like copy of the gene, and five have a copy from an unknown donor, i.e., they form a clade distinct from any of the monogenomic genera. All six of the individuals have a *Taeniatherum*-like copy of the starch synthase gene, a result supported by 99% bootstrap support (not shown).

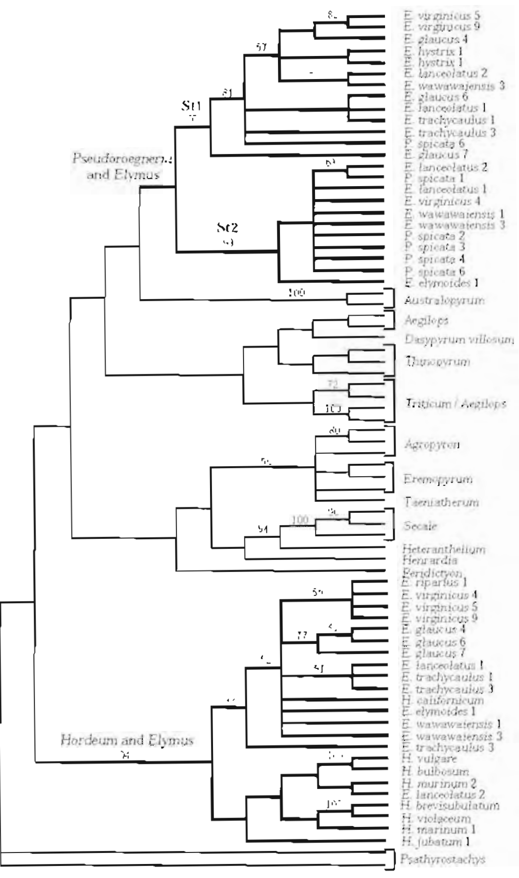

Figure 1: Phylogeny of North American *Elymus* and the monogenomic Triticeae based on cpDNA data from restriction sites, from rpoA gene sequences, and from trnT, trnF-3', trnF-5', and trnL gene and intervening spacer sequence. A strict consensus tree is shown; numbers above nodes represent bootstrap support greater than 50%. Numbers following taxon names differentiate individuals within species.

For *Elymus repens*, the results of the cpDNA analysis look very similar to those from North American *Elymus*; *Pseudoroegneria* appears to be the most likely genome donor to *E. repens*. As with the tetraploids, all of the *E. repens* individuals are grouped within a paraphyletic *Pseudoroegneria* grade.

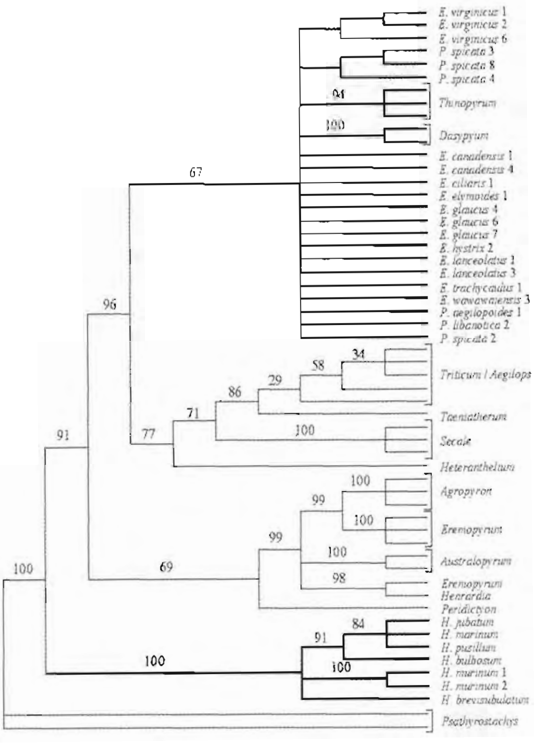

Figure 2: Phylogeny of North American *Elymus* and the monogenomic Triticeae based on starch synthase gene sequences. The tree represents a strict consensus of about 96% of all shortest trees. On the remaining 4% of the trees, the two **St**-genome clades fail to form a monophyletic group. Numbers above the nodes indicate bootstrap values greater than 50%. Numbers following taxon names differentiate individuals within species.

Table I. Presence of genome markers in *Elymus repens.*

Genome Marker	Individual #
cpDNA-*Pseudoroegneria*	1,2,3,4,5,6
Starch synthase-*Pseudoroegneria*	1
Starch synthase-*Hordeum*	1,4,6
Starch synthase-Unknown donor	1,2,4,5,6
Starch synthase-*Taeniatherum*	1,2,3,4,5,6

DISCUSSION

The identities of the genomes in allopolyploid members of the Triticeae have been widely addressed using cytogenetic data, but few molecular phylogenetic studies have included these taxa. The goal of this report is to illustrate the utility of molecular phylogenetic data for clarifying these relationships, using the North American species of *Elymus* as an example.

In the case of North American *Elymus*, it appears that the genome-pairing data have accurately identified the two genome donors, *Hordeum* and *Pseudoroegneria*. However, even though the phylogenetic results are consistent with cytogenetic data, they raise some new questions. For example, the starch synthase tree do not strongly support the North American **St** genomes as a single monophyletic group, a result that has not been reported in the cytogenetic literature. The cpDNA data fail to distinguish *Thinopyrum* and *Dasypyrum* from *Pseudoroegneria*, a result that had been previously noted (Mason-Gamer and Kellogg 1996). Therefore, while the data are consistent with the cytogenetic data, they do not rule out other possibilities.

The results from *Elymus repens* are less straightforward than those from the North American allotetraploid species. One interesting result is that, although the chloroplast genome appears to have been derived from *Pseudoroegneria*, a *Pseudoroegneria*-like starch synthase gene is lacking from five of the six individuals. This could be a sampling artifact, but because between 15 and 20 clones were sampled from each individual, it is unlikely that the *Pseudoroegneria*-like gene is present in all of them and was simply missed by chance alone. The lack of a **St**-like copy of the gene is in agreement with the findings of Ørgaard and Anamthawat-Jónsson (2001). In their genome in situ hybridization analysis, probes derived from *Pseudoroegneria spicata* did not hybridize strongly to the putative **St** chromosomes of *E. repens*.

With a *Hordeum*-like copy of the gene in three of the six individuals, it is harder to dismiss sampling artifacts. At this point, the conclusion can only be that *Hordeum* has played a role in the origin of *E. repens*.

The presence of gene copies from an apparent unknown donor was an unexpected result. Perhaps more unexpected was the presence of a *Taeniatherum*-like sequences in all of the individuals examined. I am unaware of any published suggestion that *Taeniatherum* is related to *E. repens*; however, as far as I know, chromosome pairing has not been studied in *Taeniatherum* X *E. repens* hybrids. Although *Taeniatherum* has apparently played a role in the evolution of *E. repens*, it does not necessarily mean that an entire genome is derived from *Taeniatherum*. Given the apparently highly reticulate pattern of evolution in the tribe as a whole (Kellogg et al. 1996; Mason-Gamer and Kellogg 1996), it is possible that small portions of genomes have been acquired by introgression. If this is the case, then it may be misleading to think of entire genomes as coordinated packages that always travel together.

Together, the results from North American *Elymus* and *E. repens* illustrate the utility of molecular phylogenetic data. As in the case of the native North American species, they show that genome pairing data can reflect phylogeny. Further, as illustrated by *E. repens*, molecular data may expand upon cytogenetic data, providing evidence of relationships not previously suspected.

REFERENCES

[1] Barkworth ME: Changing Perception of the Triticeae. In: Jacobs SWL, Everett J (eds) Grasses: Systematics and Evolution. CSIRO Publishing (2000).

[2] Barkworth ME, Dewey DR: Genomically Based Genera in the perennial Triticeae of North America: Identification and Membership. American Journal of Botany 72: 767-776 (1985).

[3] Cauderon Y, Saigne B: New Interspecific and Intergeneric Hybrids Involving Agropyron. Wheat Information Service 12: 13-14.

[4] Dewey DR: Derivation of a New Forage Grass from Agropyron repens X Agropyron spicatum Hybrids. Crop Science 16: 175-180 (1976).

[5] Dewey DR: Genomic and Phylogenetic Relationships among North American Perennial Triticeae. In: Estes JR (ed) Grasses and Grasslands. Oklahoma University Press (1982).

[6] Dewey DR: The Genomic System of Classification as a Guide to Intergeneric Hybridization within the Perennial Triticeae. In: Gustafson JP (ed) Gene Manipulation in Plant Improvement, Proceedings of the 6th Stadler Genetics Symposium. Columbia University Press (1984).

[7] Hitchcock AS: Manual of Grasses of the United States, Second Edition, revision by Chase A. United States Government Printing Office (1951).

[8] Kellogg EA, Appels R, Mason-Gamer RJ: When Gene Trees Tell Different Stories: the Diploid Genera of the Triticeae. Systematic Botany 21: 321-347 (1996).

[9] Mason-Gamer RJ: Origin of North American Elymus (Poaceae: Triticeae) Allotetraploids Based on Granule-Bound Starch Synthase Gene Sequences. Systematic Botany In Press.

[10] Mason-Gamer RJ, Kellogg EA: Testing for Phylogenetic Conflict Among Molecular Data Sets in the Tribe Triticeae. Systematic Biology 45: 524-545 (1996).

[11] Mason-Gamer RJ, Kellogg EA: Chloroplast DNA Analysis of the Monogenomic Triticeae: Phylogenetic Implications and Genome-specific Markers. In Jauhar PP (ed) Methods of Genome Analysis in Plants. CRC Press (1996).

[12] Mason-Gamer RJ, Weil CF, Kellogg EA: Granule-Bound Starch Synthase: Structure, Function, and Phylogenetic Utility. Molecular Biology and Evolution 15: 1658-1673 (1998).

[13] Ørgaard M, Anamthawat-Jónsson K: Genome Discrimination by In Situ Hybridization in Icelandic Species of Elymus and Elytrigia (Poaceae: Triticeae). Genome 44: 275-283 (2001).

[14] Seberg O, Petersen G: A Critical Review of Concepts and Methods used in Classical Genome Analysis. The Botanical Review 64: 372-417 (1998).

[15] Swofford DL: PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates (2000).

HOW GOOD ARE REPETITIVE DNA SEQUENCES FOR TRITICEAE PHYLOGENY?

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ABSTRACT: Huge part of the genomes of most Triticeae species is formed by the different classes of the repetitive DNA sequences. These sequences are widely used for studying of phylogenetic relationships since even one probe marks a significant piece of genome. In most cases, due to their dispersed distribution across genomes, retrotransposons depict the existent Triticeae phylogeny complementary and make the new contribution to the understanding of the generation of genetic biodiversity. Another class of repeats, tandemly organised DNA sequences, as a rule, cluster at the functionally important regions of chromosomes, centromeres and telomeres. According to the common criteria - homology of primary structure and copy number - the distribution of a number of tandem DNA families in Triticeae could not be placed into the frame of generally accepted phylogenetic relationships. We suggest that the tremendous variation in copy numbers of these class of repeats have been associated with a formation of specific chromosomal regions which are important for chromosome behaviour and stabilization of species-specific karyotype. Therefore, the evolution of these regions may occur largely independently of the evolution of gene-rich regions.

INTRODUCTION

The clear tendency in evolution of grasses at the genome level was a steady increase in genome size in the Pooideae, leading to the very large genomes in the Triticeae. The ancenstral grass genome is calculated to have had about 3.5 pg of DNA per 2C nucleus, while ancestral Pooideae genome had 4.8 pg and the Triticeae ancestor 10.7 pg (1). It is generally agreed that the Triticeae is a single lineage (is monophyletic) and within Triticeae, rye (*Secale*) shows the largest genome which is nearly two times greater than the Triticeae ancestor.

It is well established that the quantity of repetitive DNA sequences is the main factor that determines genome size. Their relative representation can be quite variable and hence they responsible for the C-value paradox. This is most marked for Triticeae where repetitive sequences make up the bulk of genomes. Traditional and still useful) classification divides repetitive DNA sequences into two major classes according to genomic organization: 1) dispersed repetitive sequences (or interspersed with genes) and 2) tandemly arrayed. Among both classes there are a few sequences with known function, such as rDNA repeats or telomeric repeats among tandem repeats and gene family or tRNA among dispersed. The functional or genic sequences, such as rDNA and 5S DNA have been frequently used for sequence alignment and suggested relationships between species and genera of Triticeae which are generally congruent with relationships based on other characteristics. Here, we analyzed those repetitive DNA sequences, either interspersed and tandemly organized, the functions of which are not defined and brought into focus the main question: whether these sequences have co-evolved according to generally accepted Triticeae phylogenetic relationships. In other words, whether these sequences have co-evolved with functional, genic DNA sequences either low copy or repeated.

LTR-RETROTRANSPOSONS HAVE CO-EVOLVED WITH BULK *HORDEUM* GENOME

It been shown that most of the interspersed repetitive DNAs are mobile DNAs, mainly LTR-retrotransposons (2). We isolated from barley several families belonging to main retrotransposon groups: LTR-containing (*gypsy* and *copia*-like) and non-LTR (LINE group) and exploited them for comparative examination of the genomic organization and distribution in eight *Hordeum* species containing the four basic genomes designated **I**, **X**, **Y** and **H** (3). Each of four *gypsy*-like probe encoding a distinct RT (reverse transcriptase) domain yielded distinct hybridization pattern of fragments for each basic genome, also indicating that the four clones represented different *gypsy*-like families. Two clones, HvGyp1 and HvGyp5, hybridized strongly to barley and its ancestor, *H.spontaneum*, but only HvGyp1 was abundant in the third species with the **I** genome, *H.bulbosum* (Fig.1) which revealed a different hybridization pattern after *Hae*III digestion. HvGyp3

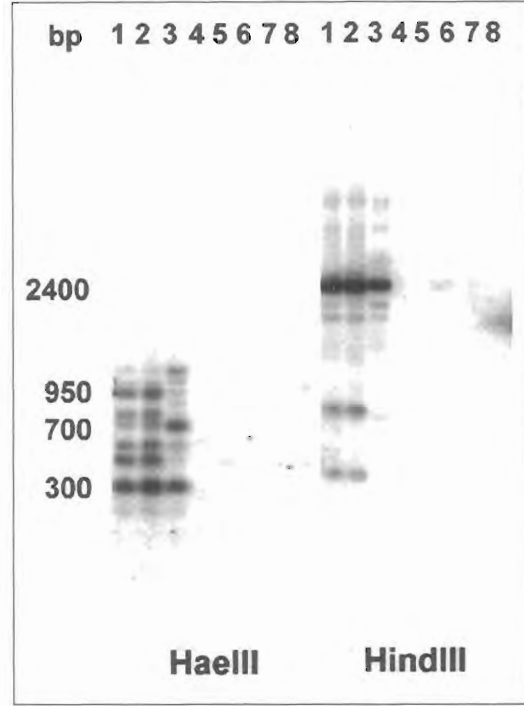

Figure 1. Southern hybridization patterns of *gypsy* clone HvGyp1 to DNA of barley species, containing four basic genome: 1-*H.vulgare*, 2-*H.spontaneum*, 3-*H.bulbosum* (all **I** genome), 4-*H.murinum* (**Y** genome), 5-*H.marinum* (**X** genome), 6-*H.roshevitzii*, 7-*H.chilense*, 8-*H.brachyanterum* (all **H** genome).

and HvGyp13 were approximately equally represented in the **I**, **X** (*H.marinum*) and **Y** (*H.murinum*) genomes, and present in the **H** genomes (data not shown).

In situ hybridization with HvGyp1 as a probe showed multiple sites of hybridization in all studied species (Fig.2), but more in *H.vulgare* than in *H.bulbosum* and *H.murinum* in agreement with blot hybridization data. In *H.vulgare*, copies were distributed mostly uniformly along all chromosomes with a tendency to concentrate in broad centromeric regions. *Copia*-like elements are dispersed over the all chromosomes with gaps near centromeres and nucleolar organizer regions. The copy numbers of the *gypsy*-like families studied are lower than those of the *copia*-like element *BARE-1* (4). *H.vulgare* accumulated significantly more copies compared with wild barley species, with *H.bulbosum* as intermediate.

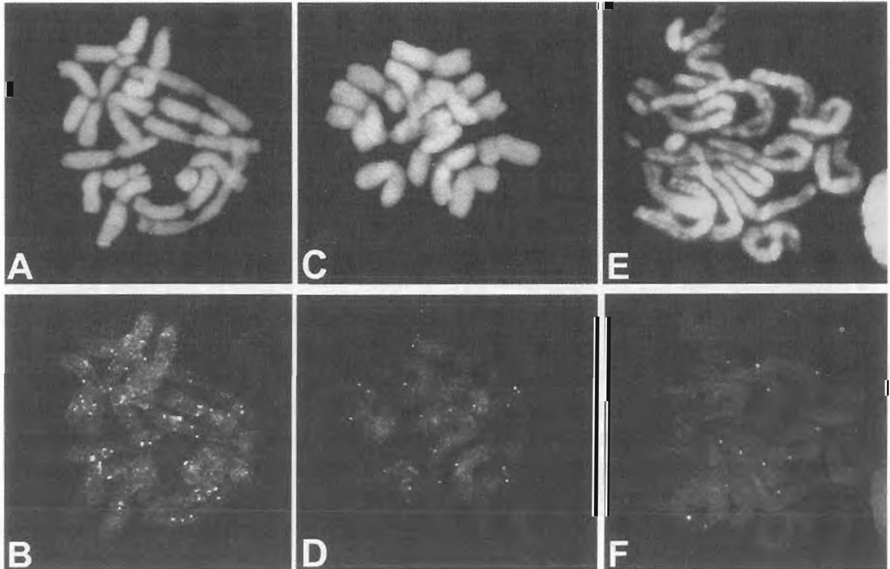

Figure 2. *In situ* hybridization showing the localization of *gypsy* elements on chromosomes of *H.vulgare* (A,B), *H.bulbosum* (C,D) and *H.murinum* (E,F). A,C,E metaphase chromosomes stained with DAPI. B,D,F the same chromosomes after *in situ* hybridization with HvGyp1.

H.vulgare and the wild *Hordeum* species showed contrasting patterns of accumulation of *gypsy*-like and LINE retrotransposons. When we used the probes HvLI3 and HvLI8 isolated from RT domain of LINE, the wild species, *H.bogdanii*, and, particularly, *H.murinum*, yielded substantially more copies dispersed along most chromosomes than *H.vulgare* (data not shown) which gave 3-4 dots after hybridization with HvLI3 and approximately 10-fold more after hybridization with HvLI8. *H.bulbosum* showed an intermediate number of HvLI3 sites between wild species and *H.vulgare*, and contained a similar copy number of HvLI8 to *H.vulgare*.

From these data we can conclude that the LTR contining retrotransposons of *copia*-like and *gypsy*-like groups show similar patterns of diversity: they clearly differentiate four basic *Hordeum* genomes. However, we found further polymorphism in I genome: the *H.vulgare-H.spontaneum* complex and *H.bulbosum* showed polymorphism with HvGyp1 (*HaeIII* digestion), HvGyp13 and, particularly, HvGyp5. Previously, we have found the differences between these two species using the probe from *BARE-1* (5). Thus, both groups of LTR-retrotransposon show a high level of diversity within I genome species. This pattern is in agreement with commonly accepted phylogenetic relationships within *Hordeum* (3) and implies that the two LTR- retrotransposon groups have co-evolved with bulk *Hordeum* genome. On the other hand, non-LTR retrotransposons or LINEs have accumulated to higher copy numbers in wild species compared to *H.vulgare* and have a more heterogeneous primary structure. This indicates that the LINEs have evolved independently from LTR-retrotransposons and, probably from their hosts DNA. Hence, the different retrotransposon groups have competed for the genome space during genus *Hordeum* evolution. This competition resulted in different abilities to increase their copy number and, correspondingly, in their individual contributions to the shape of each basic *Hordeum* genome.

MYSTERIOUS DYNAMICS OF TANDEM DNA EVOLUTION

Retrotransposons propagate across genomes through mechanism of reverse transcription and encode their own reverse transcriptase. Their LTR sequences are involved in the regulation of their propagation. Another major class of repetitive DNA where basically identical monomers are tandemly organized in long arrays are generally supposed to be generated *de novo* by the joint action of mutation, unequal crossing over, slippage replication and/or rolling circle amplification. In marked contrast to retrotransposons, there are no indications that the primary structure of these sequences or nucleotide order are significant for their propagation. Most likely these sequences are just passive, like stones rolling down from the peak of mountain due to an initial push.

The general characteristics of several tandemly organized sequences isolated from different Triticeae species are well documented and are presented in the Table 1. All of these sequences have a huge copy numbers in those genomes from where they have been isolated. However, the distribution of these sequences across tribe is intricately. For example, dpTa1 isolated from wheat, is most abundant in D genome. It is presented in most Triticeae species under low stringency blot hybridization, but we found just a faint signal after hybridization to rye. pSc119.2 is abundant in wheat, rye and most wild barley species, but has not been detected in cultivated barley, *H.vulgare*, by either blot and *in situ* hybridization. pSc200 and pSc250 are localized mostly in the subtelomeric regions of rye chromosomes with a several small intercalary sites of pSc200. These two families comprise up to 5% of large rye genome (6). Whereas *D.villosum* has a pSc200 copy number close to rye, the content of pSc200 and pSc250 in *D.breviaristatum* and *A.cristatum* and pSc250 in *D.villosum* is reduced significantly, although easily detectable by blot and *in situ* hybridization. However, pSc200 and pSc250 have a few copies in wheat and barley genomes as revealed by PCR, but not Southern and *in situ* hybridizations and their genomic location is unknown (Table 1). Summarizing these data, we can conclude that distribution of tandemly organized DNA sequences is not consistent with the generally accepted Triticeae phylogeny. These data also imply that evolutionary changes of the pSc200 and pSc250 families occur at a different rate to that of the genome regions enriched for genes and these families were amplified or reduced to different extents in the genomes after speciation. Each repetitive family appears to be an independent unit of evolution and, perhaps, different mechanisms have been involved in amplification of these sequences. The evolution of the pSc200 family is of particular interest. In rye, we have shown that pSc200 is present in head-to-tail arrays as a tandem repeat, but a fraction of the monomers is organized in pairs with the dimers oriented head-to-head and tail-to-tail, most probably as a result of chromatid type breakage-fusion-bridge cycles (6). Single primer PCR analysis of wheat and barley DNA gave no products, implying that pSc200-like sequences are not present as inverted repeats in the species. Single primer, located at the border of the monomer, amplified a single fragment from *D.villosum*, *D.breviaristatum* and *A.cristatum*, indicating the absence of the dimer units and the presence of only single monomers with inverted orientation.

Secale and *Dasypyrum* chromosomes are characterized by large bands of subterminal heterochroma-

tin where the pSc200 and pSc250 signals are localized. As has been suggested for sequences to be capable of forming heterochromatin, they should reach a threshold amount and concentration in corresponding region of genome (7). There were a several suggestions about some biological significance of heterochromatin, for example, to chromosome pairing (8). We can also speculate that the tremendous variation in copy numbers of tandem repeats are associated with a formation of specific chromosomal regions which are important for chromosome behaviour and stabilization of a species-specific karyotype. Many short direct, inverted repeats and short palindromes are present in both pSc200 and pSc250. Such structures are characteristic features of

Table 1. Characteristics of well-studied tandemly organized repetitive DNA sequences of Triticeae

Sequence	Species of origin	Length of monomer	Copy number	Chromosomal location	Detection in Triticeae genomes					
					by hybridization			by PCR		
					wheat	barley	rye	wheat	barley	rye
dpTa1 or pAS1	wheat	340 bp	10^4-10^6	mostly on D-genome, large and small intercalary bands	+	+	faint	+	+	+
pSc119.2	rye	118 bp		mostly subtelomeric but more proximal than pSc200 and pSc250, a number of intercalary sites	+	only wild species	+	+	+	+
pSc200 or 350-family	rye	379 bp		large bands on the ends of all chro mosomes, small intercalary bands	-	-	+	+	+	+
pSc250	rye	550 bp		large bands on the ends of 18 chromosome arms	-	-	+	+	+	+
HvRT	barley	118 bp		large subtelomeric bands, some small intercalary sites	-	+	-	unknown		

repetitive DNA sequences and can provide a potential source for homologous recombination allowing creation of rearrangement. The observation that each species has specific karyotype, combined with evidence that chromosomal rearrangements might reduce the fertility of heterozygous hybrids, has led to argue for a causative role for chromosomal changes in speciation. The opposing and more widely held view is that the accumulation of chromosomal differences between populations is largely incidental to speciation. Meanwhile, the answers to the main questions: 1) what leads to the origins of tandemly organized DNA repeats? and 2) what are the factors which define the dynamics of repetitive arrays? are still hard to find. We can confirm that the evolution of chromosome regions of some Triticeae species enriched in tandem repeats (heterochromatic regions) is largely independently of the evolution of gene-rich regions reflecting, at least, the differences in the dynamic properties of tandem and dispersed repeats.

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REFERENCES

[1] Kellogg EA: Relationships of cereal crops and other grasses. Proc. Natl. Acad. Sci. USA 95: 2005-2010 (1998).

[2] Bennetzen JL: Transposable element contributions to plant gene and geno me evolution. Plant Mol. Biol. 42: 251-269 (2000).

[3] Bothmer R von, Flink J, Landstrom T: Meiosis in interspecific Hordeum hybrids. I.Diploid combinations. Can. J. Genet. Cytol. 28: 525-535 (1986).

[4] Manninen I, Schulman AH: BARE-1, a copia-like retroelement in barley (Hordeum vulgare L.). Plant Mol. Biol. 22: 829-846 (1993).

[5] Shcherban' AB, Vershinin AV: BARE-ID, a representative of a family of BARE-like elements of the barley genome. Genetica 100: 231-240 (1997).

[6] Vershinin AV, Schwarzacher T, Heslop-Harrison JS: The large-scale genomic organization of repetitive DNA families at the telomeres of rye chromosomes. Plant Cell 7: 1823-1833 (1995).

[7] Kunze B, Weichenhan D, Virks P, Traut W, Winking H: Copy numbers of a clustered long-range repeat determine C-band staining. Cytogenet. Cell Genet. 73: 86-91 (1996).

[8] Hennig W: Heterochromatin. Chromosoma 108: 1-9 (1999).

ELYMUS DAHURICUS COMPLEX (POACEAE): VARIATION, CROSSABILITY, TAXONOMY

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ABSTRACT: The status of the taxa *Elymus dahuricus* Turcz. ex Griseb., *E. excelsus* Turcz. ex Griseb., *E. woroschilowii* Probat. and *E. tangutorum* (Nevski) Hand.-Mazz. in the SHY-genomic complex of *Elymus dahuricus* s. l., has been investigated using morphological, molecular (DNA-AFLP) variation characteristics and hybridological analysis. Populations include accessions from Siberia, Far East (Russia), Tien-Shan (Kirghizia), Tibetan and Sichuan Provinces (China). Variation of morphological characteristics have been found in all populations of the complex. DNA variation was very low within populations but different among populations. The analysis of seed fertility of artificial hybrids and F2-F3 progenies in 37 combinations was carried out. All biotypes studied form a distinct recombination genepool (RGP). The reproductive compatibility of a hybrid combination decreases along with geographical distance of the origin of the parental biotypes. Differences in genetic variation drawn from DNA-AFLPs did not reflect morphology and taxonomic status. Phenotypical segregation in F2 on series of diagnostic characters does not support the taxonomic treatments of the *E. dahuricus* complex both in Southern Russia,and Central-Asian area. The *E. dahuricus* complex seems to be a polymorphic species having a wide geographical range with a wide genetic variation. In our opinion the erected species in this complex do not deserve specific or even infraspecific recognition.

1. INTRODUCTION

The *Elymus dahuricus* species complex occurs in a number of morphological forms from Iran to Japan and from Southern Siberia to the central provinces of China. The genomic formula of all species of the complex seems to be **SSHHYY** (2n = 6X = 42) [1]. According to recent taxonomic treatments the complex includes 6 species: *E. dahuricus* Turcz. ex Griseb.; *E. excelsus* Turcz. ex Griseb.; *E. tangutorum* (Nevski) Hand.-Mazz.; *E. cilindricus* (Franch). Honda; *E. purpuraristitatus* Wang ex Yang; *E. villifer* Wang [2].

In Russia and in the states of the former USSR three species of the complex were recognized: *E. dahuricus* s.str., *E. excelsus* and *E. woroschilowii* Probat. [3]. In the "Flora of Siberia" [4] G. Peshkova recognized two species - *E. dahuricus*, distributed in the South of East Siberia, north-eastern China, north-eastern Mongolia, and *E. ex_elsus*, widespread in Western and Middle Siberia, Central Asia, Western Mongolia, Western China. The basis for the morphological character on which these species differ is based on whether the lemma awns are divergent - or nondivergent and on the lemma awns to lemma body ratio and on differences in glume lengths. Besides this Peshkova [5] considers the populations from Northern China and the Far East relegated to *E. franchetii* Kitag. which has equeal lemmas and glumes lengths in contrast to *E. excelsus* which has unequal length in opinion of G.Peshkova. Another species related to *E. dahuricus* is *E. tangutorum* (Nevski) Hand.-Mazz., which differs morphologicaly from the first one by the short upward awns and which is distributed in the Tibetan province of China and in the Alpine regions of Central Asia. Peshkova includes *E. dahuricus* var. *brevisetum* Ohwi. and *E. cylindricus* var. *micranthus* Meld. to synonyms of *E. tangutorum and E. franchetii* Kitag = *E. dahuricus* var. *cylindricus* Franch. = *E. cylindri-*

cus (Franch.) Honda [5]. Probatova [6] subdivides taxa from the Russian Far East into three species: *E. dahuricus, E. excelsus* and *E. woroschilowii* (= *E. dahuricus* ssp. *pacificus*) all characterized by divergent lemma awns, and *E. dahuricus* and *E. excelsus* differring by glabrous leaf sheaths in the former and hairy in the latter. In Probatova's opinion E. woroschilowii is confined to the coastal territory of the Far East, and is characterized by glaucous colour throughout.

We present the results of morphological, hybridization experiments, and DNA analysis aimed at elucidating the taxonomy of this species complex.

2. MATERIALS AND METHODS

2.1. Population sampling

Taxonomic identification of accessions was made following Probatova [6] for Far-Eastern accessions, and Peshkova [4,5] for the Siberian and other Asiatic accessions. The morphological analysis was conducted on live plants greenhouse grown and on an exhibition field of the CSBG SB RAS from natural seeds, collected within of Russia and Kirghizia (Western and Central Tien Shan), and also from a collection of the Swedish Agricultural University, kindly obtained from Prof. R. von Bothmer). Some characters used for the analysis are shown in the Table 1. Ten plant were grown from ten different accessions collected in different localities (Table 1) of the area of the *E. dahuricus* complex. Seeds were germinated and leaf material was collected from each of 100 plants.

2.2. DNA extraction, AFLP amplification and data analysis

Ten plants were grown from ten different accessions collected in different localities (Table 1) of the area of the *E. dahuricus* complex. Seeds were germinated and leaf material was collected from each of 100 plants. DNA from each of the 100 plants was digested using restriction enzymes EcoRI and Mse1. AFLPJ [7] was carried following the protocol using six primer pair combinations. Different sets of AFLP primer pair combinations were compared by Mantel tests between the individual similarity matrices computed from the AFLP data, the other by a number of consensus indices obtained by pairwise comparison of the trees of the 10 populations resulting from the input of the 10 genetic distance matrices [8] into the neighbor-joining algorithm [9]. Pairwise distances between all the haplotypes were computed from the Fst statistics over all loci, i.e. the unique polymorphic band profiles found among the 100 plants. Pairwise squared Euclidean distances and Euclidean distances (not squared) were obtained. A minimum spanning tree [10] was computed from each distance matrix and superimposed on a principal coordinate analysis [11] also computed from each of the Euclidean distance matrices. genetic structure of populations, and as a rough approximation, pairwise Fst=s between the populations over all loci are computed, and tested against the null hypothesis that there is no difference between the populations. The genetic structure of populations was subjected to analysis of variance of gene frequencies [12, 13, 14] which takes into account the number of differences, i.e. mutations, between haplotypes. Finally, geographical distances between populations, in all pairwise combinations, were obtained from their coordinates on the map and assembled into a distance matrix. Tests of association between the geographic distance matrix and the Nei [8] genetic distance matrix obtained above, were conducted by subjecting the two matrices to a Mantel test [15].

2.3. Hybridization

Hybridization was carried out according to [16]. The hybridity of F1 individuals was estimated by morphological markers and confirmed by SDS-electrophoretic technique. Seed fertility was estimated as the ratio of filled seeds to the total number of flowers in a spike [17].

3. RESULT AND DISCUSSION

3.1 Morphological analysis

The analysis of 39 accessions shows that morphological differentiation, exists between individuals from distant places and were therefore recognized as different species. For example, the main diagnostic character of *E. woroschilowii* - glaucous wax colour on all parts of plant - has a various degree of expression even for individuals, collected in the same localities. Glaucous coloured plants of the VLA-0016 accession were less expressed than in VLA-8642 plants, both collected in Vladivostok. Besides this, plants having this character were collected in Sichuan Province of China (H 8068, H 8107) and in the Altai mountains (GAL-8924, a less-gloucous collection). Diagnostic characters vary even outside the stated distribution of the

species. For instance, nondivergent lemma awns, a diagnostic character of *E. dahuricus* sensu G.Peshkova, were found on some individuals in Kirghizia (accession BAR-8818), Amurskiy region (ZEJ-9817, near Zeya town) and Primorskiy region (VLA-0015, Vladivostok). The rest of studied characters should be considered as Mendelian (hairy / glabrous leaves, its margins and sheaths; dark gray / yellow spikes; long / short lemma awns) and more or less adaptive (plant highness; leaves width).

3.2. Molecular analysis

Only 36 haplotypes were found from among the 100 plants sampled. The haplotypes were not shared among populations. The relationships between the haplotypes is visualized on a three dimensional graph of the principal coordinate plot with the minimum spanning tree connecting them (Fig. 1). Since the haplotypes were not shared between populations, a minimum spanning tree linking populations has also been drawn (top Fig. 1)

An examination of the pairwise Fst's between the populations over all loci indicates that in most populations it is high, with some exceptions, such as between population 1 and 2 or 3 and 4, but the test (not shown) rejected that populations 1 and 2 are different. The AMOVA results were summarized in two parts (Table 2). The first deals with the entire haplotypic data and the ten populations (Table 2 under A) where the amount of genetic variation among populations is a little over 2.5 times the amount of variation within populations. The fixation index was greater than 0.25 which indicates very great differentiation between populations. The second deals with the haplotypic data divided into four groups (Table 2 under B), i.e. the putative species in the complex, in which the variation among populations within groups was 3.5 times the amount of variation within populations, and in which the variation among groups was nil. Here too, the fixation indices among and within grouped populations was greater than 0.25 which indicates very great differentiation between populations whereas that among groups was negative, i.e. nil, indicating no genetic differentiation within any population in complex. Although all Mantel tests between haplotypes were significant, i.e. there was an association that was considered significant between all pairwise comparisons.

Mantel tests between geographical distances and the genetic distances between populations indicated the following. The correlation between the two matrices was a moderate 0.499, and the the hypothesis that there is no relationship between the geographic and genetic distances was rejected. In other words, there is a significant (p=0.9996) association between geographic distance and genetic distance between the 10 populations. None of the trees, including the tree obtained from the combined data, was congruent with the taxonomy of the species complex. We have seen, for example in the combined data which covers the greatest number of loci and haplotypes, that population 9, identified as. *E. dahuricus* was placed among *E. woroschilowii* populations 5 and 10; and that population 1, identified as *E. excelsus*, was placed among *E. dahuricus* populations 2 and 3. If we assume that populations were not misidentified according to [4, 6] It therefore appears that the differences do not support taxonomic status at the species level, and thus that the complex is but one species made of a gradient of isolated and different local populations. The only substantial justification for species status might be *E. tangutorum*, supported by a remarkable number of loci varying from the rest of the complex.

3.3. A nalysis of hybrid seed fertility

The preliminary data have been presented earlier that some genotypes of *E. dahuricus* complex form an united recombination gene pool [17]. Detail data in seed fertility of different hybrids are presented in the Table 3. Complete sterile were detected in 3 of 37 cross combinations of F_1 plants (2 of these 3 were reciprocal parental pair GAL-8924 - H 8068 from considerably distant regions) The combination H 8107 x PRA-8602 was formed by most differing morphologically parental individuals. In the F_2 population of this hybrid (25 plants) just one plant was completely sterile. Other shown a range of fertility 5,1-61,0 %. This population demonstrated ability of the hybrid for recombination of parental characteristics and consisted plants with most combinations of basic morphological characters for *E. dahuricus* complex: plant height, color of a plant, leaf width, hairiness of leaf surface and leaf-sheaths,

Table 1. Geographical and morphological characteristics of accessions of the *E. dahuricus* complex. **Bold** - accessions included in hybridization

Accession number	Identification	Leaves				Collection site	Popul Number	Group Taxon number
		Width, mm	Surface above	Margins	Lower sheaths			
				Siberia				
GAC-8914	*E. excelsus*	10-15	glabrate	hairy	Glabrate	Altai mts, near Ust-Sema, Katun river	1	1
GAL-8924	*E. excelsus*	8-13	glabrate	glabrate	Glabrate	Altai mts, near Inya		
CHI-8635	*E. dahuricus*	10-15	hairy	hairy	Hairy	Chitinskij region, near Zabaykalsk	3	2
				Primorskiy region of the Russian Far East				
MES-8709	*E. dahuricus*	12-18	Thin hairy	glabrate	glabrate	Near Posyet		
MES-8660	*E.woroschilowii*	12-18	glabrate	glabrate	glabrate	Near Posyet	10	4
POP-8403	*E. dahuricus*	12-18	hairy	glabrate	glabrate	Popov islandt		
VLA-8412	*E. excelsus*	15-20	hairy	hairy	Hairy	Vladivostok		
VLA-8642	*E.woroschilowii*	12-18	glabrate	glabrate	glabrate	Vladivostok	5	4
VLA-8684	*E. dahuricus*	15-20	glabrate	glabrate	glabrate	Vladivostok	9	2
ANI-8625	*E. excelsus*	15-22	hairy	hairy	hairy	Near Partizansk	4	1
PAR-9205	*E. excelsus*	15-20	hairy	hairy	hairy	Near Partizansk	8	1
PRA-8602	*E. excelsus*	15-20	hairy	hairy	glabrate	Near Ussurijsk		
ARS-8706	*E. dahuricus*	10-15	glabrate	glabrate	glabrate	Near Arsenyev		
				Kirghizstan				
BAR-8818	*E. dahuricus*	8-14	glabrate	glabrate	glabrate	Central Tien-Shan, near Barskaun		
CUR-8827	*E. dahuricus*	8-14	glabrate	glabrate	glabrate	Central Tien-Shan, near Kajy-Sie	2	2
BUD-8704	*E. dahuricus*	8-14	glabrate	glabrate	glabrate	Western Tien-Shan, near Talas		
				China				
H 8068	*E. dahuricus* var. *micranthus*	8-12	glabrate	hairy	glabrate	Province Sichuan		
H 8107	*E. dahuricus* var. *micranthus*	8-12	glabrate	thin hairy	glabrate	Province Sichuan		
H 8113	*E. tangutorum*	8-12	glabrate	glabrate	glabrate	Province Tibet	6	3
H 8363	*E. tangutorum*	8-12	glabrate	glabrate	glabrate	Province Tibet	7	3

Table 2. AMOVA results, hierarchical analysis of variance on the Euclidean distance, one group of populations.

A1: Among populations; A2: within populations; and four groups of populations, i.e. species as identified within the complex. B1: Among groups; B2: Among populations within groups; B3: Within populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
A1	7	1934.962	26.93253	71.94
A1	71	746.000	10.50704	28.06 FST=0.71936
Total	78	2680.962	37.43957	100
B1	3	872.260	0.73623	1.93 FCT=-0.01930
B2	6	1859.479	30.41066	79.72 FSC=0.78207
B3	89	754.200	8.47416	22.21 FST=0.77786
Total	98	3485.939	38.14859	100

lengths and thickness of spikes, lengths of lemma awns, etc. Similar phenomena have occurred in F_2 populations in cross combinations GAC-8914 x H 8107, PRA-8602 x VLA-8642 and GAC-8914 x CHI-8635.

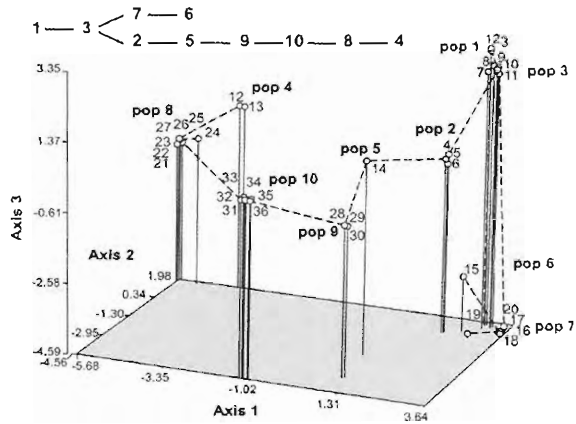

Figure 1: Principal coordinates plot of the 36 haplotypes in the Elymus dahuricus complex study. Minimum spanning tree superimposed, linking the 36 haplotypes, i.e. their points, on the principal coordinates plot. Variance explained: 87.9% axis 1; 10.6% axis 2; 1.1% axis 3.

Table 3. Seed fertility of hybrids in *E.dahuricus*-complex.

No	Cross combination	Highest value of seed set in generation %			
		F_1	N_{F1}*	F_2	N_{F2}*
1	GAC-8914 x GAL-8924	89,6	1	-	-
2	GAC-8914 x BAR-8818	43,5	3	-	-
3	GAC-8914 x CHI-8635	58,1	3	86,2	19
4	GAC-8914 x VLA-8642	5,1	3	85,1	5
5	GAC-8914 x VLA-8612	16,9	2	54,6	2
6	GAC-8914 x H 8107	22,2	3	89,7	20
7	GAL-8924 x H 8068	0	1	-	-
8	BAR-8818 x BUD-8704	41,1	2	-	-
9	BAR-8818 x VLA-8442	8,3	3	88,4	3
10	CUR-8827 x BAR-8818	90,4	2	-	-
11	CUR-8827 x H 8068	79,3	2	82,8	2
12	BUD-8704 x GAL-8924	20,0	2	-	-
13	PRA-8602 x GAC-8914	14,4	1	66,8	2
14	PRA-8602 x POP-8403	22,6	2	-	-
15	PRA-8602 x VLA-8642	31,1	2	86,4	26
16	ARS-8706 x BUD-8704	1,1	1	25,6	4
17	ARS-8706 x VLA-8412	6,1	3	26,7	3
18	ARS-8706 x CHI-8635	0,25	1	4,0	2
19	ARS-8706 x H 8363	0,2	3	0	2
20	ARS-8706 x H 8107	0,8	3	-	-
21	VLA-8642 x ANI-8615	13,3	2	59,0	3
22	POP-8403 x VLA-8642	69,0	3	94,0	5
23	MES-8709 x CHI-8635	4,8	2	45,9	3
24	MES-8709 x BUD-8704	8,0	2	51,2	6
25	H 8068 x GAL-8924	0	1	-	-
26	H 8107 x H 8068	69,1	3	91,0	29
27	H 8107 x PRA-8602	3,4	2	61,1	25
28	H 8107 x MES-8709	3,8	1	15,1	3
29	H 8113 x GAC-8914	1,3	1	-	-
30	H 8113 x BUD-8704	14,0	1	-	-
31	H 8113 x BAR-8818	37,7	3	95,8	3
32	H 8113 x ARS-8706	3,6	1	-	-
33	H 8113 x H 8068	50,9	3	92,8	3
34	H 8363 x GAL-8924	0,4	1	46,4	3
35	H 8363 x BAR-8818	48,2	1	-	-
36	H 8363 x VLA-8412	0	2	-	-
37	H 8363 x H 8113	83,7	3	-	-

*N_{F1} number of plants analized in F_1
N_{F1} number of plants analized in F_2
C_r sexual compatibility of genotypes

Thus, our study demonstrates that *E. dahuricus* complex can be considered a polymorphic taxon having a wide geographical range with a wide genetic variation on most characteristics. The different taxonomic species need to be relegated to infraspecific rank inside *E. dahuricus* Turcz. ex Griseb.

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REFERENCES

[1] Dewey DR: The genomic system of classification as a guite to intergeneric hybridization with the perennial Triticeae. In: Gustafson JP (ed.) Gene manipulation in plant improvement. New-York, Plenum Publ. Co. 209-279 (1984).

[2] Lu B-R: Biosystematic Investigations of Asiatic Wheatgrasses - *Elymus* L. (*Triticeae: Poaceae*), Alnarp, Sweden. 1-57 (1993).

[3] Cherepanov S K: Vascular Plants of Russia and Contiguous States. - St. Petersburg, Mir i semya 95. 992. (1995).

[4] Peschkova GA: Elymus L. In: Malyschev LI and Peschkova GA (eds.) Flora Siberiae 2, Poaceae (Gramineae). Novosibirsk, Nauka, p. 17-32 (1990).

[5] Peschkova GA: De generis Elymus L. (Poaceae) speciebus nonnulis e Sibiria [About some Siberian species of the genus Elymus L.] In: Novosti sistematiki vischikh rasteniy. Nauka, Leningrad 22: 39-43 (1985).

[6] Probatova NS: *Elymus* L. In: Harkevicz SS (ed.) Sosudistie rastenia Sovetskogo Dalnego Vostoka [Higher planst of the Soviet Far East]. Nauka, Leningrad 1: 110-126 (1985).

[7] Vos P, Hogers R, Bleeker M, Reijans M, van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M: AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407-4414 (1995).

[8] Nei M: Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA 70: 3321-3323 (1973).

[9] Saitou N, Nei M: 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425 (1987).

[10] Prim RC: Shortest connection networks and some generalizations. Bell System. Tech. J. 36: 1389-1401 (1957).

[11] Gower JC: Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325-338 (1966).

[12] Weir BS, Cockerham CC: Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370 (1984).

[13] Excoffier L, Smouse PE, Quattro JM: Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479-491(1992).

[14] Weir BS: Genetic data analysis II. Methods for discrete population genetic data. Sinauer Associates, Sunderland, Mass. (1996).

[15] Mantel N: The detection of disease clustering and a generalized regression approach. Cancer Res 27: 209-220 (1967)

[16] Lu B-R, Bothmer R von: Intergeneric hybridization between *Hordeum* and Asiatic *Elymus*. Hereditas 112: 109-116 (1990).

[17] Agafonov AV: The Principle of Recombination Gene Pools (RGP) and Introgression Gene Pools (IGP) in the Biosystematic Treatment of Elymus Species. In: Proc. 2nd Int. Triticeae Symp., Logan, Utah, USA. 254-260 (1994).

GENEPOOLS IN SH-GENOMIC ELYMUS SPECIES IN BOREAL EURASIA

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ABSTRACT: Siberia is the area with highest species diversity for the genus *Elymus* in Russia. According to the Floras, there are about twenty SH genome containing species distributed this region. Based on the principle of recombination gene pools (RGP) and introgression genepools (IGP), a biosystematic study of these taxa was performed. More than 250 infraspecific and interspecific cross combinations were made and the offsprings were checked for the degree of seed fertility in F1 to F3 generations. The results clearly show that many taxa are not well isolated from each other and hybridization may lead to introgression between the different taxa. One example is *E. caninus* (L.) L. and *E. mutabilis* (Drob.) Tzvel., where typical forms are both morphologically and ecologically well-defined species but where introgression orlimited recombination occurs when they grow together. In some cases free genetic recombination occur between "species". Thus, genetic analysis of the diagnostic characters in the species group *E. subfibrosus* (Tzvel.) Tzvel., *E. charkeviczii* Probat. and *E. mutabilis* from Kamchatka displayed simple Mendelian segregations, and this group corresponds to a distinct local RGP. The introgressive processes between biotypes of the *E. macrourus* group and *E. sibiricus* have been registered in crossing experiments. Based on the results obtained in this study, a model of the existing genepools among the SH genome species in Siberia is presented and discussed

1. INTRODUCTION

Elymus species - perennial self-fertilizing Triticeae taxa having different combinations of basic genomes. Apomixis has been identified in some Australian Elymus species only [1], and *Elymus* seeds are resulted of normal meiosis. Great areas, number of isolated habitats and tendency for cross polination in most Elymus species lead to easilier microevolutional differentiation (read, speciation). Self-pollination makes possible to pass through natural selection any new character or its new combination during just a few generations

According to recent data [2]) there are 72 Elymus species in the former Soviet Union territory. Not less than 28 of these have SH-genomic constitution, about 20 species of this group are distributed in Siberia and Russian Far East [3, 4, 5]. It was suggested, that some taxonomical species differ in morphological characters, which correspond to the notion of variability in heterogeneous Mendelian populations. The principle of recombination (RGP) and introgression (IGP) gene pools has been suggested for investigation of relationships in Elymus species [6]. Polymorphic endosperm storage proteins (prolamine-gluteline complex) as genetic markers are most suitable for express identification of natural and hybrid biotypes and populations in the genus Elymus [7, 8]. The main advantage of grain protein as genetic markers is that one does not need living material for analysis since proteins keep electrophoretic properties for many years.

2. MATERIALS AND METHODS

Living material of 20 Elymus species was collected in the wild or obtained from Swedish and North-American Collections. Accessions of different taxa were cultivated in the greenhouse and outdoors. Inter- and intraspecific hybridization of concrete biotypes in different combinations was carried out with morphological or electrophoretic control of reality of crossing. Taxonomic identification of accessions was made following [3] for Siberian accessions, and [4] for Far-Eastern ones. Hybridization was carried out according to [9]. The hybridity of F1 individuals was estimated by morphological markers and confirmed by SDS-electrophoretic technique. Seed fertility was estimated as the ratio of filled seeds to the total number of flowers in a spike [6]. Accessions included to interspecific hybridization and their origin are shown in the Table 1.

3. RESULT AND DISCUSSION

Based on the results obtained in hybridological study, an empiric scheme of influence of pollination type on plant seed fertility (SF) in *Elymus* populations is presented at the Fig. 1. The model of the existing gene-pools among the SH genome species in Siberia has been created coming from the SF data of hybrids F_1-F_3 in different intra- and interspecific cross combinations.

Brief comments are following for studied species relationships:

1. E. alaskanus s.l. The range of morphological and geographical differentiation and SF of hybrids show complicated structure within the complex. North European accessions form fertile hybrids between each other and some Siberian biotypes, but the accession *E. kronokensis* KES-9603 from Kamchatka demonstrated absolute post-zygotic isolation barrier with all Eurasian accessions of the this complex and those of the close related species *E. transbaicalensis*. Sexual compatibitity of the latter with the Scandinavian accession H 10356 have been registrated as **a2**-level.

2. ***E. caninus* - *E. mutabilis*** relationships. Both species normally form individual RGPs. Even the accessions from marginal populations of *E. caninus* such as Is 9404 (Iceland) and H 4111 (Pakistan) have a sexual compatibility with Siberian ones, and some hybrid plants in the F_2 reached a value of SF more than 30 %. Analysis of interspecific hybrids in three cross combinations showed that all plants were more or less fertile. Highest value of SF in the generations F_2 were 58,6 - 78,9 % for different combinations, and plants within populatins showed a recombination of morphological characteristics of parental accessions (species). Easy genetic exchange between species is corroboraited by a number of nutural accessions which combaine some morphological characters of species. For instance, our morphological study of the original herbaria in the Altaic Botanical Garden (Leninogorsk, Kazakhstan) makes a basis to assume that species *E. marmoreus* Kotuch., *E. sibinicus* Kotuch. [10] and *E. ubinica* Kotuch. [11] represent *E. caninus* **x** *E. mutabilis* hybrid individuals which have more or less value of SF. Thus, both species form united gene-pool which correspond to RGP at least for Siberian populations.

3. ***E. jacutensis* - *E. macrourus*** complex. It was shown that two species form a single RGP [12]. Some hybrids F_1 demonstrated a reduced SF such as in cross combination *E. macrourus* JAC-8922 x *E. jacutensis* JAC-8901. Seed fertility of hybrid plant F_1 was 3,8 % in free pollinations mode. One plant of the population F_2 morphologicaly looked like additional spontaneous hybrid x ***E. sibiricus***. Just 2 grains have been collected from 16 spikes. Plants F_3 had SF 0,0% and 26,0% respectively. In the F_4 an indidvidual having seed fertility close to normal (54,6%) have been obtained. It combines morphological characteristics of *E. macrourus* and *E. sibiricus* and was given a code JMS-9500. A new hybrid F_1 (F_5) JMS-9500 x *E.sibiricus* KAM-9217 was criated which had SF = 7,6% and a habitus like a typical *E. sibiricus* but pilose lemmas and shorter lemma awns. Range of SF in the F_2 (12) was determined as 0 - 70,1%. Segregation of morphological characteristics of three parental species has been ocured.

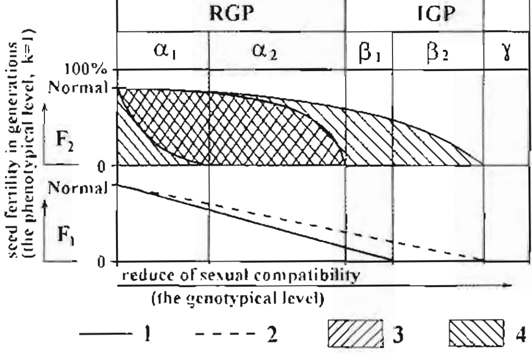
4. Five interspecific hybrids between ***E. komarovii*** and ***E. transbaicalensis*** have been analyzed. The level of fertility in F_1 was very low in three combinations, with SFmax values of less than 1%, indicating that sterility barriers exist between the two taxa [13]. However, two F_1 hybrids had an SF value as high as 5,8% and 49%, highest value of SF in F_2 populations were 52,5 and 49,2 respectively. Recombination of diagnostic characters of two species has been occurred. It is why both species are placed to the same RGP at the current model.

5. Kamchatic species (***E. mutabilis, E. subfibrosus, E. charkeviczii, E. kamczadalorum,***) form two separate RGP. Electrophoretic analisis of endosperm proteins showed a distinct specificity of the first species and the group of accessions named as different taxa. Possibility of mutual introgression between RGPs probably is pointed by protein band of REM-26 which is a characteristic of the second RGP (Fig. 2).

Table 1. Eurasian SH-genome Elymus species studied by intraspecific crossing and origin of accessions included to interspecific hybridization

Species	Number of intraspecific cross combinations	Accessions for interspecific hybridization	Origin
E. alaskanus s.l.	8	Sv 9335S Is 9401	weden, Norrboten province, Kopparasen Iceland Eyjafjardarsysla, Akureyri
E .caninus	46	ELC-8306 GAC-8921 H 7550 GAT-9210 BEL-9308	Russia: Novosibirsk, Eltzovka Russia: Altai mts, Katun river, near Ust-Sema China: province Xinjiang Russia: Altai mts, Teletzkoe lake, north bank Russia: Altaiskiy kraj, Belokurikha river
E. charkeviczii	-	KES-9632	Russia: Kamchatka, near Esso
E. fibrosus	2	H 10339 BSK-9303	Finland: Pelkosniemi, Kairala Russia: Altaiskiy kraj, Bijsk
E. hyperarcticus		TUV-9509	Russia: Tuva, Ular river
E. jacutensis	4	GAC-8958 JAC-8901 AMU-9001	Russia: Altai mts, Katun river, near Ust-Sema Russia: Jacutia, Timpton river Russia: Amurskaya oblast, Zeya river
E. kamczadalorum		KSO-9605	Russia: Kamchatka, near Elyzovo, Sosnovka
E. komarovii	14	ACH-8920 GAL-8425 GUK-8903	Rassia: Altai mts, Chike-Taman pass Russia: Altai mts, Kurieskij range, Bashkaus Russia: Altai mts, near Ust-Koksa
E. kronokensis		KES-9603	Russia: Kamchatka, near Esso
E. macrourus	3	JAC-8922 GAL-8959	Russia: Jacutia, Timpton river Russia: Altai mts, Ongudaiskij district
E. pubiflorus	-	JAC-8919 SLU-9702	Russia: Jacutia, Timpton river Russia: Irkutskaya oblast, near Sludjanka
E. sibiricus	36	SH-236 KAM-9217	Awnless artificial biotype Russia: Kamchatka, near Lazo
E. subfibrosus	-	KES-9612 KES-9634	Russia: Kamchatka, near Esso Russia: Kamchatka, near Esso

6. ***E. ircutensi*** seems to be an assembly species. An accession from classic place (Burjatia, Irkut river, Mondi) bilongs to ***E. macrourus*** - ***E. jacutensis*** complex as hybridological analysis was resulted. Other studiedaccessions are morphologicaly deviating biotypes of ***E. transbailensis*** (GAC-8923, TUV-9937) and ***E. caninus*** (GAC-9821, ALP-9734). a -level is on the whole a recombination gene pool (RGP). Demarcation between a**1** (free recombination) and a**2** (limited recombination) levels can be determined in F2 population were completely sterile plant have to be occured for a**2**-level.



1 - highest seed fertility (SF) of self-pollinated hybrids in F_1

2 - highest SF of free-pollinated hybrids F_1

3 - range of SF variation in self-pollinated F_2 population

4 - range of SF variation in free-pollinated F_2 population

Figure 1: Empiric scheme of the RGP-IGP principle for the genus Elymus; k=1 means optimal environmental conditions excluding spontaneous aberrations.

a**1**-level. A gene pool with free recombination. The meiosis is normal. Segregation of the Mendelian characters in F_2 is in accordance with the general laws of cytogenetics. Highly correct genetic analysis of alternative characters can be done.

a**2**-level. A gene pool with limited recombination. The meiosis proceeds with some or other disturbances. The segregation of qualitative characters in F_2 may have some deviations from the Mendelian laws. Self-pollination provide a range of SF from zero to closely normal.

b -level is on the whole an introgression gene pool. Seed (read sexual) reproduction is possible after back-crossing or cross pollination(one or more) in which introgressive phenomena are observed

b **1**-level. SF of self-pollinated F_1 hybrids is above zero, but F_2 plants are completely sterile in self-pollination

b **2**-level. F_1 hybrids in self-pollination are sterile,. The border between b**1**- and b**2**-levels is not clear and can be observed as SF on hybrids F1.

g -level Hybrids exist in vegetative forms. The sexual process can be restored by means of special procedures only (doubling the chromosome set and so on).

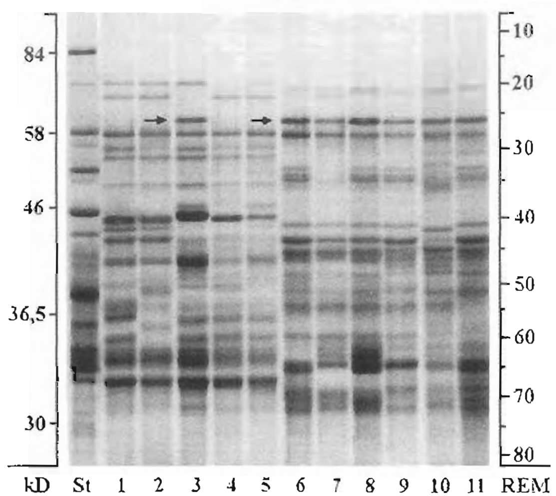


Figure 2: SDS-PAGE polypeptide spectra of endospem proteins in Kamchatic *Elymus* species.Variants +Me. St - *Elymus sibiricus*, ALT-8401.

1-5. *E. kamzchadalorum*; 1.KAM-9201; 2. KAM-9204; 3. KAM-9205; 4. KTO-9609; 5. KSO-9605;
6. *E. subfibrosus* KSO-9603; 7. *E. charkeviczii* KAD-9614; 8. *E. subfibrosus* KES-9634;
9. *E. charkeviczii* KES-9632; 10. *E. mutabilis* (?) KES-9633; 11. *E. mutabilis* (?) KES-9639.

7. All studied biotypes of ***E. sibiricus*** (36 cross combinations) form a big RGP widespreaded from Europe to Far East (**a1**- and **a2**-levels). Some natural biotypes of the species which have anormal hairy lemmas and tendency for modifications of the character "2 spiklets / node" to "single spiklets", usually are recognized as *E. pubiflorus* (accessions SLU-9702 and ACH-8918). Beside these, the accession have been collected (JAC-8919) which formed complete sterile hybrids with an accession of *E. sibiricus* SH-236.
8. Eurasian accessions of ***E. trachycaulus*** complex including ***E. novae-angliae*** showed relations as RGP in geographical range from Europe to China and Russian Far East.
9. ***E. viridiglumis*** was studied as accessions ACH-8950 (= H 3529, Altai Moutain) and SBI-9544 (Western Sajan). This species represents a long awned (up to 15mm) form of ***E. mutabilis***. The character is controlled by a single dominant gene. Relationships of *E. mutabilis* and *E. transbaicalensis* in detail: see Kostina et al., this volume.

10 ***E. fibrosus* x *E. caninus*** hybrids obtaned in 2001 showed comlpete sterility in the combination H 10339 x BSK-9302 and SF=2,6% in the combination BEL-9308 x BSK-9302.

11. *E. confusus*, *E. sajanensis*, *E. vassiljevii* are studied currently as well as a gpoup of Siberian *Elymus* accessions which can not be recognized using a stadard procedure.

* The most number of RGPs are united to general IGP. We came from that it is sufficient to exist some biotypes of separate RGPs to provide genetic introgression betwwen ones, i.e. some inter-IGP hybrids should possess low seed fertility after back crossing. For example, ***E. lineicus*** Kotuch. have been discribed as hybrid combination of *E. sibiricus* and *E. transbaicalensis* [11]. Two alive seeds were found out from the original herbarium. One plant (conditional F_2) was sterile, but another one was resulted 14 seeds. Three plants F_3 were ferlile in a SF range 2,6-8,0%., keeping mophological intermediate or discreete characteristics of parental species.

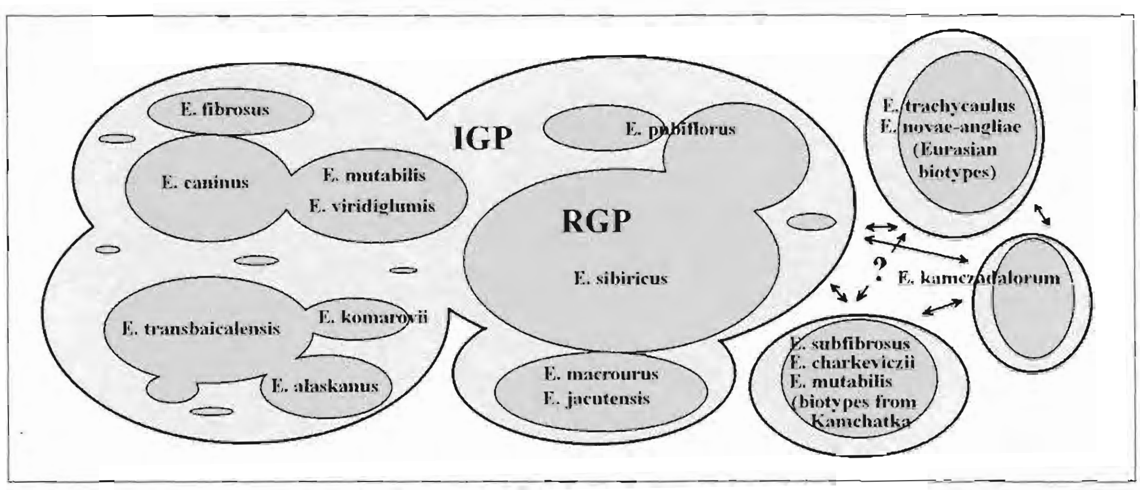


Figure 3: A current model of genepools in SH-genomic Elymus species in boreal Eurasia.*

REFERENCES

[1] Liu W-Z, Wang RC, Carman JG: Hybrids and backcross progenies between wheat (Triticum aestivum L.) and apomictic Australian wheatgrass: Karyotypic and genomic analysis. Theor. Appl. Genet. 89: 599-605 (1994).

[2] Cherepanov SK: Vascular Plants of Russia and Contiguous States. - St. Petersburg, Mir i semya 95. 992. (1995).

[3] Peschkova GA: Elymus L. In: Malyschev LI and Peschkova GA (eds.) Flora Siberiae 2, Poaceae (Gramineae). Novosibirsk, Nauka, p. 17-32 (1990).

[4] Probatova NS: Elymus L. In: Charkevicz SS (ed.) Plantae Vasculares Orientalis Extremi Sovietici 1. Leningrad, Nauka, p. 110-126 (1985).

[5] Lu B-R: Biosystematic investigations of asiatic wheatgrasses - *Elymus* L. (*Triticeae: Poaceae*), Alnarp, Sweden (1993).

[6] Agafonov AV: The Principle of Recombination Gene Pools (RGP) and Introgression Gene Pools (IGP) in the Biosystematic Treatment of Elymus Species. In: Proc. 2nd Int. Triticeae Symp., Logan, Utah, USA. 254-260 (1994).

[7] Kostina EV, Agafonov AV, Salomon B: Electrophoretic properties and variability of endosperm proteins of *Elymus caninus* (L.) L. In: Jaradat AA (ed.) Triticeae III. Enfield, New Hampshire, Science Publishers, USA p. 265-272 (1998).

[8] Agafonov AV, Baum BR: Variation of endosperm proteins in the complex of Elymus trachycaulus. In: Jaradat AA (ed.) Triticeae III. Enfield, New Hampshire, Science Publishers, USA. p. 273-282.(1998).

[9] Lu B-R: Biosystematic Investigations of Asiatic Wheatgrasses - *Elymus* L. (*Triticeae: Poaceae*), Alnarp, Sweden. 1-57 (1993).

[10] Kotukhov Ju: New species of the genus *Elymus* (*Poaceae*) from Eastern Kazakhstan. Botanical Journal (S-Petersburg)., v.77, 6: 89-93 (1992).

[11] Kotukhov Ju: The new species of the genus Elymus L. (Poaceae) from Western Altai. Turczaninowia, v.2, 4: .5-10 (1999).

[12] Agafonov AV, Agafonova OV, Salomon B, Lu B-R: Reproductive compatibility of biotypes of *Elymus macrourus* and *Elymus jacutensis*, and genetic analysis of the diagnostic character of awnedness. Siberian Journal of Ecology, III, 6: 525-530 (1996).

[13] Agafonov AV, Salomon B, Kostina EV, Diaz O: Biosystematic relationships between *Elymus komarovii* (Nevski) Tzvel. and related species. In: Jaradat, A.A. (Ed.). Triticeae III. Science Publishers, Enfield, New Hampshire, USA p. 77-84 (1998).

INFERRING RELATIONSHIPS THROUGH THE STUDY OF MULTICOPY GENES, WITH SPECIAL EMPHASIS ON THE TRITICEAE (POACEAE)

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ABSTRACT: Sequence information from multicopy genes has been widely used for phylogenetic inference. The 5S rRNA genes as well as the two internal transcribed spacers (ITS1 and ITS2) and the intergenic spacer (IGS) of the 18S-26S rDNA genes have been used at the specific, generic, familial and tribal levels. Direct sequencing of PCR products is a widespread approach. Here we argue in favor of an alternative approach, namely the sequencing of individual clones followed by careful alignment to discern groups of putative orthologous sequences. Based upon our work on the 5S rDNA genes from *Triticum* and *Hordeum*, and a re-analysis of existing data from the ITS1, ITS2 and IGS regions, we are able to identify groups of putative orthologous sequences, named Aunit classes@, that are useful for the inference of relationships among species and genera. Furthermore, putative orthology is a prerequisite for the inference of phylogenetic relationships and serves as a test of real orthology. Comparisons of paralogous sequences from different unit classes can used to infer evolutionary relationships among unit classes. In several cases, further analysis allowed the assignment of unit classes to specific haplomes.

INTRODUCTION

The reconstruction of the phylogenetic history of taxa by inferring relationships among genes within them, requires the comparison of orthologous genes [12].To conduct a phylogenetic analysis with DNA data, one assembles sets of sequences of putative orthology to compare individual nucleotide positions, i.e. positional homology [12], via alignments and infers from these alignments phylogenetic relationships.

Direct sequencing of PCR amplimers is a common experimental strategy, however, this strategy raises the likelihood that for multicopy genes a mixture of sequences is observed in a single run. Unless there is extensive gene conversion or essentially a single sequence type exists, the use of such sequence data for phylogenetic analysis is inappropriate as it assumes that all the sequences are orthologous. Orthology is an hypothesis that is tested by phylogenetic analysis and must first be demonstrated. Hence sequencing of individual clones is a prerequisite for obtaining the potential sequence variants which are likely to be found among multicopy genes. Potentially these variants may be grouped into several paralogous types.

We argue that the sequencing of large numbers of individual PCR generated clones is necessary in order to assign individual sequences into putative orthologous groups prior to conducting phylogenetically sound analysis. This approach depends upon careful sequence alignments involving iterative cycles of computer assisted alignment followed by visual adjustments, i.e. manual intervention to determine patterns, since no ideal multiple local alignment algorithm exists today. The process determines assumed orthologous groups based upon these alignments. While more laborious than the direct sequencing of PCR products, the cloning and sequencing of different repeat units is necessary in order to recover information useful for phylogenetic estimation [18].

We demonstrate the utility of our strategy by reference to the 5S rDNA genes in *Triticum* [3, 4, 5; 6, 7, 8, 9, 10]. Then, we re-analysed published data from the intergenic spacer (IGS) region of the 18-26S rDNA [19], and from the internal transcribed spacers (ITS1 and ITS2) also of the 18-26S rDNA [22] both from *Triticum* and *Aegilops*. Our approach will show that these cloned sequences can be assigned to putative orthologous groups from which useful phylogenetic inferences can be drawn.

MATERIALS AND METHODS

2.1. Data

1) 5S rDNA sequences: Baum and Bailey [5] described 115 repeat unit clones and assorted them into 9 orthologous groups or Aunit classes@. Additional sequences, derived form single clones, were available from GenBank7. 2) IGS sequences: 25 sequences from *Triticum* and *Aegilops* [19] were retrieved from GenBank7 3) ITS1 and ITS2 sequences: we identified in GenBank7 23 ITS1 and 25 ITS2 sequences from *Triticum* and *Aegilops* [22]. The non-transcribed spacer (NTS) of 5S rDNA, the IGS and the two ITS regions demonstrate greater variability than their respective gene sequences and thus are the more useful regions for phylogenetic purposes.

The following nomenclature was used: an IGS sequence denoted Au07_T_pal refers to a sequence that resulted from PCR amplification of *Triticum paleocolchicum* DNA with the Au primers described in Sallares and Brown [19]. An ITS1 sequence with the name ASH242406 (the GenBank7 accession) indicates a sequence resulting from PCR amplification of *Aegilops sharonensis* DNA.

2.2. Methods

To identify putative orthologous groups, we employ an iterative approach consisting of repeated cycles of sequence analysis by alignment, finding the most similar sequences in GenBank7 using BLAST (Basic Local Alignment Search Tool [1]), and making adjustments by visual inspection [13], until common patterns are found. Previously we demonstrated that the assessment of putative orthology does not require complete nucleotide similarity, and that differences in size between sequences, including large deletions, do not automatically indicate assumed paralogy.

Alignments generated by CLUSTAL W [20] were refined by manual editing (GeneDoc8 2.4.002, [17]). To infer unit classes, i.e. putative orthologous groups, alignments were visually inspected. Apparently similar clones were separately re-aligned in repetitive cycles manually and by computer until they could be subdivided into similar groups, called unit classes. The different patterns recognized by visual inspection [13] can be used to distinguish among unit class. An optimal subdivision implies that sequences assigned to a unit class share patterns of similarity defined by multiple stretches of identical sequences, regardless of the lengths of its members. Once deduced, the grouped sequences were pulled together and realigned to visualize the similarities among the different cloned sequences within the unit classes and portray the differences between the unit classes. Subsequently, for each unit class a consensus sequence was drawn. A more detailed discussion of issues related to this approach can be found in Baum et al.[11]

Representatives of each Aunit class@ were matched by computer searches for Ahomology@ (nucleotide pattern similarity) with the sequences in GenBank7, using the NCBI (National Center for Biotechnology Information) BLAST network service and programs [1]. The identified sequence was aligned *in toto* with each representative unit class or with all sequences in the unit class.

3. RESULTS

3.1. 5S rDNA

Consensus sequences of nine unit classes [5] were obtained. The relationships between them (Fig 1) showed that the short unit classes form a group separate from the long unit classes and that they differ among themselves as much as from the long unit classes. Direct sequencing of the PCR amplimers would not have allowed the detection of these groups.

Symbols have been assigned previously to different haplomes in the *Triticeae* [15]. Based upon these designations, and the distribution of unit classes among species, we have been able to tentatively name the unit classes to reflect the presence of these haplomes. For example, two unit classes found in the diploid species *T. monococum* L. were designated the short A1 and the long A1 unit classes to reflect the **A** haplome assignment. For similar reasons, the unit classes found in *Aegilops searsii* M. Feldman et M. Kislev ex Hammer, *A. sharonensis* Eig and *A. speltoides* Tausch were assigned to the **S** haplome. The unit classes found in *A.tauschii* Cosson, also a diploid species, were designated as short D1 and

long D1 to reflect the **D** haplome [16]. This approach requires sufficient sampling of the sequence diversity present in the genome.

Thus, we were able to identify several different groups of putative orthologous sequences and to tentatively assigned them to haplomes, as we had done before in *Hordeum*. Without cloning and sequencing, groups would never have been detected.

3.2. IGS

An alignment of all 25 sequences reported by Sallares and Brown [19] was performed, however, unlike these authors we did not subdivide the sequences into regions. These authors employed specific primers to generate PCR-amplimers that were directly sequenced. Our analysis confirmed their findings, but also revealed additional groupings (Fig. 2). A group of ten orthologous sequences may be assigned to the **Au** haplome based on the use of the *Au*-specific primers. A second, orthologous group (Am01, 03, 04, 05) may be assigned to the **Am** haplome, based upon the use of *Am*-specific primers. A third group comprises Am02, Am07, Am08, Au10 and Am06 derived from both primer sets. The fourth group, from Ss01 down to G01, is slightly heterogeneous. Possible subgroups can be identified, e.g., Ss01 with Si01; B01-B03; G01 group, althought there may be too few sequences to justify them with confidence. Members of group 4 may be assigned to the **B** haplome. Fig. 2 shows the relationship between the four orthologous groups.

3.3. ITS1 and ITS2

Alignment of the ITS1 sequences of Zhang et al. [22] reveals four possible groups. Group 1 is assignable to the **G** haplome in *T. turgidum*, group 2 is assignable to the **B** haplome in *T. timopheevii*, . Group 3 contains sequences from five *Aegilops* species (**S** haplome) and group 4 contains sequences that differ from those of the other groups. Since group 1 has **G** haplome sequences, group 4 has **A** haplome sequences. This leaves two sequences assignable to the **D** haplome found in *A. tauschii*. These relationships are depicted in Fig. 3.

The ITS2 alignment depicts five orthologous groups (Fig. 4) assignable to the **G** haplome, the **S** haplome, the **B** haplome and the **A** haplome. A single clone, from *A. tauschii* may belong to the **D** haplome.

In brief, we are able to identify different groups of putative orthologous sequences based upon their different patterns in the alignments, and to assign them to haplomes. Moreover, our approach is more general and does not require specific primers [19] nor knowledge of primer sites.

4.DISCUSSION

To infer the correct phylogenetic history between taxa, based upon DNA sequence analysis, the key issue is whether the sequences to be studied are orthologous or paralogous. Analysis that group them together can be confounded by the different histories of the sequences being compared. Orthology is determined by reference to an explicit phylogenetic hypothesis. There is a need to distinguish between putative orthologous and paralogous sequences in multicopy genes prior to conducting an alignment followed by a cladistic analysis. Each unit class needs to be analyzed separately as an assumed orthologous group. And if the goal is to carry out a phylogenetic analysis among the related genes in the multigene family, then paralogous genes are needed, as in the 5S rDNA example (Fig 1). When paralogues are used haphazardly, without knowledge of their status, the resulting phylogenetic analysis might yield erroneous results.

Thus, for the phylogenetic analysis of multicopy genes, there is a need to routinely clone and sequence a large number of individual repeats and to classify them into putative orthologous groups by the methods we have described. The cases described in this paper and our previous publications, namely the 5S rDNA, IGS, ITS1 and ITS2 regions in the Triticeae, are examples of such an effort that yielded unequivocal results when different unit types were distinguished through sequencing of clones. Direct sequencing of PCR amplimers would have yielded mixtures of sequences. Moreover, the groups that have been identified may be tentatively assigned to haplomes.

The sequences that we have investigated are tandemly repeated and all or part of them may be subject to homogenization due to concerted evolution that can result from unequal crossing-over, gene conversion or both. Concerted evolution has been proposed to explain the similarity of tandem repeats found at the same locus (intralocus homogenization) and tandem repeats found in arrays located at different loci (interlocus homogenization) [2,13]. Our ability to discern orthologous groups indicates that the identity of the classes was maintained. Either the forces of concerted evolution were weak or genetic barriers preventing frequent chromosomal exchanges must exist, thus leading to the development and maintenance of

chromosome-specific arrays [14]. In such situations we maintain that sampling of a large number of clones is necessary for determining the unit classes present in each genome and inferring their evolutionary histories.

We are aware of the possibility that only one array-type (unit class) may be detected, e.g., the ITS1 and ITS2 of *Gossypium* [21], due to homogenization or deletion and/or expansion of units at a particular locus or some other reason such as preferential PCR amplification. In these situations, direct sequencing is a valid approach. However the *a priori* assumption of one array type, without experimental validation, may lead to erroneous phylogenetic inference. We are also aware of the possibility that template switching during PCR amplification is a possible complication. The analysis of large numbers of clones should reduce the influence of a sequence artificially generated *in vitro* by PCR

REFERENCES

[1] Altschul SF, Gish W, Miller W, Myers EW,Lipman DJ: Basic local alignment search tool. Jour. Mol. Biol. 215: 403-410 (1990).

[2] Arnheim N: Concerted evolution of multigene families. *In* Evolution of genes and proteins. In: Nei M, Koehn RK (eds) Sinauer, Sunderland, MA, U.S.A. pp. 38-61 (1983).

[3] Baum BR, Bailey LG: The molecular diversity of the 5S rRNA gene in *Kengyilia alatavica* (Drobov) J.L. Yang, Yen & Baum (Poaceae: Triticeae): potential genomic assignment of different rDNA units. Genome 40: 215-228 (1997).

[4] Baum BR, Bailey LG: The 5S rRNA gene diversity in *Kengyilia rigidula* (Keng & S.L. Chen) J.L. Yang, Yen & Baum (Poaceae: Triticeae): possible contribution of the H genome to the origin of Kengyilia. Genome 43: 79-85 (2000)

[5] Baum BR, Bailey LG: The 5S rRNA gene sequence variation in wheats and some polyploid wheat progenitors (Poaceae: Triticeae). Genet. Res. Crop Evol 48: 35-51 (2001).

[6] Baum BR, Johnson DA: The molecular diversity of the 5S rRNA gene in barley (*Hordeum vulgare*). Genome 37: 992-998 (1994).

[7] Baum BR, Johnson DA: The 5S rRNA gene units in ancestral two-rowed barley (*Hordeum spontaneum* C. Koch) and bulbous barley (*H. bulbosum* L.): sequence analysis and phylogenetic relationships with the 5S rDNA units of cultivated barley (*H. vulgare* L.). Genome 39: 140-149 (1996).

[8] Baum BR, Johnson DA: The 5S rRNA gene in sea barley (*Hordeum marinum* Hudson sensu lato): sequence variation among repeat units and relationship to the X haplome in barley (*Hordeum*). Genome 41: 652-661 (1998).

[9] Baum BR, Johnson DA: The 5S rRNA gene in wall barley (*Hordeum murinum* L. sensu lato): sequence variation among repeat units and relationship to the Y haplome in the genus *Hordeum* (Poaceae: Triticeae). Genome 42: 854-866 (1999).

[10] Baum BR, Johnson DA, Bailey LG: Analysis of 5S rDNA units in the Triticeae: the potential to assign sequence units to haplomes. In: Jaradat AA (ed) Triticeae III. Science Publishers Inc., Enfield, NH, USA (1998). pp. 85-96.

[11] Baum BR, Johnson DA, Bailey LG: Defining orthologous groups among multicopy genes prior to inferring phylogeny, with special emphasis on the Triticeae (Poaceae). Can. Jour. Bot. Submitted. (2001).

[12] Hillis DM, Moritz C, Porter CA, Baker RJ: Evidence for biased gene conversion in concerted evolution of ribosomal DNA. Science 251: 308-310 (1991).

[13] Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA: Nucleic Acids IV: sequencing and cloning. In: Hillis DM, Moritz , Mable BK (eds) Molecular Systematics, 2nd ed. 321-381. Sinauer Associates Inc., Sunderland, Mass. USA (1996).

[14] Kellogg EA, Appels R: Intraspecific and interspecific variation in the 5S RNA genes are decoupled in diploid wheat relatives. Genetics 140: 325-343 (1995).

[15] Kimber G, Sears ER: Assignment of genome symbols in the Triticeae. In: Proc. 6th Int. Wheat Genet. Sym. Kyoto, Japan. pp. 1195-1196 (1983).

[16] Löve Á: Conspectus of the Triticeae. Feddes Repertorium 95: 425-521 (1984).

[17] Nicholas KB, Nicholas JB Jr: GeneDoc8: A tool for editing and annotating multiple sequence alignments. V.2.4.002, distributed by the authors (1997).

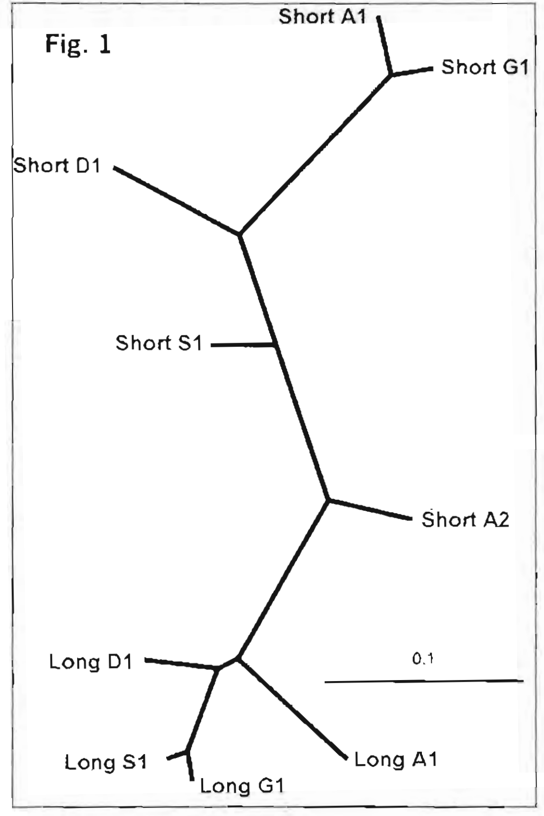
[18] Ritland CE, Ritland K, Straus NA: Variation in the ribosomal internal transcribed spacers (ITS1 and ITS2) among eight taxa of the *Mimulus guttatus* species complex. Mol. Biol. Evol. 10: 1273-1288 (1993).

[19] Sallares R, Brown,TA: PCR-based analysis of the intergenic spacers of the Nor loci on the A genomes of Triticum diploids and polyploids. Genome 42:116-28 (1999).

[20] Thompson JD, Higgins DG, Gibson,TJ: CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673-4680 (1994).

[21] Wendel JF, Schnabe, A, Seelanan T: An unusual ribosomal DNA sequence from *Gossypium gossypoides* reveals ancient, cryptic, intergenomic introgression. Mol. Phyl. Evol. 4: 298-313 (1995).

[22] Zhang WJ, Qu,LJ, Gu HY. Chen ZL:. ITS1 and ITS2 sequences of four possible donors to bread wheat genome and their phylogenetic relationships. Acta Bot. Sinica 40: 994-1000 (1998).



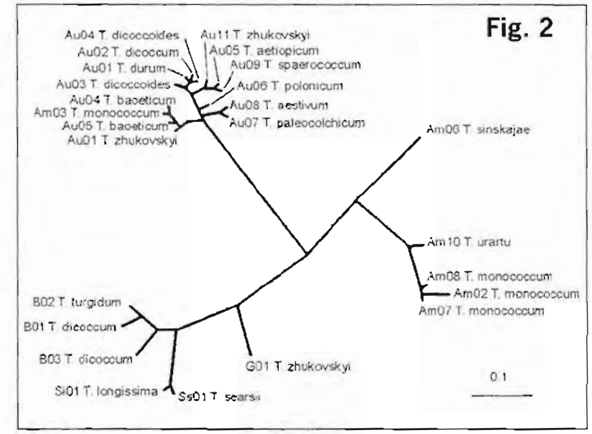
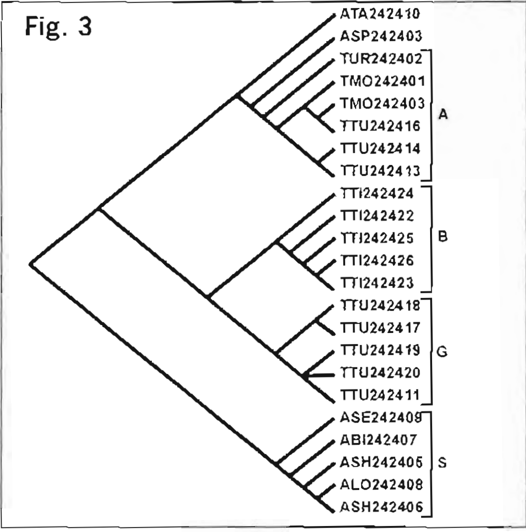


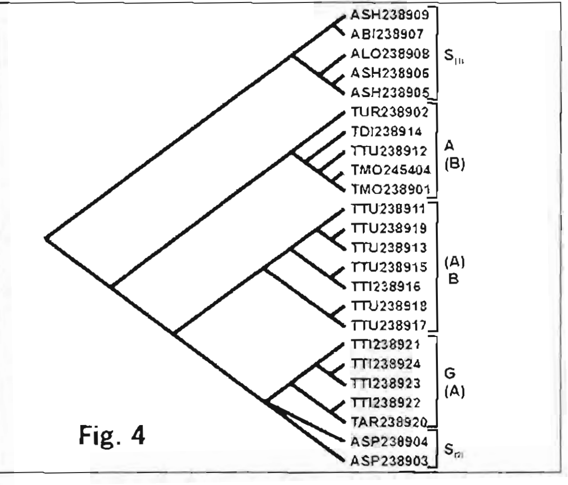
Figure 1. Alignment relationships between 5S unit classes of wheat and progenitors. The meaning of the scale bar is 0.1 nucleotide length substitutions on a scale to 1.

Figure 2. Alignment relationships between the four IGS assumed orthologous groups of wheat progenitors. The meaning of the scale bar is 0.1 nucleotide length substitutions on a scale to 1.

Figure 3. Alignment relationships between the four ITS1 assumed orthologous groups of wheat and progenitors.

Figure 4. Alignment relationships between the five ITS2 assumed orthologous groups of wheat and progenitors excluding the single sequence assigned to the D haplome.





ENGLISH TRANSLATION OF THE 1979 RUSSIAN TAXONOMIC MONOGRAPH OF TRITICUM L. BY DOROFEEV ET AL.: PROJECT PROGRESS REPORT

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ABSTRACT: Dorofeev *et al.* (1979), the taxonomic monograph of *Triticum* L. published by the N. I. Vavilov In-stitute of Plant Industry (VIR) is an important work which is largely unknown outside of Russia due to the language barrier. A morphological treatment, this monograph is at odds with current genetic concepts of wheat taxonomy and is therefore controversial. It is the only complete modern catalogue of wild and domesticated forms of *Triticum* spe-cies. For germplasm diversity and intellectual property rights, Dorofeev *et al.* can prove its value as an authoritative source that can be consulted for issues relating to the validity of proprietary claims on wheat genes and genetic lines that rightfully belong within the public domain. In 1999, an international collaboration was formed to support the translation of Dorofeev *et al.* into English. A project fund that was started by a $5000 donation from CIMMYT has been established at the Society for the Support of Research on Cultivated Plants in Gatersleben (GFK). The project is currently seeking additional matching funds. Dr. Irina Sokolova of the Komarov Botanical Institute has completed the text translation. Dr. Anna Filatenko, one of the original authors, is currently reviewing the translated copy, and adding necessary details and corrections. English editing will be underway in 2002. Publication of an affordable translation is projected for 2003. Internet copies of the taxonomic keys and descriptions also are planned.

1. INTRODUCTION

The Russian taxonomic monograph of *Triticum* L. by Dorofeev *et al.* (1), published by the N. I. Vavilov In-stitute of Plant Industry (VIR) in 1979 and known in Russia as the "Wheat Flora", is an important modern taxonomic treatment that is not widely known. It rep-resents the result of a cumulative effort that began in the early 20th century under the guidance of Vavilov. This monograph stands today as a significant research contribution that is unrecognised for its value by most of the non-Russian speaking wheat research community. The only equivalent work in English is the 1921 monograph of the wheats by John Percival, *The Wheat Plant* (2), a volume that predates the modern genetic concept of the wheats.

Since its publication, Dorofeev *et al.* has been unavail-able to the majority of the wheat research community due to the Russian language barrier. As a traditional treatment largely based in morphology, this obscure work has been eclipsed by the genetic concepts of *Triticum* and *Aegilops* L. Since Bowden's so-called phylogenetic treatment of the wheats published in 1959 (3), wheat taxonomy has been in the hands of geneticists. While many important genetic contribu-tions on the evolutionary relationships within *Triticum* and *Aegilops* have been offered in the past half cen-tury, the taxonomic handling of the wheats has suf-fered from competing genetic concepts and an acrimo-nious debate over circumscription of the wheat genera and their species.

In actuality, the differences between Dorofeev *et al.* and the genetically based classifications, which are represented as more phylogenetically accurate, are largely differences of nomenclature. Very little in the overall concept of *Triticum* has changed since Schulz organised the genus into the morphological groups of einkorn, emmer, and dinkel wheats (4), which proved later to follow the ascending polyploid series that traces the evolutionary development of *Triticum* spe-cies. Dorofeev *et al.* is no less modern than the clas-si-fications of the Bowden school. In fact, the differences are not in phylogenetic treatment but in the ran-king and handling of the domesticated wheats. In Dorofeev *et al.*, they are given specific status and are fully de-scribed; in the Bowden-type classifications, they are named as infraspecific taxa but poorly descri-bed (5), or they are eliminated entirely and thereby lost (6). The prevailing genetic classification concepts have distracted attention away from infraspecific diversity that is the basis for species variation. If infras-pecific forms are not named and described systematically in the tradition of "modern" taxonomy, their diver-sity cannot be exploited and is at risk of being lost.

Many researchers, particularly younger members of the wheat research community who are unaware of the un-derlying history, have recognised the need to bring or-der into the chaos that *Triticum* taxonomy has become. One effort is the *GrainTax* project which provides up-dated on-line information about taxono-mic classifica-tions and wheat synonymy (http://wheat.pw.usda.gov/ ggpages/GrainTax/description.html) (7, 8). Another such effort is the Dorofeev *et al.* Translation Project (DTP) which was undertaken in 1999 as an interna-tionally based volunteer project to produce an English-language version of the 1979 Dorofeev *et al. Triticum* monograph (9).

2. IMPORTANCE OF THE TRANSLATION OF DOROFEEV ET AL.

There are several pressing reasons for re-examining the importance of Dorofeev *et al.*

(1) This monograph is the only modern catalogue of wild and domesticated wheat species and their in-fraspecific diversity.
(2) As a work built on a long historical research base, it represents a compilation of information that would be extremely difficult to reproduce in to-day's research funding climate.
(3) A detailed classification provides a guide for dealing with the tremendous diversity of the genus *Triticum* existing in natural and man-made habi-tats as well as preserved in genebanks. It is essen-tial to catalogue international genebank collections with a standardised classification system such as found in Dorofeev *et al.*

3. BENEFITS OF THE TRANSLATION

Although wheat geneticists have relegated traditional taxonomic practice to a relatively minor role, taxon-omy now promises to take on a new status as an authoritative resource for establishing the boun-daries for protection of germplasm diversity and intellectual property rights. In the rapidly developing mar-kets of biotechnology, the potential threats on the public own-ership of wild and domesticated wheat diver-sity can be challenged with the documentation already present in the comprehensive treatment that Dorofeev *et al.* em-bodies. Its detailed morphological classification of do-mesticated landraces and pre-modern cultivars will be useful in establishing the extent of diversity across the geographic range of wheat distribution, particularly for Asia, Eastern Europe, and North Africa. Dorofeev *et al.* also will be useful in all aspects of biodiversity and plant genetic resources research, including preserva-tion, cataloguing, and uti-lisation.

Dorofeev *et al.* is the only modern catalogue of all known infraspecific taxa of the domesticated and wild wheat species. It serves as an authoritative reference that can be consulted for identification of dis-tinct forms of the wild and domesticated wheats and for es-tablishing public ownership of wheat genes or genetic forms in the face of proprietary ownership challenges by private companies.

4. INFORMATIONAL VALUE OF THE MONOGRAPH

The monograph describes 27 *Triticum* species (cf. Table 1). By contrast, genetic classifications usually rec-ognise only four or five species. While the appropri-ateness of giving specific status to domesticated forms whose genetic variation is minimal is a point of con-troversy, the detailed descriptions of domesti-cated spe-cies provided in Dorofeev *et al.* represent an important contribution. The full range of wheat diver-sity cannot be described by the abbreviated species concepts (6, 10, 11).

An English version of Dorofeev *et al.* will open a body of information to interested plant breeders, gene-ticists, genebank managers, botanists, archaeobotanists, and others working with wheat diversity. There are 1,242 infraspecific taxa that are catalogued with botanical descriptions, synonymy, taxonomic keys, geographic distribution, disease traits, origin, and history. As an example, the translation of the description

of *Triticum urartu* is presented in Appendix 1. Over 3,000 names for the wheats are listed in the monograph's index. While the original Russian-language publication provided only two pages of references, the translation also will include a full reference list with numerous publications not generally known outside of Russia.

5. FUNDING OF THE TRANSLATION PROJECT

Initial funding for DTP was provided by CIMMYT as a US$ 5,000 donation for fees associated with the Rus-sian to English translation. The project is actively seeking other donations from public and private research institutions to fund the costs of scientific edit-ing and publication (Morrison *et al.*, 2000). The project fund is maintained at the Society for the Support of Research on Cultivated Plants in Gatersleben (GFK).

Interested donors can contribute as follows (bank-ing instructions can be requested from H. Knüpffer, knupffer@ipk-gatersleben.de.):

- **Non-EEC citizens:** by cheque payable to GFK-Gatersleben;
- **EEC citizens:** by Eurocheque payable to GFK-Gatersleben;
  German citizens: by Eurocheque payable to GFK-Gatersleben, or via bank transfer.

Table 1. System of the genus *Triticum* L. according to Dorofeev *et al.* (1)

Subgenus	Section	Group of species	Species	2n	Genomes	Number of different genomes
Triticum	*Urartu* Dorof. et A. Filat.	Small spelts	*T. urartu* Thum. *ex* Gandil.	14	A^u	1
	Dicoccoidea Flaksb.	Emmer wheats	*T. dicoccoides* (Koern. *ex* Aschers. et Graebn.) Schweinf.	28	A^uB	2
			T. dicoccum (Schrank) Schuebl.	28		2
			T. karamyschevii Nevski	28		2
			T. ispahanicum Heslot	28		2
		Naked tetraploids	*T. turgidum* L.	28	A^uB	2
			T. jakubzineri Udacz. et Schachm.	28		2
			T. durum Desf.	28		2
			T. turanicum Jakubz.	28		2
			T. polonicum L.	28		2
			T. aethiopicum Jakubz.	28		2
			T. persicum Vav. (*T. carthlicum* Nevski)	28		2
	Triticum	Spelt wheats	*T. macha* Dekapr. et Menabde	42	A^uBD	3
			T. spelta L.	42		3
			T. vavilovii (Thum.) Jakubz.	42		3
		Naked hexaploids	*T. compactum* Host	42	A^uBD	3
			T. aestivum L.	42		3
			T. sphaerococcum Perciv.	42		3
			T. petropavlovskyi Udacz.. et Migusch.	42		3
Boeoticum Migusch. et Dorof.	*Monococcon* Dum.	Small spelts	*T. boeoticum* Boiss.	14	A^b	1
			T. monococcum L.	14		1
		Naked diploid	*T. sinskajae* A. Filat. et Kurk.	14	A^b	1
	Timopheevii A. Filat. et Dorof.	Emmer wheats	*T. araraticum* Jakubz.	28	A^bG	2
			T. timopheevii (Zhuk.) Zhuk.	28		2
			T. zhukovskyi Menabde et Ericzjan	42	A^bA^bG	2
		Naked tetraploid	*T. militinae* Zhuk. et Migusch.	28	A^bG	2
	Kiharae Dorof. et Migusch	Spelt wheat	*T. kiharae* Dorof. et Migusch.	42	A^bGD	3

Donations should be addressed to:
Dorofeev Translation Project Fund, Mr. B. Eise
Gemeinschaft zur Förderung der Kulturpflanzen-forschung e.V. (GFK)
Corrensstr. 3
D-06466 Gatersleben, Germany

6. PUBLICATION PLANS

The 323 text pages have been completely translated. Scientific editing by one of the monograph's authors, Anna Filatenko, is currently underway. A revised ref-erence list also is in process. Linguistic editing is scheduled to start in November 2001. Final editing and revisions will start in January 2002 and continue through the Fall of 2002. The projected publication date is set for early 2003.

The DTP plans to publish an affordable volume that will cost no more than $50. A publisher has not yet been chosen. Arrangements are planned to allow elec-tronic publication of the taxonomic keys and other relevant portions of the translation on the *GrainTax* website.

The DTP has obtained permission from VIR to publish the translated version and to use photographs and fig-ures from the original.

7. PROJECT VOLUNTEERS AND STAFF

The translation project was initiated in the summer of 1999 during the Percival Symposium *"Wheat - Yester-day, Today and Tomorrow"* at the University of Reading, UK (12). The committee overseeing the project includes the following individuals:

- Helmut Knüpffer - Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
- Anna Filatenko (retired, VIR) - St. Petersburg, Russia
- Laura Morrison - Oregon State University, Corvallis, Oregon, USA
- Karl Hammer - Universität Gesamthochschule Kassel, Witzenhausen, Germany
- Alexei Morgounov - CIMMYT, Almaty, Ka-zakhstan
- Iva Faberová - Research Institute of Crop Produc-tion, Prague, Czech Republic

Irina Sokolova of the Komarov Botanical Institute (St. Petersburg, Russia) prepared the Russian to English translation of the text. Editorial respon-sibilities are allocated as follows: scientific editing by A. Filatenko; linguistic editing by Charles Jeffrey (St. Petersburg); final editing by A. Filatenko, K. Hammer, and L. Morrison; editorial supervision by H. Knüpffer.

ACKNOWLEDGEMENTS

The contribution of CIMMYT to the project fund is gratefully acknowledged. We also thank the "Gemeinschaft zur Förderung der Kulturpflanzenforschung" (GFK), Gatersleben, for hosting and maintaining the project fund. The work of A. Filatenko was carried out under the sponsorship of the Cooperative Agricultural Sciences Programme between Germany and the Rus-sian Federation and funded by the German Ministry of Agriculture.

REFERENCES

[1] Dorofeev VF, Filatenko AA, Migushova EF, Uda-czin RA, Jakubziner MM: Wheat. In: Dorofeev VF, Korovina ON (eds) Flora of Cultivated Plants, vol. 1. Leningrad, Russia, Kolos. 346 pp. (1979) (in Russian).

[2] Percival J: The Wheat Plant. Duckworth & Co, London, 463 pp. (1921).

[3] Bowden WM: The taxonomy and nomenclature of the wheats, barleys, and ryes and their wild rela-tives. Can. J. Bot. 37: 657-684 (1959).

[4] Schulz A: Die Geschichte der kultivierten Getrei-de. Halle, Verlag Louis Neberts (1913).

[5] Mac Key J: A plant breeder's aspect on taxonomy of cultivated plants. Biol. Zentralbl. 107: 369-379 (1988).

[6] Kimber G, Sears ER: Evolution in the genus Triti-cum and the origin of cultivated wheat. In: Heyne EG (ed) Wheat and wheat improvement. 2nd ed. Madison, Amer. Soc. Agron., pp. 154-164 (1987).

[7] Morrison LA, Raup WJ: GrainTax synonymy table project: June 1999 progress report. Wheat Inform. Serv. 88: 52-56 (1999).

[8] Morrison LA, Raup WJ: GrainTax synonymy ta-bles project: June 2000 Progress Report. Wheat In-form. Serv. 90: 54 (2000).
[9] Morrison LA, Faberová I, Filatenko AA, Hammer K, Knüpffer H, Morgounov A, Rajaram S: Call to support an English translation of the 1979 Russian taxonomic monograph of Triticum by Dorofeev et al. Wheat Inform. Serv. 90: 52-53 (2000).
[10] Morris R, Sears ER: The cytogenetics of wheat and its relatives. In: Quisenberry KS, Reitz LP (eds) Wheat and wheat improvement. Madison, Amer. Soc. Agron., pp. 19-87 (1967).
[11] Kimber G, Feldman M: Wild wheat: an introduc-tion. Special Report 353. Columbia, Coll. Agric., Univ. Missouri (1987).
[12] Caligari PDS, Brandham PE (eds): Wheat taxon-omy: the legacy of John Percival. The Linnean, Special Issue No. 3 (2001).

APPENDIX 1. T. URARTU Thum. EX GANDIL.

(extracted from the English translation of Dorofeev et al. (1979) (1))
Russian-to-English Translation: I. Sokolova, St. Petersburg
Editing: A. Filatenko, St. Petersburg; L. Morrison, Corvallis, Oregon, USA; H. Knüpffer, Gatersleben, Germany

T. urartu Thum. ex Gandil. 1972, Bot. Zhurn. 57, 2: 176; Tumanian 1938, Tr. Arm. Fil. AN SSSR, Ser. Biol. 2: 211, descr. ross. - *T. armeniacum* Thum. ex Flaksb. 1939, Opred. Nast. Khlebov: 27, nom. nud., non *T. armeniacum* Nevski 1933, non *T. armeniacum* Jakubz. 1938 et non *T. armeniacum* Makusch. ex Menabde 1945. - *T. monococ-cum* subsp. *urartu* (Thum.) A. et D. Löve 1961, Bot. Not. (Lund) 114, 1: 49, nom. illeg. - *T. boeoticum* Boiss. subsp. *urartu* (Thum.) Vav. 1964, Mirov. Resursy Khlebn. Zla-kov: 21, nom. illeg. - *T. boeoticum* subsp. *urartu* (Thum.) V. Dorof. 1968, Euphytica, 17: 453, nom. illeg. - *T. monococcum* subsp. *michaelii* An. Fed. (Theod.) et Takht. ex Zhuk. 1968, Bot. Zhurn. 53, 4: 442, nom. nud. - *T. michaelii* Zhuk. 1971, Kult. Rast. Sorod.: 96, nom. nud.

Type: Armenian Republic, southeastern outskirts of Erevan near Vokhchaberd and Gekhadir villages, 30. VI. 1968, P. A. Gandilyan.

General characteristics. Seedlings violet or dark green. Plant prostrate at base. Culm thin, flexible, up to 145 cm high. Stem nodes violet. Leaves velvety pubescent or glab-rous, linear-lanceolate (15 cm long, 0.7-1.0 cm wide). Leaf sheaths velvety pubescent or glabrous. Auricles white, ciliate. Spikes elongate (Fig. 3, *a*), narrow (7-9 cm long, 0.6-0.7 cm wide), fragile, spontaneously disarticu-lating. Spikelets two-awned, one-grained (less often two-grained). Lateral (two-rowed) side of spike approximately twice as wide as frontal side. Pubescence of rachis inter-node segments the same as for *T. boeoticum*. Glumes scabrous-tuberculate; more delicate than for *T. boeoticum*,. Keel tooth long with a broad base. Tooth of main lateral glume vein considerably less distinct than keel tooth and 8-10 times shorter than in *T. boeoticum*. Anthers short, 2.0-2.7 mm (Gandilyan, 1972).

T. urartu has a weaker stem than *T. boeoticum*: stem fracture strength at first internode is 456 g versus 2,000-3,000 g for *T. boeoticum* (Gradchaninova, 1967).

In Armenia, found on dry slopes of foothills.

All forms of *T. urartu* are winter forms. Heading stage is long when sown under irrigated conditions in Tashkent. Both in Armenia and in other native habitats in the Near and Middle East, *T. urartu* only occurs where *T. boeoti-cum* is present.

Compared to *T. boeoticum*, *T. urartu* shows less morning flowering. It also flowers in the evening (Zhukov, 1969). In Tashkent, *T. urartu* starts flowering before full emer-gence of the spike from the flag leaf (data of E. F. Migu-shova). In Armenia, its native land, *T. urartu* begins later than *T. boeoticum* (Araratyan and Surmenyan, 1939). *T. urartu* is separated from other diploid species by anther morphology as well (Dhaliwal and Johnson, 1976). Its an-thers are unusually short (on the average 2.2mm, versus 3.6mm in *T. boeoticum*). They dehisce by a longitudinal cleft twisting into a spiral after flowering. Anthers of *T. urartu* are placed at the same level as the stigma; the lemma and palea open with difficulty (Araratyan and Surmenyan, 1939).

T. urartu, like other wild wheat species, stands out be-cause of its high seed protein content (23.7-25.0%) (Ko-narev *et al.*, 1971). Lysine content is relatively high: 2.67-2.48% of total protein; 0.632-0.681mg per 100g of grain. On the average for three years, content of protein in grain of this species (reproduction in lowland Daghestan under irrigation) made up 19.1-23.7%, that of starch 53.0-58.0% (Jakubziner and Pokrovskaya, 1971).

Immunity. *T. urartu* is distinguished from *T. boeoticum* by its strong susceptibility to yellow (stripe) rust (Gulkan-yan, 1938; Vavilov, 1957, 1964; Jakubziner, 1969). In the Tashkent region during 1968-1973, stripe-rust infections reached number 3 (on a 5-point scale) powdery mildew in-fections reached number 4 (Migushova, 1976). Sukhan-berdina (1977) also found severe powdery mildew infec-tions both in the seedling and adult plant stages.

Resistance to infection by races of brown and stem rust in the seedling stage proved to be stronger (type IV) than in *T. boeoticum* (Grigoryeva, 1975). Adult plants in field conditions were essentially resistant. M. M. Jakubziner (unpublished information) has noted that in his joint ex-periment with G. S. Turov in Southern Kazakhstan the de-gree of infection of adult plants by brown rust did not ex-ceed 10%, although response to infection by the fungus was characterised as being of type IV.

Artificial infection by loose smut races, f. *aestivi* and f. *duri*, caused infection of 10.0 and 11.5% of spikes respec-tively (Yamaleev, 1974, Krivchenko *et al.*, 1976). The fungal mycelium infected all the embryonic organs of the caryopsis. These researchers concluded that *T. urartu* was

distinctly different from *T. boeoticum* in having no embryo race-specific resistance to loose smut in all growth stages.

In general, *T. urartu* is not resistant to fungal diseases.

Geographical distribution. The species distribution range in Armenia is at southeastern outskirts of Erevan and around the environs of the villages Shorbulak, Gekha-dir, Vokhchaberd, and Atsavan in the Abovyan district (after Gandilyan, 1976, refined).

In recent years, forms of *T. urartu* were reported from Iran (Jaaska, 1974), as well as from Turkey, Iraq and Lebanon (Johnson, 1975).

Origin. A number of researchers envision the phylogenetic development of wheat as forming a branched tree, which originated from a common ancestor. According to Flaks-berger (1935), the ancestor is the progenitor for the diploid and polyploid wheat species. Nevski (1933) advanced the idea that the genus *Triticum* originated from the ancestral tribe *Protohordeae*, or more particularly from plants which had a paniculate inflorescence with 2-3 spikelets grouped at each rachis node. Sinskaya (1955) believed that the tribe *Protohordeae* consisted of perennial biotypes (2n = 14), which evolved and turned to annual growth habit, while gaining features of xerophily. Tzvelev (1976) con-cluded that the coasts of the ancient Tethys ("Ancient Mediterranean") were a region where highly specialised annuals evolved from festucoid grasses. These grasses then gave rise to the first diploid wheat. In our opinion, this ancestor could have been an ancient form of *T. urartu*.

Taxonomy. *T. urartu* was found by M. G. Tumanian in 1934 who named and published the species description in Russian in 1938. Its spikes were brought into the VIR collection by N. I. Vavilov, who identified them as *T. ar-meniacum* Thum. This name was published in 1939. The *T. armeniacum* epithet is illegitimate, since *armeniacum* was assigned to another species of two-grained wheats in 1933. In 1938 and 1945, the name *T. armeniacum* was used as a later homonym of *T. araraticum*. The epithet *michaelii* suggested by Fedorov and Takhtajan (1968) is also a later homonym of *T. urartu*. Thus, a certain confu-sion was introduced into nomenclature.

The diagnosis of *T. urartu* was published by Gandilyan (1972). For many years in the botanical and genetic lit-erature, *T. urartu* has been regarded as a subspecies of *T. boeoticum* (Vavilov, 1964) or included in *T. monococcum* (Löve and Löve, 1961).

Key to the determination of varieties of *T. urartu* Thum. ex Gandil.

Glumes						Awns		Variety	Number of variety
white	white with black margins	red	red with black margins	black on background white		same as glumes red	black		
+	-	-	-	-	-	+-	-	*spontaneoalbum*	1
-	+	-	-	-	-	+	-	*binartulutriru*	2
-	-	+	-	-	-	+	-	*spontaneorubrum*	3
	–	-	+	-	-	-	+	*urartu*	4
-	-	-	-	+	-	-	+	*albinigricans*	5
-	-	-	-	-	+	-	+	*nigrum*	6

Var. **albinigricans** Thum. *ex* Dorof. et A. Filat. 1979, in Dorof. *et al.*, Kult. Fl. 1: 38. - T. urartu var. albonigricans Thum. 1938, Tr. Arm. Fil. AN SSSR, Ser. Biol. 2: 214, descr. ross. (5). A *T. urartu* var. *spontaneo-album* glumis aristisque nigris differt.

Typus: Armenia. Reproductio stationis Daghestanskajae fulcracea, WIR, k-33871, 3. VI. 1968, leg. Soskov (WIR).

Geographical distribution. Armenia.

Var. **binartulutriru** Gandil. *ex* Dorof. et A. Filat. 1979, in Dorof. *et al.*, Kult. Fl. 1: 38. - T. urartu var. binartulutriru Gandil. 1975, Tr. Arm. NIIZ, Ser. Pshenitsa (Echmiadzin), 3: 74, descr. ross. (2). Spica alba marginibus glumarum nigris.

Typus: Armenia, prope pagum Gegadir, 30. VI. 1968, leg. P. A. Gandiljan (WIR).

Geographical distribution. Armenia.

Var. **nigrum** Thum. *ex* Dorof. et A. Filat. 1979, in Dorof. *et al.*, Kult. Fl. 1: 38. - T. urartu var. albonigricans Thum. 1938, l. c.: 214, descr. ross. (6). A *T. urartu* var. *urartu* glumis nigris differt.

Typus: Armenia. Reproductio stationis Daghestanskajae fulcracea, WIR, k-33870, 29. VI. 1968, leg. Soskov (WIR).

Geographical distribution. Armenia.

Var. **spontaneoalbum** Thum. *ex* Dorof. et A. Filat. 1979, in Dorof. *et al.*, Kult. Fl. 1: 38. - T. urartu var. sponta-neoalbum Thum. 1938, l. c.: 214, descr. ross. (1). Spica aristaque albae.

Typus: Armenia. 30. VI. 1968, leg. P. A. Gandiljan (WIR).

Geographical distribution. Armenia.

Var. **spontaneorubrum** Thum. *ex* Dorof. et A. Filat. 1979, in Dorof. *et al.*, Kult. Fl. 1: 38. - T. urartu var. spontaneo-rubrum Thum. 1938, l. c.: 214, descr. ross. (3). Spica aristaque rubrae.

Typus: Armenia. Reproductio stationis Daghestanskajae fulcracea, WIR, k-33869, 3. VI. 1968, leg. Soskov (WIR).

Geographical distribution. Armenia.

Var. **urartu**. - *T. urartu* var. *binarturutriru* Gandil. 1972, Bot. Zhurn. 57, 2: 177. (4).

Geographical distribution. Armenia.

A NEW MOLECULAR TOOL FOR STUDYING THE NUCLEAR GENOME IN THE TRITICEAE

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ABSTRACT: A barley cDNA encoding a nuclear DNA sequence highly homologous (80%) to the soybean chloroplast translation elongation factor was successfully used for phylogenetic studies. The DNA is closely linked to the *vrs1* locus (2-/6-rowed spike) of barley. Exon regions of the DNA are highly conserved in the Triticeae, and primers designed for the exons were able to amplify single DNAs. The intron of the DNA is more variable than the exons. By using nuclear DNA sequences we separated diploid taxa of the genus *Hordeum* into 4 clades corresponding to the 4 basic genomes **H**, **I**, **Xa**, and **Xu**. *H. vulgare* and *H. marinum* exhibited an unexpected range of variation within each species. In polyploids, *Hordeum marinum* ssp. *gussoneanum* 4x generated 2 classes of DNA clones; 1 was identical with ssp. *gussoneanum* 2x and the other was unique, indicating that the tetraploid has a segmental alloploid origin. *H. brachyantherum* 6x generated 3 DNA fragments, 2 of which originated from *H. brachyantherum* 4x and 1 of which originated apparently from *H. marinum* ssp. *gussoneanum* 2x. This indicates that the hexaploid carries the **HHHHXaXa** genome combination. Tetraploid *Elymus* species containing the 2 basic haplomes **S** and **H** were analyzed to elucidate the origin of the **H** haplomes. The results indicated a diploid *Hordeum* species as the donor of the **H** haplomes, and provided evidence for a multiple origin of the **H** haplomes present in *Elymus*.

1. INTRODUCTION

The genus *Hordeum* comprises 32 species (45 taxa), including diploid, tetraploid, and hexaploid forms with a basic chromosome number of x = 7. Study of meiotic pairing in diploids shows 4 genomes: **I** (*H. vulgare* and *H. bulbosum*), **Xu** (*H. murinum*), **Xa** (*H. marinum*), and **H** (all others). The 4 genomes are supported by cytological, biochemical, and molecular marker analyses (15). *Hordeum* is thus a good case study for evolutionary patterns. We present views of the diversity and phylogeny in the genus *Hordeum* that we have obtained from a series of recent studies using a new nuclear DNA sequence (7, 8, 12). We also present the evolutional pattern of *Elymus*, which suggests multiple origins of the tetraploid taxa.

2. EF-G HOMOLOGUE FOR PHYLOGENETIC STUDIES

cMWG699 is a barley cDNA encoding a nuclear DNA highly homologous (80%) to the soybean chloroplast translation elongation factor (*EF-G*) gene (2). Exon regions of the DNA are highly conserved in the Triticeae, and primer pairs designed for this region generated single-copy DNA fragments from most genera in the Triticeae (Fig. 1). The DNA size was about 930 bp when generated with an outer primer pair (T3-2 and T7-2) and 490 bp when generated with an inner primer pair (T3-3 and T7-3). Restriction digestion of the amplified DNA provided a quick view of the variation among tested materials. The restriction analysis also identified clones of different origins in polyploids. After the restriction analysis, representative taxa were further analyzed by DNA sequencing for phylogenetic studies. Each DNA included an intron of about 370 bp. The intron region was more variable than the exons (11). This variability provides information for studying evolutionary patterns in the Triticeae.

The cMWG699 locus is closely linked (0.1 cM) to the *vrs1* locus, which predominantly controls the formation, development, and fertility of lateral spikelets of *H. vulgare* (6). The possession of three 1-flowered

spikelets (a triplet) at each rachis node is a diagnostic morphological character of *Hordeum*. The central spikelet is always fertile, while fertile lateral spikelets occur only in some forms of *H. vulgare* and in some forms of wild species, such as *H. bogdanii*. The fertility of the lateral spikelets is one of the diagnostic genetic traits for the domestication process of barley. Under domestication, 6-rowed barley (*vrs1vrs1*) appeared from 2-rowed barley (*Vrs1Vrs1*) by a single recessive mutation (15).

Phylogenetic analysis of 20 diploid *Hordeum* species indicated the 4 genomes to be diverged into 2 clades, one containing **I** and **Xu** and the other containing **H** and **Xa** (8). **I** and **Xu** were resolved well, and within the **I** genome *H. vulgare* and *H. bulbosum* formed sister groups. Separation of **H** and **Xa** was less clear than that of **I** and **Xu**, and parsimony analysis suggested a close relationship between them. *H. murinum* (**Xu**) is most closely related to **I** genome species. This agrees with a morphological, ecological, and distributional study that classified *H. murinum*, *H. vulgare*, and *H. bulbosum* in the same section (13). Several biochemical and molecular marker studies supported the concept of 4 genomes (for review see 15), but none indicated **I** and **Xu** to be in the same section. This was, however, the case in this study probably because our DNA marker was closely linked to a gene controlling spike morphology.

3. I. GENOME GROUP

Tanno et al. (12) used the nuclear DNA sequence to study the origin of 6-rowed barley (*H. vulgare*). This study included 280 landraces (ssp. *vulgare*) from Eurasia and Africa, consisting of 204 six-rowed and 76 two-rowed accessions, and 183 wild or weedy forms (ssp. *spontaneum*) from the Middle East and Morocco. After restriction analysis with *Taq*I, 33 representative accessions were used for DNA sequencing. Sequence analysis showed the wild and weedy forms to have a larger variation than cultivated barley had. Moreover, 2-rowed barley had a larger variation than 6-rowed barley, in which only 2 haplotypes were found.

The major type (Type A) of 6-rowed barley had the same DNA sequence as that of a wild barley collected in Turkmenistan. This material had intermediately developed lateral spikelets with elongated awns (the var. *proskowetzii* form). Because the genus has somewhat developed lateral spikelets and the awns of those spikelets are elongated (or at least the lemmas are tip-pointed), the common progenitor of barley has been postulated to have had intermediately developed lateral spikelets like the var. *proskowetzii* form (10, 13). The results obtained from this study strongly support that hypothesis.

Figure 1. PCR amplification of *EF-G* homologues in Triticeae.

Aegilops tauschii	2x
Aegilops tauschii	2x
Agropyron cristatum	2x
Australopyrum pectinatum	2x
Australopyrum velutinum	2x
Dasypyrum villosum	2x
Eremopyrum bonaepartis	4x
Henrardia persica	2x
Leymus alaicus	4x
Lophopyrum nodosum ssp. *dorudicum*	4x
Peridictyon sanctum	2x
Psammopyrum athericum	6x
Psammopyrum pungens	8x
Psathyrostachys juncea	2x
Secale cereale	2x
Secale segetale	2x
Stenostachys gracilis	4x
Taeniatherum caput-medusae	2x
Taeniatherum caput-medusae	2x
Thinopyrum bessarabicum	2x
Pseudoroegneria ferganensis	2x
Elymus alaskanus	4x
Elymus caninus	4x
Hordeum vulgare	

The other 6-rowed type (Type D) was minor and originated from some European countries, including Bulgaria, Germany and Spain, and from Morocco. This type had the same DNA sequence as that of a Moroccan weedy barley discovered in a barley field by Molina-Cano et al. (9). The divergence time between Types A and D was calculated as more than 100 000 years, much longer than the history of barley cultivation (12). Therefore, the 2 types probably pre-existed in wild barley, which suggests a diphyletic origin of the recessive alleles (*vrs1*) of 6-rowed barley, generated from 2-rowed barley (*Vrs1*) by independent mutations. Barley inbreeds, and the linkage between the marker (cMWG699) locus and the *vrs1* locus is very close (0.1 cM). Therefore, the generation of Type D 6-rowed barley by introgression followed by recombination is less likely.

4. XA-GENOME GROUP

4.1. Diploid H. marinum

The same methodology was used to study the genetic variability of 65 diploid accessions of *H. marinum* (7). Restriction analysis revealed a diagnostic *Taq*I site that distinguished ssp. *marinum* and ssp. *gussoneanum*, and an *Msp*I site that separated ssp. *marinum* into 2 types showing allopatric distributions: type 1 (Turkmenistan to France and Mallorca) and type 2 (Iberian Peninsula). The 2 types seem to be separated by the Pyrenees, but this must be further studied by including more populations from Spain and France.

To elucidate the variation in DNA sequences, 11 accessions of type 1 and 4 accessions of type 2 were chosen to represent the maximum geographical distribution within the native area. The 2 types showed clear differences in DNA sequences. All type 1 accessions shared an identical DNA sequence in spite of the wide distribution. In contrast,

the type 2 accessions revealed considerable variation in the
DNA sequence. The 2 types may represent different ecotypes not previously recognized. This result implies that ssp. *marinum*, especially in the Iberian Peninsula, might include nucleotide variations that we did not detect in this study.

Twenty-two accessions of *H. marinum* ssp. *gussoneanum* 2x were analyzed, but they did not show any variation in DNA sequence. In cladistic analysis, ssp. *marinum* and ssp. *gussoneanum* 2x were not resolved well.

4.2. Tetraploid H. marinum

Twenty accessions of *H. marinum* ssp. *gussoneanum* 4x collected in the area from Turkey to Afghanistan were analyzed (7). All materials generated 2 classes of DNAs, 931 and 915 bp, which were clearly distinguished by *Taq*I digestion. Four accessions were used for the DNA sequencing. All accessions shared exactly the same DNA sequences in both clones, indicating a low level of diversity in the tetraploid taxon. The 915-bp fragment was identical to that of the diploid ssp. *gussoneanum*, indicating that the diploid is probably the immediate donor of one of the haplomes in the tetraploid.

The result is in accordance with earlier results obtained by isozyme studies (4, 5). The tetraploid cytotype occurs naturally from Turkey to Afghanistan, while the diploid cytotype of ssp. *gussoneanum* is widely distributed from the Mediterranean area to Southwest Asia (14, 15). The distributions of the tetraploid and diploid cytotypes overlap in the Middle East area, mainly in Turkey, and thus it is a reasonable hypothesis that the diploid cytotype might have contributed to the formation of the tetraploid cytotype in the region.

Although the sequencing analysis could not identify a diploid donor of the 931-bp fragment, phylogenetic analysis showed that it is confined exclusively to the *H.marinum* group. The 931- and 915-bp fragments probably differentiated from a common ancestor of the *H. marinum*
group. Our result is consistent with the explanation that the tetraploid cytotype has a segmentally alloploid origin, and

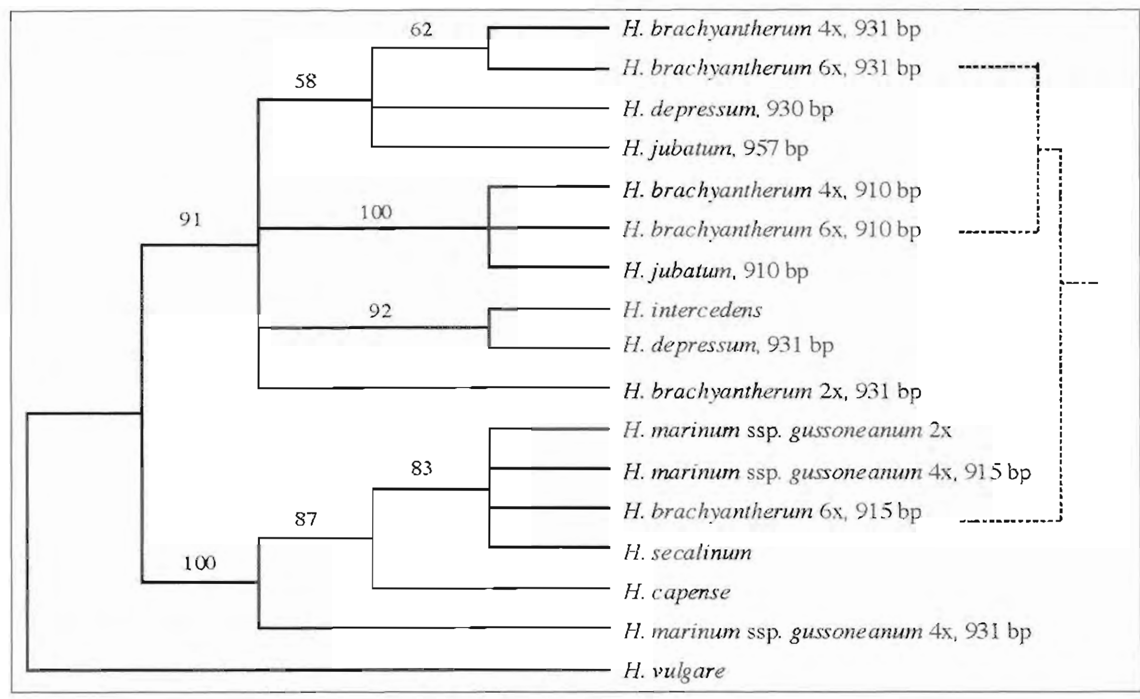

Figure 2. Phylogeny of *Hordeum brachyantherum* and related species based on cMWG699 sequence data. A strict consensus of the 4 most parsimonious trees is shown. Bootstrap values obtained from 1000 replicates are shown above branches.

that diploid populations (ssp. *marinum* or *gussoneanum*) that have the 931-bp fragment still exist in Southwest Asia (Turkey in particular), where the tetraploid is currently distributed. It is possible that the donor of the second haplome is extinct, but the hypothesis that it still exist will be explored in future studies when further materials from Southwest Asia become available.

Our preliminary data suggest that the meiosis of the tetraploid cytotypes is under genetic regulation, which suppresses homeologous pairing (von Bothmer and Salomon, unpubl.). This would explain the exclusive bivalent formation in the meiosis of the tetraploid as well as the observation of high autosyndetic pairing in tetraploids and inter and intraspecific hybrids.

5. H-GENOME GROUP

We studied the phylogenetic pattern of North American species by the same method (Fig. 2). We were especially interested in the formation of the hexaploid cytotypes of *H. brachyantherum*. Most plant material was collected in California. We also included *H. secalinum* and *H. capense* because of their morphological resemblance to *H. brachyantherum*.

Restriction analysis with *Taq*I and *Msp*I identified 3 classes of clones in *H. brachyantherum* 6x, and 2 classes in *H. marinum* ssp. *gussoneanum* 4x, *H. brachyantherum* 4x, and *H. jubatum* (Fig. 2). Because *H. depressum*, *H. secalinum*, and *H. capense* did not show restriction polymorphism, 8 independent clones were analyzed for these tetraploid cytotypes to detect different classes of clones. This analysis identified 2 classes of DNA clones in *H. depressum*, but only 1 class in each of *H. secalinum* and *H. capense.*

The cladogram (Fig. 2) shows that the analyzed taxa are separated into 2 large groups. One group consisted of the North American species, and the other contained Eurasian species and a South African species. The latter group included a 915-bp fragment of the hexaploid *H. brachyantherum*. This fragment shares an identical sequence with diploid and tetraploid *H. marinum* ssp. *gussoneanum* and *H. secalinum*. The cladogram (Fig. 2) showed that *H. brachyantherum* 6x has 2 haplomes originated from *H. brachyantherum* 4x and 1 haplomes originated from *H. marinum* ssp. *gussoneanum* 2x. The diploid *H. marinum* ssp. *gussoneanum* was introduced to California and has widely distributed there, where the hexaploid *H. brachyantherum* was discovered. Distribution of *H. secalinum* in North America is not yet reported. We therefore conclude that *H. brachyantherum* 6x (**HHHHXaXa**) most probably evolved by a crossing between *H. brachyantherum* 4x (**HHHH**) and *H. marinum* ssp. *gussoneanum* 2x (**XaXa**) followed by chromosome doubling.

Crosses were made between synthetic hexaploids (H. marinum ssp. gussoneanum 2x ¥ H. brachyantherum 4x) and H. brachyantherum 6x, and the hybrids had about 30% seed set (3). The result was in accordance with the evolutional pattern of the hexaploid H. brachyantherum. Sequencing analysis of chloroplast DNAs revealed that the hexaploid has cytoplasm of H. marinum ssp. gussoneanum 2x, indicating that this diploid was a maternal parent for the evolution of the hexaploid (Nishikawa et al. This issue).

6. ORIGIN OF THE H HAPLOME IN ELYMUS

In *Elymus*, tetraploid, hexaploid, and octoploid cytotypes have been identified; the tetraploids carry the **SSHH** and **SSYY** genome combinations. The **S** haplome is assumed to have originated from *Pseudoroegneria*, the **H** haplome from *Hordeum*, and the **Y** haplome from an unknown ancester (1). The origins have been deduced from some morphological studies and from biosystematic data. We studied 9 tetraploid accessions of the **SSHH** genome combination collected from Eurasia, North America, and South America in order to elucidate the origin of the **H** haplomes.

The tetraploid *Elymus* cytotypes generated 2 DNA fragments. The DNA sequences showed that the variable site was about 1% within the **S** and **H** haplomes, but as high as 15% between the **S** and **H** haplome. DNA sequences of the **H** haplomes of different *Elymus* species were superimposed on the data matrix of diploid species of *Hordeum*. All **H** haplomes were included in the **H** clade of *Hordeum*. **H** haplome of *E. sibiricus* was most closely related to *H. roshevitzii*, indicating that the **H** haplome of *E. sibiricus* and the **H** genome of *H. roshevitzii* are likely to have a common origin. The geographical distribution of the two species supports this assumption. The **H** haplomes of *E. caninus*, *E. fibrosus*, and *E. canadensis* formed a small clade.

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REFERENCES

[1] Dewey D: The genomic system of classification as a guide to intergenetic hybridization with the perennial triticeae. In:Gustafson J (ed), Gene Manipulation in Plant Improvement. Plenum Publishing Corporation, 209-279 (1984)

[2] Hernandez TJ, Breitenberger CA, Spielmann A, Stutz E: Cloning and sequencing of a soybean nuclear gene coding for a chloroplast translation elongation factor EF-G. Biochim Biophys Acta 1174: 191-194 (1993).

[3] Hysing SC: Relationship in the Hordeum murinum complex and Hordeum brachyantherum (6x) (Poaceae, Triticeae)-crosses between synthetic polyploids and their corresponding putative taxa. Master's Dissertation, Department of Crop Science, Swedish University of Agricultural Science (2000).

[4] Jaaska V, Jaaska V: Isoenzyme variation in the barley genus Hordeum L. 1. Alcohol dehydrogenase and superoxide dismutase. Biochem Physiol Pflanzen 181: 301-320 (1986).

[5] Jørgensen RB: Relationships in the barley genus (Hordeum): an electrophoretic examination of proteins. Hereditas 104: 273-291 (1986).

[6] Komatsuda T, Li W, Takaiwa F, Oka S: High resolution map around the vrs1 locus controlling two- and six-rowed spike in barley, Hordeum vulgare. Genome 42: 248-253 (1999).

[7] Komatsuda T, Salomon B, Bryngelsson T, von Bothmer R: Phylogenetic analysis of Hordeum marinum Huds. based on nucleotide sequences linked to the vrs1 locus. Pl Syst Evol 227: 137-144 (2001).

[8] Komatsuda T, Tanno K, Salomon B, Bryngelsson T, von Bothmer R: Phylogeny in the genus Hordeum based on nucleotide sequences closely linked to the vrs1 locus (row number of spikelets). Genome 42: 973-981 (1999b).

[9] Molina-Cano JL, Gomez-Campo C, Conde J: Hordeum spontaneum C. Koch as a weed of barley fields in Morocco. Z. Pflanzenzüchtg 88: 161-167 (1982).

[10] Takahashi R, Tomihisa Y: Genetic approach to the origin of two wild forms of barley, lagunculiforme Bacht. and proskowetzii Nabelek (Hordeum spontaneum C. Koch emend. Bacht.). Barley Genetics II: 51-62 (1970).
[11] Tanno K, Takaiwa F, Oka S, Komatsuda T: A nucleotide sequence linked to the vrs1 locus for studies of differentiation in cultivated barley (Hordeum vulgare L.). Hereditas 130: 77-82 (1999).
[12] Tanno K, Taketa S, Takeda K, Komatsuda T: A DNA marker closely linked to the vrs1 locus (row type gene) indicates multiple origins of six-rowed cultivated barley (Hordeum vulgare L.). Theor Appl Genet (in press).
[13] von Bothmer R, Jacobsen N: Origin, taxonomy, and related species. In Rasmusson DC (ed) Barley - ASA Agronomy Monograph 26: 19-56 (1985).
[14] von Bothmer R, Flink J, Jacobsen N, Jørgensen RB: Variation and differentiation in *Hordeum marinum* (Poaceae). Nord J Bot 9: 1-10 (1989).
[15] von Bothmer R, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I: An Ecogeographical Study of the Genus *Hordeum*, 2nd edition. Systematic and Ecogeographic Studies on Crop Genepools, 7. International Plant Genetic Resources Institute, Rome (1995).

BIOSYSTEMATIC RELATIONSHIPS BETWEEN ELYMUS MUTABILIS AND E. TRANSBAICALENSIS (POACEAE) AS INDICATED BY MORPHOLOGY, GRAIN PROTEINS, AND CROSSABILITY

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ABSTRACT: The relationships between *Elymus mutabilis* and *E. transbaicalensis* was studied by using biosystematic and molecular methods. Our results revealed a specificity for these taxa in their electrophoretic patterns of endosperm proteins. In general, *E. mutabilis* and *E. transbaicalensis* form two large separate recombination genepools (RGP). They should, hence, be considered as separate species. However, there are indications that some genetic exchange or gene flow between the RGPs may occur, especially in secondary habitats where the two taxa meet. Thus, the two RGPs seem to form a united introgression genepool (IGP) just like is the case with many other SH genome species.

1. INTRODUCTION

Elymus mutabilis (Drob.) Tzvel. is a perennial, self-fertilizing allotetraploid species with the SH genome. It has a wide distribution area in boreal Eurasia extending from northern Norway to the Russian Far East [1,2]. *Elymus transbaicalensis* is a taxon, which closely resembles *E. mutabilis* in morphology. Its distribution is restricted to Siberia, and adjoining areas in Mongolia and NW China [1,3]. The systematic position of *E. transbaicalensis* is somewhat ambiguous. It was first described as *Agropyron mutabile* var. *pilosum*. Later, S.A. Nevski described it as a separate species [4], and this is how it has usually been treated until 1973 when N.N. Tzvelev reduced it to a subspecies of *E. mutabilis* [5]. However, this new treatment was not accepted in the Flora of Siberia where it is still regarded as a separate species [3].

The objective of this study was to find out whether these taxa are conspecific or not, by using biosystematic methods. The specific aims were *(i)* to study whether they are morphologically discrete, *(ii)* to study their grain protein specificity and signs of introgression, and *(iii)* to estimate the potential for genetic exchange between the two taxa by studying the fertility in artificial hybrids.

2. MATERIALS AND METHODS

2.1. Plant material

Accessions of *E. mutabilis* and *E. transbaicalensis* from different regions of Siberia were used in this study (Table. 1). Some accesions were analyzed to clarify their taxonomic position. Seeds of natural plants were used. Preliminary species determination of natural accessions was conducted using the keys of Tzvelev [1] and Peshkova [3]. Two accessions of *E. komarovii* (Nevski) Tzvel. were included in the study for comparison. An aberrant accession from Kamchatka (KES-9640), belonging to *E. mutabilis* following the Flora of Soviet Far East [6], was also included in the crossing program.

2.2. Electrophoresis

Procedures and SDS-electrophoresis were carried out according Laemmli's method [7] with some modifications [8]. A scale of relative electrophoretic mobility (REM) was used for more precise comparative analysis of phoregramms.

2.3. Numerical analysis

Matrices for cluster analysis were constructed on the basis of data on prolamines. Presence or absence of a band were scored as 1 and 0, respectively. Each matrix was treated by «complete link» cluster analysis using the Manhattan distance coefficients. In «complete link» method, the distance between clusters are determined by the greatest distance between any two objects in different clusters, i.e. by the "furthest neighbors". The City block (Manhattan distance) is the average difference across dimension. All calculations were carried out using the program Statistica 5.0.

2.4. Hybridization

All procedures of prove hybridity to be true, and estimation of seed fertility of hybrids in F1-F3 generations were carried out as discribed (Agafonov, 1994).

3. RESULTS AND DISCUSSION

3.1. The morphological analysis

In their typical forms appearing in Southern Siberia, the two taxa could be described as follows. *Elymus mutabilis*: The leaves are usually hairy and the spikes are green or purple-green. The lemma is short hairy or bristly, and if it is awned, the awn is slightly bent. The glume is almost without scarious margins and it is gradually tapering. The inner side of the glume is hairy, at least near the base. *Elymus transbaicalensis*: The leaves are usually scabourus and the spike is glaucous or green. The lemma is short pilose and if it is awned, the awn is straight. The glume has prominent scarious margins and it is more or less abruptly tapering. The inner side of the glume is glabrous.

Our morphological analysis of living and pressed plant materials of *E. mutabilis* and *E. transbaicalensis* has shown that some specimens are intermediate in one or several of the above mentioned characters. Especially when the two taxa are growing intermingled. However, the glume characteristics seem to be reasonably stable charactersTable 1. Origin of accessions used in hybridizations (**bold**) and electrophoretic studies

Table 1. Origin of accessions used in hybridizations (**bold**) and electrophoretic studies

Accessions code	Species	Origin
GUK-9330, **GAJ-8918**	*E. mutabilis*	Altai, Ust-Kanskij district
GAM-9115	*E. mutabilis*	Altai, Schebalinskij district
ACH-8905, ACH-8932, ACH-8950, ATK-9902	*E. mutabilis*	Altai, Ongudaiskij district
H 10060_, GAE-8994	*E. mutabilis*	Altai, Ulaganskij district
AKU-9806, AUK-9824, AUK-9826	*E. mutabilis*	Altai, Kosch-Agachskij district
SAU-9501, SAU-9525, **SAU-9545, SAU-9546**	*E. mutabilis*	Krasnojarskij region, W. Sajan, Us river
SBI-9508, SBI-9510, SBI-9544	*E. mutabilis*	Krasnojarskij region, W. Sajan, Bilelig river
Ru 9511-36	*E. mutabilis*	Krasnojarskij region, W. Sajan, Bilelig river
Ru 9511-42	*E. mutabilis*	Krasnojarskij region, W. Sajan, Bilelig river
SON-9955	*E. mutabilis*	Krasnojarskij region, Ona river
IRC-9304	*E. mutabilis*	Irkutskiy region, near Cheremkhovo town
BAI-9202	*E. mutabilis*	Burjatia, Eastern coast of Baikal lake
KES-9640	*E. mutabilis*	Kamchatka peninsula, near Esso town
CUR-8803	*E. mutabilis*	Tien-Shan, 5 km S Issik-Kul lake, Kurbu mts
GAC-8923, GAC-8926	*E. transbaical.*	Altai, Schebalinskij district
ACH-8933	*E. transbaical.*	Altai, Ongudaiskij district
GAA-8904, GAA-8993, **H 10120*, H 10124, H 10125*, H 10127**	*E. transbaical.*	Altai, Ulaganskij district
AUK-9807, AUK-9812, AKU-9819, AKU-9820,		
AOR-9922	*E. transbaical.*	Altai, Kosch-Agachskij district

CONTINUACIÓN →

SAU-9502, **SAU-9507**, SAU-9513, **SAU-9523** (=**SAU-9509=H 10386**), **SAU-9551**,SAU-9552, H 10382, H 10387b	*E. transbaical.*	Krasnojarskij region, Western Sajan, Us river
SAI-9916	*E. transbaical.*	Krasnojarskij region, W. Sajan, Sajanskij pass
H 10376	*E. transbaical.*	Krasnojarskij region, W. Sajan, Aradan
SON-9947	*E. transbaical.*	Krasnojarskij region, W.Sajan, Ona river
SBI-9519, SBI-9547, Ru 9512-01, Ru 9512-10, Ru 9512-20, Ru 9512-30, Ru 9512-40, Ru 9512-50	*E. transbaical.*	Krasnojarskij region, W. Sajan, Bilelig river
RC-9301	*E. transbaical.*	Irkutskiy region, near Cheremkhovo toun
BUR-9730	*E transbaical.*	Western Burjatia, near Mondi
H 10390 (=SBI-9517), H 10394 (=SBI-9511)	*E. komarovii*	Krasnojarskij region, W.Sajan, Bilelig river
ALT-8401	*E. sibiricus*	Altai, Schebalinskij district

Most materials are easily identified by: *(i)* hairy vs. glabrous inner surface of the glume and *(ii)* narrow vs. wide membranous margins of the glume. These characters, in combination with the other characters, show, in our opinion, that there isn't a continuous variation between the two taxa even when growing together. Hence, they are better treated on the species level than on a subspecies level. Still, the intermediate specimens indicate that hybridization and possibly introgression occur.

3.2 Variability by seed storage protein

Accessions of all populations studied were characterized by significant individual variability in the protein spectra. Groups of protein components (60-70 REM) are present in samples of both *E. mutabilis*, and *E. transbaicalensis*. Some characteristic patterns were found in the prolamine and high molecular weight (HMW) gluteline subunits, which could be diagnostic for the two taxa. The most clear-cut visual distinctions were observed in variant + Me (fig. 1). A specific group of quickly migrating components with a relative electrophoretic mobility (REM) of 77 (about 30 kD) was present in all samples of *E. transbaicalensis*, irrespective of their geographical origin. All accessions of this taxon had also a component (or its allelic variant) with REM of 45. One characteristic of the *E. transbaicalensis* spectra were the absence of proteins in the range with REM of 37-44. In the *E. mutabilis* samples components were present in this range. Distinctions were also observed in HMW gluteline subunits, characterized by greater REM in most *E. transbaicalensis* accessions.

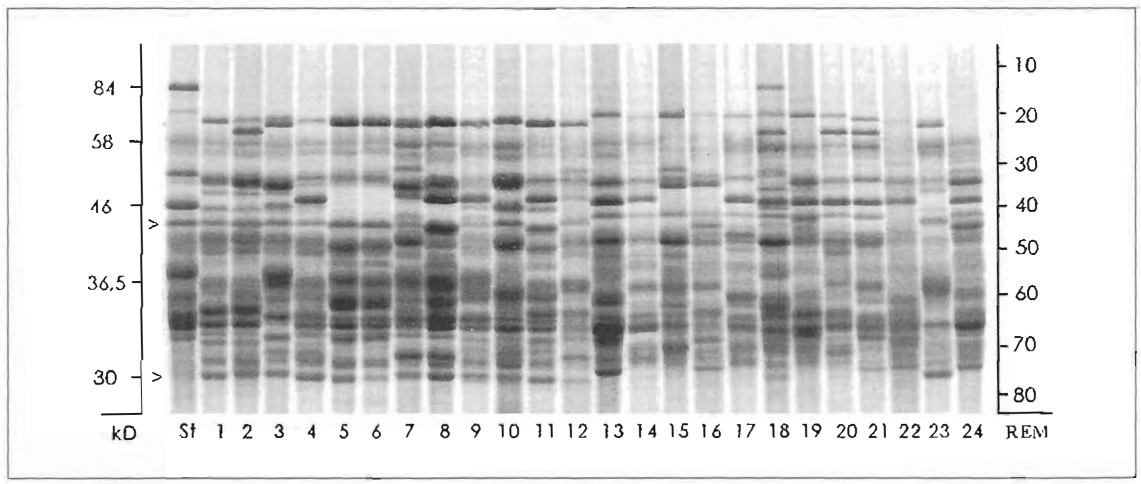

Figure. 1. SDS-PAGE of endosperm protein of *Elymus transbaicalensis* and *Elymus mutabilis*. Singe seed of Altai accessions in +Me variant. St - *Elymus sibiricus*, ALT-8401. ***E. transbaicalensis*****: 1.** GAC-8923; **2.** GAC-8926; **3.** ACH-8933; **4.** GAA-8904; **5.** GAA-8993; **6.** H 10120; **7.** H 10125; **8.** AOR-9922; **9.** AKU-9819; **10.** AKU-9820; **11.** AUK-9807; **12.** AUK-9812. ***E. mutabilis*****: 13.** GAM-9115; **14.** ATK-9902; **15.** GAI-8918; **16.** GUC-9330; **17.** ACH-8950; **18.** ACH-8905; **19.** ACH-8932; **20.** GAE-8994; **21.** H 10060a; **22.** AKU-9806; **23.** AUK-9828; **24.** AUK-9824.

The Sajan accessions Ru 9512-01, Ru 9512-10, Ru 9512-20, Ru 9512-30, Ru 9512-40 and Ru 9512-50 had identical protein patterns. Probably, these plants are offspring of a single genotype. The test on heterozygosis (2 seeds from a spike) revealed distinctions in 3 of the 12 Sajan accessions studied. Patterns of Ru-9511-42 seeds differed a little in the range REM of 32-35.

The prolamine analysis (variant -Me) of the Altai accessions did not reveal a clear visual distinction between the species, while the distinctions were more definite between Sajan accessions. In dendrograms made by the «complete link» method using «Manhattan distances» coefficients, all accessions were divided into two large clusters, each including accessions of only one taxon (fig. 2 and 3). The exception was AKU-9806 (Altai) referred by morphological characteristics to *E. mutabilis*, but included in cluster I and forming a pair with *E. transbaicalensis* AKU-9820. Probably, this is caused by introgression because of these two accessions were growing together in the same biotope. For the Sajan accessions, a clear separation of species was also found. One accession of *E. komarovii* clusterd with *E. transbaicalensis* (fig. 3). This support previous data on affinity between these two taxa that they form a uniform RGP [5]. Thus, both the Altai and Sajan accessions of each taxa (*E. mutabilis and E. transbaicalensis*) form separate groups on dendrograms.

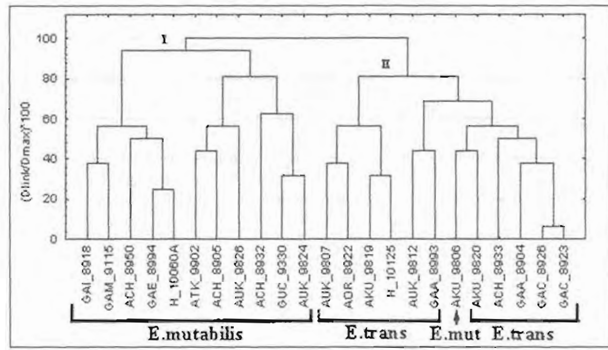

Figure 2. «Complete link»-dendrogram obtained from the prolamine data of same (fig. 1) natural accession from Altai ***E. mutabilis*** and ***E. transbaicalensis.*** The scale indicates levels of dissimilarity.

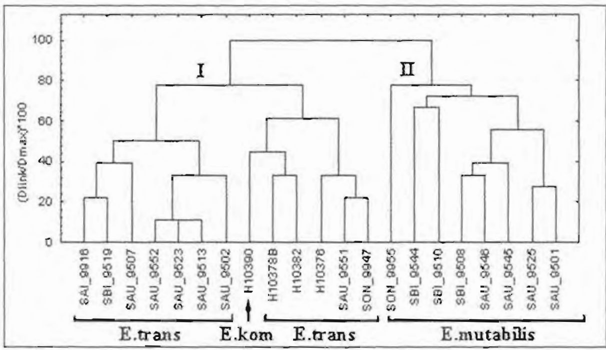

Figure 3. «Complete link»-dendrogram obtained from the prolamine data of the natural accessions from Sajan ***E. mutabilis*** and ***E. transbaicalensis.***

3.3. Hybridological analysis

As is shown in tables 2 and 3, both species generaly form two independent recombination gene pools, inside of which interchange of genes is possible after spontaneous cross pollinations.

In *E. mutabilis*, only two out of 20 hybrid combinations were completely sterile. The combination BAI-9202 x CUR-8803 is characterized by the considerable geographic distance of the parental accessions. However, this should not influence the fertility, especially since accession CUR-8803 from the Tien-Shan range is interfertile with other South Siberian accessions. Possibly, the observed sterility is due to some unfavorable genetic combination. The second sterile hybrid combination, BAI-9202 x KES-9640, is the only combination with the accession from Kamchatka in the Russian Far East. It is an aberrant form which we tentatively included in *E. mutabilis*. However, our preliminary analyses of this and other taxa from Kamchatka indicate that it should belong to another species [10]. In the light of those results, it is not surprising that this combination is sterile. All other hybrid combinations in *E. mutabilis* were interfertile, showing that they belong to the same RGP.

Differentiation in sexual compatibility was observed in *E. transbaicalensis* too (Table 3). All 18 hybrid combinations were interfertile. It is noteworthy that the four combinations with the lowest fertility, all included the accession SAU-9523. This accession possesses a protein pattern which is in common with the other accessions of *E. transbaicalensis* (fig. 3), but it is possible that it has a hybrid origin.

Seven out of the eight hybrid combinations between *E. mutabilis* and *E. transbaicalensis* were completely sterile (ACH-8905 x ACH-8933; ACH-8950 x GAC-8926;

Table 2. Seed fertility of hybrids in ***E. mutabilis.***

Cross combination	Highest value of seed set %of				Level C_r*
	F_1	N_{F1}*	F_2	N_{F2}*	
ACH-8905 x CUR-8803	13,5	2	82,3	3	α2
ACH-8905 x ACH-8950	86,7	2	87,0	34	α1
ACH-8905 x BAI-9202	7,6	1	84,6	6	α2
ACH-8932 x ACH-8905	63,8	1	87,9	3	α1
GAJ-8918 x CUR-8803	14,5	2	71,4	10	α2
GAJ-8918 x SBI-9510	45,2	2	-	-	α1
GAJ-8918 x SAU-9545	50,7	2	-	-	α1
CUR-8803 x SBI-9510	8,2	2	92,2	4	α2
SAU-9545 x GAJ-8918	75,3	2	-	-	α1
SAU-9545 x SBI-9510	54.1	3	-	-	α1
SAU-9546 x SAU-9545	14,6	2	68,2	6	α2
SBI-9508 x BAI-9202	47,4	2	-	-	α1
SBI-9510 x CUR-8803	21,7	3	-	-	α2
SBI-9544 x ACH-8950	12,3	1	86,6	3	α2
IRC-9304 x GAJ-8918	13,9	3	-	-	α2
IRC-9304 x CUR-8803	14,7	2	82,4	4	α2
IRC-9304 x SBI-9510	89,4	2	-	-	α1
BAI-9202 x SBI-9510	58,6	4	-	-	α1
BAI-9202 x CUR-8803	0	1	-	-	ß?
BAI-9202 x KES-9640	0	1	-	-	ß?

Table 3. Seed fertility of hybrids in ***E. transbaicalensis.***

Cross combination	Highest value of seed set set %of				Level C_r*
	F_1	N_{F1}*	F_2	N_{F2}*	
GAA-8904 x GAC-8923	87,5	2	-	*	α1
GAA-8993 x GAA-8904	39,6	2	88,9	12	α1
GAA-8993 x ACH-8933	18,3	3	41,6	7	α1
H 10120 x SBI-9519	26,9	1	-	-	α2
GAC-8923 x ACH-8933	83,3	4	-	-	α1
GAC-8923 x H 10125	63,5	2	-	-	α1
GAC-8926 x GAA-8904	88,2	1	87,5	2	α1
IRC-9301 x GAC-8923	58,3	3	72,2	16	α1
SAU-9507 x SBI-9519	62,9	3	-	-	α1
SAU-9551 x GAA-8993	5,9	3	12,6	2	α2
SBI-9519 x GAC-8923	51,2	2	-	-	α1
H 10124 x SAU-9523	31,7	3	30,0	4	α2
H 10125 x SAU-9523	3,5	2	0,8	4	ß
SAU-95023x H 10125	4,2	3	1,0	2	ß
SAU-9523 x GAC-8923	2,8	3	0	2	ß?
SAU-9523 x SAU-9518	6,3	3	48,5	5	α2
SAU-9523 x H 10127	2,0	1	0	6	ß?
SAU-9523 x BUR-9730	23,8	2	93,5	6	α2

* N_{F1} - number of plants analized in F_1

N_{F2} - number of plants analized in F_2

C_r - sexual compatibility of genotypes

SAU-9552 x SBI-9510; SBI-9547 x SBI-9510; CUR-8803 x GAA-8904; GAA-8904 x BAI-9202; GAA-8904 x ACH-8905). Just in one vigorous hybrid F_1 plant of ACH-8932 x GAA-8904 had a low seed set, which had not more than 2 grains / spike. Furthermore, an expected increase and stabilization of seed fertility could not be observed even in the F_4 generation, where it has a value 3-4%.

In conclusion, our results demonstrate that *E. mutabilis* and *E. transbaicalensis* in southern Siberia are morphologically discrete, reproductively isolated, and they are genetically different as indicated by their seed storage protein patterns. Hence, the two taxa are best treated as two species between which a weak gene flow is probably taking place.

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REFERENCES

[1] Tzvelev NN: Tribus 3. Triticeae Dumort. In: Fedorov AA (ed) Poaceae URSS. Leningrad. Nauka. p. 105-206 (1976).

[2] Hultén E, Fries M: Atlas of North European vascular plants North of the tropic of Cancer. Koelts Sci. Books, Königstein. p. 144 (1986).

[3] Peschkova GA: Elymus L. In: Malyschev LI and Peschkova GA (eds.) Flora Siberiae 2, Poaceae (Gramineae). Novosibirsk, Nauka, p. 17-32 (1990).

[4] Nevski SA: Zur Systematik der Gattung Agropyrum Gaertn. Izv. Bot. Sada AN SSSR 30: 607-635 (1932)

[5] Tzvelev NN: Conspectus Specierum Tribus Triticeae Dum. Familiae Poaceae in Flora URSS. Nov. Syst. Pl. Vasc. 10: 19-59 (1973).

[6] Probatova NS: Elymus L. In: Charkevicz SS (ed.) Plantae Vasculares Orientalis Extremi Sovietici 1. Leningrad, Nauka, p. 110-126 (1985).

[7] Laemmly VK: Cleavage of structural proteins during the assemly of the head of Bacteriophage T 4. Nature 227: 5259, 680-685 (1970).

[8] Kostina EV, Agafonov AV, Salomon B: Electrophoretic properties and variability of endosperm proteins of *Elymus caninus* (L.) L. In: Jaradat AA (ed.) Triticeae III. Enfield, New Hampshire, Science Publishers p. 265-272 (1998).

[9] Agafonov AV, Salomon B, Kostina EV, Diaz O: Biosystematic relationships between *Elymus komarovii* (Nevski) Tzvel. and related species. In: Jaradat AA (ed.) Triticeae III. Enfield, New Hampshire, Science Publishers p. 77-84 (1998).

MITOCHONDRIAL RPS2 OF BARLEY HAS A C-TERMINAL EXTENSION DIFFERING FROM THAT OF OTHER CEREALS BUT IS HIGHLY CONSERVED IN HORDEUM SPECIES

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ABSTRACT: Genes for ribosomal protein S2 (*rps2*) examined so far in higher plant mitochondria have C-terminal extensions as compared to *rps2* genes in lower plants. In this study, the structure of the *rps2* gene and its genetic diversity were examined in barley. The barley *rps2* gene has a C-terminal extension which is different from that of the counterpart gene in wheat, rice and maize mitochondria. Their whole N-terminal portions are highly conserved. Therefore, their C-terminal regions seem to have been acquired independently among these cereals. To establish the genetic diversity of the *rps2* sequence, portions of the *rps2* gene from the genera *Hordeum* and *Psathyrostachys* were amplified by PCR and their nucleotide sequences were determined. There was no nucleotide difference among them. This result strongly suggests that the C-terminal region of the *rps2* gene is diverged among cereals but highly conserved within the genus *Hordeum* and closely related species.

1. INTRODUCTION

Plant mitochondria have several unique characters when compared to their counterparts, e.g. animals. One of the discrepancies is that the size of plant mitochondrial genome is much larger than that from animals and yeast (see, [1] for a review). The large size of plant mitochondrial genome is mainly due to presence of repeated sequences and foreign DNAs of chloroplast and nuclear origins. Frequent recombination events across long and short repeated sequences also seem to increase their genome complexity, resulting in generation of multiple subgenomic molecules, a so-called "multipartite structure". Another feature of the plant mitochondrial genome is presence of additional genes, which are absent from animal mitochondrial genomes. In addition to gene content, the encoded genes often have additional sequences within their coding regions: the *atp6* genes from higher plant mitochondria have extensions at their N-terminal regions ([2] and references therein); the *rpl2* gene of rice [3] and the *rps4* genes of *Arabidopsis* [4], and rice and rapeseed [5] have insertions in their internal regions. These additional regions would have been acquired after split of higher plant species because such additional regions are not observed in lower plants.

Genes for ribosomal protein S2 (*rps2*) was found to be another example of such additional sequence. The *rps2* gene has been found in two higher plants, wheat and rice [6, 7] as well as in lower plants, liverwort and chlorophyte alga [8, 9]. Deduced open reading frames of the higher plant *rps2* genes have extension at their C-terminal regions when compared to those of the lower plants. The C-terminal extension of the wheat *rps2* gene shows no homology to any other known sequences while that of rice *rps2* gene contains a sequence homologous to part of *atpA* coding region. This evidence leads us to suppose that the C-terminal extensions of the *rps2* genes are variable among higher plant species.

In this study, we report the isolation of the *rps2* gene from barley. The *rps2* gene of barley also contains a C-terminal extension which differs from that of rice and wheat. We analyzed the genetic diversity of the *rps2* gene using a portion of the *rps2* gene in genus *Hordeum* and its closely related genus *Psathyrostachys*.

2. MATERIALS AND METHODS

2.1. Plant materials and DNA isolation

Young seedlings of barley (*Hordeum vulgare* L. ssp. *vulgare*, cv. Kanto Nakate Gold), 13 wild barley species and *Psathyrostachys juncea* were used for plant materials. Total DNAs were isolated as described previously [10].

2.2. Construction and screening of a mitochondrial DNA library

A mitochondrial DNA library was constructed from barley mitochodnrial DNA as described previously [11]. Preparation of the probe DNA and screening of the library were performed as described in Kadowaki et al. [11].

2.3. Thermal asymmetric interlaced (TAIL)-PCR

The 3' flanking sequence of barley *rps2* gene was amplified by TAIL-PCR [12, 13]. Primers T1 [5'-GATTTATCTCCAACCATGGATATG-3'] and T2 [5'-CTACCATTCATGTACCTCTTAGC-3'] were used as specific primers. Primers T1 and T2 were designed from the internal region of barley *rps2* sequence. Primer D1 [5'-NGTCGA(G/C)(A/T)GANA(A/T)GAA-3'] was used as a degenerate primer based on the report of Liu et al. [12]. PCR amplification was performed by the method of Liu et al. [12] with slight modifications. Amplified DNA fragments were directly sequenced.

2.4. Amplification of mitochondrial DNA fragments

Part of *rps2*-coding region was amplified by PCR with primer pair P1 [5'-GGTTATTCTGAATGCAGATAGA-3'], P2 [5'-TGCAAACTTAAAGGTATAGCTG-3']. Primer P1 was designed from the evolutionarily conserved region among cereal *rps2* genes. Primer P2 anneals to the 3' coding region specific for barely *rps2* gene. PCR reaction was performed in 20 ml volume with 20 ng of total DNA, 0.4 mM of each primers and 0.5 units of AmpliTaq GOLD DNA polymerase (PE Biosystems, USA). Other conditions were followed by the manufacturer's instructions. Reaction was performed in a GeneAmp 9700 thermalcycler (PE Biosystems) as follows: pre-incubation at 95°C for 9 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min, and the final extension at 72°C for 5 min. Amplified DNA fragments were directly sequenced.

2.5. DNA sequencing and data analyses

DNA was sequenced by the dideoxy chain termination method using Dye Terminator Cycle Sequence Kit according to the manufacturer's instruction (PE Biosystems). Nucleotide and deduced amino acid sequences were analyzed as described previously [11].

3. RESULTS AND DISCUSSION

3.1. Isolation of rps2 gene from barley mitochondrial genome

The *rps2* genes have been found in the mitochondrial genomes of two monocotyledonous plants, wheat and rice. Interestingly, rps2 gene is absent from those of several dicotyledon plants [6, 7]. Southern blot analysis was performed using the rice *rps2* gene as a probe to investigate presence or absence of *rps2* gene in another monocotyledon plant, barley. Signals were detected in the mitochondrial DNAs of barley (data not shown). This result indicates that an *rps2*-homologous sequence is also present in barley mitochondrial genome. To isolate the *rps2*-homologous sequence, a barley mitochondrial *Hin*dIII-DNA library was constructed and screened using the rice *rps2* gene as a probe. Two positive clones were obtained from 392 colonies of the library and they were found to be identical clones by restriction mapping and sequencing. Because the isolated *Hin*dIII fragment did not cover a complete open reading frame, the remaining region was amplified by TAIL-PCR [12, 13]. The complete nucleotide sequence from the isolated clone together with the TAIL-PCR products showed 98% and 83% nucleotide sequence identity to the *rps2* genes from wheat and rice, respectively. This result shows that *rps2* gene is encoded in the mitochondrial genome of barley.

3.2. The barley rps2 gene has a C-terminal extension which differs from those of other cereals

The barley *rps2* gene contains a single open reading frame capable of encoding 562 amino acids. The predicted amino acid sequence of barley *rps2* gene has a C-terminal extension as compared to RPS2 sequence from lower plants. To know its sequence origin, DNA and protein database search analyses were performed. At position 1967-2274, 67% nucleotide sequence identity was found with a spacer region bet-

ween *atpA* and *atp9* genes from common wheat, durum wheat and rye mitochondria [14-16] but there were no homology to any known protein sequences. In addition, two copies of *rps2* genes from maize mitochondria have been identified and deposited in the GenBank nucleotide sequence database under accession nos. AF273103 and AF273104. However, their C-terminal sequences are not homologous among the four cereals (Figure 1), suggesting different origins of their C-terminal extensions.

3.3. Sequence analysis of a part of rps2 gene among Hordeum species

In order to know genetic diversity of the *rps2* sequence, part of the *rps2* gene were amplified by PCR among genus *Hordeum* and its closely related genus *Psathyrostachys*. By comparison of nucleotide sequences of amplified PCR products, there was no nucleotide difference among them. Therefore, the C-terminal region of the *rps2* gene is diverged among cereals but highly conserved within the genus *Hordeum* and, at least, its closely related species. It seems due to the fact that mutation rate of mitochondrial genes are much lower than that of nuclear and chloroplast genes in higher plants as reported by Wolfe et al. [17].

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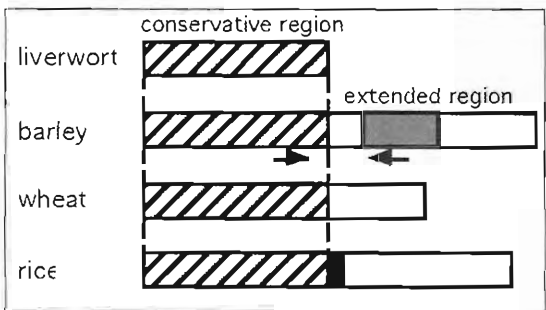

Figure 1: Schematic representation of *rps2* genes

Hatched boxes indicate regions homologous to liverwort *rps2* gene. Gray and black boxes represent sequences homologous to *atpA-atp9* spacer and *atpA* coding regions, respectively. Arrows show locations of PCR primers for sequence analysis.

REFERENCES

[1] Wolstenholme DR, Fauron C-M R: Mitochondrial genome organization. In: Levings III CS, Vasil IK (eds) The Molecular Biology of Plant Mitochondria. Kluwer Academic (1995).

[2] Marienfeld J, Unseld M, Brandt P, Brennicke A: Genome recombination of the mitochondrial *atp6* gene in *Arabidopsis thaliana* at the protein processing site creates two different presequences. DNA Res 3:287-290 (1996).

[3] Kubo N, Ozawa K, Hino T, Kadowaki K: A ribosomal protein L2 gene is transcribed, spliced, and edited at one site in rice mitochondria. Plant Mol Biol 31:853-862 (1996).

[4] Lippok B, Brennicke A, Unseld M: The *rps4* gene is encoded upstream of the *nad2* gene in *Arabidopsis thaliana* mitochondria. Biol Chem Hoppe-Seyler 377:251-257 (1996).

[5] Handa H, Kubo N, Kadowaki K: Genes for the ribosomal protein S4 protein encoded in higher plant mitochondria are transcribed, edited, and translated. Mol Gen Genet 258:199-207 (1998).

[6] Vitilingom M, Stupar M, Grienenberger J-M, Gualberto JM: A gene coding for an RPS2 protein in the mitochondrial genome of several cereals, but not in dicotyledons. Mol Gen Genet 258:530-537 (1998).

[7] Itadani H, Wakasugi T, Sugita M, Sugiura M, Nakazono M, Hirai A: Nucleotide sequence of a 28-kbp portion of rice mitochondrial DNA: the existence of many sequences that correspond to part of mitochondrial genes in intergenic regions. Plant Cell Physiol 35:1239-1244 (1994).

[8] Oda K, Yamato K, Ohta E, Nakamura Y, Takemura M, Nozato N, Akashi K, Kanegae T, Ogura Y, Kohchi T, Ohyama K: Gene organization deduced from the complete sequence of liverwort *Marchantia polymorpha* mitochondrial DNA. A primitive form of plant mitochondrial genome. J Mol Biol 223:1-7 (1992).

[9] Wolff G, Plante I, Lang BF, Kück U, Burger G: Complete sequence of the mitochondrial DNA of the chlorophyte alga *Prototheca wickerhamii*. J Mol Biol 237:75-86 (1994).
[10] Komatsuda T, Nakamura I, Takaiwa F, Oka S: Development of STS markers closely linked to the *vrs1* locus in barley, *Hordeum vulgare*. Genome 41:680-685 (1998).
[11] Kadowaki K, Kubo N, Ozawa K, Hirai A: Targeting presequence acquisition after mitochondrial gene transfer to the nucleus occurs by duplication of existing targeting signals. EMBO J 15:6652-6661 (1996).
[12] Liu Y-G, Mitsukawa N, Oosumi T, Whitter RF: Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J 8:457-463 (1995).
[13] Liu Y-G, Whitter RF: Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. Genomics 25:674-681 (1995).
[14] Bégu D, Graves PV, Litvak S, Araya A: Nucleotide sequence of the F_0-ATPase subunit 9 genes from two lines of wheat. Nucleic Acids Res 17:9491 (1989).
[15] Bégu D, Graves PV, Litvak S, Araya A: Nucleotide sequence of the F_1-ATPase subunit a genes from two lines of wheat. Nucleic Acids Res 17:9492 (1989).
[16] Laser B, Oettler G, Kück U: RNA editing of the mitochondrial *atpA/atp9* co-transcript of Triticale, carrying the *timopheevi* cytoplasmic male sterility cytoplasm from wheat. Plant Physiol 107:663-664 (1995).
[17] Wolfe KH, Li W-H, Sharp PM: Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proc Natl Acad Sci USA 84:9054-9058 (1987).

VARIATION IN CHLOROPLAST MICROSATELLITE LOCI AMONG WILD AND CULTIVATED SPECIES OF EMMER WHEAT

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ABSTRACT: It is generally accepted that cultivated Emmer wheat (*Triticum dicoccum* Schübl.) originated from its wild ancestor (*T. dicoccoides* Körn.) by domestication about ten thousand years ago, somewhere in the Fertile Crescent. To study the origin of the cultivated Emmer wheat, allelic diversity in eight microsatellite loci of the chloroplast genome was investigated using 100 accessions of *T. dicoccum* and 72 accessions of *T. dicoccoides*. Five accessions of *T. paleocolchicum* Men., two of *T. durum* L., and one each of *T. aestivum* L., *T. carthlicum* Nevski, *T. ispahanicum* Heslot, *T. polonicum* L. *and T. turgidum* L. were analyzed as references. The number of repeats in each locus was determined using PCR followed by PAGE. Polymorphic band patterns were obtained at seven out of the eight loci. As for the polymorphic microsatellites, the number of alleles per locus ranged from two to five with an average of 2.92. Nei's allelic diversity was estimated for each locus within each species. The average allelic diversity of *T. dicoccoides* (0.349) was about 3.2 times larger than that of *T. dicoccum* (0.109). One plastotype (chloroplast haplotype) representing 80 % of the *T. dicoccum* accession and all accessions of references was identical to that *of T. aestivum* cv Chinese Spring. In contrast, this major haplotype of the cultivated wheat was identified only in six accessions of *T. dicoccoides* collected in one region in the northwestern Fertile Crescent. In addition to this major maternal lineage, we have identified a minor lineage from the wild Emmer to the cultivated Emmer wheat. These results suggest that there are at least two maternal lineages in the cultivated Emmer wheat, and that the domestication of Emmer wheat with the major haplotype occurred in the north western Fertile Crescent.

INTRODUCTION

Wheat is one of the most important cereal crops, providing a staple food for over one-third of the world population. The domestication of wheat and barley was one of the most important steps toward the establishment of farming communities which later led to the civilisation in Mesopotamia (1). Genetic and morphological evidences indicate that cultivated species of Einkorn, Emmer, and Timopheevi groups were domesticated, respectively from the wild species *Triticum boeoticum* Boiss. (AA), *T. dicoccoides* Körn (AABB) and *T. araraticum* Jakubz. (AAGG). Among those species the cultivated Emmer wheat had been an important crop for several thousand years in the agricultural history in the Near East. Cultivated Emmer wheat is morphologically polymorphic. *T. dicoccum* and *T. paleocolchicum* are non-free threshing type and retain primitive characteristics, while rest of the species, e.g. *T. durum* Desf. and *T. turgidum* L. are free threshing type and have some advanced characteristics.

Paleoethnobotanical studies suggests that significant association between wheat and human began in the Middle East more than 10,000 years ago (2). Remains of *T. dicoccoides* and wild barley, *Hordeum spontaneum* C. Koch, were recovered from an Early Epi-Paleolitic site dated 17,000 BC at the south shore of the Sea of Galilee (3). The earliest signs of the cultivated Emmer wheat were obtained from the archa-

eological sites of 8th millennium BC. The remains of *T. dicoccum* appeared in Tell Aswad in Syria, dated 7800 -7600 BC, but no wild Emmer wheat were found there (4). The remains of *T. dicoccum* also appeared in Tell Abu Hureyra (6) and in contemporary Pre-Pottery Neolithic B Jerico (2, 5), both sites dating to about 7500 BC. Those facts suggest that cultivation of Emmer wheat was already successful in Levant at the early 8th millennium BC (2). The wild progenitor of Emmer wheat (*T. dicoccoides*) has a limited natural distribution area and only grows in the Near East. Therefore the findings of *T. dicoccum* from the archaeological settlements outside the Near East provide the good evidence for the domestication of Emmer wheat within this region and subsequent spread from this area. However the precise location, time and genetic process of the domestication are still unknown.

Recently we have identified 24 chloroplast microsatellite loci having more than ten mononucleotide repeats in the complete sequence of the chloroplast genome of *T. aestivum* cv Chinese Spring (7). The development of highly polymorphic microsatellite markers enables us to examine the molecular variation of the chloroplast genomes within the species in *Triticum*. In this study, allelic diversity at eight chloroplast microsatellite loci was examined within each of the wild and cultivated Emmer wheat. By comparing the geographical distribution and frequency of plastotypes (chloroplast haplotypes) between the species, location of the domestication of the Emmer wheat will be discussed.

MATERIALS AND METHODS PLANT MATERIALS

Seventy two accessions of the wild Emmer wheat (*T. dicoccoides*) whose sampling locality covers most of its natural distribution area, and 100 accessions of the cultivated Emmer wheat (*T. dicoccum*) collected from all over the world were used for the analysis (Table 1). In addition to those materials, five accessions of *T. paleocolchicum* Men., two accessions of *T. durum* L., single accession each of *T. ispahanicum* Heslot., *T. turgidum* L. and *T. carthlicum* Nevski were used as the references (Table 1).

Table 1. Plant materials used for chloroplast microsatellite analysis

Species[1)]	Type[2)]	Origin (number of accessions)
dcd	wild/N	Israel (25), Syria (4) Iraq (17), Iran (4), Turkey (21)
dcm	cult/N	Yugoslavia (7), Bulgaria (2), Portugal (1), Spain (11), Germany (3), Poland (1), Romania (1), Hungary (2), Swiss (1), Austria (1), Belgium (1), Italy (2), Czech (2), Armenia (2), Georgia (5), Former USSR (6), Turkey (2), Iran (19), Ethiopia (16), Eritrea (1), Morocco (1), Syria (1), Palestine (1), ,Saudi Arabia (1), Oman (2), China (1), India (4), USA (2),
plc	cult/N	Former USSR (5)
isp	cult/N	Iran (1)
crt	cult/F	Turkey
drm	cult/F	Italy (1), Ethiopia (1)
pln	cult/F	Iran (1)
trg.	cult/F	Ethiopia (1)

1) *dcd*: *T. dicoccoides* Körn, *dcm*: *T. dicoccum* Schübl., *plc*: *T. paleocolchicum* Men., *isp*: *T. ispahanicum* Heslot, *crt*: *T. carthlicum* Nevski, *drm*: *T. durum* L., *plc*: *T. polonicum* L., *trg*: *T. turgidum* L.
2) N: non-free threshing type, F: free threshing type

Analysis of the chloroplast microsatellite

Total DNA was extracted from fresh leaves according to the method of Liu et al. (1990) (8). Among 24 microsatellite loci found by Ishii et al. (2001) (7), allelic diversity was examined in the following eight loci, *WCt1*, *WCt2*, *WCt3*, *WCt4*, *WCt5*, *WCt6*, *WCt9* and *WCt12*. PCR, electrophoresis, and allele scoring were performed according to Ishii et al. (2001) (7).

DATA ANALYSIS

The allelic diversity of the eight chloroplast micro satellites was calculated according to the gene diversity value described by Nei (1987) (9) as follows:

$$Hi = 1 - \sum_{j=1}^{n} x_{ij}^2$$

where x_{ij} is the frequency of the jth allele for marker i and summation extends over n alleles.

Cluster analysis among the haplotypes was performed based on the similarity of chloroplast microsatellite allele sizes. Numbers of total and common amplified fragments were scored for all the haplotype pairs. The proportion of common amplified fragments between each haplotype was used as a similarity index. Based on the similarity index, a dendrogram showing genetic relationship among the haplotypes was constructed by the UPGMA method (10).

RESULTS AND DISCUSSION

Allelic diversity of the chloroplast microsatellites among the wild and cultivated Emmer wheat

Allele size at each locus was determined based on the differences in the nucleotide length from the standard variety, *T. aestivum* cv Chinese Spring. Among the eight microsatellite loci examined, polymorphisms were observed at six loci in 183 accessions of Emmer wheat. The number of alleles and the diversity values (H) were estimated within the wild Emmer wheat (*T. dicoccoides*) and the cultivated Emmer wheat (*T. dicoccum*) (Table 2). The number of alleles at the six polymorphic microsatellite loci ranged from 2 to 5. The diversity values ranged from 0.059 to 0.613. There was no significant correlation between the microsatellite loci and the diversity value in *T. dicoccoides* and *T. dicoccum*. An average diversity value of the wild Emmer wheat (0.349) was about three times larger than that of the cultivated Emmer wheat (0.109). This fact suggests that the genetic diversity of chloroplast genome in the wild progenitor is much larger than that of cultivated species and might reflect the bottle neck effect during the domestication process of the Emmer wheat.

Table 2. Number of alleles and diversity indices (H) obtained for eight chloroplast microsatellite loci

	Wild Emmer (T. dicoccoides)		Cultivated Emmer (T. dicoccum)	
Locus	No. of alleles	H	No. of alleles	H
WCt1	1	0.000	1	0.000
WCt2	5	0.613	3	0.218
WCt3	2	0.198	3	0.183
WCt4	4	0.334	3	0.039
WCt5	3	0.486	2	0.211
WCt6	3	0.478	3	0.059
WCt9	2	0.129	1	0.000
WCt12	3	0.551	2	0.164
Average	2.9	0.349	2.3	0.109

Chloroplast haplotypes detected among Emmer wheat

Based on the size variation at all the microsatellite loci, the chloroplast haplotype of each accession was determined. Twenty seven haplotypes (Type A to AB) were detected among 183 accessions of Emmer wheat, (Table 3). The number in this table indicates the size difference (in base pairs) from the standard allele (Type A) of *T. aestivum* cv Chinese Spring. Genetic relationship among the haplotypes was examined by the cluster analysis. The UPGMA tree was constructed based on the similarity index (Fig. 1). The tree indicates that haplotype AB and K found in *T. dicoccoides* are distantly related to the rest.

Geographical distribution of the haplotypes in the wild and cultivated Emmer wheat and the possible site of domestication

Geographical and frequency distribution of the haplotyes were summarised in Table 4. Twenty-one haplotypes were found in the wild Emmer wheat (*T. dicoccoides*), whereas only 10 haplotypes were detected in the cultivated Emmer wheat (*T. dicoccum*). Type A that is identical to the haplotype of *T. aestivum* cv Chinese Spring showed the highest frequency in Emmer wheat and this type was found both in the wild and cultivated species. Type B, C, D, E, G and J were found only in *T. dicoccum* and not in *T. dicoccoides*. All of these six haplotypes except for Type G are closely related to Type A (Fig. 1), suggesting that those types were derived from Type A and fixed in the *T. dicoccum* population.

Table 3. Twenty seven chloroplast haplotypes (plastotypes) found in Emmer wheat

Haplotype	Microsatellite loci							
	WCt1	*WCt2*	*WCt3*	*WCt4*	*WCt5*	*WCt6*	*WCt9*	*WCt12*
A	0	0	0	0	0	0	0	0
B	0	-1	0	0	0	0	0	0
C	0	0	0	0	0	-1	0	0
D	0	0	0	-1	0	0	0	0
E	0	0	1	0	0	0	0	0
F	0	-3	-1	0	1	0	0	-1
G	0	0	0	-6	1	0	0	-1
H	0	0	0	0	1	1	0	0
I	0	0	0	0	1	0	0	0
J	0	0	1	0	1	0	0	0
K	0	2	-1	-1	1	-1	0	-1
L	0	-3	0	1	2	0	0	-1
M	0	1	0	0	2	0	0	0
N	0	1	0	-1	2	0	0	0
O	0	0	0	0	2	0	0	1
Q	0	0	0	1	1	0	0	0
R	0	0	0	0	1	-1	0	0
S	0	0	0	0	2	0	0	0
T	0	0	0	0	1	0	1	0
U	0	1	0	0	1	0	0	0
V	0	0	0	0	2	0	1	-1
W	0	0	0	18	2	0	0	0
X	0	1	-1	0	1	0	0	0
Y	0	-1	0	0	1	-1	0	1
Z	0	0	0	0	1	-1	1	1
AA	0	1	0	0	1	1	0	1
AB	0	2	0	-1	1	-1	0	-1

Interestingly, 80% of the *T. dicoccum* accessions and all accessions of *T. paleocolchicum, T. durum T. turgidum, T. ispahanicum, T. carthlicum* and *T. polonicum* had the Type A haplotype (Table 4). In contrast, this major haplotype of the cultivated wheat was identified only in the six accessions of *T. dicoccoides* collected from a single location in the northwestern Fertile Crescent. This result suggests that there is a major maternal lineage in the cultivated Emmer wheat and that the domestication of Emmer wheat with the

major lineage occurred in this region. In addition to the major haplotype A, we have identified two minor haplotypes (Type F and I) that are shared in common between *T. dicoccoides* and *T. dicoccum* (Table 4). Type I has only one nucleotide difference from Type A and might have been derived from Type A (Table 4). However Type F has the specific alleles at the four loci (*WCt* 2, 3, 5 and 12) and clearly differentiated from Type A (Table 4). Type F was identified in four accessions of *T. dicoccoides* collected in the northeastern Fertile Crescent, and eight *T. dicoccum* accessions from Europe. This type was also found in two accessions of common wheat (7). The observations suggest that there is a second maternal lineage in Emmer and common wheat and this lineage might have originated in the northeastern Fertile Crescent. It was also strongly suggested that the number of the maternal lineage in the cultivated Emmer wheat is limited. The RFLP analysis of the nuclear DNA in the wild and cultivated Emmer wheat also supports this hyposthesis (11). However archaeological and paleoethnobotanical studies suggest that wheat domestication occurred in multiple location in this area (2). If we take all these findings in account, we could propose the following two hypotheses about the domestication of Emmer wheat. 1) Initially the domestication occurred in parallel in many locations but most of the lineage had been lost by chance during subsequent spread of the Neolithic agriculture from the core Near East region. 2) Once the cultivated Emmer wheat originated, this cultivated form spread in relatively short time in the Near East and consequently, only those lineages were fixed in cultivated Emmer wheat population. To test these hypotheses further fingerprinting studies using the DNAs from the archaeological remains will be necessary.

Table 4. Distribution of the 27 chloroplast haplotypes in *T. dicoccoides* and *T. dicoccum*

Haplotype	Wild Emmer (*T. dicoccoides*) No. acc.	Country	Cultivated Emmer (*T. dicoccum*) No. acc.	Country
A	6	Turkey	80	Rest of all
B	0	-	4	Turkey, Romania, Austria
C	0	-	2	Armenia, Germany
D	0	-	1	Ethiopia
E	0	-	1	Former USSR
F	4	Turkey, Iran	8	Spain, Swiss Czech Rep.
G	0	-	1	Bulgaria
H	5	Iraq	1	Germany
I	16	Israel, Syria, Turkey, Iraq	1	Italy
J	0	-	1	Palestine
K	3	Israel	0	-
L	4	Iraq	0	-
M	3	Israel	0	-
N	3	Iraq	0	-
O	2	Israel	0	-
Q	2	Israel	0	-
R	1	Israel	0	-
S	4	Israel	0	-
T	1	Israel	0	-
U	1	Israel	0	-
V	1	Unknown	0	-
W	1	Syria	0	-
X	1	Israel	0	-
Y	9	Turkey	0	-
Z	3	Iraq	0	-
AA	1	Turkey	0	-
AB	1	Israel	0	-

CONCLUSIONS

1) Majority (80%) of the non-free threshing cultivated Emmer wheat in the world has a single chloroplast haplotype and the domestication of the Emmer wheat with this haplotype might have occurred in the northwestern Fertile Crescent.
2) There exists another maternal lineage in the cultivated Emmer wheat and this minor lineage might have originated in the northeastern Fertile Crescent.

The number of the maternal lineage in the cultivated Emmer wheat might be limited.

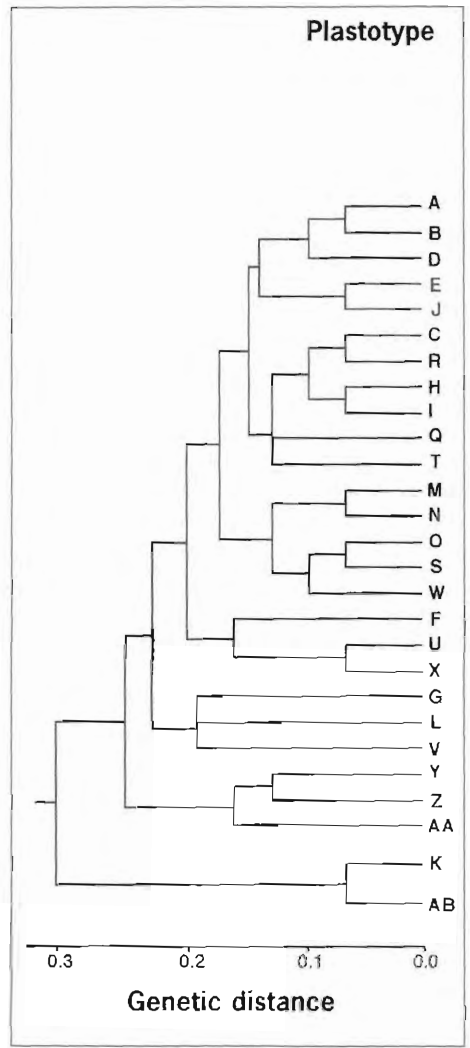

Figura 1. A UPGMA dendrogram showing the genetic relationship among the 27 plastotypes.

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REFERENCES

(1) Harlan JR: Crops and Man. American Society of Agronomy, Inc., Crop Science Society of America Inc., Madison, WI. (1992).

(2) Zohary D and Hopf M: Domestication of Plants in the Old World (2nd ed.). Clarendon Press, Ozford (1993).

(3) Kislev ME, Nadel D and Carmi I: Epipalaeolithic (19,000 BC) cereal and fruit diet at Ohalo II, Sea of Galilee, Israel. Rev. Palaeobot. Palynol. 73: 161 - 166 (1992).

(4) Van Zeist W and Bakker-Heeres JAH: Archaeological studies in the Levant 1. Neolithic sites in the Damascus Basin: Aswad, Ghoraifé, Ramad. Palaeohistoria 24: 165 - 256 (1985).

(5) Hopf M: Jericho plant remains. In: Excavations at Jericho, Vol. 5 (eds. K. M. Kenyon and T. A. Holland), pp. 576 - 621. British School of Archaeology in Jerusalem, London.

(6) Hillman G: The plant remains from Tell Abu Hureyra: A preliminary report. *Proc Prehist. Soc.* 41: 3 - 70 (1975).

(7) Ishii T, Mori N and Ogihara Y: Evaluation of allelic diversity at chloroplast microsatellite loci among common wheat and its ancestral species. Theor. Appl. Genet. (in press).

(8) Liu YG, Mori N and Tsunewaki K: Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genome DNA library construction and RFLP analysis in common wheat. Jpn. J. Genet. 65: 367 - 380 (1990).

(9) Nei M: Molecular Evolutionary Genetics. Columbia University Press, New York. (1987).

(10) Sneath PHA and Sokal RR: Numerical Taxonomy. Freeman, San Francisco. (1973).

(11) Mori N, Moriguchi T and Nakamura C: Rflp analysis of nuclear DNA for study of phylogeny and domestication of thetraploid wheat. Genes Genet. Syst. 72: 153 - 161 (1997).

THE DETECTION AND CHARACTERISATION OF WIS 2-1A-LIKE SEQUENCES IN *TRITICUM AESTIVUM* L. AND *XTRITICOSECALE* WITTMACK, AND AN EXAMINATION OF THEIR COPY NUMBER IN RELATED TRITICEAE

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ABSTRACT: Retrotransposons and other mobile elements are major components of the repeated DNA fraction in higher plant genomes. They have undoubtedly played an important role in higher plant genome evolution. The present work details the detection and characterisation of a WIS 2-1A-related sequence in direct wheat relatives, and discusses the prevalence and evolution of its copy number in their genomes. An increase in copy number is detected when following the natural hybridisation processes that gave rise to bread and durum wheats. However, the opposite is observed in the development of the synthetic hybrid, triticale.

INTRODUCTION

Retrotransposons are the most abundant mobile genetic elements, and are found in all eukaryote orders (1). They are major constituents of plant genomes and have functional and structural characteristics similar to retroviruses, though they themselves are not infectious. Heterogeneous families of more or less related retrotransposons have been described in over 100 higher plant species (*Avena, Brassica, Triticum, Vicia, Vitis, Zea*, etc.). Some regions of known retrotransposons have been used in phylogenetic studies and also in the development of different strategies for studying variability (2) (3).

Retrotransposons show the ability to replicate and insert new copies of themselves to new chromosomal locations via RNA intermediates. This particular mode of replication can rapidly increase their copy number and significantly increase the size of plant genomes (4)(5), and suggests they may have played an important role in the genomic organization and genetic structure of their host species, raising also interesting questions about the existence of reaction mechanisms that control and reduce the size of the genome (4).

Harbert et al. (6) reported the discovery of WIS 2-1A (AN:X63184), the first retrotransposon found in wheat. Its phylogenetic distribution was characterised by Southern hybridisation using sixteen species representing 11 genomes of the *Triticeae* tribe (7). Homologous sequences of the retrotransposon were found in all taxa tested. There were high levels of interspecific variability and almost no intraspecific differentiation. This suggests that WIS 2-1A is an ancient element probably present in the unknown common ancestor of the *Triticeae*, and that only under rare circumstances does it become active. The molecular characterisation of WIS 2-1A showed the insertion site to be flanked by a 5 bp duplication, that it contains an internal domain of about 5500 bp, and has terminal repeats (LTR) of over 1755 bp (8).

This paper reports the isolation, cloning, sequencing and analysis of the molecular organisation of a region of WIS 2-1A detected during the analysis of random amplified microsatellite polymorphisms (RAMPs) (9) in segregant *xTriticosecale* plants. The aims were to study the presence of this element in the genomes of *xTriticosecale* and related species and to assess its degree of distribution and conservation during the evolution of *Triticeae.*

MATERIALS AND METHODS

Plant material

The experimental material consisted of 7 species representing the genomes A, B, D and R of the following cultivated and wild *Triticeae* species: *Triticum monococcum* L. (2n=14, genome A) [accession Nº 158-1 GermplasmBank Gatersleben]; *Secale cereale* L. (2n=14, genome R) cv. 'Petkus'; *Triticum speltoides* (Tausch) Godron (2n=14, genome B) [accession Nº 01/87 GermplasmBank Gatersleben]; *Triticum squa-*

rrosa (Coss.) Schmalth. (2n=14, genome D); *Triticum turgidum* L. (2n=28, genomes AB) cv 'Jerez 137'; *Triticum aestivum* L. (2n=42, genomes ABD) cv 'Chinese Spring' and cv 'Pané 247'; x*Triticosecale* Wittmack (2n=42, genomes ABR) cv 'Torote' and cv 'Presto'.

DNA PURIFICATION AND ANALYSIS OF RANDOM AMPLIFIED MICROSATELLITE POLYMORPHISMS (RAMPS).

Genomic DNA from wheat was extracted from leaves of single plants about 4-6 weeks old as described by Sharp et al. (10). Seventy PCR primer sets combining different sequences 10 bases long (Operón Technologies) and a microsatellite $GT(CA)_4$ were used to perform RAMP experiments on genomic DNA of the triticale lines 'Torote' and 'Presto'. Amplification products were separated by non-denaturing electrophoresis in 7 % acrylamide gels and visualised using SYBR-GREEN I (FMC).

ISOLATING AND CLONING THE AMPLIFIED PRODUCT T-8(2)

Polymorphic bands from the RAMP experiments were cut from the acrylamide gels, reamplified using the same set of primers from which they were obtained, ligated into a Bluescript pSK+ vector and transformed into competent *Escherichia coli* DH10B cells. Different clones for each fragment were selected for subcloning and sequencing. The consensus sequences were deduced using the Sequence Navigator programme (Applied Biosystems).

DNA SEQUENCE-ALIGNMENT ANALYSIS AND DESIGN OF PRIMERS

Nucleotide sequences were compared to databases using the WuBLAST and FASTA programmes (www.ebi.ac.uk), and aligned by the PILE-UP and PRETTY functions (GCG package- University of Wisconsin). A set of primers was designed from the sequences of T-8(2) and BARE-1 barley retrotransposon (AN: Z17327) .(forward primer: 5´-CTACGTATTCCACCGATCGTCC-3´; reverse primer: 5´-ACCATGTCCACATGCTAGGC-3´). These primers were used on 'Torote' and 'Chinese Spring' DNA to amplify a fragment of about 800 pb of expected size.

COPY NUMBER CALCULATIONS OF PTO818

Slot blots were prepared by transfer onto charged nylon membranes of a 1:2 diluted series of the plasmid carrying the insert of 818 bp, (=pTo818) as standard, and 1:2 diluted series of genomic DNA of the different plant materials. Sequence pTo818 was digoxigenin-labelled and used as a probe to hybridise the filters. Chemiluminiscent hybridisation signal was detected by autoradiography. Signal intensity was quantitated with the volume analysis function of the Molecular Analyst programme (BioRad).

RESULTS

Several RAMP markers obtained in an ongoing study concerning a molecular map were cloned and sequenced in order to investigate their nature. One of the sequences coming from Torote , corresponding to the polymorphic fragment designated T-8(2) (AN:304463), showed significant homology to part of the leader region of the polyprotein domain (bases 2258-2582) of the barley retrotransposon BARE-1 (11). This encouraged work designed to characterise this sequence and to compare it with other retrotransposons previously described in cereals.

To this end a set of primers was designed using the known sequences of the T-8(2) fragment and the *Gag* region of BARE-1 and a PCR experiment was performed to amplify from genomic DNA of x*Triticosecale* and wheat. Electrophoretic analyses of PCR reactions performed on genomic DNA from x*Triticosecale* cv 'Torote' and *T. aestivum* cv 'Pané 247' resulted in the clear production of three DNA bands of 818 bp, 1400 bp and 1865 bp in 'Torote', and 882 bp, 1400 bp and 1865 bp in 'Pané 247' (Fig. 1).

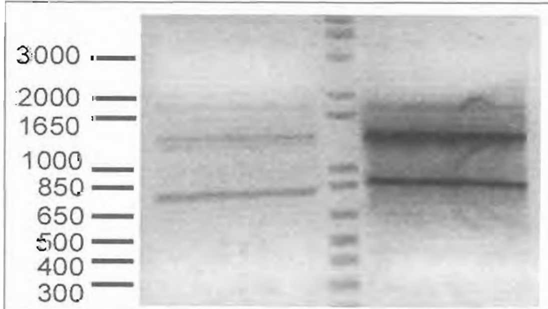

Fig. 1.-Amplified products using the designed primers to detect sequences homologous to known retrotransposons of cereals. From left to rigth: xTriticosecale cv 'Torote', PM: 1Kb+ (Amersham); T. aestivum cv 'Pané 247'. Marker sizes are shown adjacent to the gel.

Characterization of the amplified products

A cross-hybridisation experiment (not shown) resulted in the recognition of all the amplified products by hybridisation with the 818 bp Torote fragment, so the amplification products of 1865 bp from 'Torote' and 'Pané 247', 818 bp from 'Torote' and 882 bp from 'Pané 247' were isolated and cloned. Two clones from each product were sequenced to get a consensus for each product. Comparison with GenBank revealed that, in all cases, these sequences (from now on identified by their origin and size) are homologous to those of retrotransposons of wheat and related species (Table I). All sequences showed homology to WIS 2-1A of wheat, and were located between the 5´ LTR and the protease motif.

Cloned sequence	Description	Topology & % homology
pTo818 (818bp) Complete sequence EMBLE GenBank accession number AJ291717	Region 369-1060bp of WIS 2-1A, associated with CEREBA	1–147: 77% homology to **CEREBA**; 147–818: 90% homology to **WIS 2-1A**
pTo1865 (1865bp) Complete sequence EMBLE GenBank accession number AJ301642	Region LTR and 1-1060bp ofWIS 2-1A associated with the gene Acc-1,1 (acetyl-CoA carboxylase)	1–264: 83% homology to **Acc-1,1**; 264–1865: 78% homology to **WIS 2-1A**
pPané882 (882bp) Complete sequence EMBLE GenBank accession number AJ303051	Varicus regions of BARE-1 and minorregions of WIS 2-1A	1–882: 85% mean homology **BARE-1**
Pané 1865 (1865bp) Complete sequence EMBLE GenBank accession number AJ304464;AJ304465	Region LTR and 607-1060bp of WIS 2-1A associated with the gene Acc-1,1 (acetyl-CoA carboxylase)	1–257: 83% homology to **Acc-1,1**
		1865: Terminal ends, 79% mean homology to **WIS 2-1A**

TableI: Similarity of the cloned sequences obtained by PCR using genomic DNA of *xTriticosecale* cv. 'Torote' and *T. aestivum* cv. 'Pané 247'.

Sequences pTo1865, pPané1865 and pPané882 were associated with the promoter region of the wheat gene *Acc-1,1* which codes for an acetyl-coenzyme A carboxylase (AN: AF029897).

ESTIMATION OF THE NUMBER OF COPIES OF PTO818 IN WHEAT, TRITICALE AND RELATED SPECIES

The number of copies of pTo818 was estimated in the genomes of all species analysed using Slot-blot. The results are shown in Fig. 2 and Table II. The data in the table refer to the contribution of the region pTo818 of the retrotransposon to the total genome. The sequence pTo818 was present in all the diploid species with a relatively high number of copies for genome size. The results indicated that *T. squarrosa* has between 2.31x and 2.65x more copies of pTo818 than *T. monococcum* and *T. speltoides*, the putative donors of the A and B genomes respectively In contrast, it has the smallest genome ($4.9x10^9$ bp) of all the diploids examined. It was also observed that the part of the genome composed of the sequences equivalent to retrotransposons in allopolyploid species is 3-4 times *higher* than expected according to their diploid genomic composition. The only exception is the synthetic amphyploid *xTriticosecale* ($475x10^3$ copies; 2.3%) which exhibits a lesser contribution of the WIS 2-1A region of the retroelement compared to *T. turgidum* ($516x10^3$ copies; 3.6 %) and *T. aestivum* ($740x10^3$ copies; 3.4 %). It should be borne in mind that *xTriticosecale* has a genome size similar to that of *T. aestivum* and approximately three times those of each of the diploid species.

Table II:-Number of copies of pTo818 in the genomes of different species

Species	Genome/s	Genome size (C) x 10^9 bp	N° copies x 10^3	% genome
T. monococcum		6.0	61.5	0.83
T. speltoides	B	5.6	65	0.95
T. squarrosa	D	4.9	133	2.2
S. cereale	R	8.5	103	0.99
T. turgidum	AB	11.7	516	3.6
T. aestivum	ABD	17.3	740	3.4
x-Tricosecale	ABR	16.8	475	2.3

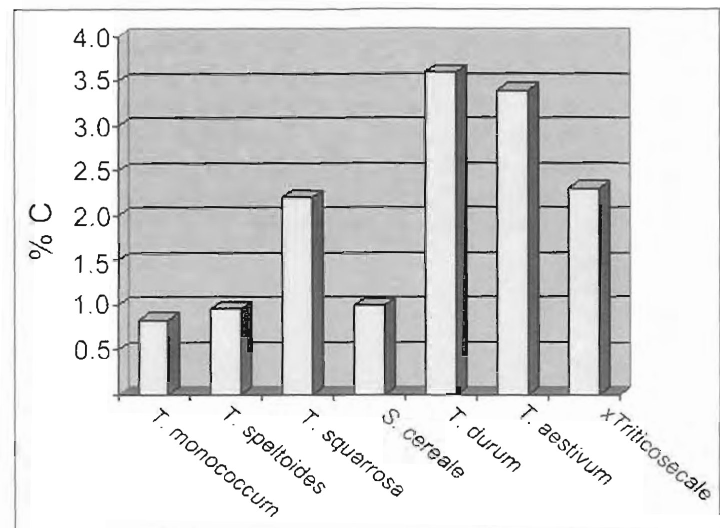

Fig. 2:-Relative contribution of pTo818 to the genome size ofthe seven species analysed.

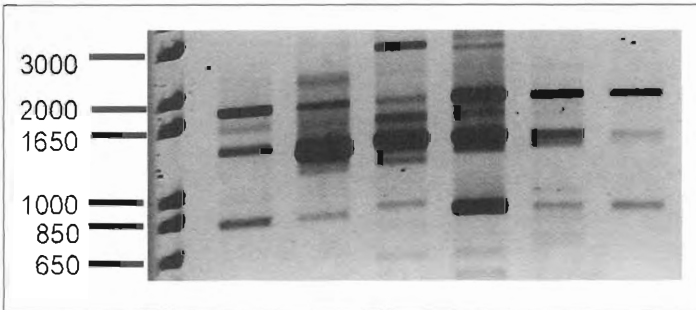

Fig. 3: -Banding patterns of amplified products on the genomic DNA from the *Triticum* species. From left to right: 1 Kb ladder; *T. monococcum*; *T. speltoides*; *T. squarrosa*; *T. turgidum*; *T. aestivum*; *xTriticosecale*. Marker sizes in base pairs are shown adjacent to the gel

Sequences homologous to pTo818 in the donor species of the wheat, rye and triticale genomes

Once the identity of the sequence pTo818 was determined, corresponding sequences were sought in the genome donor species. Genomic DNA from the species used in Slot blot experiments was used as template for PCR with the same set of primers. No amplification was obtained from rye DNA. Amplification patterns were very similar in all the *Triticum* species, except for the presence/absence of some minor bands (Fig. 3). The amplification fragments of about 800 bp from *T. monococcum*, *T. speltoides*, *T. squarrosa and T.turgidum* were purified from agarose gels, cloned, completely sequenced and compared to pTo818 (EMBL GenBank, DDBJ accession numbers AJ291715 for *T. monococcum*, AJ300268 for *T. speltoides*, AJ291716 for *T. squarrosa*, and AJ300653 for *T. turgidum*). The sequences were highly homologous (>95%) to that of pTo818 obtained in the first experiment.

DISCUSSION

The presence of retrotransposons and other transposable elements as families of high copy number in the genomes of many plants and animals (5) allows the conclusion that they were already present in ancient species. Even though certain evidence indicating the possibility of horizontal transmission of retrotransposons in plants has been reported (12), simple inheritance appears to be the most frequent mode of transmission (13). The *Ty-1-copia* retrotransposons, which have been studied in some detail in many plant species, are highly heterogeneous populations within the genome. During their coexistence in the host genome, natural selection must have regulated the copy numbers for the benefit of the receptor organism (5). The number of copies of retrotransposons or retrotransposon components commonly found in existent species of plants must be the consequence of selective pressure on the host genome to support them.

Both the ubiquity of WIS 2-1A throughout the euchromatic regions and its increased copy number in the allopolyploid species (with respect to the diploid genome donors), suggests that this element does not contribute towards reduced fitness. On the contrary, the present analysis suggests that fragments of WIS 2-1A make up nearly three times more of the genomic DNA than expected on the basis of the addition of the equivalent regions in the diploid species

Different hypothesis have been put forward to explain the minimizing of retroelelments´ negative effects and their promotion in the genomes of polyploids. These involve mutations that provide new regulatory properties for certain genes, contributions to DNA reparation, stabilisation of the behaviour of the different genomes in allopolyploids, and inactivation of other retrotransposons by inserting within them, etc. (14)(5).

On the contrary, the synthetic allopolyploid triticale, shows the opposite trend. The cultivar 'Torote' shows no increase in the number of copies of the fragment pTo818, but rather a slight reduction - 2.3% compared to an expected 2.5% (assuming a 20% reduction of the theoretical genome size reckoned by the addition of the rye and durum wheat genomes). Surprisingly, this reduction has come about in very few generations since the chromosome doubling of the wheat and rye hybrids, i.e., during the adaptive process of the newly coexisting genomes.

The comparative analysis of the region of about 818 bp amplified, cloned and sequenced from genomic DNA of *T. monococcum, T. speltoides, T. squarrosa, T. turgidum* and *xTriticosecale*, showed 98% homology amongst them. This high degree of conservation cannot be explained by natural selection of functional elements, as WIS 2-1A is assumed to be inactive. However, the primers, designed using the BARE-1 sequence, did not amplify elements composed only of this retrotransposon or WIS 2-1A. Rather, they generated different fragments, some of which showed truncated regions of WIS 2-1A associated with BARE-1, or a Ty3/*gypsy* retrotransposon-like element named CEREBA found in the centromeric regions of cereal chromosomes (15). The extremely high degree of conservation of the 818 bp fragment in all the species studied could be explained if this sequence took up part of the centromere and assumed some functional role in this region. Notwithstanding, no experimental evidence of this is available.

Other fragments of the retroelement (pTo1865, and pPané1865) have been associated with the intergenic region before gene *Acc-1,1* [acetyl-CoA carboxylase], located on the long arm of homoeologous group-1 chromosomes of wheat (16) (17). This copy of the retroelement is arranged head-to-head with respect to the gene. The presence of retrotransposon fragments within the regulatory regions of genes suggests that they could have evolved into a form involved in specific gene regulation (18).

The present results report the localization of two copies of a WIS 2-1A retrotransposon-like sequences in triticale. This Ty-1/*copia* retroelement was earlier described in wheat. The data show that WIS 2-1A has been maintained as an important component of the genome of the tribe *Triticeae* in spite of its defects in the ability to encode all necessary transposition functions (owing to insertions, deletions and degenerated sequences) (8). Further, the presence of this retroelement increased after natural hybridisation took place to form the cereal crops used by man. The increasing presence of the complete sequence of these retroelements - or parts thereof - could have been controlled by trans-acting factors, due to the presence of other active retrotransposons or retroviruses in host genomes.

Finally, a reduction was seen in the presence of retroelements and general genome size when passing from wheats and rye to the synthetic crop triticale. The possibility that changes in DNA content in triticale may be induced by underreplication of certain sequences, molecular drive, allosyndetic end-to-end pairing during meiosis and mobile elements has been previously discussed (19) (20). A loss of inactive sequences of DNA can be forced by selective pressure, or be a consequence of adjusting the cell cycle timing over the generations (21).

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REFERENCES

[1] Ellis T.H.N., Ambrose M.J. 1998. Polymorphism of insertion sites of Ty1-copia class retrotransposons and its use for linkage and diversity analysis in pea. Mol. Gen. Genet. 260,9-19.

[2] Kalendar R., Grob T., Regina M., Suoniemi A., Schulman A. 1999. IRAP and REMAP: two new retrotransposon-based fingerprinting techniques. Theor. Appl. Genet. 98, 704-711.

[3] Gribbon B. M., Pearce S.R., Flavell A.J. 1999. Phylogeny and transpositional activity or TY1-copia group retrotransposons in cereal genomes. Mol. Gen. Genet. 261, 883-891.

[4] Vicient C.M., Suoniemi A. and Schulman A. 1999. Retrotransposon BARE-1 and its role in genome evolution in the genus *Hordeum*. The Plant Cell vol 11, 1769-1784.

[5] Kumar A., Bennetzen J.L. 1999. Plant retrotransposons. Ann Rev Genet 33,479-532.

[6] Harbert N.P., Flavell R.B., Thompson R.D., 1987. Identification of a transposon-like insertion in a *Glu-1* allele of wheat. Mol. Gen. Genet. 209: 326-332.

[7] Monte.J.V., Flavell.R.B., Gustafson. J.P. 1995. WIS-2-1A: an ancient retrotransposon in the *Triticeae* tribe. Theor. Appl. Genet. 91, 367-373.

[8] Moore G, Lucas H, Batty N, Flavell R.B. 1991. A family of retrotransposons and associated genomic variation in wheat. Genomics 10: 461-468.

[9] Davila J.A., Loarce Y., Ferrer E. 1999. Comparison of RAMP and SSR markers for the study of wild barley genetic diversity. Hereditas 131, 5-13.

[10] Sharp P.J., Desai S., Gale M.D. 1998. Isozyme variation and RFLPs at the â-amylase loci in wheat. Theor. Appl. Genet. 76, 691-699.

[11] Manninen I., Schulman A. 1993. BARE-1, a copia-like retroelement in barley (*Hordeum vulgare L..).* Plant Molecular Biology. 22, 829-846.

[12] Jordan I.K., Matyunina, L.V., McDonald, J.F. 1999. Evidence for the recent horizontal transfer of long terminal repeat retrotransposon. Proc. Nat. Acad. Sci. 96: 12621-12625.

[13] Murphy G.J.P., Lucas H., Moore G., Flavell R.B. 1992 Sequence analysis of WIS-2-1A, a retrotransposon-like element from wheat. Plant Molecular Biology. 20, 991-999.

[14] SanMiguel P., Gaut B.S., Tikhoniv A., Nakajima Y., Bennetzen J.L. 1998. The paleontology of intergene retrotransposons in maize. Nat. Genet. 20: 43-45.

[15] Presting G., Malysheva L., Schubert I.A. 1998. Ty3/*gypsy* retrotransposon-like sequence localizes to the centromeric regions of cereal chromosomes.. The Plant Journal 16(6), 721-728.

[16] Gornicki P., Podkowinski J., Haselkorn R. 1994. Wheat acetyl-CoA carboxylase: cDNA and protein structure. Proc. Nat. Acad. Sci. 91, 6860-6864.

[17] Gornicki P., Faris J., King I., Haselkorn R. 1997. Plastid-localized acetyl-CoA carboxylase of bread wheat is encoded by a single gene on each of the three ancestral chromosome sets. Proc. Nat. Acad. Sci. 94 , 14179-14184.

[18] White S.E., Habera L.F., Wessler S.R. 1994. Retrotransposons in the flanking regions of normal plant genes: a role for copia-like retroelements in the evolution of gene structure and expression. Proc. Nat. Acad. Sci. 91, 11792-11796.

[19] Gustafson, J.P., Lukaszewski, A.J., Robertson K. 1985. Chromosome substitution and modifications in hexaploid triticale: a re-evaluation. Eucarpia Meeting on Triticale. Clermont-Ferrand (France): 15-27.

[20] Jouve N., Soler C. 1996. Triticale genomic and chromosomal history. In: Triticale: Today and tomorrow (Guedes-Pinto, H., Darvey, N., Carnide, V., Eds.), Kluwer Academic Publ, Dordrecht, pp. 91-118.

[21] Petsova E.G., Goncharov N.P., Salina E.A. 1998. Elimination of a tandem repeat of telomeric heterochromatin during the evolution of wheat. Theor. Appl. Genet. 97, 1380-1386.

THE ORIGIN OF TETRAPLOID WHEATS

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ABSTRACT: The origin of tetraploid wheats, *Triticum turgidum* (2n = 4x = 28, AABB) and *T. timopheevii* (2n = 4x = 28, A^tA^tGG) is analysed in the light of their structural chromosome differentiation and the affinity of the S genome of *Aegilops speltoides* to the B and G genomes. Meiotic configurations observed in interspecific hybrids between tetraploid wheats and genomic *in situ* hybridisation reveal that both tetraploid species have undergone independent and distinct evolutionary chromosomal rearrangements. Translocation 4AL/7BS and two inversions of chromosome 4A, which occurred in *T. turgidum*, do not exist in *T. timopheevii*, where four different translocations, 6A^{t}S/1GS, 1GS/4GS, 4GS/4A^{t}L and 4A^{t}l/3A^{t}L, appeared. The level of meiotic pairing between genomes B and S and G and S was assessed in interspecific hybrids of tetraploid wheats with *Ae. speltoides*. A higher degree of closeness between G and S than between B and S was concluded. Structural and non-structural chromosome differentiation support independent origins of *T. turgidum* and *T. timopheevii* with a more recent formation of the timopheevi lineage.

1. INTRODUCTION

Tetraploid wheats (2n = 4x = 28) are divided into two groups: the emmer wheat group (*Triticum turgidum*) with the genome formula AABB and the timopheevi group (*T. timopheevii*) with the genome formula A^tA^tGG. Cultivated *T. turgidum* subsp. *durum* and *T. timopheevii* subsp. *timopheevii* are domesticated forms of the wild wheats *T. turgidum* subsp. *dicoccoides* and *T. timopheevii* subsp. *araraticum*, respectively. Diploid species *T. urartu* (2n = 2x = 14, AA) and *Aegilops speltoides* (2n = 2x = 14, SS) were identified as the progenitors of tetraploid wheats (1-6).

Early cytogenetic studies based on chromosome pairing analysis in interspecific hybrids revealed translocation differences between *T. turgidum* and *T. timopheevii* (7, 8), which were confirmed later by C-banding (9, 10). Structural chromosome differentiation occurred in both evolutionary lineages. Two translocations, 5AL/4AL and 4AL/7BS, and a pericentric inversion of chromosome 4A were identified in polyploid wheats *T. turgidum* and *T. aestivum* from the analysis of homoeologous pairing (11, 12). The construction of genetic maps confirmed these structural changes and revealed the occurrence of an additional paracentric inversion on chromosome 4AL (13-16). *T. timopheevii* shares translocation 5AL/4AL with *T. turgidum*. Both species inherited this translocation from the diploid progenitor *T. urartu* (17, 18). Species-specific translocations, 6A^{t}S/1GS and 1GS/4GS were identified in *T. timopheevii* after sequential N-banding and genomic *in situ* hybridisation (GISH) (19). Two other translocations, 4A^{t}L/3A^{t}L and 4A^{t}L/4GS, were identified in cultivated lines of *T. timopheevii* (10, 18). The four translocations of *T. timopheevii* above mentioned were proposed to occur in the sequence: 6A^{t}S/1GS, 1GS/4GS, 4A^{t}L/4GS and 4A^{t}L/3A^{t}L (18).

Two different hypotheses have been advanced regarding the origin of *T. turgidum* and *T. timopheevii*. One hypothesis assumes that both species diverged at the tetraploid stage after a single hybridisation event (8, 20, 21). The second hypothesis postulates that the emmer and timopheevi wheats originated from two independent hybridisation events. Wheat phylogenies determined by restriction fragment analyses of chloroplast, mitocondrial and nuclear DNAs (2-5, 22, 23) as well as structural differences between both species support the diphyletic origin.

Meiotic pairing between chromosomes from different genomes analysed by C-banding is a useful tool by which to investigate the chromosome structure of different species as well as the degree of affinity between different genomes (24). In this work, meiotic pairing and GISH are used to analyse the chromosome structure and origin of tetraploid wheats. It summarises results obtained in hybrids between *T. turgidum* and different cultivated and wild accessions of *T. timopheevii*, which demonstrate the occurrence of independent chromosome rearrangements in both species (25), and in *T. turgidum* x *Ae. speltoides* and *T. timopheevii* x *Ae. speltoides* hybrids, which reveal a closer relationships between genomes G and S than between B and S (26).

2.MATERIAL AND METHODS

Structural differentiation between *T. turgidum* and *T. timopheevii* was studied by C-banding analysis of meiotic pairing in hybrids of *T. turgidum* subsp. *durum* 'Cappelli' with four accessions of *T. timopheevii* subsp. *araraticum* from Iran (TIA02), Iraq (TIA06), Azerbaijan and Nakhichevan and accession PI 221421 of *T. timopheevii* subsp. *timophevii*. The different types of hybrids were designated AA^tBG02, AA^tBG06, AA^tBGAZ, AA^tBGNK and AA^tBGT, respectively. Anthers of the hybrids were fixed in acetic acid-alcohol (1:3), stored at 0-4°C and stained using a C-banding technique (27). A number of 150 or more pollen mother cells (PMCs) at metaphase I, and from 86 to 232 PMCs at anaphase I, were scored in each type of hybrids. For chromosome identification at metaphase I, the somatic C-banding karyotype of all accessions of *T. timopheevii* and *T. turgidum* was determined from root tip cells. Genomic *in situ* hybridisation of the five accessions of *T. timopheevii* was carried out to confirm the presence of intergenome translocations. For *in situ* hybridisation, genomic DNA isolated from *T. boeoticum* was labelled with digoxigenin-11-dUTP and mixed with blocking DNA isolated from *Ae. speltoides* (25).

The relationships between the G and S genomes were studied in four A^tGS hybrids of *T. timipheevii* subsp. *timopheevii* (accession CI14133) and *Ae. speltoides* from Askhelon (Israel). The relationships between the B and S genomes were analysed in two ABS hybrids obtained in the cross *T. turgidum* ssp *durum* 'Cappelli' x *Ae. speltoides* from Askhelon. A total number of 300 PMCs at metaphase I in the A^tGS hybrids and 150 PMCs in the ABS hybrids were scored (26).

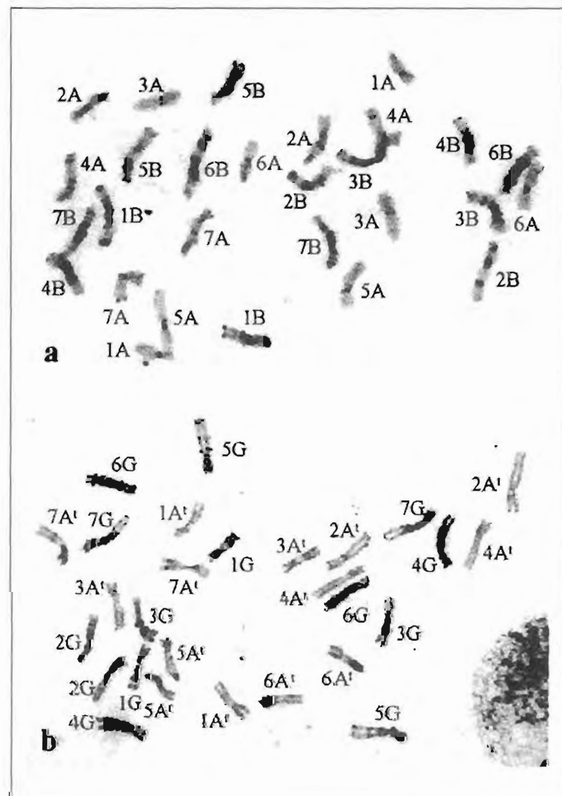

Figure 1: C-banding pattern of chromosomes from tetraploid wheats. a) *T. turgidum*. b) *T. timopheevii*

3.RESULTS

T. turgidum chromosomes were identified in somatic cells according to previous reports (18, 28). The chromosomes of *T. timopoheevii*, which showed a distinctive C-banding pattern in root tip cells in all varieties studied, were assigned to the seven homoeologous groups by virtue of pairing with *T. turgidum* chromosomes in the interspecific hybrids. The C-banding pattern of chromosomes with the standard structure of both species is shown in Fig. 1.

GISH analysis of the five accessions of T. timopheevii demonstrated that three chromosome pairs, 6At, 1G and 4G, of the standard karyotipe of T. timopheevii are involved in intergenome translocations (Fig. 2). Identification of such chromosomes was based on morphology and pairing affinities. The short arm of chromosome 6At carries a long translocated segment from the G genome, chromosome arm 1GS carries a short subdistal At segment and chromosome 4G carries a short terminal At segment.

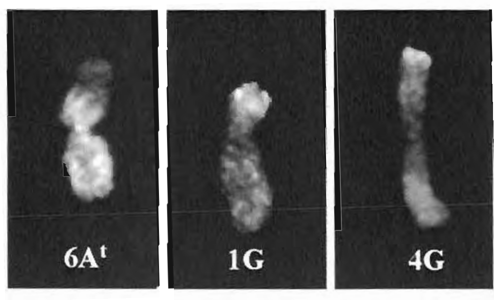

Figure 2: Chromosomes of *T. timopheevii* with intergenome translocations detected by GISH

All associations between individual chromosome arms of *T. turgidum* and *T. timopheevii* were identified at metaphase I of the hybrids (Fig 3). Only associations that identify structural differences between both species, and their frequencies, are listed in Table 1. Association 4A^{t}L-3AL, which appeared in all of the hybrids studied, demonstrates that the 4A^{t}L arm carries a translocated segment from 3A^{t}L. The low frequency of 4A^{t}S-4AS association, which was very distal, in addition to the occurrence of an interstitial association between 4A^{t}S and 4AL in the AA^{t}BGNK hybrids confirm that chromosomes 4A and 4A^t differ in a pericentric inversion. Associations 6A^{t}S-1BS, 1GS-4BS and 4AL-7GS, which indicate that 6A^{t}S carries a translocated segment from 1GS, 1GS carries a translocated segment from 4GS and 4AL carries a translocated segment from 7BS, respectively, were observed in all of the hybrids. This supports that translocations 6A^{t}S/1GS and 1GS/4GS are specific of *T. timopheevii* while translocation 4AL/7BS is specific of *T. turgidum*. The 7BS arm, which carries a translocated segment from 5AL, paired in all of the hybrids with 4GS. Thus, the standard 4GS arm carries a translocated segment from 5A^{t}L. The arms 4A^{t}L and 4AL, which were associated at metaphase I with a relatively low frequency (1.3%-6.4%), formed a bridge plus an acentric fragment configuration at anaphase I in the AA^{t}BGT (1.7%), AA^{t}BG02 (1.7%) and AA^{t}BG06 (1.0%) hybrids. This result suggests that the paracentric inversion of 4AL is absent in the 4A^{t}L arm.

Table 1. Frequency (%) of associations that identify structural differences between *T. turgidum* and *T. timopheevii* in five interspecific hybrids

Structural difference	Association	Hybrids	Frequency
T 3A^{t}L/4A^{t}L	3AL-4A^{t}L	All	> 69.3
Inv 4A	4AS-4A^{t}S	All	< 3.3
4AL-4A^{t}S	All	< 0.8	
T 6A^{t}S/1GS	1BS-6A^{t}S	All	> 9.9
T 1GS/4GS	4BS-1GS	All	> 2.6
T 4AL/7BS	4AL-7GS	All	> 5.2
T 4GS/4A^{t}L	7BS-4GS	All	> 5.2

In the *T.timopheevii* x *Ae. speltoides* (A^{t}GS) hybrids, associations at metaphase I occurred preferentially between chromosomes of the G and S genomes, although A$_t$-S and A$_t$-G associations were also observed. In the *T. turgidum* x *Ae.* speltoides (ABS) hybrids, associations of the types A-B and B-S were more frequent than associations of the A-S type. Table 2 shows the average frequency of the different pairing types between chromosomes of the S genome and those of *T. timopheevii* and *T. turgidum*. Chromosomes of the G and S genomes in A^{t}GS hybrids pair more often than chromosomes of the B and S genomes in ABS hybrids. The level of G-S pairing in A^{t}GS hybrids duplicates that of B-S pairing in ABS hybrids.

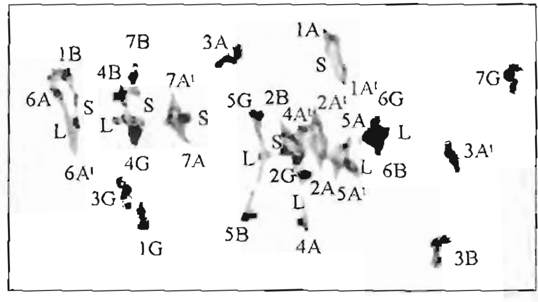

Figure 3: Cell at metaphase I from AA^{t}BGT hybrids. The arms associated are identified

Table 2. Average frequency (%) of the different association types in *T. timopheevii* x *Ae. speltopides* (AtGS) and *T. turgidum* x *Ae. speltopides* (ABS) hybrids

Hybrids	Association type		
	At-S or A-S	At-G or A-B	G-S or B-S
AtGS	9.3	11.2	55.8
ABS	8.6	19.2	28.8

4. DISCUSSION

In situ hybridisation analysis has shown that chromosomes $6A^t$, 1G and 4G of *T. timopheevii* are involved in intergenome translocations in all the accessions studied. Meiotic pairing of the hybrids between *T. turgidum* and *T. timopheevii* demonstrates that the four wild populations and the cultivated line of *T. timopheevii* carry translocations $6A^tS$/1GS, 1GS/4GS, $4A^tL$/4GS and $4A^tL/3A^tL$. Taking into account the different geographical distribution of such wild populations and previous reports (19, 29) the conclusion can be drawn that these four translocations are fixed in *T. timopheevii*. On the other hand, the results of meiotic pairing confirm that neither translocation 4AL/7BS nor the pericentric inversion of chromosome 4A exist in *T. timopheevii*. Bridge plus an acentric fragment configuration at anaphase I involving chromosome arms 4AL and $4A^tL$ supports the standard arrangement on $4A^tL$ for the segment that is inverted on 4AL. Thus, both tetraploid species suffered independent chromosome rearrangements.

The formation of new polyploids is often accompanied of extensive genomic modifications within a short period of time (30). Accordingly, rapid elimination of low copy DNA sequences was reported in wheat (31). The evolutionary chromosome rearrangements produced in tetraploid wheats probably arose at a very early stage because no transient structure was found. Most likely, these structural changes are only part of the overall genome reorganisation produced immediately after the polyploid formation. The occurrence of different chromosome rearrangements in *T. turgidum* and *T. timopheevii* support their diphyletic origin.

The degree of meiotic pairing is considered a good measure of the degree of affinity between different genomes. The reduction of the level of B-S pairing in the ABS hybrids with regards to G-S pairing in the A^tGS hybrids strongly suggests a higher differentiation between genomes B and S than between genomes G and S. By contrast, genomes A and A^t show similar affinities to the S genome. Taking into account that tetraploid wheats had independent origins, a higher degree of closeness between genomes G and S than between genomes B and S agrees with the proposal that *T. timopheevii* originated more recently than *T. turgidum* (3, 23). *Ae. speltoides* has a much larger intraspecific variation than *T. urartu* (6). It is possible that the evolutionary lineages of *T. urartu* and *Ae. speltoides* underwent a different degree of divergence in time, which caused a higher differentiation between B and G than between A and A^t relative to the current S genome. Pairing between chromosomes of the A and B genomes in the ABS hybrids was more frequent than pairing between chromosomes of the A^t and G genomes in the A^tGS hybrids. This might be related with a higher time for coevolution of genomes A and B in *T. turgidum* than genomes A^t and G in *T. timopheevii*. Repetitive sequences of the B (ancestral S) genome that spread through the A genome provide evidence of interactions between genomes in *T. turgidum* (32).

5. ACKNOWLEDGEMENTS

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REFERENCES

[1] Dvorák J, di Terlizzi P, Zhang HB, Resta P: The evolution of polyploid wheats: identification of the A genome donor species. Genome 36: 21-31(1993).

[2] Ogihara Y, Tsunewaki K: Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. Theor Appl Genet 76: 321-332 (1988).

[3] Dvorák J, Zhang HB: Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc Natl Acad Sci USA 87: 9640-9644 (1990).

[4] Terachi T, Ogihara Y, Tsunewaki K: The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops*. 7. Restriction endonuclease analysis of mitochondrial DNA from polyploid wheats and their ancestral species. Theor Appl Genet 80: 366-373 (1990).

[5] Miyashita NT, Mori N, Tsunewaki K: Molecular variation in chloroplast DNA regions in ancestral species of wheat. Genetics 137: 883-889 (1994).
[6] Sasanuma T, Miyashita NT, Tsunewaki K: Wheat phylogeny determined by RFLP analysis of nuclear DNA. 3. Intra and interspecific variations of five *Aegilops* Sitopsis species. Theor Appl Genet 92: 928-934 (1996).
[7] Lilienfeld F, Kihara H: Genomanalyse bei *Triticum* und *Aegilops*. *Triticum timopheevii* Zhuk. Cytologia 6: 87-122 (1934).
[8] Feldman M: Identification of unpaired chromosomes in F1 hybrids involving *Triticum aestivum* and *T. timopheevii*. Can J Genet Cytol 8: 144-151 (1966).
[9] Hutchinson J, Miller TE, Jahier J, Shepherd KW: Comparison of the chromosomes of *Triticum timopheevii* with related wheats using the techniques of C-banding and in situ hybridization. Theor Appl Genet 64: 31-40 (1982).
[10] Gill BS, Chen PD: Role of cytoplasm specific introgression in the evolution of the polyploid wheats. Proc Natl Acad Sci USA 84: 6800-6804 (1987).
[11] Naranjo T, Roca A, Goicoechea PG, Giráldez R: Arm homoeology of wheat and rye chromosomes. Genome 29: 873-882 (1987).
[12] Naranjo T: Chromosome structure of durum wheat. Theor Appl Genet 79: 397-400 (1990).
[13] Anderson JA, Ogihara Y, Sorrels ME, Tanskley SD: Development of a chromosomal arm map for wheat based on RFLP markers. Theor Appl Genet 83: 1035-1043 (1992).
[14] Liu CJ, Atkinson MD, Chinoy CN, Devos KM, Gale MD: Non-homoeologous translocations between group 4, 5 and 7 chromosomes within wheat and rye. Theor Appl Genet 83: 305-312 (1992).
[15] Devos KM, Dubcovsky J, Dvorák J, Chinoy C.N, Gale MD: Structural evolution of wheat chromosomes 4A, 5A and 7B and its impact on recombination. Theor Appl Genet 91: 282-288 (1995).
[16] Mickelson-Young L, Endo TR, Gill BS: A cytogenetic ladder-map of the wheat homoeologous group-4 chromosomes. Theor Appl Genet 90: 1007-1011 (1995).
[17] Jiang J, Gill BS: New 18S-26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. Chromosoma 103: 179-185 (1994).
[18] Maestra B, Naranjo T: Structural chromosome differentiation between *Triticum timopheevii* and *T. turgidum* and *T. aestivum*. Theor Appl Genet 98: 744-750 (1999).
[19] Jiang J, Gill BS: Different species-specific translocations in *Triticum timopheevii* and *T. turgidum* support the diphyletic origin of polyploid wheats. Chromosome Res. 2: 59-64 (1994).
[20] Wagenaar EB: Studies on the genome constitution of *Triticum timopheevi* Zhuk. I. Evidence for genetic control of meiotic irregularities in tetraploid hybrids. Can J Genet Cytol 3: 47-60 (1961).
[21] Tanaka M, Kawahara T, Sano J: The evolution of wild tetraploid wheats. In: Ramanujan S (ed) Proc 5th Int Wheat Genet Symp. Indian Soc Genet Plant Breeding, Kapoor Art Press, New Delhi, India, pp 73-80 (1978).
[22] Dvorák J, Zhang HB, Kota RS, Lassner M: Organization and evolution of the 5S ribosomal RNA gene family in wheat and related species. Genome 32: 1003-1016 (1989).
[23] Mori N, Liu YG, Tsunewaki K: Wheat phylogeny determined by RFLP analysis of nuclear DNA. 2. Wild tetraploid wheats. Theor Appl Genet 90: 129-134 (1995).
[24] Maestra B, Naranjo T: Genome evolution in Triticeae. In: Olmo E, Redi CA (eds) Chromosomes Today 13. Birkhäuser Verlag, Basel, pp 155-167 (2000).
[25] Rodríguez S, Perera E, Maestra B, Díez M, Naranjo T: Chromosome structure of *Triticum timopheevii* relative to *T. turgidum*. Genome 43: 923-930 (2000).
[26] Rodríguez S, Maestra B, Perera E, Díez M, Naranjo T: Pairing affinities of the B- and G-genome chromosomes of polyploid wheats with those of *Aegilops speltoides*. Genome 43: 814-819 (2000).
[27] Giráldez R, Cermeño MC, Orellana J: Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye *Secale cereale* L. Z Pflanzenzuecht 83: 40-48 (1979).

[28] Simeone R, Perrone V, Blanco A: C-banding of tetraploid wheat trisomics. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Bath Press, Bath, UK, pp 443-448 (1988).

[29] Badaeva ED, Badaev NS, Gill BS, Filatenko AA: Intraspecific karyotype divergence in *Triticum araraticum (Poaceae)*. Pl Syst Evol 192: 117-145 (1994).

[30] Leitch IJ, Bennett MD: Polyploidy in angiosperms. Trends Plants Sci 2: 470-476 (1997).

[31] Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM: Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. Genetics 147: 1381-1387 (1997).

[32] Belyayev A, Raskina O, Korol A, Nevo E: Coevolution of A and B genomes in allotetraploid *Triticum dicoccoides*. Genome 43: 1021-1026 (2000).

MOLECULAR PHYLOGENY IN THE GENUS *HORDEUM* USING THREE SEQUENCES OF CHLOROPLAST DNA

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ABSTRACT: Phylogenetic relationships among species in the genus *Hordeum* were inferred from nucleotide sequence variations based on regions of chloroplast DNA, *matK*, *atpB-rbcL* and *trnL-trnF*. In the phylogenetic tree, I and Y genome were monophyletic, while X and H genomes did not show monophyletic relationships clearly. *H. capense* was closely related to *H. secalinum*, supporting the hypothesis that *H. capense* was introduced by the first settlers from Europe. The hexaploid cytotype of *H. brachyantherum* had identical sequences with that from the diploid of *H. marinum* ssp. *gussoneanum* in the region analyzed, and they and the tetraploid of *H. brachyantherum* are distributed in the same area. Therefore, it is likely that hexaploid of *H. brachyantherum* was formed by crossing of tetraploid as a pollen parent and the diploid of *H. marinum* ssp. *gussoneanum* as the maternal parent. Regarding to *H. marinum* ssp. *gussoneanum*, *H. murinum*, and *H. brachyantherum*, tetraploid cytoplasm belonged to the same clade with diploid cytoplasm, it is supports the hypothesis that occurrence of polyploidy might be autoploid or segmental alloploid.

1. INTRODUCTION

The genus *Hordeum* contains ca. 30 related species in temperate areas of Eurasia, South Africa, and North, Central, and South America. Based on their morphology, it was divided into four sections, *Hordeum*, *Anisolepis*, *Stenostachys*, and *Critesion* (1). Cytogenetic and biochemical data showed that relationships within the genus are not completely reflected in the morphology (2). On the other hand, genus *Hordeum* was divided into four types by the genome relationships at the diploid level, genome I, X, Y and H, but the genome pattern is much more complicated at the polyploid levels (4x and 6x) (2) because of the presence of plural polyploidy system. The objectives of this study are to clarify genetic relationships within genus *Hordeum* and to estimate origin of polyploidy based on the sequence data of three chloroplast DNA regions, *matK* gene, *atpB-rbcL*, and *trnL-trnF*.

2. MATERIALS AND METHODS

Twenty-two taxa included 34 individuals were analyzed in the present study. *Psathyrostachys juncea* was used as outgroup. DNA was extracted from fresh leaves, and used them as template DNA for amplifying three chloroplast DNA regions with PCR. DNA sequences were determined using cycle sequencing, and they were multiply aligned using Clustal X version 1.81 (3). Phylogenetic analysis was performed as a maximum parsimony phylogeny estimation using the PAUP* version 4.0b8 (4) using 1,000 bootstrap replications.

3. RESULTS AND DISCUSSION

3.1. The matrix of sequences in three chloroplast regions.

Aligned sequences used were 1536 bp in *matK* gene, 738 bp in partial *atpB-rbcL*, and 332 bp in *trnL-F*, respectively. Plural equivocal regions were excluded from following analysis in two intergenic spacer regions. The parsimonious tree constructed from them was showed in Fig.1.

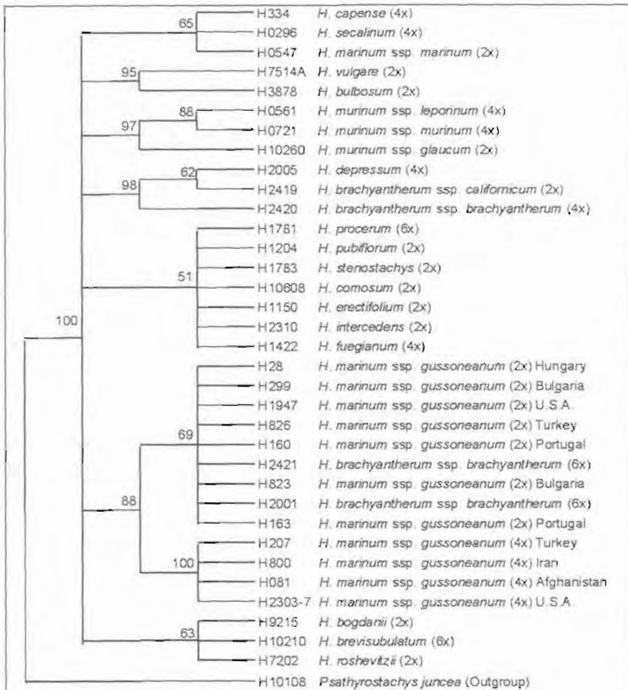

Fig. 1. Phylogetetic tree of genus *Hordeum* based on sequence data of three choloroplast DNA regions using the maximum parsimony method. The tree is strict consens us of the 486 most parsimonious trees. Tree length = 139, Consistercy index (CI) = 0,8921, Retention index (RI)=0,8929, Rescañed consistency in dex (RC)=0,7965. The numbers above the nodes represent boots trap value expressed as percentage of 1,00 bootstrap replications

3.2. Phylogenetic relationships of each genome type

Genome I, which consist of *H. vulgare* and *H. bulbosum*, was monophyletic. *H. murinum* is only species of genome Y, and it was also monophyletic. Only *H. marinum* has X genome, and it consists of two subspecies, *marinum* and *gussoneanum*. But those subspecies were not monophyletic, because *H. marinum* ssp. *marinum* belongs to the clade of *H. capense*. This result suggested the two subspecies might be differentiated very much although they showed high meiotic pairing (5). The most of species possess Genome H except species mentioned above, and they were also not monophyletic.

3.3. Origin of H. capense

H. capense is only species distributed in southern Africa, and its origin was considered to be introduction of *H. secalinum* from Europe by the first settlers (6). *H. capense* and *H. secalinum* showed close relationship in the most parsimonious tree, so that this result supported the hypothesis that *H. secalinum* is the origin of *H. capense*.

3.4. Origin of the hexaploid cytotype of H. brachyantherum

The hexaploid cytotype of *H. brachyantherum* was closely related to the diploid cytotype of *H. marinum* ssp. *gussoneanum* rather than other cytotype (diploid and tetraploid) of *H. brachyantherum* (Fig. 2), because they had identical sequences each other in this study. *H. marinum* ssp. *gussoneanum* was not distributed in North America originally, but it was later introduced and distributed widely now. The hexaploid cytotype of *H. brachyantherum* were found in California. The diploid cytotype of *H. marinum* ssp. *gussoneanum* and the tetraploid cytotype of *H. brachyantherum* also grow in California. Formation of the hexaploid was estimated by crossing between diploid *Hordeum* and tetraploid of *H. brachyantherum* based on C-banded karyotypes (7). Therefore, it is probable that the hexaploid cytotype of *H. brachyantherum* was formed from a crossing combination of tetraploid *H. brachyantherum* as the pollen parent and diploid *H. marinum* ssp. *gussoneanum* as the maternal parent.

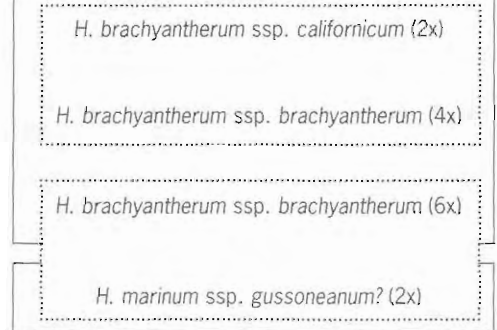

Fig. 2. Similarity of cytoplasm between *H. brachyantherum* (6x) and *H. marinum* ssp. *gussoneanum* (2x).
Solid boxes show classification mainly by morphology. Broken boxes show classification by chloroplast sequence analysis.

3.5. Relationships among three Asian species

It is noteworthy that *H. bogdanii*, *H. brevisubulatum*, and *H. roshevitzii*, all collected from Asia, were grouped in a same clade, confirming their close genetic relationship.

3.6. Origin of other polyploids in the same species

The tetraploid of *H. marinum* ssp. *gussoneanum*, *H. murinum*, and *H. brachyantherum* belongs to the clades with where diploid of the same species present, respectively. It is supports the hypothesis that they are likely either to be autoploids or segmental alloploids.

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REFERENCES

[1] Bothmer R von, Jacobsen N: Origin, taxonomy, and related species. In: Rasmusson D. (eds) Barley: ASA Monograph No. 26: 19-56 (1985)

[2] Bothmer R von, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I: An ecogeographical study of the genus *Hordeum* 2nd edition. IPGRI (1995)

[3] Tompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG: The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25(24): 4876-4882 (1997).

[4] Swofford DL: PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts (1998).

[5] Bothmer R von, Flink J, Landstöm T: Meiosis in interspecific *Hordeum* hybrids. I. Diploid combinations. Can J Genet Cytol 28: 525-535 (1986a).

[6] Bothmer R von, Jacobsen N: A taxonomic revision of *Hordeum secalinum* and *H. capense*. Bot. Tidsskr. 74: 223-235 (1980)

[7] Linde-Laursen I, Bothmer R von, Jacobsen N: Relationships in the genus *Hordeum*: Giemsa C-banded karyotypes. Hereditas 116: 111-116 (1992)

DIPLOID CYTOTYPE OF *DASYPYRUM BREVIARISTATUM*: ITS REDESCOVERY AND SIGNIFICANCE IN THE PHYLOGENY OF THE GENUS *DASYPYRUM*

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ABSTRACT: Three diploid plants of *Dasypyrum breviaristatum* were recently rediscovered from a population located 28 km south of Sefrou on the way to Ifrane at an elevation of 1490 m above sea level in the northern foothills of the Moyen Atlas Mountains of Morocco. One of the diploid plants was artificially crossed with *D. villosum* and tetraploid *D. breviaristatum*. Chromosome pairing at first metaphase (MI) of meiosis in pollen mother cells (PMCs) and fertility were examined in the F_1 hybrids and the parental plants. Mean pairing configuration and mean chiasma frequency in the *D. villosum-D. breviaristatum* (2x) hybrids were $11.12_{I}+1.44_{II}$ and 1.49 per cell, respectively, and they were almost completely sterile. In the *D. breviaristatum* (4x)-*D. breviaristatum* (2x) hybrids, up to seven trivalents were formed in PMCs, and the mean pairing configuration was $3.38_{I}+3.20_{II}+3.74_{III}+0.005_{IV}$. Seven bivalents and seven univalents were formed in the *D. villosum-D. breviaristatum* (4x) hybrids. Based on the chromosome pairing and fertility in those F_1 hybrids, we come to the following two conclusions: 1) Diploid cytotype of *D. breviaristatum* is distantly related to *D. villosum*, and they have different basic genomes; and 2) Tetraploid cytotype of *D. breviaristatum* is an autotetraploid with the doubled genome sets homologous with that of the diploid cytotype of *D. breviaristatum*. Based on these conclusions and the geographical distribution of the *Dasypyrum* species, we suggest that tetraploid cytotype of *D. breviaristatum* was derived from diploid cytotype of the species through chromosome doubling, and that annual *D. villosum* was differentiated from perennial diploid *D. breviaristatum* as the Mediterranean climate was established in the Quaternary.

1. INTRODUCTION

The genus *Dasypyrum* (Coss. et Dur.) T. Durand is a member of the tribe Triticeae. It morphologically comprises two species, *D. villosum* (L.) Cand. and *D. breviaristatum* (Lindb. f.) Frederiksen (1). The former is an annual diploid species ($2n$ = 14) distributed over the Mediterranean basin from Morocco to the Caspian Sea region. The latter consists of two cytotypes, a diploid ($2n$ = 14) and a tetraploid ($2n$ = 28). The tetraploid cytotype occurs in two geographically isolated mountainous regions higher than 1000 m above sea level, Mt. Taygetos in Greece and the Atlas Mountains in northwest Africa (1,2,3,4,5). It was suggested to be an autotetraploid, but its genomes were concluded not to be related to the genome of *D. villosum* (6,7,8,9,10,11,12). A diploid cytotype of *D. breviaristatum* was once reported in a Moroccan population without subsequent recomfirmation (6). Recently, the members of a cooperative collection mission (GSEM95) between INRA, Morocco and Gifu University, Japan rediscovered three diploid plants of *D. breviaristatum* at a natural population in the Moyen Atlas Mountains in Morocco (20). In the present study, one of the diploid plants of *D. breviaristatum* was crossed with the tetraploid cytotype and *D. villosum* to clarify the genome relationships in the genus *Dasypyrum*, and the chromosome pairing at the first metaphase (MI) of meiosis in pollen mother cells (PMCs) and fertility in the F_1 hybrids are presented.

2. MATERIALS AND METHODS

Collection localities of the materials used in the present cross experiment are shown in Table I. One plant of diploid *D. breviaristatum*, three of tetraploid *D. breviaristatum* and one of *D. villosum* were used. Among three plants of tetraploid *D. breviaristatum*, two were originally collected by one of the present authors (S. O.) from the same population in Morocco that the diploid cytotype was collected (13), and the other was collected in Mt. Taygetos in Greece (4) and kindly provided by Prof. S. Sakamoto, Ryukoku University, Japan. The material of *D. villosum* was collected in Sicily, Italy by S. O. and Y. F. in 1990 (5,14). All the materials are now preserved at Fukui Prefectural University, Japan.

Two cytotypes of *D. breviaristatum* and *D. villosum* were crossed reciprocally after hand-emasculation of female parents about two to four days before anthesis.

Table I. Materials used in the present study

Species and Plant no.	Collection locality (Elevation)
D. breviaristatum (2x)	
99008-17	28 km S of Sefrou to Ifrane, Morocco **(1490 m)**
D. breviaristatum (4x)	
99008-9	28 km S of Sefrou to Ifrane, Morocco (1490 m)
99008-10	"
99023-4	Mt. Taygetos, west of Anogia, Peloponnesos, Greece (1080 m)
D. villosum	
99028-2	17 km N from Piazza Armerina to Enna, Sicily, Italy (560 m)

Obtained F_1 hybrid seeds were sown on wet filter paper in petri-dishes incubated at 20 C, and the seedlings germinating were transplanted into pots in an unheated greenhouse. Anthers including PMCs at MI of meiosis were fixed in Farmer's solution (ethyl alcohol 3 : acetic acid 1) and prepared by aceto-carmine squash technique for cytological observation of chromosome pairing. Pollen grains were sampled twice from each F_1 plants at two-days interval. After staining in dilute aceto-carmine solution, pollen grains with well-developed starch grains and three normal nuclei were regarded as normal. At least 2000 pollen grains were observed for each sample and the mean was calculated.

3. RESULTS

3.1. Cross Ability

Interspecific and inter-cytotypic F_1 seeds were easily obtained. Mean percentage of seed set after artificial crosses varied from 11.1 % in the cross combination of *D. breviaristatum* (4x) x *D. breviaristatum* (2x) to 58.5 % in *D. breviaristatum* (2x) x *D. villosum*. To the contrary, germination was drastically different between the reciprocal cross combinations. In the cross between the two cytotypes of *D. breviaristatum*, the mean germination rate was 50.0 % when tetraploids were used as female parents, while none of the F_1 seeds germinated when diploids were used as female parents. In the interspecific crosses between *D. breviaristatum* (2x, 4x) and *D. villosum*, F_1 seeds germinated only when *D. villosum* was crossed as a female parent. The germination rates of the *villosum-breviaristatum* (2x) and *villosum-breviaristatum* (4x) hybrid seeds were 30.0 % and 85.7 %, respectively.

3.2. Chromosome Pairing at MI and Pollen Fertility

Mean configuration and frequency of chromosome pairing at MI and pollen fertility in the F_1 hybrids and their parental plants are shown in Table II.

3.2.1. Parental plants

Chromosome pairing in a diploid *D. breviaristatum* and a *D. villosum* plants was normal and seven bivalents were observed in more than 92 % of PMCs. Pollen fertility in diploid *D. breviaristatum* was 70.6 % and in *D. villosum* 95. 0 %. In tetraploid *D. breviaristatum*, 3.08 to 4.16 quadrivalents per cell were obser-

ved. Frequencies of multivalents higher than quadrivalents were very low, being 0 and 0.04, in two of the three plants observed. Slightly higher frequency of multivalents (0.04_V+0.14_{VI} per cell) were found in the other plant, 99008-9. Pollen fertility in tetraploid *D. breviaristatum* ranged from 70.0 % to 79.1 %.

3.2.2. *D. villosum-D. breviaristatum* (2x) hybrid

A total of 150 cells from three F_1 plants were analyzed. In 51 cells (34.0 %), 12 univalents plus a bivalent were formed, and 10 univalents plus two bivalents were observed in 47 cells (31.3 %). Fourteen univalents were observed in 30 cells (20.0 %). The mean configuration of chromosome pairing and the mean chiasma frequency per cell were 11.12_I+1.44_{II} and 1.49, respectively. This hybrid was highly sterile with 0.1 % of pollen fertility.

3.2.3. *D. villosum-D. breviaristatum* (4x) hybrid

Nine plants from two cross combinations involving a Greek and a Moroccan tetraploid *breviaristatum* plants and a *villosum* plant were analyzed. Of 250 cells observed in the five plants involving a Greek *breviaristatum* plant, 99023-4, seven univalents plus seven bivalents were observed in 167 cells (66.8 %).

Table II. Mean configuration of chromosome pairing and mean chiasma frequency at MI of meiosis of PMCs and pollen fertility in the F_1 hybrids among the two cytotypes of *Dasypyrum breviaristatum* and *D. villosum* and in their parental plants

Species, Cross combination and Plant no.	No. of cells observed 1)	Chromosome pairing								Pollen fertility (%) 2)
		Univ.	Biv. total	Biv. rod	Biv. ring	Triv.	Quad.	Others	X'ta/cell	
D. breviaristatum (2x), 2*n* = 14										
99008-17	50 (1)	0.08	6.96	0.24	6.72	-	-	-	13.68	70.6 (+)
D. breviaristatum (4x), 2*n* = 28										
99008-9	50 (1)	0.74	6.62	1.50	5.12	0.22	3.08	0.18	24.18	70.0 (+)
99008-10	50 (1)	0.04	5.54	0.66	4.88	0.08	4.16	-	26.06	79.1 (+)
99023-4	50 (1)	0.18	7.54	2.86	4.68	0.04	3.10	0.04	23.34	77.6 (+)
D. villosum, 2*n* = 14										
99028-2	50 (1)	0.04	6.98	2.52	4.46	-	-	-	11.44	95.0 (+)
D. villosum x *D. breviaristatum* (2x), 2*n*=14										
99028-2 x 99008-17	150 (3)	11.12	1.44	1.38	0.06	-	-	-	1.49	0.1 (-)
D. villosum x *D. breviaristatum* (4x), 2*n* = 21										
99028-2 x 99008-9	200 (2)	8.75	5.51	3.11	2.40	0.25	0.12	-	8.76	2.2 (±)
"	200 (2)	9.49	5.68	2.76	2.92	0.05	-	-	8.70	0.1 (-)
99028-2 x 99023-4	250 (5)	7.28	6.64	2.00	4.64	0.14	0.004	-	11.58	1.0 (-)
D. breviaristatum (4x) x *D. breviaristatum* (2x), 2*n* = 21										
99008-10 x 99008-17	400 (3)	3.38	3.20	1.09	2.11	3.74	0.005	-	13.96	17.2 (±)

1) Figures in parentheses indicate the number of plants observed.

2) +: anthers normally dehiscent, ±: anthers partially dehiscent, and -: anthers indehiscent, respectively.

Their mean pairing configuration and chiasma frequency per cell were 7.28_I+6.64_{II}+0.14_{III}+0.004_{IV} and 11.58, respectively. A trivalent or a quadrivalent was observed in 37 % of cells observed in two of the four F_1 hybrid plants involving a Moroccan *breviaristatum* plant, 99008-9, and the mean frequencies of trivalents and quadrivalents per cell were 0.25 and 0.12, respectively, while only 0.05 trivalents were observed in the other two plants from the same cross combination. Their mean chiasma frequencies per cell were 8.70 and 8.76, respectively. Several anthers in one of the F_1 hybrids dehisced at anthesis, and the pollen fertility of the F_1 hybrids ranged from 0.1% to 2.2 %.

3.2.4. *D. breviaristatum* (4x)-*D. breviaristatum* (2x) hybrid

A total of 200 cells in four F_1 plants from a cross combination were analyzed. The mean configuration of chromosome pairing and chiasma frequency per cell were 3.38_I + 3.20_{II} + 3.74_{III} + 0.005_{IV} and 13.96, res-

pectively. The pairing configuration observed most frequently was $3_I+3_{II}+4_{III}$ (28.5 % cells), and even seven trivalents were observed in four cells (2.0 %). The F_1 hybrids were partially fertile with 17.2 % of pollen fertility.

4. DISCUSSION

Based on the evidence from chromosome pairing, karyotype analysis and *in situ* hybridization, it has been suggested that a tetraploid cytotype of *D. breviaristatum* is an autotetraploid but that its genomes are not homologous with that of *D. villosum* (6,7,8,9,10,11,12). The mean quadrivalent frequencies per cell (3.08 to 4.16) observed in *D. breviaristatum* (4x) in the present study were within the ranges of the quadrivalent frequencies previously observed in artificially synthesized autotetraploids of Triticeae species (15,16,17,18). Furthermore, the mean pairing configuration of $3.38_I+3.20_{II}+3.74_{III}+0.005_{IV}$, up to seven trivalents and partial fertility were observed in the present F_1 hybrids between the two cytotypes of *D. breviaristatum*. Judging from these present results, it can be concluded that the tetraploid cytotype of *D. breviaristatum* is an autotetraploid with the doubled genome sets homologous with that of the diploid cytotype of *D. breviaristatum*.

In contrast to the close homology among the two genomes of *D. breviaristatum* (4x) and the genome of *D. breviaristatum* (2x), a very low frequency of chromosome pairing was observed in the F_1 hybrid between *D. villosum* and *D. breviaristatum* (2x) in the present study. This result clearly indicates that the genomes of these two species are distantly related with each other, and almost complete sterility in their F_1 hybrid supports no homology between their genomes. Seven bivalents and seven univalents were characteristically observed in the present F_1 hybrids between *D. villosum* and *D. breviaristatum* (4x). Judging from no homology between the genomes of *D. villosum* and *D. breviaristatum* (2x) and the close relationship among the genome of *D. breviaristatum* (2x) and the two genomes of *D. breviaristatum* (4x), it can be concluded that seven bivalents observed in the *villsum-breviaristatum* (4x) hybrid are autosyndetic bivalents between the two genomes of *D. breviaristatum* (4x), and that seven univalents are the chromosomes of *D. villosum* as concluded by the previous studies (7,10,11). Based on these results and discussions, we can conclude that tetraploid cytotype of *D. breviaristatum* was derived from diploid cytotype of the species through chromosome doubling.

Fifteen genera of the tribe Triticeae were classified into two groups, Mediterranean group and Arctic-temperate group, according to their geographical distribution and ecological characteristics (19). The latter group consists of perennial genera, and the former mainly consists of annual genera and was differentiated from the latter group as the Mediterranean climate was established in the Quaternary (19). The genus *Dasypyrum*, which belongs to the former group, consists of both annual and perennial species. The perennial species, *D. breviaristatum*, occurs in mountainous regions higher than 1000 m above sea level of two isolated area, the Atlas Mountains in North Africa and Mt. Taygetos in Greece, while annual species, *D. villosum*, is distributed over the Mediterranean basin at low elevation. Based on their geographical distribution and ecological characteristics, it may be suggested that annual *D. villosum* was differentiated from diploid perennial *D. breviaristatum* as the Mediterranean climate was established in the Quaternary.

So far, only Greek materials of *D. breviaristatum* have been investigated in detail. The Greek population, however, is a small, isolated one and massive stands of *D. breviaristatum* occur in the Atlas Mountains of northwest Africa (3). In the present study, higher frequency of multivalents ($0.04_V+0.14_{VI}$ per cell) were found in one of tetraploid *D. breviaristatum* from Morocco (99008-9), and a segregation in multivalent frequency was observed in *villosum-breviaristatum* (4x) hybrids when this plant was used as a parent. This result indicates that this plant (99008-9) was a heterozygote for a reciprocal translocation of chromosome segments. This suggests that variation in chromosome structures occurs in natural populations of tetraploid *D. breviaristatum* in the Atlas Mountains where its massive stands occur. Detailed investigations using Moroccan materials including both diploid and tetraploid *D. breviaristatum* are essential for the elucidation of the phylogeny and biodiversity in the genus *Dasypyrum*.

REFERENCES

[1] Frederiksen S: Taxonomic studies in *Dasypyrum* (Poaceae). Nord J Bot 11: 135-142 (1991).

[2] Halacsy E de: Conspectus florae graceae 3. Sumptibus Guilelmi Engelmann (1904).

[3] Maire R: Flore de l'Afrique du Nord 3. Paul Lechevalier (1955).

[4] Sakamoto S, Kobayashi H: Variation and geographical distribution of cultivated plants, their wild relatives and weeds native to Turkey, Greece and Romania. In: Tani Y (ed) Preliminary report of comparative studies on the agrico-pastoral peoples in southwestern Eurasia, II. Research Institute for the Humanistic Studies, Kyoto Univ (1982).

[5] Ohta S, Zine Elabidine F, Morikawa T, Tominaga T, Mellas H, Furuta Y: Report on a cooperative cereal germplasm collection mission in Morocco. Al Awamia 97: 51-64 (1997).

[6] Sarkar P: A new diploid form of *Haynaldia hordeacea* Hack. Wheat Inform Serv 6: 22 (1957).

[7] Sakamoto S: Genome analysis of polyploid form of *Haynaldia hordeacea* in the tribe Triticeae, Gramineae. In: Li Z, Swaminathan MS (eds) Proc 1st Intern Symp on Chromosome Engineering in Plants: 52-53 (1986).

[8] Bothmer R von, Claesson L: Production and meiotic pairing of intergeneric hybrids of *Triticum* x *Dasypyrum* species. Euphytica 51: 109-117 (1990).

[9] Linde-Laursen I, Frederiksen S: Comparison of the Giemsa C-banded karyotypes of *Dasypyrum villosum* (2x) and *D. breviaristatum* (4x) from Greece. Hereditas 114: 237-244 (1991).

[10] Blanco A, Simeone R, Resta P, De Pace C, Delre R, Caccia R, Scarascia Mugnozza GT, Frediani R, Cremonini R, Cionini PG: Genomic relationships between *Dasypyrum villosum* (L.) Candargy and *D. hordeaceum* (Cosson et Durieu) Candargy. Genome 39: 83-92 (1996).

[11] Galasso I, Blanco A, Katsiotis A, Pignone D, Heslop-Harrison JS: Genomic organization and phylogenetic relationships in the genus *Dasypyrum* analysed by Southern and in situ hybridization of total genomic and cloned DNA probes. Chromosoma 106: 53-61 (1997).

[12] Uslu E, Reader SM, Miller TE: Characterization of *Dasypyrum villosum* (L.) Candargy chromosomes by fluorescent in situ hybridization. Hereditas 131: 129-134 (1999).

[13] Furuta Y, Ohta S: A preliminary report of 'The Gifu University Scientific Exploration in the Mediterranean Region in 1995 (GSEM95)'. Faculty of Agriculture, Gifu Univ (1996).

[14] Furuta Y, Ohta S: A preliminary report of 'The Botanical Exploration in Southeastern Europe by Gifu University (SEEG)' in 1990 and 1991. Plant Germ-plasm Inst, Kyoto Univ (1993).

[15] Cua LD: Chromosome pairing in some autotetraploid cereals. Seiken Ziho 5: 54-57 (1952).

[16] Morrison JW, Rajhathy T: Chromosome behaviour in autotetraploid cereals and grasses. Chromosoma 11: 297-309 (1960).

[17] Yen Y, Kimber G, Metzger RJ: Meiotic analysis of autotetraploid *Triticum umbellulatum*. Cereal Res Commun 16: 101-104 (1988).

[18] Yen Y, Kimber G: Meiotic behaviour of induced autotetraploids in *Triticum* L. Genome 33: 302-307 (1990).

[19] Sakamoto S: Patterns of phylogenetic differentiation in the tribe Triticeae. Seiken Ziho 24: 11-31 (1973) Ohta S, Koto M, Osada T, Matsuyama A, Furuta Y: Rediscovery of a diploid cytotype of *Dasypyrum breviaristatum* in Morocco. Genet Resour Crop Evol (accepted).

COMPARATIVE ANALYSIS OF A-, B- AND D-GENOMES OF *TRITICUM AESTIVUM* AND ITS DIPLOID AND POLYPLOID RELATIVES BY COMBINING GENOME-SPECIFIC PCR AND DNA SEQUENCING

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ABSTRACT: Bread wheat (*Triticum aestivum*) consists of three related genomes A, B and D, that have originated from wild diploid species which have given rise to tetraploid and hexaploid wheats through natural hybridisation. We have developed a strategy for the construction of genome-specific PCR primers that allow for the separate analysis of each of the A-, B-, and D-genomes. The approach, which is based on the detection and use of orthologous variation, has successfully been applied on coding (e.g. annotated genes, cDNA probes) and non-coding (e.g. mapped gDNA probes) single-copy sequences as well as on tandem repeat families (e.g. 5S rDNA). In combination with direct DNA sequencing, the genome-specific PCR primers allow to perform detailed comparative analysis of related *Triticum* and *Aegilops* genomes. We are currently applying this concept to gain more insight into the ancestry of the A-, B- and D-genomes of bread wheat and the affinity between common, timopheevi, Einkorn and related *Aegilops* wheat species. So far, the results from suchlike analysis at two distinct, single-copy loci indicate that: (i) *A. squarrosa* is narrowly related to the D-genome of *T. aestivum*, (ii) *T. urartu* is closer to the A-genome of *T. aestivum* than the other Einkorn species, (iii) *A. speltoides* is the only *Sitopsis* species that clusters with the B-genome, (iv) next to *A. speltoides* also *A. searsii* is distinct from the other members of section *Sitopsis*, (v) the B- and G-genomes are closely related. Further, interspecific variation between the cultivated *T. monococcum* and its wild relative *T. thaoudar* as well as considerable intraspecific variation among *A. speltoides* accessions was found. Extension of these analyses at other loci, dispersed over the wheat genomes is however necessary to confirm these preliminary findings and to take firm conclusions regarding the evolutionary and systematic relationships of *Triticum* and *Aegilops* species.

1. INTRODUCTION

Bread wheat (*Triticum aestivum*, with genome constitution AABBDD) is a natural allopolyploid, being the hybrid of tetraploid emmer wheat (*T. dicoccum*, AABB) and diploid *Aegilops squarrosa* (also *T. tauschii*, DD). The wild progenitor of emmer wheat, *T. dicoccoides*, is itself an allopolyploid between a diploid member of the Einkorn group (AA) and an as yet unidentified B-genome species. The Einkorn group comprises the cultivated *T. monococcum*, its wild relative *T. boeoticum* (ssp. *aegilopoides* and ssp. *thaoudar*) and the wild *T. urartu*, but it is debated which of these species is the donor of the A-genome of emmer and timopheevi (AAGG) wheats. Also the identity of the diploid donor of the B-genome has been the subject of intensive cytogenetic studies, but is still controversial. Data from a variety of experimental approaches have implicated different species from the section *Sitopsis* (*A. bicornis*, *A. sharonensis*, *A. longissima*, *A. searsii* and *A. speltoides*) as potential B-genome progenitors of polyploid wheats.

We aim at gaining more insight into the ancestry of the A-, B- and D-genomes of bread wheat and the affinity between related *Triticum* and *Aegilops* species by comparative analysis of orthologous DNA sequences. A prerequisite for this approach is the identification of A-, B- and D-orthologues in *T. aestivum*, for which we have developed a strategy based on the detection and use of orthologous-specific DNA sequence variation via PCR on wheat nulli- tetrasomic lines. The work presented here is instrumental to the development of orthologous-specific primer sets at diverse loci, dispersed over the wheat genomes that altogether should allow for a detailed analysis of the phylogenetic and ancestral relationships of *Triticum* and *Aegilops* species

2. GENOME-SPECIFIC PRIMER SETS

2.1 Strategy

The strategy for the development of genome-specific primer sets can be outlined as follows:

1. Consensus primers are designed on the basis of DNA sequence information from e.g. probes, DNA databases.
2. The consensus primers are tested for amplification of an orthologous set of sequences on nulli-tetrasomic lines.
3. The consensus amplicon is cloned and sequencing reveals the A-, B- and D-orthologues.
4. Orthologous sequence variation is assessed via multiple sequence alignment (Fig. 1)
5. Orthologous-specific primers are designed such that at least the 3' ultimate base of the primer is placed at a site that is unique for a particular orthologue (Fig. 1).
6. Chromosome and hence genome assignment of the orthologues is done by PCR on nulli-tetrasomic lines (Fig. 2).

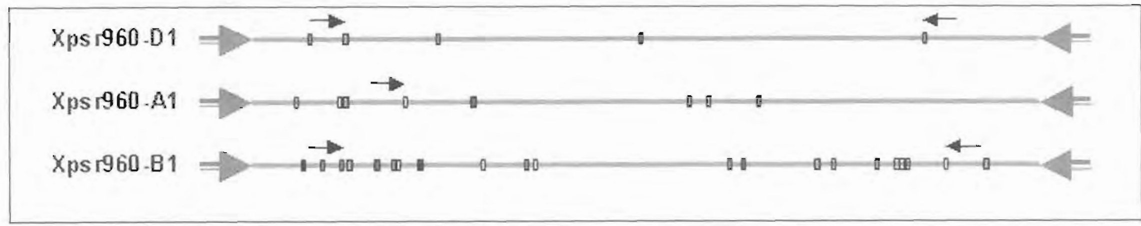
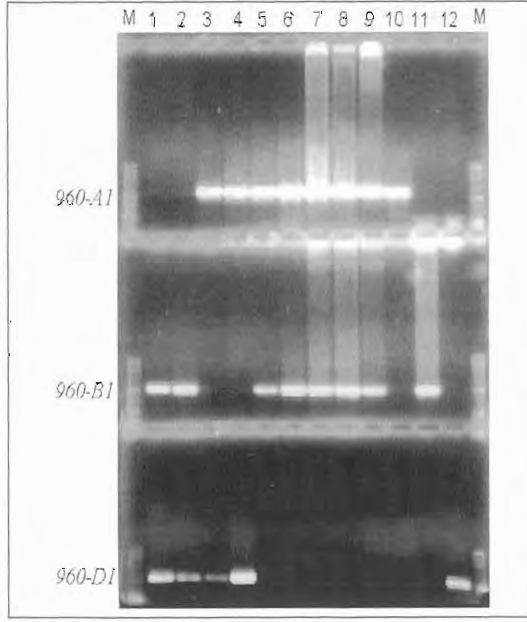


Figure 1: Schematic representation of the orthologous variation displayed by *Xpsr960* sequences amplified with the corresponding consensus primer set. The designed orthologue-specific primers are indicated by internal arrows.



2.2 Applicability of the strategy

Using this strategy, specific primer sets have been constructed for each of the A-, B- and D- orthologues of the *Xpsr960* (Fig. 2), *Xpsr161*, *Pur-1* (1), *5S-Rrna-1* (2, 3) and *5S-Rrna-2* loci.

The general applicability of the strategy emerges from the fact that orthologous primer sets have thus been derived from a single-copy cDNA probe (psr161), a single-copy gDNA probe (psr960), a single-copy gene from the public DNA databese (type-I thionin) and high-copy tandem repeated sequences (5S rDNA). As illustrated for the *Xpsr960-A1*-specific amplification (Fig.1, Fig. 2), the orthologous-specific primers can be used in combination with consensus primers without loosing their specificity. In the case of tandem repeated families, one fixed orthologous nucleotide difference in the units was found to be sufficient for selective amplification via head-to-head oriented primers (3).

Figure 2: PCR products amplified by orthologous-specific primer sets directed to *Xpsr960-A1* (top), *Xpsr960-B1* (middle), *Xpsr960-D1* (bottom) on: *1* N1AT1B; *2* N1AT1D; *3* N1BT1A; *4* N1BT1D; *5* N1DT1A; *6* N1DT1B; *7 T. dicoccoides*; *8 T. durum*; *9 T. timopheevi*; *10 T. urartu*; *11 A. speltoides*; *12 A. squarrosa*; *M*: size marker.

3. COMPARATIVE ANALYSIS OF TRITICUM AND AEGILOPS GENOMES

The use of the consensus and genome-specific PCR markers in conjunction with direct DNA sequencing allows for a detailed comparative analysis of *Triticum* and related *Aegilops* genomes. Recently, we have used this approach on the *Xpsr960* and *Xpsr161* loci of common, timopheevi and Einkorn wheats as well as on *Aegilops squarrosa* and the *Sitopsis* species. In both cases, none of the sequences of contemporary diploids was found to correspond exactly to one of the A-, B- or D-genome sequences of *T. aestivum*.

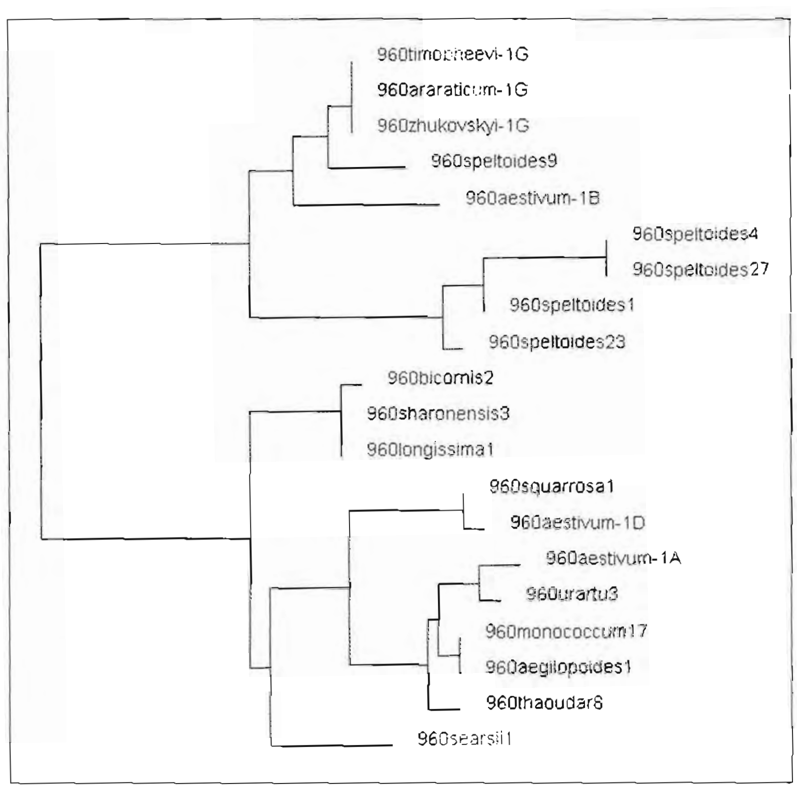


Figure 3: Neighbour joining tree derived from the Clustal W alignment of Xpsr960 sequences.

The comparative DNA sequence analyses, which for the *Xpsr960* data is visualised under the form of a dendrogram in Figure 3, established the following genome relationships: (i) *Aegilops squarrosa* is narrowly related to the D-genome of *T. aestivum*, (ii) *T. urartu* is closer to the A-genome of *T. aestivum* than the other Einkorn species, (iii) *A. speltoides* is the only *Sitopsis* species that clusters with the B-genome, (iv) next to *A. speltoides* also *A. searsii* is distinct from the other members of section *Sitopsis*, (v) the B- and G-genomes are closely related.

Further, interspecific variation between the cultivated T. *monococcum* and its wild relative *T. thaoudar* as well as considerable intraspecific (allelic) variation among *A. speltoides* accessions was observed.

4. CONCLUSION

The general applicable strategy presented here provides PCR tools for the genome-specific analysis of polyploid wheats. In conjunction with direct DNA sequencing they can be used for detailed comparative analyses of related *Triticum* and *Aegilops* genomes in order to gain more insight into the ancestry of the A-, B- and D-genomes of *T. aestivum*. In depth analysis at two loci showed that direct assignment of one of the diploids as progenitor is not possible. However, the analyses indicate that the A-genome evolved from *T. urartu* and the D-genome from *A. squarrosa*, while the B-genome probably originated from a relative of *A. speltoides*. These results are in line with the results of some recent non-molecular studies (4, 5). However, seen the fact that polyphyly of the B-genome (as a result from the hybridisation and introgression among related S genomes from different *Sitopsis* species) can not be ruled out (6); our results need to be confirmed by extension of these kind of analyses at other loci dispersed over the wheat genomes (in first instance the *Pur-1* and *5S-Rrna* loci). Further, the approach allows for the assessment of intra- and interspecific genetic variation for phylogenetic analysis of *Triticum* and *Aegilops* relationships. Other applications can be considered when these single-copy PCR markers are combined with mutation detection techniques like DGGE (denaturing gradient gel electrophoresis) and SSCP (single-strand conformational polymorphism) as this should allow for the screening of molecular variation in polyploid wheat populations.

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REFERENCES

[1] Van Campenhout S, Sági L, Vander Stappen J, Volckaert G: Characterisation of type-I thionin loci from the A, B, D and R genomes of wheat and rye. Theor Appl Genet 96: 80-86 (1998).

[2] Van Campenhout S, Aert R, Volckaert G: Orthologous DNA sequence variation among 5S ribosomal RNA gene spacer sequences on homoeologous chromosomes 1B, 1D, and 1R of wheat and rye. Genome 41: 244-255 (1998).

[3] Van Campenhout S, Vander Stappen J, Volckaert G: The specific isolation of complete 5S rDNA units from chromosome 1A of hexaploid, tetraploid and diploid wheat species using PCR with head-to-head oriented primers. Genome 44: 529-538 (2001).

[4] Ciaffi M, Dominici L, Lafiandra D: Gliadin polymorphism in wild and cultivated einkorn wheats. Theor Appl Genet 94: 68-74 (1997).

[5] Maestra B, Naranjo T: Homoeologous relationships of *Aegilops speltoides* chromosomes to bread wheat. Theor Appl Genet 97: 181-186 (1998).

[6] Ciaffi M, Dominici L, Umana E, Tanzarella O, Porceddu E: Restriction Fragment Length Polymorphism (RFLP) for protein disulfide isomerase (PDI) gene sequences in *Triticum* and *Aegilops* species. Theor Appl Genet 101: 220-226 (2000).

ORIGIN OF *TRITICUM PETROPAVLOVSKYI* UDACZ. ET MIGUSCH., AN ENDEMIC HEXAPLOID WHEAT IN WESTERN CHINA

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ABSTRACT: The Chinese wheat landrace, Xinjiang rice wheat (*T. petropavlovskyi* Udacz. et Migusch., 2n=42), known as 'Daosuimai' or rice-head wheat is characterized by its long glumes, and was found in the agricultural areas in the west part of Talimu basin, Xinjiang, China in 1948. From linkage analysis, the gene (tentatively named P_3) for long glume from *T. petropavlovskyi* was found to be located approximately 36.8 cM from the *cn-A1* locus, which controls the *chlorina* trait. Long glume was independent of purple culm controlled by the genes (Pc_1 and Pc_2). Significant deviation from a 3:1 ratio in the F_2 of CS dt 7AS/*T. petropavlovkyi* confirmed that the long glume can be controlled by a gene (P_3) located on the long arm of chromosome 7A. The gene P_3 is located approximately 11 cM from the centromere on the long arm of chromosome 7A. It is considered that P_3 is an allele on the P_1 locus. It is speculated that *T. petropavvloskyi* originated from a spontaneous interspecfic hybridization between *T. aestivum* and *T. polonicum*

1. INTRODUCTION

The long glume phenotype is one of the key characters used to classify *Triticum* species. The Chinese wheat landrace, Xinjiang rice wheat (*T. petropavlovskyi* Udacz. et Migsch), known as 'Daosuimai' or rice-head wheat is characterized by long glumes similar to those of *T. turgidum* ssp. *polonicum*, *T. ispahanicum* Heslot. *T. petropavlovskyi* was found in the agricultural areas in the west part of Talimu basin, Xinjiang, China in 1948. It was brought to Russia by A.M. Gorski. It was thought that Xinjiang rice wheat was a mutated form of *T. polonicum* [1], however, its chromosome number is 2n=42 and it was later named as *T. petropavlovskyi* Udacz. et Migusch. [2]. Chen et *al.* [3] considered, from the cytological analysis of crosses with common wheat and tetraploid species, that the species was derived from natural hybridization between *T. aestivum* and *T. polonicum*. Chen et *al.* [4] also suggested introgression from *T. polonicum* or some other wild emmer wheat as the mechanism leading to unusual characteristics of *T. petropavlovskyi*. A sterile plant that resembled very much in terms of spike morphology was collected in Xinjiang. It is considered as F_1 of *T. polonicum* and *Aegilops tauschii*. Yang et *al.* [5] suggested that it originated from an independent hybridization event, because of its *polonicum*-like spike morphology of *T. petropavlovskyi* and its relatively greater chromosomal differentiation.

T. petropavlovskyi, *Triticum carthlicum* Nevski and Iran spelt (*T. spelta subsp. kuckuckianum* Goekg) have common features, the presence of an awn-like appendage on the glume (four awns on the apical spikelet) and absence of continuous pubescence on its outer surface. *T. petropavlovskyi* resembles Iran spelt in the fragility of the ear and its shape, but not in the ease with which it threshes. Gandilyan[6] considered that *T. petropavlovskyi* is a "*carthlicoid*" of Iranian spelt. The role of the gene affecting awn formation on the glume has not been well characterized. From the word "*tetraristatus*" (four awned), it was named as the *t* gene to distinguish it from the B_2 gene [6]. The *t* gene affects awn formation on the glume and strengthens the rachis.

The objectives of the present study were to determine the inheritance and chromosomal location of the gene for long glume from *T. petropavlovskyi*.

2. MATERIALS AND METHODS

2.1. Linkage analysis

Koval [7] characterized the allelic states of certain genes of *Triticum aestivum* L cv. Novosibirskaya 67 (N67). ANK-32A is a near-isogenic line, marked with a *chlorina* trait, which was introduced from *chlorina-1* mutation controlled by the *cn-A1* locus located on the long arm of chromosome 7A. ANK-32B is also a near-isogenic line, also marked with a *chlorina* trait, which was introduced from a mutant of AN-215 controlled by the *cn-D1* locus located on the long arm of chromosome 7D. To assess the linkage relationship between the *chlorina* and the long glume phenotype, K51764, a line of *T. petropavlovskyi*, was crossed with ANK-32A and ANK-32B. The NIL ANK-30A, marked with the gene for long glume P_1 was also crossed with ANK-32A. ANK-30A, ANK-32A and ANK-32B were kindly provided by Dr. S.F. Koval, Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia. K51764 was kindly provided by the N. I. Vavilov Institute of Plant Industry, St. Petersburg, Russia. The genes (Pc_1 and Pc_2) for purple culm are located on short arm of chromosome 7B [8] and on the short arm of chromosome 7D [9], respectively. A chromosome substitution line, CS(Hope 7B) used as a marker line for Pc_1, and Lutescens 62 for *Rc2*. K51764was crossed with CS(Hope 7B) and Lutescens 62. A description of pedigree of the set of BC_9 near-isogenic lines and their complete catalogue is given in Koval[7]. Profile of spikes and spikelets of Novosibirskaya 67, a near-isogenic line ANK-30A and *T. petropavlovskyi* K51764 are shown in Fig. 1.

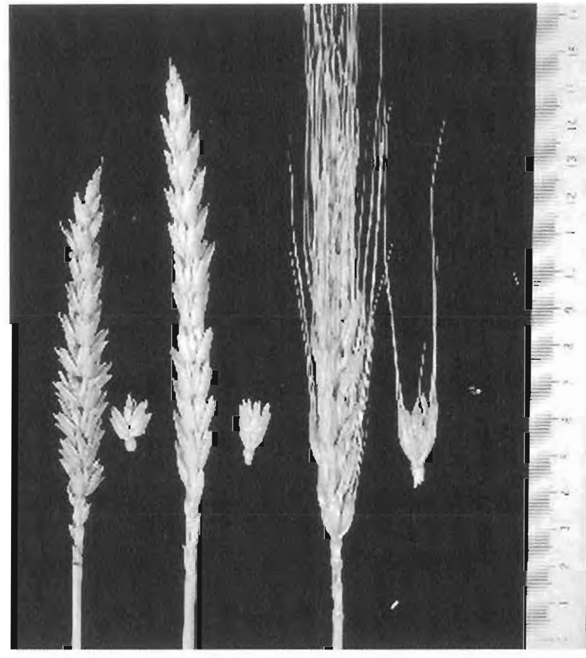

Figure 1: Profile of spikes and spikelets of *Triticum aestivum* cv Novosibirskaya 67, a near-isogenic line ANK-30A and *T. petropavlovskyi* K51764.

The F_2 plants from the six crosses were grown in the field, and classified to allow recombination to be calculated. Seedling colour was classified three times at weekly intervals beginning at the second leaf stage. Culm colour was classified after anthesis. Map distances between loci was calculated using the Kosambi function [10].

2.2. Chromosome arm location of the gene for long glume

To determine the chromosome arm location of the genes of long glume, ANK30A as male was crossed to CS, CS ditelosomic 7AL (CS dt 7L) and dt 7AS. K51764 as male was crossed to CS, CS dt 7AL, CS dt 7AS, CS dt 7BL, CS dt 7BS and CS dt 7DS. F_2 progenies of monotelodisomic (20" + t1") F_1 plants were grown and classified for glume length for each cross. CS ditelosomic 7DL was not available for this study.

2.3. Telosomic mapping

ANK30A and K51764 as male were crossed to CS ditelosomic 7AL. The F_1 plants (2n = 41+t) were backcrossed with CS. Chromosome constitutions of all BC_1 plants were checked as described below. BC_1 plants were classified for glume phenotype after heading. Map distance between loci was calculated using the Kosambi function[10].

3. RESULTS

3.1. LINKAGE RELATIONSHIP BETWEEN LONG GLUME AND CHLORINA.

In the F_2 of cross, ANK-32A/ANK-30A, the segregation for glume length and chlorina did not fit a 9:3:3:1 ratio. This indicated that *P1* is located on chromosome 7A, because *cn-A1d* is located on long arm of chromosome 7A. The recombination between P_1 and *cn-A1d* was calculated as 36.7Å}5.1 % by the maximum likelihood method. The map distance was 37.7Å}7.5 cM. In the F_2 of the cross, ANK-32A/K51764, the segregation for glume length and chlorina also did not fit a 9:3:3:1 ratio. This indicated that *P3* is located on chromosome 7A. The map distance between P_3 and *cn-A1d* was calculated as 36.8Å}8.3 cM. In the F_2 of the cross, ANK-32B/K51764, the segregation for glume length and chlorina did fit a 9:3:3:1 ratio. This indicated that P_3 was independent of *cn-D1* (Fig. 2).

3.2. Linkage relationship between long glume and culm colour

In the F_2 of the cross, CS(Hope 7B)/K51764, segregation for glume length and culm colour fitted a 9:3:3:1 ratio. This indicated that P_3 was independent of Pc_1 locus. In the F_2 of Lutescens 62/K51764, the segregation for glume length and culm colour also fitted a 9:3:3:1 ratio. This indicated that P_3 was independent of Pc_2 locus.

3.3. Chromosome arm location of the genes for long glume

In the F_2 of ANK-30A/K51764, all plants had long glume. This shows that P_1 and P_3 are located at the same locus. It is known that P_1 is located on the long arm of chromosome 7A in tetraploid wheat [11]. The F_2 progenies in CS/ANK-30A and CS dt 7AL/ANK-30A segregated into long and normal glume phenotypes. A good fit to a 3:1 ratios were obtained for the crosses (Table I).

Table I. Segregation for glume length in the F_2 progenies f monotelodisomic (20" + t1") F_1 plants.

Cross combination	Glume length Long	Short	É'2 (3:1)
ANK-30A/K51764	205	0	-
CS/ANK-30A	132	40	0.280
CS dt 7AL/ANK-30A	167	54	0.638
CS dt 7AS/ANK-30A	160	3 2)	46.628*
CS/K51764	138	36	1.724
CS dt 7AL/K51764	145	40	1.126
CS dt 7AS/K51764	156	5 2)	41.161*
CS dt 7BL/K51764	118	40	0.008
CS dt 7BS/K51764	135	45	0.000
CS dt 7DS/K51764	112	30	1.136

1) Values for significance at P=0.05; 3.84 (df=1).

2) 2n = 40+2t.

*: Significant at P=0.05.

The chromosome constitution of three plants, which had short glumes, was ditelosomic (2n=40 + 2t). This confirmed that P_1 is located on the long arm of chromosome 7A. As shown in Table 3, the segregation of F_2 progenies CS dt 7AS/K51764 showed a significant excess of plants with long glume. The chromosome constitution of 5 plants, which had ve short glumes, was ditelosomic (2n=40 + 2t). This finding confirms that P_3 is located on the long arm of chromosome 7A.

3.4 Map distance of the P1 and P3 genes from the centromere

In the test cross progenies, (CS dt 7AL/ANK-30A)/CS, the chromosome number of plants are either 42 or 41+t and segregation was expected the ratio 1:1. Segregation for glume length was expected in the

ratio 1:1. As shown in Fig.2. distance of P_1 from the centromere was calculated as 12.4 ± 0.5 cM. In the test cross progenies, (CS dt 7AL/K51764)/CS, the chromosome number of plants (2n = 42 and 2n = 41+t) segregated in a 1:1 ratio. Segregation for glume length was also in the ratio 1:1. The map distance of P_3 from the centromere was calculated as 10.8 ±.5 cM (Fig.2). These results indicated that the P_1 and P_3 genes were located at the same locus.

4. DISCUSSION

The present study indicated that the gene for long glume (P_3) from *T. petropavlovskyi* is an allele at the P_1 locus on the long glume of chromosome 7A, although Arubzova *et al.* [12] suggested that Eg_2 controlled elongated glume of *T. petropavlovskyi* and that it was not located on chromosome 7A. Watanabe [13] found that, in the genetic background of tetraploid wheat, the gene for long glume was located on the long arm of chromosome 7A. It seems that Eg_2 is identical to P_3.

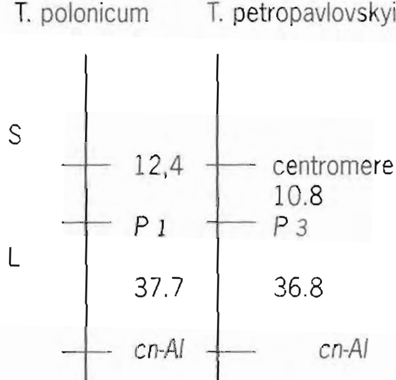

Fig. 2: Linkage map of the genes for long glume and chlorina in chromosome 7A. Figures show distance in cM.

We suggest that *T. petropavlovskyi* originated as a spontaneous hybrid between *T. aestivum* and *T. polonicum* as speculated by Chen [3]. According to Efremova *et al.* [14], *T. petropavlovskyi* has at least 13 dominant genes determining morphological and physiological chararacters and regular bivalent configuration. These authors considered that *T. petropavlovskyi* originated from *T. aestivum* through mutation rather than hybridization.

T. petropavlovskyi, *Triticum carthlicum* and Iran spelt have common features, the presence of an awn-like appendage on the glume and absence of continuous pubescence on its outer surface. Gandilyan [6] considered that *T. petropavlovskyi* is a "*carthlicoid*" of Iran spelt. *T. petropabvlovskyi* resembles Iran spelt in the fragility of the ear and its shape, though not in its threshability. We considered that hexaploid line with an awn-like appendage on the glume might be a putative parent of *T. petropavlovskyi*.

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REFERENCES

[1] Jakubtsiner, M.M.: K poznaniyu pschenits Kitaja. Bot. Zhurn. 44:1425-1436 (1959) (in Russian).

[2] Udaczin, R.A., Miguschova, E.F.: Novoe v poznanii roda *Triticum* L. Vestnik S.-Kh. Nauki 9:20-24(1970) (in Russian).

[3] Chen, Q., Sun, Y., Dong, Y.: Cytogenetic studies on interspecific hybrids of Xinjiang wheat. Acta. Agron. Sin. 11:23-28.(1985).

[4] Chen, P.D., Liu, D.J., Pei, G.Z., Qi, L.L., Huang, L.: The chromosome constitution of thee endemic hexaploid wheats in Western China. In: Miller, T.E. & R.M.D. Koebner (eds) Proc. 7th Int. Wheat Genet. Symp., Cambridge, UK, pp.75-80(1988).

[5] Yang, W.Y., Yen, C., Yang, J.L.: Cytogenetic study on the origin of some special Chinese landraces of common wheat. Wheat Inform. Serv. 75:14-20(1992).
[6] Gandilyan, P.A.: Spontaneous hybridization, mutations and some aspects of the phylogeny of wheat. Soviet Genetics (English version of Genetika), 8: 949-960(1972).
[7] Koval, S.F.: The catalogue of near-isogenic lines of Novosibisskaya 67 common wheat and principles of their use in experiments. Russ. J. Genet. 33:995-1000(1997).
[8] Law, C.N.: The location of genetic factors affecting a quantitative character in wheat. Genetics 53:487-498(1966).
[9] Maystrenko, O.I., Laikova, L.I.: Chromosomal localization and linkage relationship of the *Pan1* and *Pc2* genes controlling anthocyanin pigmentation of the anthers and culm in common wheat. In: Boerner, A. & Worland, A.J. (eds), European Wheat Aneuploid Co-operative Newsletter 1995, Institute fur Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany & Cereal Research Department, John Innes Centre, Norwich, UK, pp. 120-122(1995).
[10] Kosambi, D.D.: The estimation of map distance from recombination value. Ann Eugen 12:172-175(1944).
[11] Arbuzova, V.S., Efremova, Laikova, L.I., Maystrenko, O.I., Popova, O.M., Pshenichinikova, T.A.: Development of precise genetic stocks in two wheat cultivars and their use in genetic analysis. Euphytica 89:11-15(1996).
[12] Watanabe, N., Yotani, Y., Furuta, Y.: The inheritance and chromosomal location of a gene for long glume in durum wheat. Euphytica 91:235-239(1996).
[13] Watanabe, N.: Development and use of near-isogenic lines of durum wheat cultivar LD222. In: Pshenichnikova, T.A. & Worland, A.J. (eds), European Wheat Aneuploid Co-operative Newsletter 2001, Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia & Cereal Research Department, John Innes Centre, Norwich, UK, pp. 59-61(2001).
[14] Efremova, T.T., Maystrenko, O.I., Laikova, L.I., Arbuzova, V.S., Popova, O.M.: Comparative genetic analysis of hexaploid wheats *Triticum petropavlovskyi* Udacz. et Migusch. and *Triticum aestivum* L. Russian J. Genet. 36:1142-1148(2000).

VOUCHER SPECIMENS- A CRITICAL ELEMENT IN A SCIENTIFIC PAPER

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ABSTRACT: Voucher specimens are critical to the credibility and long-term value of scientific research involving biological entities. The best known examples of voucher specimens are type specimens, specimens that must be deposited when a new species or subspecies is being described, but voucher specimens for other kinds of scientific study are equally important and serve a similar function: they enable others to understand the meaning of the names as used by the authors of the paper and maintain the value of the research if taxonomic studies change the systematic framework prevailing at the time of the research. This is particularly important where one species or subspecies is divided into two or more entities, a common occurrence in botany and particularly in a group like the Triticeae in which the limits of many species are unclear or controversial. Voucher specimens may also affect the economic value of a study, cultivars developed from unvouchered seed collections being unacceptable in some parts of the world. Examples are presented in which the value of a paper was increased by the existence of voucher specimens and others in which it is limited because of the lack of such specimens.

1.INTRODUCTION

There has been a stream of papers over the years pointing out the need for maintaining and depositing a properly-prepared set of voucher specimens for any biological study (1-6). Each publication documents, as will this one, reasons why voucher specimens are necessary and the problems that may be encountered if they are not made. Yet it is disturbingly easy to show that most readers and listeners will nod sagely in agreement while saying to themselves that this does not really refer to *their* work. It *does* refer to your work.

2. VOUCHER SPECIMENS - WHAT THEY ARE

Voucher specimens are herbarium specimens that are made from the plants used in a research project and deposited in a recognized, active herbarium. In other words, they are well documented, pressed, dried plant specimens. All a research worker has to do is provide such specimens to an appropriate herbarium. Information on how to prepare them is in most taxonomy texts and at *http://herbarium.usu.edu/K-12/Collecting/*.

The documentation required is fairly basic: when and where the plant was collected, by whom, the name used, and the study involved. If the materials were grown from seeds in a genebank, the documentation should also include the accession number and location where the original material was collected.

A list of herbaria, with contact information, is located at *http://www.nybg.org/bsci/ih/ih.html*. Some herbaria have rather clearly defined areas of interest so you should make sure that your specimens are welcome, and will be treated appropriately, before depositing them.

Depositing specimens usually involves nothing more than giving the pressed specimens and their associated documentation to the herbarium, together with a note that they are voucher specimens for a particular study. Some herbaria will ask that the documentation be typed (many will do so if the handwriting is illegible). The herbarium staff will then prepare each specimen for inclusion in the collection. This generally involves attaching the plant specimen and a label containing its documentation to archival paper before placing it in the appropriate cabinet. At this point your vouchers become available for study by qualified individuals either in that herbarium or, through a system comparable to the International Interlibrary Loan Service, to individuals at other herbaria in any part of the world.

There is one more important step: include a sentence in your paper stating where you have deposited your voucher specimens. Deposition should, of course, precede publication of the paper. It embarrasses curators to be asked for the loan of specimens that are supposed to be in a herbarium only to find that they are still in the investigator's cabinet or, worse, non-existent. It will also embarrass the investigator because curators explain why loan requests cannot be honored.

Voucher specimens are simple and inexpensive to make, they remain scientifically valuable for literally hundreds of years. Specimens made in the 1500s are still studied; there is no inherent reason why specimens made today could not last a thousand years. Would that the importance of our papers were as long lasting!

3. NAMES: NECESSARY, BUT NOT SUFFICIENT

3.1. The function of names

The purpose of voucher specimens is to document and clarify the names that are used in a paper. It is the names that make a paper immediately useful to readers other than the author. We use scientific names assuming that this will enable others understand what plants we worked with. Papers stating that "I have obtained a wonderful forage plant from crossing two plants that were growing on my farm", or that "I have found plants with great restoration potential on a nearby hillside" have no scientific value.

Names, scientific names, are essential if our observations are to be of value to others, and having value for others is what distinguishes scientific papers from interesting stories. But the use of scientific names does not turn poor science into good science. They are useful only if authors know what such names mean.

3.2. Limitations of scientific names

Names, even scientific names, are only words. Most botanical papers are about plants, not names. Scientific names may be used to refer to individual plants, but each name actually refers to an abstract group of plants that are considered to 'belong together' or be 'the same'. Names are useful only to the extent that it is agreed what 'the same' means, in other words, how a name should be interpreted.

In the perfect world, everyone - even taxonomists - would agree on what taxa to recognize and what names to associate with them. And nobody would ever make mistakes in identification. The world is not perfect. Taxonomists are still struggling to determine what taxonomic units should be recognized. It is not simply a matter of determining to what genus a species belongs, but of determining what the species are in the first place and how they can be distinguished from other, morphologically similar, species. Some of the papers being presented at this symposium (7, 8) reflect ongoing efforts to resolve species-level problems in the taxonomy of the *Triticeae*. These papers are not final steps in resolving such problems; they are simply the next step. In addition, there are numerous other taxonomic problems awaiting resolution, some affecting well known and widespread taxa, many of which are not yet included in any taxonomic study.

Changes in taxonomic treatments that affect the circumscription of genera, species, and subspecies inevitably lead to changes in nomenclature, in other words, the meaning of names. This is frustrating, even for those making the changes, but it is essential if scientific names are to have meaning.

The changes in meaning are not always obvious. A new taxon stands out because it has a new name. It is less obvious that the name under which it used to be known immediately acquires a different meaning, one that excludes the plants included in the new taxon. Consequently, the meaning of the names in a paper is determined, in part, by the year in which the paper was published. A further complication is that some people may not learn of the new taxon for some time, or may not agree that it should be recognized. Thus, even after publication of the new taxon, some may be using the name of the older taxon in its old sense. The only way to ensure that other people can interpret the names used in a paper after it is published is by preparing voucher specimens. Specimens do not change.

Let us consider, for example, three alternative treatments of *Elymus trachycaulus* and some of its relatives. Plants that A.S. Hitchcock (9; Fig. 1) placed in four species were treated by C.L. Hitchcock (10) as

five infraspecific taxa within a single species, *E. caninus*. C.L. Hitchcock did not mention the fifth species included in the figure, *E. alaskanus*, because, as he interpreted the species, it did not occur in his area. Barkworth currently divides A.S. Hitchcock's five species among three species. As Figure 1 shows, the names *Elymus trachycaulus*, *E. alaskanus*, and *E. caninus* have rather different meanings to the three individuals. There is a core meaning to each, but there are also differences that could be significant, if not to your study then to the work of others trying to reproduce or build on your results.

If voucher specimens for studies involving these taxa have been deposited in a herbarium, others can determine to which entity they belong. If there are no voucher specimens, there is no way to determine whether the plants belonged to any of these entities, let alone which.

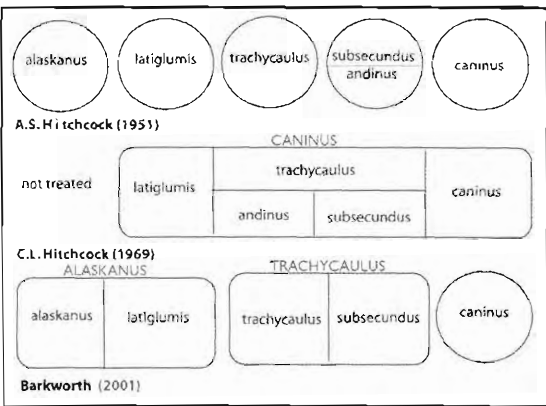

Figure 1. Treatment of *Elymus trachycaulus* and some of its allies by three taxonomists. Names above the divided containers show the specific epithet for that container.

3.3. Authorities for scientific names

Many journals ask contributors to provide the authorities the first time a scientific name is used. The practice is probably best described as scientific snobbery. If providing authorities is done without actually consulting the original publication it is tantamount to citing a reference without reading it. One can provide more information on how the name is being used by citing the reference used for identification or as a nomenclatural source. But one must do what an editor asks.

4. WHEN ARE VOUCHER SPECIMENS NECESSARY?

When is it necessary to make a voucher specimen? The answer is simple: anytime the scientific name is important. This means that voucher specimens should be an integral part of taxonomic, ecological, genetic, molecular, and plant breeding studies. They give a study repeatability and longevity because, although taxonomists may disagree over the interpretation of a name, or the meaning of a name may change over time, specimens do not change. They can also, as will be demonstrated, give a study importance beyond its immediate focus.

4.1. Existing specimens and mature plants

Studies based on existing herbarium specimens should state in which herbaria the specimens used were located. If some specimens were studied in particular detail, they should be listed individually. Some journals, primarily taxonomic journals, require a complete, or almost complete, listing of the specimens examined. Be prepared. If space is a problem, as it is with some journals, deposit a list with the journal and in the libraries of the authors' institutions and state that this has been done in the paper.

If the research involves living plants, whether cultivated or growing naturally, new specimens need to be made and a statement stating where they have been deposited placed in the paper. The specimens should be made from the same plants that are used in the study. If there are replicas of each study unit (accession, ploidy level, or whatever), it is only necessary to make specimens of representative plants.

4.2. Voucher specimens for genebank accessions.

Do specimens need to be made of plants grown from seed in genebanks? Yes. Not for every study, but at least when you first use the accession. Genebanks are run and operated by humans and humans occasionally make errors. These errors could go unnoticed for several years if those using the material assume that, because the seed came from a genebank, it must have been accurately identified. This is not always so. The cultivar 'Shoshone' was developed from particularly productive accessions of the North American *L. triticoides*. It was later determined that the accessions belonged to the Eurasian *L. multicaulis*.

More recently, Salomon was asked to confirm the identity of seeds being used in the development of a cultivar for use in revegetation in Alaska. The seed, which came from a Plant Materials Center of the USDA

was thought to belong to *E. macrourus*. Salomon identified at least four different kinds of seeds in the sample. He has since determined that some of the seed is *E. sibiricus*; identification of the three remaining entities cannot be made until they reach flowering maturity.

4.3. Cultivars

The task of writing up accounts of a region's flora, including its introduced flora falls to taxonomists who draw on their field experience and herbarium specimens in preparing descriptions and writing keys. These accounts are intended to permit identification of all established plants, including those found in artificial or improved pastures. Unfortunately, cultivars are rarely found in herbaria. They may be present, but unless they are identified as such they are probably being identified as the species to which they are most similar. This is not a significant problem if they are derived from a single species. It is a problem if their history is more complex or if they are derived from a species new to the region.

A particular specimen must be designated as the holotype [= voucher specimen] whenever a new species or infraspecific taxon is described (11). The equivalent for cultivar names are Standards (12). Standards need not be herbarium specimens but that is recommended by Recommendation 32A. We endorse the recommendation and recommend the deposit of duplicate specimens in herbaria where the cultivars are being distributed. An additional reason for making voucher specimens of cultivars and their progenitors is that some countries will not allow importation of cultivars lacking such documentation.

4.4. Identifying voucher specimens

Clearly, specimens used in a study must be identified accurately. This may require sending them to experts at other institutions. Barkworth recently attempted to identify specimens of Stipeae grown from seeds in a genebank. So far as she was able to tell, several were misidentified. The reservation in that statement is intentional. She was not familiar with the species involved, she did not have access to reliably identified specimens of them, and her literature resources for the countries of origin were limited. She recommended that the specimens be sent for identification to individuals familiar with the taxa involved and that these individuals be paid for their work. Both her recommendations were accepted.

The importance of accurate identification cannot be overemphasized, nor the difficulty of identifying plants from distant localities at institutions without appropriate resources. Errors in identification are serious because, once a paper has been published, the information in it will be cited. A correction may be published subsequently, but many readers will not see the correction.

We are not implying that failure to make voucher specimens indicates that the identifications in the study are necessarily wrong, nor that other aspects of the study have not been carried out in an exemplary fashion. But voucher specimens are essential should a question arise concerning the accuracy of an identification, the application of a particular name, or the relationship between plants exhibiting the characteristics reported in the study and those under investigation by someone else.

4.5. Voucher specimens have added value

Once voucher specimens have been reliably identified, they become part of a herbarium's resources, increasing its staff's ability to respond to requests for assistance and conduct research on the taxa in question. This is, in a sense, the other side of the coin for voucher specimens. They have greater value than other specimens simply because more is known about them. Cytological voucher specimens prepared by Bowden (12) were used by Barkworth to find characters for distinguishing between the octoploid *Pascopyum smithii* and tetraploid *Elymus lanceolatus*. An unexpected bonus was that Bowden had vouchered both the field-collected plants and their greenhouse-grown offspring. This permitted evaluation of the plasticity of the characters used to distinguish the taxa. Only one new character was found, but the study would not have been possible without the voucher specimens.

Another example involves *Persoonia* (Proteaceae). Fleur (13) produced an excellent treatment of the pollen characters in *Persoonia* but, when she did the work, the species were not well defined. Fortunately she made voucher specimens and documented her work. When Weston (14) worked on the species delimitation and relationships, he was able to reidentify Fleur's voucher specimens and, hence, make use of her observations. If Fleur had not made voucher specimens, her work would have been useless.

5. THE GOOD, THE BAD, AND THE UGLY

It is easier to come up with horror stories than good stores concerning voucher specimens simply because it is the horror stories that people recall. Nevertheless there are innumerable papers that show an

exemplary regard for the importance of voucher specimens. Van Slageren's monograph (15) is an example. One of its noteworthy features is that the lists of specimens examined include specimens made from genebank accessions, a practice that should be encouraged.

An example of the opposite kind is provided by the many papers on genetic variation for which there are no voucher specimens. Genetic variation within a species is expected; ecologically related variation is expected. If we are to be able to relate molecular and physiological variation to morphological variation, voucher specimens are needed. 'Morphological study' means detailed study and numerical analyses, not just measurements of plant height and above-ground dry-weight. It requires voucher specimens. Making specimens is time consuming, but the potential benefits are significant.

Unvouchered studies can be detrimental. For instance Hayman (16) analyzed 300 populations of *Themeda autralis* and reported clear distribution patterns for the five different ploidy levels. Vickery (17) attempted to reconcile his information with patterns of morphological variation, but was unable to match it with the chromosomal variation because Hayman made no voucher specimens. Those familiar with *T. australis* know that Hayman's work must be redone, but it is impossible to obtain support for redoing work.

Another area of concern is is ecology. Many ecologists nowadays identify plants in the field without making specimens. This precludes careful checking of the identifications and means that there is no reliable contribution to our knowledge of plant diversity. Reliability is very much an issue here. A highly regarded botanist in Oregon listed *Mitella breweri* and *M. caulescens* as being present on Steen Mountain, Oregon. Drs. Pat and Noel Holmgren spent 2 days trying to verify the report but concluded he had probably misidentified *M. pentandra* (18). With voucher specimens, it would only have taken a few minutes to verify - or refute - the report.

6. CONCLUDING REMARKS

We have, we hope, demonstrated that voucher specimens add to the credibility of a paper and its long term value. A problem for editors and referees is that few people feel that a 'serious' piece of work should be rejected because voucher specimens are not mentioned.

We suggest that, at a minimum, all papers include a statement about the existence of voucher specimens. The information provided should include, at a minimum, species name, where deposited and some means of identifying the specimen itself such as the accession number, collector's name and number, or collector's name and collection date. If there are no voucher specimens, then this should be stated.

Some studies of a general nature do not need to be vouchered and not all names mentioned in a paper will require vouchers. Inclusion of a statement such as we suggest will enable editors and reviewers to determine whether, for the study involved, there is adequate documentation of the plants used and, if the paper is accepted for publication, readers will be able to make the same judgment for themselves. Remember, voucher specimens improve the credibility and longevity of *your* work. Make them.

7. REFERENCES

[1] Hosking JR, Sainty GR, Jacobs SWL: Certainty and uncertainty in plant identification. Pp. 464-467 In: Shepherd RCH: Proceedings of the eleventh Australian weeds conference. Weed Science Society of Victoria, Melbourne (1996).

[2] Jacobs SWL: Progress in Australian aquatic plant taxonomy. Pp. 177-191 IN: Gilligan B, Maddock M, McDonald K (eds): Hunter Wetland Trust Symposium (1988).

[3] Ruedas LA, Salazar-Bravo J, Dragoo JW, Yates TI. The importance of being earnest: what, if anything, constitutes a 'specimen examined'. Molecular Phylogenetics and Evolution 17:129-132.

[4] Snow EP, Keating P: The relevance of specimen citations to conservation. Conservation Biology 13:943-944 (1999).

[5] Thomas RH: Molecules, museums and vouchers. TREE 9:413-414

[6] Wagstaff DJ, Wiersema JH, Lellinger DB: Poisonous plant vouchers. Veterinary and Human Toxicology 41:162-164 (1999).

[7] Wang S, Jacobs SWL: *Elymus* in Australasia. Pp. In: Martin A .... Proceedings of the Fourth International Triticeae Symposium.

[8] Wilson BL, Kitzmiller J, Roll W, Hipkins VD: Isozyme variation in wild, self-fertile, allotetraploid *Elymus glaucus*: implications for restoration. Pp. In: Proceedings of the Fourth International Triticeae Symposium

[9] Hitchcock AS: Manual of grasses of the United States, Ed. 2. Rev. A. Chase. U.S.D.A. Miscellaneous Publications 200 (1951).
[10] Hitchcock CL: Gramineae. Pp. 384-725 In: Hitchcock, CL, Cronquist A, Ownbey M, Thomspon JW: *Vascular plants of the Pacific Northwest*, Part 1. University of Washington Press, Seattle.
[11] Greuter W, McNeill J, Barrie FR, Burdet HM, Demoulin V, Filgueiras, TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth, DL (eds.): International Code of Botanical Nomenclature (Saint Louis Code). Adopted by the Sixteenth International Botanical Congress St. Louis, Missouri, July-August 1999. Regnum Veg. 138: 1-474 (2000)
[12] Bowden WM. In: Gillett JM, Senn HA: Cytotaxonomy and infraspecific variation of *Agropyron smithii* Rydb. Canadian Journal of Botany 38: 747-760 (1960).
[13] Feur S: Pollen morphology and evolution in the Persoonioideae, Sphalmioideae and Carnavonioideae (Proteaceae). Pollen et Spores 28:123-156 (1986).
[14] Weston PH: The western Australian species of subtribe Persoonieae (Proteaceae: Persoonioideae: Persoonieae). Telopea 6:51-165 (1994).
[15] Slageren MW van: Wild wheats: a monograph of *Aegilops* L. and *Amblyopryum* (Jaub. & Spach) Eig (Poaceae). Agricultural University, Wageningen, the Netherlands (1994).
[16] Hayman DL: The distribution and cytology of the chromosome races of *Themeda australis* in southern Australia. Australian Journal of Botany 8:58-68 (1960).
[17] Vickery JW: Gramineae. Contributions from the New South Wales National Herbarium, Flora Series No. 19, Pt. 1 (1961).
[18] Holmgren PK, Holmgren NH: Saxifragaceae. In: Cronquist A, Holmgren NH, Holmgren PK: Intermountain Flora, vol. 3a. New York Botanic Garden, Bronx, New York (1997).

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GRAIN QUALITY, YIELD AND BIOCHEMICAL MARKERS OF KAZAKHSTAN'S WINTER TRITICALE COLLECTION

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ABSTRACT: Grain quality, yeild and biochemical markers of second winter hexaploid triticale collection were studied. On the chemical and physical properties of grain the accessions were classified to 3 groups: analysis of hardness and glutenin/gliadin content ratio 23% to be perspective for breadmaking; analysis of amylose contents, extractivity, protein contents and ratio of its fractions showed 16% cvs as a suitable source for brewery. While availability of soft triticale keeps a possibility for its use in the pastry production, the majority of accessions were characterized by rather high protein contents (12,4-17,4%). The protein fractions ratio to total protein considerably vary: 33,6-53,2% for albumin+globulin, 19,1-32,7% for gliadin, and 15,1-27,2% for glutelin, respectively. The hardness index varied from 13 to 67. An analogy between analyzed breadmaking accessions and the first Kazakhstan's winter bread triticale cultivar Taza was determined: hardness 46-67; vitreousness 29-52%; protein contents 11,4-16,2; gluten contents 19,2-30,2%; glutelin/gliadine ratio 0,95-1,36, W - 20-58 e.a.; farinograph 21-27 e.t., bread volume - 530-750 ml. Electrophoresis of gliadins showed five varieties to be monomorphic and stable, while rest accessions including Taza as polimorphic those. Comparative electrophoretic analysis of a-amylase, b-amylase, esterase, acid phosphatase, 6-phoshogluconate dehydrogenase, cathodic peroxidase showed both inter- and intravarietal polymorphism of winter triticale.

INTRODUCTION

Triticale (X *Triticosecale* Wittmack), the first cereal crop which was obtained synthetically, would be one of the main grain and forage crop in future due to its high genetic potential. Yield potential of triticale under optimum crop production environments has reached nearly the same level of wheat under marginal environments (1). Triticale is currently widely used as a feed grain and forage. Most triticale breeding studies have concentrated on improving the agronomic traits, but less to the improvement of traits associated with breadmaking quality (2-4). Local collection of this untraditional cereal crop for Kazakhstan consists of winter second hexaploid forms with vegetation period from 286 to 312 days, breed by intraspecific hybridization with following cytological and field selection of 42-chromosome forms in F_2-F_{10} generations. In order to evaluate the potential of hybrid breeding in triticale, agronomic and biochemical studies as well as grain quality analyses have been started to assess the genetic diversity in the Kazakhstan triticale genepool by evaluating agronomic traits and molecular marker data.

2. MATERIALS AND METHODS

The collection of the following 73 winter second hexaploid triticale 1- T-187; 2 - T-186, 96; 3 - 103; 4 - T-561-2-3; 5 - T-529-505; 6 - T-545-2-3; 7 - 99; 8 - T-66-2-3; 9 - T-1613-51/31; 10- CD-60; 11 -T-545-2-3", 13 - T-501-2; 14 -83; 15 -T-1134-2; 16 - T-1198-4; 17 - 1428; 18 - T-1198-2; 19 - 97; 20 - T-560-2-3; 21 - T-1198-4; 22 - T-545-2-3; 23 - T-1613-51/30; 24 -10A; T-25- T-694-4; 26- 1427; 27 - 49; 28 - T-392-1; 29

- T- 198; 30 - 107 MB; 31 - T-784; 32 - T-365-1-2; 33 - T-185; 34 - St.; 35 - St., 36 - 21/22; 37 - St.; 38 - T-1136-1; 39 - T-1133-7; 40 - AD 60; 41 - 47; 42 - T-1221-1; 43 - M-121/7; 44 - T-1133-7; 45 - T-784; 46 - T-784; 47 - T-554; 48 - Krasnodarskii z/k; 49 - 81; 50 - AD 206; 51 - T-551-13-1; 52 - T-1166-1; 53 - T-16-12/14; 54 - T-553-1-2; 55 - T-597-1-23; 56 - 78; 57 - T-1613-51/29; 58 - 102; 59 - 1426; 60 - 1416; 61 - ADg; 62 - T-501-6; 53 - 100; 64 - T-1198-5; 65 -T-597-1-23; 66 - T-1221-1; 67 -T-1221-1"; 68 -Taza; 69 - 21/22; 70 - N-545-2-3; 71 - 101; 72 - 107 IB; 73 -T-635-1 were studied for identification of accession on morphological traits according to the UPOV, yield, on gliadin (5); isozymes (6) and grain quality according to the methods previously used for wheat (7,8). Taza cv was tested in 14 soil-climate poimts of Kazakhstan on all above mentioned traits in 1995-1999. Also 5 CYMMIT cultivars Kissa, Armino, Shacal, Sonni, Farhad grown in Uzbekistan was compared with local cv Taza on grain quality characteristics.

3. IDENTIFICATION OF TRITICALE COLLECTION

3.1. Morphological description of triticale collection according to UPOV

The morphological description according to the UPOV (International Union for the Protection of New Varieties of Plants) system has allowed to refer all the samples as most similar to Clercal, Carmen, Triton, and Lasko varieties on a degree of expressiveness of the most of the traits. All accessions are of winter middle-ripening second hexaploid forms with vegetation period from 286 to 312 days. Type of cereal bush has occured as following: straight standing (1) - 14.5%; semi-straight (3) -46.1%; middle (5) - 36.8%; semi lodging (7) - 2.6%. Flag leaf inclination were distributed as: absence or weak (1) - 14.6%, low (3) - 27.6%, middle (5) -26.3%, high (7) -28.9%, and very high ones (9) - 2.6% of accessions. Anthocyan color of auricles in flag leaf was as following: absence or weak (1) - 67.1%, low (3) - 10.5%, middle (5) - 9.2%, high - 13.2% of triticale samples. Heading date: very early (1) - 2.6%, early (3) - 28.9%, late (5) - 30.3%, middle (7) - 28.9%, very late (9) - 9.3% of accessions. Flag leaf length: very short (1) - 5.1%, short (3) - 32.9%, middle (5) -50.1%, long (7) - 10.6%, very long (9) - 1.3%; width: very narrow (1) - 1.2%, narrow (3) -27.5%, middle (5) - 47.3%, wide (7) - 25.0%. Straw of more than 50% of them is solid has allowed to varieties with middle height (99-110 cm) to be resistant to lodging. absence or weak (1) - 18%, weak (3) - 4%, middle (5) - 8%, high (7) - 18%, very high (9) - 52%. Stem density: open packed (3) - 5%, middle (5) - 30%, closely packed (7) - 65%. It was observed the following characteristics for spike: 1) length: short (3) - 15%, middle (5) - 61%, long (7) - 24%; 2) width: middle (5) - 96%, wide (7) -4%; 3) awn: top awn (1) - 3%, middle (2) - 8%, long (3) - 89%; 4) inner glume: 28% downy; 5) the length of the first tine of the spike inner glume: very short (1) - 6%, short (3) - 9%, middle (5) - 37%, long (7) - 29%, and very long (9) - 19% accessions; 6) second tine: absent or very small (1) - 31%, small (3) - 19%, middle (5) - 25%, large (7) - 15%, very large - 10%; 7) color: white (1) - 45%, weak colored (2) - 45%, colored (3) - 10%. Awn color: uncolored or very weak - 82%, weak -17%, middle - 1%; awn length: very short (1) - 8%, short (3) - 25%, middle (5) - 28%, long (7) -36%, very long (9) - 3%. In common morphological identification according to UPOV has allowed to attribute most accessions to the type of two standard cultivars Clercal and Carmen.

3.2. Biochemical identification of triticale collection

The local triticale collection was estimated by using biochemical markers as well. Six accessions (T-187, T-551-13-1, T-185, T-198, and T-554) were represented by 2-3 identical samples, 25 accessions had the only sample, 8 accessions (T-1221-1, T-1198-4, T-1137-7, T-545-2-3, 107IB, T-597-1-23, T-784, T21/22) by 2-4 samples non-identical on electrophoretic spectra, while Krasnodarski z/k and T-186 have characterized by unknown gliadin analog. In common it has been revealed 58 profiles of gliadin in 73 analyzed accessions, including 16 unknown those. Electrophoretic spectrum of triticale gliadin had 50 components: a - 8, b - 9, g - 9, w -24. Over 35% components occurred were common for all the analyzed set (a 98, 95; b 83, 79, 75; g 69,65, 61, 53; w 40, 36, 32, 20, 14, 10, 8, 6). The level of genetic variability of the collection of winter triticale was established by cluster analysis on gliadin components (fig.1). The first cluster combined 41 accessions, have characterized by 26 electrophoretic types with identity level 1-0,88, second cluster consisted from 32 samples and 24 profiles. In only third cluster each accession had unique rare type of spectrum (r=0,96-0,76). Electrophoresis of gliadin showed five varieties to be monomorphic and stable, while rest accessions including Taza as polimorphic those. All the accessions are hexaploid according to the HMW glutenin as in comparison with material analyzed in the frame work of CYMMIT (Pena, 2001, unpublished data) they have not 1D subunit. Comparative electrophoretic analysis of a-amylase, b-amylase, 6-phoshogluconate dehydriogenase, esterase, acid phosphatase, cathodic peroxidase showed both intervarietal and intravarietal polymorphism of winter triticale. Cluster analysis of morphological and biochemical markers has allowed to reveal a relationship of some different accessions.

4. GRAIN QUALITY AND YIELD ANALYSES

The grain quality as a criteria for techological end use as well as uniformity and stability in reproduction are very important in breeding. It is known that quality and quantity of protein and carbohydrate complexes determine the grain end use type for many of cereal crops. Calibration equations were created for express value of content of protein and its fractions, and amylose as for wheat in our previous studies (9). Samples presented all the scale on calibrated trait: protein content - 10.6-16.2%, albumiin and globulin to total protein - 5.14-7.44%, prolamin - 2.48-4.97%, glutelin - 2.04-3.90%, amylose content - 12.7-24.0% (table I). An analysis of chemical properties of grain, extractivity, hardness indecies and falling number was carried. The majority of varieties were characterized by rather high protein contents (12,4-17,4%). The ratio of protein fractions was considerably changed to respect of the total protein: from 33,6 to 53,2 % for albumin and globulin, 19,1-32,7 % for gliadine, and 15,1-27,2% for glutelin, respectively. The hardness index was varied from 13 to 67 according to data of SKCS 4100. 16% of the accessions estimated as a mixture class and 7% those as middle hardness. The results have allowed to classify 3 groups of accessions upon on end use products. 23 percents of the triticale accessions were found to be perspective for breadmaking due to analysis on hardness and glutenin/ gliadin contents ratio. These were accessions with hardness index 47-67 with glutenin predominance on gliadin (1.0-1.36) and acetic acid sedimentation level above 25 ml. Study of amylose contents, extractivity, contents of protein and ratio of its fractions has allowed to reveal 16 percents of varieties as quite suitable raw material for brewery. While availability of soft triticale keeps a possibility for its use in the pastry production, some accessions, such as T1612/14 and Krasnodarski z/k are perspective for feed and forage as lysine contents vary from 3,79 to 6,45, and tryptophan - 1,25-1,72; methionine in the range - 1,54-2,75. The variability of productivity parameters was established for productive bushiness (3-8 spikes), length of ear (6.9-15 sm); number of grains per ear (52-74), mass of a grain from the plant (6.3-8.8 g). Cluster analysis of productive features has allowe to differentiate the collection to 8 classes.

Table I. shows grain quality variation of winter triticale collection and Taza cv on the base of NIR-spectroscopy calibration (Pacific Scientific 4250)

Grain quality	Rank (1998-99) min-max	Taza 1996-99	NIR calibrations Characteristics (Pacific Scientific 4250)			
			S_{sev}	S_{sep}	CV	Mean
1. Protein content, Nx5,7%	12.4-17.4	11-4-16.2	0.31	0.33	0.98	14.3
2. Starch content, %			0.66	0.67	0.97	
3. Albumine+globuline content, Nx5,7%	5.14-7.44	5.23-6.48	0.33	0.34	0.84	6.14
4. Albumine+globuline content, % to total protein	34.4-53.2	38.2-43.4				
5. Gliadine content, Nx5,7%	2.48-4.97	2.74-3.38	0.42	0.44	0.91	3.40
6. Gliadine content, % to total protein	19.1-32.7	20.0-24.3				
7. Glutenine content, Nx5,7%	2.04-3.90	3.19-3.72	0.61	0.62	0.74	3.51
8. Glutenine content, % to total protein	15.1-27.2	22.5-27.2				
9. Gliadine/glutenine	0.59-1.36	0.95-1.36				
10. Amylose content, %	12.0-30.2		2.74	2.82	0.62	19.8
11. Falling number, Ser60-150	70-130				79	
12. Sedimentation 2% CH_3COOH, ml	12-37	19-26.5				20
13. Lysine content, % to protein		3.90-4.12				3.98
14. Hardness index (SKCS 4100)	13-67		7	8	0.88	39
15. Vitreousness, %	27-69	46-67				

Table II. The Table II shows a variation rank of grain quality characteristics of winter hexaploid triticale cultivar Taza from Kazakhstan and CYMMIT samples grown in Uzbekistan.

Quality traits	Competitive trials 1995-98 Taza cv	State trials 1998 irrigated Taza cv	State trials 1998 non irrigated Taza cv	Agronomical trials, 1999 Taza cv	CYMMIT cultivars in Uzbekistan Kissa	Armino	Shacal	Sonni	Farhad
1. Nature, g/l	707-733	630-711	665-799	661-684	796	784	775	7776	728
2. Weigt of 1000 grain, g		44.5-50.7	49.2-50.7		46.5	56.9	44.7	43.2	59.1
3. Vitreousness, %	37-39	35-52	29-52	29-47	53	55	32	62	32
4. Hardness, %	46-56	49-67	50-65	67-69	60	65	60	63	64
5. Protein content, %	11.4-16.2	11.8-15.4	12.5-15.7	11.8-13.2	12.8	13.2	11.5	13.8	14.3
6. Flour gluten content, %	26.0-30.2	19.2-27.2	22.6-28.2	19.6-25.6	22.0	21.6	13.2	22.4	22.8
7. Quality of gluten, IDK	90.105	90-100	85-90	80-95	105	95	65	95	100
8. Elasticity of dough, P, mm	34-40	46-110	49-63	37-70					
9. Elasticity/extensibility, P/L	0.73-1.62	2.4-3.5	1.8-2.1	0.7-3.5					
10. W, e.a.	33-47	20-58	36-46	30-45					
11. Dough resistance to mixing	230-250	230-260	250-260	240-270	230	250	210	240	270
12.Valorimetric evaluation, e.f.	24-27	21-27	24-26	24-30	20	28	32	26	21
13. Bread volume, ml	530-600	560-660	650-750	470-640	490	580	555	640	510
14. Overall breadmaking grade	2.9-3.3	2.9-3.4	3.5-3.8	2.6-3.6	2.74	3.60	3.46	4.14	2.24
15. Falling number, sec	60-96			71-128	245	63	296	123	72
16. Sedimentation (Zeleny), ml	2-10			1-12	11	15	82	13	19
17. Sedimentation ,2%- (2%-CH_3COOH),, ml	20-28			22.0-26.5	27.0	23.5	26.5	25.5	24.0
18. SDS-Sedimentation	20-24				22.0	22.0	24.0	26	24

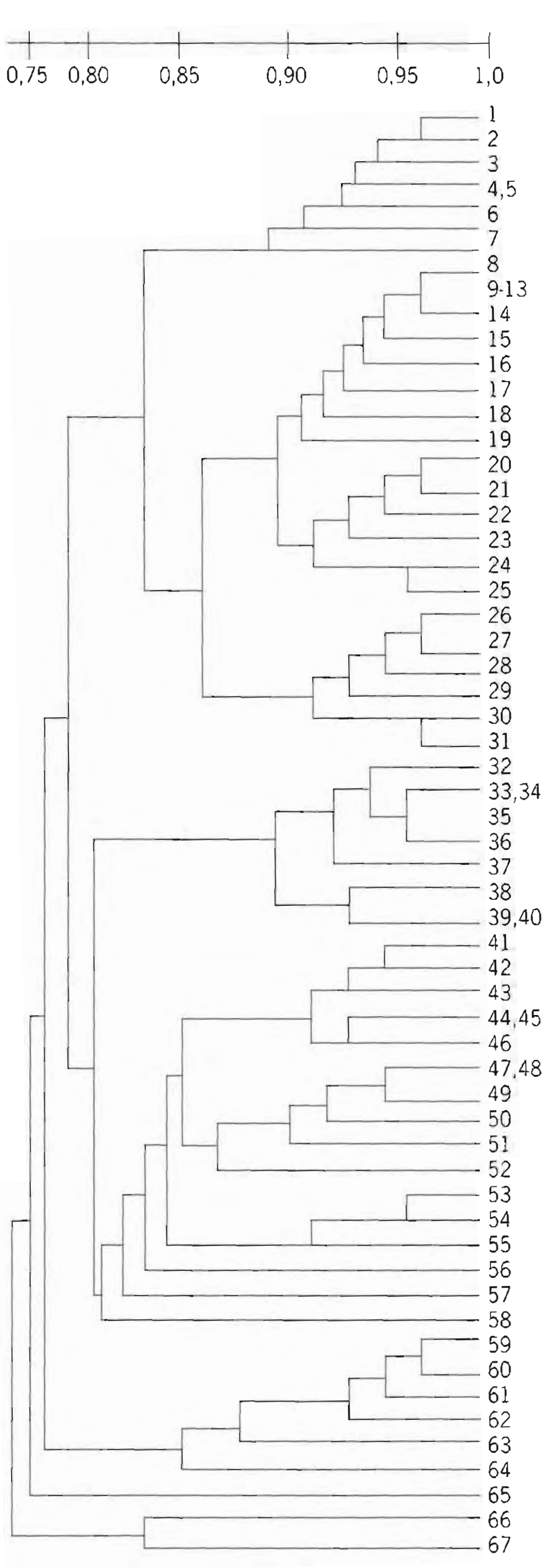

Figure 1. Classification of triticale accessions on gliadin spectra

The most "productive" cluster consisted of 16% samples with spike length 14-15 cm, number of spikelets per spike 35-38, weight of grain per spike - 4.6-5.2 g, and weight of 1000 grains more than 50g. These are accessions predominanted cultivars AD 206, AD 60, and Krasnodarski z/k - T-784, T-1612/14, T-1136-1, new cultivar Taza (table II), etc. Further study of triticale accessions using PCR-based molecular markers have been started.

REFERENCES

[1] G. Varughese, W.H. Pfeiffer, R.J. Pena, Triticale: A Successful Alternative Crop. Cereal Foods World. 1996. 41, 6:474-645.

[2] A.J. Lukaszewski, C.A. Curtis, Transfer of the Glu-D1 Gene from Chromosome 1D of Bred Wheat to Chromosome 1R in Hexaploid Triticale. Plant Breeding , 1992, 109:203-210

[3] R.J. Pena, Genetic, Biochemical, and Rheological Aspects Considered at CYM-MIT for the Improvement of Wheat and Triticale Quality, In: Background Material for the Germplasm Improvement Subprogram External Review, Cd.Obergon, Son., Wheat Program, 1994. Mexico, D.F.: CYMMIT (1994). 98-100.

[4] W.H. Pfeiffer, A.J. Lukaszewski, M. Mergoum and R.J.Pena, Industrial Quality and Agronomic Performance of Spring Triticale Substitution Lines. Agronomy Abstracts, ASA, Indianopolis, Indiana, USA. 1996.

[5] A.I. Abugalieva, Gliadin Components and Glutenin Subumits in Wheat Breeding. In.: Wheat Structure, Biochemistry and Functionality. Reading, Cambridge, UK, Ed. J.D.Shofield, 2000. 173-179.

[6] Abugalieva S.I., Skokbaev S.A., Abugalieva A.I., Turuspekov Ye.K., Dracheva L.M., Savin V.N. 1999. Classification of wheat and barley cultivars in Kazakstan. In: S.Andrews, A.C. Leslie and C. Alexander (Editors). Third International Symposium on the Taxonomy of Cultivated Plants, Royal Botanic Gardens, Kew. p.18-32.

[7] A.I. Abugalieva, .I.A. Abugaliev, Optimization of Wheat Breeding Process: protein markers, databases, and softwares. Reports of Russian Agricultural Academy Science, Moscow, 1997, 4:.5-7.

[8] A.I. Abugalieva, L.M. Dracheva, Grain Hardness of Wheat Charachteristics Grown in Kazakhstan, Reports of Russian Agricultural Academy Science, Moscow, 2001,4: 10-13.
[9] A.I.Abugalieva, S.O.Skokbayev, L.M.Dracheva, V.N.Savin, Optimization of High Quality Wheat Production Zones in Kazakhstan. In.: Spring Wheat in Kazakhstan: Current Status and Future Directions, Proceedings of the Kazakhstan – CIMMYT Conference, Shortandy, Akmola, Kazakstan, September 22-24, 1997: 49-56.
[10] Gosudarstvennyi Reestr Sortov Selskohozyaistvennyh Kultur, Dopushennyh k Ispolzovaniyu na Territorii Respubliki Kazakhstan na 2001. Almaty, 2001, 57.

GENETIC DIVERSITY AND GENE FLOW IN THE CROP-WEED COMPLEX OF WHEAT (*TRITICUM AESTIVUM* L.) AND JOINTED GOATGRASS (*AEGILOPS CYLINDRICA* HOST)

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ABSTRACT: Jointed goatgrass (*Aegilops cylindrica* Host - CD genomes) is a wild, weedy relative of wheat (*Triticum aestivum* L.- ABD genomes) brought to the USA in contaminated wheat seed from Eastern Europe. Although hybrids between jointed goatgrass and wheat are common, they have not been treated as part of the weed problem and have been assumed to be sterile. Recent discoveries of partially female-fertile hybrids in the Pacific Northwest have forced a reevaluation of hybridization potential, particularly under the pressure of possible gene flow from herbicide-resistant wheat into jointed goatgrass. Genetic marker analyses using the high molecular weight (HMW) glutenin seed proteins and microsatellites showed a low genetic diversity in jointed goatgrass. The seed protein analysis and genomic *in-situ* hybridization together has provided preliminary evidence of hybridization rates and dynamics. To date, evaluation of hybrid material from wheat-field collections and experimental work supports the potential for development of stable jointed goatgrass-like hybrid forms carrying wheat genes. On the basis of this preliminary evidence, the wheat-jointed goatgrass crop-weed complex offers an agricultural introgression model.

1. INTRODUCTION

The allotetraploid wild wheat species *Aegilops cylindrica* Host (jointed goatgrass) arrived in the USA approximately 100 years ago as a contaminant in new wheat cultivar (*Triticum aestivum* L.) introductions from Eastern Europe [1,2]. It was first identified as a weed of winter wheat fields in Kansas during the early 1920's [1,2] and has since become extensively spread from the Midwest to the Pacific Northwest, currently infesting over 3 million hectares of winter wheat cropland [3]. At the same time that jointed goatgrass was identified as a USA wheat-field weed, its ability to hybridize with wheat also was noted [1,2]. Although occasional seed production on F_1 hybrids was found [1,2], the traditional view has held that they are sterile [1,2,4]. Wheat-field jointed goatgrass x wheat hybrids have not been unique to the USA but are known from European and Asian distributions [5,6,7], with the first European report dating back to 1869 [5]. Recent discoveries of seed-bearing hybrids in the Pacific Northwest [8,9] have forced a reevaluation of the hybridization. With the imminent introduction of herbicide-resistant wheats into cultivation [8], gene-flow potential between wheat and jointed goatgrass has become a pressing issue.

Hybridization between jointed goatgrass (CD genomes) and wheat (ABD genomes) should not be unexpected. Both belong to a complex of wild and domesticated species whose allopolyploid members evolved via hybrid speciation [10]. Hybrids between wheat and other *Aegilops* species with which it comes into contact have been reported across the sympatric native and adventive distributions of the wheats [7]. While hybridization between jointed goatgrass has been considered an innocuous novelty, it is now apparent that the seed-bearing potential of hybrids must be considered as part of the weed problem in management strategies for jointed goatgrass infestations.

Experimental studies have established that F_1 hybrids have a low rate of female fertility and must be pollinated by either parent for seed production to occur [11]. Fertility increases with each successive back-

cross generation leading toward stable, self-fertile backcross derivative lines within two to three generations [11,12, 13, 14]. Additionally, genomic *in-situ* hybridization (GISH) has revealed A/B translocations to the jointed goatgrass genome in these hybrid derivatives [L Crèmieux, pers. comm]. Experiments conducted under field conditions have demonstrated development of BC_1 and BC_2 seed-producing hybrids [15,16], verifying the potential for such events in jointed goatgrass-infested wheat fields.

In Oregon, jointed goatgrass has become a serious weed pest in winter wheat fields located in the Columbia Basin, a region which stretches from the east side of the Cascade mountains to the northeast corner of the state. Wheat is grown under dryland conditions, usually in a wheat/fallow rotation. A three-year survey of this region, begun in 1998, was undertaken to assess the hybrid problem in Eastern Oregon wheat fields. Collections of jointed goatgrass and hybrid material have been characterized using the high molecular weight (HMW) glutenin seed storage proteins as genetic markers. The HMW glutenins are expressed as paired subunits, x and y, which are coded by two tightly linked genes, *Glu-1-1* and *Glu-1-2*, located on the long arm of the homoeologous group 1 chromosomes [17,18]. Jointed goatgrass and wheat both have diagnostic subunit compositions that can be used to characterize genetic diversity and distinguish between their respective contributions in hybrids.

2. MATERIALS AND METHODS

2.1. Wheat-Field Collections

Jointed goatgrass and hybrid material was collected from 50 field (winter wheat, spring wheat, spring barley, non-grain rotation crops) and 16 non-field (uncultivated) sites distributed across the geographic range of the dryland wheat region of Eastern Oregon during the 1998-2000 growing seasons. Numbered collections were randomly made in jointed goatgrass and hybrid populations. Two to four spikes of jointed goatgrass were collected at hybrid collection points or as random collections through weed populations. Hybrids spikes were collected either as single-plant (all spikes, numbered by plant) or bulk collections (selected spikes, numbered by field) with a minimum of 25 hybrids per site, unless fewer were present. Accessions of the C and D-genome species, *Ae. markgrafii* (Greuter) K.Hammer and *Ae. tauschii* Cosson were obtained from the United States Department of Agriculture National Small Grains Collection in Aberdeen, Idaho. In 1999 and 2000, the rootball also was collected and examined for remnants of the mother seed (F_1 wheat parent) or mother spikelet (F_1 jointed goatgrass parent), hereafter referred to as seed/spikelet.

2.2. HMW Glutenin Marker Analysis

In the HMW glutenin analysis, the non-embryo half of 2-6 seeds from jointed goatgrass spike samples and 856 seeds threshed from selected hybrid spike collections were cut, weighed, and ground. HMW glutenin proteins were extracted and separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (PAGE) and gels stained following Galili and Feldman [19]. Either 5-12% 8cm x 8cm handcast mini-gels or Owl PAGE-ONE pre-cast 4-20% gels, 10cm x 10cm and 10cm x 8cm cassette sizes were used and run at a constant voltage of 200v in an EC 120-4 Mini Vertical Gel System

For jointed goatgrass, identification of the respective *Glu-C1* (Cx,Cy) and *Glu-D1* (Dy only) contributions was made by comparison with the band patterns of its progenitor parents *Ae. markgrafii* (C genome) and *Ae. tauschii* (D genome). For the wheat, identification of the respective *Glu-A1* (Ax only), *Glu-B1* (Bx,By), and *Glu-D1* (Dx,Dy) contributions was made by comparison with the band patterns found in the soft white-wheat cultivars, Oregon cvs. 'Madsen' and 'Stephens' and the genetic standard in wheat research, cv. 'Chinese Spring'. Bands were scored on the basis of relative position (Fig. 1) rather than by molecular weight or by the *Glu-1* subunit assignment system for wheat developed by Payne and Lawrence [20]. Scoring was by presence ("one") or absence ("zero") of the x- and y-subunits for each of the genomes contributing to the HMW glutenin pattern. Hybrid band intensity based on female (2n) versus male (1n) parental dosage was not used in the scoring due to the expected irregular *Glu-1* expression in wheat aneuploids [21,22]. Hybrid seed with null patterns, i.e., no *Glu-1* expression or weak expression relative to other seed storage protein patterns, were dropped from the analysis.

Multiple bands in the Dx region were coded as one and scored as a $Glu\text{-}D1_{aes}$ contribution for the following reasons: 1) Dx is silenced in jointed goatgrass [23] and thus presumed to be null in the hybrids; 2) multiple $Glu\text{-}D1_{aes}$ x-subunit combinations of closely associated bands are present in some Oregon cultivars; 3) since the Ax subunit is located in the wheat Dx region, its contribution is equivalent to Dx for evaluating parental HMW glutenin contributions. In coding the Dy subunits in the hybrids, no distinction was made between the jointed goatgrass versus wheat contribution because 1) the respective Dy subunits are relatively close to each other and 2) the Dy_{cyl} is similar in position to a Dy_{aes} band that can be found in

soft-white wheat cultivars. For the pollen parentage analysis, only Cx was used to identify pollination by a jointed goatgrass parent because the Cy band is not informative given its overlap with a particular By band found in soft-white wheats (Fig. 1b). Only the presence of Dx and Bx together (and in some cases, with a By subunit, located well above the Cy position) was used to identify pollination by wheat. Dy was not used for pollen parent analysis for the reasons stated above.

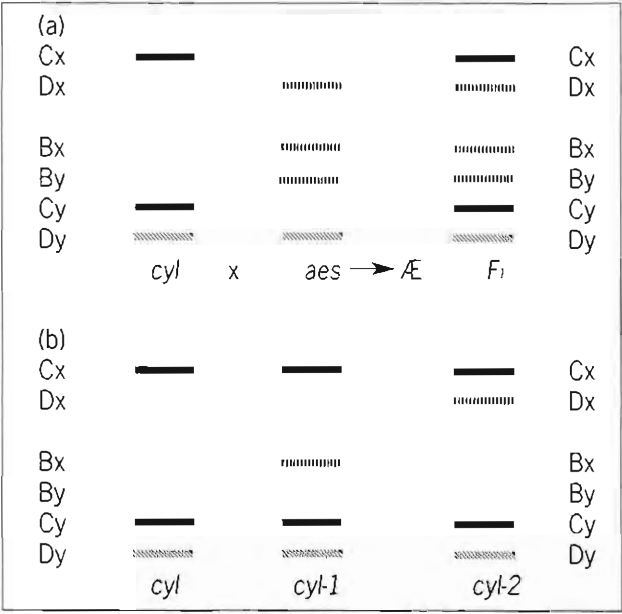

Figure 1: Coding of jointed goatgrass (*cyl*), wheat (*aes*), and F_1 hybrid HMW glutenin subunit compositions (a); jointed goatgrass-like patterns *cyl-1* (By subunit combination not shown), *cyl-2* (b). *Glu1* x- and y-subunits are represented in descending order of migration, i.e., from slowest to fastest. Solid band indicates unique jointed goatgrass *Glu-1* contribution; vertically-hatched band indicates unique wheat *Glu-1* contribution; slanted-hatched band indicates similar *Glu-1* contribution.

3. HYBRIDIZATION RATES AND PARENTAGE

3.1. Jointed Goatgrass F1s

Diversity in the three-band HMW glutenin pattern for the Oregon jointed goatgrass collections (30 sites; 2528 seeds) was extremely low and appeared only as the absence of either the Cy or Dy subunits. Extrapolating from this group 1 chromosome marker, overall species diversity was low, a finding consistent with other genetic analyses of USA and international accessions of jointed goatgrass [24, 25, 26]. Our preliminary evidence from microsatellite studies also confirms low overall genetic diversity. A finding of low HMW glutenin diversity verified the uniformity of the jointed goatgrass parental contribution in F_1 and backcross hybrids. For collections from eight winter wheat sites, the marker analysis identified 25 F_1 hybrid seed. The hybrid HMW glutenin pattern was additive, with all parental subunits represented (see Fig. 1a). Hybridization rate across the 22 field sites where this jointed goatgrass seed was collected was 1.6% (25 /1579 seeds) with a range of 0.3-8%.

3.2. Backcross Hybrids

During the three years of the hybrid survey, 5517 spikes were collected from 754 hybrid plants in 45 sites (Table I). Of these, 328 plants collected from 33 sites (44%) contained one or more seeds. An overall backcross hybridization rate was calculated at 1% (1128 seed/5517 spikes with an average 22 spikelets/spike). The seed production and hybridization data were consistent over the three years. Numbers of plants producing seed ranged from 39-49% and reflected differences in yearly collection strategies. Seed/spikelets were found for 57 seed-producing plants with a distribution of 39 jointed goatgrass (5 sites) and 18 wheat (2 sites) F_1 plants and one presumed BC_1 plant. On the basis of this seed sample and the distinctive F_1 spike morphology of most hybrid collections (long, cylindrical spikes, terminating in long apical awns), most Oregon collections were presumed to be of the F_1 generation. This F_1 female-parental distribution in the seed/spikelet plant sample, a 70% (jointed goatgrass) versus 30% (wheat), suggested that jointed goatgrass is the female parent for the majority of F_1 hybrids formed in wheat fields.

Table I. Hybrid plant collections and seed production in Oregon wheat-field hybrids. Fertile plants and spikes produced one or more seeds.

Year	Total plants	Fertile plants	Total seed	Fertile spikes	Total spikes
1998	86	42	222	165	918
1999	269	129	504	400	2280
2000	399	157	502	335	2319
Total	754	328	1228	900	5517

In the marker analysis, 590 seed (390 single-plant: 200 bulk) were analyzed. Over half of the seed had an additive F_1-like HMW glutenin pattern (343 seed: 58%), showing a retention of the intermediate hybrid condition in the BC_1 generation. This group was not informative for determinations of backcross parentage because female versus male subunit contributions could not be distinguished on the basis of dosage as mentioned above. For the subsample with diagnostic parental-like patterns, wheat was the predominating BC_1 pollen parent, i.e., - 159 seed with a wheat-like pattern (DxBx(By)Dy) versus 19 seed with two jointed goatgrass-like patterns (CxBxCyDy: 3 seeds; CxDxCyDy: 16 seeds). Pollination by wheat was evident by the absence of the Cx subunit; pollination by jointed goatgrass was determined by presence of either, but not both, of the Bx or Dx subunits. The relative distribution of parental-like patterns verified a low jointed goatgrass pollen rate of 11%, which is consistent with the expected effect of a high wheat pollen load across the field. Similar parental-like pattern distributions and pollination rates were found in the seed/spikelet subsample (136 total seed) where 5 seed had the jointed goatgrass-like patterns and 42 seed had the wheat-like pattern. With respect to potential introgression events, this marker analysis provided evidence that gene flow in the direction of jointed goatgrass is at least possible up to the BC_1 generation. The two jointed goatgrass-like patterns also have been found in experimental BC_1 lines developed from a wheat x jointed goatgrass hybrid. When taken to the BC_2S_1 generation, some of these lines have developed into jointed goatgrass-like plants with a morphology indistinguishable from the wild type and a chromosome constitution that was at the 28-chromosome number of jointed goatgrass or within a close range (29-34 chromosomes) due, at least in part, to presence of A/B-genome chromosomes from wheat [14, L Crèmieux, pers. comm].

4. CONCLUSION

This study has provided evidence for an active wheat-field hybrid zone in Eastern Oregon. Given the extensive spread of jointed goatgrass across the winter wheat-growing region of the USA [3], the Oregon hybrid survey can serve as a model for assessing gene flow risk associated with introductions of herbicide-resistant wheat into areas where jointed goatgrass is present. Hybrid seed counts and the HMW glutenin marker analyses provide a view of the hybridization dynamics at play in this hybrid zone. It is clear that jointed goatgrass is the predominating female F_1 parent. Although wheat F_1s do occur, most of them will be lost to the grain harvest. Due to the wheat pollen load, most backcrosses will be to wheat which will shift the population toward the wheat parental end, a favorable move with respect to gene flow risk. However, a low rate of backcrosses to jointed goatgrass is verified by this research. Experimental evidence confirms that the jointed goatgrass BC_1s have the potential to progress toward stable, self-pollinating forms with one or two additional backcrosses to jointed goatgrass. In this respect, the importance of managing the hybrid zone becomes an issue for risk assessment. When jointed goatgrass populations are allowed to persist continuously in and around wheat fields, they will provide a safe hybrid zone in which development of introgressed forms carrying wheat genes can develop.

5. REFERENCES

[1] Johnston CO, Parker JH: *Aegilops cylindrica* Host, a wheat field weed in Kansas. Trans. Kansas Acad. Sci. 32:80-84 (1929)

[2] Johnston CO, Heyne EG: Distribution of jointed goatgrass (*Aegilops cylindrica* Host.) in Kansas. Trans. Kansas Acad. Sci. 63:239-242 (1960)

[3] Thompson M: *Aegilops cylindrica* (jointed goatgrass) - a threat to U.S. wheat production. Executive Summary of the National Jointed Goatgrass Research Program CSREES-USDA Special Grant (http://www.ianr.unl.edu/jgg) (1999)

[4] Donald WW, Ogg AG: Biology and control of jointed goatgrass. (*Aegilops cylindrica*), a review. Weed Technol. 53:3-17 (1991)
[5] Belea A: Examination of the F_1 hybrids of *Aegilops cylindrica* Host x *Triticum aestivum* L. Acta Agron. Acad. Sci. Hung. 17:151-160 (1968)
[6] Rajhathy T: Continuous spontaneous crosses between *Aegilops cylindrica* and *Triticum aestivum*. Wheat Info. Serv. 11:20 (1960)
[7] Slageren MW van: Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub & Spach) Eig (Poaceae). Wageningen Agricultural University Papers 7(94). 513pp. (199
[8] Mallory-Smith CA, Hansen J, Zemetra RS: Gene transfer between wheat and *Aegilops cylindrica*. 2nd Intl Weed Control. Cong., Copenhagen, pg. 441-445 (1996)
[9] Seefeldt SS, Zemetra RS, Young FL, Jones SS: Production of herbicide-resistant jointed goatgrass (*Aegilops cylindrica*) x wheat (*Triticum aestivum*) hybrids in the field by natural hybridization Weed Sci 46:632-634 (1998)
[10] Kimber G, Sears ER: Evolution in the genus *Triticum* and the origin of cultivated wheat. In: 'Wheat and Wheat Improvement', Heyne EG (ed). Agronomy Monograph No. 13 ASA-CSSA-SSSA, Madison, Wisconsin (1987).
[11] Zemetra RS, Hansen J, Mallory-Smith CA: Potential for gene transfer between wheat (*Triticum aestivum*) and jointed goatgrass (*Aegilops cylindrica*). Weed Sci. 46:313-317 (1998)
[12] Wang ZN, Hang A, Hansen J, Burton C, Mallory-Smith CA, Zemetra RS: Visualization of A and B genome chromosomes in wheat (*Triticum aestivum* L.) x jointed goatgrass (*Aegilops cylindrica* Host) backcross progenies. Genome 43:1038-1044 (2000)
[13] Wang ZN, Zemetra RS, Hansen J, Mallory-Smith CA: The fertility of wheat x jointed goatgrass hybrid and its backcross progenies. Weed Sci 49:340-345 (2001)
[14] Crèmieux L: Seed protein and chromosome number analyses of experimental wheat x jointed goatgrass (*Aegilops cylindrica* Host) hybrid derivatives. M.S. thesis, Oregon State University (2000)
[15] Snyder J, Mallory-Smith C, Balter S, Hansen J, Zemetra RS: Seed production on *Triticum aestivum* by *Aegilops cylindrica* hybrids in the field. Weed Sci 48:588-593 (2000)
[16] Guadagnuolo R, Savova-Bianchi D, Felber F: Gene flow from wheat (*Triticum aestivum* L.) to jointed goatgrass (*Aegilops cylindrica* Host.), as revealed by RAPD and microsatellite markers. Theor Appl Genet 103:1-8 (2001)
[17] Payne PI, Hold LM, Law CN: Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Theor Appl Genet 60:229-236 (1981)
[18] Thompson RD, Bartels D, Harberd NP: Nucleotide sequence of a gene from chromosome 1D of wheat encoding a HMW-glutenin subunit. Nucleic Acids Res 13:6833-6846 (1985)
[19] Galili G, Feldman M: Genetic control of endosperm proteins in wheat 1. The use of high resolution one-dimensional gel electrophoresis for the allocation of genes coding for endosperm protein subunits in the common wheat cultivar Chinese Spring. Theor Appl Genet 64:97-101 (1983)
[20] Payne PI, Lawrence GJ: Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1* and *Glu-D1* which code for high molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Comm 11:29-35 (1983)
[21] Galili G, Feldman M: Intergenomic suppression of endosperm protein genes in common wheat. Can J Genet Cytol 26:651-656 (1984)
[22] Galili G, Levy A, Feldman M: Gene-dosage compensation of endosperm proteins in hexaploid wheat *Triticum aestivum*. Proc Natl Acad Sci 83:6524-6528 (1986)
[23] Wan Y, Liu K, Wang D, Shewry PR: High-molecular-weight glutenin subunits in the *Cylindropyrum* and *Vertebrata* section of the *Aegilops* genus and identification of subunits related to those encoded by the Dx alleles of common wheat. Theor Appl Genet 101:879-884 (2000)

[24] Watanabe, N, Mastui, K, Furuta Y: Uniformity of the a-amylase isozymes of *Aegilops cylindrica* Host introduced into North America: Comparison with ancestral Eurasian accessions. In: Wang RR-C, Jensen KB, Jaussi C (eds) Proc 2nd Intl Triticeae Symp., Logan, UT, June 20-24. pp 215-218 (1994)
[25] Okuno K, Ebana K, Noov B Yoshida H: Genetic diversity of central Asian and north Caucasian *Aegilops* species as revealed by RAPD markers. Genet Res Crop Evol 45:389-394 (1998)
[26] Pester TA: Winter annual grass weed management in winter wheat. Chapter 4. Genetic diversity of jointed goatgrass (*Aegilops cylindrica*) determined with RAPD and AFLP markers. Ph.D. thesis, Colorado State University., Fort Collins, CO. pp. 75-107 (2001)

ECOLOGICAL CHARACTERIZATION OF WILD *TRITICUM AND AEGILOPS* SPECLES IN SYRIA USING GIS TOOLS.

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ABSTRACT: Detailed climatic and soil information for 391 collection sites in Syria, from which ICARDA gene bank accessions were collected and geographic coordinates were known, was generated using the Almanac Characterization Tool developed by Texas Agricultural Experiment Station and ICARDA. These accessions represent 183 wild *Triticum* and 558 *Aegilops* populations belonging to four and 16 species, respectively. The data were subjected to discriminant analysis and the group centroids of the canonical discriminant functions were used for computing proximity matrix and, subsequently, hierarchical cluster analysis. Dendrograms from the analysis indicated high similarity in the ecological adaptation between *Ae. umbellulata* and tetraploid *Ae. triuncialis, Ae. biuncialis, Ae. geniculata* and, less loose clustering with *Ae. columnaris, Ae.neglecta* and *Ae. peregrina.* Similar to this, D-genome diploid *Ae. tauschii* clustered with D-genome polyploids, *Ae. crassa* and *Ae. vavilovii* and A-genome diploids *T. urartu* and *T. baeoticum* with tetraploid T *dicoccoides*. When a sub-set of wheat close relatives was analyzed separately using climatic variables, *Ae. searsii* and *T. urartu* were closely linked to *T. dicoccoides*, whereas *Ae. speltoides* and *T. baeoticum* were associated with *T. araraticum.* When all species were analyzed together, monthly precipitation and potential evapotranspiration variables were all included in the canonical discriminant functions, while only two out of 24 monthly temperature extreme variables and four of 12 precipitation/potential evapotranspiration ratio variables. Three soil variables were involved in the species discrimination. Standardized canonical discriminant coefficients indicated which ecological variables had the highest discriminating power for a given pair or group of species. The results are discussed in the context of the evolutionary relationships within the *Triticum-Aegilops* complex.

Key words: *Triticum, Aegilops*, ecological characterization, GIS

1. INTRODUCTION

Conservation and utilization of plant genetic resources are essential for food security not only in the present, but more importantly, to satisfy the future needs of the rapidly growing population in the developing countries. Those, who benefited less from the first 'Green Revolution' in the 70s of the last century, were the poor farmers typically producing their crops in the conditions of low-input subsistence agriculture under frequent biotic and abiotic stresses. Since genetic diversity for stress tolerance and specific adaptation to marginal environments may be insufficient in the cultivated species, scientists and breeders have to explore wild relatives of the primary and secondary crop gene pool. Wheat, one of the two globally most important crops, provides food for one third of the humankind. It is widely grown in many countries of the developing world. However, the yield and stability of the production in the low-input rainfed farming systems greatly depends on the genetically conditioned stress resistance and adaptation to specific environments. Wheat wild relatives have been evolving much longer in the stressful environments of the West and Central Asia and the Near East region than cultivated wheat forms and, consequently, accumulated in the natural populations many genes for stress tolerance and adaptation. As a number of techniques for transferring traits from the wild relatives to cultivated durum and bread wheats are now available, utilization of the potentially useful genes of the crop wild gene pool can be one of the means for poverty alleviation in the rural areas of the wheat-growing regions in developing countries.

One of the limitations for using wild relatives in wheat improvements is the lack of ecological data in crop databases. The ecological information could be generated by GIS from collection site geographical coordinates, latitude and longitude, if these are known. Unfortunately, the proportion of germplasm accessions with the geographical data in germplasm databases is usually very low. For example, only nine per cent out of nearly 33,000 wheat accessions in the USDA Germplasm Resources information Network database (GRIN) are with latitude-longitude, but 48% accessions have a narrative site description, for which geographical coordinated could be identified (1).

The Genetic Resources Unit at the International Center for Agricultural Research in the Dry Areas (GRU/ICARDA) which is currently holding more than 3,400 *Aegilops* and 1400 wild *Triticum* accessions in its *ex situ* genebank, has spent a lot of effort in the last ten years to fill the gaps in the passport information and identify latitude/longitude data from the collection site description. For example, out of the total 994 *Aegilops* and wild Triticum accessions of Syrian origin, 96% are with geographical coordinates. As a new GIS tool with Syrian climatic and edaphic data became recently available, this was an opportunity for generating a number of new ecological variables for Syrian wheat wild relatives and explore the ecological data in statistical analyses to specify ecological characteristics of individual species and identify patterns of variation between the species.

2. MATERIAL AND METHODS

2.1. Material

A total of 954 *Aegilops* and wild *Triticum* accessions with collection site geographical coordinates (latitude/longitude) held at ICARDA genebank and originating from Syria were the primary data source. These accessions, which represented 558 and 183 natural populations and 16 and four species of the two genera, respectively, were collected from 391 different sites. The taxonomic nomenclature used is according to van Slageren (2), but in wild *Triticum* the sub-species were, for convenience, elevated to a species level. The following is a list of species included in the analyses:

Table 1. List of species

Genus	Species
Aegilops	*biuncialis*
	caudata
	columnaris
	crassa
	cylindrica
	geniculala
	juvenalis
	kotschyi
	neglecta
	peregrina
	searsii
	speltoides
	tauschii
	triuncialis
	umbellulata
	vavilovii
Triticum	*araraticum*
	baeoticum
	dicoccoides
	urartu

2.2. Methods

A total of 71 ecological variables were generated for 391 collection sites using Almanac Characterization Tool (ACT) and Arc View 3.1 software. The ACT was developed by the Characterization, Assessment and Application Group at the Blackland Research and Extension Center, Temple, Texas, U.S.A with ICARDA's Natural Resource Management Program (NRMP/ICARDA) support. The ecological data included: 60 monthly variables - precipitation in mm (PRE), potential evapotranspiration in mm (PET), precipitation/potential evapotranspiration ratio (PPE), maximum temperature in °C (MAXT) and minimum temperature in °C (MINT), seven annual variables - rainfall in mm (RAINFALL), annual precipitation/potential evapotranspiration ratio (ANNPPE), mean maximum temperature in °C (MEANMAX–T), mean minimum temperature in °C (MEANMIN–T), absolute maximum temperature in °C (ANNMAXXT), absolute minimum temperature in °C (ANNMINIT) and annual chilling units in °C (ANNCHILLU), and four soil variables - soil texture (TEXTURE), soil layer 1 depth in cm (DEPTH–1), available water capacity in soil layer 1 (AWC–1) and top soil percentage in the soil profile (SOIL–PERC). Site altitude data were obtained from GRU/ICARDA database. Statistical analyses were performed using SPSS 10.1 software. Altitude data and species with less than 10 collection sites *(T. araraticum, Ae. caudata, Ae. cylindrica* and *Ae. juvenalis)* were not included in the multivariate statistical analyses.

3. RESULTS

3.1. Univariate analysis

One-way ANOVA results demonstrated highly significant ($P<0.001$) differences between species for all 71 ecological variables. High differences among the mean values indicated a potential of wheat wild relatives in breeding for specific adaptation to different environments and abiotic stresses. Among the most tolerant species to low annual rainfall were D-genome *Ae. tauschii, Ae. crassa* and *Ae. vavilovii*, together with *Ae. searsii, Ae. kotschyi, T. dicoccoides* and T *urartu*. However, the adaptation to low rainfall sites was in most of these species due to, at least partly, high available water capacity in soil. Wild wheats, *T. urartu* and T *dicoccoides* were most tolerant to low temperature minima in winter, while *Ae. crassa* and *Ae. tauschii* were best adapted to high temperature in summer. The between- and within-species diversity for tolerance and adaptation to different climatic and soil stresses may be particularly valuable in wheat improvement programs for stress-affected rainfed farming systems in semi-arid regions of Central and West Asia and North Africa, as well as in other parts of the world.

3.2. Multivariate analysis

Factor analysis

When all climatic and soil variables were included, the principle component analysis extracted six factors. Climatic variables were associated with the first three factors, which accounted for 84% of the total variance. Factor I involved monthly variables PRE and PPE and was interpreted as water availability, factor 2 was temperature in the winter rainy season, and factor 3 represented temperature and drought stress in the summer hot period. The other three factors were associated with soil variables and contributed together with only 10.8% to the total variance.

Discriminant analysis

A total of 642 populations belonging to 16 species of wheat wild relatives were subjected to the discriminant analysis, which resulted m seven significant canonical discriminant functions. While all monthly precipitation and potential evapotranspiration variables were included in the analysis, only four precipitation/potential evapotranspiration ratio (PPE 01, 02, 07 and 12) and two temperature extreme variables (MAXT 01 and MAXT 02) were involved. Three soil variables (SOIL1_PERC, TEXTURE_1 and AWC_I) were associated with the discriminant functions, but all annual climatic variables, except for one (RAINFALL), were excluded by the analysis. Standardized canonical discriminant coefficients indicated a strong association of the first two canonical discriminant functions, accounting for most of the variation, with some winter month PPE and PRE variables and with PET 07. The third function was strongly linked to winter temperature maxima (MAXT 01 and 02) and soil water capacity (AWC_I). The other four functions of minor discriminating power accounted together for 20% of the total variance.

Hierarchical cluster analysis All species and all environmental variables

Group centroids for the seven significant canonical discriminant functions generated by the discriminant analysis provided input for computing a proximity matrix using Euclidean squared distance as a measure. The subsequent hierarchical cluster analysis based on UPGMA method produced a dendrogram indicating species ecological relationship. The dendrogram showed a high similarity in ecological adaptation of *Ae. umbellulata* with three polyploids sharing its U-genome, Ae. *triuncialis, Ae. biuncialis* and *Ae. geniculata.* This sub-cluster was associated with S-genome *Ae. searsii* and *Ae. speltoides* and another U-genome polyploid sub-cluster of *Ae. columnaris, Ae. neglecta* and *Ae. peregrina* in a larger cluster. Wild *Triticum* species, *T. dicoccoides, T urartu and T. baeoticum* were grouped in a different cluster and D-genome species produced a very distinct loose cluster. *Ae. kotschyi* was not associated with any of the three major clusters.

All species and climatic variables

When the hierarchical cluster analysis was based on a discriminant analysis of climatic data only, then *Ae. columnaris* joined the *Ae. umbellulata* sub-cluster, *T. baeoticum* replaced *Ae. searsii* in the U-genome species cluster and the latter moved to the *Triticum* cluster, closely associated with *T. dicoccoides* and *T. urartu.*

Wheat close relatives and climatic variables

A dendrogram based on a discriminant analysis of climatic data for four wild *Triticum* species and *Ae. speltoides* and *Ae. searsii* demonstrated an ecological proximity of diploid *Ae. speltoides and T. baeoticum* with tetraploid wild wheat T. *araraticum*, as well as a close relationship of the other two diploids, T *urartu* and *Ae. searsii* wild emmer wheat, T. *dicoccoides*. However, only three sites were available for *T. araraticum.*

Discriminant analysis for two species

When ecological data for two species were analyzed, only one canonical discriminant function was generated. Again, high values of the standardized canonical discriminant coefficients indicated which ecological variables discriminated between the two species, For example, PRE 04, 09, 10 and PET 05 separated *Ae. searsii* from *Ae. speltoides*, while PRE 03 and PET 05, 07, 10, and 11 discriminated *Ae. kotschyi* from *Ae. peregrina*.

4. DISCUSSION

As was earlier suggested (1), GIS would be a useful tool with a number of applications in genetic resources management and utilization. However, geo-referencing of genebank accessions is a prerequisite for any GIS applications. Detailed ecological information generated by GIS can be used in different statistical analyses for inter- and intraspecific diversity studies to understand better the species ecology. The species relationship assessed in the present paper from multivariate analyses conforms, in principle, to the genomic concept, except for *Ae. kotschyi* and *Ae. peregrina*. There is also some similarity with results based on composite ecological variables (3). The database of newly generated ecological information may be explored in the identification of germplasm tolerant to abiotic stresses and/or adapted to specific ecologies. We also believe that the detailed ecological information on crop wild relatives and other wild species held in genebanks can stimulate their utilization in research and breeding.

REFERENCES

[1] Greene S.L., Hart T: Plant genetic resources collections: an opportunity for the evolution of global data sets. http: //www.ncgia.ucsb.edu/ SANTA_FE_CD-ROM/sf_papers/greene-stepha.../ sgreene.htm (2001).

[21 van Slageren M.V: Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poacea). Agricultural University, Wageningen, the Netherlands and ICARDA, Aleppo, Syria (1994).

[31 Valkoun J: The geographical distribution of wild wheats in their historical setting and current context. In: Caligari P.D.S, Brandham PE (eds) Wheat taxonomy: the legacy of John Percival, Academic Press, The Linnean Special Issue No 3: 81-95 (2001).

ELYMUS IN AUSTRALASIA.

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ABSTRACT: The genomic constitution of *Elymus* species in Australasia is reportedly SYW, the W genome being derived from the endemic genus *Australopyrum*. The arguments for retaining these species in *Elymus* are examined. Delimitation of the species of *Elymus* in Australia and New Zealand has been debated for over 100 years. Recent studies have included RAPD analysis and the elaboration of further micromorphological characters. The results indicate that there are six endemic species in New Zealand, three endemic species in Australia and one species native to both Australia and New Zealand (*E. multiflorus*). One of the Australian species is now established in New Zealand, but there is some debate as to which species that is. One of the Australian species (*E. scaber*) has two subspecies. There is one octoploid (*E. tenuis*) and one tetraploid (*E. enysii*) species in New Zealand, the latter only doubtfully included in *Elymus*. All the remaining species are hexaploid. The relationships between the New Zealand and Australian species are discussed. The information on breeding systems is still being accumulated, but one taxon (*E. scaber* subsp. *rectisetus*) appears to be mostly apomictic, the other species being mostly sexual. There are conflicting reports as to the level of apomixis present in the remaining species.Some level of apomixis has been suggested for most Australian species, but none for the New Zealand species.

1. INTRODUCTION

For a long time, only one species of *Elymus* was recognised as native to Australia. The morphological diversity (e.g., awn length and curvature) of *Elymus scaber* resulted in the taxon being known as the '*E. scaber* complex' (1) in Australia. For a long time only one taxon, *Agropyron scabrum*, was recognised (2), though subsequently at least three have been referred to (3,4) and occasionally more (5,6).
In New Zealand, four species were recognised in 1954 (7) and now eight are recognised (8), with two (*E. multiflorus, E. rectisetus*) recorded as shared with Australia. The New Zealand studies were based on thorough morphological, biosystematic and ecological studies.

Of the 10 taxa under consideration it seems that only one (*E. scaber* var. *plurinervis*) is treated by all authors as the same entity (1).
The aim of this study was to try and rationalise the previous studies and produce an effective treatment for the genus in Australasia.

2. CHARACTERS

Traditional characters have mostly been numeric, such as awn length, glume length, ratio of lemma length to awn length, and number of glume veins. The reproductive process was used in one case (6).
To avoid repeating previous classifications a range of new characters were used. The characters that were taxonomically useful were (9):

(i) the angle between the callus and the rhachilla, typically an angle of c. 30° but one species with an angle of c. 50°;

(ii) shape of the rhachilla apices, varying from oblate, elliptic, ovate, obovate or circular;
(iii) ndumentum of the rhachilla, length and density of the trichomes, e.g., short, long or short and long trichomes;
(iv) shape of the callus, broadly triangular, triangular, narrowly triangular;
(v) thickening or not of callus base;
(vi) dorsal or abaxial surface of the callus, whether rounded, flat, or sunken;
(vii) callus hairiness, whether glabrous, glabrescent, whether trichomes restricted to the margins or distributed over the surface;
(viii) thickening of the callus margins, whether extending to c. half way or less than that;
(ix) ventral or adaxial surface of the callus, whether raised, flat or sunken;
(x) shape of the palea apex, whether truncate, obtuse or pointed;
(xi) incision of the palea apex, whether entire, retuse or bifid; and
(xii) length of the palea tip beyond the thickened margins.

To these characters were added another set using PCR-based RAPD techniques (10,11) using slightly modified methods (1).

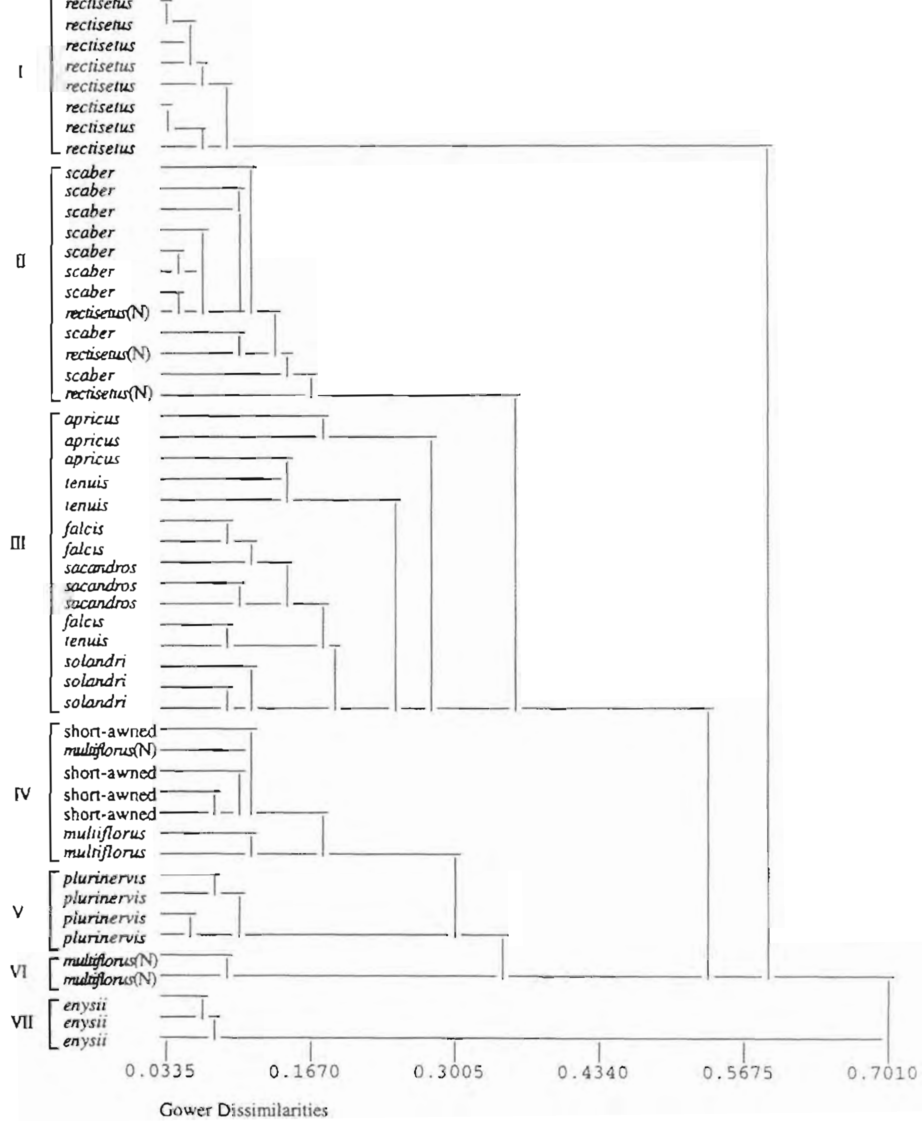

Fig. 1 (1). Dendrogram of morphological data of Australian and New Zealand (N) samples from UPGMA using the Gower Metric. I = *E. rectisetus*; II = *E. scaber* var. *scaber*; III = the New Zealand endemic species excluding *E. enysii*; IV = *E. multiflorus* + sp. B; V =*E.* sp. A; VI = New Zealand *E. multiflorus*; VII = *E. enysii*.

3. RESULTS

The molecular and morphological results were basically in agreement. Both sets suggested that:

(i) the New Zealand *E. enysii* is a distinct species but probably best excluded from *Elymus* (Fig. 1);
(ii) the remaining species are probably best retained in *Elymus*;
(iii) the New Zealand species *E. apricus, E. falcis, E. sacandros, E. tenuis* and *E. solandri* could not be reliably distinguished in this study (Fig. 1);
(iv) the Australian members of the complex could be best organised into four species with two subspecies in one of these (Fig. 2). The classification would be:

Elymus scaber
  subsp. *scaber*
  subsp. A [*E. rectisetus*]
E. sp. A [*E. scaber* var. *plurinervis*]
E. multiflorus
E. sp. B

(v) the New Zealand species referred to as *E. rectisetus* is the same as *E. scaber* subsp. *scaber* (Fig. 1);
(vi) the Australian species known as *E. rectisetus* is best treated as a subspecies of *E. scaber*.
(vii) *E. scaber* var. *plurinervis* is best treated as a distinct species (Figs 1,2);
(viii) a new species described to accommodate a taxon from southern Queensland and northern New South Wales.

4. DISCUSSION

(i) *E. enysii* has been reported as a tetraploid with the H genome but lacking the S genome (12). Morphological characters such as the shorter compact inflorescence and distinctive callus and rhachilla characters support its exclusion from *Elymus*. More information is needed as to where it is best placed.
(ii) The discussion over the best placement of the Australasian species has yet to be completed with options for *Roegneria* (13) and *Elymus* (7,8,14,15) yet to be fully resolved. On balance it seems best to retain the Australasian species in *Elymus* for now. If they were to be separated into a separate genus then the generic name would be *Anthosachne* (1,7).
(iii) While this study failed to reliably distinguish between the New Zealand endemic species *E. apricus, E. falcis, E. sacandros, E. tenuis* and *E. solandri* (Fig. 1), they were not included in the RAPD study, and not all relevant morphological characters were included in the morphological data set. There is no suggestion that these data support synonymising these names.
(iv) The morphological data and the RAPD data (Figs 1,2) corroborated conclusions for the Australian taxa.
(v) The New Zealand samples of *E. rectisetus* grouped with Australian samples of *E. scaber* subsp. *scaber* (Fig. 1). The latter is still very variable and the misidentification presumably arose from the New Zealand material looking different from the Australian material of *E. scaber*.
(vi) Arguments about the level of recognition of taxa can become somewhat arbitrary. The morphological, molecular and distribution data support the recognition of *E. rectisetus* and *E. scaber* as distinct species but breeding system studies (5,6) indicate that these two are probably facultative apomicts. The decision was made to follow an earlier study suggesting that such taxa be treated as subspecies (16).
(vii) *E.* sp. A [*E. scaber* var. *plurinervis*] has several distinct morphological characters (large glumes with broad membranous margins, upper glumes with numerous veins, glabrous and broadly triangular callus, semi-circular to transverse-circular rhachilla apex, awn entered by one vein, high ratio of palea: fruit length). These are corroborated by 13 RAPD markers, and its distinct habitat that is overlapped by the geographic range of *E. scaber*.
(viii) *E.* sp. B is morphologically distinct (erect habit, short and straight awns, glabrous and broadly triangular callus), has 11 RAPD markers, is sympatric with *E.* sp. A and readily distinguished from it and, like *E.* sp. A, has a distinct habitat that is overlapped by the geographic range of *E. scaber*.

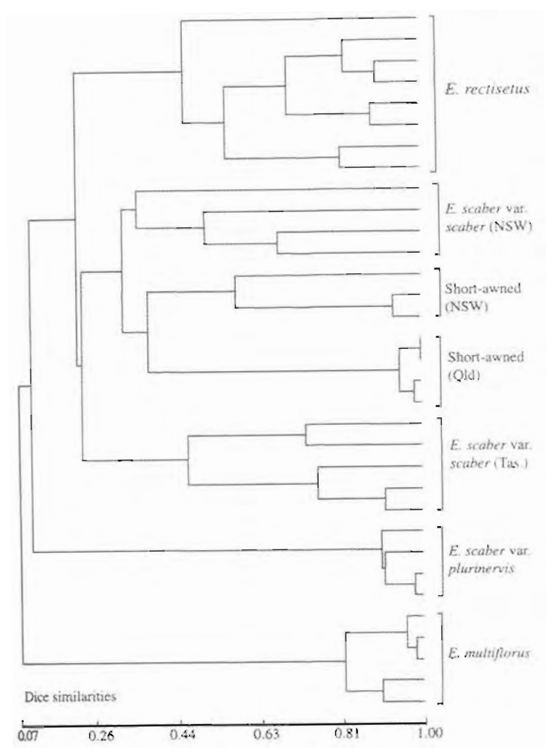

Fig. 2 (1). Dendrogram for Australian members of the *Elymus scaber* complex based on RAPD characters. Dendrogram is derived from UPGMA using the Gower Metric. The 'short-awned' samples are *Elymus* sp. B.

5. BREEDING SYSTEMS

E. scaber has been of interest to plant breeders because it was the only species of the Triticeae that was reported to be apomictic (18), although many are reported as self-pollinating (15,19,20). The reality appears to be somewhat different. Studies of species in New Zealand (7,21,22,23,24) confirm that all native species there are self-compatible and chasmogamous but cleistogamy may occur occasionally (8). *E. scaber*, reportedly introduced from Australia, is facultatively apomictic (5,8) and studies in Australia (5,6) support this finding. *E.* sp. A, *E.* sp. B and *E. multiflorus* appear to be mainly sexual (1).

As several studies have used different circumscriptions of the taxa, it will be necessary to track down voucher specimens before we can be confident about the distribution of apomixis in Australian *Elymus*.

6. KEY TO SPECIES OF ELYMUS (1,8,17) IN AUSTRALASIA

1 Plants with long, white vigorous rhizomes; lemmas acute but awnless. ***E. repens**
1* Plants caespitose; at least some lemmas usually awned. **2**
2 Inflorescence short and compact, of few-flowered shining spikelets; NZ. **E. enysii**
2* Inflorescence elongated; spikelets usually several-flowered, dull. **3**
3 Culms and inflorescences trailing along ground, prostrate or ascending; uppermost internodes greatly elongating after anthesis or not. **4**
3* Culms and inflorescences erect or drooping; uppermost internode not elongating after anthesis. **5**
4 Culms and inflorescences trailing along ground, uppermost internodes greatly elongating after anthesis; leaf blades long, linear; NZ. **E. tenuis**
4* Culms and inflorescences prostrate to ascending; leaf-blades short, variously falcate, sinuous or straight; NZ. **E. falcis**
5 Spikelets widely diverging from axis at maturity; NZ. **E apricus**
5* Spikelets erect or slightly diverging; awns may curve away from spikelet. **6**
6 Leaf-blades densely hairy at the base with hairs c. 1 mm long, becoming shorter and less dense above; NZ. **E. sacandros**
6* Leaf-blades variously hairy or glabrous, but without an area of dense hairs c. 1 mm long at the base. **7**

7 Palea apex bifid. **8**
7* Palea apex entire or retuse. **9**
8 Leaf-blades glaucous; thickened palea margins extending to apex; NZ. **E. solandri**
8* Leaf-blades green; thickened palea margins not extending to apex, leaving a distinct tip; NZ, Aust. **E. scaber**

9 Rhachilla hairs usually 0.2-1 mm long; callus usually triangular to narrowly triangular, usually distinctly hairy; NZ, Aust **E. scaber**
9* Rhachilla hairs <0.2 mm long; callus broadly triangular, glabrous to glabrescent. **10**
10 Glumes 7.7 mm or longer; lemma awn regularly curved when dry, 1-nerved at base; mature caryopsis <55% palea length; Aust. **E. sp A**
10* Glumes usually <7 mm long; lemma awn straight when dry, 3-nerved at base; mature caryopsis >60% palea length. **11**
11 Awn of lowest lemma 2-7.5 mm long; other lemma awns 4.4-15 mm long; awn: lemma length 0.7-1.3 ; dark brown basaltic soils of SE Qld and N NSW; Aust. **E. sp B**
11* Awn of lowest lemma 0-3 mm long; other lemma awns 0-11 mm long; awn: lemma length 0.2-0.8; coastal or riparian habitats; NZ, Aust. **E. multiflorus**

REFERENCES

[1] Wang S: Taxonomy of the *Elymus scaber* complex (Triticeae: Poaceae). PhD thesis, Biology Department, University of Sydney (1999)
[2] Vickery JW :Contributions to the taxonomy of Australian grasses. Contributions from the New South Wales National Herbarium 1: 322-343 (1951).
[3] Carman JG, Wang RR-C: Apomixis in the Triticeae. Apomixis Workshop, Atlanta, Georgia, USA, USDA-ARS, ARS-104 (1992).
[4] Jacobs SWL, Hastings SM: *Elymus* In: Harden GJ (ed.) Flora of New South Wales. New South Wales University Press (1993).
[5] Crane CF, Carman JG: Mechanisms of apomixis in *Elymus rectisetus* from eastern Australia and New Zealand. American Journal of Botany 74: 477-496 (1987).
[6] Murphy MA, Jones CE: Observations on the genus *Elymus* (Poaceae: Triticeae) in Australia. Australian Systematic Botany 12: 593-604 (1999).
[7] Connor HE: Studies in New Zealand *Agropyron*. New Zealand Journal of Science and Technology 35B: 315-343 (1954).
[8] Edgar E, Connor HE: Flora of New Zealand volume V. Manaaki Whenua Press (2000).
[9] Wang S, Henwood MJ: The taxonomic utility of micromorphological characters in Australian and New Zealand *Elymus* species (Poaceae) (1999).
[10] Welsh J, McClelland M: Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213-7218 (1990).
[11] Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535 (1990).
[12] Svitashev S, Salomon B, Bryngelsson T, Von Bothmer R: A study of 28 *Elymus* species using repetitive DNA sequences. Genome 39: 1093-1101 (1996).
[13] Baum BR, Yen C, Yang JL: *Roegneria*: its generic limits and justification for its recognition. Canadian Journal of Botany 69: 282-294. (1991)
[14] Löve A: Conspectus of the Triticeae. Feddes Repertorium 95: 425-521 (1984).
[15] Lu, BR: Biosystematic investigation of Asiatic wheatgrasses - *Elymus* L. (Triticeae: Poaceae). Ph.D. Dissertation Thesis, The Swedish University of Agricultural Sciences, Svalov (1993).
[16] Löve A: Biosystematics and classification of apomicts. Feddes Repertorium 63: 136-149 (1960).
[17] Wheeler DJB, Jacobs SWL, Whalley RDB: Grasses of New South Wales, 3rd edition. University of New England Press (in press).
[18] Hair JB: Subsexual reproduction in *Agropyron*. Heredity 10: 129-160 (1956).

[19] Löve A, Connor HE: Relationships and taxonomy of New Zealand wheatgrasses. New Zealand Journal of Botany 20: 169-186 (1982).
[20] Dewey DR: The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In Gustafson JP (ed.) Gene manipulation in plant improvement. Plenum Publishing (1984).
[21] Connor HE: Interspecific hybrids in New Zealand *Agropyron*. Evolution 10: 415-420 (1956).
[22] Connor HE: Breeding systems in some New Zealand grasses. New Zealand Journal of Science and Technology 38A: 742-751 (1957).
[23] Connor HE: Studies in New Zealand *Agropyron* Part 3. Intraspecific hybrids in *A. scabrum* (R. Br.) Beauv. New Zealand Journal of Science 5: 99-115 (1962).

THE *TRITICEAE* IN THE AMERICAS

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ABSTRACT: The *Triticeae* includes 300-350 species, of which 50 are native to North America and 34 to South America. No species is native to both North and South America. We recently became interested in elucidating the relationship between species on the two continents, based on morphological and anatomical information. This paper summarizes our preliminary work. We present a synopsis of the distribution of the genera and two character lists for further work, one of morphological characters and one of anatomical characters. Two sets of anatomical characters are presented, one for leaf blade cross-sections and one for glume cross sections. Subulate to narrowly linear glumes are found in all three of the most speciose American genera (*Elymus*, *Hordeum*, and *Leymus*), their anatomical structure differs substantially. Leaf anatomy appears to show less variation, but it cannot be well characterized on herbarium specimens. Informal assessment of the data obtained to date suggest that most North American species are more closely related to Asian taxa than to those in South America, a pattern similar to that which has been reported for *Hordeum*. We are unable to suggest the most probable origin of the South American taxa at this time.

1.GOALS

The *Triticeae* includes 300-350 species. Most are native to temperate regions of the northern hemisphere, but about 46 are native to the southern hemisphere (1, 2), the exact number depending on the taxonomic treatment adopted. We recently became interested in the biogeographic relationships between the North and South American members of the *Triticeae*. The goals of the work reported here were very limited:

- Summarize existing information on the distribution of the species;
- Develop morphological and anatomical character lists for assessing relationships among the American *Triticeae*; and
- Develop specific goals for future collaborative work.

Many American *Triticeae* are allopolyploids; even those with the same genomes may have different diploid progenitors (3,4). This complicates the determination of systematic relationships among the species, a necessary precursor to evaluating biogeographic relationships. In addition, there are significant questions concerning the generic and specific taxonomy on both continents (5,6). Moreover, the American *Triticeae* should not be studied in isolation from the species of other continents (4,7). We recognize these problems, but see an immediate need to obtain comparable morphological and anatomical data for American *Triticeae*.

2. MATERIALS AND METHODS

Distributional data were obtained from files developed for the *Manual of Grasses for North America* (Barkworth et al., in prep) and various published sources (6,8,9,10,11,12). Maps were developed using ArcView (13).

A character list was developed from consideration of existing treatments supplemented by our experience. A preliminary assessment of the efficacy of this list was made by scoring representative specimens of *Triticeae* obtained from UTC and US (14); *Eremium erianthus* was scored using a specimen from BA (for a complete listing, see appendix), descriptions developed for the *Manual of Grasses for North America* (Barkworth et al., in prep.), and (6,12,15).

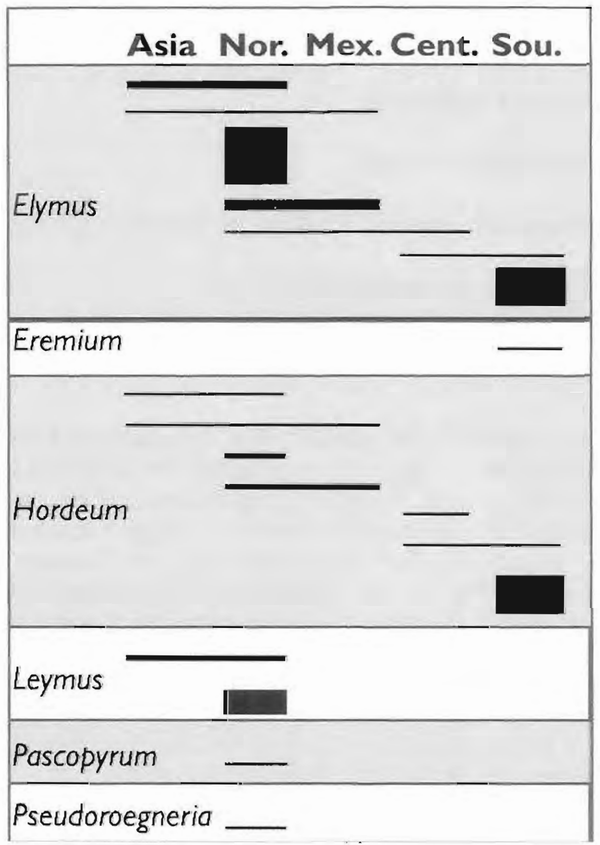

Figure 1. Distribution of *Triticeae* genera in the Americas. Bars are proportional the number of species. Nor.: North America; Mex.: Mexico; Cent.: Central America; Sou.: South America. *Eremium* has only 1 species

3. RESULTS

3.1. Distribution and cytogenetic information

North America (including Mexico) has 50 native species of *Triticeae* whereas South America has only 34. One species is native to Central America. No species are native to both North and South America (Fig. 1). The higher representation of *Triticeae* in North rather than South America is to be expected, given the larger temperate land area (approximately 1.7 times that of South America) and proximity to Asia of North America. Indeed, given these factors, the *Triticeae* might be considered relatively poorly represented in North America.

The *Triticeae* floras of the two continents are completely distinct at both the specific and the generic level. One South American species, *Elymus cordilleranus*, extends to Costa Rica, Central America, and one North American species, *E. arizonicus*, extends from the southern United States to Guatemala, Central America.

Hordeum jubatum and *Elymus trachycaulus* are the most widespread species North American species, extending from Asia through North America to northern Mexico. In South America, *Elymus angulatus*, *E. cordilleranus* and *Hordeum stenostachys* and *H. parodii* appear to be the most widespread.

The most speciose genus in North America is *Elymus* but in South America it is *Hordeum*. North America has two genera, *Pseudoroegneria* and *Pascopyrum*, that are not present in South America; South America may have one endemic genus, *Eremium*, but Dubcovsky et al. (4) provided evidence it would be more appropriately placed in *Leymus*.

Pseudoroegneria is a homogenomic genus that is most speciose in Asia. Jensen et al. (16) suggested that the North America species, *P. spicata*, is conspecific with *P. strigosa*, which would make this another species that is native to Asia and North America. *Pascopyrum* is a monotypic North American genus that is derived from an *Elymus-Leymus* hybrid.

North American species of *Elymus* are almost all tetraploid, the exception being the octoploid *E. californicus*, which some evidence suggests should be transferred to *Leymus* (5). Similarly, most of the South American species are tetraploid but *E.angulatus* and *patagonicus* are hexaploid and *E. mendocinus*, which Dubcovsky et al. (4) transferred to *Leymus*, is octoploid.

Leymus is represented in North America by 11 or 12 native species, depending on whether *Elymus californicus* is included in the genus. Most species are tetraploid, but *L. ambiguus*, *L. cinereus*, *L condensatus*, *L. salinus* subsp. *salinus*, and *L. innovatus* are also octoploid. *Elymus californicus* is known only as an octoploid. Both the two South American species that Dubcovsky et al. (4) transferred to *Leymus*, *Eremium erianthum* and *Elymus mendocinus*, are hexaploid. Polyploids are more common in American species of *Hordeum*, with five species being wholly or almost wholly polyploid on each continent (11). Because the evolution and biogeography of *Hordeum* has been well covered by others (e.g., 7,11, Figure 2), we shall not discuss it further

3.2. Morphological characters

The morphological character list that we developed, is presented in the next paragraph. It is designed for phenetic, not phylogenetic, analyses because the taxa involved appear to be recent polyploids.

Morphological characters to be scored: 1. Longevity; 2. Growth habit; 3. Culm posture; 4. Lower cauline node pubescence; 5. Basal leaf sheath pubescence; 6. Auricle length; 7. Ligule length; 8. Ligule shape; 9. Rib height compared to intercostal width; 10. Primary and secondary veins evident; 11. Blade adaxial pubescence; 12. Spike posture; 13. Rachis fragility; 14. Rachis edge indumentum; 15. Rachis surface indumentum; 16. Spikelets per node; 17. Pedicel length; 18. Spikelet length; 19. Glume basal fusion; 20. Longest glume length; 21. Shortest glume length; 22. Glume shape; 23. Glume body length; 24. Glume awn length; 25. Number of glume veins; 26. Glume flexibility; 27. Glume margins texture; 28. Lowest lemma body length; 29. Lowest lemma awn length; 30. Lowest lemma awn angle; 31. Lowest lemma pubescence; 32. Lowest lemma pubescence distribution; 33. Palea tip; 34. Anther length.

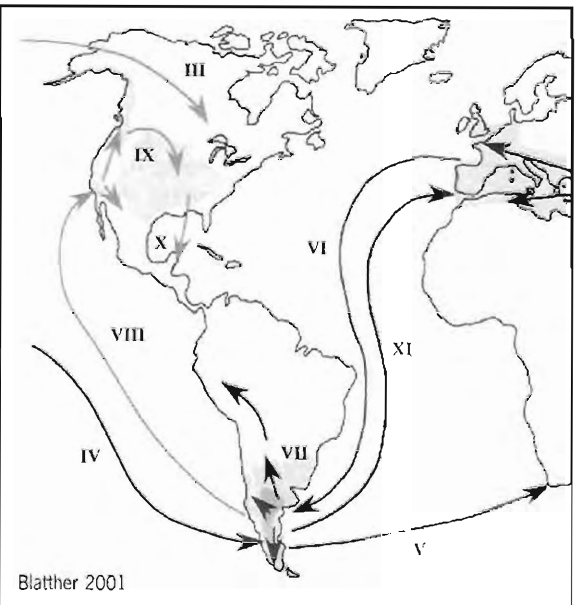

Figure 2. Origins of American *Hordeum*, from Blattner (7).

Most characters will be scored as multistate characters. Length measurements will be recorded as continuous characters but they may be divided into categories to evaluate, for instance, the ecological distribution of species with long, divergent awns.

Our preliminary investigation of morphological variation in several species convinced us that the many blade and glume characters needed to be examined using cross-sections of fresh material explicitly preserved for this purpose if the structural basis of morphologically similar species was to be accounted for appropriately. For intance, determing the pattern of primary and secondary blades within the leaves is very diffcult on herbarium specimens, particularly if the leaves are tighly convolute. Similarly, the tough, smooth surface of the glumes in some species made the confident determination of the number of veins, for instance, impossible. This led us to develop the following lists of anatomical characters.

3.3. Anatomical characters

These lists were constructed after examining sections prepared of from herbarium specimens of 10 species representing almost all the genera (not *Eremium*), but not all the infrageneric taxa, recognised on the two continents.

Blade characters: Intercostal characters are measured between the first two primary veins on one side of the midvein. Rib characters are measured on the primary vein adjacent to the midrib.

1. Blade width; 2. Number of primary vascular bundles; 3. Number of secondary vascular bundles; 4. Intercostal distance; 5. Thickness at mid-intercostal distance; 6. Thickness at first primary vein; 7. Thickness at secondary vein; 8. Rib shape; 9. Number of different adaxial pubescence types; 10. Adaxial pubescence type 1 density; 11. Adaxial pubescence type 2 density; 12. Hypodermis continuity; 13. Abaxial epidermis cell shape; 14. Nodular crystal abundance in abaxial epidermis; 15. Nodular crystal abundance in adaxial epidermis; 16. Blade curvature.

Glume characters: We shall score the first (lowest) glume of the spikelets. For *Hordeum*, we shall measure the lower glume of the functional floret. Each glume will be measured at the base and at midlength of the glume body.

1. Glume width; 2. Width of side 1; 3. Width of side 2; 3. Thickness on center line; 4. Thickness halfway between midvein and edge of widest side; 5. Number of veins; 6. Intercostal thickness between first primary vein and adjacent lateral vein; 8. Number of parenchyma layers between chlorenchyma and adaxial epidermis; 9. Type of adaxial parenchyma cells; 10. Number of parenchyma layers between chlorenchyma and abaxial epidermis; 11. Type of abaxial parenchyma cells; 12. Shape of margin; 13. Width of

sclerified portion; 14. Abaxial trichome type; 15. Abaxial trichome abundance. 16. Abundance of sclerified epidermal cells; 17. Abundance of epidermal cells with rhomboidal crystals.

Glume cross sections in the *Triticeae* have only been examined in *Thinopyrum* (17). For that reason we report our preliminary observations here even though they are based on only one specimen per species. *Pseudoroegneria spicata* glumes have a relatively of thin walled parenchyma adaxial, a definite clorenchyma layer, and non-contiguous sclerenchyma strips abaxial to the vascular bundles. By contrast, *Hordeum branchyantherum* had a single, centrally located vascular bundle.

In *Elymus trachycaulus*, there is a well developed adaxial parenchyma layer and an unbroken abaxial sclerenchyma layer below the chlorenchyma. *Elymus viriginicus* and *E. riparius*, by contrast, have a much more stongly developed adaxial parenchyma layer, a poorly developed clorenchyma layer, and the abaxial sclerenchyma is confined to strips associated with the vascular bundles. *Elymus elymoides* subsp. *brevifolius* has basic pattern similar to that of *E. trachycaulus* but the abaxial sclerenchyma associated with the midvein is much more strongly developed than that associated with the other ribs and a higher proportion of the parenchyma in other portions of the glume is more strongly sclerified. The cross section of *Elymus scabrus*, an Australian species, is much more leaf-like than that of any of the American species examined.

Glumes of *Leymus mollis* seem simlar to those of *E. scabrus*. *Leymus condensatus* and *L. innovatus* have somewhat similar glumes but with narrow, more heavily sclerified sides.

Hordeum, *Elymus elymoides*, and the inland species of *Leymus* are often described as if they have similar glumes of the three taxa in similar terms. Even close morphological examination reveals that this does not accurately reflect the variation that exists. Use of anatomical characters permits a more accurate assessment. Determining which morphologies are homologous requires developmental studies, but identification of the anatomical patterns underlying similar morphlogies is an important first step.

4. DISCUSSION

4.1. General comments

The evolutionary and biogeographic history of *Hordeum* is much better understood than that of other members of the American *Triticeae*, largely because the whole genus has been a major focus of interest for several years (18), stimulated in part by the importance of *Hordeum* to human welfare. The genus is of interest to our work primarily as the source of one of the genomes represented in *Elymus*. Two other genomes are present in the native American *Triticeae*, the **St** genome of *Pseudoroegneria* and the **N** genome of *Psathyrostachys*.

Genomes provide minimal phylogenetic information, but their ability to suggest useful evolutionary species groupings within the Triticeae is well supported. Elucidation of the systematic and biogeographic relationships between the non-*Hordeum* Triticeae of the Americas is hampered by the allopolyploid origin of most taxa but also by a lack of comparable data.

The basic premise underlying morphological studies is that comparable morphologies reflect homologous development. Our preliminary data indicate that this is not true for glume morphology in the *Triticeae*. It is not clear to what extent it is true for similar leaf blade morphologies.

The third goal of this preliminary study was to develop specific goals and testable hypotheses for future collaboration on the American *Triticeae*. These are listed below.

4.2. Future goals

- Collect 5 fresh specimens of each native taxon of American *Triticeae* (fewer of those that are rare or endangered), each specimen to consist of a herbarium specimen, blade and spikelet material preserved in alcohol for anatomical studies, and young growing tissue presevED in CTAB nucleic acid for study by others.
- Characterize each specimen in terms of its morphology and anatomy, using the character lists developed through this preliminary study.
- Conduct numerical analyses of the data.
- Analyze geographic patterns of the genomic and phenetic groups within the American *Triticeae*.
- Develop collaborations with individuals elsewhere that will enable us to extend this research to other portions of the *Triticeae* and other sources of information.

4.3. Some testable hypotheses

- Taxa within a phenetic group will belong to the same genomic group.
- Species of a genus on the same continent will exhibit greater phenetic similarity than species of the same group on different continents.

- Each of *E. californicus*, *Eremium erianthum*, and *Elymus mendocinus* will be closer, phenetically speaking, to North American species of *Leymus* than other American species of the *Triticeae*.
- The geographic distribution of phenetic groups will be similar.
- There will be a significant correlation between the geographic distribution of characters such as fragile rachises and climatic factors such as rainfall.

7. REFERENCES

[1] Gibbs Russell GE, Watson L., Koekemoer M, Smook L, Barker NP, Anderson HM, Dallwitz: Grasses of southern Africa. Memoirs of the Botanical Survey of South Africa No. 58 (1990).

[2] Wang S, Jacobs SWL: *Elymus* in Australasia. Pp. In: Martin A .... Proceedings of the Fourth International Triticeae Symposium.

[3] Svitashev S, Salomon B, Bryngelsson, von Bothmer R von: A study of 28 *Elymus* species using repetitive DNA sequences. Genome 39: 1093-1101 (1996).

[4] Dubcovsky J, Schlatter AR, Echaide M: Genome analysis of South American *Elymus* (Triticeae) and *Leymus* (Triticeae) species based on variation in repeated nucleotide sequences. Genome 40:505-520 (1997).

[5] Jensen KB, Wang R-C: Cytological and molecular evidence for transferring *Elymus coreanus* from the genus *Elymus* to *Leymus* and molecular evidence for *Elymus californicus* (Poaceae: Triticeae). International Journal of Plant Science 158:872-877 (1997).

[6] Seberg O, Petersen G: A taxonomic revision of the genus *Elymus* L. *s.l.* (Poaceae, Triticeae) in South America. Bot. Jahrb. Syst. 120:503-543 (1998).

[7] Blattner FR: Tracing alloploid speciation in *Hordeum*. Poster presented to the annual meeting of the American Society of Plant Taxonomy (2001).

[8] COTECOCA: Las Gramíneas de México., vol. 1. Secretario de Agricultura y Recursos Hidraulicos Directorio, México (1983).

[9] COTECOCA: Las Gramíneas de México., vol. 3. Secretario de Agricultura y Recursos Hidraulicos Directorio, México (1991).

[10] Davidse G, Pohl RW: Poaceae. Pp. 184-401 IN: Davidse G, Sousa SM, Chater AO: Flora MesoAmericana. Universidad Nacional Autónoma de México, México DF, México (1994).

[11] Bothmer R von, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I: An ecogeographic study of the genus *Hordeum*. Ed. 2. Systematic and ecogeographical studies on crop genepools. 7. International Plant Genetic Resources Institute (1995).

[12] Seberg O, Linde-Laursen I: *Eremium*, a new genus of the Triticeae (Poaceae) from Argentina. Syst. Bot. 21:3-15 (1996).

[13] ESRI: ArcView GIS 3.2. Earth Systems Research Institute, Redlands, California (1999).

[14] Holmgren OK, Holgren NH, Barnett LC: Index herbariorum: Part 1: the herbaria of the world. New Yok Botanical Garden, Bronx, New York (1990).

[15] Nicora EG: Flora Patagonica, vol. 3, Poaceae. Coleccion Cientifica del INTA, Buenos Aireas, Argentina (1978).

[16] Jensen KB, Curto M, Asay KH: Cytogenetics of Eurasian bluebunch wheatgrass and their relationship to North American bluebunch and thickspike wheatgrasses. Crop Sci. 35:1157-1162 (1995).

[17] Jarvie JK, Barkworth ME: Anatomical variation in some perennial Triticeae. Botanical Journal of Linnean Society, 108: 287-301 (1992).

[18] Seberg O: Prospects and need for genebanking in the Triticeae (Poaceae). Opera Botanica 113:39-42 (1992).

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9. SPECIMENS EXAMINED

Elymus albicans UTC 228139; *E. arizonicus* UTC 205786; *E. bakeri* US 368727; *E. borealis* US 101961; *E. calderi* UTC 157322; *E. californicus* UTC 107944; *E. canadensis* UTC 93705; *E. elymoides* subsp. *Brevifolius* UTC 205790; *E. glaucus* UTC 213394; *E. hystrix* UTC 228796; *E. interruptus* UTC 200592; *E. lanceolatus* UTC 105767; *E. alaskanus* subsp. *latiglumis* US 556692; *E. macrourus* US 725185; *E. multisetus* UTC 205829, 73346; *E. pringlei* US 316873; *E. riparius* UTC 215855; *E. scribneri* UTC 228137; *E. sibiricus* UTC 228857; *E. sierrae* US 556654; *E. stebbinsii* UC 774196; *E. trachycaulus* subsp. *trachycaulus* UTC 173981; *E. villosus* UTC 106261; *E. virginicus* UTC 19389; *E. wawawaiensis* UTC 58351; *Eremium erianthus* BA 55734; *Hordeum brachyantherum* UTC 212086; *H. depressum*UTC 212086; *H. jubatum* UTC 228851; *H. pusillum* UTC 23869; *Leymus cinereus* UTC 227929; *L. Condensatus* UTC 230528; *L. flavescens* UTC 138792; *L. innovatus* UTC 223523; *L. Mollis* UTC 179558; *L. Salinus* UTC 36245; *L. Simplex* UTC 171733; *L. Triticoides* UTC 113904; *Pascopyrum smithii* UTC 166338; *Pseudoroegernia spicata* UTC 183278.

BIODIVERSITY: A STORY OF SUCCESS BASED ON ITS CREATION AND UTILIZATION FOR STRESS TOLERANCE IN WHEAT

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ABSTRACT Agricultural biodiversity can produce wonders because it i) adds value to crop, ii) induces resistance, iii) contributes enormously to human foodstuff, iv) removes fear of generic uniformity and vulnerability and v) is responsible for the food security of the world. It is for the later, that NIAB initiated a program on collection, evaluation, and utilisation of agro-biodiversity related with wheat and wheat wild relatives. The program was mainly focused on addition of value to commercial cultivars in the form of stress tolerance transferred from wild species. The objectives were i) to have a source of stress tolerant germplasm continuously available, ii) to facilitate availability of such germplasm for environment friendly, profitable and sustainable agriculture on stressed lands and iii) to ensure safety of agro-biodiversity (through gene conservation) for the stability of future agriculture. During 1998-2001, we conducted field trials on wheat lines developed by using biodiversity residing in the *Aegilops* species. These trials were conducted in the area that requires stress tolerant material to be planted in the fields where cotton was already growing up to the stage of second picking. The inputs included only half the amount of recommended dose of fertilizer, approximately half of the normal irrigation, no herbicide and two applications of compost. Two of the lines tested in these trials out-yielded all existing wheat cultivars traditionally grown in this area and convinced the farmers that bio-diversity does play a role in adding value to the existing material making it suitable for specific requirement. This paper describes a success story based on significance of the material for the area, practical achievements, acceptance by the farmers and economic feasibility of the stress tolerant material developed at NIAB.

INTRODUCTION

Pakistan is blessed with a wealth of genetic diversity in wild and cultivated plants [1]. It is thus imperative that the diversity native to this country is collected, evaluated and utilized in crop improvement program in order to strengthen the basis for sustainable agricultural production especially on saline stressed lands. However, working with salinity is a difficult task and requires multidisciplinary approaches depending upon i) the gravity of the situation, ii) priorities of the agriculture sector, iii) resources of the country and iv) social acceptance of the approach in terms of economic benefits. In Pakistan, we have about 6.8 million hectares of salt affected land [2] and country's first priority in agriculture sector is to achieve wheat production target of 26.433 million tones by 2010 through a growth rate of 2.93% per annum [3]. We have 4. 1 million small farmers possessing 5-10 hectares of land that is being degraded at a rapid pace due to salinity and drought. Presently, water availability is 30% less than that in 1999-2000 and is further diminishing. The farmers want to see their lands productive with minimum possible inputs but without sacrificing food and social security of their families.

Anticipating the consequences of the prevailing situation, NIAB initiated a multidisciplinary research program in 1985 [4] through which genetic diversity for salt tolerance was to be identified and/or evolved for its possible integration into existing breeding system. The objective was to identify, evaluate or tailor low input varieties of wheat for cultivation on saline/stressed land in order to provide a source of germplasm that would remain continuously available to cope with any stress situation as and when required.

MATERIAL AND METHODS

Collection of germplasm:

NIAB has identified a range of biodiversity native to saline lands that included wild plants, land races, grasses [5] and wild relatives of wheat [6, 7]. Among these, *Aegilops* species were preferred for use in wheat improvement program because *Aegilops cylindrica, Ae. tauschii* and *Ae. geniculata* is a part of the biodiversity available in Pakistan especially in the province of Baluchistan. The species possess considerable tolerance to salinity and drought [8]. The most promising among these were accessions of *Ae. cylindrica* and *Ae. geniculata* [5]. *Ae. cylindrica* is especially characterized by profuse tillering, a character that can be exploited for crop improvement [9]. It was anticipated that material generated using *Aegilops* species would have wider adaptability and better performance on saline soils compared to the existing and available salt tolerant germplasm [10] and the same is recorded at Bangor, UK at website http://www.bangor.ac.uk/zs809/ salinity/Resources/Germplasm/htm]

Development and testing of germplasm: a brief account

The germplasm was initially screened for salinity tolerance [6, 7]. Salt tolerance was transferred to hexaploid wheat cultivars LU-26 and Pak-81 [10]. Field screening was conducted on natural and artificially salinized fields [11]. Performance of the material under stress and normal situation was tested in collaboration with private sector and Cotton Research Station, Multan [9]. Trials under saline conditions were conducted during 2000-2001 at Postgraduate Agriculture Research Station (PARS) belonging to the University of Agriculture, Faisalabad. Wheat line 1076 and Inqlab were used as test material with or without hormone treatment [12]. Experiment was conducted in randomized complete block design using three replications. Electrical conductivity of the soil ranged between 10-14 dS/m. Only two irrigations could be applied due to shortage of water and thus the crop was under drought stress through most of the growth period.

RESULTS AND DISCUSSION

Results of trials conducted for three consecutive years indicated that stress tolerant wheat line 1076 produced continuously and comparatively higher grain yield on stressed field compared to the normal wheat field (Table 1). Performance of wheat line 41 was also better than wheat cultivar Inqlab. During 2000-2001, wheat line 41 produced a grain yield of 4400 kg ha^{-1} that is 36 % and 25% higher than Inqlab and wheat line 1076, respectively, on stressed wheat fields. The higher yield of wheat line 41 was due to the highest number of tillers ever observed in any commercial wheat cultivar grown on traditional wheat growing area (unpublished data). This character has been transferred from *Ae. cylindrica* and is expressed in one of the lines (41) selected from its cross with wheat cultivar Pak-81 [10]. Stress tolerance in wheat line 41 has also been attributed to higher number of primary roots and greater root length compared to Inqlab [12]. A further increase in stress tolerance was also envisaged by soaking the seeds in hormone solution of different concentrations, which increased the root biomass of wheat line 41 especially under saline conditions, which ultimately increased the number of grains per spike.

Table 1. Grain yield (kg ha^{-1}) obtained for three wheat cultivars growing under normal and stress conditions for three consecutive years

Growth	Grain yield during the year			
Conditions	98-99	99-00	00-01	Mean
		1076		
Normal	2580	3536	3784	3300
Stress	2580	3185	3300	3021
		41		
Normal	2224	3186	3018	2809
Stress	2510	3020	4400	3110
		Inqlab		
Normal	2427	1830	1552	1936
Stress	2893	2912	2816	2873
LSD	227	245	265	191

Also transferred from *Ae. cylindrica* is the character for high K^+/Na^+ ratio (Table 2). It is evident from the results that both wheat lines 1076 and 41 possess the ability to maintain high K^+/Na^+ ratio under stress conditions, as there was no considerable difference between the two grown on stressed and normal field. Wheat cultivar Inqlab had comparatively low K^+/Na^+ ratio and there was significant difference between the ratios exhibited under stressed and normal conditions. Since K^+ plays an important role in osmotic adjustment [13] therefore, tolerance for stress in 1076, and 41 was comparatively high.

Table 2. Contents of Na^+ and K^+ and K^+/Na^+ ratio in three cultivars of wheat grown under stressed (St) and normal (Nor) conditions

	Na^+, mmol/L		K^+, mmol/L		K^+/Na^+ ratio	
	St	Nor	St	Nor	St	Nor
1076	0.63	0.45	6.6	4.7	10.5	10.4
41	0.69	0.50	5.6	4.4	8.5	8.8
Inq	0.78	0.53	5.0	5.6	6.4	10.6
LSD	0.08	0.04	0.3	0.3	0.7	0.8

Results obtained in trials conducted to assess the additional reasons(s) for stress tolerance in wheat line 1076, exhibited significantly higher straw and grain yield (42% and 37% respectively) in wheat line 1076 compared to Inqlab under control conditions [Table 3]. A further two fold increase in grain and straw yield was observed due to seed treatment with hormone that could be attributed to root modifications [12]. Probably, the hormone treatment helped the plants in mitigating the negative effect of salinity [14] thereby enhancing the stress tolerance of wheat line 1076 more than Inqlab.

Table 3. Effect of seed treatment with growth hormone on straw and grain yield of commercial (Inqlab) and stress tolerant (WL-1076) wheat

Seed treatment	Dry matter yield, g/plot			Harvest Index
	Straw	Grain	Total	
		Inqlab		
Nil	102.2a*	22.1a	124.3a	0.18a
Treated	96.4a	26.6b	123.0a	0.22b
WL-1076				
Nil	145.2b	30.3b	175.5b	0.17a
Treated	197.6c	50.6c	248.2c	0.20b
% edge of WL-1076 over Inqilab				
Nil	42.1	37.1	41.2	-5.6
Treated	105.0	90.0	101.9	-5.8

Figures in a column sharing a similar letter are not significantly different from each other at 5% level of probability

The importance of the germplasm in terms of economic feasibility is given in Table 4. In the normal field, approximately Rs. 7,100 are being spent to obtain grain yield that is worth Rs. 27,189 (32% higher) with a net profit of Rs. 20,089 to the farmer. Under stressed situation, where compost fertilizer was applied along with urea, net investment was about 3550 only and a grain yield worth Rs. 29700 was obtained with a net profit of Rs. 26150 which is 23% higher than the profit obtained form normal field. The cultivation of this material is thus economically feasible for resource starved small farmers.

Table 4. Comparison of normal and low input agricultural agricultural practices for input/output relationship of stress tolerant wheat

Input/Output	Agricultural practice	
	Normal	**Low input**
Water	100%	50%
DAP	Rs. 3200 (6 bags)	Rs. 1600 (2 bags)
Urea	Rs. 2700 (6 bags)	Rs. 1350 (3 bags)
Compost	Nil	Rs. 600 (1200 kg)
Herbicide	Rs. 1200	Nil
Total cost	Rs. 7100	Rs. 3550
Grain Yield	3020 kg	3300 kg
Total Income	Rs. 27,180	Rs. 29,700
Net profit[a]	Rs. 20,080	Rs. 26,150

All figures are on per hectare basis; price of wheat grain, Rs. 9 kg^{-1}

[a] low input farming gave 23% higher profit for stress tolerant wheat

CONCLUSION

Wheat is a major staple food of over 140 million people of the country and is being grown on about 8.3 million hectares with an annual production of about 19 million tones [3]. The government has set the target to increase wheat production and area under wheat by 2.9% and 0.79%, respectively, by the year 2010. Production of stress tolerant material and cultivation of stressed/marginal lands can help achieve this target. With this background, wheat material produced through transferring gene(s) from genome contributing species [10] and developed on saline lands would be of many fold significance due to its in-built stress tolerance. Its cultivation in the fields with standing cotton crop will revolutionize wheat production in this area. This is because Pakistan is currently producing one million tones of wheat in the rice growing and rain-fed areas and 0.9 million tones in the cotton areas where yields are low due to delayed planting made in early January after the last picking of cotton. If the material like wheat line 1076 is available for cultivation in the cotton fields during the month of November (normal wheat planting month), an additional 0.3 million tones of wheat can be obtained which may help enormously to achieve the projected targets. Since this material has been developed in the fields with very low osmotic potential, it requires very low moisture for germination and comparatively less number of irrigations than the others. A precious commodity like water can thus be economized. It requires only half the recommended close of fertilizer and is therefore an environment friendly material. If cultivation of this material can save even 50% of fertilizer without compromising yield, it will save about 9 billion rupees being spent on the import of fertilizer [3]. It can therefore, be inferred very safely, that biodiversity is a vital component of environment friendly, sustainable, and dependable agriculture which should be saved, enhanced and created in order to save the future of agriculture.

REFERENCES

[1] Anonymous: Pakistan National Conservation Strategy. The World Conservation Union, Environment and Urban Affairs Division. Government of Pakistan. Islamabad (1991).

[2] Gassemi F, Jakman AJ, and Nix AG: Pakistan. In: Salinization of land and water resources; human causes, extent, management and case studies. University of New South Wales Press Ltd., Sydney. 369-395 (1995).

[3] Anonymous: Agriculture strategies for the first decade of new millennium. Pakistan Agriculture Research Council (PARC), Ministry of Food, Agriculture and Livestock (MINFAL) and Planning and Development Division. Government of Pakistan, Islamabad (2000).

[4] Anonymous: Twenty-five years of NIAB: a fifth 5 years report of scientific activities of NIAB, Faisalabad (1997).

[5] Farooq S: Salt tolerance potential of wild resources for crop improvement. Ph.D. thesis. University of the Punjab, Lahore, Pakistan (1990).

[6] Farooq S, Aslam Z, Niazi MLK, Shah TM: Salt tolerance potential of wild resources of the tribe *Triticeae*-I. Screening of perennial genera. Pak J Sci Ind Res 31: 506-511 (1988).

[7] Farooq S, Niazi MLK, Iqbal N, and Shah TM: Salt tolerance potential of wild resources of the tribe *Triticeae*-II. Screening of species of the genus *Aegilops*. Plant & Soil 119: 255-260 (1989).

[8] Farooq S, Azam F: Drought tolerance in Triticeae: an aspect of work being conducted at NIAB. In: 4th International Triticeae Symposium held in Cordoba, Spain. September 10-12 (2001).

[9] Farooq S: Salt tolerance in *Aegilops* species: a success story from research and production to 'Proc. International Symposium on Prospects of Saline Agriculture in GCC countries, Dubai, UAE, (in press), March 2001.

[10] Farooq S, Iqbal N Asghar M and Shah TM: Intergeneric hybridization for wheat improvement-VI. Production of salt tolerant germplasm through crossing wheat with *Aegilops cylindrica* and its significance in practical agriculture. J Genet and Breed 46: 125-132 (1992).

[11] Farooq S, Asghar M, Iqbal N, Askari E, Arif M, Shah TM: Production and evaluation of salt tolerant wheat germplasm produced through crossing wheat (*Tricticum aestivum* L.) with *Ae. cylindrica*-II. Field evaluation of salt tolerant germplasm. Cereal Res Commun 23: 275-282 (1995).

[12] Gulnaz A, Iqbal J, Farooq S, Azam F: Seed treatment with growth regulators and crop productivity -I. 2,4-D as an inducer of salinity tolerance in wheat (*Triticum aestivum* L.). Plant and Soil 210: 209-217 (1999).

[13] Maathuis FJM, Amtmann A: K^+ nutrition and Na^+ toxicity: The basis of cellular K^+/Na^+ ratios. Annals of Botany 84: 123-133 (1999).

[14] Garcia AB, Engler JA, Lyer S, Gerats T, Montagu MV and Caplan AB: Effect of osmoprotectants upon NaCl stress in rice. Plant Physiol 115: 159-169 (1997).

DISCRIMINATING CHARACTERS OF DIPLOID WHEAT SPECIES

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ABSTRACT: The large collection of diploid wheats of the genebank of IPK Gatersleben, comprising almost 500 accessions, has been investigated with the aim to find new morphological characters for the discrimination of the species. Especially in large collections it is important to have good criteria to distinguish between accessions of *Triticum urartu*, *T. boeoticum* and *T. monococcum*. The newly found discriminating characters are summarised in a table.

1.INTRODUCTION

According to IPGRI databases on germplasm holdings (http://www.ipgri.cgiar.org/), international genebank col-lections maintain more than 710,000 accessions of wheats of which 8,100 belong to diploid *Triticum* spe-cies (Table 1). Comprehension of this diversity is pos-sible only with a detailed systematic investigation of the variability of each species over its entire geo-graphical range, which takes into account as many characters as possible. Thus, the structure of each spe-cies can be revealed, and its taxonomic limits and op-timum circumscription determined.

The diploid species *Triticum boeoticum* Boiss., *T. urartu* Thum. ex Gandil. and *T. monococcum* L. remain imper-fectly studied as to the botanical-geo-graphical principles of their diversity. For this reason there has been no agree-ment upon the taxonomic status of these species.

2.MATERIALS AND METHODS

All diploid wheat accessions from the IPK Genebank col-lection and additional ones from the Vavilov Institute (VIR) were investigated (cf. Table 2).
Since material from Western Asia is insufficiently rep-re-sented in these collec-tions, 120 additional accessions from the United States Department of Agricul-ture col-lection were also included. These accessions were se-lected on the basis of passport data of the Ge-netic Re-sources Information Network (GRIN, http://www.ars-grin.gov/npgs/searchgrin.html) and largely comprise material collected in the 1960's by B. Lennart John-son.

The material was studied using Vav-ilov's botani-cal-differential approach to systematics of cultivated plants and their wild relatives (1). Numerous samples collected recently (2) need to be included in fur-ther studies.

3.RESULTS AND DISCUSSION

This investigation has increased the number of diag-nostic characters available for the diploid wheat spe-cies. Up to the present, wild diploid wheat species have been differen-tiated by the extent of glume keel and lateral teeth devel-opment, anther length, and pu-bes-cence of the leaf blade. This small set of characters has obviously been insuffi-cient, especially for cataloguing large germplasm collec-tions. Examining a larger number of characters has revealed character complexes that are constant for all infraspecific forms throughout the geographical range of each diploid wheat species.

Traits of the leaf (e.g., type of pubescence, ciliation of the blade margin, colour and ciliation of auricles, shape of the leaf tip), stem nodes, spike, spikelets, and glumes can be used as diagnostic characters at various developmental stages. For example, morphological similarity of the char-acters of the first leaf - colour and ciliation of auricles, short or absent pubescence - suggests a common ancestor for all diploid species. In the young vegetative stage, the two wild species al-ready show characters of leaf xero-phyty, i.e., a smaller, more corrugated leaf blade as com-pared to the culti-vated *T. monococcum*. Dorofeev and Grad-chaninova (3) also noted their small epidermal and guard cells. At the booting stage, a further diffe-rentia-tion between the wild and domesticated diploid spe-cies can be made by pubescence traits. *T. boe-oticum* develops long, stiff hairs (1.2-1.6 mm) on the leaf blade, mainly along the veins, in addition to the short velvety pubescence ob-served in both it and *T. urartu*. The leaf of *T. mono-coccum* either has no pubescence, or it is covered with short spi-nules and very sparse short papillae.

Each species retains its own pattern of pubescence up to the development of the flag leaf, which shows a lower density of pubescence. Generally, the three spe-cies can be differentiated as follows:

T. monococcum possesses mesomorphic characters which are more primitive in the grasses. Its uni-que characters in-clude a semi-fragile spike, spikelets bearing one thin awn, rachis internodes glabrous or very slightly pubescent, a weak keel, glumes weakly tuber-culate to completely glab-rous, leaf blades soft, long (cf. Table 3).

T. boeoticum and *T. urartu* are xerophytes of dry foot-hills, which explains the strong sclerification of strengthening elements of the stem and leaves and the rigid structure of the glumes. However, the xerop-hily of spike characters in *T. urartu* is somewhat less dis-tinct as compared to *T. boeoticum*. The softer and longer leaves of *T. urartu*, cov-ered with velvety pu-bescence, are also characteristic of mesophytes. This trait correlates with the position of *T. urartu* in a den-drogram summarising the genetic diversity of 73 dip-loid wheat accessions for 25 microsatellite mark-ers (4, 5). In addition, the microsatellite marker analysis shows that the boundaries between these species at the mo-lecular level are also clearly distinct, i.e., each species forms a separate cluster.

Table 1. Genebank collections of diploid wheat spe-cies. Source: IPGRI database and online data-bases of some institutions. Data for IPK include 184 accessions newly identified in the research reported here.

Genebank	*T. urartu urartu*	*T. mono-coccum*	*T. boeoti-cum*	Total
Saskatoon, Canada	163	284	1180	1627
USDA-NSGC, USA	210	237	882	1329
UCR, USA	203	381	738	1322
MPI, Ger-many	44	193	796	1033
IPK, Germany	85	173	215	473
Bari, Italy	139		318	457
BAZ, Germany	1	196	66	263
VIR, Russia	44	109	93	246
EMBRAPA, Brazil	196	27	223	
Martonvàsàr, Hun-gary	23	189	86	198
Winnipeg, Canada	140		140	
Baghdad, Iraq		90	90	
NGB, Sweden	58	2	60	
21 additional genebanks	*2 (1)*[1]	233 (*19*)[1]	82 (*10*)[1]	317
Total accessions	**914**	**2389**	**4685**	**8088**
Total genebanks	***10***	***29***	***22***	***34***

[1]The number of different genebanks is given in paren-theses

Table 2. Summary of diploid wheat accessions of the IPK Genebank

Country of origin	*T. urartu*	*T. monococcum*	*T. boeoticum*	Total
Albania		40		40
Armenia	3		7	10
Austria		2		2
Azerbaijan		1	4	5
(Balkan)			3	3
Bulgaria		8	1	9
Europe		39	4	43
France		1		1
Georgia		4		4
Germany		5	1	5
Great Britain		1	1	
Greece		7	8	15
Iran	2		9	11
Iraq	1	1	52	54
Israel			1	1
Italy		6		6
Lebanon	40		5	45
Morocco		2		2
Portugal			1	1
Romania		8	1	9
Spain		5		5
Sweden		1		1
Switzerland		2		2
Syria	2	1		1
Turkey	33	30	87	150
USSR (former)	3		6	9
(unknown)	1	6	27	34
Total	**85**	**173**	**215**	**473**

4.CONCLUSIONS

Each diploid wheat species (*Triticum urartu, T. boeoti-cum, T. monococcum*) has its own set of unique char-acters, which provide, along with archaeological data, evidence of their ancient separation. Such natural groups deserve to be named as separate species. Fur-thermore, the application of these species circumscrip-tions in germplasm collections and scientific research such as biodiversity studies will facili-tate better communication among scientists about the ma-terial.

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REFERENCES

[1] Vavilov NI: On the knowledge of the soft wheats. Tr. prikl. bot. sel. 13 (1): 149-257 (1923) (in Rus-sian).

[2] Valkoun J, Waines JG, Konopka J: Current geo-graphical distribution and habitat of wild wheats and barley. In: Damania AB, Valkoun J, Willcox G, Qualset CO (eds) The Origin of Agriculture and Crop Domestication. ICARDA, Aleppo, Syria, pp. 293-299 (1998).

[3] Dorofeev V, Gradchaninova O: Anatomical study of stem and leaf of wheat. Tr. prikl. bot., gen. sel. 44 (1): 57-75 (1971).
[4] Korzun V, Röder M, Ganal M, Hammer K, Filatenko A: Genetic diversity and evolution of the diploid wheats *T. urartu, T. baeoticum* and *T. monococcum* revealed by microsatellite markers. Schr. Genet. Ressour. 8: 244-247 (1998).
[5] Hammer K, Filatenko AA, Korzun V: Microsatel-lite markers - a new tool for distinguishing diploid wheat species. Genet. Resour. Crop Evol. 47: 497-505 (2000).

Table 3. Comparison of morphological characters of diploid wheat species

Organ	Characteristic	*Triticum urartu*	*Triticum monococcum*	*Triticum boeoticum*
Spike	Length (cm)	6-12	6-12	6-12
	Spikelet number per spike	16-29	25-28	16-30
	Rachis fragility	fragile	semi-fragile	fragile
	Rachis internode length (mm)	3.5-4 (5)	2-3	(3) 4-5
	Rachis internode pu-bescence	strongly to very strongly present	absent to very weakly present	strongly to very strongly present
	Awns per spikelet	2	1	1-2
	Awn divergence	spreading	(parallel to spike)	parallel
	Anther length (mm)	3-4	5-6	6-7
Glume	Keel tooth, length (mm)	1.3-3.0	1.0-1.3	1.0-2.5
	Lateral vein tooth length (mm)	0.2-0.5 (1)	0.2-0.5	0.3-1.0 (1.5)
Flag-leaf blade	Length (cm)	(6) 12-18	7-18	5-13
	Width (mm)	6-11	7-14	4-9
	Pubescence, type	velvet-like	small-prickled	velvet-like short hairs and long stiff hairs
	Pubescence, density	intermediate	(absent) very weak	weak, intermediate, strong
	Hairs, length (mm)	0.05-0.1; 0.3	0.03-0.06	(0.01) 0.03-1.0 (1.6)
	Auricle, coloration	light-green	light-green violet	light-green, greenish-violet,
	Edge leaf blade ciliate	absent	absent or long	intermediate and very long
Apical stem node	Shape	cylindrical, slightly convex	spherical	cylindrical to slightly convex
	Length (mm)	1-3	1.5-4.0	2.0-6.0 (8.0)
	Pubescence, density	medium or strong	weak to medium	weak to strong
	Hair, length	uniform very short to uniform medium	uniform very short to uniform short	long upper longer than from below
	Node, coloration	green	green-brown	green-brown, violet
	Ring below the nodes, expression	weakly expressed	weakly or well expressed	expressed
	Ring below the nodes, coloration	absent or light brown	brown	violet, brown
Basal stem node	Shape	slightly convex to cylindrical	spherical	slightly convex to spherical
Basal leaf	Apex shape at tillering stage	acute	obtuse	acute to narrowly acute
	Pubescence, density	strong	absent or weak	intermediate to strong
	Hairs, length (mm)	0.07-0.3	0.03-0.06	0.02-0.8 (1.8)
	Edge leaf blade ciliate	absent	absent	absent, rare, or long over the whole length
Growth habit	At end of tillering	prostrate, semi-prostrate, semi-erect erect	semi erect,	prostrate, semi - prostrate, erect, semi-erect
	At maturity	prostrate, semi-prostrate,	erect	prostrate, semi-prostrate, bent like a knee, semi-erect, erect

WILD TRITICUM IN THE CZECH GENE BANK -GEOGRAPHIC DIVERSITY AND POTENTIAL FOR BREEDING

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ABSTRACT: The wheat collection of the Czech Gene Bank has over 10 684 accessions at present. The oldest materials are from the end of 19th century. Wild wheats were present in the collection already in the fifties and were mainly of Soviet origin. The wild *Triticeae* collection was widely grown in eighties and nineties to the present 1,503 items. Over 150 accessions belong to the genus *Triticum*. The material comes from the collection activities to Trancaucasia, Balkan and Near East and from germplasm exchanges. In the gene bank, the material was evaluated according to descriptor list for wheat on morphological traits, earliness, and other breeding valuable traits. Most of accessions were tested on resistance to a wide range of races of leaf diseases: powdery mildew - *Erysiphe graminis*, leaf, stem and stripe rusts - *Puccinia recondita, P.graminis* and *P. striiformis*. Field and greenhouse infestation tests were done for cereal aphids: *Metopolophium dirhodum, Rhopalosiphum padi* and *Sitobion avenae*. Pm and Lr resistance gene markers have been recently identified using PCR- STS technique in several *T. boeoticum* and *T. timopheevii* wheats. The germplasm evaluation revealed a great diversity in characters of the wheat accessions. Some populations were very uniform, however most of them considerably varied in characters studied within a collection site. The available resistances in the material were geographically localized according to origin of collected samples. Powdery mildew resistance from *T. boeoticum* was transferred to *T. aestivum* background via crosses with *T. durum*.

1. INTRODUCTION

Wheat is the most important crop in the Czech Republic. Since the beginning of breeding in the country (the year 1919) about 341 original cultivars has been produced in the former Czechoslovakia to the country splitting in 1992 (1). A similar attention was paid to wheat in germplasm collections from historical times. The oldest material is possible to trace to the end of 19th century. During the 1st World War The German Agricultural Board proclaimed originality of several Czech and Moravian wheats (2). Later, the Czechoslovak breeding based their programme on domestic landraces. At present the wheat collection of the Czech Gene Bank has 10,684 accessions of which 787 is of Czech and Czechoslovak origin (Faberova, pers. comm.). It includes ancient land races of einkorn -*Triticum monococcum*, emmer - *T. dicoccon*, spelt - *T. spelta, T. polonicum, T. timopheevii*, and other in the past cultivated species.

Wild wheats were present in the collection already in the fifties and were mainly of Soviet origin. The wild *Triticeae* collection, especially *Aegilops* and *Triticum*, was widely grown in eighties and nineties to the present 1,500 items. Resistance to leaf rust discovered by Valkoun (7) was later used in a commercial Czech cultivar Vlasta (6). Wild *Triticum* collection part was chosen for this contribution.

2.MATERIALS AND METHODS

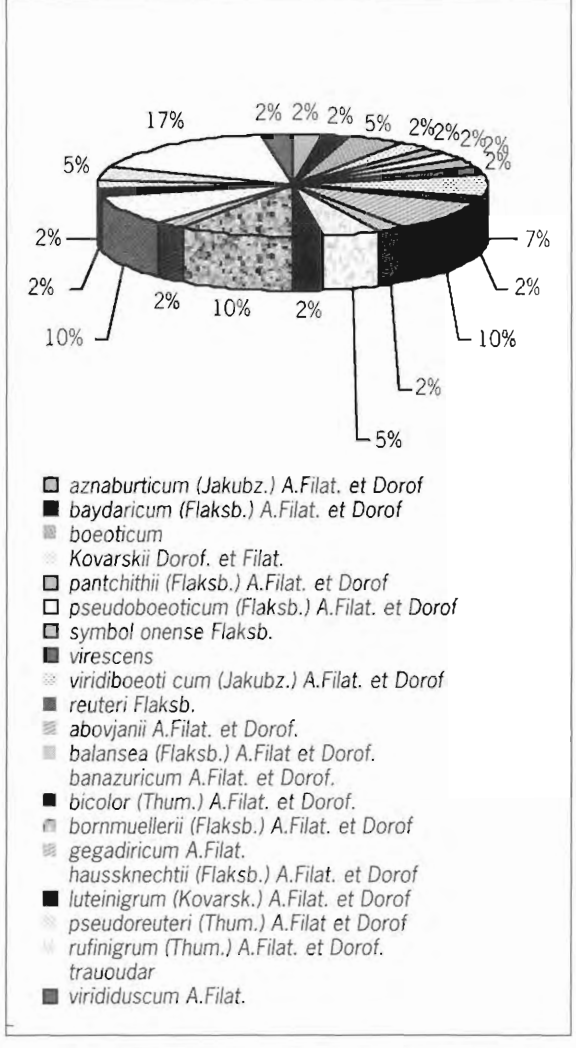
Wild *Triticeae* collection is maintained in the Czech Gene Bank, Prague -Ruzyn_ and has presently 1503 accessions.() Major part belongs to *Aegilops* and *Triticum*. The material comes from our collecting expeditions to Trancaucasia, Balkan, Near East and Central Asia and from other gene banks, namely from IPK Gatersleben, VIR Sankt Peterburg, ICARDA, WGRC - KSU Manhattan, and USDA Logan.

In the gene bank, the material was determined and evaluated according to descriptor list for wheat on morphological traits, earliness, and other breeding valuable traits. In the disease resistance evaluation, the accessions were screened for resistance to the following fungi: stem rust (*Puccinia graminis* f.sp. *tritici* ERIKSS. et HENN), leaf rust (*P.recondita* ROB. ex DESN.), stripe rust (*P.striiformis* WESTEND), and powdery mildew (*Erysiphe graminis* f.sp. *tritici* (DC.)MARCHAL). Both, stem and stripe rusts were tested on an infection field. A row of a susceptible wheat cultivar 'Michigan Amber' was inoculated into the leaf sheaths with a suspension of spores. Leaf rust and powdery mildew were tested in a greenhouse. Seedlings with about 3 leaves in plastic pots, covered with a glass cylinder for 48 hours after inoculation with spores, were evaluated after 10 days. The inoculum was prepared as a mixture of the most virulent isolates from Czech Republic.

Ifid. Additional recent observation of *Sipha* spp was included (*Sipha (Rungsia) elegans* del Guer. - (SE) - observed since 1995 and *Sipha glyceriae* (Kaltenbach) - (SG) - observed since 1996). Aphids were counted in the flowering stage on 3 to 5 plants, on about 10 to 15 fertile shoots. The average number of aphids per shoot served as the criterion for field resistance (3). Further, analysis of plant resistance (antibiosis, nonpreference and tolerance) to aphids was studied in the laboratory. Presented antibiosis was calculated as a number of larvae birthed by females on seedlings.

Table 1. Czech Gene Bank *Triticum* collection

Triticum	**xc.**
aestivum L.	9212
durum DESF.	900
dicoccum (SCHRANK) SCHUEBL	103
spelta L.	79
monococcum L.	56
boeoticum BOISS.	55
turgidum L.	55
araraticum JAKUBZ.	48
compactum HOST	48
dicoccoides (KOERN. ex ASCHERS. et. GRAEB.) SCHWEINF.	29
polonicum L.	19
carthlicum NEVSKI	16
urartu THUM. ex GANDIL.	12
sphaerococcum PERCIV.	12
macha DEKAPR. et MENABDE	6
turanicum JAKUBZ.	5
timopheevii (ZHUK.) ZHUK.	5
vavilovii (THUM.) JAKUBZ.	3
fungicidum ZHUK.	3
karamychevii NEVSKI	2
aethiopicum JAKUBZ.	2
petropavlovskyi UDACZ. et MIGUSCH.	2
zhukovskyi MENABDE et ERITZJAN	2
sinskajae A. FILAT. et KURK.	1
ispahanicum HESLOT	1
militinae ZHUK. et MIGUSCH.	1
palmovae G. IVANOV	1
kiharae DOROF. et MIGUSCH.	1
miguschovae ZHIR.	1
timonovum HESLOT et FERRARI	1
timococcum ZHUK.	1
flaksbergeri NAVR.	1
Total	**10683**

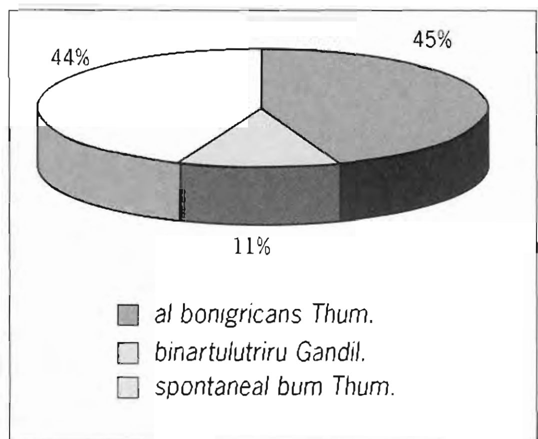


Graph 1. Determination of varieties in the *T. boeoticum* collection.

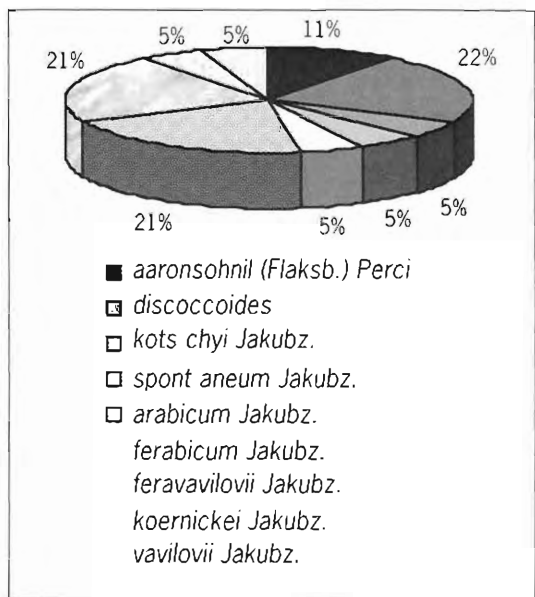
3. RESULTS AND DISCUSSION

The Gene Bank wheat collection is based on two main cultivated species *T. aestivum* and *T. durum* representing 94 %, further it includes eight less cultivated regional species, ten ancient cultivated species, four wild species and others are synthetic polyploids (Table 1).

Nearly all material is determined to subspecies and varieties. This botanical determination shows clearly morphological diversity of particular species. It is based on a combination of morphological characters of spike and grain. The varietal diversity is shown for *T. boeoticum, T. urartu* and *T. dicoccoides* in the graphs. In germplasm collections this criterion characterizes collection diversity, and when it is large, a larger diversity in other, breeding useful characters can be expected.



Graph 2. Determination of varieties in the T. urartu collection.



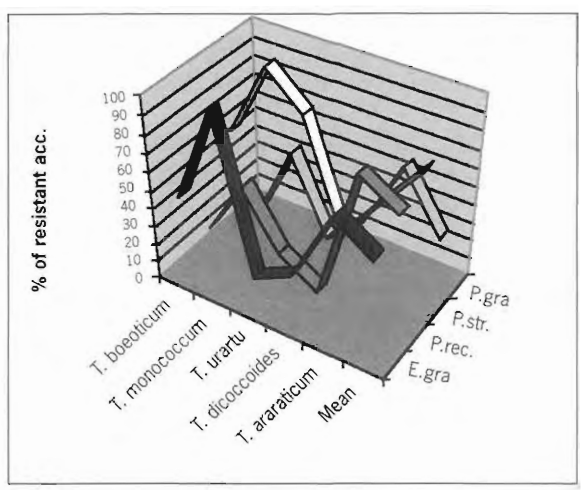
Graph 3. Determination of varieties in the T. dicoccoides collection.

Origin of wild wheats *T. boeoticum, T. urartu, T. dicoccoides* and *T. araraticum* from the gene bank collection is shown in the Maps 1 and 2. The weedy species, *Triticum boeoticum* comes from the major part of its distribution area. We believe the north-western distribution in the Bulgarian Rhodops Mts. is a northern limit of its natural distribution. However about a half of the collection habitats were secondary, along roads and around fields. The probable wheat ancestor, diploid *T. urartu* is known to be distributed within Fertile Crescent, the hypothetical place of origin of cultivated wheats. The material in the collection is available from the western part of Fertile Crescent and from Armenia and is missing from East Turkey, and Iran. Similarly, tetraploid species *T. dicoccoides* is available from western and northern part of Fertile Crescent. *T. araraticum,* a teraploid from the other evolution branch, the *Timopheevii* section, comes from most of its distribution area.

The resistance evaluation was summarized and plotted to the Map 1. with marked origin of particular wild wheat accessions. Resistance to leaf diseases in *Triticum boeoticum* accessions were found in line all across the distribution area: from Bulgaria to Turkey and Iraq. No one was in found in Transcaucasia. Resistant accessions of *T. urartu* and *T. dicoccoides* come from Syria, Lebanon and Jordan. Resistant accessions of *T. araraticum* are from all its distribution area.

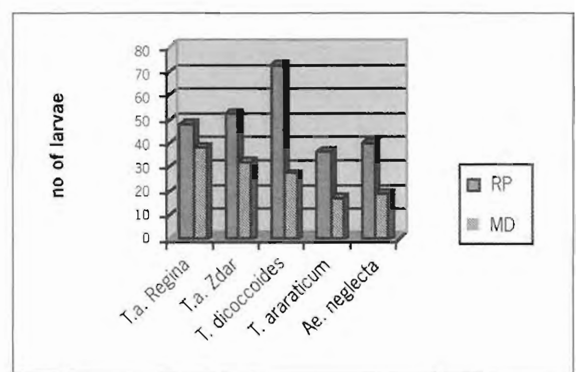
Resistance to aphids (Map 2.) were located for *T. boeoticum* only to the Western Turkey and Greece, for *T. dicoccoides* to northern Syria and adjacent Turkey, for *T. urartu* to southern Syria only and for *T. araraticum,* again to the most of its distribution area.

A higher useful level of resistance to leaf diseases was found in *T. araraticum* accessions (Graph 4). However, the highest level of the resistance was in a group of ancient cultivated *T. monococcum var. clusii* from Hungary and some *T. monococcum* lines of a Czech and German origin. It is rare to find resistancies within *T. dicoccoides* and *T. urartu,* but they are available within the collection. Resistance to a wide spectrum of races of powdery mildew was found in one accession of *T. boeoticum* from Turkey. Several Pm and Lr resistance gene markers have been recently identified using PCR- STS technique in several *T. boeoticum* and *T. timopheevii* wheats (5).



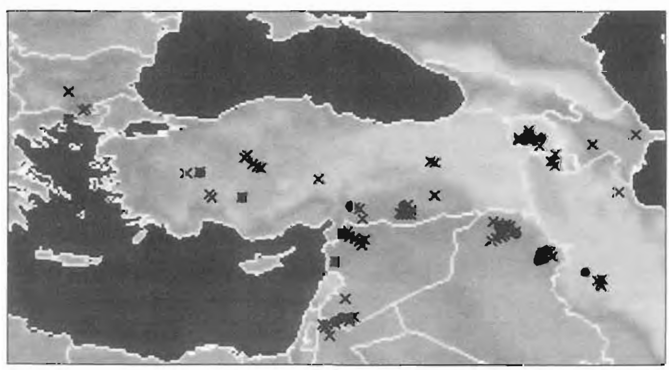
Resistance to cereal aphids *Metopolophium dirhodum* and *Rhopalosiphum padi* was studied in 5 wild wheat accessions chosen on the basis of previous evaluation (Graph 5). One accession of *Ae. neglecta* and one of *T. araraticum* had significantly higher antibiosis to aphids comparing to *T. dicoccoides* and 2 checks of *T. aestivum*. The accession of *T. araraticum* showed also a high non-preference and tolerance to the aphids.

Graph 4. Percetage of fully resistant accessions of *Triticum* spp. to stem rust (*P.gra*), stripe rust (*P.stri*), leaf rust (*P.rec*) and powdery mildew (*E.gra*).



Graph 5. The level of antibiosis for cultivated and wild wheats of *Triticum* and *Aegilops* to aphids *M.dirhodum* and *R. padi*

Map 1. Origin of *Triticum boeoticum, urartu, dicoccoides* and *araraticum* accessions from the Czech collection (cross), location of found resistances to powdery mildew and rusts (point).



Map 2. Origin of *Triticum boeoticum, urartu, dicoccoides* and *araraticum* accessions from the Czech collection (cross), location of found resistances to 3 species of cereal aphids (point).

The germplasm evaluation revealed a great diversity in characters of the wheat accessions. Some populations were very uniform, however most of them considerably varied in characters studied within a collection site. The available resistances in the material were geographically localized according to origin of collected samples. Due to a large variability of material, the number of studied accessions does not allow to come to conclusions about geographical localization of certain resistance genes. But definitely, it is possible to conclude that in some regions/sites there is much higher frequency of resistant accessions than in others. Such mapping can serve as a guide for further collecting.

Resistant accessions were used in wheat improvement programme. Leaf rust and powdery mildew resistance from *T. boeoticum* was transferred to *T. aestivum* background via crosses with *T. durum*.

4. REFERENCES

[1] Bare I. : Original and registered cultivars of wheat in Czechoslovakia in the years 1918-1992. Genetic Resources 65. RICP Praha, (1995).

[2] Chmela F.: Tasks and organization of assortment experiments. Reports of Moravian Agric. Research Inst. 68, Brno (1920).

[3] Havlíková H, and Holubec V.: Wild grasses of the genus Aegilops L. as hosts for cereal aphids. Z. Pfl Krank. Pfl Schutz., 102/6:627-6321994 1995.

[4] Holubec V., Havlí_ková, H., Hanuová, R. and Bocková, R.: Wild *Triticeae* as genetic resources of aphid, rust and powdery mildew resistance. In: A.A.Jaradat (ed.) Triticeae III. Science Publishers, Inc., USA.pp.341-350 (1998).

[5] Stepien L., Holubec V. and Chelkowski J.: Resistance genes in Wild Triticeae accession - inoculation test and STS marker analysis results. In: Broad variation and precise characterization - limitation for future. Eucarpia Abstracts, Poznan, p.144. (2001).

[6] Síp, V., Skorpík, M., Kuχcřa, L., Bobková, L., Amler, P.: Winter wheat Vlasta. Czech J. Genet. Plant Breed., 35, 1999: 91-92.

[7] Valkoun J. and Mamluk O.F.: Disease resistance and agronomic performance of durum and bread wheat lines derived from crosses with *Triticum monococcum*. In A.B. Damania: Biodiversity and wheat improvement. J.Willey & Sons, Chichester /ICARDA/IPGRI pp.141-146. (1993).

5. ACKNOWLEDGEMENT

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A STUDY OF GENETIC DIVERSITY IN BARLEY OF NORDIC AND BALTIC ORIGIN

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ABSTRACT: This study evaluates changes of genetic variation in barley of Nordic - Baltic origin over time from the end of the 19th century until today. Other aspects covered are the genetic relationship and genetic differences between material from the Nordic and Baltic countries. Two methods have been used in the analysis of the material: isozyme starch gel electrophoresis and inter-sinple-sequence-repeats (ISSRs). 290 accessions, including 160 Nordic and 80 Baltic landraces, varieties and breeding lines, and another 50 landraces of exotic origin (Central Asia) were surveyed using electrophoresis of the following isozymes: Aco, Est and Pgd. For the three isozymes studied, 23 alleles at 8 loci were observed. In the exotic material all loci were polymorphic but in the Nordic and Baltic material seven among them were polymorphic and one was monomorphic. Some of the rare alleles were detected only in material from Denmark and in exotic material. Of all Nordic-Baltic barley, 16.3% are characterised principally by the *Ge-B-Ca-Fr-At-Pi-Tn* genotype and 14.1% by the *Ge-B-Ca-Fr-At-Pi-Ps* genotype at the Aco-1 - Aco-2 - Est-1 - Est-2 - Est-4 - Est-5 - Pgd-2 multilocus. In exotic barley such big groups of accessions with identical isozyme characters were not found. The isozyme data could not be used to differentiate the material after its origin in Nordic-Baltic material, but the exotic cultivars could be distinguished from Nordic-Baltic cultivars. Only Baltic accessions has so far been analysed with ISSRs. Four ISSR primers from the University of British Colombia Biotechnology Laboratory (#888, #889, #890, #891) were used to detect polymorphism. In total 33 polymorph loci were observed. ISSR did not show differentiation in Baltic accessions after origin. As expected, the highest genetic diversity was observed in landraces and old cultivars independent of which method was used. In the modern varieties different tendencies were detected depending of the method used: isozymes showed decrease of genetic diversity during the 20th century, whereas ISSR data detected a slight increase of diversity in the varieties bred during the last decade.

INTRODUCTION

Barley is one of the most important crops in the Nordic-Baltic region. For a long time it has been under intensive selection and breeding pressure which makes it interesting for genetic diversity studies. It is well known that in the Nordic and Baltic region landraces are wholly replaced by pure line cultivars. Much of the commercial material is based on few landraces, which constitute the backbone: 'Chevalier', 'Prentice', 'Plymage', 'Hanna', 'Gotlands Barley'. Later new varieties have come from successive cycles of crosses between established pure lines, sometimes of diverse geographical origin (7).

Genes from the old, diverse landraces are still present in our modern varieties. The question is how much of the genetic variation eventually has been lost during the course of plant breeding development. In this study the changes of genetic variation in barley of the Nordic-Baltic region has been evaluated over time from the end of the 19th century until today and compared with genetic variation of cultivars from Central Asia where breeding has not been so intense. Two methods have been used in the analysis of the material: isozyme starch gel electrophoresis representing protein variability (8) and inter-sinple-sequence-repeats, ISSR, (6) (so far only Baltic in material) which is a DNA marker.

MATERIALS AND METHODS

The material -160 accessions from the Nordic and 80 from the Baltic region, including landraces, varieties and breeding lines and another 50 landraces of exotic origin (Central Asia), were obtained from gene banks in the Baltic countries, the Nordic Gene Bank and plant breeding companies. These accessions were surveyed using electrophoresis of the following isozymes: Aco (aconitate hydrotase), Est (esterase) and Pgd (phosphogluconatedehydrogenase). The procedures of enzyme extraction, starch gel electrophoresis, and enzyme staining were conducted as described by Kahler and Allard (1970) (1) for Est and Nielsen and Johansen (1986) (5) for Aco and Pgd. PCR products were obtained from 80 Baltic barley accessions using four ISSR primers from the University of British Colombia Biotechnology Laboratory (#888, #889, #890 and #891). After a separation on a polyacrylamide gel, staining was performed according to the manufacturer's protocol for the DNA Silver Staining Kit (Amersham Pharmacia Biotech AB).
Values of genetic diversity (H_T) were calculated according to Nei (4).

Table 1. Frequencies of isozyme genotypes in Nordic and Baltic material

Loci		Alleles							
Aco1-Aco2		Ge-B						Fn-B	
Pgd1-Pgd2		Ak-Tn				Ak-Ps		Ak-Tn	Other
Est1-Est2-Est4-Est-5		Ca-Fr-At-Pi	Ca-Fr-Su-Pi	Pr-Fr-Su-Pi	Ca-Fr-At-Pi	Ca-Fr-Su-Pi	Pr-Fr-Su-Pi	Pr-Fr-Su-Pi	
Region	**Group**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
	Nordic-Baltic	16.3 %	14.1 %	4.2 %	6.7 %	5. 0 %	4.2 %	3.8 %	45.8 %
	Nordic	10.0 %	11.3 %	5.6 %	8.1 %	5.6 %	4.4 %	4.4 %	50.6 %
	Baltic	18.8 %	20.0 %	1.3 %	3.8 %	3.8 %	3.8 %	2.5 %	46.3 %
	Finland	17.7 %	5.9 %	5.9 %	2.9 %	8.8 %	5.9 %	8.8 %	44.1 %
	Norway	6.1 %	18.2 %	6.1 %	3.0 %	6.1 %	3.0 %	9.1 %	48.5 %
	Sweden	20.4 %	18.5 %	5.6 %	11.1 %	3.7 %	3. 7 %	1.9 %	35.2 %
	Denmark	12.8 %	0.0 %	5.1 %	12.8 %	5.1 %	5.1 %	0.0 %	58.6 %
	Estonia	13.8 %	17.2%	3.5 %	0.0 %	0.0 %	0.0 %	6.9 %	58.6 %
	Latvia	32.3 %	3.2 %	0.0 %	6.5 %	9.7 %	6.5 %	0.0 %	41.9 %
	Lithuania	5.0 %	0.0 %	0.0 %	5.0 %	0.0 %	5.0 %	0.0 %	25.0 %

RESULTS

3.1. Isozymes

In Nordic - Baltic material for the three isozymes studied (Aco, Pgd and Est), 22 alleles at 8 loci were observed. Among them Aco-1, Aco-2, Est-1, Est-2, Est-4, Est-5, Pgd-2 were polymorphic and the locus Pgd-1 was monomorphic. In the exotic material all loci were polymorphic and 23 alleles observed. Of all Nordic-Baltic barley, 16.3% are characterised principally by the *Ge-B-Ca-Fr-At-Pi-Tn* (group A) and 14.1 % by the *Ge-B-Ca-Fr-Su-Pi-Tn* (group B) genotype at the Aco-1 - Aco-2 - Est-1 - Est-2 - Est-4 - Est-5 - Pgd-2 multilocus. In exotic barley such big groups of accessions with identical isozyme characters were not found (Tab. 1).

Isozymes showed the highest diversity in exotic material and in Nordic-Baltic landraces and old cultivars. From the middle of 20th century, the diversity gradually decreases (Fig.1)

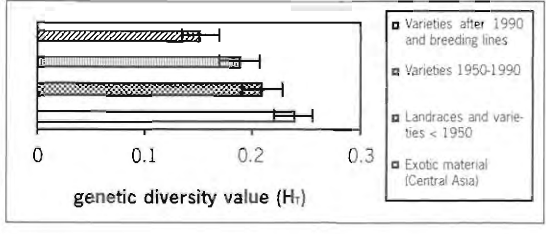

Figure 1: Genetic diversity values in Nordic-Baltic barley cultivars representing different breeding periods and compared with exotic material. Isozyme data.

If we compare the genetic diversity of the Nordic and Baltic material after its origin, the lowest allelic variation was observed in barley of Estonian origin (H_T=0.097±0.045), and the highest in accessions from Denmark (H_T=0.212±0.017). Cluster analysis of isozyme data (data not shown) did not give clear-cut separation according to the country of origin of accessions. Because isozyme markers can discover only a small portion of genetic variation we have started the analysis of barley accessions using DNA markers.

3.2. Inter-sinple-sequence-repeats (ISSRs)

Only Baltic material (80 accessions) has so far been analysed with ISSR-s. In total, 33 polymorphic loci were observed. The data showed a different tendency compared to isozyme data. After a decrease of variability in the middle of last century it increases again in modern varieties and breeding lines (Fig. 2). ISSR also did not show differentiation in Baltic accessions according to origin (data not shown).

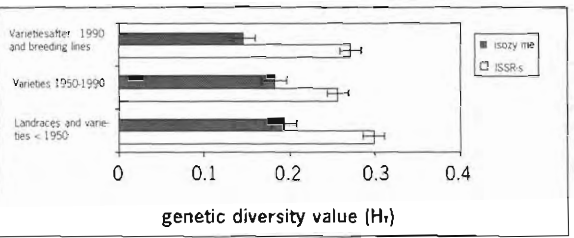

Figure 2. Genetic diversity in Baltic barley cultivars during different breeding periods. Comparison of isozyme and ISSR data.

DISCUSSION

Isoenzyme data confirm a significant difference between modern bred barley cultivars and material from Central Asia which suggest material of exotic origin to be used to widen the existent genepool. As expected, the highest genetic diversity was observed in landraces and old cultivars independent of which method was used. In the modern varieties, we detected difference depending of the method used: isozymes showed decrease of genetic diversity, but ISSR data detected slight increase of diversity in varieties bred in the last decade. Differences in isozymes and ISSR data concerning diversity in modern breeding lines could be explained by the fact that some of the isozyme loci are linked to adaptive traits (2,3). The increase of diversity detected by ISSRs is probably due to an increase of wider crosses in modern plant breeding programs. A complete data analysis of genetic diversity in the studied material will be done by comparing those markers with field trial data, disease resistance tests and microsatellie data. Only then we could draw final conclusions about the situation in barley breeding in the Nordic and Baltic countries regarding changes in genetic diversity and important agronomic characters over the years.

REFERENCES

[1] Kahler A and Allard R: Genetics of isozyme variants in barley. I. Esterase. Crop Sci. 10: 444-448 (1970)

[2] Konishi T, Kawada N, Yoshida H, Sohtome K: Linkage Relationship between Two Loci for the Barley Yellow Mosaic Resistance of Mokusekko 3 and Esterase Isozymes in Barley (Hordeum vulgare L.). Japan. J. Breed. 39: 423-430 (1989)

[3] Konishi T, Suh S, Park K and Matsuura S: Comparative Studies in Isozyme Genotypes between Korean and Japanese Barley Varieties. Crop Production and Improvement Technology in Asia, KSCS, Korea: 405-411 (1993)

[4] Nei M: Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA, 70, 3321-3323 (1973).

[5] Nielsen G and Johansen H: Proposal for the identification of barley varieties based on the genotypes for 2 hordein and 39 isoenzyme loci of 47 reference varieties. Euphytica 35: 717-728 (1986)

[6] Ratnaparkhe M, Tekeoglu M and Muehlbauer: Inter-simple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theor. Appl. Genet. 97: 515-519 (1998)

[7] Smartt J and Simmonds N: Evolution of Crop Plants. Second Edition. Longman Scientific &Technical, Singapore 140-146 (1995).

[8] Soltis D and Soltis P: Isozymes in Plant Biology. Chapman and Hall, London: 87-189 (1990).

EVALUATION OF MORPHOLOGICAL CHARACTERS IN THREE CULTIVATED WHEAT SPECIES: *TRITICUM AESTIVUM* L., *T. TURGIDUM* L. AND *T. DURUM* DESF. IN IRAN.

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ABSTRACT: 38 reproductive and 17 vegetative characters were assessed among 978 and 400 individuals respectively. They belong to 40 populations of three cultivated wheat species *Triticum aestivum*, *T. turgidum* and *T. durum*. Mean, standard deviation and coefficient of variation for each character were calculated and the three species were compared on their characters. Using Manhattan distance coefficient a UPGMA clustering was performed. The results of this study showed a relatively high morphological variation within the three species. Population comparison showed closer relationships between *T. aestivum* and *T. turgidum* than between each of them with *T. durum*.

1. INTRODUCTION

• Allopolyploid origin, along with natural and artificial hybridisation has obscured the morphological limits of the species and caused taxonomic confusion in the *Triticum - Aegilops* group. The taxonomic confusions, lectotyfication problems, and nomenclature debates in this *polyploid pillar complex* have been well discussed by Morrison (10, 11, and 12).

• Despite many years of efforts and using modern molecular techniques (e. g., 2, 3, 4, 5, 6, 7, 8, 13, 14, 18, and 19) yet, the genomic formula and the genome donor species, particularly the B - genome is a hot point and controversial subject among the polyploid species of *Triticum*. This stipulates the necessity for more hard works and serious taxonomic and biosystematics studies on the group *Triticum - Aegilops*.
ó In addition, the presence of different ploidy levels, high variation and taxonomic confusions encountered among the taxa belonging to *Triticum - Aegilops* complex necessities the urgent need for collecting and study the local germ plasm and land races of the group. For instance, Sharma et al. (16) examined 10 morphological characters among 297 genotypes belonging to *T. aestivum* and *T. durum*. They easily, documented genetic divergence among the genotypes studied.

• Vavilov (20) in his study on the centres of origin and diversity of cultivated plants referred to Iran as one of the important origins and diversity centres for different ploidy levels of cultivated wheat. Bor (1) in his taxonomic treatment on the family Poaceae, in the *Flora Iranica* area believed that 8 cultivated *Triticum* species occur in Iran: one diploid, *T. monococcum*; five tetraploids, *T. dicoccum*, *T. polonicum*, *T. orientale*, *T. turgidum* and *T. durum*; and one hexaploid, *T. aestivum*.

• Aims of this study are:

Collecting the germ plasm of the main cultivated wheat landraces, particularly from the Zagroos area,
Evaluation the inter-population morphological variation among the cultivated wheat, focusing on the three main species i.e. *T. aestivum*, *T. turgidum* and *T. durum*.
Providing materials for later biosystematics studies on the group *Triticum - Aegilops*.

2. Materials and Methods

• This investigation concerns the morphological study on 40 populations and 978 individuals belonging to three cultivated wheat species (Table 1). All the population samples were collected form the landraces grown as non-irrigated, particularly rural areas, in June and July in 1997. Randomly, 10 to 30 spike samples from each population were collected; furthermore in order to grow the accession of each population,

a bulk seed sample from 30 plants was collected. To obtain complete plants for vegetative studies, at least 100 seeds from each accession were grown along the lines in the garden

Table I. Materials examined from three wheat species.

Triticum:	**Locality (Province)**	**No. Coll. Trcs.**:	**Qty. pop. & Individuals studied**
Aestivum	Khz., Chm., Isf., Ilm., Krm.	12, 14, 17, 21, 23, 25, 28, 30, 64, 74, 90	11 & 287
Turgidum	K-Ba., Khz., Chm., Krm.	2, 3, 6, 10, 11, 13, 31, 32, 35, 36, 37, 39, 43, 80, 84	15 & 367
Durum	K-Ba., Khz., Ysj., Chm., Krm., Lrs.	4, 5, 7, 15, 16, 18, 20, 24, 26, 27, 42, 42*, 69, 89	14 & 324

K-Ba.: Koukiloyeh-Booyerahmadi, Khz: Khuzestan, Chm.Chaharmahl, Krm: Kermanshah. No. Coll. Trcs.: Collecting numbers of the wheat collection studied at Isfahan University. Randomly, 10 plants were used for leaf and stem (vegetative) characters.

• Thirty-eight spike (reproductive) characters (Table II) were measured on the spike samples (10 - 30) of each population. Seventeen leaf and stem (vegetative) characters (Table III) were studied on ten plants grown in the experimental field.

Table II. Thirty-eight morphological spike characters stu died and their **cvs**. in the three wheat spec L.: length, W.: width, sk: spike, skl: spiklet, lm: lemma, pl: palea, glm: glume, fl: floret, sd: seed, rdm: rudimentary, a: uppermost, -b: middle, -c: lowermost.

Triticum: Characters	***aestivum***	***turgidum***	***durum***
L sk.	17.54	17.23	10.09
W.sk.	17.92	13.55	16.01
No.skl./sk.	18.99	14.41	14.28
L.lm.-a	6.52	6.45	127.40
L.lm.-b	6.20	6.43	6.37
L.lm.-c	5.93	6.58	5.90
W.lm.-a	11.06	10.03	45.29
W.lm.-b	7.12	6.63	43.14
W.lm.-c	8.76	30.58	47.10
L.pl-a	8.54	5.18	6.94
L.pl-b	7.09	6.44	5.94
L.pl-c	7.69	12.66	6.78
W.pl-a	6.22	7.30	5.74
W.pl-b	6.37	6.78	14.81
W.pl-c	9.69	6.84	19.62

continuación ⟶

Triticum: Characters	*aestivum*	*turgidum*	*durum*
L.glm-a	6.52	9.45	8.14
L.glm-b	4.78	7.80	13.49
L.glm-c	3.97	6.77	13.68
W.glm-a	7.61	8.36	5.67
W.glm-b	5.41	5.02	6.35
W.glm-c	5.33	4.43	5.72
L.awn-a	43.36	47.38	25.53
L.awn-b	29.58	28.71	12.57
L.awn-c	32.83	31.61	15.25
No.fl/sk-a	18.39	13.12	21.25
No.fl/sk-b	10.79	9.93	17.61
No.fl/sk-c	22.27	20.31	148.73
L.sd-a	8.25	7.86	9.57
L.sd-b	7.90	8.42	6.29
L.sd-c	17.03	14.80	12.16
W.sd-a	4.42	8.78	4.64
W.sd-b	5.87	9.63	4.81
W.sd-c	43.54	45.09	48.61
No.sd/sk.	38.39	19.41	25.45
L.awn-glm-a	39.63	90.95	57.03
L.awn-glm-b	67.92	149.27	0.66
L.awn-glm-c	99.58	210.98	131.71
No.rdm	33.93	32.20	28.19
Total cvs.	**18.49**	**24.93**	**28.43**

• Flora Iranica (1) was used to identify the population samples taxonomically.

• Mean value and coefficient of variation for each character/population were calculated. Using above values, total mean ( ) and total coefficient of variation (cv) for each character/species was computed. Based on their values, the coefficient of variations of each species were classified into six classes of variability: 0-5, 5-25, 25-50, 50-75, 75-100 and >100. The three species were compared on their frequency percentage in each class.

• Based on the total mean of each character/species and using Manhattan metric distance (9)

$$MMdij = \sum_{k=1}^{n} |x\,ik - x\,jk|$$

A cluster analysis **(UPGMA)** was performed.

3.RESULTS

• Taxonomic treatment on the populations studied (Table I) showed that they belong to three species: *Triticum aestivum*, *T. turgidum* and *T. durum* (Table I).

• Based on the morphological assessment on the spike characters (Table II) the maximum and minimum cv values (**210.98** and **3.97**) were observed on the "length of awn of the lowermost glume (**L.awn-glm-c**)" in *T. turgidum* and "length of lowermost glume (**L.glm-c**)" in *T. aestivum* respectively. As Table III shows, the maximum and minimum cv values (**57.84** and **5.18**) on the leaf and stem (vegetative) characters are related to "total number of fertile stems (**T.frt-stm**)" in *T. durum* and "number of nodes of the longest stem" in *T. aestivum*.

Table III. Seventeen morphological leaf and stem characters and their **cvs** in the three wheat species.

Triticum: Characters	*aestivum*	*turgidum*	*durum*
T.stm	31.08	36.65	51.77
T.frt-stm	24.34	31.11	57.84
L.stm	11.90	11.37	11.72
L.spk + awn	20.16	13.41	16.13
L.spk - awn	13.79	13.71	11.23
No.nd	5.18	5.18	7.27
T.lve	27.15	27.61	42.97
L.lw-lfe	12.01	8.18	17.16
W.lw-lfe	16.24	13.83	21.26
L.lw-sht	11.01	13.86	15.56
L.mdl-lfe	12.44	16.51	15.26
W.mdl-lfe	13.57	13.69	20.50
L.mdl-sht	15.97	17.72	10.91
L.flg-lfe	24.76	23.78	26.17
W.flg-lfe	16.42	15.23	27.55
L.flg-sht	16.58	15.50	12.86
L.up-intnd	21.14	15.53	15.74
Total cvs.	**17.27**	**17.34**	**22.46**

T.: Total, stm: stem, frt: fertile, spk: spike, nd: node, lve: leaves, lw: lowermost, lfe: leaf, mdl: middle, sht: sheath, flg: flag, up: uppermost, intnd: intern ode.

4. DISCUSSIONS AND CONCLUSION

- Taxonomic treatments of the population samples showed that distinguishing the three species is difficult and ambiguous. The current taxonomic morphological keys are quiet deficient in distinction between the two species *T. aestivum* and *T. turgidum*. They are indeed hard to be taken apart from each other and their identifications have been often doubtful and time consuming. Morrison (11) in her commentary on the taxonomy of the wheats pointed out that the Floras and many of the other current taxonomic treatments of *Triticum* for the region of western and central Asia where the wheats are endemic, are lacking in consistency and informational content. This opinion is quiet in accordance with the results of this study.

- Based on the results of this study a wide range of morphological variation was observed both on spike and leaf and stem characters among the populations studied (Tables II and III). The total **cvs** (Tables II and III) showed that *T. aestivum* and *T. turgidum* are less variable on their spike and leaf and stem characters than *T. durum*. The histogram of variability classes (Figure 1) shows that the majority of characters in the three species are related to the class **II (cv 5-25)**. The least variability was observed in *T. aestivum* and the highest in *T. durum*. While the number of characters in the class **I (cv 0-5)** is 3 for *T. aestivum* it is 1 and 2 for *T. turgidum* and *T. durum* respectively **(Figure 1)**. Length of the awn of the glume is a character that is considerably variable in the three species. Length of the spike revealed least variability in *T. durum* while, number of florets/spikelet is most variable one in this species. Number of seeds/spike presented most variability in *T. aestivum*.

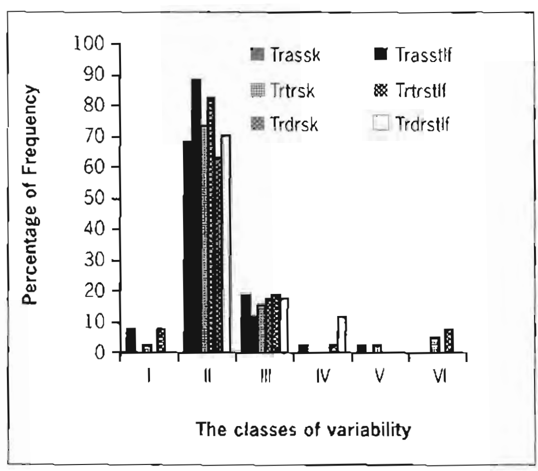

Figure 1: Histogram of frequency percentage of characters in the classes of variability (see materials and methods). Trassk: *T. aestivum* spike characters, Trasstlf: *T. aestivum* stem and leaf characters, Trtrsk: *T. turgidum* spike characters, Trtrstlf: *T. turgidum* stem and leaf characters, Trdrsk: *T. durum*: spike characters, Trdrstlf: *T. durum* stem and leaf characters.

• Based on the results of this study it can be concluded that despite high similarity among these three species, the hexaploid *T. aestivum*, showed less variability-in comparison to the two teteraploids (*T. turgidum* and *T. durum*). Considering the hexaploid nature of *T. aestivum* its less variability relative to the two other species seems unusual. In their review on the biosystematics of *Aegilops* and *Triticum*, Waines and Barnhart (21) suggested that the genomic formula of *T. turgidum* should be listed as **BBAA** rather than **AABB**. They listed the genome of *T. aestivum* as **BBAADD**; which had been reported as **AABBDD** (15). Presumably, different genomic formula along with inter-specific hybridisations in the tetraploids have caused more variability in them comparing to the hexaploid. In addition, growing wild tetraploids e.g., *T. dicoccoides* and *Aegilops cylindrica* that grow around the non-irrigated farms of tetraploid wheat species (1) has increased the possibility of natural hybridisation between these closely related species. This mechanism can be resulted in more variability in the tetraploids relative to the hexaploids that do not hybridise with their wild tetraploid relatives as easy as cultivated tetraploids.

• Cluster analysis using morphological data on the three species showed more or less high dissimilarity among the populations (Figure 2 and Table IV). Nevertheless, as the phenogram of Figure 2 shows most the populations of *T. aestivum* and *T. turgidum* are clustered together; this confirms the closer relationships between these two species. The relative distance among the latter species and *T. durum* can be interpreted as the result of its origin, which probably is around Mediterranean area (18).

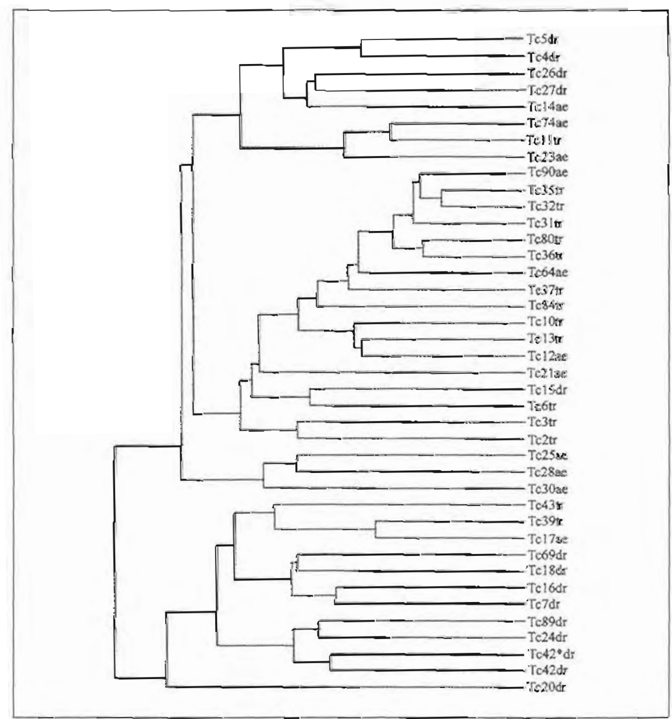

Figure 2: Phenogram of clustering analysis (UPGMA) using of 38 spike and 17 stem and leaf characters of 40 populations belonging to Triticum: aestivum (ae), turgidum (tr) and durum (dr). Tc: Triticum, numbers refer to collecting number of the wheat collection at Isfahan University.

• Zagroos area, from Azarbayjan to north of Khuzestan, includes part of the gene pool of *Triticum* (Fertile crescent) both as wild and cultivated. The high variability of the taxa belonging to the germ plasm of *Triticum* stipulates the necessity of urgent collecting and identifying *Triticum*'s taxa occurring in this area. Regarding the deficiency of the Floras in identification wheat specimens along with the wide range of variation observed among the cultivated wheat species, it seems necessary to determine an Indicator sample (a kind of
lectotype) for cultivated and wild *Triticum* taxa occurring in Iran.

Table IV: Dissimilarity values among 40 populations from: *Triticum aestivum* (**ae**), *T. turgidum* (**tr**) and *T. durum* (**dr**) in Iran (see **Figure 2**).

ND	GR 1	GR 2	DSSMLTY	No. OBJ.
1	Tc32tr	Tc35tr	20.82	2
2	Tc36tr	Tc80tr	25.28	2
3	ND 1	Tc90ae	26.01	3
4	ND 3	Tc31tr	27.75	4
5	ND 4	ND 2	32.62	6
6	Tc74ae	Tc11tr	33.21	2
7	Tc17ae	Tc39tr	37.07	2
8	Tc4dr	Tc5dr	39.90	2
9	Tc13tr	Tc12ae	40.20	2
10	ND 5	Tc64ae	41.23	7
11	N 9	Tc10tr	42.04	3
12	NE 10	Tc37tr	43.61	8
13	ND 6	Tc23ae	44.52	3
14	Tc7dr	Tc16dr	46.72	2
15	Tc42*dr	Tc42dr	47.95	2
16	Tc24dr	Tc89dr	50.74	2
17	ND 12	Tc84tr	51.20	9
18	Tc26dr	Tc27dr	51.41	2
19	Tc6tr	Tc15dr	53.17	2
20	NE 18	Tc14ae	53.36	3
21	ND 17	ND 11	55.97	12
22	Tc69dr	Tc18dr	56.21	2
23	Tc25ae	Tc28ae	56.21	2
24	Tc2tr	Tc3tr	56.25	2
25	ND 16	ND 15	57.18	4
26	ND 22	ND 14	57.62	4
27	ND 8	ND 20	59.00	5
28	ND 7	Tc43tr	61.88	3
29	ND 23	Tc30ae	64.66	3
30	ND 21	Tc21ae	65.62	13
31	ND 19	ND 30	67.49	15
32	ND 27	ND 13	70.12	8
33	ND 31	ND 24	70.30	17
34	ND 28	ND 26	71.66	7
35	NDE34	ND 25	76.02	11
36	ND 33	ND 32	81.94	25
37	ND 36	ND 29	84.83	28
38	ND 35	Tc20dr	88.56	12
39	ND 38	ND 37	101.47	40

ND: Node, GR: Group, DSSMLTY: Dissimilarity, No. OBJ.: Number of objects in fused group, Tc and the numbers followed it: Collecting numbers.

REFERENCES

[1] Bor N L: Gramineae. In: Rechinger, K. H. (ed) *Flora Iranica* 70: 147-244. Akademisch Druck-u. verlags Anstalt Graz (1970).

[2] Caldwell K A, Kasadra D D: Assessment of genomic and species relationships in *Triticum* and *Aegilops* by PAGE and differential staining of seed albumins and globulins. Theo. Appl. Genet. 52: 273- 280 (1978).

[3] Hirai A, Tsunewaki K: Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. VII. Fraction I protein of 39 cytoplasms. Genetics 99: 487-493 (1981).

[4] Johnson B L: Tetraploid wheats: Seed protein electrophoresis patterns of Emmer and timophivii group. Science 158: 131-132 (1967).

[5] Johnson B L: Seed protein profiles and the origin of the hexaploid wheat. Ame J. Bot. 59: 952-960 (1972a).

[6] Johnson B L: Protein electrophoretic profiles and the origin of the B genome of wheat. Proc. Natl. Acad. USA 69: 1398-1402 (1972b).

[7] Johnson B L: Identification of the apparent B-genome donor of wheat. Can. J. Genet. Cytol. 17: 21-39 (1975).

[8] Johnson B L: Polyploid weats and fraction I protein. Science 192: 1252 (1976).

[9] Kovach W L: A multivariate statistical package for IBM-PCs. Ver. 2.1: Kovach computing services (1993).

[10] Morrison L A: *Triticum-Aegilops* systematics: Taking an integrative approach. In: Damania A B (ed) *Biodiversity and wheat improvement:* 59-66 (1993a): John Wiley & Sons.

[11] Morrison L A: Taxonomy of the wheats: A commentary. In: Li Z S, Xin Z Y (eds) proceeding of the Eight International Wheat Genetic Symposium 1: 65-71 (1993b).

[12] Morrison L A: Lectotyfication of *Triticum turgidum* and *T. spelta* (poaceae). Taxon 47: 705- 710 (1998).

[13] Nath J, Mcnay J W, Paroda C M, Gulati S C: Implication of *Triticum searsii* at the B-genome donor to wheat using DNA hybridisations. Biochem. Genet. 21: 745-760 (1983).

[14] Nath J, Hanzel J, Thompson J P, Mcnay J W: Additional evidence implicating *Triticum searsii* as the B-genome donor to wheat. Biochem. Genet. 22: 37-50 (1984).

[15] Parker P F: The classification of crop plants. In: Stret H E (ed) *Essay in plant taxonomy*. Academic Press (1978).

[16] Sharma P K, Gupta P K, Balyan H S: Genetic diversity in a large collection of wheats (*Triticum* spp.). Indian Genet. 8(3): 271-278 (1998).

[17] Spangolettzeuli P L, Depace C, Porceddu E: Variation in durum wheat populations from three geographical origins. I. Material and spike characteristics. Euphytica 33: 563-575 (1984).

[18] Talbert L E, Smithy L Y, Blake N K: More than one origin of hexaploid wheat is indicated by sequence comparison of low-copy DNA. Genome 41: 402-407 (1998).

[19] Thompson T P, Nath J: Elucidation of the B-genome donor to *Triticum turgidum* by unique and repeated sequence DNA hybridisations. Biochem. Genet. 24: 39-50 (1986).

[20] Vavilov N L: *Origin and geography of cultivated plants*. Translated from Russian by: Löve D. Cambridge University Press (1992).

[21] Waines J G, Barnhart D: Biosystematics research in *Aegilops* and *Triticum*. Hereditas 116: 207-212 (1992).

THE GENETIC CONTROL OF FLOWERING IN CEREALS AND ITS IMPLICATIONS FOR CROP ADAPTATION

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ABSTRACT: The timing of flowering is an important adaptive character in plants. External cues such as photoperiod (day length) and periods of low temperature (vernalization) are commonly used to regulate flowering and major genes controlling these responses have been mapped in several crop and model plants. An interesting question is to what extent plants share common genetic flowering time controls and how far comparative genetic approaches can expedite the analysis of species in the Triticeae. This paper briefly discusses what is known about the genetic basis of photoperiod and vernalization in barley (*Hordeum vulgare*) and bread wheat (*Triticum aestivum*) and the ways in which this can be integrated with knowledge from other Triticeae species, other grasses including rice (*Oryza sativa*) and the model dicot *Arabidopsis thaliana*.

1. FLOWERING TIME

Flowering time is an important adaptive character for plants, ensuring that flowers are produced when the conditions for fertilization, seed maturation and dispersal are optimal. These characters are clearly important for crop species, but the selective pressures on crops are different from those of their wild relatives. Therefore, when we consider the adaptation of species such as barley and wheat in present day agriculture it is interesting to consider two questions. Firstly, what flowering time characteristics did their wild ancestors possess? Secondly, what genetic variation for flowering time can we detect in modern crop varieties? Answering these questions should provide insights into the ways in which crops evolved as their ranges expanded beyond the ecogeographical limits of their wild ancestors.

2. WHAT GENES CONTROL VARIATION IN FLOWERING TIME?

Modern barley and wheat varieties fall into two major groupings, namely winter (autumn/fall sown) or spring (spring sown) types. Winter varieties typically have a vernalization response, so that flowering is promoted after a period of exposure to low (4-10 C) temperatures. Three major genes regulating vernalization response have been identified in barley (*Sgh1*, *Sgh2* and *Sgh3* on chromosomes 4HL, 5HL and 1HL, respectively [17, 29]). These genes operate in combination and only plants with late alleles at all three loci show a strong vernalization response [34].

European winter and spring barleys also typically differ in photoperiod response, with winter forms showing a greater response to long days. Although barley is classified as a quantitative long day (LD) plant (flowering is promoted by exposure to day lengths in excess of 12h) QTL analysis shows that it also has genes that regulate flowering under short days [17]. Two major genes regulating photoperiod response have been identified (*Ppd-H1* (*Eam1*) and *Ppd-H2*). These map on chromosomes 2HS and 1HL and control flowering under long day (LD) and short day (SD) conditions, respectively [17]. They appear to act independently, as no interaction between them was detected. In addition to these genes, numerous QTL have been detected [8] but the ways in which these act are poorly understood.

An interesting question is whether the allelic variation at the major loci controlling flowering time in modern varieties was already present in the gene pool of their wild ancestors or whether variation arose during

domestication. Wild progenitors of barley and wheat (*H. spontaneum* and *T. diccoccoides*, respectively) show considerable variation for flowering time which is correlated with growing conditions [e.g. 13, 14]. However, it is not known whether this is due to variation at the same loci that differentiate cultivated types. This requires further study

3. ARE THE SAME FLOWERING TIME GENES FOUND IN DIFFERENT SPECIES?

3.1. Comparisons within the Triticeae

Comparative mapping using common markers, often cDNAs, allows the genetic maps of different grass species to be aligned (recently reviewed by [3, 6, 20] This enables the positions of mapped trait loci to be compared. When barley is compared with other Triticeae species we find that *Sgh2* has a counterpart in bread wheat, rye (*Secale cereale*) and *T. monococcum* (*Vrn1* [7], *Sp1* [23] and *Vrn-A^{m}1* [4], respectively) in terms of phenotype and comparative map location. An *Sgh1* equivalent (*Vrn-A^{m}2*) has been found in *T. monococcum* [4]. In barley and *T. monococcum* the early flowering allele of *Sgh1/Vrn-A^{m}2* is recessive. This may account for the fact that no similar gene has been identified in hexaploid wheat as homozygosity for recessive alleles at all three homoeologous loci would be needed. To date, no counterpart of *Sgh3* has been identified in wheat or rye.

Similarly, the genetic map location of *Ppd-H1* suggests that it forms a homoeologous series with the *Ppd* genes on the short arms of the group 2 chromosomes in wheat [2,].

These results suggest that wheat, barley and rye show similar variation and the adaptive significance of this has been considered for vernalization and photoperiod genes. The ecogeographical distribution of *Vrn1* alleles has been analyzed [10, 11] showing that there is regional variation in which *Vrn* alleles predominate in varieties, presumable due to differing selection pressures.

The effect of *Ppd* variation in wheat has been studied extensively using chromosome substitution methods and near-isogenic lines. These investigations have shown that different alleles confer yield advantages in different environments [37]. For example, an early flowering (day length insensitive) allele of *Ppd-D1* typically confers a small yield penalty in the UK (where cool wet summers allow a long grain filling period), a small gain in Germany and a large yield benefit in Yugoslavia (where summers are hot and dry) (Table I).

Table I. Mean percent change in plot yield associated with different *Ppd* alleles backcrossed into the variety 'Cappelle-Desprez'. Figures in brackets are the number of years testing from which these means are derived. Data from [37].

allele	UK	Germany	Yugoslavia
Ppd-D1	-1.8 (10)	+7.7 (6)	+33.0 (4)
Ppd-B1	-2.5 (3)	-9.5 (2)	not tested

The data in Table I might suggest that the avoidance of high temperatures during grain filling is the cause of the yield benefit. However, these studies also showed that early *Ppd* alleles were associated with reduction in plant height, tillering and the number of spikelets per ear. These would be expected to reduce yield, but this was offset by the formation of more grains per spikelet [37]. This shows the complexity of *Ppd* effects and the need for detailed study of the effects of flowering time genes on plant performance.

Within the groupings defined by variation at major loci governing vernalization and photoperiod response, there remains considerable variation. Genetic analysis shows that this is due to the action of larger numbers of QTL that have effects that are not directly attributable to cues such as day length or temperature. These genes (variously described as earliness *per se* or narrow sense earliness) are clearly important and worth more detailed analysis, but are currently poorly defined.

3.2. Comparisons with other plants

To date, none of the barley or wheat genes discussed above have been isolated. This contrasts with the situation in the model dicot *Arabidopsis thaliana* whose experimental advantages, including the complete genome sequence and the availability of large numbers of mutants in common genetic backgrounds, have allowed researchers to isolate many genes that affect the timing of flowering. By combining knowledge of gene action with the genetic analysis of double and triple mutants it has been possible to develop a sophisticated model of flowering time control. Four linked pathways (photoperiod, vernalization, autonomous and gibberellic acid) are recognized [reviewed by 25, 27, 28 and illustrated in Figure 1].

Recent work has shown how the pathways controlling flowering in *Arabidopsis* can be integrated by the control of genes such as *FT* and *SOC1* (*AGL20*) [26] and the meristem identity gene *LEAFY* [1]. Thus, flowering in *Arabidopsis* is a balance of promotive and repressive effects in the plant that are affected by external cues.

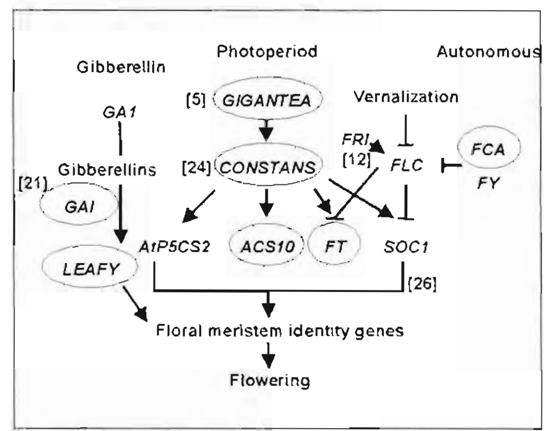

Figure 1: Flowering time control pathways in *Arabidopsis*. Arrows indicate promotion of gene expression, squared lines indicate repression. References to individual genes are shown on the figure. In this highly simplified diagram the genes shown to date to have homologues in cereals are circled. The function of the cereal genes has not been proven in all cases.

It is important to consider if these pathways are common to other plants. If they are, *Arabidopsis* can offer fast-track access to genes in crop species. The division between the dicot lineage that contains *Arabidopsis* and the magnoliid lineage (to which grasses and other monocots belong) is an ancient branch point in angiosperm phylogeny [31]. Nevertheless, these groups have been shown to use conserved genes to regulate several aspects of development. Examples include the use of phytochromes for sensing light and light quality and the use of MADS-box transcription factors homologous to the *Arabidopsis PISTILLATA*, *APETALA3* and *AGAMOUS* genes to control the organization of the flower [see 19, 36 for recent reviews].

It has also been shown that genes regulating gibberellic acid mediated growth are conserved. In this case the *Arabidopsis GAI* gene [21] was shown to be an orthologue of *Rht* in wheat and *d8* in maize [22], and *slender* in rice [9].

Arabidopsis, like barley, is a quantitative LD plant and it is therefore of interest to consider whether flowering time controls are conserved. To date, we have isolated several genes from barley including homologues of *GIGANTEA* (*GI*) and *CONSTANS* (*CO*) which are in the photoperiod pathway in *Arabidopsis*. Importantly, the *Hd1* gene of rice, a major determinant of photoperiod control [38], has recently been isolated and shown to be highly homologous to *CO* [40].

Barley has a counterpart of *Hd1*, as judged by sequence similarity and comparative map location, but neither this nor any of the other barley *GI* or *CO* like genes correspond to known flowering time loci. This may mean that the barley genes have different roles, but this seems unlikely as the barley *GI* and most *CO*-like genes have circadian expression patterns that are very similar to those seen in *Arabidopsis*. Further work is under way to assess the roles of *GI* and *CO*-like genes in barley development.

Two other flowering time genes have recently been cloned from rice and these have also been shown to correspond to genes previously known to affect flowering in *Arabidopsis*. Rice *Hd3a* is a homologue of *FLOWERING LOCUS T* (*FT*) [15], while *Hd6* is the alpha subunit of *CASEIN KINASE II* (*CKII*) [35]. The former is downstream of *CO* (Fig. 1) while the latter is thought to regulate circadian clock function by phosphorylation of the *CCA1* and *LHY* proteins, at least in *Arabidopsis* [33]. The importance of gene expression in relation to the circadian clock has emerged as an important research area in *Arabidopsis*. For example, studies of circadian expression have provided models which explain how the expression of *CO* can be used to provide photoperiodic control of flowering [32].

The results from rice described above are particularly interesting for two reasons. Firstly, they show that there are shared elements of flowering control in monocots and dicots. Secondly, they show that there is conservation between LD plants (*Arabidopsis*) and SD plants (rice). This is also supported by studies of a *CO* homologue from the SD plant *Pharbitis nil* [18]. Thus, LD and SD plants use common genes to regulate flowering. Exactly how LD and SD control is achieved in these different systems remains to be established.

Recently, there have also been major advances in understanding major determinants of vernalization response in *Arabidopsis*. Two important genes are *FLC* [30] and *FRIGIDA* (*FRI*) ([12]; Figure 1). *FRI*, which shows no significant homology to other known proteins, is needed for the expression of *FLC*, which is a MADS-box transcription factor [30]. Loss of *FRI* function, or vernalization, reduces *FLC* expression, thereby removing an inhibitor of flowering (Figure 1). Variation at the *FRI* locus is an important determinant of the flowering behaviour of *Arabidopsis* ecotypes, with loss of function conferring early flowering. Sequence analysis of the *FRI* gene shows that early flowering alleles have evolved a least twice [12].

To date it is not known if the mechanism controlling vernalization response in *Arabidopsis* is conserved in other plant groups. This can only be answered by isolating the relevant genes and this may soon be possible either by direct map-based cloning [39] or by comparative approaches.

4. FUTURE AREAS

The recent advances in understanding the control of flowering in rice [see 41 for recent review] were achieved by the utilization of naturally occurring variation and by use of map-based cloning rather than gene sequence homology. Map-based approaches in rice are likely to predominate because of its small genome size and the emergence of the complete rice genomic sequence. However, comparative sequence homology based approaches remain attractive for the Triticeae because of their large genome sizes (for example, the haploid genome size of barley is about 5400 mb compared to 450 mb in rice).

A weakness of comparative approaches in cereals is that relatively few crosses in each species have been analysed in detail for flowering time variation. Therefore it would be valuable to known more about individual crops, their wild relatives, and other species within the Triticeae. One of the interesting observations from comparative mapping is that major photoperiod and vernalization genes appear to be common between barley, wheat and rye (see section 3.1). This suggests that variation at relatively few genes is compatible with high performance in agriculture. It would be interesting to know if it is these genes that determine flowering time variation in other Triticeae species. This analysis will be greatly helped by comparative maps that provide markers closely linked to major flowering time genes. Comparative studies relating Triticeae species to other cereals and dicots will also help to build up a wider picture of the ways in which flowering is regulated in plants and the role of flowering time variation in adaptation.

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REFERENCES

[1] Blázquez MA, Weigel D: Integration of floral inductive signals in *Arabidopsis*. Nature 404: 889-892 (2000).

[2] Börner A, Korzun V, Worland AJ: Comparative genetic mapping of loci affecting plant height and development in cereals. Euphytica 100: 245-248 (1998).

[3] Devos KM, Gale MD: Genome relationships: The grass model in current research. Plant Cell 12: 637-646 (2000).

[4] Dubcovsky J, Lijavetzky D, Appendino L, Tranquilli G: Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. Theor Appl Genet 97: 968-975 (1998).

[5] Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Putterill J: *GIGANTEA*: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. EMBO J 18: 4679-4688 (1999).

[6] Gale MD, Devos KM: Plant comparative mapping after 10 years. Science 282: 656-659 (1997).

[7] Galiba G, Quarrie SA, Sutka J, Morgounov A, Snape JW: RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. Theor Appl Genet 90: 1174-1179 (1995).

[8] GrainGenes database - http://wheat.pw.usda.gov/

[9] Ikeda A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J: *slender* rice, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the height-regulating gene *GAI/RGA/ RHT/D8*. Plant Cell 13: 999-1010 (2001).

[10] Iwaki K, Nakagawa K, Kuno H, Kato K: Ecogeographical differentiation in east Asian wheat, revealed from the geographical variation of growth habit and *Vrn* genotype. Euphytica 111: 137-143 (2000).

[11] Iwaki K, Haruna S, Niwa T, Kato K: Adaptation and ecological differentiation in wheat with special reference to geographical variation of growth habit and *Vrn* geneotype. Plant Breed 120: 107-114 (2001).

[12] Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C: Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. Science 290: 344-347 (2000).

[13] Kato K, Mori Y, Beiles A, Nevo E: Geographical variation in heading traits in wild emmer wheat, *Triticum dicoccoides*. I. Variation in vernalization response and ecological differentiation. Theor Appl Genet 95: 546-552 (1997).
[14] Kato K, Tanizoe C, Beiles A, Nevo E: Geographical variation in heading traits in wild emmer wheat, *Triticum dicoccoides*. II. Variation in heading date and adaptation to diverse eco-geographical conditions. Hereditas 128: 33-39 (1998).
[15] Kojima S, Monna L, Fuse T, Sasaki T, Yano M: Map-based cloning of the rice photoperiod sensitivity QTL, *Hd3a*. Plant & Animal Genome IX, San Diego, abstract P116 (2001).
[16] Laurie DA: Comparative genetics of flowering time in cereals. Plant Mol Biol 35: 167-177 (1997).
[17] Laurie DA, Pratchett N, Bezant JH and Snape JW (1995). RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter x spring barley (*Hordeum vulgare* L.) cross. Genome 38: 575-585.
[18] Liu JY, Yu JP, McIntosh L, Kende H, Zeevaart JAD: Isolation of a *CONSTANS* ortholog from *Pharbitis nil* and its role in flowering. Plant Physiol 125: 1821-1830 (2001).
[19] Ng M, Yanofsky MF: Three ways to learn the ABCs. Curr Opin Plant Biol 3: 47-52 (2000).
[20] Paterson AH, Bowers JE, Burow MD, Draye X, Elsik CG, Jiang CX, Katsar CS, Lan TH, Lin YR, Ming RG, Wright RJ: Comparative genomics of plant chromo-somes. Plant Cell 12: 1523-1539 (2000).
[21] Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP: The *Arabidopsis GAI* gene defines a signalling pathway that negatively regulates gibberellin responses. Genes & Dev 11: 3194-3205 (1997).
[22] Peng JR, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP: 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400: 256-261 (1999).
[23] Plaschke J, Börner A, Xie DX, Koebner RMD, Schlegel R, Gale MD: RFLP mapping of genes affecting plant height and growth habit in rye. Theor Appl Genet 85: 1049-1054 (1993).
[24] Putterill J, Robson F, Lee K, Simon R, Coupland G: The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80: 847-858 (1995).
[25] Reeves PH, Coupland G: Response of plant development to environment: control of flowering by daylength and temperature. Curr Opin Plant Biol 3: 37-42 (2000).
[26] Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G: Distinct roles of *CONSTANS* target genes in reproductive development of *Arabidopsis*. Science 288: 1613-1616 (2000).
[27] Samach A, Coupland G: Time measurement and the control of flowering in plants. BioEssays 22: 38-47 (2000).
[28] Simpson GG, Gendall AR, Dean C: When to switch to flowering. Annu Rev Cell Dev Biol 15: 519-(1999).
[29] Shahla A, Tsuchiya T: Genetic analysis in six telotrisomic lines in barley (*Hordeum vulgare* L.). J Hered 81: 127-130 (1990).
[30] Sheldon CC, Finnegan EJ, Rouse DT, Tadege M, Bagnall DJ, Helliwell CA, Peacock WJ, Dennis ES: The control of flowering by vernalization. Curr Opin Plant Biol 3: 418-422 (2000).
[31] Soltis PS, Soltis DE, Chase MW: Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402: (6760) 402-404 (1999).
[32] Suárez-López P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G: *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature 410: 1116-119 (2001).
[33] Sugano S, Andronis C, Ong MS, Green RM, Tobin EM: The protein kinase CK2 is involved in regulation of circadian rhythms in *Arabidopsis*. Proc Natl Acad Sci USA 96: 12362-12366 (1999).

[34] Takahashi R, Yasuda S: Genetics of earliness and growth habit in barley. In: Proc 2nd International Barley Genetics Symposium, ed Nilan RA. Washington State University Press, pp 388-408 (1971).
[35] Takahashi Y, Shomura A, Sasaki T, Yano M: *Hd6*, a rice quantitative trait locus involved in photoperiodic sensitivity, encodes the _ subunit of protein kinase CK2. Proc Natl Acad Sci USA 98: 7922-7927 (2001).
[36] Theissen G, Becker A, Di Rosa A, Kanno A, Kim JT, Munster T, Winter KU, Saedler H: A short history of MADS-box genes in plants. Plant Mol Biol 42: 115-149 (2000).
[37] Worland AJ, Börner A, Korzun V, Li WM, Petrovic S, Sayers EJ: The influence of photoperiod genes to the adaptability of European winter wheats. Euphytica 100: 385-394 (1996).
[38] Yamamoto T, Kuboki Y, Lin SY, Sasaki T, Yano M: Fine mapping of quantitative trait loci *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97: 37-44 (1998).
[39] Yan L, Muzzi G, Basso C, Lijavetzky D, Marcussi-Poltri S, Tranquilli G, Dubcovsky J: High-density map of vernalization gene *Vrn2* in the Triticeae. Plant & Animal Genome IX, San Diego, abstract P423 (2001).
[40] Yano M, Katayose Y, Ashikara M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T: *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12: 2473-2483 (2000).
[41] Yano M: Genetic and molecular dissection of naturally occurring variation. Curr Opin Plant Biol 4: 130-135 (2001).

DOCUMENTATION AND EVALUATION OF BARLEY GENETIC RESOURCES IN EUROPE

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Thirty-five European Institutions participate in the EU-funded GENRES CT-98-104 project and its ECP/GR-funded complementary Non-EU module (see *http://barley.ipk-gatersleben.de).* Documentation of collections holding barley genetic resources in Europe, and the standardised evaluation of *Hordeum* spp. germplasm for resistance against biotic and abiotic stress factors are the objectives of this initiative. The International Barley Core Collection (BCC) is the major focus of the screening for stress resistance. Resistances against *Pyrenophora teres* and *Rhynchosporium secalis* and the virus diseases (BaYMV complex, BYDV) have been observed in accessions of the BCC. Resistance to *Erysiphe graminis* and *Puccinia hordei* is limited in the *H. vulgare* germplasm of the BCC and mainly restricted to well characterised cultivars. It appears that the BCC subset from ICARDA (incl. *H. vulgare* ssp. *spontaneum*) includes promising germplasm for resistance against leaf rust and powdery mildew. Observations made during pre-screening are being verified by multi-location testing. The European Barley Database is evolving into an information system providing geo-referenced passport, characterisation and evaluation data.

1. INTRODUCTION

The EU GENRES CT-98-104 project [1] is concerned with improved access to, and utilisation of, barley germplasm in Europe. The three year project began in 1999 with 28 partners (Breeders, Genebanks, Public Research Institutions).

In 2001 an additional 7 partners from non-EU countries, including several EU candidates, joined the project in its final phase (Fig. 1).

Our activities are focussed on two areas:

- Development of an information system for European barley collections comprising passport, characterisation and evaluation data.
- Evaluation of barley germplasm for resistance against biotic and abiotic stresses

2. THE EUROEPAN BARLEY DATABASE

The basis for the information system is an updated version of the European barley database (EBDB)[2, 3]. This is being developed as a backbone to link with information related to individual accessions.

The database is an inventory for barley germplasm held in 35 genebanks and for the barley core collection (BCC, 1126 accessions), totalling 137445 accessions. Two collections from outside of Europe, those of the International Center for Research in the Dry Areas, ICARDA and the Australian Winter Cereals Collection, Tamworth are also included.

For the passport data emphasis is being placed on improvement of geo-referencing (Fig. 2), standardisation of accession names according to published cultivar inventories [4, 5, 6] and compilation of synonymous accession numbers.

Accession numbers are stored in a maximum of three columns, separating prefix, numerical part and, if present, suffix. This format allows flexibility in linking with data files containing characterisation and evaluation information from individual collections und thus provides the primary key to related information about an accession.

3. EVALUATION OF THE BARLEY CORE COLLECTION FOR RESISTANCE AGAINST AND TOLERANCE TO BIOTIC STRESSES

The BCC has been conceived as a representative sample of the genetic diversity in the barley gene pool to provide a manageable set of genetically well defined accessions (maximum 2000 entries) for genetic, characterisation, and evaluation studies.

Screening for resistance against, in the European context, economically important fungal and viral diseases was carried on its complete East Asian, American, European, and partial South-West Asian subsets.

The aim of this study is to provide an overview of the available genetic diversity for disease resistance and to pinpoint particular parts of the genepool for further detailed evaluation.

For the years 1999, 2001 and 2000, 23899 observations were provided by partners of project GENRES CT98-104, funded by the European Union. These data were collected during three spring and two winter season. 22571 observations on BCC material were used as the basis for a first analysis. Two further seasons observations (winter 2000 and spring 2001) have been carried out but were not completly included in the present analysis.

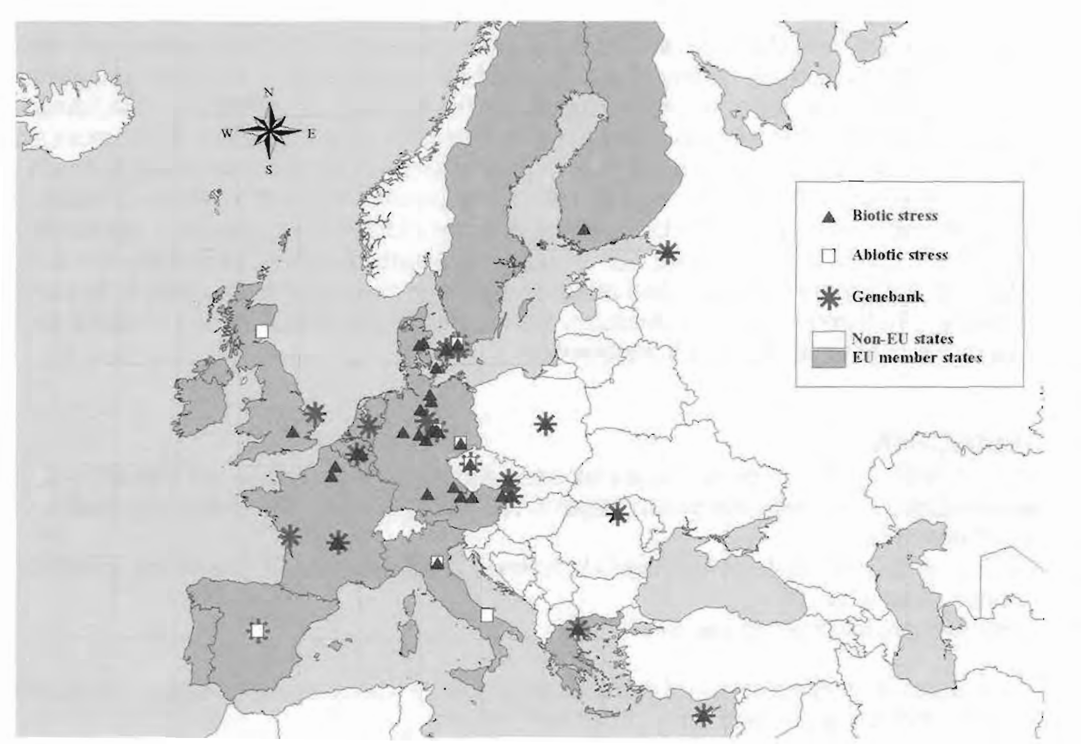

Fig 1. Distribution of Evaluation sites and Partner Institutes of the EU barley project in 2001

3.1. Methods

The methodology [7] used for field observations of fungal diseases is based on multiple assessments of the percentage of infected leaf area during the course of infection. The discrete observations and time intervals between them are then used to calculate a disease progression curve, from which an average ordinate is derived. Finally an average score is calculated (logarithmic intervals 1=0% infection, 9=100% infection) With this scoring system it is possible to detect genotypes with effective combinations of minor resistance genes, the so called horizontal or quantitative resistance. This type of resistance is considered more durable in the long term. The potential utility of such genetically broadly based resistance provides the stimulus to carry out the more labour intensive multiple scoring method.

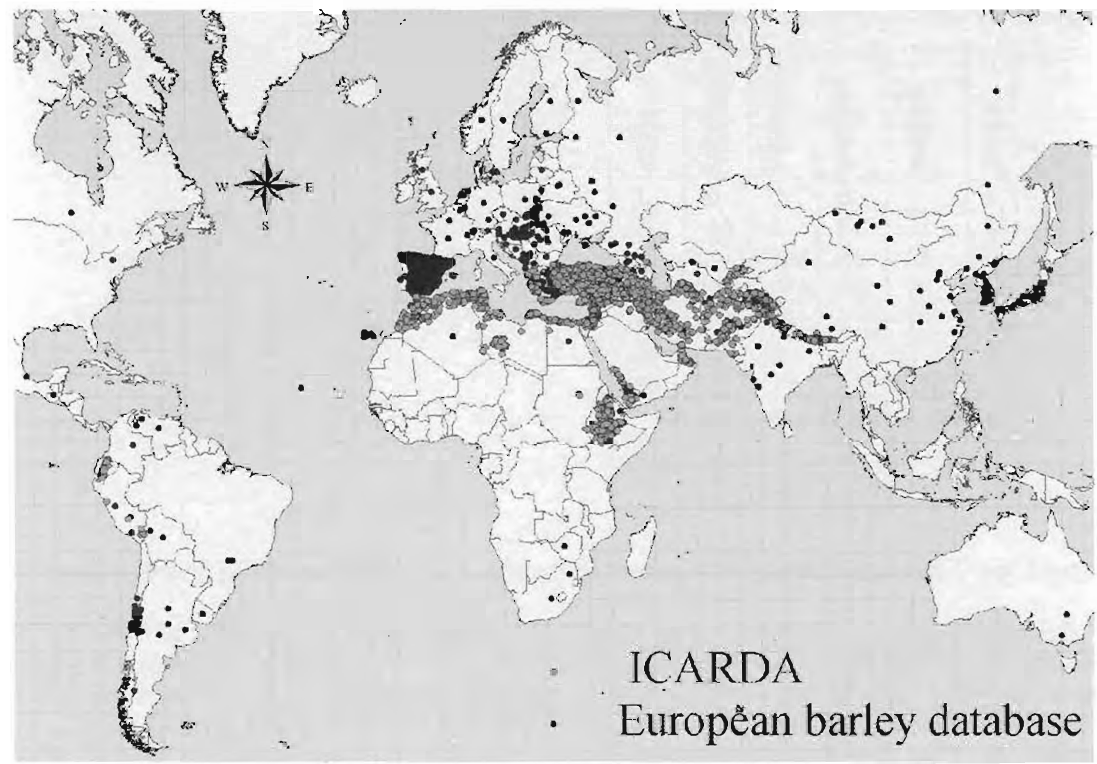

Fig 2. Geo-referenced accessions in the European barley database

The number of genotypes evaluated by individual partners varied from 60 to 400. In the first season the available accessions were distributed so that each would be evaluated at at least three different sites for each disease. The most promising material was provided to each experimenter during the second season. With capacities available to screen 300 to 400 accessions at some sites for several diseases, rapid pre-screening was possible.

Not all the planned activities regarding evaluation for particular resistances could be carried out. This is due to lack of natural infection pressure by some diseases as influenced by climatic conditions.

3.2. Fungal diseases

Differences between spring and winter barley, with higher frequency of susceptible scores for spring types, appear to relate more to increased infection pressure than to lower resistance levels.

Table 1. Frequency of infection scores in BCC winter barley accessions

Score	BaMMV	BYDV	leafrust	mildew	netblotch	scald	BaMMV + BaYMV_1	BaMMV + BaYMV_1+ BaYMV_2
1	61	0	0	0	2	25	57	82
2	0	1	2	0	107	7	11	13
3	12	1	31	54	136	44	12	3
4	0	7	84	158	64	90	26	9
5	0	62	111	114	6	124	13	19
6	0	98	88	40	0	116	7	4
7	0	80	8	8	0	40	19	51
8	0	41	0	2	1	20	10	7
9	27	9	0	0	0	5	32	111
Σ	100	299	324	376	316	471	187	299

Table 2. Frequency of infection scores in BCC spring barley accessions

Score	leafrust	mildew	netblotch	ramularia	scald
1	7	6	0	6	1
2	10	13	6	14	4
3	14	18	361	17	36
4	37	64	456	13	270
5	91	209	64	3	216
6	178	218	8	4	148
7	210	225	1	1	60
8	205	127	0	0	16
9	19	29	0	0	0
Σ	771	909	896	58	751

Tables 1 and 2 show the distribution of scores from evaluations of winter and spring barleys, respectively.

Resistances against *Pyrenophora teres* (netblotch) and *Rhynchosporium secalis* (scald) and the virus diseases (BaYMV complex, BYDV) have been observed in accessions of the BCC. Resistance to *Blumeria graminis* (mildew) and *Puccinia hordei* (leafrust) is limited in the *H. vulgare* germplasm of the BCC and mainly restricted to well characterised cultivars. It appears that the BCC subset from ICARDA (incl. *H. vulgare* ssp. *spontaneum*) includes promising germplasm for resistance against leaf rust and powdery mildew.

3.3. Viral diseases

Some genotypes with tolerance against BYDV were identified. No completely resistant forms were found so far.

A different picture has emerged for soil borne viruses belonging to the BaMMV - BaYMV complex. A high portion of genotypes with qualitative resistance could be identified. amongst these several with combined resistance against BaMMV, BaYMV type 1 and BaYMV type 2.
These accessions are now being assessed for the presence of known resistance genes using established molecular markers.

Detailed results, including accession numbers are to be published following 12 months after completion of the project.

REFERENCES

[1] Evaluation and conservation of barley genetic resources to improve their accessibility to breeders in Europe
http://barley.ipk-gatersleben.de

[2] Enneking, D. and Knüpffer, H.: The European Barley Database. In: Knüpffer, H., R. von Bothmer, M. Ambrose, R. Ellis, A. M. Stanca, D. Enneking, L. Maggioni & E. Lipman (compilers). Report of a Working Group on Barley. Sixth Meeting, 3 December 2000, Salsomaggiore, Italy. IPGRI, Rome (2001).

[3] The European barley database
http://barley.ipk-gatersleben.de/ebdb.php3

[4] Arias, G.; Reiner, A.; Penger, A., and Mangstl, A.: Directory of Barley cultivars and lines. Stuttgart: Eugen Ulmer (1983).

[5] Baum, B. R.; Bailey, L. G., and Thompson, B. K.: Barley register. Canada Governm. Publ. Centre. Cat. No. A53-1783/1985 (1985).

[6] Baumer, M. and Cais, R.: Abstammung der Gerstensorten. Freising: Bayerische Landesanstalt für Bodenkultur und Pflanzenbau (2000)
http://www.stmelf.bayern.de/lbp/forsch/pz/gerstenstamm.pdf

[7] Evaluation methods to screen barley for resistance to abiotic and biotic stresses
http://barley.ipk-gatersleben.de/EU_methods.htm

THE SPANISH BARLEY CORE COLLECTION

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ABSTRACT: Seven thousand years of barley cultivation under the environmental hardships typical of the Mediterranean climate have generated genetic singularity of the Spanish barleys, consistently reported in the literature. From the Spanish National Collection (BNG) of 2289 accessions, a Core subset with 159 landraces and 16 old varieties was constituted.

Comparisons of genetic diversity were performed with the use of the Shanon-Weaver index with the conclusion that the Core is a good representation of the existing diversity in the BNG. Comparisons with results of studies on Spanish materials from other collections seem to indicate that the Spanish diversity is not well represented in World Collections.

Twenty seven characters were evaluated for the Core collection to define the structure of the diversity. Through the use of principal component analyses, distinction between two- and six-row cultivars is very clear, and also between landraces and commercial varieties. Phenology and aptitude to get a good grain filling seem to be the main cause of the variation.

Geographical origins of the landraces were correlated with grain yield, heading and filling period, and growth class. In relation with diseases, altitude plays an important role on the resistance to *Erysiphe graminis and Puccinia hordei*. For *Puccinia* all the resistant landraces come from low altitude origins.

These geographical gradients seem consistent with prior knowledge about barley adaptation, and would confirm the real agreement between passport data and true adaptive origin of these landraces from a geographical point of view. Thorough agronomic evaluation, of the Spanish Core Collection, under differential environments and studies of diversity patterns based on molecular markers are currently undergoing, by a consortium of breeders and geneticists from three Spanish public research institutes.

INTRODUCTION

Barley has been cultivated in Spain for over 7000 years, under the environmental hardships typical of Mediterranean climate. Genetic singularity of Western Mediterranean barleys, including Spanish, has been consistently reported in the literature (1,2). These facts suggest the possible existence of specific adaptation traits in autochthonous barleys.

The Spanish National Germplasm Bank (BNG) holds a collection of 2289 barley accesions from which 1805 are landraces recollected in Spain prior to extensive introduction of modern varieties.

There is a great concern among barley breeders in Europe about the genetic narrowness of their germplasm (3), but in the other hand barley is a good example of a crop which could benefit from more extensive use of the so called non conventional germplasm that has evidenced contribution to the improvement of current cultivars (4) and in some environments it has not been overpassed by new varieties, with the persistence of landraces still under cultivation (5).

In Spain, since 1984 an extensive evaluation of these landraces, under semiarid conditions, has been carried out with breeding purposes. Recently a Spanish Core Collection has been assembled (5). The procedure followed the recommendations given by van Hintum (6). To maximize the potential genetic diversity, we followed a four steps system:

a) Classification of Spanish provinces into agro-ecological groups: similar yield levels, and consistency of yields over time (1948-1994).
b) Stratified sampling on agro-ecological groups, weighed by historical importance for barley cultivation (area cultivated in 1930-1960). Use of logarithmic proportionality.
c) Allocation of entries to provinces within agro-ecological groups proportional to province historical hectarage.
d) Maximizing diversity of entries within provinces, taking account of passport data and agronomic evaluations.

The objective of this presentation is to offer a progress report on the Spanish Core Collection.

MATERIAL AND METHODS

The Core Collection is constituted of 159 landraces plus 16 old varieties that have been extensively use in the country. The selected landraces were collected from 1931 till 1954, with a most extensive collection in 1944. Geographical distribution is shown in Figure 1.

Agronomic evaluation of 1650 entries of the BNG has been carried out under semi-arid conditions in the Ebro valley, with an average rainfall of 412 mm/year and extreme values of 232.2 and 699.5 mm. Evaluation was carried out with sets between 120 and 250 entries/year. Data were transformed into classes by using yearly averages and standard deviations.

Figure 1: Number of entries selected per province.

The components of the core collection have been characterized and disease evaluated, in addition, in the 1999-2000 season in Valladolid and Gerona. The characters evaluated are listed in Table I.

Table I. Characters evaluated

Qualitative characters	Qualitative characters
Growth class	Plant height
Row number	Heading
Spike density	Spikelet number
Awn roughness	1000 kernel weight
Rachilla hairs	Test weight
Kernel covering	*Puccinia hordei*
Growth Habit	*Erysiphe graminis*
Stem colour	Tillering
Purple auricle	Photoperiod sensit.
Leaf hairiness	Grain filling period
Glume and glume awn	Spike length
Awn colour	Kernels per spike
Waxiness	Grain Yield
	Lodging

For the measurement and comparison of phenotypic diversitiy, the Shannon-Weaver information index (H') was used, normalized as described by Tolbert et al. (1).

Differences of trait means among agro-ecological regions were checked by analysis of variance, using the variance of accessions within regions as error. Altitude and latitude effects on trait distributions were assessed by covariance analysis. Type III sums of squares were used to assess the relative contribution of the two covariables and the agro-ecological regions to each trait's variance. The entries of the core collection were classified using principal component analysis. This was done separately for qualitative and quantitative traits, as they may represent sets of loci with different selection history. All calculations were performed with PROC GLM and PROC PRINCOMP of SAS (9).

RESULTS AND DISCUSSION

Table II presents the H' values for comparison of genetic diversity. In one hand the comparison is between the Spanish Barley Collection (BNG) and its Core collection. Values show that there is a good representation of the

Spanish diversity in the Core subset. Last column shows the H' values for what we could call a good representation of the world diversity contained in the Germplasm Resources Information Network (GRIN) (8).

Table II. Genetic diversity (H')

Characters	BNG	Core	USDA
Row number	0,249	0,363	0,903
Spike density	0,848	0,787	0,663
Awn roughness	0,385	0,169	0,342
Rachilla hairs	0,750	1,000	0,712
Kernel covering	0,030	0,000	0,561

Comparisons with frequencies and H' values of Spanish landraces included in other studies, such as Cross (7) with 20 entries from Morocco, Portugal and Spain from the New Zealand Collection, and Tolbert et al. (1) with 63 Spanish landraces from the USDA Collection, suggest that the Spanish diversity present in some of the so called World Collections is not a good representation of the existing diversity.

Table III. Evaluation of Spanish landraces (H')

Characters	Cross	Core	Tolbert
Growth class	0,000	0,640	0,610
Row number	0,722	0,363	0,530
Spike density	0,937	0,787	
Awn roughness	0,000	0,169	0,220
Rachilla hairs	0,881	1,000	
Kernel covering	0,469	0,000	0,000
Growth Habit	0,000	0,861	
Stem colour	0,000	0,999	
Purple auricle	0,779	0,951	
Awn colour	0,934	0,701	

To analyse the structure of the genetic diversity among the set of accessions that constitutes the Spanish Core collection we performed principal component analysis. In spite of the fact that similar results were obtained when qualitative and quantitative variables were analysed together, we prefer to present separate results showing in one side quantitative characters that have been subject to direct human pressure and in the other qualitative characters, that could be defined as neutrals from a selective point of view.

In Figure 2 we can observe the two principal components calculated for the quantitative characters, responsible of 43% of the variance.

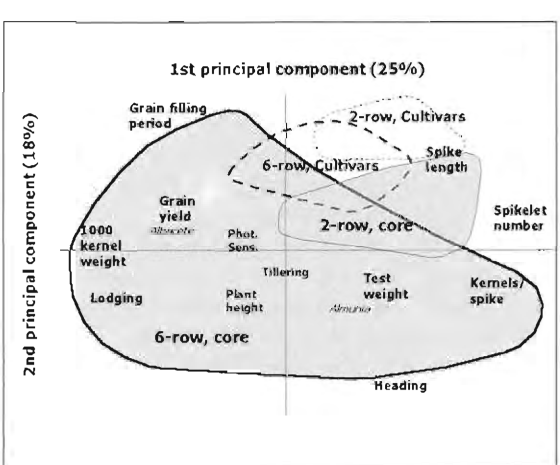

Figure 2: Quantitative characters (PCA).

Characters related with spike and yield present the largest loadings on the first component, and phenology for the second component. Some conclusions could be extracted for these material, first of all is that yield and lodging are very related, and secondly that with these material the way to get yield is not to have more grains but to get a good grain filling. At the same time early flowering and long filling periods result in a better behaviour of the materials. Distinction between six- and two-rows is very clear, and also between landraces and commercial varieties, with the exception of Albacete and Almunia, clearly due to its origins from local populations. Commercial and two-row material occupy the North-East quadrant of the graph. Six- row landraces covered the rest of the quadrants in a clear proof of wide diversity.

In Figure 3 we observe the two principal components calculated for the qualitative characters, which were responsible for 39% of the variance. Number of rows was not included in the analyses, but is very clear again the distinctiveness of both groups with the exception of the Alpha variety that looks to show its six-row origin.

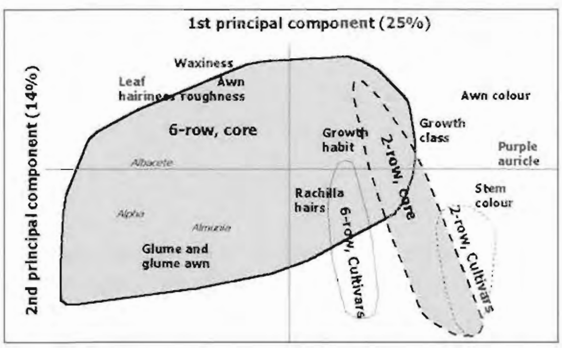

Figure 3: Qualitative characters (PCA).

Pigmentation characters seem to pull in the direction of the two- row material, and the explanation must be either the isolated evolution of the two types of material, or the presence in the same arm of chromosome 2 of the pigmentation genes and the gene *vrs1*, main responsible of the number of rows.

Possible geographical influences were analysed, working with Latitude and Altitude. Results are presented in Table IV.

Table IV. Geographical influences

LATITUDE	ALTITUDE	R^2 (%)
	Growth habit (-)	6
Heading (+)		18
Growth class (-)		10
Spikelet number (+)		8
Grain filling (-)	Grain filling (-)	13
Spike density (-)		6
Grain yield (-)		18
Kernel weight (-)		6
Test weight (+)		8
E. graminis (+)	*E. graminis* (+)	18
	P. hordei (+)	25

(+,-; sense of the correlation)

Grain yield, heading and filling period, and growth class were correlated with the geographical origin of the landraces. In relation with diseases, altitude plays an important role on the resistance to *Erysiphe graminis* and *Puccinia hordei*. For *Puccinia* all the resistant landraces come from low altitude origins (Figure 4).

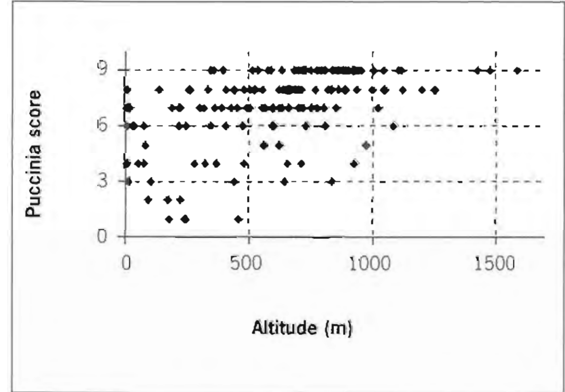

Figure 4: Altitude and *Puccinia* susceptibility.

These geographical gradients seem consistent with prior knowledge about barley adaptation, and would confirm the real agreement between passport data and true adaptive origin of these landraces from a geographical point of view.

Also significant differences were found among agro-ecological regions on some of the characters as leaf hairiness, awn colour, susceptibility to *Erysiphe graminis* and grain yield. On this last trait, it is possible to observe how these differences were not related with the potential yield of each region (Table V). The behaviour of groups of accessions coming from different origins gave differential responses when tested in a common region like, in this case, in Ebro-Centre.

Table V. Yield and origin

Agro-ecological region	Historic yield	Core evaluation
South	1.086	1.737
Ebro-Centre	1.576	1.498
Castilla-Leon	1.876	1.437
High yield	2.056	1.737

Our work is currently focused in a very thorough agronomic evaluation of the Spanish barley Core collection under differential environments, and in studies of diversity patterns based on molecular markers. About fifty microsatellites showing consistent results and good discrimination among barley cultivars, and a small number of RFLP probes will be tested.

A representative set of six-row European cultivars will be included in the analysis for comparative purposes. These results, together with the morphological and agronomic evaluations, will provide a more consistent picture on the nature and distribution of genetic diversity in Spain.

Finally, the authors of this work, as plant breeders, want to express that the use of this landraces in our breeding programme has produced good results for semi-arid areas. By direct crossing, some varieties like *Candela* have been obtained, and the recombination of some of these landraces through the use of populations and male sterile facilitated recurrent selection could be of real interest.

REFERENCES

(1) Tolbert D.M., Qualset C.O., Jain S.K., Craddock J.C.: A Diversity Analysis of a World Collection of Barley. Crop Sci. 19:789-794 (1979).

(2) Peeters JP: The emergence of new centres of diversity: evidence from barley. Theor. Appl. Genet., 76:17-24 (1988).

(3) Melchinger A.E., Graner A., Singh M., and Messmer M.M.: Relationships among European Barley Germplasm: I. Genetic Diversity among Winter and Spring Cultivars Revealed by RFLPs. Crop Sci. 34:1191-1199 (1994).

(4) Hadjichristodoulou A: Evaluation of barley landraces and selections from natural outcross of *H. vulgare* ssp. *Spontaneum* with ssp. *vulgare* for breeding in semi-arid areas. Genetic Resources and Crop Evolution 42:83-89 (1995).

(5) Igartua E., Gracia M.P., Lasa J.M., Medina B., Molina-Cano J.L., Montoya J.L., Romagosa I: The Spanish Barley Core Collection. Genetic Resources and Crop Evolution 45:475-481 (1998).

(6) Van Hintum Th.J.L.: Core collections in germplasm conservation: evaluation and use. In: Scoles G, Rossnagel B (eds) Proc. V Internat. Oat Conf, & VII Internat. Barley Simp. Univ. Saskatchewan, Saskatoon (1996).

(7) Cross R.J.: Geographical trends within a diverse spring barley collection as identified by agro/morphological and electrophoretic data. Theor. Appl. Genet. 88:597-603 (1994).

(8) USDA, ARS, National Genetic Resources Information Network - (GRIN) (Online Database) National Germplasm Resources Laboratory, Beltsville, Maryland (2001).

(9) SAS Institute Inc.: SAS/STAT User's Guide, Release 6.03 Edition. Cary, NC (1988).

SYNTHETIC HEXAPLOIDS FOR BREAD WHEAT IMPROVEMENT

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ABSTRACT: The accessions of the primary gene pool diploid wheat relative, *Aegilops tauschii* (2n=2x=14, DD); syn. *Ae. squarrosa, Triticum tauschii*; constitute a unique source of novel genetic variability for bread wheat improvement because of their diversity and global distribution. The germplasm has the potential of providing resistance/tolerance to several biotic/abiotic stresses that reduce the crops productivity in several wheat growing environments. Due to the stress sreening constraints, *Ae. tauschii's* winter habit, and its tendency for grain shattering, the accessions were indiscriminately hybridized with elite durum wheat (*T. turgidum*) cultivars, producing 800 synthetic hexaploids (SH), 2n=6x=42, AABBDD) to date. Reported here is the current status of stress resistances identified in these synthetics, and also the transfer of the traits to elite but stress susceptible bread wheats. The stresses are *Fusarium* head scab, *Helminthosporium sativum, Neovossia indica, Septoria tritici*, and salinity. Gene pyramiding involving different *Ae. tauschii* accessions is addressed, and the necessity of DNA fingerprinting of a mapping population with multiple stress resistances (MDR) mentioned.

INTRODUCTION

In conventional improvement of bread wheat (*Triticum aestivum* L.; 2n=6x=42; AABBDD), breeders have normally made crosses between varieties. Such crosses have few constraints and invariably all associations of parental traits and segregation are based on genetic recombination. The next step in bread wheat improvement is to tap the varied gene pools of other *Triticum* species. For swift outputs from such crosses, breeders utilize the numerous alien accessions of species whose genomes are similar to the A, B, or D genomes of bread wheat. These crosses allow for relatively easy alien gene transfers, are compatible with normal field research, and set the stage for the successful introgression of several genes simultaneously by homologous exchange. Elucidated here is the interspecific hybridization area where the focus is on the D genome diploid *Aegilops tauschii* (Syn. *Ae. squarrosa, Triticum tauschii*).

The procedures used to incorporate such alien variability and the choice of genome to work will differ among researchers. Presented is the utilization of the *Ae. tauschii* (goat grass) diversity. The accessional diversity provides a unique opportunity for exploiting new genetic variability that encompasses a wide range of resistances or tolerances to biotic or abiotic stresses. Some of these outputs are described and their potential in wheat improvement documented for various stress constraints.

MATERIALS AND METHODS

Germplasm Development

Elite durum wheat (*Triticum turgidum* L. s.lat.) cultivars were crossed with several hundred *Aegilops squarrosa* accessions. Embryos were rescued, plated in artificial media, differentiated, and yielded F1 hybrids (2n=3x=21, ABD) that upon colchicine treatment produced 2n=6x=42, AABBDD synthetic hexaploids (SH). Accession acquisition and procedures for SH production have been described (3). These SH wheats are maintained by increasing seed of each combination under controlled conditions by glassine bagging at least 50 spikes per combination at each increase cycle. Based upon growth performance in two Mexican locations (Ciudad Obregon and El Batan) an elite set of 95 SH entries has been assembled for global distribution. Distribution of this elite SH set is handled by CIMMYT's Genetic Resources Group.

Germplasm Screening

The SH germplasm was screened for Helminthosporium sativum (Cochliobolus sativus), Fusarium graminearum (Head Scab), Septoria tritici, and Tilletia indica (Karnal Bunt) in Poza Rica, Toluca (Scab and Septoria), and Obregon, Mexico, respectively over three to five years which forms the basis of conclusions made in this paper. All evaluations were under field conditions. The synthetics were planted in hill plots . The evaluations protocols were similar to those earlier reported by (4) for H. sativum, (6) for Fusarium, (5) for S. tritici, and (7) for N. indica.

Abiotic stress screening for salinity was conducted under greenhouse conditions at El Batan, Mexico according to the hydroponic protocol of (1) and (9) based upon K:Na discrimination after a 21 day growth of the seedlings at 50mM NaCl

Identification and utilization of resistant SH germplasm

From data of the above five stress tests resistant SH's were identified and hybridized with elite but stress susceptible bread wheat cultivars. The hybrids were advanced by the pedigree method with the focus being on making selections for plant type, maturity, height and specific plus multiple disease resistance keeping the global cultivation scenario into consideration. Protocols for evaluation and the test sites were similar to those described for the SH germplasm above. The SH/BW or BW/SH entries from all biotic stress related germplasm were field planted in 2 to 3m double rows, tested and led to desired selections which were stabilized using the maize based doubled haploid protocol (2).

Germplasm Distribution

For each of the four biotic stresses SH wheats and their advanced derivatives from crosses onto BW have been distributed to wheat breeding programs upon request and also registered as germplasm stocks in Crop Science with samples of each entry reported deposited in the US germplasm bank, and the Gene Bank of CIMMYT in Mexico. Small amounts (3g) of the samples could further be obtained from this papers author on a one time basis only.

Mapping Population Development

The main biotic stress priority currently is *F. graminearum* head scab in bread wheat. Some superior advanced Bread Wheat (BW)/SH derivatives have been identified that are being used by breeders in CIMMYT, USA, and some National breeding programs for wheat improvement. One such line (Mayoor//TKSN1081/*Ae. tauschii* 222) possesses Scab resistance across all four categories; Type I (Penetration), Type II (Spread), Type III (Toxin-DON), Type IV (Test Weight losses). This line also possesses multiple disease resistance for all three rusts, *H. sativum*, *S. tritici* and *N. indica*. A bread wheat cultivar Flycatcher is susceptible to all scab types and the other six stresses. This has formed the basis of developing a mapping population which involves crossing the resistant and the susceptible line. The F1 derivatives were crossed with maize and 170 doubled haploids produced that form the mapping population for molecular study and phenotyping currently underway in Mexico.

RESULTS and DISCUSSION

SH Status

From our working collection of *Ae. tauschii* accessions that currently come to about 600 we have randomly hybridized them to elite durum wheat cultivars. So far 800 synthetics have been produced over the last decade and from these one elite set of 95 entries has been compiled and globally distributed. The set has some descriptors and can be accessed on the web site **http//www.KSU.edu/wgre/ germplasm/synthetics.html** jointly structured by Dr B.S. Gill's group in Kansas State University, Kansas, USA and our group in CIMMYT, Mexico. Not all the 600 accessions have been combined, but attempts are in progress to accomplish this. There are three categories of SH's : 1) Same durum wheat with different accessions, 2) different durums with the same accession, and 3) durum wheat/an accession and its reciprocal cross. In addition beyond the 800 synthetics are SH's from a gene pyramiding program where SH wheats with *Ae. tauschii* accessions of diverse nature involved in bestowing stress resistance were hybridized, and highly resistant F2 plants (superior resistance than their individual SH's) used for producing stable DH derivatives. Sub-sets of SH's involved in the various biotic and abiotic stresses have been prepared and are the subject for DNA fingerprinting using D genome microsatellites; an aspect underway with collaborators in the molecular biology area.

Selection Criteria for Resistant/Tolerant SH's and their derivatives

The criteria set for identifying resistance or tolerance to biotic/abiotic stress for traits of this study were set from data existant in literature and cited in the earlier section of the materials and methods. The limits set were stringent for the germplasms to be advanced for pre-breeding objectives. These were not to exceed a 3-3 double digit score for *H. sativum* and 2-2 for *S. tritici*, 15.0% or less Type 11 infection score for *F. graminearum*, and less than 3.0% infected kernels for *N. indica*. For salinity, tolerant germplasms were to possess K:Na discrimination values of greater than 2.0 where values close to 1.0 were associated with non-tolerance to the stress.

Synthetic related germplasm for biotic stress resistances
***Fusarium graminearum* (Head scab).**

Resistance in synthetic hexaploid wheats: The SH wheats (*T. turgidum* x *Ae. tauschii*) most resistant (less than 15% infection) to *Fusarium graminearum* (Type II) are presented in Table 1. Resistant BW check Sumai 3 scored around 15% or slightly less, while the moderately susceptible BW check 'Flycatcher' always had over 20% infection and the durum wheat 'Altar 84' over 40%. After several cycles of testing some advanced BW/SH scab resistant entries were selected for Type II resistance (Table 1). These derivatives generally also possessed resistance to leaf rust, stripe rust and *Septoria tritici*. Each scab resistant entry selected had a disease score of less than 15% across each test year. Sumai-3 averaged 12% over the three test years.

The most promising entries from the BW/SH combinations were further tested for the other three scab categories (I, III, IV). Four were found to possess combined resistance to all four types of scab. These are currently being used in bread wheat breeding at CIMMYT and in the collaborative activity with the US Scab Initiative.

The combination Mayoor//TK SN 1081/*Ae. tauschii* (222) and several of its sister lines exhibited superior scab resistance across its four categories and also possess resistance to *S. tritici*, *N. indica*, and *H. sativum*. One line was crossed with 'Flycatcher' (susceptible to all the above stresses), and the F_1 seed used to produce 170 doubled haploids (DH) for molecular mapping/phenotyping.

Table 1. Promising D genome synthetic hexaploids and some SH and BW derivatives screened for head scab (Type II) at Toluca, Mexico; mean values across years.

Germplasm pedigree	Infection Type II(%)
SYNTHETICS	
YUK/ (217)†	11.6
68.111/RGB-U//WARD/3/FGO/4/RABI/5/ (629)	10.9
68.111/RGB-U//WARD/3/FGO/4/RABI/5/ (878)	12.7
68.111/RGB-U//WARD/3/FGO/4/RABI/5/ (882)	12.3
SORA/ (884)	13.2
68.111/RGB-U//WARD/3/FGO/4/RABI/5/(890)	12.7
CETA/ (895)	12.0
GAN/ (180)	10.8
LCK59.61/ (313)	11.8
SCOOP 1/ (358)	12.9
YUK/ (217)	11.6
TRN/ (700)	13.5
DOY1/ (333)	12.5
DVERD_2/(1027)	13.1
MAYOOR//TK SN1081/ (222)	8.7
FLYCATCHER (Susceptible)	33.8
SUMAI-3 (Resistant)	12.0
ALTAR 84	40.8

CONTINUACIÓN ⟶

Germplasm pedigree	Infection Type II(%)
SYNTHETICS	
PRE-BREEDING DERIVATIVES	
BCN//DOY1/ (447)	12.5
BCN/DOY1/ (447)	12.0
ALTAR 84/ (224)//2*YACO	10.1
OPATA/6/RGB-U//WARD/3/FGO...(878)	11.6
SABUF/5/BCN/4/RABI//GS/CRA/3/(190)	11.2
SABUF/3/BCN//CETA/(895)	11.0
MAYOOR//TK SN1081/(222)	8.9
FLYCATCHER (Susceptible)	33.8
SUMAI-3 (Resistant)	12.0

* Percentage score means from 10 spikes tested.
† Numbers in parenthesis = *Aegilops tauschii* accessions in wide crosses working collection.

Helminthosporium sativum (Spot blotch)

The disease effects wheat crops across several environments from Latin America, Africa, Asia and South East Asia with Bangladesh being represented as a major disease location. Our Mexican screening site is the severest of all. Several SH/BW germplasms were evaluated at this location based upon damage recorded progressively (79 to 96d) on leaves and grain (Table 2). All lines possessed superior *C. sativus* resistance as compared with 'Mayoor' a resistant check, and 'Ciano 79', a susceptible check.

The synthetic hexaploids represent diverse accessional gene pyramiding and were developed by intercrossing several different *T. turgidum/Ae. tauschii* involving different *Ae. tauschii* accessions. From segregating F_2 populations, spot blotch resistant plants were selected and hybridized with *Zea mays*. The resulting haploids (n=3x=21, ABD) were colchicine treated (3) to yield homozygous doubled derivatives (2n=6x=42, AABBDD). The seven bread wheat germplasms were derived from various SH lines crossed with the spot blotch susceptible bread wheat cultivars 'Bacanora', 'Opata', and 'Yaco'.

Some of the best lines (SH and advanced lines) are in Table 2. The disease scores does not exceed 3-2 and grain finish is less than 2 versus a susceptible score of 9-9 and 4 respectively.

Table 2. Characteristic of five double haploid synthetic hexaploid wheats, and seven bread wheat germplasm lines resistant to *H. sativum*; mean values across years

Pedigree	Infection Score (days)	Grain finish‡
Synthetic hexaploids		
Gan/(236)†//Doy 1/ (447)	2-2	1
Gan/(236)//Ceta/(895)	2-2	2
Scoop 1/(434)//Ceta/(895)	3-3	1
Doy 1/(447)//Ceta/ (895)	3-3	2
68.111/Rgn-u//Ward/3/ Fgo/4/(629) /5/Ceta/(895)	3-3	1
Ciano 79 (Susceptible)	9-9	4
LSD 0.01	0.4	
Pre-breeding advanced derivatives		
Altar/(224)//2*Yaco	3-2	1
Sabuf//Altar/(224)/3/Yaco/Croc_1/ (205)	2.2	2

CONTINUACIÓN ⟶

Pedigree	Infection Score (days)	Grain finish‡
Bcn//Sora/(323)	2-2	2
Opata/3/Sora// (323)	3-3	2
Bcn/4/68.111/Rgb-u//Ward/3/ (325)	2-1	1
Bcn//Doy/(447)	3-2	1
Mayoor (Resistant)	9-3	2
Ciano 79 (Susceptible)	9-9	4
LSD 0.01 -	0.4	

† Numbers in parenthesis = *Aegilops tauschii* accessions in wide crosses working collection.
‡ Infection scores rated on a double digit modified scale: the first digit indicates the height of infection where 1=lowest leaf; 5=up to mid plant; and 9=up to flag leaf, the second digit indicates Disease severity on infected leaves, where 1=10% coverage; 5=50% and 9=90% coverage
‡ Grain finish scale of 1 to 5, where 1=low grain infection and 5=severely infected.

Septoria tritici *(Leaf blotch)*

Septoria leaf blotch limits wheat production in high rainfall areas across 10.4 million hectares globally. *S. tritici* (syn. Mycosphaerella graminis) resistant synthetic hexaploids (SH) crossed with the *S. tritici* wheat cultivars Seri M82, Yaco, Borlaug M95, Opata M85, Kauz, Papago M86, and the moderately resistant cultivar Bagula, gave advanced lines with good leaf blotch resistance (5).

Ratings of *S. tritici* resistance were based upon leaf damage recorded at water, milk, and dough growth stages according to (10) using a double digit modified scale (8). The disease ratings of each of the germplasms indicated their superiority over the bread wheat check cultivars (scores of 2-1 or 1.1 vs. 4.1 to 8.9). All germplasms have a good agronomic plant type and were high yielding under optimum disease free environments.

Pedigrees, disease scoring, and agronomic phenotype descriptor details of some of these registered lines are in (5).

Neovossia. indica *(Karnal bunt)*

Several of the *Ae. tauschii* accessions in our working collection were identified as sources of Karnal bunt resistance. These accessions were randomly hybridized with *T. turgidum* cultivars to yield SH wheats and resistant entries identified. The bread wheat germplasm lines were derived from the karnal bunt resistant synthetic hexaploids crossed with karnal bunt susceptible bread wheat cultivars Flycatcher, Kauz, Yaco, Borlaug and Papago M86. Segregating generations of the crosses were advanced by pedigree method. The mean agronomic performance of the germplasm lines over five years of field tests demonstrates an acceptable phenotype; this is an asset for breeding use.

The disease score was based on the number of infected and healthy kernels at maturity in each plot. Synthetic hexaploid and bread wheat germplasm line infections ranged from 0% up to 1.97% compared with a 30% mean infection of 'WL711', the susceptible bread wheat check cultivar. The durum wheats in the pedigrees had infection levels from 0.3 to 1.6%, while the SH wheats were immune. These germplasms offer genetic diversity of the *Ae. tauschii* accessions as well as the A and B genome diversity of the durum cultivars in the SH pedigrees.

Abiotic stress *(Salinity tolerance)*

K^+/Na^+ discrimination is a trait which enhances salinity tolerance in bread wheat compared to durum wheat, and is present in the wheat ancestor *Aegilops tauschii*. K^+/Na^+ ratios were lower in the durum parents (close to 1.0) than in the elite synthetics (over 1.0 and greater than 2.0 being selected for breeding use). This confirmed that the K^+/Na^+ trait was present in the synthetics, and demonstrated its expression in durum wheats. The best-performing synthetics with high K^+/Na^+ ratios are in Table 3.

CONCLUSION

- *Ae. tauschii* is a valuable source of genetic diversity for resistance to biotic stresses that can contribute to bread wheat improvement. These resistances are distributed over several accessions.
- Fusarium head scab is a priority constraint currently.
- Synthetic hexaploid wheats derived from *T. turgidum* x *Ae. tauschii* crosses express moderate levels of diversity for scab resistance equivalent to resistance levels in the best bread wheat cultivars.
- This resistance has been transferred to elite-but-susceptible bread wheat cultivars.
- The most promising line- the multiple disease resistant Mayoor//TK SN1081/*Ae. tauschii* (222)-has been crossed with Flycatcher (susceptible) and a DH population developed from the F_1 progeny for molecular mapping for several stresses and for phenotyping.
- Germplasm registrations of material other than scab made in Crop Science (2000-2001) allow access to global germplasm use by collaborators.

Table 3. Best synthetic hexaploids from the Elite set of 95 entries with high K:Na Ratios as determined from leaf tissue sampled from growth of seedlings in hydroponics in 50 mMol NaCl

Pedigree	K:Na
68.111/Rgb-U//Ward Resel/3/Stil/4/ (781) †	4.5
68.111/Rgb-U//Ward/3/Fgo/4/Rabi/5/ (883)	3.0
68112/Ward// (369)	3.2
Altar 84/ (224)	4.9
Altar 84/ (502)	5.2
Altar 84/ (220)	3.0
Altar 84/ (211)	3.5
Altar 84/ (Bangor)	3.7
Ceta/ (1027)	3.5
Ceta/ (895)	3.9
Croc 1/ (224)	3.9
D67.2/P66.270// (220)	3.5
D67.2/P66.270// (213)	3.4

† = *Ae. tauschii* with accession number/source in parenthesis

REFERENCES

[1] Gorham J, Hardy C, Wyn Jones RG, Joppa L and Law C. 1987. Chromosomal location of a K+/Na+ discrimination character in the D genome of wheat. Theor. Appl. Genet. 74:584-588.

[2] Mujeeb-Kazi A. 2000. An analysis of the use of haploidy in wheat improvement. *In:* Kohli MM and Francis M *ed.* Application of Biotechnologies to Wheat Breeding. Proc. of a Conference at La Estanzuela, Uruguay, 1998, Uruguay. pp. 33-47.

[3] Mujeeb-Kazi A, Rosas V, Roldan S. 1996a. Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. Non L.) in synthetic hexaploid wheats (*T. turgidum* L. s.lat. x *T. tauschii*; 2n=6x=42, AABBDD) and its potential utilization for wheat improvement. Genet. Res. Crop Evol. 43:129-134

[4] Mujeeb-Kazi A, Villareal RL, Gilchrist L, Rajaram S. 1996b. Registration of five wheat germplasm lines resistant to *Helminthosporium* leaf blight. Crop Science 36: 216-217.

[5] Mujeeb-Kazi A, Gilchrist LI, Villareal RL, Delgado R. 2000a. Registration of 10 wheat germplasms resistant to *Septoria tritici* leaf blotch. Crop Science 40:590-591.

[6] Mujeeb-Kazi A, Delgado R, Cano S, Rosas V and Cortes A. 2000b. Alien genetic diversity for wheat improvement: Focus on scab resistance. 2000 National Fusarium Head Blight Forum Procedings, *ed.* Ward RW, Canty SM, Lewis J and Siler L, Cincinnati, Ohio, USA. Pp 220-224.

[7] Mujeeb-Kazi A, Fuentes-Davila G, Villareal RL, Cortés A, Rosas V, Delgado R. 2001c. Registration of 10 synthetic hexaploid wheat and six bread wheat germplasms resistant to karnal bunt. Crop Science (In press).

[8] Saari EE, Prescott JM. 1975. A scale of appraising the foliar intensity of wheat diseases. Plat Dis. Rep. 59:377-380.
[9] Shah S, Gorham J, Forster B and Wyn Jones RG. 1987.Salt tolerance in the Triticeae: the contribution of the D genome to cation selectivity in hexaploid wheat. J. Exp. Bot. 38:254-269.
[10] Zadoks JC, Chang TT, Konzak, CF. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415-421.

GENE FLOW AND HYBRIDIZATION BETWEEN WHEAT (*TRITICUM AESTIVUM*) AND ITS WILD WEEDY RELATIVE JOINTED GOATGRASS (*AEGILOPS CYLINDRICA*)

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ABSTRACT: The possibility of gene flow between a cultivated species and their wild relatives is a concern, especially in herbicide-resistant crop systems. In the Midwestern and Western United States, jointed goatgrass (*Aegilops cylindrica*: CD genome) is a major weed of winter bread wheat (*Triticum aestivum*: ABD genomes). Weed control, particularly selective herbicide treatment, is difficult because wheat and jointed goatgrass are closely related. Wheats with herbicide-resistance offer a promising control method. Because these two species share the D genome, development strategies for transgenic wheat are focusing on the A and B genomes. Traditionally, hybrids between wheat and jointed goatgrass were believed to be sterile, but partially fertile hybrids and backcross derivatives have been found under field conditions and have been reproduced experimentally. This fact presents a challenge to evaluate the possibility of intra- and intergenomic gene flow between these species and to study the implications of positive herbicide selection in the fixation of herbicide-resistance genes in jointed goatgrass. Several projects are underway to study gene flow and hybridization between jointed goatgrass and wheat. These projects include evaluating the effect of herbicide-resistance transgene location on the wheat genome, evaluating the genetic diversity of jointed goatgrass across its native and adventive distributions, constructing a linkage map of jointed goatgrass, determining mating patterns of wheat and jointed goatgrass in the field, and evaluating seed dormancy and plant vernalization requirements in hybrids and backcrosses. Information from these projects will be used to design management strategies to prevent gene flow from herbicide-resistant wheat.

1. INTRODUCTION

Crops that have been documented to naturally hybridize with their wild relatives include wheat (*Triticum aestivum*) rice (*Oryza sativa*), maize (*Zea mays*), soybean (*Glycine max*), cotton (*Gossypium hirsutum*), sorghum (*Sorghum bicolor*), bean (*Phaseolus vulgaris*), rapeseed (*Brassica napus*), sunflower (*Helianthus annuus*), and sugar cane (*Saccharum officinarum*) [1]. The potential for gene flow between crops and weeds has been demonstrated in cases involving sunflower [2] sorghum [3], maize [4], and *Cucurbita* [5]. Long-term establishment of crop genes in sunflower also has been documented [6]. The sexual transfer of transgenes from a crop to a weed or potential weed is considered to be the greatest risk of developing and releasing transgenic crops [7]. The transfer of genes or transgenes that confer traits such as herbicide-resistance could produce a plant that can no longer be controlled easily and may be more aggressive than the wild type. Such a transfer would result in the loss of a herbicide-resistant crop as a management tool and the possible creation of an even greater management problem, a herbicide-resistant weed.

In the Midwestern and Western United States, jointed goatgrass (*Aegilops cylindrica*) is a major weed infesting an estimated 3 million hectares of winter wheat (*T. aestivum*) [8]. Weed control, particularly selective herbicide treatment, is difficult because wheat and jointed goatgrass are closely related. Jointed goatgrass is an allotetraploid with 28 chromosomes. Each chromosome set (genome) contains 7 chromosomes pairs that originated from two wild species designated by C and D [9]. Jointed goatgrass and wheat

share a common ancestor, *Ae. tauschii*, the donor of the D genome [9]. In addition to the D genome, wheat, an allohexaploid, also contains the A and B genomes donated by two other wild wheat species. The similarity of the D genomes of wheat and jointed goatgrass is reflected by the level of D-genome chromosome pairing during meiosis in wheat x jointed goatgrass hybrids [10]. Besides weed control issues, the close relationship between wheat and jointed goatgrass could also allow for the transfer of herbicide-resistance into jointed goatgrass via a hybrid bridge. Hybrids between wheat and jointed goatgrass produced from controlled crosses [11] and cross-pollination under field conditions [12, 13] have been well documented. In a recent study, Guadagnuolo et al. [14] reported that the mean hybridization rate between jointed goatgrass and wheat under field conditions in Switzerland was 3%. Similar results were obtained in a survey of 23 wheat field sites in Oregon (USA) where the mean hybridization rate was 1.6% (Morrison LA and Mallory Smith CA, pers. comm.). Even though the hybridization rate between these two species is relatively low, the presence of hybrids in field sites is significant due to the extensive area of their overlapping distribution. In Oregon (USA), 48% of 104 wheat field and non-field sites that have been surveyed contained wheat x jointed goatgrass hybrid populations (Morrison LA and Mallory-Smith CA, pers. comm.).

2. REPRODUCTIVE BEHAVIOR OF WHEAT X JOINTED GOATGRASS F1 HYBRIDS

Historically, wheat x jointed goatgrass hybrids have been described as being sterile [12, 15]. However, the production of viable seed on hybrid plants under field [16, 17, 18] and greenhouse [19] conditions suggest that hybrids have partial female fertility. Zemetra et al. [19] and Wang et al. [20] reported that experimental wheat x jointed goatgrass F_1 hybrids were male sterile but had about 1 to 2% female fertility. In a two-year field trial, Snyder et al. [18] observed that mean hybrid fertility ranged from 2 to 4% in different years. An analysis of about 5500 hybrid spikes from 50 field sites in Oregon (USA) showed that the fertility of hybrids under field conditions was 1% (Morrison LA and Mallory-Smith CA, pers. comm.). Evidence from artificial and natural cross-pollination has shown that viable seed can be produced on hybrids with either jointed goatgrass or wheat as a pollen donor. What determines the preponderant pollen donor under field conditions is still unclear but studies are underway to address this issue.

The cytogenetic basis for female fertility in wheat x jointed goatgrass hybrids has not been described. However, an examination of the chromosome composition of BC_1 generation plants from various studies [19, 17, 21, 22] suggest that partial female fertility in hybrids in the greenhouse or in the field is due to the production of both restitution and non-restitution gametes. The female gamete population has a bimodal distribution with peaks in the ranges of 22 to 23 and 32 to 33 chromosomes (Fig. 1). Gametes with more than 28 chromosomes probably resulted from some form of meiotic restitution while gametes with fewer than 28 chromosomes probably arose from chromosome segregation.

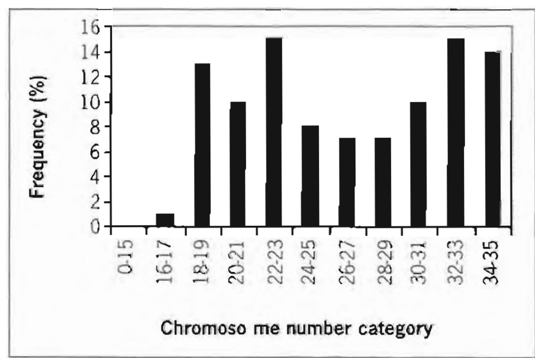

Figure 1: Distribution of female gamete chromosome numbers in *T. aestivum* x *Ae. cylindrica* hybrids. Data compiled from Zemetra et al. [19], Cremieux [21], Seefeldt et al. [17], and Wang [22].

3. REPRODUCTIVE BEHAVIOR OF BACKCROSS DERIVATIVES

In greenhouse studies, Zemetra et al. [19] found that female fertility in hybrid derivatives increased with each subsequent backcross (BC_1 - 5%; BC_2 - 26%) with partial self-fertility restored in the BC_2 generation (21%) (Fig. 2). In a similar study, Wang et al. [20] showed that, on average, a BC_1 plant had 2% male fertility and 4% female fertility. The average male, female, and self-fertility of the BC_2 generation were 9, 18, and 7%, respectively (Fig. 2). These findings suggest that BC_1 and/or BC_2 plants could serve as either the male or female parent in subsequent crosses. Both of these studies showed that the chromosome number decreased with each successive backcross, approaching the 28-chromosome number of the recurrent parent (jointed goatgrass). Individuals in the BC_2S_1 and $BC2S_2$ generations were observed with chromosome numbers ranging from 29 to 34. In the field, natural backcrossing has also been observed where BC_2 seed was produced on field-derived BC_1 plants [18]. This finding indicates that BC_2 plants can be produced naturally in wheat fields. Based on greenhouse studies, partial self-fertility should be observed in some of these BC_2 plants.

4. GENETIC AND CHROMOSOMAL BARRIERS TO GENE MOVEMENT

Map-based studies suggest the existence of genic as well as chromosomal barriers to introgression in sunflower [23]. If these differences are minimal between the crop and weed species, long-term establishment of crop genes in the weed population can occur [6]. The movement of a wheat gene or transgene in the A or B genomes to jointed goatgrass will be limited by the lack of chromosome pairing and recombination. On the other hand, the movement of genes in the D genome shared by these two species is expected to take place. In a recent study, the normal inheritance and retention of 14 wheat D genome-specific microsatellite markers in BC_1, BC_2, and BC_2S_1 derivatives supports this conclusion [24].

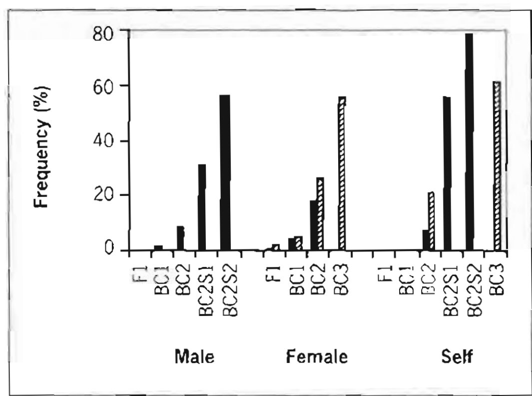

Figure 2: Male, female, and self-fertility of T. aestivum x Ae. cylindrica F1 hybrids and their derivatives. Black solid bars show data from Zemetra et al. [19] and striped bars show data from Wang et al. [20].

Even though A- and B- genome chromosomes are gradually eliminated by recurrent backcrosses to jointed goatgrass and subsequent selfing, cytological studies [20, 25] have shown that some (up to three) A/B genome chromosomes can be retained in advanced derivatives like BC_2S_1 and BC_2S_2 plants (Fig. 3). Since these derivatives are self-fertile, fixation of A/B genomes chromosomes is possible. In addition, Wang et al. [25] observed a translocation involving A/B and C/D chromosomes in a BC_2S_2 plant with 30 chromosomes. This indicated that A- or B-genome chromatin may be retained even after restoration of the recurrent parent chromosome number. Of relevance here is the fact that inter- and intragenomic chromosomal rearrangements have played a significant role in the evolution of hexaploid wheat [26, 27, 28, 29]. Thus, intergenomic translocation events in hybridizations between jointed goatgrass and wheat may be another mechanism of gene or transgene movement.

aestivum x *Ae. cylindrica* BC_2S_2 progenies tracing back to 29- (solid black bars) and 30-chromosome (striped bars) BC_2S_1 progenitors. Plants with more than 28 chromosomes contain additional A- or B-genome chromosomes. Data modified from Wang [22].

5. WORK IN PROGRESS

At this point, information on the inheritance and retention of wheat herbicide-resistant333 transgenes in jointed goatgrass is limited. The retention of herbicide-resistance transgenes on the A, B, or D genomes of wheat will be evaluated in wheat x jointed goatgrass backcross derivatives with and without herbicide selection. We are also constructing a molecular marker linkage map of jointed goatgrass to address the effect of genomic architecture on gene flow. In order to obtain additional evidence for gene flow between wheat and jointed goatgrass, studies of the genetic diversity in jointed goatgrass and wheat are being undertaken using both nuclear and chloroplast SSR markers. Patterns of variation for common molecular markers and a history of the association of particular genotypes might allow the assessment of introgression between these two species. Other weed management issues that will be addressed by future studies include seed dormancy and plant vernalization requirements in wheat, jointed goatgrass, their hybrids and backcross derivatives. This information will be critical for the development of appropriate weed management recommendations.

6. CONCLUSION

Studies under controlled and field conditions suggest that gene flow in sympatric populations of wheat and jointed goatgrass is likely. Thus, the release of herbicide-resistant wheat must be accompanied by a strong management plan to delay or prevent the occurrence of herbicide-resistant jointed goatgrass. This management plan will need to be aimed at blocking gene escape and also prevent selection of a naturally occurring herbicide-resistant biotype.

The F_1 hybrid is essential for production of the first backcross generation and is totally dependent on pollination from an outside source to set seed. Thus, elimination of the F_1 hybrid from the field through tilla-

ge or crop rotation would prevent the production of the first backcross generation that is essential for gene transfer. Some strategies to minimize the production of hybrids include: 1) using certified wheat seed free of jointed goatgrass; 2) applying herbicide for maximum efficacy to kill jointed goatgrass in the field and its borders before flowering; and 3) delayed planting of wheat combined with mechanical control of jointed goatgrass before planting. These strategies coupled with crop and herbicide rotation can also interrupt the path of gene flow. If a herbicide-resistant wheat is grown in the first year, followed by two years of a broadleaf crop, hybrids could be killed in the second year using a grass-specific herbicide. In the third year, hybrids that lie dormant would be controlled. If BC_1 seeds were produced in the second year, they would be controlled also in the third year. Without F_1 hybrids and BC_1 derivatives, the gene flow process can be blocked.

REFERENCES

[1] Ellstrand NC, Prentice HC, Hancock JF: Gene flow and introgression from domesticated plants into their wild realtives. Annu. Rev. Ecol. Syst. 30:539-563 (1999)

[2] Rieseberg LH, Beckstrom-Sternberg S, Doan K: *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius*. Proc. Natl. Acad. Sci. USA 87:593-597 (1990)

[3] Arriola PE, Ellstrand NC: Crop to weed gene flow in the genus *Sorghum* (Poaceae): Spontaneous interspecific hybridization between johnsongrass, *Sorghum halepense*, and crop sorghum, *S. bicolor*. Amer. J. Bot. 83:1153-1160 (1996)

[4] Doebley J: Molecular evidence for gene flow among *Zea* species. BioScience 40:443-448 (1990)

[5] Wilson HD: Gene flow in squash species. BioScience 40:449-455 (1990)

[6] Whitton J, Wolf DE, Arias DM, Snow AA, and Reiseberg LH: The persistence of cultivated alleles in wild populations of sunflowers five generations after hybridization. Theor. Appl. Genet.95-33-40 (1997)

[7] Goodman RM, Newell N: Genetic engineering of plants for herbicide resistance: status and prospects, p. 47-53. In 'Engineering Organisms in the Environment: Scientific Issues', Halverson HO, Pramer D, and Rogul M (Ed.). American Society of Microbiology, Washington D.C. (1985)

[8] Thompson M: *Aegilops cylindrica* (jointed goatgrass) - a threat to U.S. wheat production. Executive Summary of the National Jointed Goatgrass Research Program CSREES-USDA Special Grant (http://www.ianr.unl.edu/jgg) (1999)

[9] Kimber G, Sears ER: Evolution in the genus *Triticum* and the origin of cultivated wheat. In: 'Wheat and Wheat Improvement', Heyne EG (ed.). Agronomy Monograph No. 13 ASA-CSSA-SSSA, Madison, Wisconsin (1987)

[10] Kimber G, Zhao YH: The D genome of the Triticeae. Can. J. Genet. Cytol. 25:581-589 (1983).

[11] Priadcencu AL, Miclea C, Moisescu L: The local form of the species of *Aegilops cylindrica* Host. and its genetic importance. Revenue Roumanian Biologie Botanique 12(6):421-425 (1967)

[12] Belea A: Examination of the F1 hybrids of *Aegilops cylindrica* Host x *Triticum aestivum* L. Acta Agron. Acad. Sci. Hung. 17:151-160 (1968)

[13] Slageren MW van: Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub & Spach) Eig (Poaceae). Wageningen Agricultural University Papers 7(94). 513pp. (1994)

[14] Guadagnuolo R, Savova-Bianchi D, Felber F: Gene flow from wheat (*Triticum aestivum* L.) to jointed goatgrass (*Aegilops cylindrica* Host.), as revealed by RAPD and microsatellite markers. Theor Appl Genet 103:1-8 (2001)

[15] Donald WW, Ogg AG: Biology and control of jointed goatgrass. (*Aegilops cylindrica*), a review. Weed Technol. 53:3-17 (1991)

[16] Mallory-Smith CA, Hansen J, Zemetra RS: Gene transfer between wheat and *Aegilops cylindrica*. 2nd Intl. Weed Control. Cong., Copenhagen, pg. 441-445 (1996)

[17] Seefeldt SS, Zemetra RS, Young FL, Jones SS: Production of herbicide-resistant jointed goatgrass (*Aegilops cylindrica*) x wheat (*Triticum aestivum*) hybrids in the field by natural hybridization (1998)

[18] Snyder J, Mallory-Smith C, Balter S, Hansen J, Zemetra RS: Seed production on *Triticum aestivum* by *Aegilops cylindrica* hybrids in the field. Weed Science 48:588-593 (2000)

[19] Zemetra RS, Hansen J, Mallory-Smith CA: Potential for gene transfer between wheat (*Triticum aestivum*) and jointed goatgrass (*Aegilops cylindrica*). Weed Sci. 46:313-317 (1998)

[20] Wang ZN, Zemetra RS, Hansen J, Mallory-Smith CA: The fertility of wheat x jointed goatgrass hybrid and its backcross progenies. Weed Science 49:340-345 (2001)

[21] Cremieux L: Seed protein and chromosome number analyses of experimental wheat x jointed goatgrass (*Aegilops cylindrica* Host) hybrid derivatives. M.S. thesis, Oregon State University (2000)

[22] Wang ZN: Gene introgression between wheat (*Triticum aestivum* L.) and jointed goatgrass (*Aegilops cylindrica* Host); an implication of potential risk of releasing herbicide resistant wheat cultivars. Ph.D. dissertation, University of Idaho (2000)

[23] Rieseberg LH, Baird SJE, Gardner KA: Hybridization, introgression, and linkage evolution. Plant Molecular Biology 42:205-224 (2000)

[24] Kroiss L: Retention of wheat alleles in Imidazolinone-resistant wheat x jointed goatgrass recurrent backcross generations. M.S. thesis, Oregon State University (2001)

[25] Wang ZN, Hang A, Hansen J, Burton C, Mallory-Smith CA, Zemetra RS: Visualization of A and B genome chromosomes in wheat (*Triticum aestivum* L.) x jointed goatgrass (*Aegilops cylindrica* Host) backcross progenies. Genome 43:1038-1044 (2000)

[26] Naranjo T, Roca P, Goicochea PG, Giraldez R: Arm homoeology of wheat and rye chromosomes. Genome 29:873-882 (1987)

[27] Naranjo T: Chromosome structure in durum wheat. Theor. Appl. Genet. 79:397-400 (1990)

[28] Liu CJ, Atkinson MD, Chinoy CN, Devos KM, Gale MD: Nonhomeologous translocations between group 4, 5, and 7 chromosomes within wheat and rye. Theor. Appl. Genet. 83:305-312 (1992)

[29] Nelson JC, Sorrells ME, VanDeynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA: Molecular mapping of wheat. Major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721-731 (1995)

DEVELOPMENT OF WHEAT D-GENOME INTROGRESSION LINES ASSISTED BY MICROSATELLITE MARKERS

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ABSTRACT: New wheat introgression lines were obtained which contain different segments of individual chromosomes of *Aegilops tauschii* in the *Triticum aestivum* cv. 'Chinese Spring' background. The introgression lines were developed to examine various subsets of alleles from the wild grass in the genetic background of common wheat. As starting point substitution lines of 'Chinese Spring' in which single chromosomes of the D genome had been replaced by homologous chromosomes of a synthetic wheat were used. Synthetic wheat had been obtained earlier from a cross between the tetraploid emmer (genomes AABB) and wild grass *Aegilops tauschii* (genome DD). The seven wheat chromosome substitution lines carrying different chromosomes of *Ae. tauschii* were crossed twice to *T. aestivum* cv. 'Chinese Spring'. In total 259 BC1-progeny plants (37 plants for each chromosome) were analysed. Genotypic analysis was carried out using microsatellite markers previously mapped on the chromosomes of the D genome of wheat. Sixty-five microsatellite markers were found to be polymorphic between wheat cv. 'Chinese Spring' and synthetic wheat, and these were used to test the genetic composition of the produced lines. During this analysis recombinant lines carrying different segments of *Ae. tauschii* chromosomes were detected. Plants containing small introgressions of the alien genetic material were selfed and plants carrying large pieces of the donor chromosome were backcrossed again to get smaller introgressions. BC1 progeny were evaluated for different phenotypic traits such as plant height, spikelet number, peduncle length, flowering time, spike length, tiller number, grain weight per ear, fertility and thousand kernel weight.

1. INTRODUCTION

Wild relatives of wheat represent a high potential source for crop improvement. In the past decades the vast majority of such accessions was collected in gene banks throughout the world. However, the existing collections are not actively utilized particularly with respect to complex traits such as yield and nutritional quality.

Despite their inferior phenotypes, wild accessions contain genes that can improve quantitative traits (quantitative trait loci, or QTLs) (1). A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines (advanced backcross QTL analysis) was proposed by Tanksley and Nelson (2). This method involves the use of molecular linkage maps and a breeding technique and allows to examine subsets of alleles from the wild species in the genetic background of an elite cultivar.

The availability of a set of *Triticum aestivum* cv. 'Chinese Spring' / *Aegilops tauschii* chromosomal substitution lines provided us the opportunity to use the method of advanced backcross QTL analysis for the study of QTLs specific for individual chromosomes.

New wheat introgression lines were developed by backcrossing the chromosomal substitution lines with wheat 'Chinese Spring' in order to obtain different segments of individual chromosomes of *Ae. tauschii* in the common wheat background. The development of the lines was accompanied and confirmed by microsatellite marker analysis.

2. MATERIALS AND METHODS

2.1. Plant material

Substitution lines of 'Chinese Spring' in which single chromosomes of the D genome had been replaced by homologous chromosomes of a synthetic wheat were developed and kindly provided by A.J. Worland (John Innes Centre, Norwich, UK). Synthetic wheat used for the production of the substitution lines had been obtained earlier from a cross of tetraploid emmer and wild grass *Aegilops tauschii* (3). The seven wheat D-genome chromosome substitution lines carrying different chromosomes of *Ae. tauschii* were crossed twice to *T. aestivum* cv. 'Chinese Spring'. In total 259 BC1-progeny plants (37 plants for each chromosome) were analysed.

2.2 Genotypic analysis

DNA was isolated from the leaf material at the seedling stage according to a modified procedure of Plaschke et al. (4). Genotypic analysis was carried out using microsatellite markers previously mapped on the chromosomes of the D genome of wheat (5, 6). The chromosomal location of 65 markers polymorphic between the synthetic wheat and cv. Chinese Spring are shown in Fig. 1. Unpublished primer sequences are available upon request. Polymorphic microsatellites were used to test the genetic composition of the produced BC1 lines.

2.3 Phenotypic analysis

BC1-progeny plants were grown in green-house. Ten phenotypic traits such as flowering time, plant height, spikelet number, peduncle length, spike length, tiller number, grain weight per ear, grain weight per plant, fertility and thousand kernel weight were evaluated. The association between phenotype and marker genotype data was investigated using the QGENE software application (7).

3. RESULTS AND DISCUSSION

3.1. Microsatellite analysis of substitution lines

Microsatellite markers previously mapped on the chromosomes of the D genome of wheat (5, 6) were tested for polymorphism between the synthetic wheat and 'Chinese Spring' and 65 markers were found to be polymorphic (Fig. 1). The number of polymorphic markers per chromosome varied from 5 on chromosome 4D to 14 on chromosome 2D, with an average of 9.3 per chromosome. Using this set of markers all seven substitution lines were checked for the presence of the 'synthetic' (donor) chromosome. Sixty markers revealed the expected fragment of the donor chromosome while five markers amplified the fragments specific to the recipient chromosome. The markers demonstrated that incorrect substitution were located in the distal regions of the chromosomal arms 1DL, 4DL and 7DS. The occurrence of recipient sequences on the terminal part of the donor chromosome was shown earlier in the set of substitution lines Saratovskaya 29/Yanetzkis Probat (8). The reason for that illegitimate recombination in the distal regions of substituted chromosomes is still not clear.

3.2. Genotypic analysis of BC1-progeny

Sixty microsatellite markers which revealed the correct substitution were used to test the genetic composition of the produced BC1 generation. Due to relatively even

distribution of the markers along the chromosomes we were able to trace the different segments of the donor and the recipient chromosomes in the progeny. Only chromosome 4D carries a scarce number of available markers (4) and, therefore, the analysis of the BC1-progeny for the chromosome was less effective.

It is interesting to note that different chromosomes of the D genome are characterized by diverse frequencies of recombination. The highest number of recombination events was detected for chromosome 5D and the lowest number for chromosome 1D (chromosome 4D was omitted from the consideration).

The results of the analysis of 37 BC1-progeny plants from the cross between 'Chinese Spring' and substitution line 3D are presented in Fig. 2. We could distinguish homozygous plants carrying the 'Chinese Spring' alleles and heterozygous plants carrying both 'Chinese Spring' and *Ae. tauschii* alleles. Three plants were found to contain the intact chromosome of 'Chinese Spring', and three plants were heterozygous in all studied loci. Hence these two classes represented the parent genotypes. All other plants fell into 14 recombinant classes carrying the different segments of *Ae. tauschii* chromosome 3D. The plants containing large pieces of the donor chromosome were backcrossed again to get smaller introgressions of the alien genetic material. The classes with small pieces of the *Ae. tauschii* chromosome (like I, II and VIII, Fig. 7) will be used for producing of first homozygous introgression lines. Microsatellites assisted selection will be performed to find such individuals in the BC1F2-progeny. We are looking forward to get a complete set of introgression lines representing the whole *Ae. tauschii* genome.

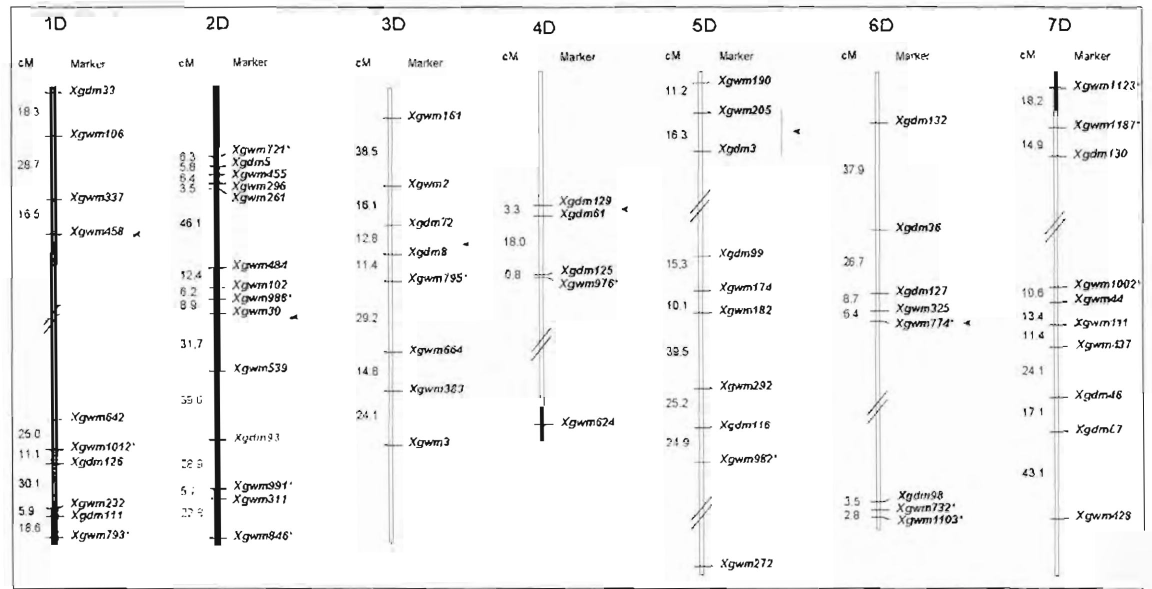

Figure 1: Schematic chromosomal location of polymorphic microsatellite markers between 'Synthetic' and 'Chinese Spring' based on the genetic microsatellite map of wheat (5). Unpublished microsatellite markers are marked with an asterisk and available upon request. Short arms of the chromosomes are on the top. Arrows show the position of the centromeres. The chromosomal pieces with detected incorrect substitution are coloured in black.

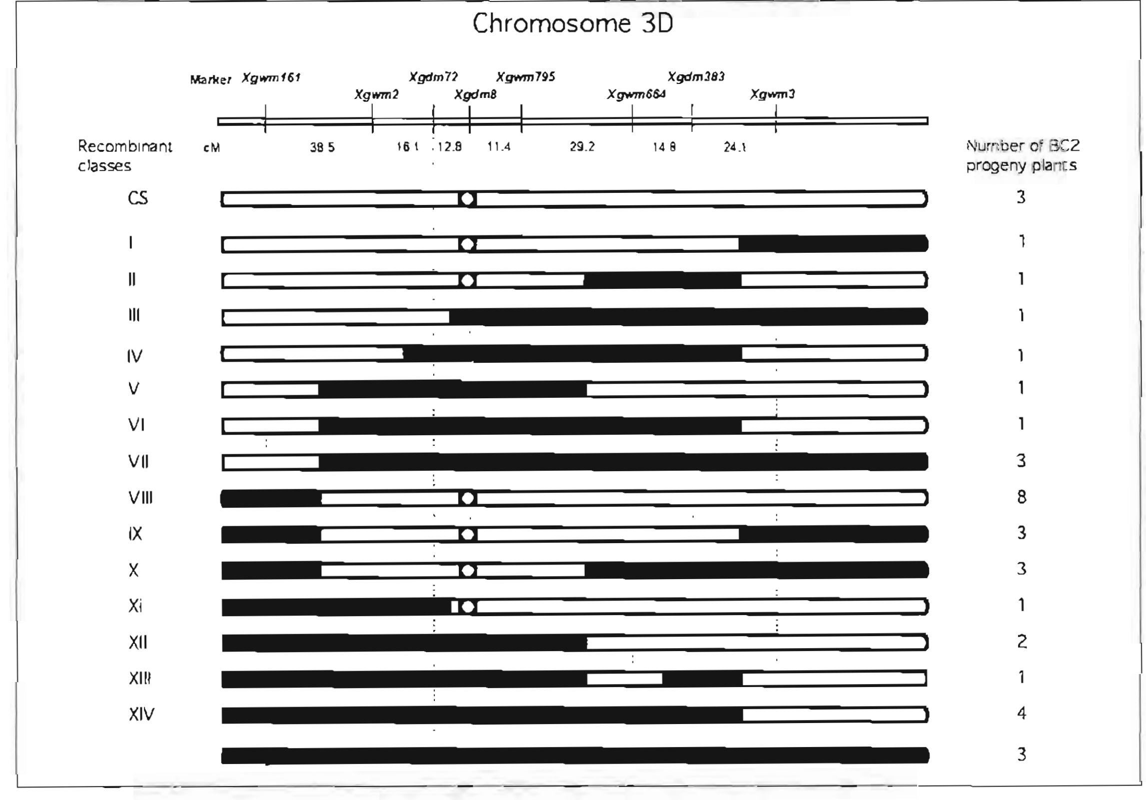

Figure 2: Scheme of genotypic analysis of 37 BC2-plants from the cross between *T. aestivum* 'Chinese Spring' and 'Chinese Spring/Synthetic' substitution line 3D. The genetic composition of the plants was analysed by eight microsatellite markers specific to chromosome 3D. Homozygous parts of the chromosome carring 'Chinese Spring' alleles are designated in white, heterozygous parts of the chromosome carring 'Chinese Spring' and *Ae. tauschii* alleles are designated in black.

3.3. Phenotypic analysis

BC1 progeny were evaluated for different phenotypic traits (flowering time, spike length, tiller number, plant height, spikelet number, peduncle length, grain weight per ear, grain weight per plant, fertility and thousand kernel weight) and the search for putative QTLs was performed. It has been found that chromosome 5D of synthetic wheat has a strong negative effect on flowering time (substitution line 5D has 14 days delay in flowering). Due to that late flowering time most of the traits, especially fertility and grain weight per ear, were negatively influenced. That could be partly explained by pleiotropic effects of vernalisation gene *Vrn3* located on the long arm of chromosome 5D. Slight positive effect of synthetic chromosome 7D was found for grain weight per ear and thousand grain weight. Further extended studies using the homozygous introgression lines are necessary to confirm the results and to detect further agronomical important QTLs.

REFERENCES

[1] Tanksley SD, McCouch SR: Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277: 1063-1068 (1997).

[2] Tanksley SD, Nelson JC: Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92: 191-203 (1996).

[3] McFadden ES, Sears ER: The genome approach in radical wheat breeding. J Amer Soc Agron 39: 1011-1026 (1947).

[4] Plaschke J, Ganal MW, Röder MS: Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91: 1001-1007 (1995).

[5] Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW: A microsatellite map of wheat. Genetics 149: 2007-2023 (1998).

[6] Pestsova EG, Ganal MW, Röder MS: Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43: 689-697 (2000).

[7] Nelson JC: QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3: 239-245 (1997).

[8] Pestsova E, Salina E, Boerner A, Korzun V, Maystrenko OI, Roeder MS: Microsatellites confirm the authenticity of inter-varietal chromosome substitution lines of wheat (*Triticum aestivum* L.). Theor Appl Genet 101: 95-99 (2000).

MOLECULAR CHARACTERIZATION OF WILD AND CULTIVATED TETRAPLOID WHEAT OF THE NEAR EAST ORIGIN

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ABSTRACT: AFLP marker technique has been used to evaluate and to study the evolution and the geographical distribution of different sub-species of *Triticum turgidum* mainly *T. dicoccoides, T.dicoccon* and the cultivated durum wheat. Four primer combinations were used to discriminate between all the subspecies. The AFLP technique has detected a significant level of polymorphism and was able to cluster separately most of the subspecies. The results using unrooted tree showed also different clustering based on the origin of the accessions as illustrated by *T. dicoccoides* from the West Asia region. The AFLP results are suitable for assessing phylogenetic relationships in tetraploid wheat.

INTRODUCTION

Cultivated tetraploid wheats of BBAA genomic constitution have played an import role in human food production, since the origins of agriculture in the Near East till the present. Farmer-assisted evolution of these wheats started with the domestication of wild emmer and continues to the present, when durum wheat landraces are still grown on a significant area, particularly in low-input rain-fed farming systems of developing countries with Mediterranean climate. In spite of the achievements of archeological research, some questions relating to tetraploid wheat domestication and evolution remain unanswered, e.g. in which part of the Near East the wild emmer domestication occurred, was it a single or multiple event or what is a relationship between emmer wheat and naked tetraploid wheat taxa? Recently, Heun *et al.* (1997) demonstrated that DNA fingerprinting based on AFLP markers applied to a set of wild progenitor and cultivated einkorn accessions could pinpoint the site of einkorn wheat domestication and the study also provided further insight into within-taxa evolution. Therefore, we have taken a similar approach to analyze the evolutionary relationships among tetraploid wheats with BBAA genome.

MATERIAL AND METHODS

Plant material

Ninety-six accessions of five tetraploid wheat taxa were selected for the study using geographical information of GRU/ICARDA database. Selection of *T. dicoccoides* and *T. durum* is representative for the whole area of geographical distribution, while *T. durum* and *T. carthlicum* is limited to the Near East region and only two *T. polonicum* accessions originating from Algeria were available. Seed material of the selected accessions was provided by ICARDA genebank. Three plants were analyzed for each accession.

AFLP analysis

Total DNA was extracted from 200 mg of fresh leaves of each individual plant with the CTAB method (Muray and Thompson 1980). 500 ng total DNA per sample was completely digested with PstI and MseI and ligated. The ligated DNA was preamplified using preselective primers for PstI and MseI (Pharmacia). Preamplification PCR was conducted for 20 cycles. The product of preamplification was amplified using four selective primer combinations. The primer combinations used in this study were PstI-51 and MseI-48; PstI-51 and MseI-50; PstI-12 and MseI-88; PstI-73 and MseI-88.The products of selective amplification were electrophoresed in 6 % polyacrylamide gel for 2 hours at 65 W. The fragments in the gel were detected using DNA Silver staining.

Estimation of genetic diversity

Bands were scored using cross-checker software and checked also manually. The approximate fragment length was estimated by comparison to a 100 bp ladder (Pharmacia). The range of the bands scored was from 100 bp to 300 bp.110 polymorphic bands were used for estimationg the genetic diversity.

Phylogenetic tree

To clarify the relationship among populations and between species, phylogenetic tree was reconstructed by Neighbor-joining method (Saitou and Nei, 1987). Using the estimates of Nei's coefficient as the genetic distance, phylogenetic trees were reconstructed with the computer software PHYLIP 3.57c (Felsenstein 1995).

RESULTS

Triticum dicoccoides

Accessions of *T. dicoccoides* are grouped into three major clusters in a tree outgrouped by an accession from southern Syria. The first cluster, located in lower part of the tree, includes dicoccoides originating mostly from southwestern part of the Fertile Crescent (four accessions from southern Syria, three from Jordan and two from Lebanon), one accession was collected in southeastern Turkey and another comes from Iraq. The second dicoccoides group is located in a major cluster consisting of two very distinct sub-clusters, a dicoccoides one which includes three accessions from Palestine, one from Jordan and one from southeastern Turkey, and a cultivated wheat one having a mixed sub-cluster of *T. dicoccum, T. carthlicum* and one *T. dicoccoides* from southern Syria, which makes a secondary sub-cluster with two *T. dicoccum* from southeastern Turkey. The remaining dicoccoides are grouped in a major cluster, which splits into two distinct sub-clusters. One contains accessions mostly from the northern part of the Near East arc, four were collected in northwestern Syria and three in the neighboring Gaziantep Province in Turkey and one accession is a weedy race from Tafila Province of Jordan. The second sub-cluster is more heterogeneous being composed of three accessions from northwestern Syria, two originating in southeastern Turkey, another two come from southern Syria and one was collected in Jordan.

Triticum dicoccum

The cultivated wheats are located in three major clusters and *T. dicoccum* is present in all of them. The first in sequence in the tree and phenogram outgrouped by a *T. dicoccoides* from southern Syria, is the mixed one referred to in the above paragraph. The cultivated wheat sub-cluster is further split into a group containing all three dicoccum accessions from Oman, one from Armenia and, somewhat surprisingly, an accession of Spanish origin. The other group begins with *T. dicoccoides* from Jebel Al Arab region in southern Syria from which two *T. dicoccum* originating from southeastern Turkey branch. *T. dicoccoides* is the only species represented in the second major cluster that splits in the tree and phenogram from the main evolutionary line immediately after the first cluster with cultivated wheats. It can be further divided in three sub-clusters, one with a single Palestine and Greek dicoccum accessions in lower branches and two Iranian, one Armenian and two Ethiopian accessions in the upper part. The Three accessions of Balkan origin (two from Bosnia-Herzegovina and one from Yugoslavia) and a single German dicoccum are found in the second sub-cluster. The last one has two branches, one with a Palestinian dicoccum at the base from which a single Georgian and Russian dicoccum evolve, while the other starts with an Iranian accession and extends much further with a Chinese accession. The third cluster of *T. dicoccum* accessions separates from the northwestern Syria/southeastern Turkey dicoccoides cluster at the end of the evolutionary line. It consists of three *T. dicoccum*, one durum wheat and one *T. polonicum* wheat sub-clusters. The first dicoccum one, which shares the evolutionary line with durum and polonicum wheats, includes accessions of Ethiopian and Indian origin, two of each country, and an accession from Slovakia. Nine accessions of different geographical origin are grouped in the second cluster, three are from Russia, and a single accession comes from Bulgaria, Portugal, Iran, Yugoslavia, Ethiopia and an Afghani dicoccum extends the sub-cluster far from the others. The last sub-cluster has *T. carthlicum* at the lowest branch, the next in the evolutionary line is a Jordanian dicoccum, followed by two Russian and one Italian *T. dicoccum* accessions.

Triticum durum

Contrary to *T. dicoccum*, durum wheat accessions of the Near East origin and an improved variety 'Moa S' all were allocated to a single cluster that is considered a sub-cluster of the third major cluster of cultivated wheats. Durum wheat landraces from Iraq, Yemen (2) and Palestine are at placed at the lower part, followed by landrace accessions from Jordan, southern Syria and southeastern Turkey, and finally, at the

end of the durum wheat evolutionary line are landraces from northeastern Syria, southeastern Turkey and the improved cultivar 'Moa S'.

Triticum polonicum

This species was represented by only two accessions from Algeria. Both produced a distinct sub-cluster in the third major cluster of cultivated wheats. It creates a branch immediately after the separation of durum wheat from the evolutionary line of *T. dicoccum*.

DISCUSSION

Archeologists placed the origins of agriculture in the southern and central Levant, where three PPNA sites yielded cereal remains interpreted as domestic. These crops were two-row barley, emmer and einkorn (Harris 1998). The dominance of *T. dicoccoides* originating from Palestine, Jordan, southern Syria and Lebanon in the first dicoccoides cluster and association of Palestinian and Jordanian wild emmer with *T. dicoccum* and *T. carthlicum* in the first cluster of cultivated wheat corroborates this hypothesis. The presence of cultivated emmer accessions of the Near East and Oman origin in this cluster indicates that these wheats of 20th century retained, at least partly, the genetic structure of the ancient cultivated ancestors and of the wild progenitor. The same is true for *T. carthlicum*, because all accessions but one are located in this cluster. Mandy (cit. Dorofeev *et al.* 1979) dates the origin of this species to 8500-8000 BP. Since the genetic basis of the free-threshing and non-brittle rachis is monogenic (Q factor) in *T. carthlicum* and polygenic in *T. durum* (Dorofeev *et al.* 1979), one can speculate that the first naked wheats found in the Neolithic sites belonged rather to the former than the latter wheat species. Clustering with *T. dicoccum* in the evolutionary oldest group of cultivated wheats confirms both the ancient origin of *T. carthlicum* and its close relationship to cultivated emmer. This cluster of cultivated wheat also includes a dicoccoides wheat accession originating from Jebel Al-Arab in southern Syria. When we inspected spikelets of this accession we found some 'dicoccum' types, which resembled those collected and described by the late Professor Gandilian in a collection mission to this region in 1992 (Gandilian, unpublished results). Possibly, these are remnants of an old introgression from times, when *T. dicoccum* was still grown in remote mountain areas of southern Syria. However, we have not found any evidence in *T. dicoccoides* for introgressions with *T. durum*, which were reported by Blumler (1998) for populations from the Upper Jordan Valley. The second dicoccum cluster branches from the main line close to the first one and is highly heterogeneous regarding the geographical origin and in the respect it is similar to the three dicoccum sub-clusters of the third major cluster of cultivated wheats. Contrary to Kawahara and Taketa (2000), who suggest a monophyletic origin of Ethiopian tetraploid wheat, our results indicate a polyphyletic origin, since five Ethiopian accessions were scattered in three different clusters. As Table 1 shows, wild and cultivated emmer, the main objective of our study, were well represented regarding the geographical origin, therefore the evolutionary relationships revealed may indicate a more general pattern within and between the two taxa.

In contrast to emmer wheat, our results indicate a monophyletic origin of the Near East *T. durum* from a single group of *T. dicoccum*. A more detailed study based on a globally representative set of accessions would be needed to find out, whether this true for durum wheat as a whole. The proximity of *T. polonicum* to durum wheat in the tree confirms their close evolutionary relationships as suggested by a number of authors including Vavilov (Dorofeev *et al.* 1979), but again, this finding should be supported by a more representative set of Polish wheat.

Zohary (1999) arrived at the conclusion that most crops of the Near East origin, including emmer wheat were "very likely taken into domestication only once or - at most - a very few times" and Heun *et al.* (1997) brought an evidence on the monophyletic origin of einkorn wheat. Our results corroborate, in principle, Zohary's conlusions, that plant domestication in the Near East was a unique or rare event.

REFERENCES

[1] Blumler, M.A. 1998. Introgression of durum wheat into wild emmer and the agricultural origin question. In: *The origin of agriculture and crop domestication,* A. B. Damania, J. Valkoun, G. Wilcox, C.O. Qualset, Eds., ICARDA, Aleppo, Syria, pp. 252-268.

[2] Dorofeev, V.F., A. A. Filatenko, E.F. Migushova, R.A. Udachin and M.M. Jakubziner. 1979.
Wheat. Flora of cultivated plants. Vol.1. Kolos, Leningrad, USSR, p. 17, p. 19, p. 181, pp. 137-138 (in Russian).

[3] Harris, D.R. 1998. The origins of agriculture in South West Asia. Review of Archeology, 19: 5-11.

[4] Heun, M., R. Schäfer-Pregl, D. Klawan, R. Castagna, M. Accerbi, B. Borghi, and F. Salamini. 1997. Site of einkorn wheat domestication identified by DNA fingerprinting. Science 278: 1312-1314.

[5] Hilman, G.C. and M.S. Davies. 1999. Domestication rate in wild wheats and barley under primitive cultivation. In: *Prehistory of agriculture. New experimental and ethnographic approaches*. P.C. Anderson, Ed., Monograph 40, Institute of Archeology, University of California, Los Angeles, pp. 70-102.

[6] Kawahara, T. and S. Taketa. 2000. Fixation of translocation 2A-4B infers the monophyletic origin of Ethiopian tetraploid wheat. Theor. Appl. Genet. 101: 705-710.

[7] Morrison, L. A. 1998. Report on the 2nd IWGS Taxonomy Workshop.http:/wheat.pw.usda.gov/ggpages/ DEM/9IWGS/taxonomy.html

[8] Nesbitt, M. and D. Samuel. 1996. From staple crop to extinction? The archeology and history of the hulled wheats. In: *Hulled wheats. Promoting the conservation and use of underutilized and neglected crops. 4.* S. Padulosi, K. Hammer, J. Heller, Eds., Proceedings of the First International Workshop on Hulled Wheats, 21-22 July 1995, Castelvecchio Pascoli, Tuscany, Italy. International Plant Genetic Resources Institute, Rome, Italy.

[9] Valkoun, J. 2001. Wheat pre-breeding using wild progenitors. Euphytica 119: 17-23.
Vardi, A. and D. Zohary. 1967. Introgression in wheat via triploid hybrids. Heredity 22: 541-560.

[10] Willcox, G. 1998. Archeobotanical evidence for the beginnings of agriculture in Southwevst Asia. In: *The origin of agriculture and crop domestication*, A. B. Damania, J. Valkoun, G. Wilocox, C.O. Qualset, Eds., ICARDA, Aleppo, Syria, pp. 25-38.

[11] Zohary, D. 1999. Monophyletic vs. polyphyletic origin of the crops on which agriculture was founded in the Near East. Genetic Resources and Crop Evolution 46: 133-142.

INTERGENOMIC REARRANGEMENTS INDUCED BY *PH* MUTATION IN NEWLY SYNTHESIZED *TRITICUM TURGIDUM* - *AEGILOPS OVATA* AMPHIPLOIDS

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ABSTRACT: Two newly synthesized *Triticum turgidum* - *Aegilops ovata* amphiploids (genome constitution $AABBU^{o}U^{o}M^{o}M^{o}$) either carrying and lacking *Ph1* gene were obtained and their S_2 offspring karyotypes were scored for intergenomic translocations by genomic in situ hybridization. Remarkable difference in the frequency of exchange events between both progenies was found. So the *ph1* family (12 plants), obtained from a *ph1c* mutant wheat line, showed nine translocations, whereas only one translocation was detected in the *Ph1* family (9 plants), obtained from a *Ph1* wheat line. All exchanges were terminal, their estimated size ranged from 3% to 36%, and none was in homozygous condition. Some of them involved M^{O} and wheat genomes, but no U^{O}-wheat translocation was observed. The analysis demonstrates that mutation at Ph1 locus can be used to promote homoeologous meiotic pairing between wheat and related genomes in successive selfing generations of the original amphiploids to increase the chance for stable introgression of beneficial traits from wild relatives into crop wheat, and suggests that success for the introgression of *Ae. ovata* chromatin into durum wheat is genome-dependent.

1. INTRODUCTION

Interspecific hybridization has been of great importance for the transfer of genes of agronomic interest, mainly involved in pathogen resistances, into durum and bread wheat. Usually, duplication of the hybrids leads to the obtaining of amphiploids that are subsequently backcrossed to the wheat parent. Wild species chromosomes in monosomic state are quite quickly eliminated early during the backcrossing process. If homoeologous recombination between wheat and the alien species chromosomes takes place, intergenomic translocations are produced. These chromosome constructions facilitate the stable introgression of alien chromatin into wheat.

Some breeding programmes have recourse to mutant lines where the multigenetic system that ensures the diploid-like meiotic behaviour of wheat is disrupted by inactivity of its major locus, namely *Ph1*. Mutations at this locus are available in both hexaploid wheat (mutation *ph1b*) (1) and tetraploid wheat (mutation *ph1c*) (2). When a mutant line is used as the wheat parent of the initial hybrid, homoeologous pairing and recombination between wheat and the related species is promoted. This increases the probability of success in the incorporation of beneficial foreign genes into the wheat genome that can be further transferred by backcrossing to any other wheat cultivar of agronomic value (3).

Because of the recessive nature of *ph1* mutations, the promotion of homoeologous exchanges is restricted to meiotic events prior to the backcrossing process. It is then expected that selfing of the original amphiploids obtained from *ph1* mutant lines increases the frequency of intergenomic exchanges.

Genomic in situ hybridisation (GISH) is currently the most suitable technique for cytological identification of individual genomes in complex multigenomic materials (e.g., ref 4) and, particularly, in the Triticeae it is being routinely employed with many different purposes such as characterization of alien chromatin introgressed into wheat.

2. Materials and Methods

2.1. Plant material

Two *T. turgidum ssp durum* ¥ *Ae. ovata* hybrids (2n=28; genome constitution $ABU^{o}M^{o}$) were obtained by crossing Primadur, a french commercial cultivar homozygous *Ph1/Ph1*, and the mutant line Creso *ph1c/ph1c* (kindly provided by J. Jahier, INRA, Rennes) with two different accessions of *Ae. ovata* both of Moroccan origin. These hybrids gave rise to two amphiploids (2n=56) either by spontaneous doubling (*Ph1* amphiploid) or by colchicine treatment (*ph1* amphiploid). The original amphiploids were selfed and S_1 grains were bulked. Nine individuals of the second generation of selfing (S_2) from the *Ph1* amphiploid (*Ph1* family) and twelve S_2 individuals from the *ph1* amphiploid (*ph1* family) have been examined in this study.

2.2. Preparation of cells

Plants were grown in glasshouse and pots were lying on sand to allow good draining. Once roots were out of the pot, they were cut off between 1-2.30 p.m. (solar zenith) and immediately placed in melting ice until the following morning to synchronise cell divisions. Tips were then fixed in ethanol : acetic acid (3 : 1) and stored at 4°C for 2-4 months. Then root tips were heated in 1% acetic carmin and squashed in 45% acetic acid. Slides were then stored at 4°C prior to genomic *in situ* hybridisation (GISH).

2.3. GISH procedure

U, M and AB genomic DNAs were isolated from young leaves of *Aegilops umbellulata* (2n=2x=14, genome constitution UU), *Ae. comosa* (2n=2x=14, genome constitution MM) and *Triticum turgidum ssp durum* cv. Langdon (2n=4x=28, genome constitution AABB), respectively, following standard protocols. Then U and M genome DNAs were mechanically sheared to 10-12Kbp pieces and labelled with digoxigenin-11-dUTP (U_{digo} and M_{digo} probes) or biotin-16-dUTP (U_{bio} and M_{bio} probes) by nick translation (Boehringer Mannheim).

Hybridization mixtures contained differentially labelled *Ae. umbellulata* and *Ae. comosa* (U_{digo} and M_{bio} or U_{bio} and M_{digo}) genomic probes, to a final concentration of 8ng/ml each. Unlabelled sonicated durum wheat genomic DNA (60-fold in excess the labelled probe concentration) was also added in order to block the DNA sequences common to A and B genomes. GISH protocol was as described earlier (4).

Digoxigenin-labelled probes were revealed with 5ng/ml goat antidigoxigenin antibody conjugated with fluorescein isothiocyanate (FITC, Boehringer Mannheim) whereas biotinylated probes were detected with 5ng/ml avidin conjugated with Cy3 dye (Amersham) (see Fig. 1). Slides were screened using an Axiophot epifluorescent microscope (Zeiss) equipped with different sets of filters and photographed with Kodak Ektachrome film ASA/ISO 400. The slide films were scanned and processed for contrast and brightness before grey-scale printing.

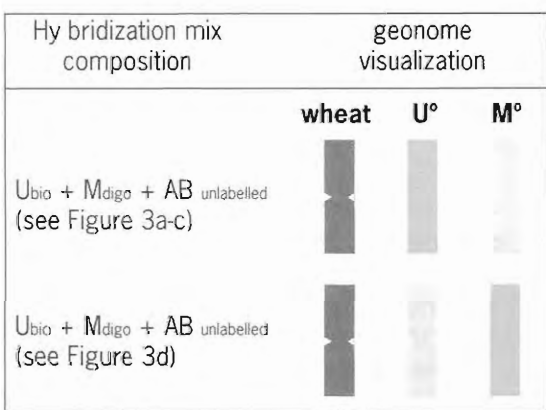


Figure 1. Grey-scale visualization of wheat, Uo and Mo chromatin by GISH.

3. RESULTS

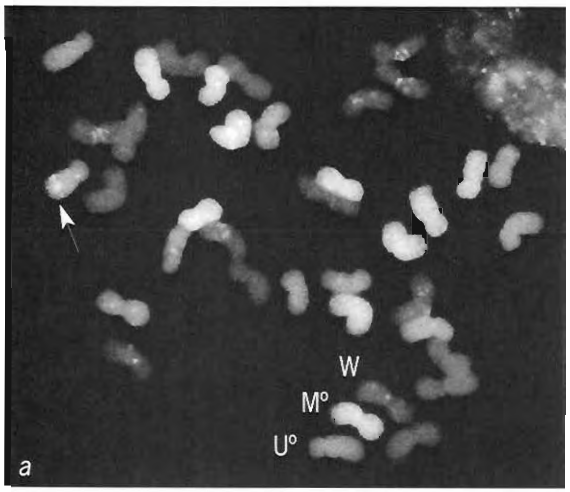
Fig. 2 shows mitotic figures obtained in the *T. turgidum- Ae. ovata* amphiploid progenies after GISH.
The frequency and genome composition of the intergenomic exchanges detected in *Ph1* and *ph1* families are given in Tables I and II.

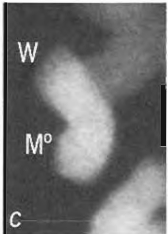
Table I Distribution of S2 individuals according to the number of intergenomic exchanges.

Family	Intergenomic exchanges per plant				
	0	1	2	total	mean
Ph1	8	1	0	1	0.11
ph1	5	5	2	9	0.75

Table II. Genomic composition of exchanges. Data on their relative size, calculated on 6-8 mitotic figures, are included.

Family	Whea- U°	Wheat- M°	U° -M°
Ph1	-	-	1 5%
ph1	-	4 5%-36% mean= 16%)	5 3%-31% mean= 9.6%)





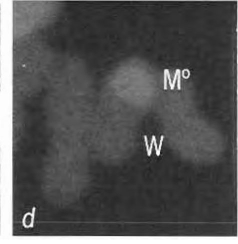


Figure 2. Microphotographies of intergenomic exchanges. The composition of the hybridization mix is indicated in Figure 2. In the naming of translocations, the centromeric genome is noted first. ***a*** Partial mitotic metaphase showing wheat, U^o and M^o genome discrimination after GISH. ***b*** Magnification of the U^o/M^o translocation arrowed in *a*. ***c*** M^o/wheat translocation. ***d*** Wheat/M^o translocation.

4. DISCUSSION

Liu et al. (5, 6) used RFLPs as molecular markers to examine the early genomic evolution of distinct wheat-alien synthetic amphiploids. These authors reported no differences in the rate of changes at the DNA level between S_5 progenies from *T. aestivum* - *Ae. kotschyi* amphiploids that differed by the activity/inactivity of the *Ph1* locus. However, our GISH analysis provides direct and confident evidence on the advantage of using *ph1* mutations, at least in durum wheat, to generate intergenomic translocations (Table I; Fig. 2). These chromosome constructions could further be used as intermediate materials in wheat introgression programmes as proposed bellow.

None of the exchanges involved wheat and the U^o genome (Table II). The sample is small but it seems that the success for introgression of beneficial genes from *Ae. ovata* into durum wheat could depend on the genomic origin of the gene responsible.

Wheat - *Ae. ovata* translocations have been detected in 4 out of the 12 individuals belonging to the *ph1* family examined (Table II). Since the homoeologous pairing promoting effect of the *ph1* mutation is expected to affect any meiotic event prior to the backcrossing to a *Ph1* wheat line, the frequency of interspecific exchanges will likely increase in successive selfing generations.

An alternative strategy for the use of amphiploids in wheat introgression programmes could proceed as follows: 1) Production of the wheat ¥ wild species hybrid by using a *ph* mutant line as wheat parent. 2) Obtaining of the amphiploid by chromosome doubling. 3) Selfing of the original amphiploid for a number of generations to create a large population composed by many distinct variants of the amphiploid, some of which will carry intergenomic translocations. 4) Screening of that population (either by GISH, by genetic or molecular markers) to select those lines that actual or potentially have stably incorporated genes of agronomic interest from the wild species. 5) Use of these introgressed lines as bridge materials to transfer the favourable traits to any wheat line of agronomic value by the backcrossing methodology.

REFERENCES

[1] Sears ER (1977) An induced mutant with homeologous pairing in common wheat. Can J Genet Cytol 19: 585-593

[2] Giorgi B (1978) A homeologous pairing mutant isolated in *Triticum durum* cv. Capelli. Mutant Breed Newsl 11: 4-5

[3] Jauhar PP, Chibbar RN (1999) Chromosome-mediated and direct gene transfers in wheat. Genome 42: 570-583

[4] Sanchez-Moran E, Benavente E, Orellana J (1999) Simultaneous identification of A, B, D and R genomes by genomic *in situ* hybridization in wheat-rye derivatives. Heredity 83: 249-252

[5] Liu B, Vega JM, Segal G, Abbo S, Rodova H, Feldman M (1998 b) Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy noncoding DNA sequences. Genome 41: 272-277

[6] Liu B, Vega JM, Feldman M (1998 a) Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. II. Changes in low-copy coding DNA sequences. Genome 41: 535-542.

TRANSLOCATIONS BETWEEN WHEAT AND *Hordeum chilense* CHROMOSOMES INDUCED BY GAMETOCIDAL FACTORS.

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ABSTRACT: Chromosomal structural changes have been obtained in common wheat by means of gametocidal factors present in chromosome 2C from *Aegilops cylindrica* Host (2n=4x=28, CCDD) [1]. Using the *Ae. cylindrica* system for inducing rearrangement between *H. chilense* and wheat chromosomes, we have crossed hexaploid tritordeum (the fertile amphiploid between *H. chilense* and *Triticum turgidum*, 2n= 6x=42, $AABBH^{ch}H^{ch}$) with the disomic addition line for chromosome 2C of *Ae. cylindrica* in 'Chinese Spring'. The F1 plants were backcrossed to tritordeum followed by two generations of selfing. The descendence was screened for chromosomal structural aberrations by fluorescence in situ hybridization (FISH). Total genomic *H. chilense* DNA probe was used to identify intra- e intergenomic translocations and repetitive DNA sequences (pAs1 and GAA-satellite) were used to identify chromosomes involved in these translocations. Centromeric, intercalar and dicentric translocations between *H.chilense* and wheat genomes were obtained, most of them involving D-genome chromosomes.

1. INTRODUCTION

The interchange of genetic material between different species of the tribe Triticeae has a great potential for broadening genetic basis of crop plants. Different approaches have been used to induce the interchange of genetic information between relative species. Translocation, substitution and recombination lines have been obtained in this tribe by using radiations, manipulations of the *ph* genes or tissue culture. Recently, gametocidal chromosome 2C of *Ae. cylindrica* introduced into common wheat was found to induce high frequencies of cromosomal structural changes such as deletions and translocations [2].

The FISH technique is powerful tool to detect translocations involving chromosome of different genomes.

The present work we shows the detection and identification by FISH of intergenomic translocations involving wheat and *H. chilense* chromosomes induced by chromosome 2C of *Ae. cylindrica*.

2. MATERIAL AND METHODS

Plant Material.

Tritordeum (the amphiploid between *H. chilense* and *T. turgidum*, 2n=6x=42, $\mathbf{AABBH^{ch}H^{ch}}$) was crossed with the disomic addition line for chromosome 2C of *Ae. cylindrica* in wheat conv. 'Chinese Spring' (CS). The disomic addition line was produced by Endo [1] and kindly provided by the Wheat Genetics Resource Center, Department of Plant Pathology, Kansas State University U.S.A. F1 plants ($\mathbf{AABBDH^{ch}}$+ **C**) monosomic for 2C were backcrossed once to tritordeum followed by two generations of selfing.

FISH.

Analysis of chromosome complements of somatic cells was carried out in root-tip cells pretreated for 3h in 0.05 % of colchicine solution at 25 ^{O}C and fixed in 100 % ethanol-

acetic acid 3:1 (v/v) for at least two week. The barley clone pHvG38 [3] containing the GAA-satellite sequence was

kindly provided by Dr. S.K. Rasmussen from the Riso National Laboratory, Roskilde (Denmark) and the pAs1 probe (1kb) isolated from *T.tauschii* by Rayburn and Gill [4] was kindly provided by the Wheat Genetics Resource

Centre, University of Kansas, USA. The GAA-satellite and pAs1 probes were labeled with digoxigenin-11-dUTP and biotin-11-dUTP respectively, by nick-translation, and mixed to a final concentration of 5ng/µl in the hybridization solution. In situ hybridization and post- hybridization washes were performed as previously described by Cabrera *et al.* [5].

After examination of metaphases hybridized with the repetitive probes, preparations were re-probed with total genomic *H. chilense* DNA. Biotin-labeled pAs1-probe and digoxigenin-labeled GAA-satellite sequence were detected with Streptavidin-Cy3 conjugate (Sigma) (50ng/µl), and anti-digoxigenin-FITC (10ng/µl), respectively. Chromosomes were counterstained with DAPI (4´,6-diamidino-2-phenylindole) (2.5 ng /ml) and mounted in Vectashield. Signals were visualized using a Leica epifluorescence microscope. Images were captured with a SPOT CCD camera using the appropiate SPOT 2.1 software (Diagnostics Instruments, Inc., Sterling Heights, Michigan, USA) and processed with PhotoShop 4.0 software (Adobe Systems Inc.). Images were printed on a Hewlett Packard DeskJet HP 840C Printer after conversion to gray scale.

3. RESULTS AND DISCUSION.

Plants with chromosome numbers ranging from 38 to 45 were screened by FISH using the pAs1 and GAA sequences as probes. The pAs1 identified D- and H^{ch}-genome chromosomes [4, 6] and the GAA probe detected multiple hybridization sites on B- and some minor sites on A-genome chromosomes, respectively [3].

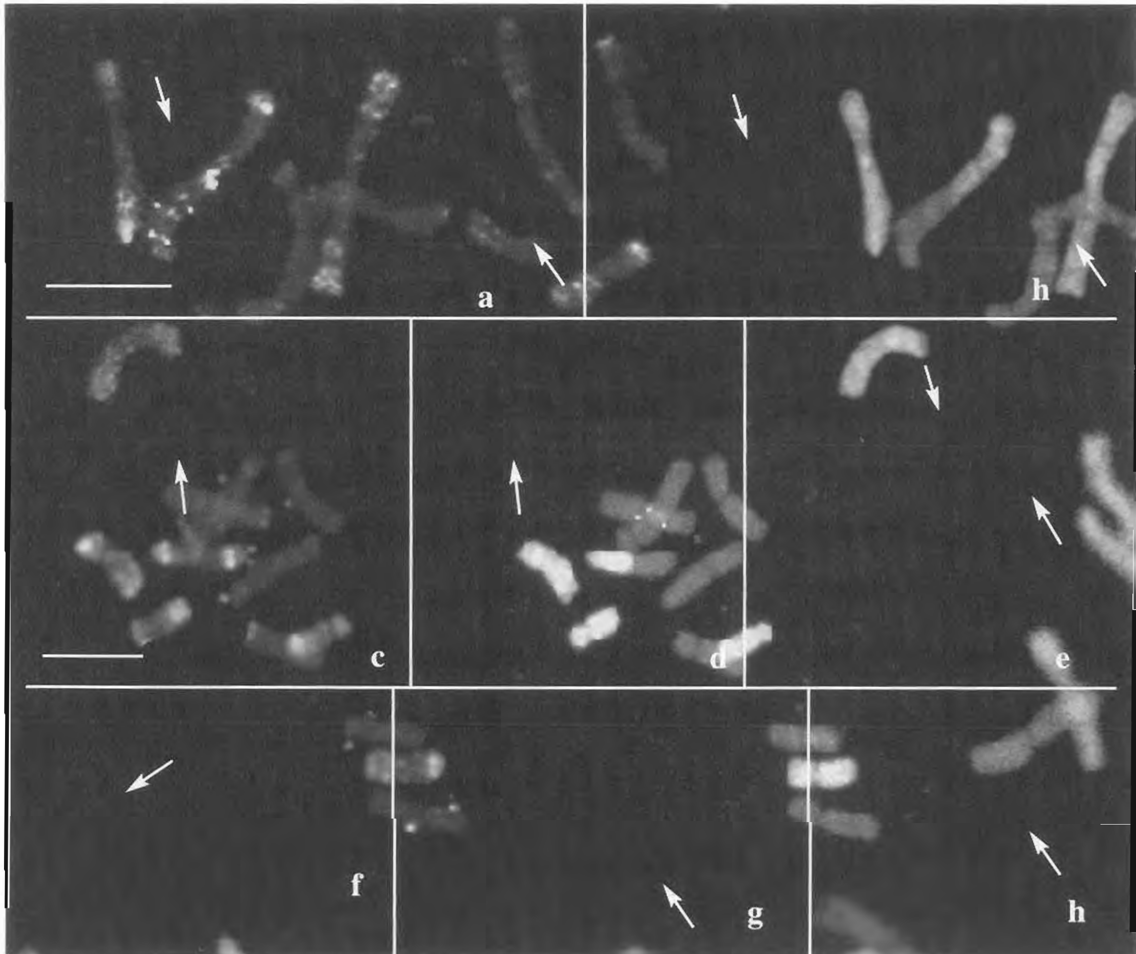

Figure 1. FISH to metaphase spreads showing different intra- and intergenomic translocations in the tritordeum plants analyzed. Heterozygous 2DL-3HchL//2DS-3HchS translocations identify with pAs1 and genomic H. chilense respectively (a,b). 1HchL-1DL translocation identify with pAs1 and genomic H chilense respectively (c,d). 2DL-3HchL translocation and intercalar translocation of H. chilense in wheat (e). Dicentric H. chilense -wheat (f). Hch-Hch (g) and wheat-wheat (h) chromosome detected with genomic H. chilense respectively. Bars =10 µm (a,b, e and g are the same; c,d,f and h are the same).

Centromeric, intercalar and dicentric chromosomes were observed involving wheat and *H. chilense* chromosomes. All centromeric translocations obtained were between D- and H^{ch}- genome chromosomes. Identified translocations were $3H^{ch}L$-2DL, $3H^{ch}S$-2DS, (Figure 1a,b) $1H^{ch}L$-1DL, (Figure 1c,d) and $6H^{ch}L$-1DS some of them obtained in homozygous conditions after selfing. One intercalar translocation of *H. chilense* into one unidentified A- or B-genome chromosome was also obtained (Figure 1e). Translocation lines are of potential value for introgressing D-genome chromosomes into hexaploid tritordeum which is of interest for breeding breadmaking quality of this amphiploid.

Dicentric translocations involving wheat-wheat, H^{ch}-H^{ch} and wheat-H^{ch}-genome chromosomes were frequently observed (Figure 1f,g,h). Chromosome 2C is known to cause single breakage in the chromosome arms producing terminal deletions [1]. If fusion occurs between two centric fragments, it gives rise to a dicentric chromosome.

The healing of the broken chromosome ends achieved by *de-novo* additions of telomeric repeats lead to deficiencies [7]. Different deletions lines have been obtained in common wheat [2], barley [8] and rye [7] using chromosome 2C from *Ae. cylindrica.* Deficiencies in *H. chilense* chromosomes have been also isolated using this genetical system (data not shown).

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REFERENCES

[1] Endo, T.R. 1988. Induction of chromosomal structural changes by a chromosome of *Aegilpos cylindrca* L. in common wheat. J. Hered. **79**:366-370.

[2] Endo, T.R. & Gill B.S. 1996. The deletion stock of common wheat. J. Hered **87**: 295-307.

[3] Pedersen, C.; Rasmussen, S.K. & Linde-Laursen, I., 1996. Genome and chromosome identification in cultivated barley and related species of the Triticeae (Poaceae) by in situ hybridization with the GAA-satellite sequence. Genome **39**:93-104.

[4] Rayburn, A.L. & Gill, B.S. 1986. Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa.* Plant Mol Biol Rep **4**:102-109.

[5] Cabrera, A.; Ramirez, M.C. & Martín, A. 1999. Aplication of C-banding and in situ Hybridization of the trisomic of *Hordeum chilense.* Euphytica **109**:123-129.

[6] Cabrera, A.; Friebe, B.; Jiang, J. & Gill, B.S. 1995. Characterization of *Hordeum chilense* chromosomes by C-banding and in situ hybridization using highly repeated DNA probes. Genome **38**: 435-442.

[7] Friebe, B.; Kynast, R.G. & Gill, B.S. 2000. Gametocidal factor-induced structural rearrangements in rye chromosomes added to common wheat. Chromosome Research **8**: 501-511.

[8] Schubert, I.; Shi, F.; Fuchs, J. & Endo, T. R. 1998. An efficient screening for terminal deletions and translocations of barley chromosomes added to common wheat Plant J **14**:489-495.

MOLECULAR MARKERS FOR THE GENETIC ANALYSIS OF WILD BARLEY, *HORDEUM CHILENSE*, AND THEIR APPLICATION IN WHEAT AND TRITORDEUM BREEDING

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ABSTRACT: The wild South American barley species *Hordeum chilense* Roem. et Schult. contains interesting genes for biotic and abiotic stress resistance, as well as important quality traits such as carotene content and seed storage proteins, many of which are expressed in a wheat background. The use of this wild species to increase wheat genetic resources will be greatly facilitated by marker-assisted introgression. In recent years, the search for the most suitable DNA marker system for tagging *H. chilense* genomic regions in a wheat background has lead to the development of RAPD and SCAR markers for this species. RAPDs represent an easy way of quickly generating suitable introgression markers, but their use is limited in heterogeneous wheat genetic background. SCARs are more specific assays, suitable for automatation or multiplexing. Direct sequencing of RAPD products is a cost-effective approach that reduces labour and costs for SCAR development. Practical applications of the different marker approaches for the obtention of derived introgression products are described.

1. *HORDEUM CHILENSE* AS A SOURCE OF GENETIC VARIABILITY FOR WHEAT BREEDING

The wild South American barley species *Hordeum chilense* Roem. et Schult. contains interesting genes for biotic and abiotic stress resistance, as well as important quality traits such as carotene content and seed storage proteins, many of which are expressed in a wheat background (1). Tritordeum, the barley-wheat amphiploid, is the basic genetic material for using *H. chilense* genetic variability in wheat breeding (2). The use of this wild species to increase wheat genetic resources will be greatly facilitated by marker-assisted introgression. To do this, molecular markers that enable tracking of *H. chilense* chromatin in a wheat background are needed.

2. APPROACHES FOR THE DEVELOPMENT OF COST-EFFECTIVE MOLECULAR MARKERS

The practical application of molecular markers in a breeding programme requires simple and economic methods due to the high numbers of individuals that need to be characterized every generation. The advent of PCR-based molecular markers has made molecular tools accessible for breeder use. However, when the breeder needs to use molecular tools for a wild species like *H. chilense*, two more constraints are usually faced: a lack of sequence information available for the wild species genome, and strong economic limitations to marker development when working with a non-crop species. For these reasons, RAPDs were the first DNA molecular markers to be developed for *H. chilense*.

RAPDs represent an easy way of quickly generating markers suitable for introgression (3, 4), but their use is limited when the wheat genetic background changes. SCARs (5) are more specific assays, suitable for automation or multiplexing. To reduce labour and costs for SCAR development, direct sequencing of RAPD products is the preferred approach for their development (6). The former approach is based on a modification of the standard RAPD protocol, consisting of the use of 10-mer primers in pairwise combina-

tions, instead of a single decamer. In this way, mixtures of RAPD amplification products flanked by the same or by two different primers are obtained. After a simple selection of RAPD products flanked by two different RAPD primers, products can be directly sequenced using dye-terminator cycle sequencing, thus avoiding the cost and time-consuming cloning step, usually required for SCAR development (6).

An alternative approach for direct sequencing of RAPD products is the use of a single primer for amplification (the standard RAPD technique) and the 4 possible sets of 3'-extended oligonucleotide primers for dye terminator cycle sequencing (7). The cloning step is also avoided. So far, the development of SCAR markers using the obtained sequences has not been reported.

There is a major difference in the nature of the sequence amplified by SCARs obtained by cloning or by direct sequencing of RAPD products. When cloning the selected RAPD fragment: the original RAPD polymorphism can be reproduced. When directly sequencing the selected RAPD markers, the sequence within the RAPD fragment is used for priming instead of the immediate 3' extensions including the original primers. Such an amplification of sequences inner to the original RAPD maintains the polymorphism, due to a significant sequence divergence between the wheat and barley genomes.

3.THE UTILITY OF WHEAT AND BARLEY SSR AND STS PRIMERS

Wild species like *H. chilense* can benefit from marker resources developed in related Triticeae crop species such as barley and wheat. For example, a subset of SSR and STS primers developed for wheat and barley have an additional application for marker-assisted *H. chilense* introgression into wheat.

There is little information available to predict transferability of SSR or STS markers, but their ease of assay enables them to be tested empirically. As large numbers of SSR and STS markers are being developed in wheat and barley, even a relatively low level of transferability provides a valuable marker resource. In the present case, more than 50% of wheat and barley primer pairs amplified products from *H. chilense* DNA and would be applicable for mapping and germplasm surveys. Some of the *H. chilense* products failed to amplify in tritordeum or the addition lines, probably because of preferential amplification of the wheat sequences, but 36% of wheat and 29% of barley markers could be used for analysis of tritordeums and derived introgression lines. These figures clearly show that wheat and barley SSRs constitute an efficient and cost effective source of molecular markers for *H. chilense* (8). About 90% of the wheat and barley STS primers tested amplified the *H. chilense* genome. About 10% of them have proven useful for genetic analysis of tritordeums and derived introgression lines (9).

4. MARKER-ASSISTED SELECTION OF DERIVED INTROGRESSION PRODUCTS

Traditionally, marker-assisted selection of basic breeding material has been accomplished using morphological or biochemical markers. The advent of DNA molecular markers widens the possibilities of application. Marker-assisted selection of basic breeding material has been carried out, in a first approach, with the development of chromosome-specific markers for *H. chilense*, detectable in a wheat background. These markers are currently being used to obtain new addition lines of *H. chilense* accessions carrying agronomically interesting traits. The hybrid bread wheat ¥ hexaploid tritordeum (**AABBH^{ch}D**) is in some varietal combinations self-fertile and seed set can always be obtained after backcrossing with either of the parents (wheat or tritordeum). In this way, tritordeum can be used as a bridge for transferring desirable traits from *H. chilense* into bread wheat (1). Short-term goals are the introgression of genetically or chromosomally characterized traits. For example, high carotenoid content, interesting for durum wheat breeding, is conferred by the addition of chromosome 7H^{ch} (10) and resistance to carnal bunt is located on 4 H^{ch} (1). With this aim, *H. chilense* chromosome-specific SSR, STS and SCAR markers will be used.

Additional goals are the marker-assisted selection and chromosomal characterization of tritordeum nullisomic lines. This will be carried out using wheat SSRs specific for the A and B genome chromosome arms, as well as SSRs, SCARs and STSs marking the *H. chilense* chromosome arms.

Finally, barley STSs and SSRs non-transferred to *H. chilense* are detected in the primer screening process, and constitute valuable markers for the marker-assisted selection of tritordeum addition and substitution lines, in conjuction with *in situ* hybridisation techniques (11).

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REFERENCES

[1] Martin A, Martin LM, Cabrera A, Ramirez MC, Gimenez MJ, Rubiales D, Hernández P, Ballesteros J: The potential of *Hordeum chilense* in breeding Triticeae species. In: Jaradat AA (ed) Triticeae III, pp. 377-386. Science Publishers, Enfield, NH, USA (1998).

[2] Martín A, Martínez-Araque C, Rubiales D, Ballesteros J: Tritordeum: triticales's new brother cereal. In: Guedes-Pinto H, Darvey N, Carnide VP (eds) Triticale: Today and Tomorrow. Kluwer Academic (1996).

[3] Hernández P, Rubio MJ, Martín A: RAPDs as molecular markers for the detection of *Hordeum chilense* chromosomes in wheat addition lines and in tritordeum. Chromosome Research 3: 100 (1995).

[4] Peil A, Schubert V, Schumann E, Weber WE: RAPDs as molecular markers for the detection of *Aegilops markgrafii* chromatin in addition and euploid introgression lines of hexaploid wheat. Theor Appl Genet 94: 934-940 (1997).

[5] Paran I, Michelmore R: Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet. 85: 985-993 (1993).

[6] Hernández P, Martín A, Dorado G: Development of SCARs by direct sequencing of RAPD products: a practical tool for the introgression and marker-assisted selection of wheat. Molecular Breeding 5: 245-253 (1999).

[7] Mitchelson K, Drenth J, Duong H, Chaparro J: Direct sequencing of RAPD fragments using 3'-extended oligonucleotide primers and dye terminator cycle-sequencing. Nucleic Acids Research electronic article e28 (1999).

[8] Hernández P, Laurie DA, Martín A, Snape JW: The utility of barley and wheat Simple Sequence Repeat (SSR) markers for the genetic analysis of *Hordeum chilense* and tritordeum. Theor Appl Genet, *in press*.

[9] Hernández P, Dorado G, Martín A: The utility of barley Sequence Tagged Sites (STS) markers for genetic analysis of *Hordeum chilense* and tritordeum, *submitted*.

[10] Alvarez J, Martin A, Martin L: Chromosomal location of genes for carotenoid pigments using addition lines of *Hordeum chilense* in wheat. Plant Breeding 117: 287-289 (1998).

[11] Hernández P, Dorado G, Cabrera A, Laurie DA, Snape JW, Martín A: Rapid verification of wheat-*Hordeum* introgressions by direct staining of SCAR, STS and SSR amplicons. Genome, *in press*.

EMPLOYING ESTS AS A RESOURCE FOR THE DEVELOPMENT OF NOVEL MARKERS IN BARLEY (*HORDEUM VULGARE* L.)

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ABSTRACT The progress of genome sequencing projects of model plants combined with the recent advances in DNA sequence analysis and the establishment of high throughput assays have provided the framework for large-scale discovery and analysis of DNA sequence variation. With the development of EST sequencing projects, a wealth of sequence information is being generated allowing the possibility for potentially mapping these ESTs. In order to initiate a systematic approach to develop an EST-map of barley (*Hordeum vulgare* L.), we have employed RFLP, SSR and SNP assays to achieve our goals. RFLPs and SSRs are being identified using conventional techniques while SNPs are being analysed employing the denaturing high-performance liquid chromatography (DHPLC) for checking the segregating patterns in a doubled-haploid (DH) mapping populations. To this end, SNPs between the parental genotypes were identified using a direct sequencing approach. Once a SNP was established between the parents, the DH lines were analysed for the presence of either of the alleles. Employing the three assays mentioned above, we plan to place over 1000 genes onto a consensus EST map of barley with preliminary results being presented.

1 INTRODUCTION

The analysis of DNA sequence variation is of major importance in genetic studies. In this context, molecular markers represent a major tool for genome mapping and have revolutionised the genetic analysis of crop plants. Several approaches have been pursued to detect sequence polymorphism in barley, including RFLP (restriction fragment length polymorphism; eg. 1), RAPD (random amplified polymorphic DNA; eg. 2), SSR (simple sequence repeat; eg. 3) and AFLP (amplified fragment length polymorphism; eg. 4). With the development of EST sequencing projects, a wealth of sequence information is being generated allowing the possibility for detecting and genotyping them. ESTs provide valuable but incomplete information. However, because they represent expressed genomic regions, ESTs are thought to identify the parts of the genome with the most biological significance. An EST map can provide the chromosomal location for genes whose functions are currently unknown. For identifying agronomicaly important genes, the map can also suggest a set of candidate genes to test whether the approximate location of these genes has been mapped by molecular marker techniques. Based on the conservation of expressed genes, mapped ESTs provide a platform for the systematic analysis of synteny between the genomes of rice and barley.

RFLP is a variation of hybridisation-based assays and has been most widely used because of their reproducibility. This assay utilises cDNAs or cloned fragments of total genomic DNA as probes on DNA which has been digested with restriction enzymes, to detect polymorphic DNA fragments between different individuals. The RFLP assay has several advantages such as the detection of co-dominant markers although this assay suffers from being relatively laborious and requires large amount of DNA to obtain good results. Several detailed RFLP maps have been constructed in barley, which together comprise more than 1000 different RFLP markers (5).

Microsatellites [SSRs or hypervariable sequences] are arrays of short motifs of 1-4 base pairs in length (3). These single-locus markers are characterised by their hypervariablility, abundance, reproducibility, mendelian inheritance and co-dominant nature. SSR markers require the design of primers for the conserved

flanking regions of the microsatellite, and the PCR amplification of the repeat region. In the past, SSRs have been expensive to develop thus often limited to applications in larger commercial crops. Therefore, the identification of SSRs from EST databases might represent a less costly approach.

SNPs are genetic markers, which are highly abundant and less prone to mutations than SSRs (6). In addition, many assays have been developed to type SNPs in an automated fashion and many yield simple positive or negative results that can be interpreted easily (7). Among these assays, DHPLC has significantly reduced the processing time of detection and analysis of SNPs (6,8). This technique represents a highly sensitive and fully automated assay for high-throughput SNP analysis, which is based on an ion-pair reversed-phase high performance liquid chromatography method (9).

The barley genome is well characterised with respect to classical genetics and cytogenetics, and in many regards acts as a model crop for mapping studies in cereals. Based on the DNA sequence information obtained from the barley EST database (B-EST; http://pgrc.ipk-gatersleben.de/), we have investigated the usefulness of ESTs as a resource to develop and map a comprehensive set of novel markers for the barley genome

2 Materials and Methods

2.1. Genetic analysis

To screen for markers, barley mapping populations "Igri" x "Franka", "Steptoe" x "Morex", and "Oregon Wolfe Dom" x "Oregon Wolfe Rec" were employed. A doubled-haploid mapping population, which was derived from these crosses was kindly supplied by P. Hayes (Oregon State University, USA). Total genomic DNA was extracted from leaf material as described in Graner et al. (1). Mapping of RFLP, SSR and SNP markers were undertaken using JoinMap using a LOD score of 3.0 (11).

2.2. RFLP analysis

A set of six restriction enzymes (*Bam*HI, *Hind*III, *Eco*RI, *Eco*RV, *Xba* and *Dra*I) was used to digest genomic DNA. Southern blotting and probe labelling was carried out according to Graner et al. (1). Autoradiography was performed by exposure of hybridised blots to imaging plates (Fuji Photo Film, Japan) and subsequent signal detection on a phosphoimager (Fuji, Japan).

2.3. SSR analysis

For development of EST derived SSR markers the IPK barley EST database (B-EST) consisting of 13109 entries was searched for all possible dimeric (minimum 6 repeats), trimeric and tetrameric microsatellites (both minimum 5 unit repeats) with the help of a PERL5 script. The search revealed 367 non-redundant SSRs, for which flanking primers were designed with the Primer3 software (Whitehead Institute). PCR was done in 10µl reactions containing 20 ng genomic DNA, 1x PCR buffer (incl. 1.5 mM $MgCl_2$), 0.2 mM dNTPs, 2.5 pmol of each primer and 1/4 U *Taq* DNA Polymerase (Qiagen, Germany). For all microsatellites the following touchdown PCR profile was used: 3 min at 94°C; 10 cycles of 30 sec at 94°C, 30 sec at 60°C minus 0.5°C/cycle, 30 sec at 72°C; 25 cycles of 30 sec at 94°C, 30 sec at 55°C, 72°C at 72°C; and 5 min at 72°C for final extension. PCR products were separated on 10% denaturing polyacrylamide gels (7M urea) and visualized by silver staining.

2.4. DHPLC analysis

To perform the PCR, primers were designed to amplify ESTs derived from cDNA libraries constructed using *H. vulgare* cv. Barke. All primers were designed using the Primer Express software (Perkin-Elmer, USA). PCR reactions consisted of 1 x PCR buffer (incl. 1.5 mM $MgCl_2$), 1.5 mM $MgCl_2$, 0.5 mM of each primer, 1.0 U of *Taq* DNA polymerase (Qiagen, Germany), 250 mM dNTPs and 25 ng genomic DNA, in a volume of 20 ml. The touch down PCR cycling profile included an initial denaturing step at 94°C for 2 minutes followed by a profile of: 1 cycle of 30 sec. at 94°C, 1 min. at 65°C and 1 min. at 72°C followed by 9 cycles over which the annealing temperature was decreased by 1°C per cycle, followed by 40 cycles of 30 sec. at 94°C, 1 min. at 56°C and 1 min. at 72°C.Denaturing high-performance liquid chromatography was carried out on automated HPLC instrumentation equipped with a DNASep column as described in Kota et al. (10).

2.5. Direct sequencing

To identify SNPs, PCR products amplified among the parental genotypes were sequenced in both directions on an ABI 377 automated sequencer using big dye-terminator chemistry (Perkin-Elmer, USA). DNA sequence data was checked for sequencing errors using the "Sequencher" software (Gene Codes Corporation, USA). Sequences were aligned using GCG pileup program (13), and polymorphisms between the parents were identified manually.

3 Results and Discussion

EST mapping in recent times has gained momentum, especially with the wealth of sequence information that is being generated in various EST sequencing programs. Mapping ESTs using RFLPs among the three populations resulted in the mapping of 62 ESTs in "Igri" x "Franka", 58 in"Steptoe" x "Morex", and 95 in "Oregon Wolfe Dom" x "Oregon Wolfe Rec" mapping populations respectively. While employing the SSR technique, we have so far mapped 16 ESTs in "Igri" x "Franka", 11 in"Steptoe" x "Morex", and 61 in "Oregon Wolfe Dom" x "Oregon Wolfe Rec" mapping populations respectively. As ESTs represent expressed sequences, the frequency of SSR occurrence and the allelic variability is lower than in genomic sequences. Although RFLP and SSR assays have been successful in mapping ESTs, approximately 38% of the EST-RFLPs and 68% of the EST-SSRs were monomorphic. Hence alternate marker assays need to be considered for mapping a greater number of barley ESTs. One such assay is the single nucleotide polymorphism (SNP).

The screening of SNPs was performed by DHPLC, based on the differential retention of homo- and heteroduplex DNA molecules under conditions of partial denaturation. In the present work, optimal temperatures for discrimination was determined by employing the algorithm available with the WAVEmaker software (Transgenomics Inc., USA). Although, the algorithms are fairly accurate, we have been analysing our samples in 1-3°C increments over the range of predicted melting temperatures to achieve greater sensitivity. In addition to temperature, other parameters, which include PCR primer design, PCR profile and separation gradient are also important for optimal SNP analysis (9). In cases where the sequence of the PCR fragment is not known, samples will have to be run at several temperatures making it a time-consuming process (14). Considering this factor, we decided to sequence the PCR fragments generated from the parental genotypes, as this method in our experience has proven to be a faster and more efficient way to identify potential SNPs rather than trying to identify them directly on the DHPLC.

To test the reproducibility of the results obtained from the DHPLC, primers were designed to amplify ESTs that have been previously tested as RFLP markers. Results from these ESTs revealed identical data sets for both marker types, confirming the accuracy and reproducibility of the DHPLC assay (Fig. 1). In addition, those ESTs that were monomorphic at the RFLP level could now be mapped using the DHPLC approach. Therefore, the DHPLC analysis represents a sensitive tool to maximise the number of ESTs that can be mapped, as it will be required for the construction of a comprehensive transcript map of barley. Employing the above process, we identified 51 SNPs among the three mapping populations, of which 36 have been mapped so far.

Compared to most primer extension procedures applied to detect SNPs, a major advantage of the procedure described in this study is the savings in labour costs associated with purifying PCR products, setting up and loading of sequencing reactions and reading the sequencing gels. This holds true in our case where only a few sequencing reactions were necessary to reveal the chemical nature of a mutation.

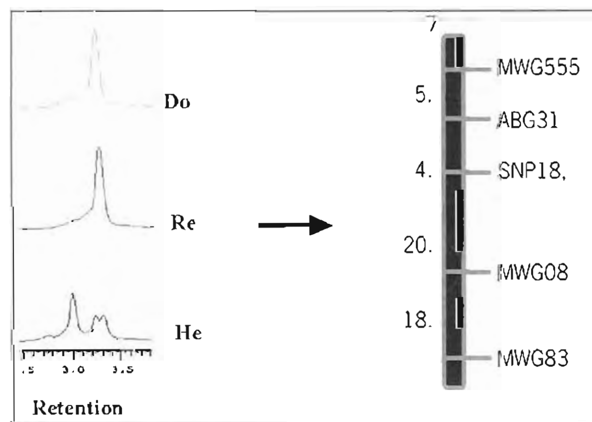

Figure 1: Elution profile and a partial map of barley chromosome 7H displaying the genetic position of marker SNP18 and RFLP marker HY05C04 from barley in the "Dom" x "Rec" DH population. "Het" represents a mixture of PCR products from "Dom" and "Rec" in equimolar ratio. The above figure shows the congruency of the results of RFLP and DHPLC analysis of the SNP markers and its corresponding RFLP probe.

Our results demonstrate the high reproducibility of the DHPLC, simultaneously providing us with the possibility of mapping monomorphic RFLPs and SSRs. These advantages and other useful attributes such as low reagent costs and ease of operation make DHPLC a suitable assay for routine large-scale SNP analysis. We are currently using these techniques (RFLP, SSR and SNP) to construct a high density EST map of barley (Fig. 2). While mapping these ESTs in barley would be useful as they represent expressed genes, data generated from these studies could be employed for syntenic studies with rice and other related species. Since sequence information from rice is available, synteny information can be accessed rapidly by sequence alignments. In conclusion, an EST map will enable workers to align existing linkage maps in barley and would enable ESTs that are associated with traits of interest to be more efficiently identified.

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We thank P. Hayes for providing the DH lines of barley mapping populations "Steptoe" x "Morex" and "Oregon Wolfe Dom" and "Oregon Wolfe Rec". We also thank Ulrike Beier, Sylvane Stegmann and Jacqueline Pohl for their technical assistance. This work was funded by the BMBF (grants 0312271A and 0312278C).

REFERENCES

[1] Graner A, Jahoor A, Schondelmaier H, Siedler K, Pillen K, Wenzel G, and Herrmann RG: Construction of an RFLP map of barley. Theoretical and Applied Genetics. 83: 250-256 (1991).

[2] Weyen J, Bauer E, Graner A, Friedt W, and Ordon F: RAPD-mapping of the distal portion of chromosome 3 of barley, including the BAMMV/BAYMV resistance gene ym4. Plant Breeding. 115: 285-287 (1996).

[3] Pillen K, Binder A, Kreuzkam B, Ramsay L, Waugh R, Forster J, and Leon J: Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. Theoretical and Applied Genetics. 101: 652-660 (2000).

[4] Waugh R, Bonar N, Baird E, Thomas B, Graner A, Hayes P, and Powell W: Homology of AFLP products in three mapping populations of barley. Molecular and General Genetics. 255: 311-321 (1997).

[5] Kleinhofs A, and Graner A: An integrated map of the barley genome. In: I. Vasil (ed) DNA markers in plants. Kluwer Academic (1996).

[6] Giordano M, Oefner PJ, Underhill PA, Luca Cavalli Sforza L, Tosi R, and Richiardi PM. Identification by denaturing high-performance liquid chromatography of numerous polymorphisms in a candidate region for multiple sclerosis susceptibility: Genomics. 56: 247-253 (1999).

[7] Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, and Lander ES: Large-scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. Science. 280: 1077-1082 (1998).

[8] Oefner PJ and Underhill PA. DNA mutation detection using denaturing high performance liquid chromatography (DHPLC): In: Current Protocols in Human Genetics. Wiley and Sons (1998).

[9] Kuklin A, Munson K, Gjerde D, Haefele R, and Taylor P: Detection of single nucleotide polymorphisms with the WAVE DNA fragment analysis system. Genetic Testing. 1: 201-206 (1997/1998).

[10] Kota R, Wolf M, Michalek W, and Graner A: Application of DHPLC for mapping of single nucleotide polymorphisms (SNPs) in barley (*Hordeum vulgare* L.). Genome. 44: 523-528 (2001).

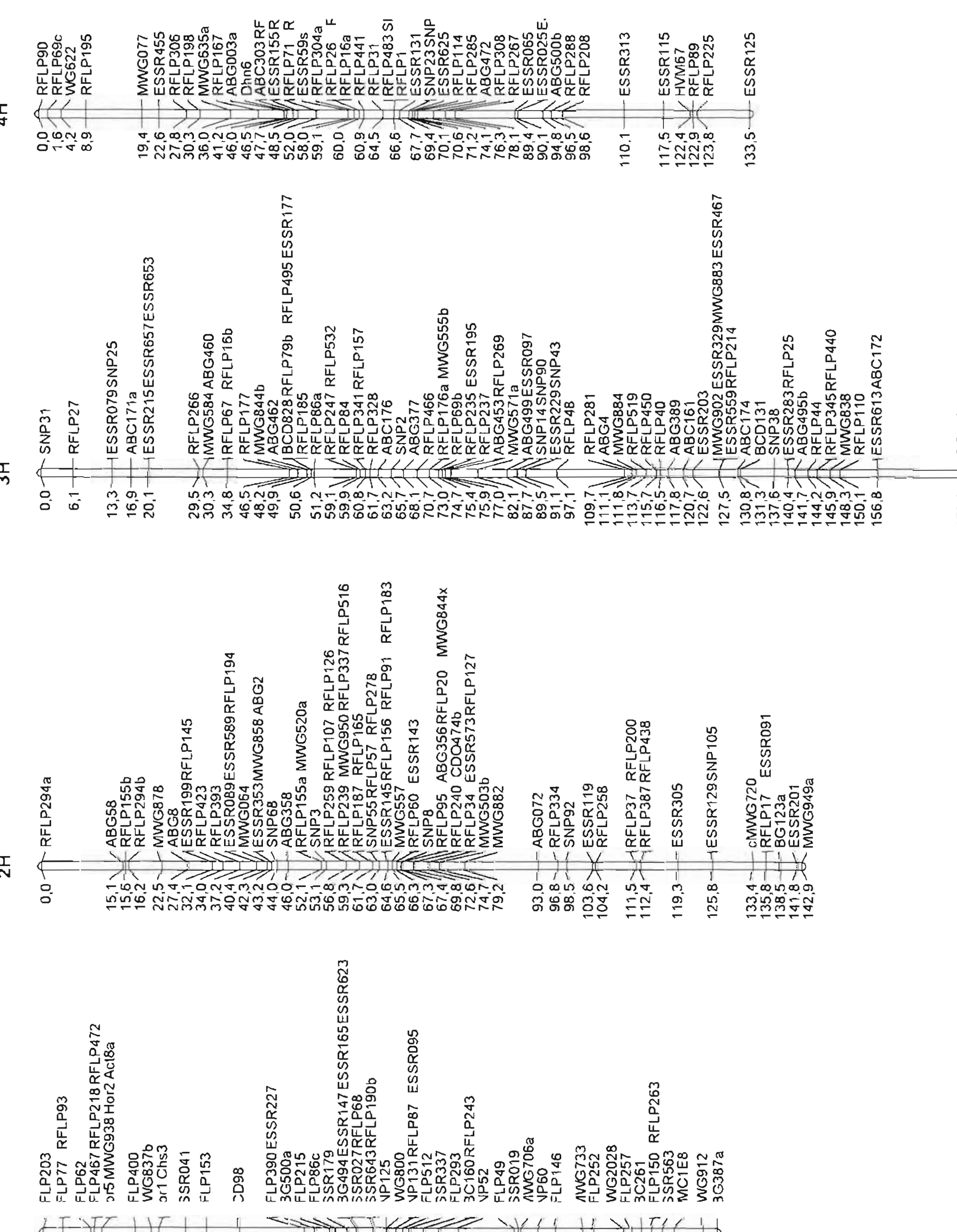
[11] Stam P: Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. The Plant Journal. 3: 739-744 (1993).

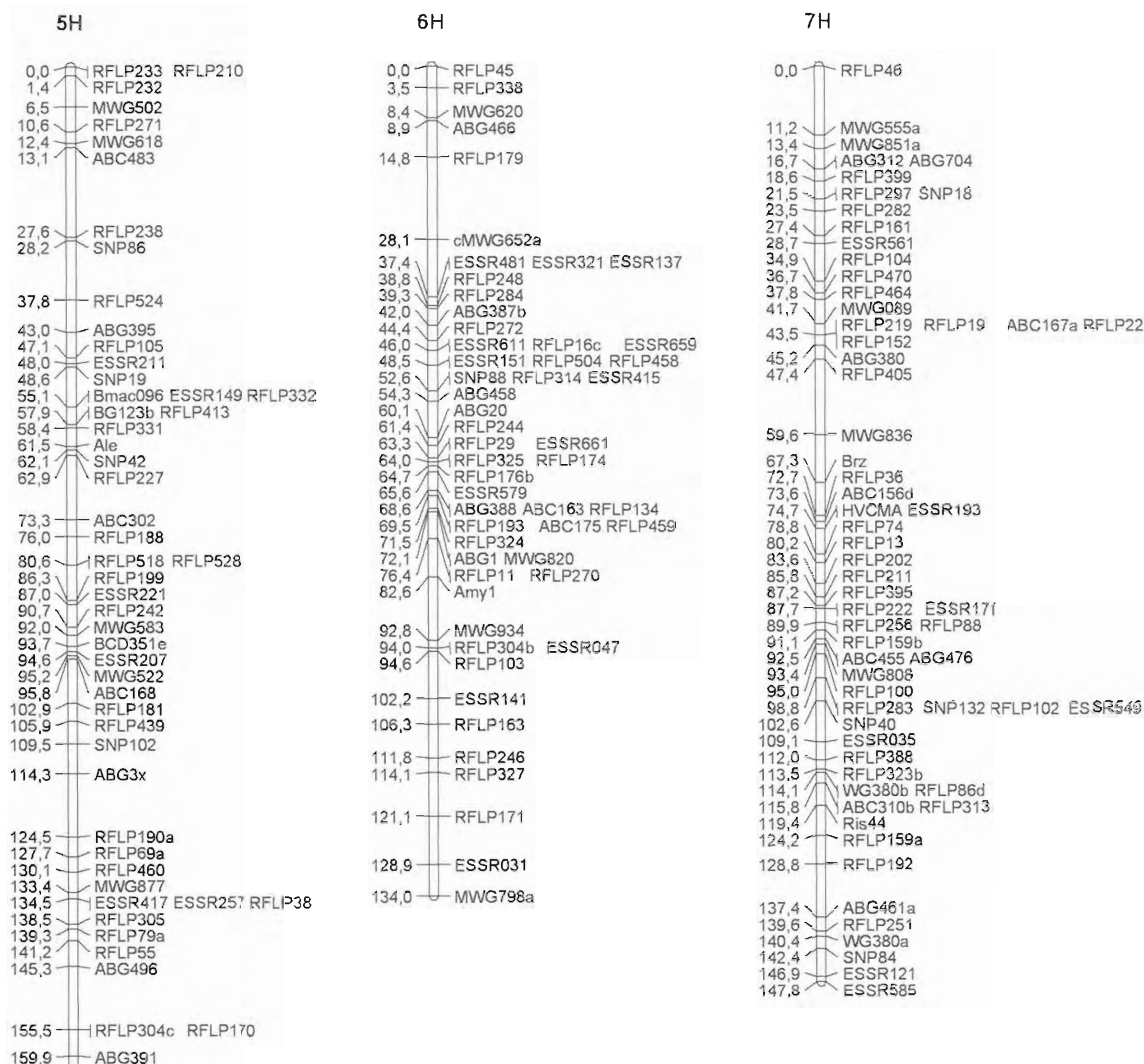
[12] Huber CG, Oefner PJ, and Bonn GK: High-resolution liquid chromatography of oligonucleotides on nonporous alkylated styrene-divinylbenzene copolymers. Analytical Biochemistry. 212: 351-358 (1993).

[13] Gribskov M, Devereux J, and Burgess RR: The codon preference plot: graphic analysis of protein coding sequences and prediction of gene expression. Nucleic Acids Research. 12: 539-549 (1984).

[14] Wagner T, Stoppa-Lyonnet D, Fleischmann E, Muhr D, Pagès S, Sandberg T, Caux V, Moeslinger R, Langbauer G, Borg A, and Oefner P: Denaturing high performance liquid chromatography (DHPLC) detects reliably *BRCA1* and *BRCA2* mutations. Genomics. 62: 369-376 (1999).

Figure 2: Current state of consensus map of barley illustrating the various ESTs mapped so far employing RFLPs, SSRs and SNPs.





5H
0,0 RFLP233 RFLP210
1,4 RFLP232
6,5 MWG502
10,6 RFLP271
12,4 MWG618
13,1 ABC483
27,6 RFLP238
28,2 SNP86
37,8 RFLP524
43,0 ABG395
47,1 RFLP105
48,0 ESSR211
48,6 SNP19
55,1 Bmac096 ESSR149 RFLP332
57,9 BG123b RFLP413
58,4 RFLP331
61,5 Ale
62,1 SNP42
62,9 RFLP227
73,3 ABC302
76,0 RFLP188
80,6 RFLP518 RFLP528
86,3 RFLP199
87,0 ESSR221
90,7 RFLP242
92,0 MWG583
93,7 BCD351e
94,6 ESSR207
95,2 MWG522
95,8 ABC168
102,9 RFLP181
105,9 RFLP439
109,5 SNP102
114,3 ABG3x
124,5 RFLP190a
127,7 RFLP69a
130,1 RFLP460
133,4 MWG877
134,5 ESSR417 ESSR257 RFLP38
138,5 RFLP305
139,3 RFLP79a
141,2 RFLP55
145,3 ABG496
155,5 RFLP304c RFLP170
159,9 ABG391
163,8 ABG390
164,5 RFLP323a
168,7 ESSR015
169,3 ESSR583 RFLP86b
177,0 MWG851b
180,5 ABC309
181,4 MWG813a
182,6 RFLP97
189,9 MWG602a
6H
0,0 RFLP45
3,5 RFLP338
8,4 MWG620
8,9 ABG466
14,8 RFLP179
28,1 cMWG652a
37,4 ESSR481 ESSR321 ESSR137
38,8 RFLP248
39,3 RFLP284
42,0 ABG387b
44,4 RFLP272
46,0 ESSR611 RFLP16c ESSR659
48,5 ESSR151 RFLP504 RFLP458
52,6 SNP88 RFLP314 ESSR415
54,3 ABG458
60,1 ABG20
61,4 RFLP244
63,3 RFLP29 ESSR661
64,0 RFLP325 RFLP174
64,7 RFLP176b
65,6 ESSR579
68,6 ABG388 ABC163 RFLP134
69,5 RFLP193 ABC175 RFLP459
71,5 RFLP324
72,1 ABG1 MWG820
76,4 RFLP11 RFLP270
82,6 Amy1
92,8 MWG934
94,0 RFLP304b ESSR047
94,6 RFLP103
102,2 ESSR141
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7H
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11,2 MWG555a
13,4 MWG851a
16,7 ABG312 ABG704
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21,5 RFLP297 SNP18
23,5 RFLP282
27,4 RFLP161
28,7 ESSR561
34,9 RFLP104
36,7 RFLP470
37,8 RFLP464
41,7 MWG089
43,5 RFLP219 RFLP19 ABC167a RFLP22
RFLP152
45,2 ABG380
47,4 RFLP405
59,6 MWG836
67,3 Brz
72,7 RFLP36
73,6 ABC156d
74,7 HVCMA ESSR193
78,8 RFLP74
80,2 RFLP13
83,6 RFLP202
85,8 RFLP211
87,2 RFLP395
87,7 RFLP222 ESSR171
89,9 RFLP256 RFLP88
91,1 RFLP159b
92,5 ABC455 ABG476
93,4 MWG808
95,0 RFLP100
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102,6 SNP40
109,1 ESSR035
112,0 RFLP388
113,5 RFLP323b
114,1 WG380b RFLP86d
115,8 ABC310b RFLP313
119,4 Ris44
124,2 RFLP159a
128,8 RFLP192
137,4 ABG461a
139,6 RFLP251
140,4 WG380a
142,4 SNP84
146,9 ESSR121
147,8 ESSR585

RYE AND *Hordeum chilense* CHROMATIN DISTRIBUTION IN SOMATIC CELLS OF INTERGENERIC HYBRID COMBINATIONS

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ABSTRACT: GISH technique was used to analyse spatial relationships between homologous chromosomes of *Hordeum chilense* and *Secale cereale* in different somatic cells of *Triticum aestivum* x tritordeum (genome constitution $AABBDH^{ch}$), *T. turgidum* x tritordeum ($AABBH^{ch}$), triticale x *T. aestivum* (AABBDR) and *T. aestivum* x *S. cereale* (ABDR), hybrid combinations. Rye and barley chromatin could be easily distinguished from wheat chromatin in anthers binucleated tapetal cells. The number of *H. chilense* and rye chromatin domains and its localisation was quite variable in all types of cells analysed. In all hybrid combinations, the spatial distribution of alien chromatin in the binucleated cells of the tapetum was very similar. Since the discrimination of the two species chromosomes domains was possible, we made the comparison between the distribution of the number of alien domains per cell with those expected according with a mathematical model assuming random association between the rye or barley chromosomes in the nucleus. The results indicated that labelled chromatin is randomly located in the nucleus and there is neither evidence to assume the existence of parental genome separation nor a significant tendency of labelled chromosomes to occupy a specific region into the nucleus. The influence of Ph1 gene activity on the spatial organisation of rye chromosomes and chromatin condensation during the cell cycle is also discussed.

1. INTRODUCTION

The existence of an interphase nuclear architecture is a polemic topic [1]. Most of cellular activities take place at interphase, when chromosomes are decondensed. Nuclear organisation at interphase has been extensively investigated to understand chromosomes behaviour and to explore relationships with gene location or expression [2]. Chromatin seems to appear apparently mixed at interphase stage, but using genomic in situ hybridisation techniques, it has been demonstrated that chromatin of each chromosome was located in a discrete region, called chromosome territory [3].

Different methods, involving cytogenetic studies and mathematical models, have shown contradictory results and interpretations. Some authors have reported that there is a parental genome separation in hybrid combinations [2] and in other occasions a fixed chromosome location of all chromosomes of an haploid complement has been described [4, 5].

In wheat, the spatial distribution of genomes and chromosomes could be controlled, at least in part, by the Ph1 locus, that also is involved in homeologous pairing behaviour. It has been pointed out that Ph1 locus has a clear influence on chromatin and nuclear matrix interactions, and subsequently on spatial chromatin distribution [6].

The aim of this work is to study *Hordeum chilense* and *Secale cereale* chromosome distribution in the interphase nucleus of somatic cells in wheat-tritordeum, wheat-triticale and wheat-rye hybrid combinations in the presence as well as in the absence of the Ph1 gene activity, by using GISH technique.

3. RESULTS AND DISCUSSION

H. chilense and rye chromatin were easily distinguished from wheat chromatin using GISH technique. With these materials we could analyse simultaneously the existence of parental genome separation, and Ph1 gene influence on rye chromatin arrangement in somatic interphase cells.

During cereal meiosis, tapetal nuclei divide mitotically. Binucleated tapetum cells constitute an ideal material since:

1.- Cellular division time is well known.
2.- Both nuclei are derived from the last division.
3.- Stages of meiosis can be used as a way to analyse the temporal evolution of chromosomes associations.

We have used binucleated cells of the tapetum through the meiosis stages. It is well known that such cells are originated from a karyokinesis without cytokinesis division, which takes place during leptotene stage. In this study we adopted the term domain to assign the territory of a specific chromosome. So, in this material, it was possible to study the distribution of labelled domains in both nuclei using the meiosis stages as a way to measure the course of time. From direct observations, labelled chromosomes did not appear in a specific region of the nucleus, but also they were found dispersed or grouped, indistinctly. So, we can conclude that there is no reason for accepting the existence of parental genome separation organisation in our material.

The possibility to distinguish *H.chilense* and rye labelled domains allowed us to develop a mathematical model to ascertain if labelled chromosomes are distributed in a fixed position in the nucleus or, on the contrary, they are randomly distributed.

For an haploid complement of 7 chromosomes, as occurred for rye and H. chilense genomes, we can observe cells from 7 to 1 domain(s). The number of domains per cell depends on the number of chromosome territories that has been fused. Thus, a nucleus with one domain has been originated by 6 events of fusion, 2 domains by 5, ... 6 domains by 1 fusion event and 7 domains by 0 fusion events.

If all labelled chromosomes are randomly distributed in the interphase nucleus, all of them have the same opportunity to be fused and therefore, the number of nucleus with 6, 5, ... and 0 fusions should be derived from the binomial distribution $(x+y)^6$ where x is the probability of fusion for any chromosome and y the probability of no fusion. The parameters x and y would be easily obtained from the sample as follows: If *a* is the number of total numbers of domains and *N* the total nuclei analysed, then

$x = \frac{a}{6?N}$ and consequently $y = 1 - x$

This model is based in several premises such as:

a) All chromosomes have the same probability to be fused.
b) There is no interchromosome interaction that modifies the probability of fusion of each chromosome.
c) There is cellular homogeneity, i.e., the probabilities described above are maintained in all cells.
d) Both nuclei in the binucleated cells are independent.

The numbers of sites in which rye and H. chilense chromatin was located in the nuclei ranged from 1 to 7 nuclear regions in every hybrid combination (Fig. 1).

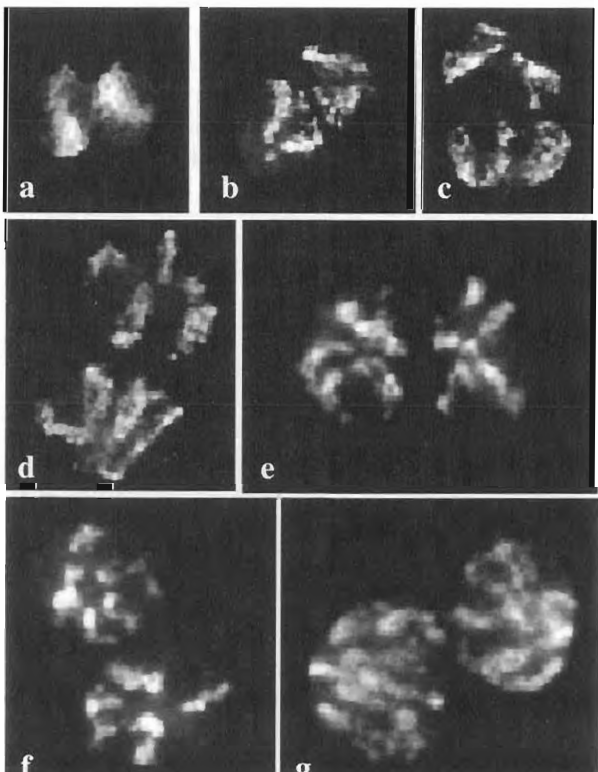


Figure 1: Binucleated tapetal cells with different number of domains from ABDR hybrid combination. Rye chromatin domains range from 1 to 7 nuclear regions (a-g). Each image shows cells with the same number of domains in both nuclei. nucllei.

In most cases, it was observed a very good fit between the expected and observed data when x^2 tests were performed. This result seems to indicate that probably labelled chromosomes are located at random in the interphase nucleus.

However, the situation seems to be different when ph1b mutants were studied, because the differences between the observed and expect values were significant in the 30% of the analysis. This can be explained by the fact that the number of cells with 2 or 3 domains increases to expenses extreme classes (1,5,6). This different behaviour would be explained by the activity of Ph1 gene. Thus the Ph1 activity suppression can affects chromatin organisation as it has been reported by Mikhailova [6]. However, we found no differences between Ph1 and ph1b hybrids neither in chromatin organisation of domains, nor in the mean number of domains per nucleus in both types of hybrids (Fig. 2).

No significant deviations with respect to the mean number of domains per nucleus were observed at different meiotic stages when t-Student tests where performed (Fig. 3).

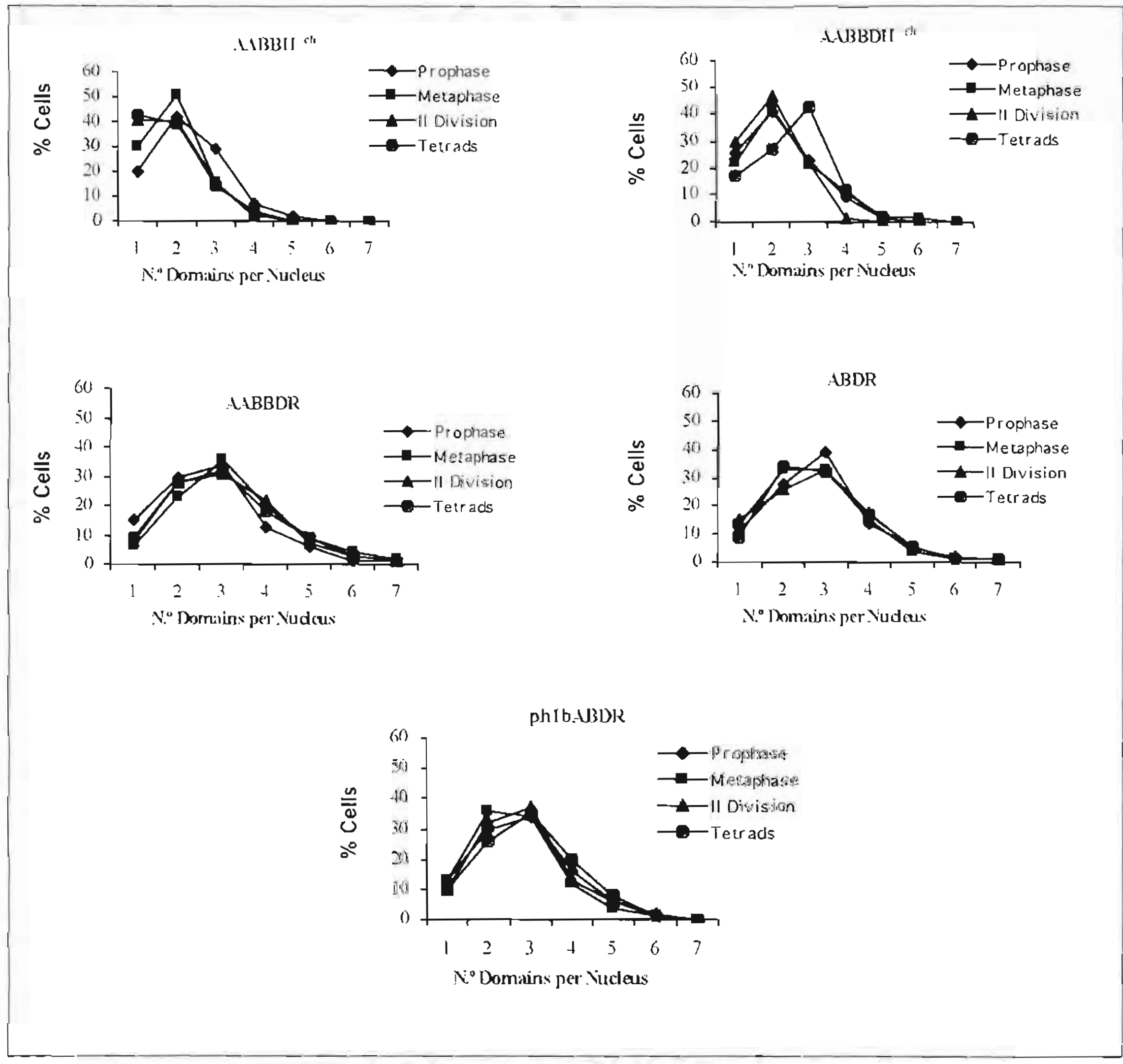


Figure 2: Binucleated cells with 1, 2, 3, ...7 domains per nucleus observed in each hybrid combination (AABBHch, AABBDHch, AABBDR, ABDR and ph1bABDR) in different meiotic stages (prophase, metaphase, I' division and tetrads).

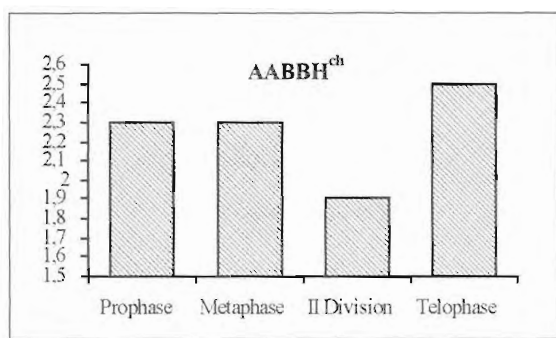
Moreover, we found that binucleated cells with the same number of domains in both nuclei were almost the double than expected under the premises of the model (Table I), in all meiosis stages and hybrids analysed.

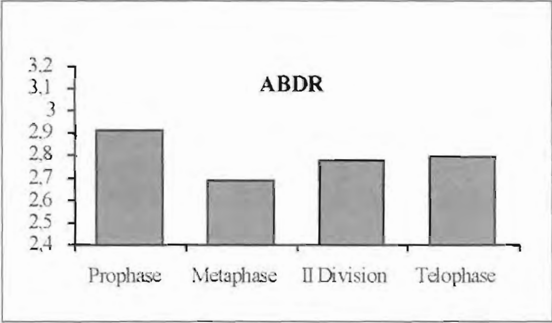
These findings indicated that the number of domains through different meiotic stages is rather constant and the position of chromosome territories are maintained quite well from the last mitotic division.

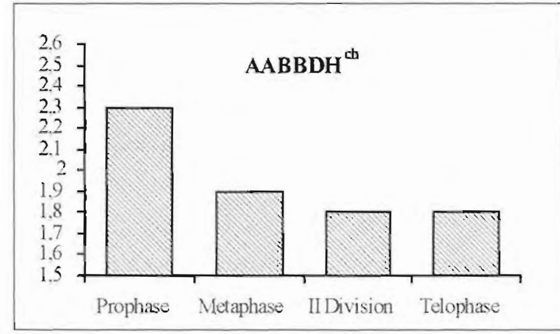
In addition, these results seem to indicate that the distortion of chromatin organisation due to squash procedure should not be very high.

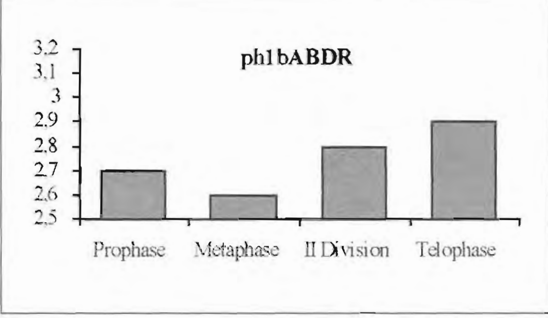
Table I. Frequency of binucleated cells with the same number of domains in both nuclei (N1=N2) observed and expected in each hybrid combination.

	P (N1=N2)	
Hybrid Combination	**Observed**	**Expected**
AABBHch	0.66	0.29
AABBDHch	0.60	0.29
AABBDR	0.55	0.24
ABDR	0.55	0.26
ph1b ABDR	0.56	0.26









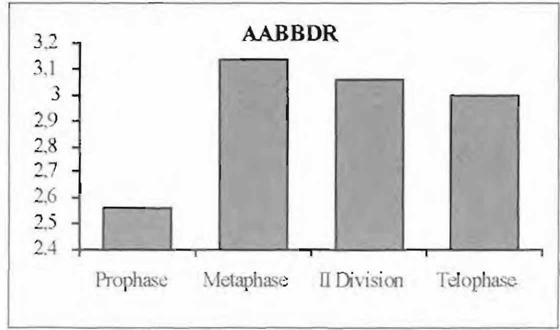


Figure 3: Mean number of domains per nucleus observed in each hybrid combination (AABBDHch, AABBHch, AABBDR, ABDR, ph1bABDR) during the different meiotic stages (prophase, metaphase, II division and telophase).

ACKNOWLEDGEMENTS

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REFERENCES

[1] Lewin R: Do chromosomes cross-talk? Science 214: 1334-1335 (1981).

[2] Leitch A R, Mosgoller W, Schwarzacher T, Bennett M D, Heslop-Harrison J S: Genomic in situ hybridization to sectioned nuclei shows chromosome domains in grass hybrid. J. Of Cell Science 95: 335-341 (1990).

[3] Schardin M, Cremer T, Hager H D, Lang M: Specific staining of human chromosomes in Chinese hamster x man hybrid cell lines demonstrates interphase chromosomes territories. Human Genetics 71: 281-287 (1985).

[4] Bennett M D: Kew Chromosome Conference II (ed.) P.E. Brandham & M.D. Bennett. London, George Allen & Unwin. 71-79 (1983).

[5] Heslop-Harrison JS: Chromosome disposition in *Aegilops umbellulata*. Kew Chromosome Conference II (ed.) P.E. Brandham & M.D. Bennett. London, George Allen & Unwin. 63-70 (1983).

[6] Mikhailova E I, Naranjo T, Shepherd K, Wennekes-Van Eden J, Heyting C. de Jong H: The effect of the wheat Ph1 *locus* on chromatin organisation and meiotic pairing analysed by genome pairing. Chromosoma 107: 339-350 (1998).

[7] Fernández-Calvín B, Benavente E, Orellana J.: Meiotic pairing in wheat-rye derivatives detected by genomic *in situ* hybridization and C-banding- a comparative analysis. Chromosoma 103: 554-558 (1995).

EVALUATION AND IMPORTANCE OF DEVELOPMENTAL MUTANTS FOR NON-STANDARD SPIKE MORPHOLOGY IN WHEAT (*Triticum aestivum* L.)

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A survey of existing genetically controlled differences in spike morphological structure of wheat *Triticum aestivum* L. is presented. Attention is given to new morphotypes such as multirow spike (MRS) and spikes with long glumes (LG). MRS is characteristic of a higher number of spikelets (up to 10) with a few florets that grow out of individual nodes of spike rachis close to each other and above each other. The new MRS-lines exhibit higher grain weight per spike, a markedly increased grain number per spike, and harvest index on the level of current varieties. Yield potential is lower until now than in currently grown cultivars. Lines exhibiting improved state of health and tillering ability are supposed to be developed. LG were transferred into common wheat using wide hybridisation with *T. polonicum* L. The LG are considered to be important for the prolongation of assimilation activity of the spike at the end of ripening and for improved starch pasting properties. The yielding ability of new LG-lines is comparable with some current cultivars. This allelic variant predicts the breadmaking quality. The importance of the developmental mutant genes for increasing spike sink capacity is discussed.

1. INTRODUCTION

One possible way how to affect assimilate distribution is to increase grain weight through an increasing grain number [6]. For this purpose, wide genetic variability in distinctions of spike morphological structure in wheat can be used. While particular elements of spike productivity are of a multigenic character of inheritance, spike morphological structure is a character which is easily measured and, at the same time, controlled by a lower number of genes. The objective of this study was to analyse spike productivity in selected groups of winter wheat genotypes (*Triticum aestivum* L.) differing in spike morphological structure. A collection of wheat gene resources exhibiting different morphological structure of the spike which is maintained at the Agricultural Research Institute Kroměříž Ltd. (ARI) was used in the study. The greatest part of original genetic material was provided by the research institutes in Zagreb [8] and Novi Sad (Yugoslavia) [3], VIR in Sankt Petersburg (Russia), and CIMMYT (Mexico). These materials were mostly derived from wide crosses of *T. aestivum* L. with tetraploid species (*T. turgidum* L.). At the ARI this material has been investigated since 1986 and crossed with selected Czech and foreign cultivars.

Classification of spike morphological structure

Basing on the number of spikelets growing out from the individual nodes of the spike rachis we can divide the genetic deviations in the morphological structure of the spike into two groups: a) normal spike arrangement (one spikelet per node of the spike rachis) and b) supernumerary spikelet (SS) where more spikelets go to one node of the spike rachis [3, 8, 12].

1. **Normal spike structure (NS),** a single spikelet is situated in one node of the spike rachis. We can divide this group as follows:

1.1. Standard morphotypes (SM) that are common for most of grown cultivarss (Fig. 1a).

1.2. Multispikelet forms, an increased number of spikelets per spike rachis and a considerably longer spike rachis. The long multispikelet spikes are sometimes also designated as "gigas forms" [3].

1.3. Multifloret forms with a higher number of florets in spikelets, some of them are associated with fan-like arrangements of florets, "flabellum" [5] (Fig. 1b). Some of the present high-yielding cultivars have a high number of florets in the spikelets (e.g. Record from Germany).

1.4. Screwedness of spike rachis (SCR). It is a recessive mutation [14].

1.5. Long glumes (LG). There are some wheat forms (T. aestivum L.) in which a larger size of glumes was transferred by wide crosses with T. polonicum L. (Fig. 1c). LG are characteristic for T. polonicum [1] (as well as hexaploid T. petropavlovskyi Udacz. et Migusch). In the T. polonicum LG are controlled by the P gene on the long arm of chromosome 7A. P gene significantly contributed to modified starch pasting properties in tetraploid and hexaploid wheat [16]. LG were transferred in common wheat using wide hybridisation with T. polonicum (cultivar Buitre Cometa obtained from CIMMYT, Mexico) (Fig. 1b).There is also information available on the isogenic line ANK 30 with LG which was derived from the spring wheat cultivar Novosibirskaya 67 [9].

1.6. Hulled forms (spelta spike types) - characterised by hulled grain. In hexaploid wheat the main representatives are *T. spelta* and *T. macha* Dekapr et Menabde.

1.7. Sham ramification of the vavilovi type is typical for T. vavilovi (Tum.) Jakubz (2n = 6x = 42). Characteristic is the lengthening of the spikelet axis (rachilla) [5] (Fig. 1d). This ramification is probably implicated through the interaction of alleles for the branchiness of the spike and homozygote recessive genes qq (on chromosome 5A) conditioning hulled grain.

2. Supernumerary spikelet (SS) where one node of the spike rachis has a number of spikelets. The SS character of bread wheat (*T. aestivum* L.) is abnormal spike morphology expressing extra spikelets per spike [3, 7, 8, 12].

2.1. Vertical sessile spikelets (VSS), sometimes designated as "banana twin spikelets" or "duospiculum", when two or three spikelets grow up vertically in a spike rachis node [4] (Fig. 1e). The VSS expression is controlled dominantly [4, 5] or recessively (unpublished).

2.2. Tetrastichon sessile spikelets (TSS), when two (or three) spikelets are sessile close to each other in a horizontal position in a spike rachis node [5] (Fig. 1f).

2.3. Floribunda, a high number of spikelets grow up in a common spike rachis node close to each other and, at the same time, above each other [5]. This morphotype is characteristic of little space among spikelets, which often hampers the development of inflorescence organs. That may result in reduced spike fertility [10]. According to the fertility level we can devide it into:

2.3.1. Fully sterile or with considerably reduced fertility

2.3.2. Fully fertile spikes, which are designated as "fertile floribunda" [5]. They occur only rarely in the F2 generation of crosses of branched and standard forms of wheat (*T. aestivum* L.). Fertile forms are named Multirow Spike (MRS) [11]. MRS is characteristic of a higher number of fertile spikelets (up to 10) per spike rachis node. Some of glumes can be missing in some spikelets, i.e. a glume number does not correspond with a spikelet number. Another rachilla of different length without a basal flower grows instead of an opposite glume and carries 3 to 4 florets that are partly sterile. The highest number of spikelets is developed in the lower third of the spike (Fig. 1g). In the central and upper thirds of the spike there are mostly groups with three spikelets when each of them has up to three florets. The distribution of spikelets is similar to that in six-row barley in this part, however, the spikelets have more florets. The terminal part of such a spike is similar to the standard spike. The MRS is a recessive trait [10, 11]. It was transferred in *T. aestivum* from the resource Ra 1 that was provided by the VIR in Sankt Petersburg.

2.4. Genuine branching of the turgidum type. Branchiness of the turgidum type was incorporated in *T. aestivum* L. using wide crosses with branching forms of *T. turgidum* [3, 8] (Fig. 1h). Spike branching has been studied by many authors [3, 5, 7, 8, 10, 12, 13, 17]. A common feature of these studies is that spike branching is controlled by one or more *bh* genes. [3, 12, 17]. Some authors divide branchiness into: long branch (LB) and short branch (SB) [7, 12].

The objective of this study was to analyse spike productivity in selected groups of winter wheat (*T. aestivum* L.) genotypes differing in spike morphology.

The focus is more on the MRS and LG morphotypes.

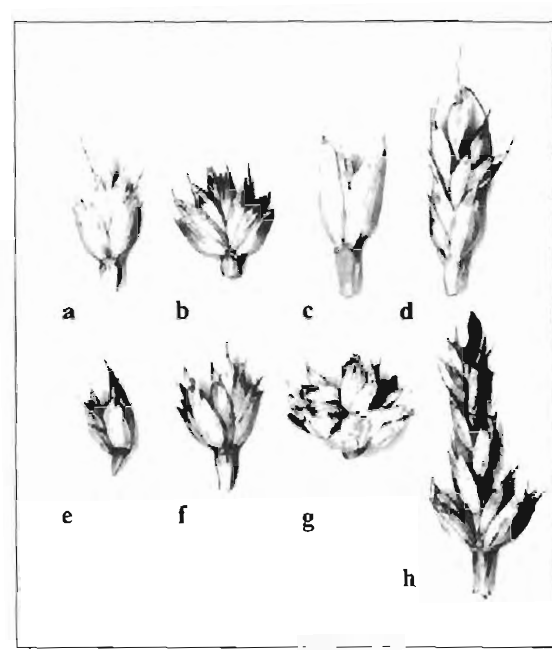

Figure 1: Arrangement of the spikelets on rachis node a - SM, b - flabellum, c - long glumes, d - sham ramification (vavilovi type), e - VSS, f - TSS, g - MRS, h - genuine ramification (turgidum type)

2. MATERIAL

Selected genotypes with different spike morphology were selected for the evaluations (NS, VSS, branchy spike, MRS, LG) from the ARI collection. The MRS and LG lines have originated mostly in 2 to 4 cycles of hybridisation with standard cultivars.

3. METHODS

The samples were cultivated at the ARI Kroměříž.

Results of the collection of genetic sources from the growing seasons 1995/96, 1996/97, 1998/99 and 2000/01 were presented.

In 1995/96, altogether 170 genotypes were evaluated and divided into groups based on the morphological structure of the spike. Differences among these groups were found (Table I). Data were obtained from hand-sown experiments (row width 12 cm, plant distance in a row 4 cm). Progenies of selected plants were grown in plots of 1.0 m^2 without replications. A two hundred seeds were sown per plot. Thirty spikes of each plot were used to analyse elements of spike productivity and their correlations.

In 1996/97, the selected genotypes were then cultivated in 5-m^2 plots at the seeding rate of 4 million germinable seeds per hectare to investigate the yields (Table II).

Our attention gradually turned to morphotypes designated as LG and MRS. In 1998/99, detailed evaluations of the characteristics of spike productivity were carried out in selected genotypes with the highest grain weight. In 5-m2 plots sown in the same way as in 1995/96 (200 grains per 1.0 m2), the MRS and LG lines were evaluated together with check cultivars with standard (SM) spike morphotype, i.e. Ilona, Alana, Astella and Contra (Table III).

In order to estimate the progress in breeding Table II also presents the yields of genotypes with MRS and LG spikes from the 2001 harvest. The cultivars registered in the Czech Republic were used as checks, i.e. Siria, Astella, and Hana.

All experiments were conducted after the forecrop oilseed rape, with split applications of N at a rate of 40 $kg.ha^{-1}$ in autumn and 40 $kg.ha^{-1}$ in spring. P_2O_5 and K_2O were applied at 45 $kg.ha^{-1}$ each.

4. RESULTS

Table I shows that the highest values of grain weight per spike were recorded in the SCR, VSS, and NS morphotypes (2.39, 2.21 and 2.14 g, respectively). The grain weight in check cultivars did not exceed 2 g. Increasing the grain weight per spike did not lead to an adequate increase in the harvest index.

Table I. Characteristics of wheat gene resources differing in spike morphology (harvest 1996)

Spike morpho-type	Grain weight per spike (g)	1000-grain weight (g)	Grain number per spike	Spikelet number per spike
SCR	2.39±0.22	50.3±4.5	47.4±4.1	23.0±0.5
VSS	2.21±0.17	43.8±3.2	50.1±2.8	26.5±0.7
NS+)	2.14±0.06	45.3±2.1	47.4±1.0	21.2±0.3
TSS	1.87±0.18	37.9±4.7	49.9±4.7	27.8±0.9
LG	1.78±0.20	46.3±3.7	38.1±2.7	20.6±0.4
MRS	1.41±0.10	33.3±1.8	42.4±1.0	40.4±2.3
TFS	1.40±0.21	36.3±2.5	34.3±2.9	30.0±0.9
Check cultivars (SM)				
Siria	1.99	37.4	44.2	20.7
Astella	1.57	39.0	36.8	20.7
Hana	1.59	38.4	40.2	19.9
LSD (0.05)	0.69	10.6	10.8	12.9

+) "gigas forms" with long spikes

The yielding ability of the selected genotypes, which were sown in the experimental plots in the successive year (Table II), usually did not reach the yields of the check cultivars. In most cases the reason was the lower tillering ability and a smaller number of spikes per unit area.

After regulated selection for spike productivity the weight of grains in the spike increased in the MRS and LG lines (Table III) and also the yielding ability increased (Table II). In MRS lines the high grain weight per spike was due to a high grain number per spike that was about 20 grains higher than in check cultivars. MRS lines had 42 spikelets per spike while LG and NS produced only 24 and 22 spikelets, respectively. Rather high differences were found in mean grain weight. In MRS lines, grain weight ranged from 29 to 44 mg, in LG from 29 to 49 mg, and in NS from 36 to 54 mg. MRS were characterised by a high spike density (a high spikelet number per 100 mm of spike rachis), while LG had a low spike density. MRS and LG lines had 4.7 and 5.5 spikes per plant. Check cultivars with standard spike had the highest number of spikes per plant (6.25). The tested lines did not differ too much in harvest index (HI) values. The current MRS and LG lines did not greatly differ in heading and ripening dates from check cultivars. MRS lines were more susceptible to Fusarium head blight and Septoria glume blotch, which is obviously caused by an increased spikelet density. Also, a high spike density in MRS was usually associated with lower values of falling number, which indicates a higher alpha-amylase activity.

Table II. Yields of genotypes differing in spike morphology

Morpho-type	Harvest	Yield ($t.ha^{-1}$)		
		Mean	Max.	Min.
SCR	1997	6.61	7.25	6.12
VSS	1997	6.82	8.21	5.44
NS+)	1997	7.47	8.24	5.02
TSS	1997	4.68	5.82	3.40
LG	1997/2001	5.50/7.13	6.74/7.54	4.74/5.42
MRS	1997/2001	3.41/5.39	5.28/7.58	2.30/3.30
TFS	1997	2.82	3.12	2.27
Siria	1997/2001	7.79/7.73		
Astella	1997/2001	8.26/7.87		
Hana	1997/2001	5.73/5.76		

+) "gigas forms" with long spikes

Table III. Comparison of groups of MRS, LG and SM-standard morphotypes (harvest 1999)

Trait	MRS	LG	SM
1.	6.44±0.69	8.24±1.443	8.71±0.68
2.	0.81±0.09	0.84±0.04	0.83±0.12
3.	4.7±1.3	5.5±1.2	6.3±0.9
4.	2.52±0.16	1.93±0.43	1.81±0.38
5.	72±13	50±4	43±15
6.	1.76±0.48	2.09±0.18	2.00±0.80
7.	42±7	24±2	22±2
8.	35.7±6.0	38.8±8.5	43.8±7.1
9.	3.1±0.4	2.5±0.1	2.3±0.1
10.	104±8	135±12	106±15
11.	41±9	18±1	21±3
12.	0.36±0.01	0.35±0.04	0.38±0.05
13.	13.9±0.9	13.4±0.9	13.4±1.1
14.	30.3±4.9	27.5±4.6	25.8±1.7
15.	49.5±2.9	53.3±3.1	54.8±4.9

1. Yield ($t.ha^{-1}$). 2. Plant length (m), 3. Spike number per plant, 4. Grain weight per spike, 5. Grain number per spike, 6. Grain number per spikelet, 7. Spikelet number per spike, 8. Mean grain weight (mg), 9. Stem diameter below spike (mm), 10. Spike rachis length (mm), 11. Spike density, 12. Harvest index, 13. Protein content (%), 14. Wet gluten content (%), 15. SDS-sedimentation value (ml)

A number of days to maturity showed a higher correlation with a node number per spike rachis (r = 0.61**) than with spikelet number per spike (r = 0.27*). It suggests that it would be possible to partly limit the undesired lateness by selecting forms with a high grain number in spikelets or those with an increased number of spikelets growing up in common nodes of spike rachis. Significant correlations between grain number per spike and stem diameter (r = 0.38**) show the importance of the size and number of vascular bundles in stem for spike productivity.

A relatively lower grain weight in MRS lines seems to be accompanied by higher protein content in grain due to an increased proportion of aleurone layers in grain. In some MRS (the lines KM 58-00 and KM 68-00) very high protein content was detected - 16.4 and 15.1 %, respectively. Gliadin and HMW-glutenin polymorphism was determined by electrophoresis according to the PAGE ISTA and SDS-PAGE [2]. To predict breadmaking quality, "Glu-score" was used. In some genotypes the allelic block GLD 1B3 (marker of poor breadmaking quality) and/or the rye translocation T1RL.1BS (marker of poor breadmaking quality) were detected. Allelic blocks GLD 1B1 wwere confirmed in all MRS genotypes, heterogeneity was expressed by 1D5 and 1D2 blocks. The 1B1-gliadin block and GLU D1 5+10 are considered as markers of excellent breadmaking quality. These analysed genotypes are used for further crosses and breeding.

5. DISCUSSION

Breeding activities in wheat have led towards a considerable increase in the genetic yield potential associated with changes in plant proportions. Most models of yield formation in wheat consider the grain number per unit area as the most important yield component. It can be expected in the future that yields will increase due to increasing grain weight per spike rather than increasing spike numbers. Besides traditional approaches aiming at breeding wheat with the standard spike morphotype, alternative methods of affecting assimilate distribution using donors with changed morphological spike structure are investigated. Our findings suggest that the MRS morphotype could be used to increase the grain number per spike. Due to recurrent crosses with registered cultivars there is some progress in increasing the yield ability and transfer of MRS in the genetic background of present cultivars. It is assumed that the LG lines could be important for the prolongation of the assimilation activity of the spike at the end of maturation. Preliminary results of amylographic assessments confirmed the connection between the occurrence of LG and higher values of starch viscosity.

6. REFERENCES

[1] Al-Hakimi A, Monneveux P, Galiba G: Soluble sugars, proline and relative water content (RWC) as a trait for improving drought tolerance and divergent selection for RWC from *T. polonicum* into *T. durum*. Journal of Genetics and Breeding 49: 237-245 (1995)

[2] Bednář J, Vyhnánek T, Martinek P: Prediction of bread-making quality in multispikelet winter wheat (*T. aestivum* L.) donors using protein signal genes. Acta Univ. Agric. et Silvic. Mendel. Brun. (Brno), XLVII: 83-88 (1999)
[3] Denčić S: Genetic analysis of different structures of sink capacity in wheat. Proc. 7th Int. Wheat Genet. Symp., Cambridge: 499-502 (1988)
[4] Foltýn J: Transfer of the duospiculum trait to winter forms of wheat (*Triticum aestivum* L.) Genet. a Šlecht. 25: 117-123 (1989)
[5] Foltýn J: Evolution form homology of cultural wheat ear (genus Triticum): homogeny and heterogeny traits. Genet. a Šlecht. 26: 13-22 (1990)
[6] Frederick JR, Bauer PJ: Physiological and numerical components of wheat yield., Chapter No. 3 In: Satorre E H, Slafer G A (eds) Wheat Ecology and Physilology of Yield Determination, Food Products Press: 45-65 (2000)
[7] Huang G, Yen C: Studies on the development of multiple spikelet per spike in wheat. Proc. 7th Int. Wheat Genet. Symp., Cambridge: 527-532 (1988)
[8] Korić S: Study of branched-ear gene complex of *Triticum aestivum* ssp. *vulgare* and its significance for wheat breeding. J. Sci. Agric. Res., Zagreb 41: 271-282 (1980)
[9] Koval SF: Near-isogenic lines of spring common wheat Novosibirskaya 67 with short and long glume. Wheat Information Service 88: 37-42 (1999)
[10] Martinek P: Branchiness of the turgidum type spikes, its heredity and utilization in wheat (*Triticum aestivum* L.). Genet. a Šlecht. 30: 61-74 (1994)
[11] Martinek P, BednářJ, Provazníková E, Kruličková K: A new gene multispikelet resource of winter wheat (*Triticum aestivum* L.) with non-standard arrangement of spikelets in spike. Proceeding of the Conference ·Agronomická fakulta a vývoj poľnohospodárstva na Slovensku", section B: 47-53 (1996)
[12] Peng ZS, Yen C, Yang JL: Chromosomal location of genes for supernumerary spikelet in bread wheat. Euphytica 103: 109-114 (1998)
[13] Pennell AL, Halloran GM: Inheritance of supernumerary spikelets in wheat. Euphytica 32: 763-776 (1983)
[14] Smoček J: Screwedness of the spike rachis - A new phenomenon in wheat spike morphology. Rostlinná Výroba 37: 507-514 (1991)
[15] Watanabe N, Miura H: P gene effects on the starch pasting properties of tetraploid and hexaploid wheat Cereal Research Communications 25: 963-968 (1997)
[16] Watanabe N, Yotani Y, Furuta Y: The inheritance and chromosomal location of a gene for long glume in durum wheat. Euphytica 91: 235-239 (1996)

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CHARACTERISTICS OF TRITORDEUM (*xTritordeum* Ascherson et Graebner) AND ITS POTENTIAL USE IN THE CZECH REPUBLIC

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Twelve genotypes of hexaploid tritordeum (HT31-1, HT31-2, HT31-3, HT31-4, HT31-5, HT115, HT119, HT135, HTC1323, HTC1324, HTC1331, and HTC1380) were sown in spring together with check varieties of spring wheat (*T. aestivum*) Sandra and Saxana at the Agricultural Research Institute Kromeríz, Ltd. (Czech Republic) in 1999, 2000 and 2001. Tritordeum sown in spring matured extremely late. In 1999/00 and 2000/01, tritordeum sown in autumn was cultivated with check varieties of winter wheat Astella and Brea. It was not damaged by frost due to mild winter and duration of the growing season was comparable with check varieties of winter wheat. Tritordeum sown in autumn produced higher yields (1999/00 - 2.04±0.46 $t.ha^{-1,}$ 2000/01 - 3.92±0.84 $t.ha^{-1}$) than tritordeum sown in spring (2000 - 1.48±0.31 $t.ha^{-1}$, 2001 - 2.44±0.33 $t.ha^{1}$). Tritordeum sown in spring had higher resistance to leaf rust and powdery mildew of wheat, high protein content (1999 - 17.6%, Saxana - 13.8%; 2000 - 21.2%, Saxana - 14.6%; 2001 - 19.7%, Saxana - 13.0 %), higher content of cystine amino acid in protein (1999 - 5.8%, Saxana - 2.3%; 2000 - 3.3%, Saxana - 1.7%). Incidence of ergot (*Claviceps purpurea*) was found in some lines. The breadmaking characteristics of hexaploid tritordeums were inferior to those of bread wheat. Potential use of tritordeum under conditions of Central Europe is discussed. Wider use of winter forms of tritordeum, if they were developed, is assumed.

1. INTRODUCTION

Tritordeum (*xTritordeum* Ascherson et Graebner) is an amphiploid species derived from crosses of wheat (*Triticum* spp.) and wild perennial barley (*Hordeum chilense* Roemer et Schultese; 2n = 2x = 14, $H^{ch}H^{ch}$) that was developed in Spain [2]. Some hexaploid and octoploid forms have been obtained for research purposes in the Czech Republic (CR). Since 1999, hexaploid tritordeum has been subjected to field evaluation and analyses of grain quality. Potential hybridisation of tritordeum with wheat and triticale, detection of derived hybrids using molecular and genetic methods and experiments with *in vitro* embryogenesis are tested. This paper presents mostly results of field experiments and data on grain quality.

2. MATERIALS

A total of 12 genotypes of hexaploid tritordeum were used for analyses: HT31-1, HT31-2, HT31-3, HT31-4, and HT31-5 (= progenies of plants derived from HT31), HT115, HT119, HT135, HTC1323, HTC1324, HTC1331, HTC1380. Depending on a sowing date, they were compared with spring wheat varieties Sandra (bread quality - B) a Saxana (baking - A) and winter wheat varieties Astella (bread quality - B) and Brea (elite baking quality - E) registered in the CR. They were also compared (orientation study only) with well-known winter donors of high protein content, Nap Hal (IND), Atlas 66 (USA) and Lancota (GBR).

3. METHODS

The experiments were conducted in the Agricultural Research Institute Kroměříž , Ltd. located in central Moravia (CR). Characteristics of the location: 235 m above see level, mean of day temperature 8.7 °C, sum of rainfall per year 599 mm, soil type luvi-haplic chernozem, sugar beet production region. Hexaploid tritordeum was sown a) in spring and b) in autumn.

a) Tritordeum genotypes were sown in spring 1999, 2000 and 2001 together with the check varieties Sandra and Saxana after the forecrop sugar beet. They were sown in plots of 2.5 m^2 in size (without replicates) in 1999, in plots of 10 m^2 (1 to 4 replicates depending on seed amount available) in 2000, and in plots of 10 m^2 in 4 replicates in 2001. The seeding rates of 5.0 and 4.5 mio germinable seeds per hectare were used for tritordeum and spring wheat (Sandra and Saxana), respectively. A total nitrogen rate of 70 $kg.ha^{-1}$ (nitrate fertiliser in split application) was applied after emergence and at tillering.

b) In the growing seasons 1999/00 and 2000/01, tritordeum was sown also in autumn together with check varieties Astella and Brea and donors of high protein content (Nap Hal, Atlas 66, Lancota). In both years, they were sown in plots of 10 m^2 in size without replicates after the forecrop oilseed rape. The seeding rates of 4.5 and 4.0 mio germinable seeds per hectare were used for tritordeum and winter wheat, respectively. Nitrogen rates of 33 $kg.ha^{-1}$ were applied prior to sowing and 33 $kg.ha^{-1}$ in spring in the form of nitrate fertiliser.
36 $kg.ha^{-1}$ P^2O^5 and 36 $kg.ha^{-1}$ K^2O were applied to both spring and autumn sown cereals.

Basic growth characteristics were evaluated during the growing season. Field resistance to diseases was scored according to the 1-9 scale (9 - most resistant, 1 - most susceptible). After harvest, characteristics of grain quality were evaluated. Crude protein content (Kjejdahl´s method), content of individual amino acids using an amino acid analyser AAA 331, wet gluten content using a washing instrument according to Spidla and Hýža, falling number on the Falling Number 1600 instrument, SDS-sedimentation value according to the methodology of Axford, gluten index on Glutomatic 2200, and farinographic values (water absorption, dough development, stability and softening) were analysed. Alveographic evaluation was performed for some samples only. A baking test was carried out based on 100 g flour.

Results of obtained characteristics of tritordeum genotypes are given in tables as mean values ± standard error of mean and/or ranges of values.

4. RESULTS

4.1. Field experiments

In all field experiments, tritordeum was rather late in emergence (by about 5 days) in comparison with wheat.

Tritordeum sown in spring had a date of heading approximately comparable with check varieties of spring wheat but markedly late maturation. It is probably caused by effects of the genome of wild perennial species *H. chilense*. It also induces a high level of rejuvenation of tillers in tritordeum, which results in large unevenness in spike maturation on different stems of one plant. Considerably late maturation of tritordeum probably led to delayed incidence of fungal diseases in comparison with wheat. All genotypes of hexaploid tritordeum in both spring plantings demonstrated high field resistance to leaf rust (*Puccinia recondita* f. sp. *tritici*) (9) vs. varieties Sandra (7) and Saxana (6) (Table I). Resistance to powdery mildew (*Blumeria graminis*) in tritordeum ranged on the level of checks from 6 to 8 in 1999. Infection pressure of powdery mildew in 2000 was lower than in 1999 and 2001. Field resistance to Septoria leaf blotch (*Septoria tritici*) was somewhat higher than in check wheat varieties. There were big differences in resistance to Septoria glume blotch (*St. nodorum*) in 1999 and 2000 among individual genotypes. Mmore susceptible genotypes were HTC1323 and HTC1324 (3) and more resistant HTC1331 (7); check varieties Saxana and Sandra were scored with 7. This disease did not occur in 2001. It is interesting that most tested genotypes of tritordeum were naturally infected by ergot (*Claviceps purpurea*). In both years, extreme infection was found in HT135 and increased infection in HT119.

Severe drought during the second half of April and early May in 2000 resulted in considerable yield loss, decrease in stem length, reduction of average grain weight and increase in grain protein content. This year, yields of tritordeum ranged from 0.64 to 2.20 $t.ha^{-1}$, which was about a quarter of yields produced by check varieties (Sandra 6.25 $t.ha^{-1}$, Saxana 6.41 $t.ha^{-1}$). Yields of tritordeum from 2.05 $t.ha^{-1}$ (HT119) to

3.12 t.ha^{-1} (HTC1380) were obtained in 2001 when weather conditions were more favourable. Check varieties Sandra and Saxana gave 7.12 and 7.37 t.ha^{-1}. A disadvantage of tritordeum sown in spring was its lateness in comparison with spring wheat. It was hardly possible to predict a date of maturation because of continuous growth of late tillers. So, besides mature spikes there was about a third of immature spikes at the milk to waxy stage during the harvest. The harvested grain was necessary to dry.

Considering this fact, we tested a possibility of eliminating the late maturation by autumn sowing and vernalisation during winter. Tritordeum was sown in autumn 1999 and 2000 (Table II). The stands were not damaged by frost during the mild winter in 1999/00 and 2000/01. There was a very similar course of growth and development in tritordeum and wheat plants. This is confirmed by the approximately identical date of heading and maturation. Occurrence of powdery mildew found on tritordeum sown in autumn was comparable with check wheat varieties Astella and Brea. In both years, infection of wheat by leaf rust was scored with 7-9 in tritordeum and 6-7 in wheat. So, absolute resistance of tritordeum sown in autumn to this pathogen was not confirmed as it was assessed in tritordeum sown in spring in 1999 and 2000.

In 2000, yields of tritordeum were very low and ranged from 1.62 t.ha^{-1} (HT119) to 2.98 t.ha^{-1} (HTC1380) whereas yields of check varieties Astella and Brea were 9.27 and 7.45 t.ha^{-1}, respectively. Higher yields were obtained in 2001 ranging from 3.86 t.ha^{-1} (HT31-1) to 5.01 t.ha^{-1} (HTC1380); yields of check varieties Astella and Brea were 8.26 and 6.74 t.ha^{-1}, respectively.

4.2. Quality tests

All genotypes of tritordeum were characteristic of very high crude protein content. In tritordeum sown in spring 1999, protein content ranged from 16.3 % (HTC1331) to 18.7 % (HT31-3), in 2000 from 18.8 % (HTC1331) to 23.8 % (HT129) and in 2001 from 16.1 % (HTC1380) to 18.5 % (HT31-1). These results are in contrast to protein content assessed in check wheat varieties that is considerably lower. Hexaploid tritordeum exceeds well-known wheat donors Atlas 66 (17.1 %), Nap Hal (16.7 %) and Lancota (15.7 %) that were evaluated in 2000.

High protein content in tritordeum also expressed in very high values of wet gluten.

Table I. Characteristics of hexaploid tritordeum - sown in spring (a - 1999, b - 2000, c - 2001)

Trait		tritordeum	wheat	
			Sandra	Saxana
Powdery mildew (9-1)	a	6.7±0.9	6	8
	b	7.9±1.0	8	8
	c	6.8±0.9	7	8
$^{+)}$ **Leaf rust (9-1)**	a	8 (7 - 9)	7	6
Septoria - spike (9-1)	a	5.1±1.2	7	7
	b	7.2±1.0	7	7
Septoria - leaf (9-1)	a	6.4±0.8	6	7
	b	6.8±1.2	7	7
	c	7.2±0.4	7	7
Stem length (m)	a	0.95±0.14	0.94	0.90
	b	0.73±0.11	0.70	0.68
	c	0.71±0.06	0.86	0.75
Date of heading in June	a	03. - 07.	03.	02.
	b	05. - 11.	02.	01.
	c	11. - 18.	12.	12.
1000-kernel weight (g)	a	37.6±2.1	43.2	42.7
	b	28.7±2.6	30.7	32.7
	c	38.8±3.8	45.7	44.8
Yield (t.ha^{-1})	b	1.48±0.31	6.25	6.41
	c	2.44±0.33	6.89	7.02

CONTINUACIÓN →

Trait		tritordeum	wheat	
			Sandra	Saxana
Protein content (%)	**a**	17.6±0.7	13.4	13.8
	b	21.2±1.8	15.7	14.6
	c	17.7±1.1	13.2	13.0
Lysine content in grain DM (mg.g^{-1})	**a**	5.4±0.8	6.0	6,2
	b	5.7±0.5	4.0	3,9
Lysine content in protein (%)	**a**	3.1±0.4	4.5	4.5
	b	2.7±0.3	2.5	2.7
Cystine content in grain DM (mg.g^{-1})	**a**	5.8±0.9	2.3	2.3
	b	6.8±0.7	3.4	3.3
Cystine content in protein (%)	**a**	3.3±0.5	1.6	1.7
	b	3.2±0.4	2.1	2.2
Falling number (sec)	**b**	118±61	88	122
	c	185±82	192	215
SDS-sedimentation value (ml)	**b**	54.5±6.7	82	87
	c	44.3±5.5	55	64
Wet gluten content (%)	**b**	43±4	31	27
	c	38±6	25	24
Gluten index	**b**	46.4±29.3	96	99
Water absorption (ml)	**b**	56.8±1.1	56.2	59
Peak of development time (min)	**b**	2.3±0.8	2.5	2
Dough stability (min)	**b**	2.7±1.3	9.5	9
Degrees of softening (BU)	**b**	107±30	60	50
Loaf volume (cm3)	**b**	260±60	350	443

$^{+}$) in 1999 and 2000, all samples were resistant

Results of technological analyses suggest that tritordeum grain is less acceptable for baking as compared with wheat (Table I and II). In the experiment with tritordeum sown in spring 2000, a SDS-sedimentation value ranged from 40 ml (HT135) to 63 ml (HT31-4) and on average it was lower vs. checks (Saxana 82 ml and Sandra 87 ml). Also, values of gluten index indicate worse breadmaking properties of gluten protein of tritordeum vs. wheat. It was confirmed by dough evaluation using a farinograph. Alveograms show relatively low values of energy W and relatively low values of P. The dough of tritordeum was mostly very extensible. Some samples could not be evaluated by alveograph due to extreme dough extensibility and stickness. Results of the baking test also confirmed unsuitability of tritordeum for breadmaking purposes even though there were considerable differences in loaf volume among individual tritordeum samples (Fig. 1). It documents lower loaf volume vs. check varieties. Tritordeum is characteristic of carotenoides in grain endosperm, which results in yellowish colour of flour and crumb. Despite the obtained results show that tritordeum is less suitable for baking purposes, extremely high protein content could be used for feeding.

Table II. Characteristics of hexaploid tritordeum - sown in autumn (growing seasons: a - 1999/2000, b - 2000/2001)

Trait		tritordeum	wheat	
			Astella	Brea
Powdery mildew (9-1)	a	7.6±1.3	6	7
	b	8.1±0.5	6	7
Leaf rust (9-1)	a	7.9±0.4	7	7
	b	7.6±0.6	5	5
Septoria - spike (9-1)	a	7.2±0.4	7	8
Septoria - leaf (9-1)	a	6.8±0.3	7	6
	b	6.2±0.4	6	7
Stem length (m)	a	0.82±0.04	0.84	0.92
	b	1.05±0.07	0.90	0.85
Date of heading in May	a	08. - 10.	11.	15.
	b	21. - 25.	21.	27.
Date of maturing in July	a	10. - 16.	13.	15.
	b	13. - 18.	16.	20.
1000-kernel weight (g)	a	33.1±5.0	40.1	43.4
	b	40.3±4.3	41.4	44.6
Yield ($t.ha^{-1}$)	a	2.04±0.46	9.27	7.45
	b	3.92±0.84	8.26	6.74
Protein content (%)	a	19.8±1.8	10.8	12.0
	b	18.6±1.5	9.7	11.8
Falling number (sec)	a	214±26	166	148
	b	225±32	245	261
SDS-sedimentation value (ml)	a	57.3±8.8	42	61
	b	55.2±7.2	40	57
Wet gluten content (%)	a	41.5±1.6	23.6	28.0
	b	37.3±1.4	21.2	24.2
Gluten index	a	46±24	86	90
Water absorption (ml)	a	57.7±2.2	50.6	56.0
Peak of development time (min)	a	2.7±0.2	1.0	1.0
Dough stability (min)	a	1.7±0.2	1.5	4.5
Degrees of softening) (BU)	a	103±21	100	90

Interesting results were obtained by comparing content of individual amino acids in tritordeum and wheat assessed from the harvest of spring planting in 1999 and 2000. There was no considerable difference in content of amino acid lysine in wholemeal DM in tritordeum vs. check wheat varieties. In the content of some other amino acids there were differences in their proportions in sample DM and protein. The most marked difference was assessed in cystine that contains sulphur. In 1999, average content of cystine in tritordeum was 5.8 $mg.g^{-1}$ and in 2000 6.8 $mg.g^{-1}$ whereas in wheat it was 2.3 and 3.3 $mg.g^{-1}$, respectively. However, these differences must be confirmed by further analyses.

GENETIC VARIATION IN SPANISH POPULATIONS OF THE GENUS AEGILOPS REVEALED BY AFLPS

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ABSTRACT: Amplified Fragment Length Polymorphism (AFLPs) DNA markers were used to analyse genetic variability and relationships in wild species of the genus *Aegilops*. Fifty populations, which included the species *Aegilops biuncialis* (UUMM), *Ae. neglecta* (UUMMNN), *Ae. ovata* (UUMM), *Ae. ventricosa* (DDNN) and *Ae. triuncialis* (UUCC) were selected. These populations are distributed in the Iberian peninsula and Balearic islands. Five AFLP selective primer combinations generated a total of 527 amplification products of which 517 (98.10%) detected polymorphisms. Genetic relationships within the populations were evaluated by generating a similarity matrix based on the Jaccard index. In the resulting phenogram Ae. ventricosa appears segregated from the other species, probably owing to the influence of the D genome. The species sharing the U genome are located in the main cluster. The branching pattern of the U genome group reflects the proximity of the species sharing the M genome. *Ae. biuncialis* and *Ae. ovata* are clearly separated suggesting that the super index system should be used to differentiate the M genomes of both species. The variation among populations within species in relation to their geographical origin and the results obtained with RAPDs and biochemical markers are discussed.

1. INTRODUCTION

The species of the genus *Aegilops* probably represent the main genetic reserve for the improvement of the wheat cultivars. *Aegilops* species are mostly annual and autogamous. Polyploid forms exist, arising by evolutionary convergence of ancestral diploid species. The distribution of the genus extents from south-west and central Asia to the Mediterranean basin with Turkey as the centre of diversification. The genus has adapted in such a way that all the species are annual and survive the extreme Mediterranean summers as seeds. Spain is especially rich in wild populations of the polyploid species *Aegilops biuncialis* Vis. (UU$\underline{MM}$), *Ae. triuncialis* L. (UUCC), *Ae. Ovata* L. (UU$\underline{MM}$) and *Ae. ventricosa* Tausch (DDNN), all of which are allotetraploids, and *Ae. neglecta* Req. ex Bert. (UUMM/UUMMNN) which is found naturally in both tetraploid and hexaploid forms [3]. The hexaploid variant (*Ae. neglecta* subsp. Recta Zhuck and Hammer) was selected for the present study since the tetraploid is not found in the Iberian peninsula. *Aegilops ovata* and *Ae. triuncialis* are widely distributed in Spain except in the north and northwest, areas which are typified by cool, damp summers. *Aegilops neglecta*, *Ae. biuncialis* and *Ae. ventricosa* may be found in more restricted areas [9]. *Aegilops caudata L.* (CC), *Ae. commosa* Sibth. And Sm (MM), *Ae. uniaristata* Vis. (NN), and *Ae. squarrosa* Coss. (DD) are proposed diploid ancestors of the species mentioned above and were used as phylogenetic markers.

Over several years, the present authors have explored the Iberian peninsula and collected wild populations of *Aegilops* with the aim of introducing native material into plant breeding programmes, mainly those involving wheat and *Triticale*. Special care must be taken to preserve the original structure of natural populations. The collection method of Hawkes [5] increases the genetic value of the material harvested. Indeed, if the genetic individuality of a plant is maintained, genes of interest may be detected in individual plants, and their allelic frequencies calculated for each population.

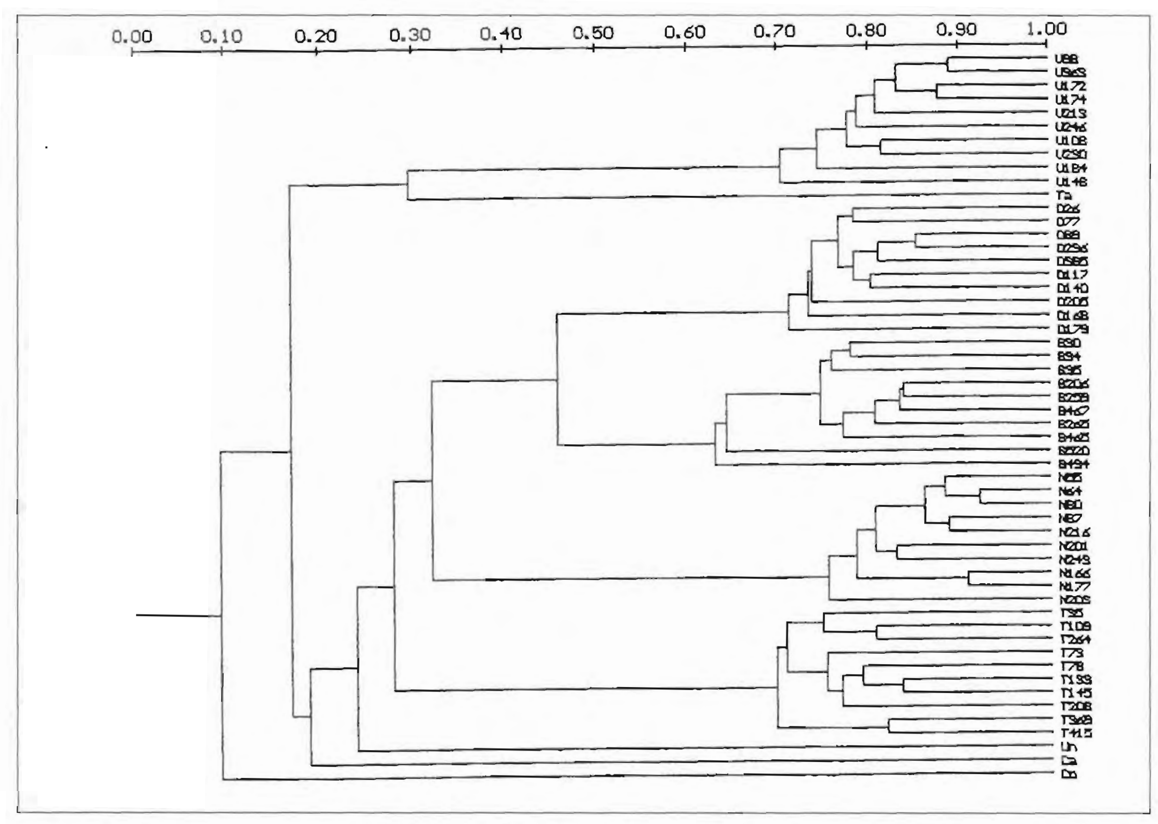

Figure 2. Dendrogram generated from the AFLP data by NTSYS and UPGMA. The Jaccard coefficient was used to estimate genetic distances.

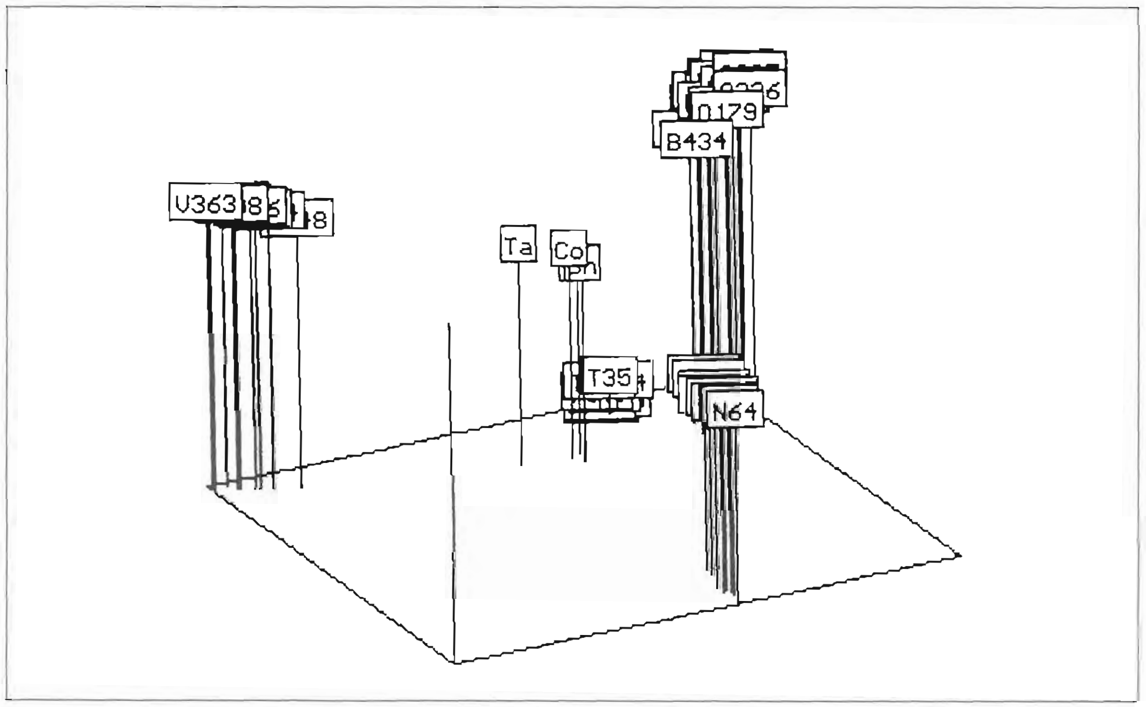

Figure 3. 3D plot of the Principal Coordinates Analysis of the AFLP data.

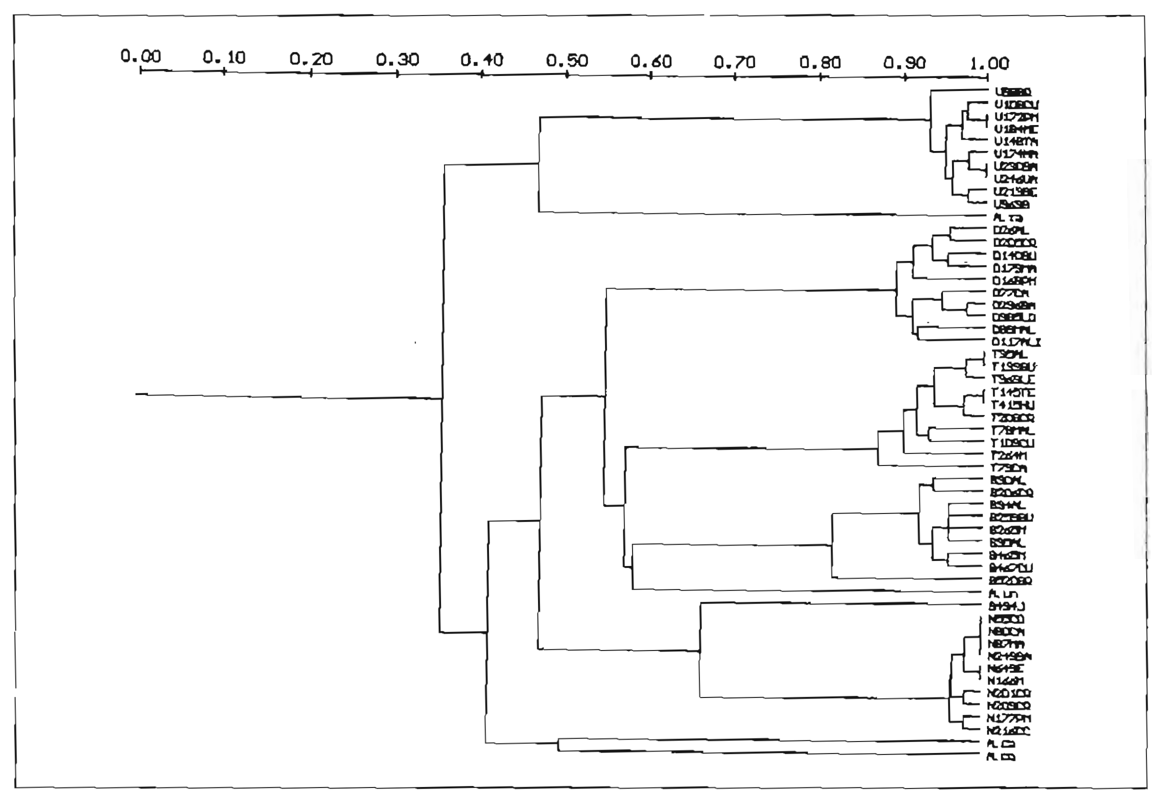

Figure 4. RAPD dendogram constructed using the Jaccard coefficient and UPGMA.

Table II. Number of RAPD products generated (diagonal) and shared (remaining data) by 5 primer+3 combinations in 9 *Aegilops* species. The number of specific markers found in each taxonomic unit is indicated in brackets.

	VE	OV	TR	BI	NE	CA	CO	SQ	UN
Aegilops ventricosa	75 (36)	30 (0)	27 (1)	30 (1)	40 (10)	20 (2)	10 (0)	47 (20)	26 (0)
Agilops ovata		127 (33)	61 (0)	82 (12)	50 (1)	29 (0)	22 (5)	22 (0)	19 (0)
Agilops triuncialis			167 (47)	72 (11)	47 (3)	54 (22)	21 (3)	23 (0)	40 (2)
Aegilops biuncialis				117 (64)	54 (1)	31 (0)	24 (8)	12 (0)	34 (4)
Aegilops neglecta					126 (35)	27 (2)	16 (2)	27 (0)	37 (1)
Aegilops caudata						107 (34)	15 (0)	19 (1)	28 (0)
Aegilops commosa							88 (35)	13 (0)	14 (1)
Aegilops squarrosa								111 (48)	22 (0)
Aegilops uniaristata									75 (14)

3. RESULTS AND DISCUSSION

The five primer+3 combinations used generated 527 bands of which 517 (98.10%) were polymorphic. Recent reports showed that the application of fluorescence-labelled primers generates a large number of scorable AFLP fragments [4]. The combinations E-ACC and M-CAA, E-AGC and M-CAG, E-ACG and M-CAA, E-AGG and M-CAA, and E-AGC and M-CTG; yielded 156, 124, 63, 64, and 110 polymorphic markers respectively. For each species were generated between 75 and 167 bands. *Aegilops neglecta* showed the least variation with 32.54% of polymorphic bands in contrast with *Ae. biuncialis* that presented the highest degree of polymorphism (71.79%). The remaining values were 38.32% (*Ae. triuncialis*), 44% (*Ae. ventricosa*) and 37.00% (*Ae. ovata*) (Table II). The relatively low level of variation detected in *Ae. neglecta* is consistent with the tendency of that species to colonise similar ecological habitats in different geographical locations of the Iberian Peninsula [9]. AFLP fragments sizes ranged from 50 to 500 base pairs. The number of bands decreased as the size increased. Meanwhile 69.26% of the total markers were scored under

the 150 bp size, only a percentage of 7.40% were detected above the 400 bp range. The dendrogram generated was of sufficient resolution to separate all the species and populations (Fig. 2). The species sharing the U genome formed a main cluster and *Ae. ventricosa*, intensely associated with *Ae. squarrosa* (DD), was clearly segregated from the other species. This is probably due to the influence of the D genome. This genome has undergone less divergence than other diploid genomes during evolution and therefore appears to be less modified and is well separated within the

Triticeae [1]. *Ae. neglecta* showed to be more associated with *Ae. Ovata* and *Ae. biuncialis* than *Ae. triuncialis*. Given that *Ae. neglecta* is hexaploid and share the genomes U and M with *Ae. ovata* and *Ae. biuncialis*, the AFLP markers seem to reflect more differences at the genomic level than those involving variation at the ploidy numbers. Although *Ae. ovata* and *Ae. biuncialis* cluster together according to their genomic constitution (both are UUMM), both species are clearly separated in the dendrogram. This suggests that despite the latest genome nomenclature for the *Aegilops* group [11], super index should be used to differentiate the M genomes of both species. This has been proposed in the past by Kihara [6]. All diploid species, except *Ae. squarrosa*, appeared segregated from the main cluster. Although in the past, the N genome of *Ae. uniaristata* was regarded a modification of the M genome of *Ae. commosa* [6], the present findings according with recent data [1] suggest that N should be considered a different genome.

Figure 3 shows a 3-dimensional PCO plot of the *Aegilops* AFLP data set. 14.80% of the total variation is represented on the x axis, the next 13.06% on the y axis, and the next 11.72% on the z axis. The populations plotted are labelled according to their abbreviated names as listed in the tree (Fig. 2). Meanwhile *Ae. ventricosa* and the group *Ae. biuncialis-Ae. ovata* were clearly separated in the x axis, the hexaploid *Ae. neglecta* was intensely segregated from the rest on the y axis. The separation of *Ae.triuncialis* from the *Ae.biuncialis-Ae.ovata* group was only resolved at the Z axis level probably reflecting differences involving the C genome of the former. The four diploid markers used clustered together in the first two dimensions.

The interpopulational variation patterns of *Ae.*

ventricosa and *Ae. biuncialis* shown by the tree are

in agreement for most of the populations with the geographical separation of the taxa. Those relationships indicate that the populations have adapted to local ecogeographical conditions during many generations. Since similar associations were poor or almost absent in the other species, the data suggest that *Ae. ventricosa* and *Ae. biuncialis* have been the first *Aegilops* species to colonise the Iberian peninsula advancing from the east of the Mediterranean basin in ancient times.

RAPD analysis yielded 146 fragments of which 131 (89.70%) were polymorphic. The resulting phenogram showed poor resolution when compared with the AFLP tree (Fig. 4). The distances scored among populations presented lower values and differences between many populations were not resolved. As result, the intraspecific relationships generated completely different branching patterns of those obtained with AFLPs. Despite those disadvantages, the tree was able to discriminate the D species from the U cluster taxa, although in this case *Ae. neglecta* appeared separated from the other U species probably due differences at the ploidy level. *Ae. biuncialis* appeared associated with *Ae. triuncialis* and relatively separated from *Ae.ovata*. The diploid ancestors were not clearly segregated from the polyploids as in the AFLP dendrogram due to the relatively low number of polymorphisms detected by the markers. Although the use of more RAPD primers would surely improve the quality of these results, the AFLP tree was built using a third part of the time required to obtain the presented RAPD data and about a four times higher number of DNA markers were generated. In addition, the possibility offered by AFLPs to score and analyse really small size bands that can not be safely account in RAPD analyses, increases the number of polymorphisms detected and provides higher resolutions.

Studies made in the past by our group using isozymes and endosperm proteins (data not shown) generated a dendrogram of sufficient resolution to clearly separate all populations [2]. The tree showed a main cluster formed by *Ae. ovata, Ae. biuncialis, Ae. neglecta*, and *Ae. ventricosa*. *Ae. triuncialis* appeared segregated from that group probably due to the influence of the C genome. In concordance with the AFLP phenogram, *Ae. biuncialis* and *Ae. ovata* cluster together and positive relationships were found between the geographical origin of the populations and their genetic distances in the case of *Ae. biuncialis*. The fact that *Ae. ventricosa*, a species clearly separated within the *Aegilops* group at the botanical level, was associated with the species sharing the M genome could be related to the relatively restricted genomic location of the markers used. Low genomic coverage could produce that variation involving the D genome was underestimated or almost undetected.

The AFLP combination of two different sources of variation leads to highly reproducible, efficient, and reliable results that make this procedure an extremely useful tool for molecular variability studies in plant breeding programs.

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REFERENCES

[1] Badaena ED, Friebe B, Gill BS: Genome differentiation in Aegilops 2. Physical mapping of 5S and 18S-26S ribosomal RNA gene families in diploid species. Genome 39: 1150-1158 (1996).

[2] Gonzalez S: Recursos fitogenéticos silvestres: Estudios de variabilidad en poblaciones españolas del género *Aegilops* L. Tesis Doctoral, Universidad de Alcalá de Henares, (1992).

[3] Gupta PK, Baum BR: Nomenclature and related taxonomic issues in wheats, triticales and some of their wild relatives. Taxon 35: 144-149 (1986).

[4] Hartl L, Seefelder S: Diversity of selected hop cultivars detected by fluorescent AFLPs. Theor Appl Genet 96: 112-116 (1998).

[5] Hawkes JG: Crop genetic resources, Field collection manual for seed crops, root and tuber crops, tree fruit crops and related wild species. IBPGR, Eucarpia, Rome (1980).

[6] Kihara H, Tanaka M: Addendum to the classification of the genus *Aegilops* by means of genomic-analysis. Wheat Information Service 30: 1-2 (1970).

[7] Monte JV, Casanova C, Soler C: Genetic variation in Spanish populations of the genus *Aegilops* revealed by RAPDs. Agronomie 19: 419-427 (1999).

[8] Rolhlf JF: NTSYS.pc: numerical taxonomy and multivariate analysis system for the IBM pc microcomputer (and compatibles). Setavek, NY, Applied Biostatistics INC (1989).

[9] Ruíz-Fernández J, Casanova C, Soler C: Collecting Spanish populations of the Genus *Aegilops* L. Genetic Resources and Crop Evolution 42: 339-345 (1995).

[10] Soler C, Ruíz-Fernández J, Monte JV, de Bustos A, Jouve N: The assessment of variability in Spanish populations of wild relatives of cereals. Bocconea 7: 107-119 (1997).

[11] Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M: AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407-4414 (1995).

[12] Wang RCC, von Bothmer R, Dvorak J, Fedak G, Linde-Laursen I, Muramatsu M: Genome symbols in the Triticeae (poaceae). In: Wang RCC, Jesen KB, Jaussi C (eds) Triticeae, 2nd International Symposium. Logan, Utah, USA (1994).

INTERGENERIC HYBRIDS IN WHEAT: CURRENT STATUS IN CIMMYT

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ABSTRACT. Most of the tertiary gene pool species when hybridized with wheat yield F1 hybrid combinations that do not exhibit intergenomic chromosome pairing. The classical advance route of such hybrids involves production of fertile amphiploids, disomic addition lines, establishing homoeologous relationships, stress screening, selective advance for desired disomic substitution line generation, and use of protocols that promote alien introgression into the wheat genomes for the appropriate stress trait. The CIMMYT wide cross intergeneric program has also used this classical route involving several *Thinopyrum* and *Leymus* species. The current emphasis however is given to exploiting a protocol shift for a *Triticum aestivum /Th. bessarabicum* combination with multiple stress resistance for a major disease (*Fusarium graminearum*) and one abiotic stress (salinity). Intergenomic chromosome pairing between wheat and *Thinopyrum* chromosomes was not observed in the F1 hybrid and the derived backcross 1 plants. In order to promote this from the initial phase when all the alien chromosomes exist, a strategy was developed around the existant ***ph*** genetic stock. The derived ***phph*** progeny from this strategy has produced abundant recombination events. The protocol used is described and the translocation products cytologically validated.

INTRODUCTION

Genetic diversity of the tertiary gene pool species is a potent resource for gene pyramiding which contributes towards stress durability and addresses sustainable agricultural aspects. The conventional classical protocols of introgressing alien genetic diversity into wheat are complex, and long-term in generating farmer usable products. The gene transfer procedures are further complicated when the stress trait has multigenic control associated with several alien chromosomes. Our current approach has incorporated a novel strategy for promoting alien chromosome introgression involving wheat/alien homoeologous as well as non-homoeologous chromosomes. The protocol comprises of hybridizing the ***PhPh*** based amphiploid with the ***phph*** Chinese Spring wheat genetic stock to yield heterozygote ***Phph*** derivatives. From selfing of the heterozygotes or from their derived haploids via wheat/maize crosses the ***ph*** derivatives are identified by a PCR diagnostic. The ***ph*** seedlings form the reservoir of wheat/alien chromosome translocations which are identified by Giemsa C-banding/ fluorescent *in situ* hybridization (FISH). Plants with translocations are step-wise advanced by backcrosses to elite wheat cultivars, ascertaining the presence of the translocated chromosomes in the derivatives, reach the euploid status (2n=6x=42) and stabilize the translocation output. Presented here are results that have emerged by applying this protocol to the *T. aestivum/Th. bessarabicum* amphiploid.

MATERIALS AND METHODS

Germplasm

Amphiploid of *Triticum aestivum* cv. Chinese Spring/*Thinopyrum bessarabicum* (2n=8x=56, AABBDDJJ) as the material to be cytologically manipulated.

T. aestivum cv. Chinese Spring (2n=6x=42, AABBDD, *phph*) as the cytogenetic stock for crossing onto the amphiploid and generating the Phph heterozygote progeny. (Source: Dr. E. R. Sears, University of Missouri, Columbia, Missouri. USA).

Wheat cultivars PDW34, Kharchia 65, Sumai-3 and Flycatcher obtained from the Wheat Germplasm Bank, CIMMYT, Mexico, served as experimental controls for the salinity and scab evaluations.

Stress Evaluation

Salinity. The protocols of Gorham (1) Shah (5) were the basis for testing the amphiploid and appropriate control wheats for K and Na levels 21 days after culture in 50mM NaCl in hydroponic growth conducted under controlled greenhouse conditions of 14 h day, 24/14 C day/night temperatures and about 65% R.H., in El Batan, CIMMYT, Mexico.

Fusarium (Head Scab). Head scab isolates from Mexico locations were the source for inoculating the test germplasm under field conditions in Toluca, Mexico with a concentration of 50,000 spores/ml of water using the cotton inoculation method as described by Mujeeb-Kazi (3). Inoculated spikes were evaluated 30-35 days thereafter.

Genetic Manipulation

The schematic of Figure 1 elucidates the *Phph* heterozygote backcross 1 production. Its hybridization with Zea mays (2) yields *Ph* or *ph* based haploids(1:1), or upon selfing give segregates in a 3:1 *Ph:ph* ratio. PCR diagnostics (4) identified the haploid ph or selfed *phph* seedlings. Haploids with ph were colchicine doubled (DH) and all *phph* derivatives subjected to cytological analyses comprising of mitotic/meiotic Giemsa C-banding and fluorescent in situ hybridization (FISH). Plants with wheat/alien chromosomal translocations were selected . These plants were backcrossed, cytologically checked for continued translocation presence and to ultimately yield 42 chromosome euploids. Euploids were then stabilized, seed increased and became candidates for stress screening.

RESULTS and DISCUSSION.

Stress Evaluation. The test data (Table 1) indicated the T. aestivum/Th. bessarabicum amphiploid to be a promising resource for addressing salinity tolerance and scab resistance (Type II); both traits for which additional genes are needed in the wheat germplasms. A greater than 1.0 K:Na discrimination value and less than 10.0% Type II scab infection score allows this interpretation.

Table 1. Test data for salinity tolerance (K:Na discrimination) and Fusarium head scab (Type II).

Germplasm	K+:Na+	Type II (%)
Salinity		
Chinese Spring (CS)	9.6	-
CS/Th. bessarabicum	14.5	-
PDW 34 (Susceptible)	1.0	-
Kharchia 65 (Tolerant)	3.1	-
Fusarium		
CS/Th. bessarabicum		6.5
Sumai 3 (Resistant)	-	14.4
Flycatcher (Susceptible)	-	40.5

Genetic Manipulation. Our earlier studies provided supportive data of the contribution of several *Th. bessarabicum* chromosomes for salinity tolerance and Type II scab resistance when their disomic addition lines were similarly evaluated. Also previously established was the observation that *Th. bessarabicum* chromosomes did not associate with those of the A, B, and D genomes of wheat; due to the presence of the dominant pairing regulator gene/s. This led us to initiate a *Ph* gene manipulation strategy from a stage when all *Th. bessarabicum* chromosomes were present; as in the 56 chromosome amphiploid. The Figure 1 schematic elucidates the production of *phph* derivatives that are the potential source of promoting wheat/alien chromosomal exchanges. The *ph* influence is observed in the high meiotic chromosome pairing of the *phph* derivatives (Figs. 2a and b). FISH diagnostics contributes to demonstrate wheat/alien chromosome pairing in bivalent and trivalent associations; the source of eventual translocation products that upon further prebreeding yield stable 42 chromosome euploids. Table 2 categorizes some translocations generated via the projected multi-technique integrated strategy of genetic manipulation.

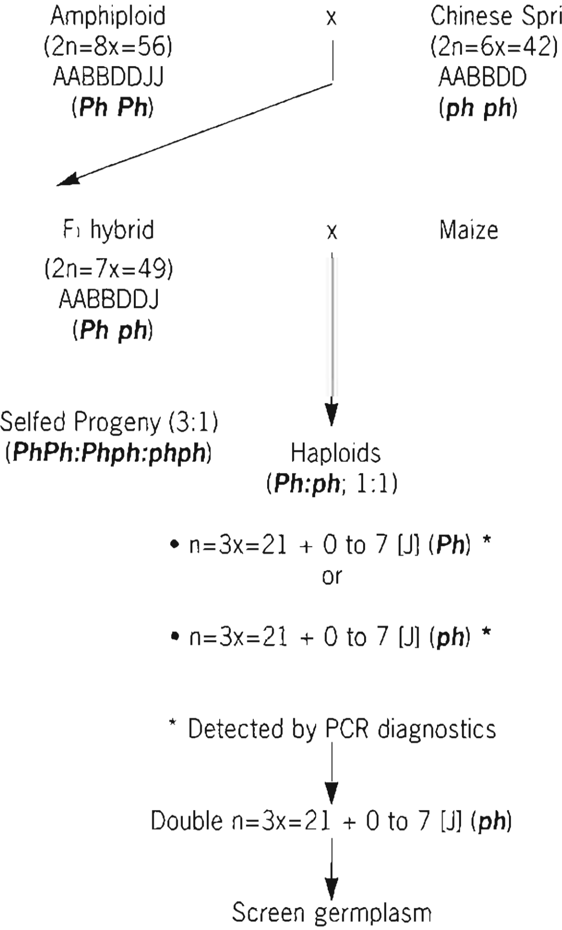
CONCLUSIONS

1. A novel cytogenetic manipulation protocol is described that facilitates wheat/alien homoeologous and non-homoeologous translocations.

2. The protocol uses the Chinese Spring ***phph*** genetic stock and can be exploited by crossing onto ***PhPh*** based amphiploids or other genetic stocks.

3 The ***Phph*** heterozygote derivatives from the initial cross yield ***ph*** haploid or selfed progeny which becomes the source of obtaining wheat/alien chromosomal translocations.

4. The translocations are Robertsonian or of a smaller magnitude, which then are so far terminally located on the wheat chromosomes.



(A)

Figure 1. Schematic demonstrating the use of the ph manipulation strategy for producing *phph* germplasms with possible wheat/alien chromosomal translocations

3.2. Morphology of the F1 hybrid plants

a. Spike morphology

In general, the hybrid plants at the vegetative stage resembled the female *Elymus* parent species. In spike morphology, the hybrids had a single spikelet per rachis node, as was the case in the *Elymus* species. On the other hand, by the number of floret per spikelet not many resembled the *Hystrix* parent. Besides these fundamental positions of the florets, the florets toward rachis were different between the two genera. In *H. longe-aristata* florets are positioned such a way that inner glumes face the rachis just like in the *Hordeum* species, whereas in *E. ciliaris* and *E. yezoensis* the lateral side of the floret is toward the rachis. In the hybrids, however, the position of the florets was just intermediate between the two parent genera.

b. Anthers

E. ciliaris x *H. longe-aristata*

Anthers of *E. ciliaris* are small and the length is about *1.4 mm* whereas those in *H. longe-aristata* are larger in comparison with the present *Elymus* species; it was 5.1mm on average when measured in during the 2001 season. In the F1 the anther length was *2.2 mm* showing a tendency to be small and that resembled to the *Elymus* parent.

E. yezoensis x *H. longe-aristata*

The anthers were rudimentary and degenerate in the early stage. The length of those degenerated anthers was about *1.0 mm*, thin with needle like shape, and gray brownish in color.

3. 3. CYTOLOGICAL OBSERVATION IN THE F1

a. *Anomalous meiosis and anther degeneration*

The F1, *E. ciliaris* x *H. longe-aristata*, was characterized by very anomalous meiosis. Sporocytes are irregular in size and do not show synchrony of occurrence of the stages at meiosis. There were cells that were very large in comparison with the normal sporocyte, while others were much smaller.

(1) *E. ciliaris* x *H. longe-aristata*

Within the same anther there are sporocytes at various meiotic stages. Apparent disturbance of both karyokinesis and cytokinesis was observed. Presumably abnormal cells resulted from the instability of cytokinesis that occurred in the cell divisions of the sporogenesis. Since no such abnormal cell was observed in anther tissues, somatic cell division as far as observed was completely normal. (Fig. 1)

(2) *E. yezoensis* Çò *H. longe-aristata*

Cytological observation of sporocytes was tried repeatedly for years. However, it was not successful, because I was unable to find cells that were thought to be sporocyte (Fig.1). Although the F1 plants were carefully cultivated and placed either in shade or under enough sunlight, anthers did not show any further development and degenerated.

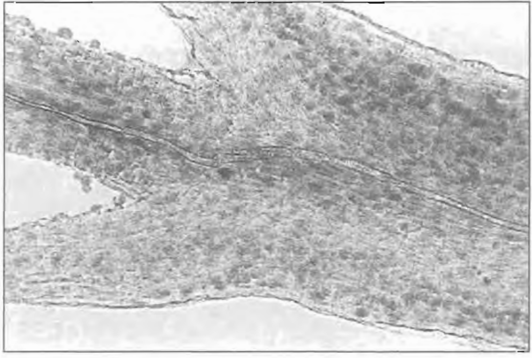


Figure 1. Base part of the anther of the F1 hybrid between *Elymus yezoensis* and *Hystrix longe-aristata*. Notice that there is no microsporocyte.

b. *Chromosome pairing*

Chromosome number and chromosome pairing were extremely variable between cells. There were large sporocyte cells with higher chromosome numbers than that expected from somatic number of $2n=28$. On the other hand, in cells with small size the number tended to be smaller. Besides bivalents, quadrivalents and trivalents were often observed. However, those multivalents tended to appear in cells with higher chromosome number. This tendency that smaller the cell size, less the chromosome association indicated abnormal and irregular sporogenesis that occurred at pre-meiotic cell division. Cells with higher and lower chromosome numbers than expected at meiosis resulted apparently from abnormal tokinesis. Absence of cytokinesis results in the large cells by

putting two nuclei formed after karyokinesis together into one meiotic cell. Non- coincidence of karyokinesis and cytokinesis may partition irregularly a set of chromosomes into variable numbers in sister cells. That may result in chromosome doubling, duplication and structural rearrangement. Because of this abnormality, it was difficult for us to determine the degree of chromosome homology between the two genera. However, the possibility is high that there is *little homology betweenÅ@HÅDlonge-aristata and either of the two Elymus species involved.*

Because as far as I observed there is very small limited chromosome pairing in the meiotic cells of the plants with 2n=28, indicating that chromosome homology is very limited between *Elymus* and *Hystrix*. Ms Tomoko Nikaido (personal communication) observed in average, 0.3 bivalents and 27.3% univalents in selected cells with 2n= 28 chromosomes.

3.4. Fertility

Since there was no normal pollen at all, anthers showed degeneration. For studying female fertility back-cross pollination by *H. longe-aristata* pollen, or by the *Elymus* parent plant or putting both F1 and *Elymus* spikes together into the same bag, were made. In either case seed set never occurred. I conclude that the female side is also completely sterile.

3.5. Conclusion

a. There is little homology between the Japanese species of *Elymus* and *Hystrix* in point of view of chromosome pairing.

b. Anomalous sporogenesis that occurred in F1, produced by complementary genetic effect brought from both parents might be responsible for this observation.

c. Reproductive isolation between genera had long been taking place through geological ages.

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5. REFERENCES

[1] Ohwi J: Flora of Japan. Smithsonian Institution, Washington, D.C., pp 1067 (1965)

[2] Koyama T: Grasses of Japan and its Neighboring Regions. Kodansha, Tokyo pp. 570 (1987)

[3] Osada T: Illustrated Grasses of JapanÅ@Enlarged Edition. Heibonnsha, Tokyo pp.777 (1993)

[4] Dewery, D R: The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: (ed., GUSTAFSON J. P.) Gene Manipulation in Plant Improvement. Proc. 16th Stadler Genetics Symp. Columbia 1984, p. 209-279 (1984)

ANTHER CULTURE RESPONSE OF TRITORDEUM (*XTRITORDEUM* ASCHERSON ET GRAEBNER) AND ITS COMPARISON WITH WHEAT (*TRITICUM AESTIVUM* L.) AND BARLEY (*HORDEUM VULGARE* L.)

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Five genotypes of hexaploid tritordeum (2n=6x=42, $AABBH^{ch}H^{ch}$) and one genotype of octoploid tritordeum (2n=8x=56, $AABBDDH^{ch}H^{ch}$) were tested for androgenic responsibility and regenerated capacity and compared with barley and wheat. In the experiments the time of the effect of cold pre-treatment (10, 20, 30 days at 4 °C) and influence of two different media MN6 and modified C17 were observed. The androgenesis was induced in all tested genotypes of the tritordeum. The frequency of androgenic anthers ranged from 5.7 to 23.9% and the frequency of green regenerants ranged from 0.6 to 5.9% of cultured anthers. The highest regeneration rate, 7.6 green plants per 100 cultivated anthers, was reached at 20 days cold pre-treatment in octoploid tritordeum HT109. The modified medium C17 gave the higher frequency of calli and embryos formation, but more green plants regenerated on the medium MN6. Androgenic response and regeneration capacity of tritordeum genotypes were low in comparison to barley genotypes cultivated on medium MN6 and also wheat genotypes cultivated on modified medium C17. The longer time of the cold pre-treatment in tritordeum is more suitable for anther culture responsibility than in barley and wheat.

INTRODUCTION

The development of haploid plants and subsequent production of homozygous lines is of great interest to plant geneticists and breeders. The practical use of double haploid plants of cereals has been demonstrated in barley, wheat and rice. One way how to improve androgenic capacity is to optimize culture conditions by testing the genotypes under study in a wide range of culture media using right pre-treatment [1].

Our experiments are focused on the testing of androgenic responsibility in different genotypes of tritordeum. We studied the time of the effect of cold pre-treatment and influence of two different media - MN6 and modified C17. The androgenic responsibility and regeneration capacity from anther culture of tritordeum were compared with barley and wheat.

MATERIALS AND METHODS

Plant material: Five genotypes of hexaploid tritordeum (2n=6x=42, $AABBH^{ch}H^{ch}$) HT129, HT327, HTC1323, HTC1380, HT31-4 and one genotype of octoploid tritordeum (2n=8x=56, $AABBDDH^{ch}H^{ch}$) HT109 were used as experimental materials. Donor plants were grown in the growth chamber (20/18 ^{O}C, 16/8 h-day/night). *Anther culture:* Spikes were collected when microspores reached the middle to late uninucleate stage of development and then pre-treated for 10, 20, 30 days at 4oC in the dark. After extirpation, the anthers were placed on an induction medium and cultivated in the dark at 26°C (MN6) or for three days at 32°C and subsequently at 28°C (C17).

Culture media: The anthers were cultured on modified induction medium MN6 - for barley or C17 - for wheat [2]. MN6 medium is based on N6 medium [3] with maltose (80 g l-1), 2,4-D (2.0 mg l-1), NAA (0.5 mg l-1) and KI (0.5 mg l-1). Modified C17 medium is based on C17 medium [5] with maltose (120 g l-1), 2,4-D (1.5

mg l-1) and KI (0.5 mg l-1). Induced pollen embryos and microsporial calli were transferred to regeneration media 190-2 and 190-4 [5].

A total of 7,405 anthers in hexaploid tritordeum and 1,501 anthers in octoploid tritordeum were cultivated. The following traits were scored and evaluated: the responsive anthers rate (androgenesis induction ability) as the number of anthers with embryos or calli per 100 anthers plated, the plant regeneration rate as the number of regenerated plants (total, green and albino) per 100 anthers plated.

RESULTS AND DISCUSSION

Androgenesis was induced and plant regenerated from anther cultures of all tested genotypes of tritordeum.

The frequency of androgenic anthers in hexaploid tritordeum (2n=6x=42, $AABBH^{ch}H^{ch}$) ranged from 5.7 to 16.1% of cultured anthers. The highest number of responsive anthers (23.9%) was obtained in one genotype of octoploid tritordeum (2n=8x=56, $AABBDDH^{ch}H^{ch}$).

The frequency of green regenerants in hexaploid tritordeum ranged from 0.6 to 1.6% of cultured anthers and in octoploid tritordeum 5.9% of cultured anthers.

The frequency of albino regenerants from hexaploid tritordeum was higher than that of green plants (Fig. 1, 2 and 3).

The modified medium C17 gave the higher frequency of calli and embryos formation, but more green plants regenerated on the medium MN6.

The effect of cold pre-treatment on regeneration of green plants depended on tested genotypes. The highest regeneration rate, 7.6 green plants per 100 cultivated anthers, was reached at 20 days cold pre-treatment in octoploid tritordeum HT109 (Fig. 4).

Androgenic response and regeneration capacity of wheat, barley and tritordeum genotypes (with high responsible capacity) was compared. In wheat cv. Florida 7- and 14-day cold pre-treatment significantly enhanced a number of responsive anthers and green plants. In barley cv. Igri the highest number of responsive anthers/100 cultured anthers was obtained without any pre-treatment. Cold pre-treatment of 14 days caused a significant decrease in androgenesis induction. The regeneration of green plants showed the highest value in variant with 3 days of cold pre-treatment. In tritordeum HTC1323 the highest number of responsive anthers/100 cultured anthers and green regeneration capacity was obtained 21 days of cold pre-treatment.

Based on our results, the androgenic response and regeneration capacity of tritordeum genotypes were low in comparison to barley genotypes cultivated on medium MN6 and also wheat genotypes cultivated on modified medium C17. In all the three cereal crops (barley, wheat and tritordeum), the optimal duration of cold pre-treatment probably depends on the genotype that is used for anther cultures. The longer time of the cold pre-treatment in tritordeum is more suitable for anther culture responsibility than in barley and wheat.

It is known that heterozygotes (hybrids) have the higher androgenic responsibility than their homozygous plants. Our experiments demonstrated that on a level of whole genome substitution it could not be the same. Simultaneously time, the genetic specificity of pollen embryogenesis can be strongly enhanced.

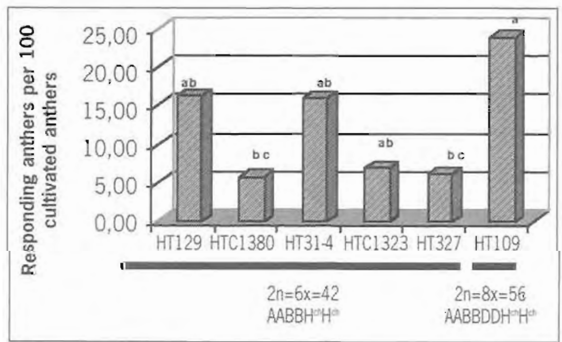

Figure1: Frequency of pollen calli and pollen embryoids in tritordeum

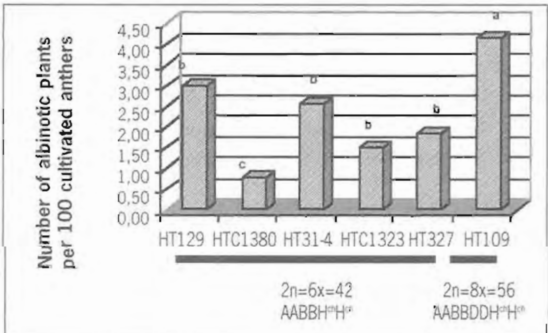

Figure 2: Frequency of albino regenerants obtained in tritordeum

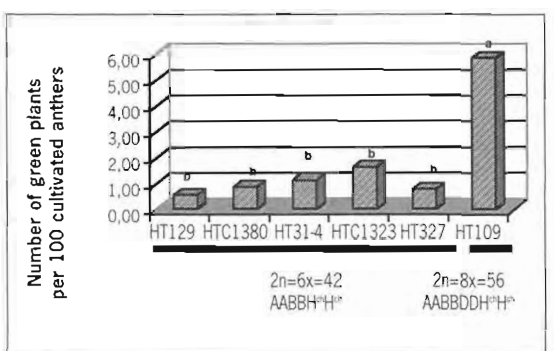

Figure 3: Frequency of green regenerants obtained in tritordeum

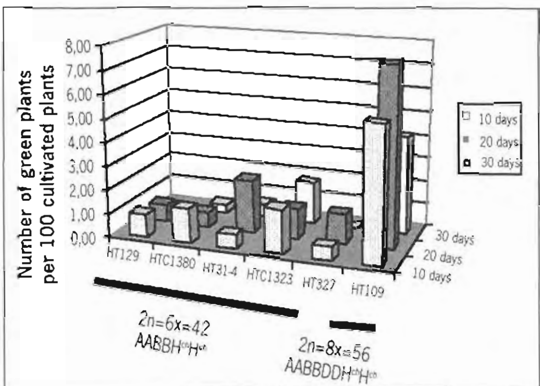

Figure 4: Effect of cold pre-treatment on regeneration of green plants in tritordeum

REFERENCES

[1] Zamani I, Gouli-Vavdinoudi E, Roupakias DG: Production of doubled haploids in wheat (Triticum aestivum L. In: Bohanec, B, Ed. Biotechnological Approaches for Utilization of Gametic Cells. Office for Official Publications of the European Communities, Luxembourg. 43-51 (2001).

[2] Vagera J, Ohnoutková L: In vitro induction of androgenesis in wheat and barley. (In Czech) Rostl. Výr. 39: 97-114 (1993).

[3] Chu CC: The N6 medium and its applications to anther culture of cereal crops. In: Proceedings of Symposium Plant Tissue Culture, 43-45 Science Press, Beijing (1978).

[4] Henry Y, DeBuyser J: Effect of the 1B/1R translocation on anther culture ability in wheat *(Triticum aestivum* L.). Plant Cell Rep. 4: 307-310 (1985).

[5] Pauk J, Manninen O, Mattila I, Salo Y, Pulli S: Androgenesis in hexaploid spring wheat F2 populations and their parents using a multiple-step regeneration system. Plant Breed. 107: 18-27 (1991).

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COLLECTION AND CHARACTERISATION OF *TRITICEAE* GENETIC RESOURCES AND ACTIVITIES OF CONSERVATION IN CENTRAL ITALY

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ABSTRACT: One hundred and six landraces (i.e. farmer's varieties) belonging to 7 species of the *Triticeae* taxon were collected in Central Italy by DBVBA of the University of Perugia, DIBIAGA of the University of Ancona and the Agricultural Development Agency of Abruzzo region (ARSSA) in different individual and joint missions. A few number of accessions was supplied by private and other public organisations. The main part of accessions was collected in the Abruzzo and Umbria regions. *Triticum dicoccum* Schubler (emmer) is the most widespread species, followed by *T. aestivum* L. (common wheat) and *T. monococcum* L. (einkorn), *T. spelta* L. (spelt), *T. turgidum* var. *durum* Desf. (durum wheat), *Secale cereale* L. (rye) and *Hordeum vulgare* L. (hulled and naked barley). Besides the presence of materials reproduced on farm over generations, information related to on farm management and to adaptative, agronomic, qualitative and organoleptic traits of landraces found as well as information related to the use, local names, traditions and social context were gathered during the missions.

Most of the seed collected is conserved in the DBVBA germplasm bank under long term storage conditions and the information relative to each accession is stored in a specific relational database.

The great part of the accessions was characterised by morphological and phenological traits and molecular markers.The results of this work show the presence of morpho-phenologic and genetic differences among landraces and the importance of some species in the agricultural systems and food customs of the investigated area. Particularly for emmer three well distinct landraces are present, "Farro Italia Centrale", "Farro della Garfagnana" and "Italia Meridionale". Other interesting and traditional landraces are the "Solina" common wheat in Abruzzo and the "Orzo mondo" naked barley in Marche. Many populations are still cultivated, frequently in marginal lands and under low input or organic agronomic conditions, but also near bred variety and under modern agricultural techniques.

1.INTRODUCTION

Italy, like the other countries of the Mediterranean basin, is an area rich on crop biodiversity. Central Italy (Toscana, Umbria, Marche, Lazio, Abruzzo, Molise regions) is very representative in this context because it is characterised by a high diversity of climatic, edaphic, agronomical and historical conditions.

Until the 50ies agricultural activity was based on a large number of local varieties in all these regions and mainly in the inland areas near the mountains. Landraces of seed propagated crops, constituted by a complex of different genotypes each with different tolerance to biotic and abiotic stresses, are characterised by a specific adaptation to the environmental conditions of the area of cultivation and by relatively low but constant yields (1).

In traditional agriculture the main task was to produce many different products, making it possible for the farmer's family and the local community to have a stable food supply. The development of modern agriculture, which was aimed to satisfy the needs of a larger market and was characterised by high energy inputs and by massive breeding activity, produced, on one hand, the disappearance of the spectre of hunger and, on the other hand, a sharp decrease in the Italian genetic resources. A large quantity of landraces has already completely disappeared and, for this reason, in the last few years some initiatives aimed to collect and preserve genetic resources have started at the regional level.

We report the salient data relative to our efforts in collecting and characterising *Triticeae* germplasm of Central Italy.

2.MATERIALS AND METHODS

Since the beginning of the 90ies DBVBA of Perugia University, DIBIAGA of Ancona University and the Agricultural Development Agency of Abruzzo region collected landraces belonging to different *Triticeae* taxa in Central Italy in individual and joint missions. Other seed samples were kindly supplied by other Institutions and private donors. Figure 1 shows the collection area.

Farmers were approached in a friendly manner, the reason for the visit was explained and an interview followed during which information on farm management and on adaptative, agronomic, qualitative and organoleptic traits of landraces found as well as information related to the use, local names, traditions and social context were gathered during the missions.

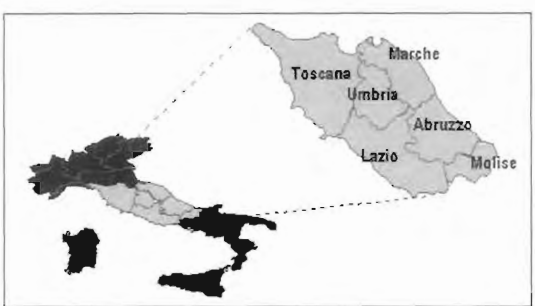

Figure 1: Landraces collection area: the regions of Central Italy are highlighted.

Most of the seed collected, dehydrated to reach an average moisture content of 7-8% and vacuum sealed in aluminum packets, is stored at -18°C in the DBVBA germplasm bank.

Part of the seed of several accessions, particularly of emmer and common wheat, was used in programs of characterisation and evaluation. Recently, besides usual morpho-physiological observation (2, 3), a genetic characterisation has been conducted on collected samples.

Information relative to each accession is stored in a specific relational database (FILEMAKER-PRO) which also assures an easy export of data. Even though the department has no official duty of maintaining and providing germplasm, a small sample of seed is freely donated to all *bona fide* users, when enough seed of the requested accession is present in the collection.

3.RESULTS

A total of 106 accessions of different *Triticeae* taxa were collected (Table I) and are currently stored in DBVBA germplasm bank.

Table I. Number of collected accessions per region and per species.

Species/Region	**Toscana**	**Umbria**	**Marche**	**Lazio**	**Abruzzo**	**Molise**	**Total**	%
H. vulgare	0	2	4	0	4	0	**10**	9,4
S. cereale	0	2	0	0	4	0	**6**	5,7
T. aestivum	0	2	1	0	17	0	**20**	18,9
T. dicoccum	3	23	1	8	16	8	**59**	55,7
T. monococcum	0	1	0	0	1	0	**2**	1,9
T. spelta	0	3	0	0	0	1	**4**	3,8
T. turgidum ssp. durum	0	0	0	0	5	0	**5**	4,7
Total	**3**	**33**	**6**	**8**	**47**	**9**	106	100
%	2,8	31,1	5,7	7,5	44,3	8,5	100	

The co-operation and support of local technicians and many private donors were precious during collection missions, both for the wide knowledge of the investigated area and for the successful approach with the farmers.

Some species are still important in the agricultural systems and food customs of this area, particularly for emmer and bread wheat. Many populations are still cultivated, frequently in marginal lands and under low input or organic agronomic conditions, but also under modern agricultural techniques, near bred varieties in the same farm.

Also morpho-phenologic and genetic diversity among landraces was detected.

The highest number of accessions was collected in Abruzzo (44.3%) and Umbria (31.1%).

Landraces of *Triticum dicoccum* Schubler (emmer) and *T. aestivum* L. (common wheat) were most frequentely found (55.7% and 18.9% of total accessions collected, respectevely). Landraces belonging to *T. monococcum* L. (einkorn), *T. spelta* L. (spelt), *T. turgidum* var. *durum* Desf. (durum wheat), *Secale cereale* L. (rye) and *Hordeum vulgare* L. (hulled and naked barley) were rarely found with percentages ranging from 1.9 to 9.4%.

3.1. Triticum dicoccum

In Central Italy the area cultivated under this species increased during the last decade due to a renewed interest in natural and healthy food.

Since this species was not submitted to modern breeding, the landraces were the only genetic material available, so that their acreage increased specially in marginal land since emmer suits well low input agronomic systems (4).

Table II shows the value of means, standard deviation and range of growth habit, heading date and plant height of the evaluated accessions.

Table II. Emmer: mean, standard deviation and range for some morpho-phenological traits in the 3 different groups.

Trait/ Emmer group	Mean			Standard deviation			Minimum value			Maximum value		
	Garfagnana	Italia Centrale	Italia Meridionale	Garfagnana	Italia Centrale	Italia Meridionale	Garfagnana	Italia Centrale	Italia Meridionale	Garfagnana	Italia Centrale	Italia Meridionale
GH (1)	3,0	4,1	3,8	0	0,7	0,7	3,0	3,0	3,0	3,0	5,0	5,0
HD (2)	48	47	50	1,7	3,0	4,8	46	39	45	50	53	59
PH (3)	136	127	132	1,5	6,8	10,4	135	115	115	138	140	145

(1) GH, Growth Habit, scale 1-5: 1=very prostrate; 2=prostrate; 3=semi-prostrate; 4=semi-erect; 5=erect.
(2) HD, Heading Date: days from April 01.
(3) PH, Plant Height: cm from soil to the tip of spike.

According to characterisation data relative to growth habit, heading date and plant height and data relative to geographic area of cultivation (collection) the emmer materials can be classified into three group types (5, 6):

- "Garfagnana" (Tuscany) including winter types, characterized by medium-late vegetative cycle, tall plant, semi-prostrate growht habit, big spike with or without awns and by big kernel with floury fracture;
- "Italia Centrale" (Umbria, Lazio and Abruzzo) including spring types, characterized by medium cycle, medium tall plant, ranged from semi-prostrate to erect growth habit, small spike with awns and medium-small kernel usually with vitreous fracture;

- "Italia Meridionale" or "Farro Molisano" (South Abruzzo, Molise and other South Italy regions) including winter types, characterized by late vegetative cycle, tall plant, from semi-prostrate to erect plant type, big spike with long awns, big kernel with both flour and vitreous fracture.

The different kind of kernel fracture determines the different final use of the product, as whole or pearled kernels, flour, broaken kernels (named "tritello di farro") to make soups, pasta, biscuits and many other oven products. Each product is related to the specific geographic area and to the traditional use and food customs of this area.

Emmer, durum and common wheat landraces collected in Abruzzo and some other landraces were characterised by RAPD (Random Amplified Polymorphic DNA) molecular markers as described by Barcaccia and Rosellini (7).

Figure 2 shows the UPGMA dendrogram based on Dice's genetic similarity (8) estimates and 13 RAPD marker loci.

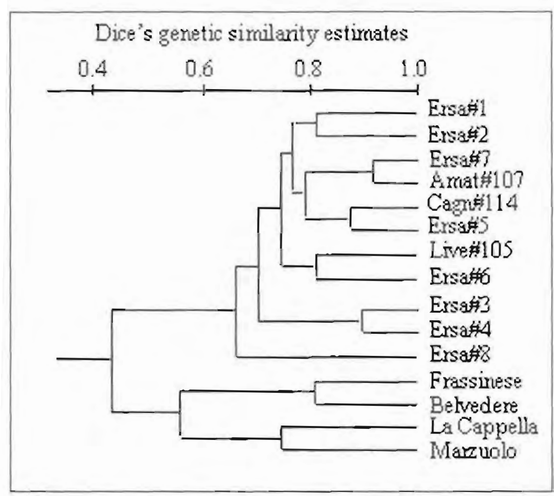

Figure 2: Dice's genetic similarity estimates in emmer, common and durum wheat accessions collected in Abruzzo Region.

Landraces from Central and Southern Italy showed distinctive molecular traits. In particular, local varieties classified as "Italia Centrale" types were characterized by a common set of RAPD markers and proved to be distinguishable from either the "Italia Meridionale" or the "Garfagnana" accessions. This result agrees with the characterisation based on morpho-phenological traits.

Local varieties belonging to the "Italia Centrale" group were much less variable and differentiated among each other than those of the "Italia Meridionale" group. The "Garfagnana"-type local variety had genetic similarities and distances intermediate between the two main group types (9).

3.2. *Triticum aestivum*

More than 80% of common wheat landraces was gathered in Abruzzo Region. These landraces are still cultivated by farmers in mountainous areas (800-1000 m a.s.l.) for their adaptability to marginal areas and yield stability (10).

"Solina" is the most diffused local variety because of its agro-environmental adaptability and the technological characteristics of the flour which is particularly appreciated to make traditional home made bread (11).

Interesting historical information on "Solina" and some other common wheat landraces has been discovered on public and private archives. This information shows that these landraces have been cultivated since the XVI and XVIII Century.

Some old variety bred in Italy in the first decade of XX Century are still grown in Central Italy: "Frassinese" (probabily equivalent to variety Frassineto), "Belvedere" and "Generoso".

Table III shows mean, standard deviation and range of growth habit, heading date and plant height of collected landraces, including all accessions of "Solina", "Frassinese" and "Belvedere".

Table III. Common wheat: mean, standard deviation and range of some morpho-phenological traits.

Trait	Mean	Standard deviation	Min	Max
GH (1)	2,5	0,5	2	3
HD (2)	44	2,1	40	47
PH (3)	126	18,5	72	140

(1) GH, Growth Habit, scale 1-5: 1=very prostrate; 2=prostrate; 3=semi-prostrate; 4=semi-erect; 5=erect.
(2) HD, Heading Date: days from April 01.
(3) PH, Plant Height: cm from soil to the tip of spike.

Wheat landraces show a growth habit from prostrate to semi-prostrate, a medium-late vegetative cycle, tall plant with a wide range (from 72 to 140 cm), prevalently awned spikes of red or white colour.

"Frassinese" and "Belvedere" are clearly distinguished from emmer and durum wheat by the molecular analysis reported in Figure 2.

3.3. *Triticum turgidum subsp. durum*

Two of the 5 accessions of durum wheat are probably derived, as common wheat, from varieties bred in the first half of XX Century by Nazareno Strampelli at the Plant Breeding Station of Rieti (close to the area where the materials were collected). In particular, the accession "La Cappella" could have been derived from the variety "Senatore Cappelli". The one named "La Ruscia" and "Marzuolo" have not a clear origin.

The three accessions show (Table IV) a semi-erect growth habit, early cycle, tall culms with red ("La Ruscia") or white spikes ("La Cappella" or "Cappella") (10, 11).

Table IV. Durum wheat: mean values of some morpho-phenological traits.

Name of landrace	GH(1)	HD (2)	PH (3)
LA CAPPELLA	5	40	135
LA RUSCIA	4	43	145
MARZUOLO	5	49	135

(1) GH, Growth Habit, scale 1-5: 1=very prostrate; 2=prostrate; 3=semi-prostrate; 4=semi-erect; 5=erect.
(2) HD, Heading Date: days from April 01.
(3) PH, Plant Height: cm from soil to the tip of spike.

These materials were molecularly characterised, as shown in the previous subsection (Figure 2), and are well separated from emmer and common wheat groups.

3.4. Other species

Hordeum vulgare (hulled and hulless barley), *Secale. cereale, Triticum monococcum; Triticum spelta.*

The accessions of these species have been recently collected and they will be included in new characterisation and evaluation activities in the next future.

In this paper we would like only to report some observations:

- Naked barley landraces were largely cultivated until the 60ies because they were used to make the "caffè d'orzo", the drink prepared with toasted kernels. In the recent years, as for emmer, the interest in this crop increased and presently some breeding programmes are carried out using landraces (12, 13).
- Rye local varieties still remain in the high-lands where they are used for forage; in the past its grains was milled and utilised in mixture with wheat flour.
- Spelt and einkorn collected accessions have been probably introduced in the area from other places. In particular, spelt accessions probably are bred varieties.

3. CONCLUSION

Some landraces are still maintained and managed on farm because of resistance to harsh climatic conditions, traditional reasons or organoleptic peculiarities which make them highly valued. On farm conservation strategies should rely on the above mentioned facts provided that problems concerning present seed legislation and trade are overcome (14).

This study has shown that in Central Italy it is still possible to find a wide genetic variation for several cultivated species of relevant importance.

The need to encourage an ecological agriculture aimed at promoting the human resources in the area through the production and marketing of high quality food from landraces is recognised.

The detected materials should be safeguarded in *ex situ* collection and better characterised and evaluated in order to check their possible value in future breeding programs.

To strengthen the relationships between local cultures (in the sense of knowledge) and crops, could lead to develop products well paid by market because of their intrinsec quality. So, the product could well contribute to farmer's income. As a consequence an on farm conservation of genetic resources could be achieved.

REFERENCES

[1] Harlan, J.R. Crops and Man. Second edition. ASA-CSSA. Madison, Wisconsin, USA (1992).

[2] UPOV. Guidelines for conduction of tests for distinctiveness, homogeneity and stability. Wheat (*Triticum aestivum* L., *Triticum durum* Desf.) TG/1/1, Geneve (1974).

[3] IBPGR. Revised descriptor list for wheat (*Triticum* spp.). IBPGR Secretariat, Rome (1985).

[4] Porfiri O., D'Antuono L. F., Perrino P. Stato della ricerca e della sperimentazione sui frumenti vestiti in Italia, con particolare riferimento al farro medio (*Triticum dicoccum* Schubler). In: A. Troccoli, P. De Vita & N. Di Fonzo (Eds), Prospettive di una moderna cerealicoltura alle porte del terzo millennio, VI Intl. Workshop on Durum Wheat, April 30-May 2, 1998, Foggia, Italy, pp. 327-351 (1998).

[5] Porfiri O., Papa R., Veronesi F. Il farro nel rilancio delle aree marginali umbro-marchigiane. In C. Papa (Ed), Il farro. Saperi, usi e conservazione delle varietà locali. Quaderni del CEDRAV 1: 58-67 (1998).

[6] Porfiri, O., D'Antuono L.F., Codianni P., Mazza L., Castagna R. Genetic variability of a hulled wheats collection for agronomic and quality characteristics. In: A.A. Jaradat (Ed.) Proc. of the 3rd Intl. Triticeae Symposium, May, 4-8, 1997, Aleppo, Syria, pp. 387-392 (1998).

[7] Barcaccia G., Rosellini D. A quick method for the isolation of plant DNA suitable for RAPD analysis. J. Genet. & Breed. 50: 177-180 (1996).

[8] Dice, L.R. Measures of the amount of ecological association between species. Ecology 26: 297-302 (1945).

[9] Barcaccia G., Molinari L., Porfiri O., Veronesi F. Molecular characterisation of emmer (*Triticum dicoccum* Schubler) Italian landraces. Submitted to GRACE (2001).

[10] Porfiri O. Alla riscoperta di vecchi frumenti e di altri cereali abruzzesi. Atti Convegno ARSSA 6 novembre 2000 "Possibilità di diffusione dell'agricoltura sostenibile in Abruzzo". EU Project P.O.M.- Action 3.2: 11-22 (2000).

[11] Silveri D., Dalla Ragione I., Porfiri O., Torricelli R., Tosti N., Veronesi F. Collection, evaluation, and conservation of plant genetic resources in the Abruzzo Region, Central Italy. IPGRI, Genetic Resources Newsletter (in press).

[12] Bellucci, E., Bulfon, D., Cappella, G., D'Amico, T., Ferradini, N., Nanni, L., Piermattei, S., Porfiri, O., Rossi, M., Papa, R. Le risorse genetiche vegetali nelle Marche. Regione Marche Agricoltura, 1: 20-22 (2001).

[13] Porfiri O., Petrini A., Giorgi B. L'uso di popolazioni locali in programmi di miglioramento genetico dell'orzo nudo. In Proceedings "Gestione delle risorse agroforestali in aree protette", Ancona. February 19-21, 1999 (in press).

[14] Negri V., Becker H., Onnela J., Sartori A., Strajeru S., Lalibertè B. A first inventory on-farm conservation and management activities in Europe including some examples of co-operation between the formal and informal sector. Report of the ECP/GR In situ/on farm Conservation Network Task Forces Meeting. Isola Polvese. Lalibertè B., Maggioni L., Maxted N. and Negri V. (compilers). Perugia, May 19-21, 2000: 14-30 (2000).

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ANALYSIS BY FISH OF *HORDEUM VULGARE* SUBSTITUTION LINES IN TRITORDEUM

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ABSTRACT: Fertile amphiploids (named tritordeums) between *Hordeum chilense* and diploid, tetraploid and hexaploid *Triticum* species have been obtained. Hexaploid tritordeum (2n = 6x = 42, **AABBH^{ch}H^{ch}**), obtained after chromosome doubling of the hybrid between *H. chilense* and *T. turgidum* conv. *durum*, is the objetive of a breeding program to develop a new crop. With the aim of obtaining *H. vulgare* substitution lines in hexaploid tritordeum we crossed *H. vulgare* addition lines in bread wheat (*T. aestivum* var. "Chinese Spring") with hexaploid tritordeum, following some backcrosses with the tritordeum parental or self-fertilization. Fluorescence in situ hybridization (FISH) using total genomic DNA from both *H. vulgare* and *H. chilense* and repetitive DNA probes (pAs1 and GAA-satellite) have been used to analyse the substitution lines for chromosomes 2I, 3I, 4I, 5I, 6I and 7I obtained. The introgression into tritordeum of specific characters from *H. vulgare*, for example the tenacity of the rachis which map on chromosome 3I, has been done is this way.

1. INTRODUCTION

The interest in genetic exchange between *Hordeum* and *Triticum* species led to the development of several hybrids and amphiploids between this genera. _*Tritordeum* Ascherson et Graebner, were obtained only when wild relatives of *H. vulgare*, as for example *H. chilense*, were used. *H. chilense* Roem. et Schult. is a wild South American diploid barley included in the Section Anisolepis. It occurs exclusively in Chile and Argentina and it is highly polymorphic both morphologically and biochemically (3).

The first amphiploid between *H. chilense* and *T. aestivum* (cv. Chinese Spring) was obtained at the former Plant Breeding Institute in Cambridge (UK) by Martín and Chapman(16). This octoploid tritordeum (2n=8x=56, **H^{ch}H^{ch}AABBDD**) show poor initial growth and high frequency of aneuploids.

Hexaploid tritordeum (*H. chilense* _ *T. turgidum* conv. *durum*, 2n=6x=42, **H^{ch}H^{ch}AABB**) showed good fertility, chromosome stability and plant morphology similar to that of wheat. These and other favourable agronomical traits such as high biomass yield, number of spikelets/spike, seed size, or protein content, made us consider the potential of this amphiploid as a possible new crop (14). The breeding program of tritordeum is based on the genetic variability generated by synthesising new amphiploids with a range of lines of *H. chilense* and wheat. The genetic variability available in the primary tritordeums for most of the traits of breeding value is enormous. For some of them such us heading date, plant height, biomass yield, spikelets per spike, kernel size, resistance to biotic and abiotic stresses the range of variability is even larger than on wheat (18). Nevertheless, for some others traits, e.g. threshing related characters, the available variability is insufficient for gaining response to selection on the short term. Tough rachis (one component of threshability) variants have not been found in *H. chilense*, neither has been possible to obtain them after mutagenic treatment . *H. vulgare* could be a source of this kind of traits and hybrids between barley and tritordeum were produced (15), but progeny from these crosses were not obtained.

The availability of chromosome addition lines of *H. vulgare* in wheat offers the possibility of transferring individual chromosomes from barley to tritordeum. Disomic addition lines of *H. vulgare* in hexaploid wheat

(**AABBDD** + 1 pair **II**) were crossed with hexaploid tritordeum (**H^chH^chAABBDD**). The hybrid **AABBDH^ch** + 1**I** is in some combinations fertile and always some seeds can be obtained after backcrossing to tritordeum.

Genomic in situ hybridization (GISH), in which total genomic DNA is labeled and used as probe, has been successfully applied to identify parental genomes in hybrids (24,1) and allopolyploids (2, 20). Furthermore, GISH was used to detect alien segments in translocations (9 ,24) to study genome organization during cell cycle (13) and in the analysis of homologous and homoeologous chromosome pairing (12, 6, 19).

In this report, GISH analysis using total genomic DNA from *H. chilense* and *H. vulgare* was used to identify *H. chilense* and *H. vulgare* chromosomes present in *H. vulgare* substitution lines in tritordeum.

2. MATERIAL AND METHODS

Advanced lines of the cross between *Hordeum vulgare* addition lines in *T. turgidum* (**AABBDD** + 1 pair **II**) (10) with hexaploid tritordeum (**AABBH^chH^ch**) have been analysed. Root-tip cells were pretreated for 4 hours in a 0.05% colchicine solution at 25∞C and fixed in 100% ethanol-acetic acid, 3:1 (v/v) for analysis of chromosome of somatic cells. Pre-hybridization washed were performed as previously describe by (5).

H. chilense and *H. vulgare* DNA was extracted from young frozen leaf tissue using the CTAB method(22) with some modification (8).

H. chilense and *H. vulgare* DNA used as probes were labelled by nick translation with biotin-11-dUTP or digoxigenin-11-dUTP (Boehringer Mannheim). The hybridization mixture consisted on 50% formamide, 2 x SCC, 5 ng of biotin or digoxigenin-labeled probe, 10% dextran sulfate, 0.14 mg of yeast tRNA, 0.1 mg of sonicated salmon sperm DNA and 0.005 mg of glycogen. In situ hybridization and post-hybridization washed were performed as previously described(5).

For detection of *H. vulgare* and *H. chilense* chromosomes, streptavidin-sulforhodamine and antidigoxigenin-FITC (fluorescein isothiocyanate, Boehringer Mannheimn) in PBS (phosphate-buffered saline) were used respectively. The slides were mounted in an antifade solution (11) containing 0.4 mg/ml 4',6-diamidino-2-phenylindole

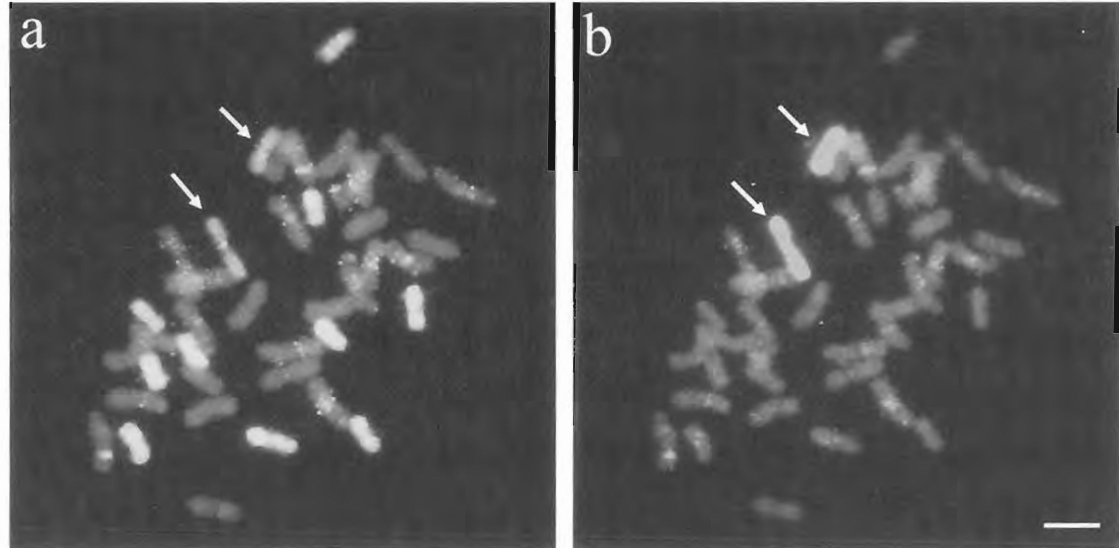

Figure 1: Genomic in situ hybridization (GISH) to *H. vulgare* substitution line in tritordeum (THSI) using total genomic *H. chilense* (a) and *H. vulgare* DNA (b) as probes detected with fluorescein (FITC) and rhodamine respectively. *H. vulgare* chromosomes are indicated by arrows. Note the bright *H. chilense* chromosomes and the faded wheat chromosomes in (a). Scale bar = 10 mm.

(DAPI) or propidium iodide (0.25 mg/ml) when sulforhodamine detection or FITC detection was used respectively. Probe hybridization sites were visualized with a Leica epifluorescence microscope following excitation using appropiate light filters. Photographs were taken with Kodak Ecktachrome Elite 400 colour print film. Colour prints were scanned and printed from PhotoStyler after conversion to gray scale.

1. RESULTS AND DISCUSSION

Advances lines from the cross between *H. vulgare* addition lines in *T. aestivum* (**AABBDD** + 1II) with hexaploid tritordeum (**AABBH^{Ch}H^{ch}**) have been analised by FISH (fluorescence in situ hybridization). Total genomic *H. chilense* and *H. vulgare* DNA probes were used to hybridize metaphase chromosomes of H. vulgare substitution lines for chromosomes 2I, 3I, 4I, 5I, 6I and 7I.

We have analysed more than one hundred lines and we have found a wide variability in the high number of substitution lines developed for each *H. vulgare* chromosome. Monosomic or disomic *H. vulgare* substitution lines for the *H. vulgare* substituted chromosome have been obtained (Fig. 1).

We have also found a high number of intergeneric translocation chromosome arms by FISH analysis using total genomic DNA from *H. chilense* and *H. vulgare*. Translocations involving wheat - *H. vulgare*, wheat - *H. chilense* and *H. chilense* - *H. vulgare* have been detected. Translocations between *H. chilense* and wheat are the most frequent translocations (data not showed). FISH analysis using pAs1 probe or GAA-satellite probe prior to GISH in the same metaphases makes it possible to determine which arm of barley, *H. chilense* or wheat are involved in translocations (data not showed).

Genomic digoxigenin-labeled *H. chilense* DNA probe can produce hybridization bands on A- and B- genome chromosomes present in *H. vulgare* substitution lines, with one to a few bands on the A- genome chromosomes and multiple bands on B- genome chromosomes as described by

(7). These hybridization bands are even found on *H. chilense* chromosomes.

Correspondence between the in situ hybridization sites observed with total genomic *H. chilense* DNA and the in situ hybridization patterns observed with the GAA-satellite sequence previously reported by (23) has been describe by (7). This correspondence allowed the identification of wheat and *H. chilense* chromosomes present in *H. vulgare* substitution lines in tritordeum. For identification and analysis of some lines of interest, pAs1 (4) and GAA-satellite sequence (23) were also used as probes (data not showed).

The combination of FISH and GISH analysis offers a reliable method of seedling selection for introgressions of *H. vulgare* chromosomes, translocations between wheat - *H. vulgare*, wheat - *H. chilense* and *H. chilense* - *H. vulgare* chromosomes or deletions in tritordeum. This approach offers a suitable tool to detect and analysed a high number of lines with chromosome modifications of interest in a plant breeding program in which species from different genera are involved.

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2. REFERENCES

[1] Anamthawat-Jónsson K, Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS : Discrimination between closely related Triticeae species using genomic DNA as a probe. Theor Appl Genet 79: 721-728 (1990).

[2] Bennett ST, Kenton AY, Bennett MD: Genomic in situ hybridization reveals the alloploid nature of *Milium montianum* (Gramineae). Chromosoma 101: 420-424 (1992).

[3] Bothmer RV, Jacobsen N, Baden C, Jorgensen RB, Linde-Laursen I: An ecogeographical study of the genus *Hordeum*, vol. 7, Rôme, pp 1-129 (1995).

[4] Cabrera A, Friebe B, Jiang J, Gill BS: Characterization of *Hordeum chilense* chromosomes by C-banding and in situ hybridization using highly repeated DNA probes. Genome 38: 435-442 (1995).

[5] Cabrera A, Ramirez MC and Martín A: Application of C-banding and fluorescence in situ hybridization for the identification of the trisomics of *Hordeum chilense*. Euphytica 109: 123-129 (1999).

[6] Fernández-Calvin B, Benavente E, Orellana J: Meiotic pairing in wheat-rye derivates detected by genomic in situ hybridization and C-banding-A comparative analysis. Chromosoma 103: 554-558 (1995).

[7] González MJ, Cabrera A: Identification of wheat and tritordeum chromosomes by genomic in situ hybridization using total *Hordeum chilense* DNA as probe. Genome 42: 1194-1200 (1999).

[8] Hernández P, Dorado G, Prieto P, Jiménez MJ, Ramírez MC, Laurie DA, Snape JW, Martín A: A core genetic map of *Hordeum chilense* and comparisons with maps of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). Theor appl Genet 102: 1259-1264 (2000).

[9] Heslop-Harrison JS, Leitch AR, Schwarzacher T, Anamthawat-Jónsson K: Detection and characterization of 1B/1R translocations in hexaploid wheat. Heredity 65: 385-392 (1990).

[10] Islam AKMR, Shepherd KW, Sparrow DHB: Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46: 161-174 (1981).

[11] Krenik KD, Kephart GM, Offord KP, Dunnette SL and Gleich GJ: Comparison of antifading agents used in immunofluorescence. J. Immunol. Methods 117: 91-97 (1989).

[12] Le HT, Armstrong KC: In situ hybridization as a rapid means to assess meiotic pairing and detection of alien DNA transfers in interphase cells of wide crossess involving wheat and rye. Mol Gen Genet 225: 33-37 (1991).

[13] Leitch AR, Schwarzacher T, Mosgöller W, Bennett MD, Heslop-Harrison JS: Parental genomes are separated throughout the cell cycle in a plant hybrid. Chromosoma 101: 206-213 (1991).

[14] Martín A: Tritordeum: the first ten years. Rachis 7: 12-15 (1998).

[15] Martin A: Cytology of a hybrid between barley (*Hordeum vulgare* L.) and tritordeum (_*Tritordeum* Ascherson et Graebner). Barley Genetics, Helsingborg, Sweden, 83-84 (1991)

[16] Martín A, Chapman V: A hybrid between *Hordeum chilense* in cereal breeding. Cerea Res Commun 5: 365-368 (1997).

[17] Martín A, Cubero JI: The use of Hordeum chilense in cereal breeding. Cereal Res Commun 9: 317-323 (1981).

[18] Martín A, Martín LM, Cabrera A, Ramirez MC, Gimenez MJ, Rubiales D, Hernández P, Ballesteros J: The potencial of *Hordeum chilense* in breeding Triticeae species. In: Jaradat AA (ed) Triticeae III. Science Publisgers, Enfield, New Hampshire, USA (1998a).

[19] Martín A, Rubiales D, Cabrera A: Meiotic pairing in a trigeneric hybrid *Triticum tauschii-Agropyron cristatum-Hordeum chilense* . Hereditas 129: 113-118 (1998b)

[20] Martín A, Cabrera A, Esteban E, Hernández P, Ramirez MC, Rubiales D: A fertile amphiploid between diploid wheat (*Triticum tauschii*) and crested wheatgrass (*Agropyron cristatum*) Genome 42: 519-524 (1999).

[21] Martín A, Cabrera A, Hernández P, Ramirez MC, Rubiales D, Ballesteros J: Prospect for the use of *Hordeum chilense* in durum wheat breeding. In: Rojo C, Nachit M, DiFonzo N, Araus J (eds) Durum wheat improvement in the Mediterranean region: new challenges Vol. 40. Options Méditerranées; Zaragoza (2000).

[22] Murray, Y H G, y Thompson, W F: Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8, 4321-4326 (1980).

[23] Pedersen C, Langridge P: Identification of the entire chromosome complement of bread wheat by two-colour FISH . Genome 40: 589-593 (1997).

[24] Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS: In situ localization of parental genomes in a wide hybrid. Ann Bot 64: 315-324 (1989).

USE OF GLIADIN PROTEIN MARKERS TO ANALYSE THE GENETIC IDENTITY OF BREAD WHEAT (*TRITICUM AESTIVUM* SSP. *VULGARE* L.) ACCESSIONS MAINTAINED IN A GENEBANK.

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ABSTRACT: Periodic regeneration of seed accessions in Genebanks is necessary to maintain optimal seed viability and replenish seed stock in the cold chambers. In the case of large size germplams collections, like those of Small Grains Cereals, this process introduces the possibility that a seed sample was incorrectly ascribed to a wrong entry. In this work, 52 accessions, conserved simultaneously in the chambers and in the spike collection, have been evaluated with different protein molecular markers (six gliadin loci). The results indicated that 75% of the accessions were right, 19% were mislabeled and 6% were solvable mistakes.

1.INTRODUCTION

- Periodic regeneration of seed accessions in Genebanks is necessary to maintain optimal seed viability and replenish seed stock in the cold chambers. Of course, during each regeneration event, genetic integrity of the accessions should be maintained, but human error introduces the possibility that samples might be incorrectly placed or labelled at any step, resulting in a seed sample being incorrectly ascribed to the wrong accession. Preservation of genetic integrity involves maintaining the joint frequency distribution of all alleles at all loci (Sackville Hamilton and Chorlton, 1997)(1). In the case of large size germplasm collections, like those of Small Grain Cereals, this objective is usually unachievable target, being more realistic to try to retain the accession identity. Comparisons of plant morphology are the simplest approach for the detection of mislabelled accessions and the assessment of genetic integrity. However, this strategy is sensitive to environmental influences and cannot always distinguish between closely related samples. In the case of wheat and related genera, a group of storage proteins of the endosperm named gliadins has proven valuable for variety identification and for checking on the nature of contaminating genotypes in pure seed. Moreover, the area of plant growth does not affect the gliadin pattern of a cultivar (Zillman and Bushuk, 1979)(2)
- In the Plant Genetic Resources Centre of Spain (CRF-INIA), the bread wheat collection conserved in the chambers has been multiplied several times since it was started collecting in 1920. The objective of this work is to know if a group of entries maintained in the CRF has been preserved accurately along the time, using gliadin proteins as genetic markers of their genetic identity

2. MATERIAL AND METHODS

2.1. Material

- Bread wheat accessions are maintained in the active and base collections stored in the Bank chambers. On the other hand, the CRF also preserves a spike collection of most of the wheat landraces stored in the chambers. This spike collection has been held without any change since the 50´s. Seed of 52 accessions, conserved simultaneously in the Active collection chamber and in the spike collection have been analysed to know if these entries have been maintained correctly.

2.2. Methods

- Gliadin storage proteins were examined using poliacrylamide gel electrophoresis techniques. Most gliadins are controlled in common wheat by six main *Gli* loci located on the chromosomes of the first (*Gli-1*) and sixth (*Gli-2*) homoeological groups. In this work gliadin alleles at the loci: *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2* and *Gli-D2* were identified in accordance to Metakovsky and Novoselskaya (1991)(3). At least five grains/accession from the Bank chamber were analysed. The two gliadin-genotypes obtained for each accession: one from the spike collection seed and other from the Bank chamber seeds, were compared to check accession identity.

3. RESULTS AND DISCUSSION

3.1. Gliadin alleles in the Spanish landraces studied

- Gliadin composition of the accessions studied using seeds from the spike collection and the Bank chamber is shown in Tables 1 and 2, respectively. No biotypes were detected in the landraces studied.

Table I. Local name and gliadin alleles of the 52 samples analysed from the Spike Collection

Entry no.	Bank number	Local name	Gli-A1 alleles	Gli-B1 alleles	Gli-D1 alleles	Gli-A2 alleles	Gli-B2 alleles	Gli-D2 alleles
1	BG-013169	BARBILLA	t	w	a	u	ag	a
2	BG-018228	BARBILLA	t	h	a	af	ag	a
3	BG-013154	BARBILLA BLANCA	k	t	b	h	?	f
4	BG-013195	BARBILLA CARBAJALES DE ALBA	t	h	d	af	ag	k
5	BG-013194	BARBILLA DE ALCAÑICES	d	h	l	u	ag	k
6	BG-018234	BARBILLA ROJA	f	h	k	af	t	k
7	BG-013130	BLANQUILLO	f	g	a	ac	r	a
8	BG-012582	BLANQUILLO	f	v	i	ai	ai	x
9	BG-013149	BLANQUILLO	d	new-1	f	f	r	a
10	BG-013762	BLANQUILLO	r	g	n	f	r	a
11	BG-013183	BLANQUILLO	f	v	f	ai	?	x
12	BG-018256	BLANQUILLO DE BARCARROTA	f	g	a	ac	r	a
13	BG-013124	CANDEAL	u	f	i	q	o	y
14	BG-013131	CANDEAL	f	f	i	af	?	a
15	BG-013132	CANDEAL	u	f	i	q	o	y
16	BG-012579	CANDEAL DE ALCALA	u	f	i	q	o	y
17	BG-013146	CANDEAL DE ALCARAZ	u	f	i	q	o	y
18	BG-012576	CANDEAL DE MINAYA	o	o	l	ae	o	a
19	BG-013129	CANDEAL DE NAVA DEL REY	f	f	i	ah	?	a
20	BG-018197	CANDEAL MOTA DEL CUERVO	o	t	a	a	?	a
21	BG-013139	CANDEAL PUEBLA ALMENARA	o	b	a	u	?	q
22	BG-013141	CANDEAL S. LORENZO DE PARRILLA	o	f	a	ae	o	a
23	BG-012591	CANDEAL VELLISCA	f	new-2	l	f	?	new-1
24	BG-018230	HEMBRILLA	o	o	a	ad	?	ad
25	BG-012876	HEMBRILLA CORTA DE ALTA MONTAÑA	m	b	i	f	o	a
26	BG-012594	HEMBRILLA DE ALFARO	k	o	a	f	?	a
27	BG-018224	HEMBRILLA DE ALFARO	f	o	a	g	?	a
28	BG-013800	HEMBRILLA DE BLECUA	o	o	b	af	?	a
29	BG-018232	HEMBRILLA DE JERGA	m	o	i	g	?	a
30	BG-013125	HEMBRILLA DE RUEDA	o	o	i	q	?	a
31	BG-013168	JEJA	o	o	d	u	?	a

CONTINUACIÓN ⟶

Entry no.	Bank number	Local name	Gli-A1 alleles	Gli-B1 alleles	Gli-D1 alleles	Gli-A2 alleles	Gli-B2 alleles	Gli-D2 alleles
32	BG-018196	JEJA CANDEAL	m	v	k	g	o	w
33	BG-013181	JEJA CAÑIFINA	p	o	f	o	ai	n
34	BG-013153	JEJA COLORADA DE CENIZATE	f	v	i	ai	ai	x
35	BG-018222	JEJA DE CIEZA	f	h	i	f	?	new-2
36	BG-012604	JEJA DE MINAYA	f	v	f	ai	?	x
37	BG-012019	NEGRETE	f	f	i	g	aj	a
38	BG-012056	NEGRETE ALTAREJOS	f	u	i	g	f	a
39	BG-012078	NEGRETE ATALAYA	f	o	i	u	aj	a
40	BG-012087	NEGRETE CAÑAVERAS	f	u	i	g	f	a
41	BG-012085	NEGRETE CUEVAS VELASCO	f	u	i	g	f	a
42	BG-012061	NEGRETE DE PRIEGO	f	o	i	u	aj	a
43	BG-012083	NEGRETE HUELVES	f	o	i	u	s	a
44	BG-012612	ROJO BOADILLA DE CAMPOS	o	o	f	f	o	new-4
45	BG-012596	ROJO DE CAMPOS	o	new-4	i	b	o	ab
46	BG-018207	ROJO DE CARAVACA	f	v	b	f	o	a
47	BG-013167	ROJO DE CARCEDO	f	o	f	new-1	ai	a
48	BG-012867	ROJO DE CARCEDO	f	o	f	new-1	ai	a
49	BG-018217	ROJO DE HUMANES	t	q	k	q	ah	k
50	BG-013197	ROJO DE PAREDES	f	o	i	g	?	a
51	BG-018214	ROJO DE VILLASECA	m	k	i	e	h	j
52	BG-013151	TRIGO DEL PAIS	t	t	a	u	ag	a

In bold= new catalogued allele; new = new but not catalogued allele; ? = allele not identified

Table II. Local name and gliadin alleles of the 52amples analysed from the Bank chamber

Entry no.	Bank number	Local name	Gli-A1 alleles	Gli-B1 alleles	Gli-D1 alleles	Gli-A2 alleles	Gli-B2 alleles	Gli-D2 alleles
1	BG-013169	BARBILLA	f	f	i	ah	h	f
2	BG-018228	BARBILLA	t	h	a	af	ag	a
3	BG-013154	BARBILLA BLANCA	f	v	i	f	new-5	ac
4	BG-013195	BARBILLA CARBAJALES DE ALBA	t	h	d	af	ag	k
5	BG-013194	BARBILLA DE ALCAÑICES	d	h	l	u	ag	k
6	BG-018234	BARBILLA ROJA	f	h	k	af	t	k
7	BG-013130	BLANQUILLO	f	new-1	a	q	o	a
8	BG-012582	BLANQUILLO	f	v	i	ai	ai	x
9	BG-013149	BLANQUILLO	d	new-1	f	f	r	a
10	BG-013762	BLANQUILLO	r	g	n	f	r	a
11	BG-013183	BLANQUILLO	f	v	f	ai	?	x
12	BG-018256	BLANQUILLO DE BARCARROTA	f	f	i	ah	new-2	a
13	BG-013124	CANDEAL	u	f	i	q	o	y
14	BG-013131	CANDEAL	f	g	a	ac	r	a
15	BG-013132	CANDEAL	u	f	i	q	o	y
16	BG-012579	CANDEAL DE ALCALA	u	f	i	q	o	y
17	BG-013146	CANDEAL DE ALCARAZ	u	f	i	q	o	y
18	BG-012576	CANDEAL DE MINAYA	o	o	l	ae	o	a

CONTINUACIÓN →

Entry no.	Bank number	Local name	Gli-A1 alleles	Gli-B1 alleles	Gli-D1 alleles	Gli-A2 alleles	Gli-B2 alleles	Gli-D2 alleles
19	BG-013129	CANDEAL DE NAVA DEL REY	u	f	l	q	o	y
20	BG-018197	CANDEAL MOTA DEL CUERVO	f	f	l	q	o	f
21	BG-013139	CANDEAL PUEBLA ALMENARA	o	h	l	f	ak	w
22	BG-013141	CANDEAL S. LORENZO DE PARRILLA	o	f	a	ae	o	a
23	BG-012591	CANDEAL VELLISCA	f	new-2	l	f	?	new-1
24	BG-018230	HEMBRILLA	o	o	a	ad	?	ad
25	BG-012876	HEMBRILLA CORTA DE ALTA MONTAÑA	m	b	i	f	o	a
26	BG-012594	HEMBRILLA DE ALFARO	k	o	a	f	?	a
27	BG-018224	HEMBRILLA DE ALFARO	f	o	a	g	?	a
28	BG-013800	HEMBRILLA DE BLECUA	o	o	b	af	?	a
29	BG-018232	HEMBRILLA DE JERGA	m	o	i	g	?	a
30	BG-013125	HEMBRILLA DE RUEDA	o	o	i	q	?	a
31	BG-013168	JEJA	f	f	i	ah	h	f
32	BG-018196	JEJA CANDEAL	m	v	k	g	o	w
33	BG-013181	JEJA CAÑIFINA	p	o	f	o	ai	n
34	BG-013153	JEJA COLORADA DE CENIZATE	f	v	i	ai	ai	x
35	BG-018222	JEJA DE CIEZA	f	h	l	f	?	new-2
36	BG-012604	JEJA DE MINAYA	f	v	f	ai	?	x
37	BG-012019	NEGRETE	f	f	i	g	aj	a
38	BG-012056	NEGRETE ALTAREJOS	f	u	i	g	f	a
39	BG-012078	NEGRETE ATALAYA	f	o	i	u	aj	a
40	BG-012087	NEGRETE CAÑAVERAS	n	v	i	new-1	new-12	a
41	BG-012085	NEGRETE CUEVAS VELASCO	f	b	i	u	h	a
42	BG-012061	NEGRETE DE PRIEGO	f	o	i	u	aj	a
43	BG-012083	NEGRETE HUELVES	f	o	i	u	s	a
44	BG-012612	ROJO BOADILLA DE CAMPOS	c	new-4	new-2	i	o	h
45	BG-012596	ROJO DE CAMPOS	o	new-4	i	b	o	ab
46	BG-018207	ROJO DE CARAVACA	f	v	b	f	o	a
47	BG-013167	ROJO DE CARCEDO	f	f	i	ah	h	f
48	BG-012867	ROJO DE CARCEDO	f	o	f	new-1	ai	a
49	BG-018217	ROJO DE HUMANES	t	q	k	q	ah	k
50	BG-013197	ROJO DE PAREDES	f	o	i	g	?	a
51	BG-018214	ROJO DE VILLASECA	m	k	i	e	h	j
52	BG-013151	TRIGO DEL PAIS	t	t	a	u	ag	a

In bold= new catalogued allele; new = new but not catalogued allele; ? = allele not identified

- Eleven alleles were identified at the *Gli-A1* locus, 14 at the *Gli-B1*, 9 at the *Gli-D1*, 17 at the *Gli-A2* locus, 14 at the *Gli-B2* and 16 at the *Gli-D2*. Therefore, the most varied sets of alleles were found at the loci of the sixth homologous group.
- At least 25 new gliadin alleles, which were not catalogued before, were found in the Spanish landraces studied (Tables 1 and 2).
- Some gliadin alleles present in the spike collection were not in the material stored in the chamber: *w* (*Gli-B1*), *a* and *h* (*Gli-A2*) and *q* and *new-4* (*Gli-D2*). Conversely, alleles *c* and *n* (*Gli-A1*), *new-2* (*Gli-D1*), *i* (*Gli-A2*), *ak*, *new-2*, *new-5* and *new-12* (*Gli-B2*), and *h* and *ac* (*Gli-D2*) were present in the accessions in the chamber but not in the spike collection. In general, gliadin variability was similar for both types of materials, although *Gli-B2* variation was greater in the samples from the chamber. For the new catalogued alleles, *Gli-B1w* was absent in the chamber whereas *Gli-B2ak* and *Gli-D2ac* did not appear in the spike collection

3.2. Authenticity of the grain stored in chambers

- Comparisons of the two-gliadin genotypes obtained for each accession (Table 1 and 2) revealed which entries had maintained their genetic identity accurately along the time. In total 39 accessions (75%) were right, 10 accessions (19%) were mislabelled and 3 were also mislabelled, but their correct iden-

tity could be discovered (solvable mistakes) (Fig. 1). So, we found that different gliadin profiles were in the spike collection and in the Bank chamber (Fig. 2): the BG-13139 'Candeal Puebla Almenara', lane A (spike) and B (chamber); the BG-13167 'Rojo Carcedo', lane J (spike) and I K (chamber); and BG-13154 'Barbilla Blanca' , lane N (spike) and O (chamber). An example of the same gliadin profile is shown in the lanes L (spike) and M (chamber) of BG-12583 'Blanquillo'.

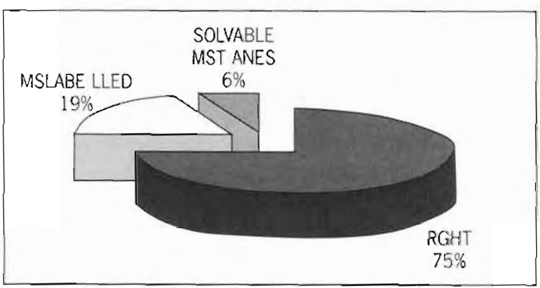

Figure 1: Results of the accessions genetic identity accuracy based on comparisons of the two-gliadin genotypes (spike collection and Bank chamber samples.

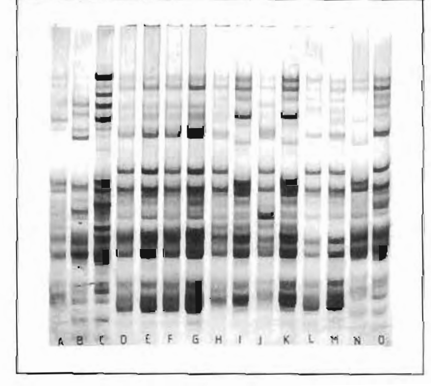

Figure 2: Gliadin electrophoretic spectra of several wheat landraces accessions.

- Gliadin analyses have also shown to be very useful to detected duplicates in the accessions studied. In Fig. 2: the BG-13103 'Blanquillo' , lane D (spike) and E (chamber) and the 'Jeja', lane F (spike) and G)chamber) presented identical gliadin composition, being duplicated in spike collection and in the Bank chamber.
- To preserve the common wheat germplasm of a country and fight erosion it is well worth the maintaining, evaluation and using in breeding of local wheat collections, which would include both old cultivars and landraces. To assure a correct maintenance in Banks genetic identity of these materials should be monitored by means of analysis of polymorphic markers. Some advantages of gliadin alleles for wheat genotype identification over up-to -date molecular markers have been discussed recently (Metakovsky and Branlard 1997)(4). Our study has confirmed that gliadin markers are an easy, cheap and powerful tool to analyse intraspecific genetic polymorphism in wheat genetic resources.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Sackville Hamilton, N.R. and K. G. Chorlton. 1997. Regeneration of accessions on seed collections: a decision guide. Handbook for Genebanks No. 5. International Plant Genetic Resources Institute, Rome, Italy.

[2] Zillman RR and Bushuk W. 1979. Wheat cultivar identification by gliadin electrophoregrams. III. Catalogue of electrophoregram formulas of Canadian wheat cultivars. Can J Plant Sci 59: 287-298

[3] Metakovsky, E.V. & A. Yu. Novoselskaya, 1991. Gliadin allele identification in common wheat. 1. Methodological aspects of the analysis of gliadin pattern by one-dimensional polyacrilamide gel electrophoresis. J. Genet. & Breed. 45: 317-324.

[4] Metakovsky, EV and G. Branlard. 1998. Genetic diversity of French common wheat germplasm based on gliadin alleles. Theor. Appl. Genet. 96: 209-218.

CROSSES BETWEEN *Triticum turgidum* AND THE AMPHIPLOID DDPP (*T. tauschii-Agropyron cristatum*): ANALYSIS BY FISH OF THE DESCENDENCE

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ABSTRACT: *Agropyron* (Gaertn) is a genus of the Triticeae which includes the crested wheatgrass complex, being *A. cristatum* (L.) a representative species containing P genome. This species is an important resistance source for improving the genetic variability of both durum and bread wheat. By crossing tetraploid wheat (*T. turgidum* conv *durum*, 2n=4x=28; AABB) with a fertile allotetraploid between *T. tauschii* and *A. cristatum* L (x*Agroticum*, 2n=4x=28; DDPP), amphiploid plants with chromosome numbers ranging from 50 to 57 were obtained. The response to wheat leaf rust and powdery mildew diseases were evaluated in these plants in adult stage under field condition. Fluorescence *in situ* hybridization (FISH) using both total genomic DNA from *A. cristatum* and the repetitive pAs1 probe, have been used to examine the genome composition of the progeny of these intergeneric combinations.

1. INTRODUCTION

The tribe Triticeae is one of the botanical groups in which interspecific and intergeneric crosses have been more successfully explored. Many genomic combinations have been obtained to be used directly as new crops or indirectly to widen the genetic basis of cultivated crops.

The tribe Triticeae includes important crops such as wheat, barley and rye, as well as forage crops within the genera *Agropyron*. Hybrids and amphiploids between both tetraploid and hexaploid wheat with *Agropyron* species have been obtained with the aim to introducing characters from *Agropyron* into both durum and bread wheat (1,2,3,4). Recently, a fertile amphiploid between diploid wheat (*T. tauschii*) and *A. cristatum* have been obtained (5). In this work we used the fluorescence *in situ* hybridization (FISH) to analyze the genome composition of the descendence from the crosses between durum wheat with the amphiploid x*Agroticum* (2n=4x=28; DDPP).

2. MATERIAL AND METHODS

The fertile amphiploid between *T. tauschii* and *A. cristatum* (2n=4x=28; DDPP) was used as male parent to pollinate emasculated spikes of durum wheat (*T. turgidum* L. var. *durum*). From 180 seeds obtained, 20 were germinated in Petri dishes and nine seedling were obtained. To evaluate chromosome numbers in mitosis, root tips were collected from each seedling when the roots had 1.5-2 cm of length, they were treated with 0.05% colchicine for about 3 hours at room temperature and directly fixed in a mixture of 3:1 (ethanol 99% and glacial acetic acid) for 20 days at least. The apexes were pre-treated one minute with acetocarmin and the number of chromosomes was determined by microscopic examination of squash preparation in one drop of 45% acetic acid. The chromosome preparations were frozen in liquid nitrogen for one minute, the coverslip removed and they were stored until used. For FISH, both the highly repetitive pAs1 sequence, isolated from *T. tauschii* by Rayburn and Gill (6) and total genomic DNA isolated from young frozen green leaves of *A. cristatum* were used as probes. Total genomic DNA from *A. cristatum* and pAs1 probes were labeled by nick translation with biotin-11-dUTP and digoxigenin-11-dUTP (Boerhinger Mannheimn), respectively. The hybridization mixture consisted of 50% formamide and 10% of dextran sulfate, 2 x SSC, 7 ng of digoxigenin-labeled *A. cristatum* DNA and 7 ng of the biotin-labeled pAs1 probe.

Double hybridization was performed with the two probes simultaneously. Antidigoxigenin-FITC (fluorescein isothiocyanate Boehringer Mannheimn) and estreptavidin-suforhdamine (Cy3) were used for the detection of digoxigenin-labeled *A. cristatum* DNA and biotin-labeled pAs1 probes, respectively. The hybridization in situ protocol was carried out according to Cabrera et al (7). The preparations were counterstained with DAPI (4´,6 - diamidino-2-phenylindole). The signals were visualized with an epifluorescence microscope Leica, images were captured with a SPOT CCD camera using the appropriate SPOT 2.1 software and processed with PhotoShop 4.0 software (Adobe Systems Inc.). Color images were scanned and printed from Adobe PhotoShop after conversion to gray scale.

3. RESULTS AND DISCUSSION

Table 1 shows the chromosome constitution of the nine plants obtained. The total number of chromosomes in these plants ranged from 50 to 57 with a number of *A. cristatum* chromosomes from 12 to 14. We assume that these amphiploids plants might have been produced naturally by the formation of unreduced gametes. FISH using both total genomic DNA from *A. cristatum* and pAs1 probes allowed simultaneous discrimination between D- and P-genome chromosomes. Figure 1 shows FISH to metaphase chromosome spreads of two plants showing 12 and 14 P-genome chromosomes from *A. cristatum*, respectively both of them having 14 D-genome chromosomes derived from *T. tauschii*.

The amphiploids were morphologically more variable than the highly uniform DDPP, upright, tall and tillered profusely, the spikes morphology were intermediate between both parents but tended to resemble *T. turgidum*.

The amphiploids were resistant to powdery mildew (*Blumeria graminis* f. sp. *tritici*) and wheat leaf rust (*Puccinia triticina*). The infection of the two diseases occurred naturally and diseases scores were based on the percentage leaf area covered by mildew and rust (Table 2). In all cases the amphiploids were more resistant than the wheat parent. These plants could be used as bridge for introgression both resistance to leaf rust and powdery mildew into tetraploid and hexaploid wheat.

Table 1. Chromosome constitution of the nine plants obtained (*T. turgidum* x DDPP)

Plant	No. of chromosomes	P	D	No. of grains
(T15xDDPP)-8	56	14	14	28
(T15xDDPP)-10	56	13	15	11
(T15xDDPP)-12	55	12	15	0
(T15xDDPP)-13	55	12	14	0
(T15xDDPP)-14	56	14	14	7
(T15xDDPP)-15	50	13	14	0
(T15xDDPP)-17	54	13	15	10
(T15xDDPP)-19	57	14	15	3
(T15xDDPP)-21	56	14	14	3

Table 2. Percentage of leaf area covered by leaf rust and powdery mildew in the amphiploids and its parents

Plant	*P. triticina*	*B. graminis*
(T15xDDPP)-8	1	0
(T15xDDPP)-10	1	0
(T15xDDPP)-13	1	0
(T15xDDPP)-14	1	0
(T15xDDPP)-15	1	0
(T15xDDPP)-17	0	0
(T15xDDPP)-19	1	0
(T15xDDPP)-21	0	0
Gerardo (T15)	20	20
A. cristatum	0	0
DDPP		

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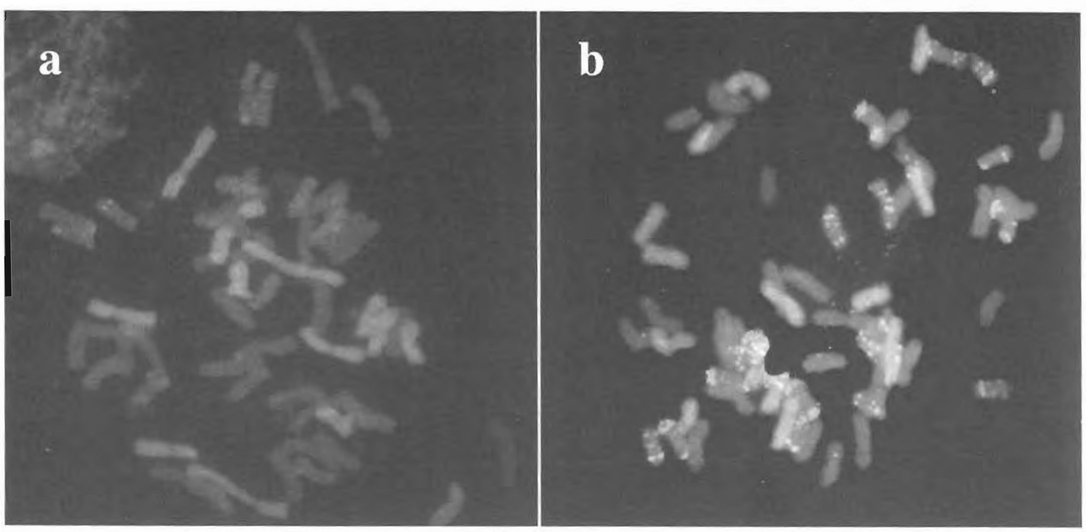

Figure 1. FISH to mitotic chromosome metaphase spreads of two amphiploids plants between *T. turgidum* and DDPP showing (a) 12 and (b) 14 P-genome chromosomes, respectively.

REFERENCES

[1] Sharma HC, Gill BS (1983) Current of wide hybridization in wheat. Euphytica 32

[2] Li LH and Dong YS, (1990). Production and study of intergeneric hybrids between *Triticum aestivum* and *Agropyron desertorum*.(In Chinese). Sci. China Ser B Chem. Life Sci. & Earth Sci.5: 492-496.

[3] Li LH and Dong YS, (1991). Hybridization between *Triticum aestivum* L. and *Agropyron michnoi* Roshev. 1. Production and cytogenetic study of F_1 hybrids. Theor. Appl. Genet. 81: 312-316.

[4] Limin AE and Fowler DB, (1990). An interspecific hybrid and amphiploid produced from *Triticum aestivum* crosses with *Agropyron cristatum* and *Agropyron desertorum*. Genome, 33: 581-584.

[5] Martín A, Cabrera A, Esteban E, Hernández P, Ramírez MC and Rubiales D, (1998). A fertile amphiploid between diploid wheat (*Triticum tauschii*) and crested wheatgrass (*Agropyron cristatum*). Genome, 42: 519-524

[6] Rayburn AL and Gill BS, (1986). Isolation of D-genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Mol Biol Rep 4: 102-109.

[7] Cabrera A, Ramírez MC and Martín A, (1999). Application of C-banding and fluorescence *in situ* hybridization for the identification of the trisomics of *Hordeum chilense*. Euphytica 109: 123-129.

SESSION D:

RSISTANCE TO BIOTIC AND ABIOTIC STRESSES.

RESISTANCE IS THE KEY TO A SUCCESSFUL REVOLUTION

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ABSTRACT: Poverty, hunger and malnutrition occur in many parts of the world despite the enormous progress that has taken place in agriculture and food production in the last century. It is estimated for wheat, that by 2020 the world will require a 60% increase in production to meet the projected requirement. This implies that agricultural scientists will have to use all the approaches available to them to increase the productivity of cereal crops. Resistance to both biotic and abiotic stresses will be critical in reaching this goal. All are familiar with the so called "Green Revolution" and many are aware that the rust resistant *Sr2* complex of genes has been attributed with stabilising wheat yields in the early years of this phenomenon. Distinct advantages accompany the use of genetic resistance to biotic and abiotic stresses. The most important is the fact that response to the stress situation occurs independently of the managerial ability, skill and resource level of the producer. Anyone can use a stress resistant crop. Immense progress has been made in the field of functional genomics and molecular manipulation. It is clear that the restraining factor in future will not be the availability of scientific techniques and tools, or for that matter, genetic resources, but the human and financial capacity to achieve the goals on a world-wide scale so that they really do make a difference to the livelihood of the poor. Triticeae play a meaningful role as staple and non-staple food crops around the world. To face the demands of the future a new revolution will be needed and again resistance to stresses will play a major role. The future demands crops with stable yield irrespective of environmental constraints, good quality and a high nutritional value; crops that are free of pesticide residues and other harmful substances.

"Revolution" is defined by the Oxford Dictionary as 'any fundamental change or reversal of conditions', and this word has been used to describe changes in circumstances from political dispensations to industrialisation. The "Green Revolution" began late in the 1960's, most here today are familiar with the broad principles of the movement which by employing improved crop cultivars, irrigation, fertiliser, pesticides and mechanisation led to an enormous increase in the availability of food. Most people here today will also be aware that three decades down the line there are many critics of the process. Some maintain that it caused an increase in the cost of production, chemical inputs in the form of pesticides, herbicides and fertilisers have damaged the environment and sometimes left the rural poor worse off than they were before - it has not eliminated famine and the world population is still increasing. In a sense there is a measure of truth in everything I have just mentioned; both the positive and the negative. The most important however, is the fact that the "Green Revolution" was a process - a genuine endeavour by dedicated people to make the world a better place; somewhere where wide spread hunger, malnutrition and poverty cease to exist. If the original goals have not yet been met it simply means that the process is bigger and more complex than what was originally anticipated. The FAO and the World Bank have both called for what boils down to a "New Green Revolution". Thirteen percent of the global population is food insecure (1) and many people still go to sleep hungry at night despite the enormous progress that has taken place in agriculture and food production in the last century. The negative impacts of political instability, poverty and social turmoil cannot be underestimated and these issues surely need to be addressed if household food security of the world population is to be attained.

As scientists there is not much we can do to solve political instability and social turmoil, but there is a major role for us to play in developing the crops that will be needed to meet the food requirement of the future. In the book 'Food in the 21st Century: From Science to Sustainable Agriculture' released in October 1999 by the CGIAR System Review Secretariat, specific mention is made of the role to be played by genetic resistance to insects, diseases and environmental stress conditions.

Most of the production constraints that producers face today are not new or unknown, in fact most have been around for many years. Environmental constraints undoubtedly cause major yield and quality losses and in some circumstances simply prevent production. These include phenomena like drought, untimely or excess heat, untimely or excess rain, waterlogging of soils, wind, extreme cold, frost, acid soils and associated aluminium toxicity, salinity, nutrient imbalances and/or shortages as well as micro-nutrient deficiencies. Technological advances of the modern world, if anything, have added a few new stresses and made others even worse. It is estimated that 1.5 to 2 million hectares are being lost each year to salinisation and waterlogging (2), a consequence of poorly managed irrigation. Air pollution from industries, mining operations and power plants has lead to the accumulation of pollutants in the soil. This happens unseen and plants easily take up heavy metals like zinc and nickel, effecting plant growth by causing phytotoxicity, or cadmium, making grain unfit for animal or human consumption (3). The competition for land from ever-increasing urbanisation results in more marginal and often more fragile land, being used for agriculture in an effort to meet the production need.

The impact of biotic stresses can be equally devastating. A multitude of diseases, insect and nematode pests occurs throughout the regions where Triticeae are produced. Every region has its own specific array of organisms fine-tuned to utilise the host plant in that specific environment. Diseases include *Septoria*, the rusts, *Fusarium*, eyespot, scab, mildew, root rots, BYDV, snow mould, bunts and smuts, to name but a few. The list of pests includes Hessian fly, Sunn pest, Greenbug, Oat aphid, Russian wheat aphid, and even nematodes like root lesion nematode and cereal cyst nematode.

The challenge that faces us as scientists is to reintroduce those genes that enable plants to cope with these adverse conditions, genes that may have inadvertently or purposefully been lost in the process of 'crop improvement' in the past. Twenty years ago Feldman & Sears (4) recognised that "modern breeding practices have reduced the genetic variability of the cultivated wheats". Skovmand & Rajaram (5) stated that "modern agriculture is characterised by homogeneity of crops and cultural practices" and it is principally to redress this situation that the targeted reintroduction of desirable plant characteristics is needed. Rajaram (6) states that yield potential *per se* should be combined with both biotic and abiotic tolerances in the breeding of future cultivars. Distinct advantages accompany the use of genetic resistance to biotic and abiotic stresses. The most important is the fact that response to the stress situation occurs independently of the managerial ability, skill and resource level of the producer. Anyone can use a stress resistant crop. It is also generally assumed that resistant crops possess more benefits than drawbacks for the environment.

Luckily there is a fairly abundant supply of resistance genes available in related Triticeae species. Feldman & Sears (4) give a list of desirable characteristics to be found in the wild relatives of wheat including increased resistance to fungal and viral diseases, to insects, drought, lodging, improved winter hardiness, heat tolerance and salt tolerance. We know from history that transferring desirable characteristics into valuable genotypes is a formidable challenge, but with the expertise of today's scientists and molecular techniques, these traits can and should be exploited to their full potential.

The tricky part of incorporating resistance into a food crop like wheat, durum, barley or rye is that the crop must meet the requirements of both the producer and end-user. This was illustrated so well when I was preparing for this presentation. Martin, Cabrera, Hernandez, Ramirez, Rubiales and Ballesteros (7) reported the array of resistance present in *Hordeum chilense*; resistance to rusts, powdery mildew, *Septoria*, common and karnal bunt, the smuts, *Pyrenophora*, *Rhyncosporium*, Russian wheat aphid, oat aphid, greenbug and nematodes; a veritable genetic treasure trove. A colleague's comment brought me back to earth with a bump. "No one's going to eat *that stuff* though". It is combining resistance with other characteristics, particularly high yield and quality that proves to be the major challenge facing breeders today. This, in essence, was probably why the resistance went missing in the first place. It wasn't easy to combine these traits then either and much of the information we have at our disposal today had yet to be

collected. Worland and Snape (8) believe the molecular tools that are now becoming available will "allow us to manipulate the wheat genome with a precision never thought possible previously". It is clear that the restraining factor in future will not be the availability of scientific techniques and tools, or for that matter, genetic resources, but the human and financial capacity to achieve the goals on a world-wide scale so that they really do make a difference to the livelihood of the poor.

In the case of the developing world many countries simply cannot afford to import food. A lack of basic infrastructure like roads means that the distribution of food is difficult and for many people this means they must grow their own food to survive. Where producers are often only aiming to meet their household needs, the value of resistance to biotic and abiotic stress becomes most apparent. Cultivars that are resistant to abiotic stress can give a more stable yield over seasons and those resistant to pests and diseases can save crop yield otherwise lost. In essence something like drought resistance may be the difference between life and death. It has been said that a hungry man only has one problem and that is where to find food. In contrast, a man who is not hungry usually has many problems.

Sometimes the needs of the producer and end-user can be conflicting. Take malting barley as an example, brewers want a uniform rapid germination while producers would like a measure of dormancy to protect against pre-harvest sprouting in the field when rain occurs in the harvest season (9). The well known 1B/1R translocation, arguably the most successful of all alien introductions to bread wheat is another example. The rye segment has, in addition to disease and insect resistance, the ability to promote a higher plant biomass and larger grain size (8). These yield-enhancing advantages which are important for the producer are counteracted by the associated "sticky dough" characteristic which reduces bread making quality (8).

There are many success stories about resistance to biotic and abiotic stress in the Triticeae. In the field of disease resistance, the rust resistant *Sr2* complex of genes has been attributed with stabilising wheat yields in the early years of the Green Revolution and rust resistance still plays a major role today in many cultivars throughout the world. *Rht* or dwarfing genes that give resistance to lodging under high fertility conditions, in addition to their ability to improve the harvest index of cultivars, have been exploited well.

Resistance to Russian wheat aphid in South Africa is another example of the positive impact resistance can have. Fifteen cultivars have been commercially released in the summer rainfall production region of the Free State Province since 1992. It is estimated that between 70 and 85% of the area planted to wheat is now under resistant cultivars. These cultivars save wheat producers approximately ZAR 120.00 (one tenth of the income per ton) per hectare by eliminating the need for chemical control. This reduction in input costs makes it easier to compete on the global market. The benefit spills over into the environment as well; all insecticides, except two namely imidacloprid and amethoxam, registered for the control of Russian wheat aphid in South Africa are broad-spectrum systemic or contact organophosphates (LD_{50} 2-70mg/kg) (10). Due to the rapid adoption of resistant cultivars the average area treated with insecticides decreased from 85% in 1990 to 30% in 1997 (11). In addition to the Russian wheat aphid resistance, which lowers not only the number of tillers infested but also the number of aphids per infested tiller, some of these cultivars show a measure of drought tolerance too. Small-scale and emerging commercial farmers, previously disadvantaged by the political dispensation in our country, have eagerly adopted these cultivars and believe that they benefit in the process.

The use of plant resistance isn't always the answer to a problem, it can also exasperate situations and as scientist we should be aware of this. Wheat cultivars with genetic tolerance to aluminium toxicity in acid soils make it possible for producers to grow a crop without applying lime. In the long run this is a practice which does nothing to counteract further acidification of the soil and may indeed lead to situations where rehabilitation of the soil becomes impracticable.

The use of resistance to biotic and abiotic stresses as a method to stabilise yields and minimise disease and pest damage seems easy in theory, until it is put into practice. Large amounts of money, effort and time are invested in breeding a resistant crop and it is of the utmost importance that the resistance is durable. The adoption of new cultivars is sometimes slow in developing countries and once adopted a cultivar may be retained for many years. Durable resistance is a prerequisite for sustainable agricultural production. Exactly what causes resistance to be durable? The truth is we often don't know. In many cases the exact genetic control of the characteristics we seek, eludes us and though it is important to figure out how

things work in order to optimise the breeding process there is a cut-off point between 'need to know' information and 'nice to know' information. 'Need to know' information includes combining the resistance with other desirable factors such as high yield, beneficial agronomic characteristics, quality and processing characteristics. It means making sure that the technology is adopted and includes on-farm evaluation of new cultivars and production techniques. 'Need to know' information also means using innovative science to meet the challenges of the future. Host plant resistance particularly to biotic stresses can supply in the demand for so-called 'safe food' with low pesticide residues and 'natural' versus 'genetically manipulated' resistance, but will production be high enough to meet the demand?

Dr Swaminathan (12) stated in his plenary lecture at the 6th International Wheat Conference in Budapest last June that "we are now in a state of transition from Mendelian to Molecular breeding". We are standing on the brink of the "Gene Revolution" with "explosive progress being witnessed in the areas of functional genomics and molecular manipulation". The techniques exist and there are research programmes currently running in many countries. The issue of genetically manipulated (GM) crops is a sticky one with public concern for bioethical issues in addition to food and environmental safety issues. Concerns related to intellectual property rights exist and the application of these crops in the developing world is hotly debated. These are all issues that will have to be resolved somehow. It is probably not a question of whether GM crops will be used or not but rather one of how these technologies will be utilised.

Triticeae play a meaningful role as staple and non-staple food crops around the world. The crux of the matter remains the same whether exploiting resistance to biotic or abiotic stresses from related or unrelated species. Crops must be developed that are in tune with the requirements of the environment, the producer and the end-user. Whatever work we do as scientists in identifying desirable characteristics and finding molecular markers, tools and procedures to manipulate them, we must make sure that the fruits of our labours are translated into cultivars that producers and end-users are eager to grow and utilise. The future demands crops with stable yield irrespective of environmental constraints, good quality and a high nutritional value; crops that are free of pesticide residues and other harmful substances.

REFERENCES

[1] Dar WD: An assessment of technology development from the green revolution to today. Presentation at the 7th JIRCAS International Symposium Tsukuba, Japan, October 2000. http://www.icrisat.org/text/research/ presentations.htm (2000).

[2] Wienhold B: Salinity and Sodicity Management to insure sustainability in irrigated agriculture. In: Fairclough AJ (ed) Sustainable Agriculture Solutions; The sustainable Agriculture Initiative Report. Novello Press, London (1999).

[3] Van der Eerden L, Kempenaar C, Tonneijck A: Air Pollution and Crop production in the developing world. In: Fairclough AJ (ed) Sustainable Agriculture Solutions; The sustainable Agriculture Initiative Report. Novello Press, London (1999).

[4] Feldman M, Sears ER: Wild gene resources of wheat. Scientific American 244(1): 98-109 (1981).

[5] Skovmand B, Rajaram S: Utilization of Genetic Resources in the Improvement of Hexaploid wheat. In: Srivastava JP, Damania AB (eds) Wheat Genetic Resources: Meeting Diverse Needs. John Wiley & Sons (1990).

[6] Rajaram S: Approaches for breaching yield stagnation in wheat. Genome 42: 629-634 (1999).

[7] Martin A, Cabrera A, Hernandez P, Ramírez MC, Rubiales D, Ballesteros J: Prospect for the use of *Hordeum chilense* in durum wheat breeding. In: Royo C, Nachit MM, di Fonzo N, Araus JL (eds) Durum wheat improvement in the mediterranean region: new challenges. Proceedings of a seminar, Zaragoza, Spain, 12-14 April (2000).

[8] Worland T, Snape JW: Genetic Basis of Worldwide Wheat Varietal Improvement. In: Bonjean AP, Angus WJ (eds) The World Wheat Book: A History of Wheat Breeding. Lavoisier Publishing, France (2001).

[9] Derera NF: The effects of preharvest rain. In: Derera NF (ed) Preharvest sprouting in cereals. CRC Press Inc, Boca Raton, Florida (1989)

[10] Nel A, Krause M, Ramautar N, Van Zyl K: A Guide for the control of plant pests. National Department of Agriculture, Republic of South Africa. (1999).

[11] Marasas C: Wheat Management Practices and adoption of the Russian wheat aphid integrated control programme in the study area. In: Socio-economic impact of the Russian wheat aphid integrated control programme. PhD Thesis, Department of Economics, Extension and Rural Development, Faculty of Biological and Agricultural Science, University of Pretoria, South Africa (1999).

[12] Swaminatham MS: A century of Mendelian breeding: Impact on Wheat. In: Bedö Z and Láng L (eds) Developments in Plant Breeding: Wheat in a Global Environment. Kluwer Academic Publishers, Netherlands (2001).

PREHAUSTORIAL AND POSTHAUSTORIAL RESISTANCE TO WHEAT LEAF RUST IN DIPLOID WHEAT

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ABSTRACT: Diploid wheat is a close relative of tetra- and hexaploid cultivated wheats and can be used as a donor species in wheat breeding. Diploid wheat comprises three (sub)species, *T. monococcum*, *T. boeoticum* and *T. urartu*, that can be distinguished by morphological and molecular characteristics. About 200 accessions per diploid wheat species were tested for resistance to the wheat leaf rust fungus *Puccinia triticina*. *T. monococcum* was almost completely resistant (98%), *T. boeoticum* and *T. urartu* were completely susceptible.

The resistance in *T. monococcum* consisted of two mechanisms: 1) prehaustorial, before the formation of haustoria by the fungus, and not associated with necrosis, and 2) posthaustorial, based on hypersensitivity after the formation of a haustorium in the plant cell. Resistant accessions with a truly high level of prehaustorial resistance were scarce. Only two percent of the accessions had at least 50% of the infection units aborted prehaustorially. All remaining infection units aborted in association with hypersensitivity.

Two mapping populations were studied for their reaction to wheat leaf rust. Both populations were derived from a cross between a susceptible *T. boeoticum* accession and a resistant *T. monococcum* accession. Both resistant accessions had a high level of prehaustorial resistance to wheat leaf rust. Several quantitative trait loci (QTLs) for resistance were identified. Some effective only in the first, others also in the fifth leaf stage. The QTLs had a pleiotropic effect, governing prehaustorial as well as posthaustorial resistance.

It is remarkable that the two resistance mechanisms in diploid wheat are controlled by the same QTLs. In barley and cultivated wheat, genes for prehaustorial and posthaustorial resistance to leaf rusts represent two different classes that map to distinct locations on the respective genomes.

1.INTRODUCTION

Diploid wheat, *Triticum monococcum* s.l. is also often referred to as a species complex of *T. monococcum* s.s. L., *T. boeoticum* Boiss., and *T. urartu* Tum. We will use this classification throughout this paper. The species, probably *T. urartu*, is the donor of the A-genome in the cultivated wheat species (6). High levels of resistance to the wheat leaf rust fungus, *Puccinia triticina* (syn. *P. recondita* f.sp. *tritici*) have been reported to occur in this species complex (2, 16, 20). To this pathogen species, at the cellular level, two types of resistance can be discerned in diploid wheat: prehaustorial and posthaustrial (12). Prehaustorial resistance prevents the formation of haustoria by the fungus. Normal haustorium mother cells are formed, but haustoria do not develop, and a papilla is often induced at the site of attempted cell wall penetration (4, 12). In genotypes with posthaustorial resistance, the plant cells die after the formation of a haustorium by the fungus (4, 8, 10). In general, race-specific hypersensitivity resistance is posthaustorial, and not durably effective.

The prehaustorial resistance would be of practical interest if it is based on a different mechanism that may be hard for the pathogen to break-down.

The purpose of the present study was to determine the frequency of resistance to *P. triticina* in the diploid wheat complex, the frequency of occurrence of prehaustorial resistance to this pathogen, and the inheritance of the pre- and posthaustorial resistance in resistant *T. monococcum* accessions.

2. MATERIALS AND METHODS

2.1. Screening of the plant collection

Two-hundred-and-eleven accessions of *T. boeoticum*, 182 accessions of *T. monococcum* s.s., and 205 accessions of *T. urartu* were kindly provided by the National Small Grains Collection, Idaho, USA. These accessions were inoculated in 8 sets, each containing approximately 80 accessions. The seeds were sown in plant boxes (39 x 37 cm) and plants were grown in a greenhouse compartment at about 19 to 26 °C, and a minimum day length of 16 hours.

2.2. Inoculation

Twelve to 13 days after sowing, first leaves of at least five seedlings per accession were fixed horizontally, and inoculated in a settling tower. Five mg of urediospores of leaf rust isolate Felix were mixed with about ten times the volume of *Lycopodium* spores, and used to inoculate each plant box. Density of inoculum amounted to about 300 spores per cm^2. Inoculated seedlings were incubated in a humidity chamber overnight.

2.3. Macroscopic observations

Ten to 12 days after inoculation, the infection type (IT) was determined according to the 0-9 scale of McNeal et al (9). Accessions showing IT 7 or higher, i.e. large pustules that might be associated with chlorosis, but not with necrosis, were considered susceptible. IT scores 3 or lower (no symptoms, flecks only, or minute pustules associated with extensive necrosis) were classified as resistant.

2.4. Microscopic observations

Four cm long leaf samples were collected six days after inoculation. Three seedlings were sampled per accession. The samples were fixed, cleared and stained for fluorescence microscopy according to the procedure described by Rohringer et al. (15), using Uvitex 2B (Ciba-Geigy) instead of Calcofluor. Fifty infection units were recorded per leaf sample. All infection units with at least one, but no more than five haustorial mother cells were considered early aborted (13). The presence or absence of autofluorescent plant cells, indicative for a hypersensitivity reaction, was recorded as well. The results were averaged over the three leaf segments.

2.5. GENETIC ANALYSIS

2.5.1. Mapping population

Two populations were used to map the loci determining the pre- and posthaustorial resistance. One population consisted of recombinant inbred lines (RILs, in F_5, F_6 or F_7), from the cross *T. monococcum* DV92 (resistant) x G3116 (susceptible), (3). The other population consisted of 224 F_2 plants from the cross *T. boeoticum* 1486 (susceptible) x *T. monococcum* Einkorn (resistant). Both resistant parents are macroscopically immune and have up to 45% early abortion without necrosis and 55% early abortion with necrosis in the seedling stage.

2.5.2. Disease screening

The material was inoculated in first and fifth leaf stage, and all methods and observations were as described before in sections 2.1 to 2.4.

2.5.3. Map construction

RIL population: AFLP markers were developed according to Qi and Lindhout (14), using five PstMse primer combinations. Seventy-seven markers were obtained, and the Joinmap version 3.0 programme (18) resulted in 6 to 10 markers on each of the seven chromosomes. An integrated linkage map was constructed using the RFLP data obtained in the F_2 by Dubcovsky et al (3) or http://wheat.pw.usda.gov/ggpages/maps) as well as the AFLP data scored in the present research. This integrated map served as a basis for QTL detection.

F_2 population: out of the 224 F_2 plants, 118 were selected to serve as mapping population. Especially plants with very high or very low level of prehaustorial resistance were represented, whereas some of the plants with intermediate levels were excluded. In this population AFLP markers were obtained by application of 16 EcoMse and 6 PstMse primer combinations. We obtained 147 markers that were specific for parent *T. boeoticum* 1486, and 126 markers for parent *T. monococcum* Einkorn. These were used to construct a maternal and a paternal map of seven linkage groups, using Joinmap version 3.0 (18). Some linka-

ge groups could be assigned to chromosomes, using 9 AFLP markers that were mapped on the RFLP-map of the RILs, and 3 AFLP markers that were mapped in hexaploid wheat (5). The difficulties inherent to the estimation of recombination between dominant markers in repulsion phase (e.g. 7) and the resulting problems in ordering such markers, has prohibited the integration of the maternal and paternal maps.

2.5.4. QTL mapping

QTL analysis was performed using the interval mapping module of MapQTL4.0 (19) with steps of 5 cM. Any peaks in the LOD profile above a threshold of 3.3 (RILs) and 3.7 (F_2) (17) were confirmed with the multiple QTL mapping (MQM) module of MapQTL4.0, taking the marker nearest to a peak as a cofactor. In addition, the Kruskal-Wallis test was performed with the same software to establish association between individual markers and the scores for infection type.

3. RESULTS

3.1. Frequency of resistance in diploid wheat

The macroscopic evaluation of the 598 accessions indicated clear differences in resistance between the diploid wheat species. All *T. urartu* accessions and all but three *T. boeoticum* accessions were fully susceptible. AFLP fingerprint analysis and morphological characterisation indicated that the three atypically resistant *T. boeoticum* accessions were not true to their taxon, and may have been derived from a cross with *T. monococcum* in the past (1). Most of the *T. monococcum* accessions were fully resistant, 6 accessions segregated for resistance and susceptibility, 24 were susceptible. The accessions that were not uniformly resistant were checked for their identity, and only four of them had typical *T. monococcum* morphology and AFLP fingerprint. The other susceptible *T. monococcum* accessions carried features of *T. boeoticum*, probably by hybridisation in the past (1). Correcting for the accessions that were not true to their taxon (Table I), we conclude that resistance to *P. triticina* in diploid wheat occurs (almost) exclusively in *T. monococcum* s.s. This subspecies is almost a nonhost to *P. triticina*.

Table I. Number of accessions per diploid wheat species that was resistant to the wheat leaf rust isolate Felix in the seedling.

Species	Nr tested*	Resistant	
		nr	%
T. monococcum	156	152	97
T. boeoticum	208	0	0
T. urartu	205	0	0

* Accessions that were intermediate between *T. monococcum* and *T. boeoticum* for AFLP and for morphology, were excluded.

3.2. Frequency of pre- and posthaustorial resistance

In the resistant *T. monococcum* accessions, the level of prehaustorial resistance was quantified by the percentage of infection units that were aborted early, without the induction of plant cell necrosis. Posthaustorial resistance, associated with hypersensitivity, is easily detected by the golden-yellow fluorescence of plant cells, associated with the infection site. The infection units that were arrested posthaustorially could be either early aborted, or could have colonised the leaf to some extent (established colonies associated with plant cell necrosis).

Twenty-nine accessions had all infection units arrested by posthaustorial resistance. In all other accessions at least some infection units were arrested by prehaustorial resistance. In none of the accessions the prehaustorial resistance arrested all infection units. Those infection units that were not arrested pre-haustorially, were stopped sooner or later in their development by posthaustorial resistance. In only three of the 152 resistant *T. monococcum* accessions, the percentage of infection units arrested prehaustorially was higher than 50 %. We conclude that *T. monococcum* accessions with very high levels of prehaustorial resistance occur, but are relatively rare (about 2% of the accessions), and the resistance invariably occurs in association with posthaustorial resistance.

3.3 Genetics of pre- and posthaustorial resistance

In both populations, the pre- and posthaustorial resistance segregated continuously (Fig. 1).

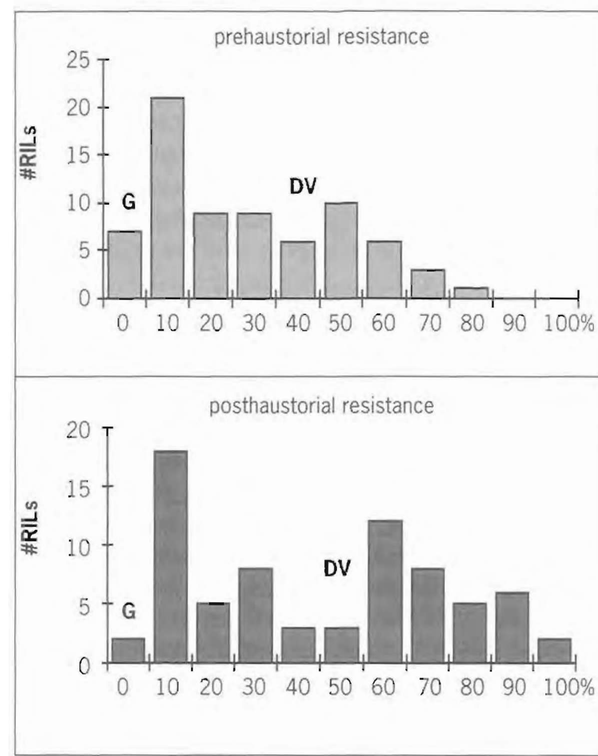

Figure 1. Frequency distribution of prehaustorially and posthaustorially early aborted infection units of *P. triticina* in first leaves of 72 lines derived from DV92 (DV, resistant) x G3116 (G, susceptible).

For the first leaf stage, transgressive segregation was observed for the percentage of prehaustorial early abortion. Ten RILs surpassed the level of resistant of the most resistant parent (Fig 1, upper panel) , and 54 of the F^2 plants. Transgressive segregation also occurred for posthaustorial early abortion, with 21 RILs (Fig 1, lower panel) and 25 F^2 plants surpassing the level of the most resistant parent. In the fifth leaf stage, data were similar, but plants tended to be more resistant.

Pre- and posthaustorial early abortion in the first leaf stage were significantly and positively correlated (r=0.75 for the RIL population; r=0.74 for the F^2 population), and both correlated negatively with the infection type and mean colony size.

One QTL was found that appeared in both populations. This QTL was located on the long arm of chromosome 5, and was significant in the RIL-map, and in both parental F^2 maps. This QTL contributed both to prehaustorial and to posthaustorial resistance. In the same region, the Kruskal-Wallis-test indicated association with the infection type. The position of this QTL is indicated in Fig 2.

In the maternal map of the F^2 population, an additional QTL was found for all infection components, except for mean colony size in the fifth leaf. That QTL mapped on a linkage group that probably corresponds with chromosome 4. Some other putative QTLs were indicated, but did not reach the significance threshold.

3. DISCUSSION

The screening of the about 600 accessions of diploid wheat for resistance against *P. triticina* indicate a qualitative difference between the three taxa, *T. boeoticum* and *T. urartu*. The latter two species do not seem to contain effective resistance genes against the isolate applied in this study, whereas *Triticum monococcum* s.s. is almost uniformly resistant. Our results confirm the report by The (16), who found 110 of 121 accessions of *T. monococcum* resistant to two isolates of *P. triticina*, and 11 with an intermediate reaction. Zitelli (20) reported that all 122 tested were resistant. Neither of these authors mention the other two taxa.

T. monococcum s.l. seems to be a unique case in the plant kingdom, where two perfectly crossable subspecies (*T. monococcum* s.s. and *T. boeoticum*) differ in host status to a pathogen.

It is remarkable, that the qualitative difference between *T. monococcum* s.s. and *T. boeoticum*, is not due to one major gene. The progeny of two crossing combinations segregated quantitatively for the resis-

tance (Fig. 1). This suggests a quantitative inheritance of the resistance. A complex inheritance, based on several genes, is also suggested by the fact that none of the RILs were as susceptible as the susceptible parent.

Some of the genes responsible for this resistance could be mapped. The fact that the QTL with the greatest effect was detected in two crossing populations, derived from different parental pairs, suggests that on a few loci nearly all *T. monococcum* accessions carry the resistance allele.

Each of these alleles would have a quantitative effect, but together they would result in complete resistance.

Another surprise is, that the QTLs that were detected, seemed to be responsible both for pre- and for posthaustorial resistance (Fig. 2). In barley and cultivated hexaploid wheat, genes for both resistance mechanisms represent two classes that map to distinct loci on the respective genomes. In those crops high prehaustorial resistance to leaf rust (*P. hordei* and *P. triticina*, respectively) is not associated with any substantial posthaustorial resistance (11) as we found here in diploid wheat.

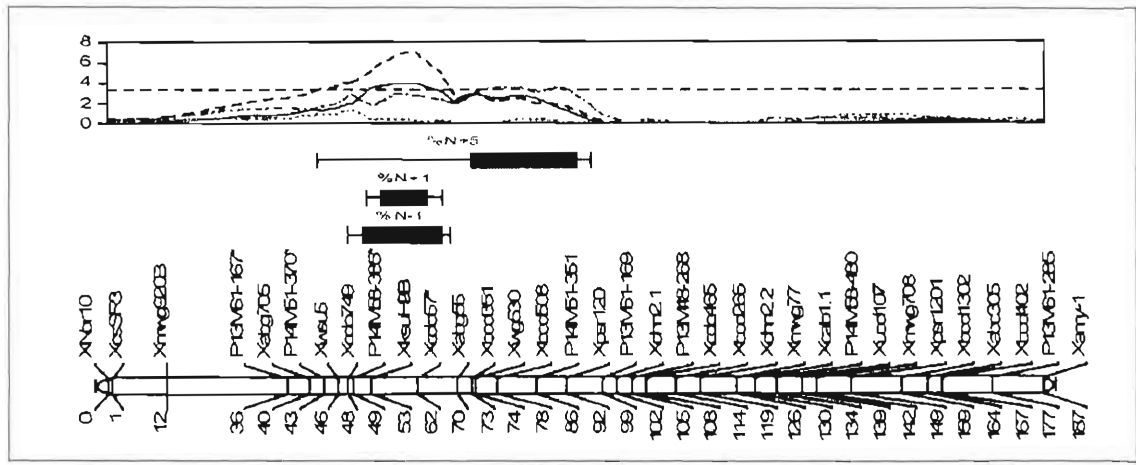

Figure 2. LOD profile for early abortion without (% N-) and with necrosis (%N+) in first (1) and fifth (5) leaf of infection units of *Puccinia triticina* on a molecular marker linkage group representing chromosome 5, constructed on the basis of 72 F_5 lines derived from diploid wheat parents DV92 (resistant) x G3116 (susceptible).

In prehaustorially resistant barley and hexaploid wheat genotypes, infection units that do succeed in the formation of at least one haustorium, hardly ever induce hypersensitivity, but rather develop into sporulating colonies. It seems that in diploid wheat the same gene(s) that hamper haustorium formation, also increase the chance on hypersensitivity after a haustorium is formed. This indicates, that the genes discovered in this study, represent a new class of resistance genes. The quantative inheritance of the resistance, and the high degree of sterility of hybrids between *T. monococcum* and hexaploid wheat complicates the short term transfer of resistance genes from diploid wheat to modern wheat germplasm.

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REFERENCES

[1] Anker CC, Buntjer JP, Niks RE: Morphological and molecular characterization confirm that *Triticum monococcum* s.s. is resistant to wheat leaf rust. Theor Appl Genet: in press.

[2] Dhaliwal HS, Gill KS: Screening and utilisation of wild-wheat germplasm for rust resistance. Wheat Information Service 54: 39-42 (1982).

[3] Dubcovsky J, Luo M-C, Zhong G-Y, Bransteitter R, Desai A, Kilian A, Kleinhofs A, Dvorak J: Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143: 983-999 (1996).

[4] Heath M: A generalized concept of host-parasite specificity. Phytopathology 71:1121-1123 (1981).

[5] Huang X, Zeller FJ, Hsam SLK, Wenzel G, Mohler V: Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. Genome 42: 298-305 (2000).

[6] Kerby K, Kuspira J, Jones BL: Biochemical data bearing on the relationship between the genome of *Triticum urartu* and the A and B genomes of the polyploid wheats. Genome 30: 567-581 (1988).

[7] Knapp SJ, Holloway JL, Bridges WC, Liu BH: Mapping dominant markers using F2 matings. Theor Appl Genet 91: 74-81 (1995).

[8] Littlefield LJ, Aronson SJ: Histological studies of *Melampsora lini* resistance in flax. Can J Bot 47: 1713-1717.

[9] McNeal FH, Konzak CF, Smith EP, Tate WS, Russel TS: A uniform system for recording and processing cereal research data. Agricultural Research Service Bulletin 34-121. United States Department of Agriculture: Washington (1971).

[10] Niks, RE: Haustorium formation by Puccinia hord-ei in leaves of hypersensitive, partially resistant, and nonhost plant genoty-pes. Phytopathology 73: 64-66 (1983).

[11] Niks, RE: Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. Physiol. Mo-lec Plant Pathol. 28: 309-322 (1986).

[12] Niks RE, Dekens RG: Prehaustorial and posthaustorial resistance to wheat leaf rust in diploid wheat seedlings. Phytopathology 81: 847-851 (1991).

[13] Niks RE, Kuiper HJ: Histology of the relation between minor and major genes for resistance of barley to leaf rust. Phytopathology 73: 55-59 (1983).

[14] Qi X, Lindhout P: Development of AFLP markers in barley. Mol Gen Genet 254: 330-336 (1997).

[15] Rohringer R, Kim WK, Samborski DJ, Gundlach AM: Calcofluor: an optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology 67: 808-810 (1977).

[16] The TT: Variability and inheritance studies in *Triticum monococcum* for reaction to *P. graminis* f.sp. *tritici* and *P. recondita*. Z Pflanzenzücht 76: 287-298 (1976).

[17] Van Ooijen JW: LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83: 613-624 (1999).

[18] Van Ooijen JW: Joinmap tm version 3.0: Software for the calculation of genetic linkage maps. *www.plant.wageningen-ur.nl/products/mapping (2000).*

[19] Van Ooijen JW, Maliepaard C: MapQTL tm version 4.0: Software for the calculation of QTL positions on genetic maps. CPRO-DLO (PRI), Wageningen (1996).

[20] <Zitelli G: Role of related species in genetic improvement of cultivated wheats. In: Proceedings of the fifth European and Mediterranean cereal rusts conference, Bari and Rome. Instituto di Patologia Vegetale,Bari, Italy p 95-103 (1980).

GERMAN NETWORK FOR EVALUATION AND USE OF DISEASE RESISTANCE IN CEREALS (EVA II)

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ABSTRACT: EVA II aims to facilitate a more effective use of genetic resources in cereal resistance breeding. Goal of the project is the transfer and spreading of new or unknown resistance genes into (commercial) breeding programs to support sustainability and genetic diversity in modern agriculture. A network of more than 20 private German plant breeders and research institutes jointly evaluates pre-selected germplasm (actual breeding material, domestic or foreign varieties, and gene bank material) of barley and wheat. The nurseries are screened in multi-site field trials for resistance to most important fungal and viral pathogens. Besides vertical resistance, horizontal resistance is searched for with repeated scoring. To characterise infestation conditions, resistant and susceptible standards are included. Virulences of the different locations will be described following laboratory examinations. Commonly used evaluation methods will be refined to be simultaneously applicable to several cereal diseases. Based on field observations, the occurrence of known PCR markers for different pathogens will be verified. An information network for an effective acquisition and provision of the data will be developed by the German Centre for Documentation and Information in Agriculture (ZADI/IGR). The acquired results will be shared first of all amongst the project partners for direct use in the respective breeding programs. The information will become freely accessible in the medium term. It is intended to collect experience and develop expertise to build up similar systems for other species. The Institute of Epidemiology and Resistance (IER, Federal Centre for Breeding Research on Cultivated Plants) co-ordinates the project; there are further associated partners. This pilot project commenced in January 2001 and is funded by the Federal Ministry of Consumer Protection, Food and Agriculture (BMVEL).

1. INTRODUCTION

The fundamental importance of genetic diversity to crop improvement has long been evident to plant breeders, conservators, agronomists, entomologists, and phyto-pathologists. To improve a crop or enhance its resistance to stresses and diseases, plant breeders have relied on the genetic diversity in their working collections, on collections of genetic resources stored in gene banks, and on those varieties maintained and selected by farmers. Today, biotechnology and genetic engineering promise to break the biological barriers imposed on conventional plant breeding by incorporating desirable genes and gene complexes from wild relatives across species (1).

Public concern has focused on the potential for plant disease epidemics caused by uniformity in the genetic base of resistance (2). Many virulence genes in pathogens occur at frequencies correlated to the frequencies of the corresponding resistance genes in the host populations. Specific resistances in such diseases as the cereal rusts contribute to a 'boom-bust' cycle of resistance and vulnerability, because the pathogen is able to mutate rapidly and form new races (3). The average duration of a resistance based on major resistance genes appeared to be only a few years (4). One can distinguish between **vertical**, race-specific, often only temporary resistance, which is mostly monogenic, and **horizontal or partial resistance**, which is race-non-specific, (more) durable, and with a polygenic base. Recently, efforts have been concentrated to enhance the horizontal resistance in crops.

The Federal Centre for Breeding Research on Cultivated Plants (BAZ) investigates the scientific base of the development of high quality and stable disease resistance in food and industrial crop plants. It scientifically supports political and administrative decisions by the Federal Ministry of Consumer Protection, Food and Agriculture (BMVEL). One major task of the Institute of Epidemiology and Resistance (IER) is the evaluation of plant genetic resources to select genotypes with new or improved resistance to important pathogens. Also, the development and optimisation of evaluation methods and resistance tests is pursued.

In the past, huge amounts of evaluation data have been collected for various species, but often data from varying sources or test-conditions cannot be compared. A joint project of several German research institutes led to the compilation of the available German evaluation data and the development of a prototype for an online database (5). A newly established project, EVA II, aims to provide plant breeders with accelerated access to resistant genotypes and thus to support the sustainability of agriculture through an increased genetic diversity in varieties. Overall the goal is a better transfer and dissemination of new resistance genes into (commercial) breeding programs. Therefore, secondary evaluations of barley and wheat are carried out and a network-information system for data acquisition, overview, and provision is in development. EVA II also pursues the refinement of standardised systems for resistance evaluation.

2. MATERIAL AND METHODS

2.1. Material

For both crops (wheat, barley) and both spring and winter forms, sets of a maximum of 100 genotypes are selected. The material is chosen according to promising former disease evaluations. Data bases from gene banks and institutes are used as well as variety lists and relevant publications. The sets consist of actual breeding material mainly from BAZ-Institutes, as well as foreign varieties, and gene bank material. The contributing partners may supply the network with their own breeding material. All tested genotypes should carry new or unknown resistance genes or combinations. At least two standards (susceptible and resistant) for every fungal pathogen are included to characterise the infestation conditions of all sites. Resistance to most important fungal pathogens in cereals (Table I) is evaluated. Additionally, virus resistance is screened at several sites.

Table I. Fungal pathogens screened and standards for experiments.

Pathogen	Standards: resistant	susceptible
Winter wheat		
Powdery mildew[1]	Cortez	Monopol
Drechslera[2]	Dream	Bussard
Fusarium ssp.	Romanus	Darwin
P. triticina[3]	Travix	Dekan
P. striiformis[4]	Cortez	Flair, Mikon
Septoria tritici	Dream	Renan
St. nodorum[5]	Petrus	Monopol
Spring barley		
Powdery mildew[6]	Alexis	Prisma, HOR7226
Drechslera teres	Annabell	Barke, Pasadena, Compana
Puccinia hordei	Barke, Hanka	Alexis, Prisma, L94
Scald[7]	Sissy	Pasadena, Lenka, Annasofie
Winter barley		
Powdery mildew[6]	Verena	Regina, HJ171
Drechslera teres	Camera	Krimhild
Puccinia hordei	Carola	Cornelia, Vg. Gold
Scald[7]	Leonie	Intro, MS Scald

1: *Blumeria graminis* f.sp. *tritici*, 2: *Drechslera tritici-repentis*, 3: *Puccinia triticina*, 4: *Puccinia striiformis*, 5: *Stagonospora nodorum*, 6: *Blumeria graminis* f.sp. *hordei*, 7: *Rhynchosporium secalis*.

Twenty-two private German cereal breeders, mostly organised in the 'German Federation of Private Plant Breeders (GFP)', Bonn, and several research institutes conduct the evaluation trials, score the material, and collect data (Figure 1).

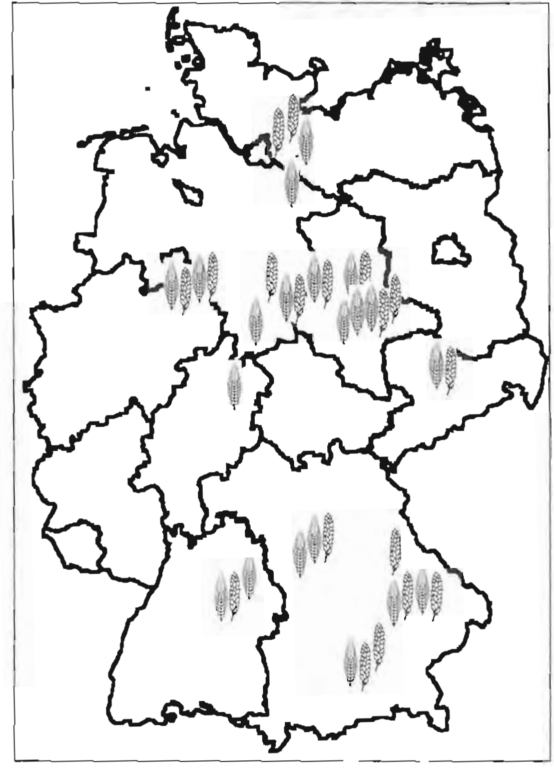

Figure 1: Test sites for resistance evaluation across Germany

Scientists of the following institutions are whether directly involved in the project or indirectly support it with scientific knowledge on the various pathogens, on obtaining, or multiplying material:

- BAZ, Institute of Epidemiology and Resistance, Aschersleben
- ZADI/IGR, German Centre for Documentation and Information in Agriculture, Bonn
- BAZ, Institute of Resistance Research and Pathogen Diagnostics, Aschersleben
- BAZ, Plant Genetic Resource Collection, Braunschweig
- Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Plant Protection in Field Crops and Grassland, Kleinmachnow
- Bundessortenamt (BSA), Hannover
- I nstitute of Plant Genetics and Crop Plant Research (IPK), Gene bank, Gatersleben

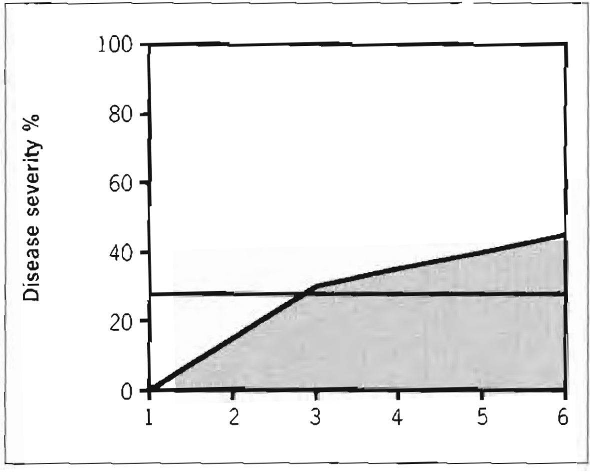

Figure 2: Area under the disease progress curve.

HORDEUM CHILENSE AS A SOURCE OF DISEASE RESISTANCE FOR CEREAL BREEDING

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ABSTRACT *Hordeum chilense* is a South American wild barley with high potential for cereal breeding given its high crossability with other members of the Triticeae. In the present paper we study the resistance of *H. chilense* to several fungal diseases and its perspectives for transference to cultivated cereals.

H. chilense is resistant to the barley, wheat and rye brown rusts, to the barley yellow rust, to the wheat, barley, rye and oat powdery mildew, to Septoria leaf blotch, to common bunt and to loose smuts. There are lines resistant to the wheat yellow rust, to the stem rust and to the *Agropyron leaf* rust, as well as moderate levels of resistance to Septoria glume blotch, tan spot and Fusarium head blight. There are several mechanisms of resistance to brown rusts operative in *H. chilense*. Some lines display a pre-appressorial avoidance mechanism to brown rust fungi. Lines differ in their response to rust and mildew fungi as expressed by the level of resistance to penetration and the frequency of plant-cell necrosis associated with the infection sites

Unfortunately, resistance of *H. chilense* to rust fungi is not expressed in tritordeum, nor in chromosome addition lines in wheat. There is a quantitative contribution of the resistance to wheat powdery mildew, Septoria leaf blotch, tan spot, loose smut and common bunt of *H. chilense* to the resistance of tritordeum. The resistance to mildew is expressed as a reduction of disease severity not associated with increased macroscopically visible necrosis. Hexaploid tritordeums are immune to Septoria leaf blotch and to common bunt although resistance is slightly diluted in octoploid tritordeums. Studies with addition lines on wheat indicate that the resistance of *H. chilense* to these diseases is of broad genetic basis, conferred by genes present on different chromosomes

1. INTRODUCTION

Hordeum chilense Roem. et Schult is a diploid wild barley that occurs exclusively in Chile and Argentina and is extremely polymorphic. After *H. vulgare* and *H. bulbosum*, *H. chilense* is the species of the genus *Hordeum* with the highest potential for cereal breeding purposes, given its high crossability with other members of the Triticeae tribe (*Triticum*, *Hordeum*, *Secale* and *Agropyron*) and its agronomically interesting characteristics (11, 12, 13).

Plant geneticists have been interested in hybridizing barley with wheat for more than 100 years with little success (10). Unfortunately no fertile wheat x *H. vulgare* amphiploids have been produced even after many attempts (5). In contrast, fertile amphiploids with wheat were easily produced using *H. chilense* instead of *H. vulgare.* Hybridisation has also been successful with *Hordeum, Secale and Agropyron* (11, 12, 13). Addition, translocation and substitution lines of *H. chilense* in wheat, and addition lines in rye have been obtained what might be used as bridges to transfer useful traits to cultivated cereals. Potentially, disease resistance is a valuable trait for transfer from *H. chilense*.

2.RESISTANCE TO RUSTS

H. chilense is resistant to the barley and wheat brown rusts (*Puccinia hordei* and *P. triticina*, respectively). *H. chilense* lines may be susceptible to *P. agropyrina*, to wheat and barley yellow rusts (*P. striiformis* ff.spp. *tritici* and *hordei*, respectively) (24) and to the wheat stem rust (*P. graminis*) (Rubiales, unpublishe.

There are several mechanisms of resistance to rusts operative in *H. chilense*. Mechanisms that hamper the ability to find and to penetrate stomata had not previously been documented in close relatives of wheat or barley. We detected however a very low appressorium formation by brown rust fungi on some *H. chilense* lines (23). The poor recognition of the signal triggering the appressorium differentiation over the stomata seems to be due to a prominent wax covering on the stomata opening (25). The trait seems to inherit quantitatively. Three QTL for avoidance have been identified that have been mapped in chromosomes 1, 3' and 5, respectively (32). Morphology and AFLP markers suggest three *H. chilense* ecotypes, one of which has a very high level of avoidance (33). Avoidance has also been detected in other *Hordeum* accessions (*H. brachyantherum*, *H. marinum*, *H. parodii* and *H. secalinum*) but not in *H. vulgare* nor in accessions of the other genera in the Triticeae (30).

The reaction of tritordeum to rust pathogens is very similar to that of the wheat parent, being resistant to the barley brown and yellow rusts. Tritordeum lines might be resistant or susceptible to races of the wheat brown, yellow and stem rusts, and to the agropyron brown rust, depending on the reaction type of the wheat parental line (18, 29, 24). The resistance of *H. chilense* to rusts was neither expressed in chromosome addition lines in wheat (Rubiales, unpublished). The effect of the resistance to rust fungi present in *H. chilense* on the resistance of tritordeum is minimal. However, when screening a large collection in the field over years, disease severities tend to be lower in tritordeum than in wheat for brown rust, but slighly higher for yellow rust. There are indications that the tritordeums from particular *H. chilense* (H7 and H17) lines tend to show lower yellow rust severities than the tritordeums from other *H. chilense* lines (H1 and H12). So, although the *H. chilense* resistance is suppressed by the wheat genome, there might still be a small, but useful, role of the *H. chilense* background in the response of tritordeum (Rubiales, unpublished).

The resistance of *H. chilense* to rust fungi is suppressed by the wheat genome, but not by the rye genome (27) being expressed in a hybrid and an amphiploid with rye as an increased hypersensitivity (reduction in the infection type from 9 to 2). Suppression of rust resistance due to intergenomic interactions has previously been reported in cereals. Suppressors may possibly be eliminated through induction of mutations as has been reported for the 7DL suppressor locus of wheat 'Canthatch' (9) that inhibited resistance to stem rust.

3. RESISTANCE TO POWDERY MILDEW

H. chilense is resistant to the wheat, barley, rye and oat powdery mildew (*Blumeria graminis* ff.spp. *tritici, hordei, secalis* and *avenae*, respectively) (16, 20). A range of different mechanisms appears responsible for resistance to mildew attack. All accessions were highly resistant to all ff.spp. studied. Relatively large differences between accessions were detected in terms of penetration resistance and the frequency with which attacked epidermal cells died. Resistance to penetration of living cells was extremely high in certain accessions (H11, H47, H49 and H51) and it appeared to be expressed more strongly in later-formed, adult plant leaves. Oat and wheat powdery mildews were generally able to penetrate *H. chilense* epidermal cells at a very low frequency whereas barley one penetrated slightly more frequently. In the majority of cases successful penetration led to death of the infected cell and cessation of fungal development. Haustorium formation is relatively uncommon, and when it occurs, most epidermal cells die soon after haustoria are formed (16).

Tritordeum is resistant (infection type 0) to the barley and rye powdery mildew (20, 27). There is a quantitative contribution of the resistance of *H. chilense* to wheat powdery mildew to the resistance of tritordeum (20). Field trials showed that disease severity was lower in tritordeum than in its wheat parent. Detached leaf inoculations with several isolates showed that there was a considerable reduction in the density of mildew colonies in tritordeum although the infection type was that of the wheat parental line. The tetraploid tritordeum had 20x reduction of infection frequency in comparison with its wheat parent; hexaploid tritordeums had a 7-14x reduction; and octoploid tritordeums a 1.5-2.5x reduction.

The resistance of *H. chilense* to wheat powdery mildew was expressed in the addition lines as varying levels of reduction of mildew severity without any visible increase in necrotic responses. Resistant factors were broadly distributed in the *H. chilense* genome, being present in chromosomes $5H^{ch}$, $6H^{ch}$ and $7H^{ch}$. In addition to those, there was factors present in chromosomes $1H^{ch}S$, $2H^{ch}a$, $4H^{ch}$ effective only to isolate H12 (17).

4. RESISTANCE TO SEPTORIA DISEASES

Both *H. chilense* and tritordeum are resistant to *Mycosphaerella graminicola* (anamorph *Septoria tritici*) the causal agent of Septoria leaf blotch (19). Inoculations both in seedlings under controlled conditions and in mature plants in the field indicated that all hexaploid tritordeums were immune whatever their stature and lateness. The seedling test included both durum and bread wheat isolates. Octoploid tritordeums displayed some disease development but considerably less than their respective bread wheat parental line (19).

Resistance is conferred by gene(s) on chromosome $4H^{ch}$ and to a minor extent by genes on chromosomes $5H^{ch}$, $6H^{ch}$ and $7H^{ch}$ (31). This resistance might be particularly valuable when transferred to wheat, particularly to durum wheat where little resistance to *M. graminicola* exists. *H. chilense* and tritordeum may be susceptible to *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*), the causal agent of Septoria glume blotch (22). Nevertheless, seedlings and field experiments showed that some tritordeums were more resistant than their wheat parental lines, although others were not. There was a slight dilution of the resistance at higher ploidy level, but not as clear as for *M. graminicola*. As for *M. graminicola*, tritordeum resistance to *P. nodorum* is associated with lateness but not with tall plant stature. It should therefore be possible to breed short octoploid tritordeum lines with resistance to both septoria diseases. Special efforts will not be needed in hexaploid tritordeums.

5. RESISTANCE TO BUNTS AND SMUTS

H. chilense is highly resistant to common bunt (*Tilletia caries*) (28). This resistance is completely expressed in hexaploid tritordeum, although slightly diluted in octoploid ones (average of 3x reduction in % of infected spikes). Resistance is conferred by gene(s) on chromosome $7H^{ch}$ and to a minor extend by such on chromosome $6H^{ch}$ (21). The resistance of *H. chilense* is partially diluted in octoploid tritordeum and in addition lines in bread wheat. We might expect that the resistance would be better expressed if the chromosome(s) were added to a durum wheat. Thus, the *H. chilense* resistance could be very helpful in durum wheat breeding.

H. chilense is also resistant to Karnal bunt (*T. indica*) and that resistance was also expressed in some tritordeum tritordeum lines (3).

H. chilense is resistant to Loose smuts of wheat and barley (*Ustilago tritici* and *U. nuda*, respectively) (14). There is a quantitative contribution of the resistance in tritordeum (Rubiales, unpublished).

6. RESISTANCE TO FUSARIOSIS

H. chilense is slightly susceptible to *Fusarium culmorum*. Tritordeum as a crop can be regarded as being susceptible to, but some tritordeums were more resistant than their wheat parent (22). The contents of ergosterol (estimates the fungal biomass) and the phytotoxic mycotoxin deoxinivalenol (DON) showed that the level of resistance to colonization by *Fusarium* is on average higher in tritordeum than in wheat. Some *H. chilense* genotypes (H7, H17, H56, H61) enhanced the wheat resistance to *F. culmorum* in its tritordeum offspring, others (H1, H11, H12, H13) did not. As there does not seem to be specialization of resistance for *F. culmorum*, *F. graminearum* or *F. nivale*, we expect a similar response of tritordeum to these other pathogenic fusariums. This is supported by preliminary data on the reaction of a few tritordeum lines to *F. graminearum* (Fedak, pers. comm.).

7. RESISTANCE TO OTHER PATHOGENS

H. chilense is moderately resistant to tan spot of wheat incited by *Pyrenophora tritici-repentis* (anamorph *Drechslera tritici-repentis*). This resistance is expressed in tritordeum with a quantitative reduction of infection (Rubiales, unpublished).

H. chilense is resistant to other diseases such as net blotch incited by *P. teres* (anamorph *D. teres*) and to scald (*Rhynchosporium secalis*) (Rubiales, unpublished) what might be useful in barley and rye breeding. Tritrodeum is resistant to these diseases.

H. chilense is moderately susceptible to Take-all (*Gaeumannomyces graminis*) (8) as well as the only 4 addition lines in wheat that were studied (6) and tritordeum (Rubiales, unpublished).

H. chilense and tritordeum are susceptible to *Pseudocercosporella herpotrichoides* (Rubiales, unpublished).

8 RESISTANCE TO APHIDS AND NEMATODES

H. chilense is resistant to the Columbia root-knot nematode (*Meloidogyne chitwoodi*) (7) and to root-knot nematode (*M. naasi*) (15) but is susceptible to *Heterodera avenae* (Rubiales, unp.). Resistance to *M. naasi* although allowing gall formation severely limited the number of nematodes which could establish within the roots and reduced their reproduction. The resistance was located on the short arm ($1H^{ch}S$) of the chromo-

some $1H^{ch}$. There was a gene dose effect, being the expression of resistance slighly higher in plants disomic for $1H^{ch}$ than in the monosomic ones, and being reduced at the octoploid level in the only 8x tritordeum studied.

Varying levels of resistance to greenbug (*Schizaphis graminum*) have been described in *H. chilense* (2). Both antixenosis, antibiosis and tolerance mechanisms were detected. Different plant characters appeared to prolong aphid developmental time and reduce the length of adult life and total fecundity. Genes with positive effects on antixenosis were located on chromosome $1H^{ch}$, those that prolonged aphid developmental time on chromosomes $5H^{ch}$ and $7H^{ch}$, those that reduced the total fecundity on $4H^{ch}$, those that accounted for host tolerance on $7H^{ch}$ (1).

H. chilense is also known to possess resistance to the aphids *Diuraphis noxia* (4) and *Rhopalosiphum padi* (34). However, it possess little resistance to Hessian fly (*Mayetiola destructor*)(Rubiales, unpublished)

9. CONCLUSION

H. chilense appears as a valuable reservoir of genes for disease resistance that can potentially been exploited in cereal breeding. Some of the resistances described in *H. chilense* seem to be of complex nature.

This broadly based resistance is most desirable in breeding for durable resistance as is likely to be more durable than resistance based on simple genetic control. Although it might be difficult to transfer the multiple genetic factors present in different chromosomes that might be governing for the several resistance mechanisms altogether to an agronomically valuable cultivar, even the transfer of a single factor governing a resistance mechanism different from the commonly used governed by single genes would be of interest in cereal breeding for resistance.

Preappressorial avoidance to rusts and prehaustorial mechanisms of resistance to rusts and mildews are particularly interesting to be transferred to cereal breeding to be used in combination with genes for hypersensitive resistance, enhancing their effects and protecting durability.

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REFERENCES

[1]. Castro AM, Martín A, Martín LM: Location of genes controlling resistance to greenbug (*Schizaphis graminum* Rond.) in *Hordeum chilense*. Plant Breeding 115:335-338 (1996).

[2]. Castro AM, Martín LM, Martín A, Arriaga HO, Tobes N, Almaraz LB: Screening for Greenburg Resistance in *Hordeum chilense* Roem et Schult. Plant Breeding 112: 151-159 (1994).

[3]. Chauhan RS, Singh BM: Resistance to Karnal bunt in *Hordeum chilense* and its amphiploids with *Triticum* species. Euphytica 96: 327-330 (1997).

[4]. Clement SL, Lester DG: Screening wild *Hordeum* species for resistance to Russian wheat aphid. Cereal Res. Comm. 18: 173-177 (1990).

[5]. Fedak G: Intergeneric hybrids with *Hordeum*. In: P.R. Shewry (Ed.), Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology, CAB International, UK, pp. 45-70 (1992).

[6]. Hollins TW, Scott PR: Take-all, pp. 102-103 In Plant Breeding Institute Annual Report 1986.

[7]. Jensen KB, Griffin GD: Resistance of diploid Triticeae species and accessions to the Columbia root-knot nematode *Meloidogyne chitwoodi*. Journal of Nematology 26 (4S):635-639 (1994).

[8]. Jorgensen JH, Jensen HP: Screening of *Hordeum* species for resistance to the take-all fungus, *Gaeumannomyces graminis*. Z. Pflanzenzüchtg. 76:200-203 (1976).

[9]. Kerber ER: Stem rust resistance in 'Canthatch' hexaploid wheat induced by a non-suppressor mutation on chromosome 7DL. Genome 34: 935-939 (1991).

[10]. Kruse A: *Hordeum* x *Triticum* hybrids. Hereditas 73: 157-161 (1973).

[11]. Martín A, Cabrera A, Hernández P, Ramírez MC, Rubiales D: Prospects for the use of *Hordeum chilense* in durum wheat breeding. Options Méditerranéennes 40: 111-115 (2000).

[12]. Martín A, Cubero JI: The use of *Hordeum chilense* in cereal breeding. Cereal Res. Comm. 9: 317-323 (1981).

[13]. Martín A, Martínez-Araque C, Rubiales D, Ballesteros J: Tritordeum: triticale's new brother cereal. *In*: H. Guades-Pinto *et al.*,(ed.), Triticale: Present and Future. Kluwer Academic Publishers, Dordrecht (1996).

[14]. Nielsen J: Reaction of *Hordeum* species to the smut fungi *Ustilago nuda* and *U. tritici.* Can. J. Bot. 65: 2024- 2027 (1987).

[15]. Person-Dedryver F, Jahier J, Miller TE: Assessing the resistance to cereal root-knot nematode, *Meloidogyne naasi* in a wheat line with the added chromosome arm 1HchS of *Hordeum chilense*. J. Genet. & Breed. 44: 291-296 (1990).

[16]. Rubiales D, Carver TWL: Cellular responses of *Hordeum chilense* genotypes to inappropriate formae specialis of the cereals powdery mildew fungus. Can. J. Bot. 78: 1561-1570 (2000).

[17]. Rubiales D, Carver TWL, Martín A: Expression of resistance to *Blumeria graminis* f.sp. *tritici* in 'Chinese Spring' wheat addition lines containing chromosomes from *Hordeum vulgare* and *H. chilense*. Hereditas 134: 53-57 (2001).

[18]. Rubiales D, Ballesteros J, Martin A: The reaction of X *Tritordeum* and its *Triticum* spp. and *Hordeum chilense* parents to rust diseases. Euphytica 54: 75-81 (1991).

[19]. Rubiales D, Ballesteros J, Martín A: Resistance to *Septori tritici* in *Hordeum chilense* x *Triticum* spp. amphiploids. Plant Breeding 108: 281-286 (1992).

[20]. Rubiales D, Brown JKM, Martín A: *Hordeum chilense* resistance to powdery mildew and its potential use in cereal breeding. Euphytica 67: 215-220 (1993).

[21]. Rubiales D, Martín A: Chromosomal location in *Hordeum chilense* of resistance to common bunt. Euphytica 109: 157-159 (1999).

[22]. Rubiales D, Nicholson P, Snijders CHA, Martín A: Reaction of tritordeum to *Septoria nodorum* and *Fusarium culmorum*. Euphytica, 88:165-174 (1996).

[23]. Rubiales D, Niks RE: Low appressorium formation by rust fungi on *Hordeum chilense* lines. Phytopathol. 82: 1007-1012 (1992).

[24]. Rubiales D, Niks RE: Histological responses in *Hordeum chilense* to brown and yellow rust fungi. Plant Pathol. 41: 611-617 (1992).

[25]. Rubiales D, Niks RE: Avoidance of rust infection by some genotypes of *Hordeum chilense* due to their relative inability to induce the formation of appressoria. Physiol. Mol. Pl. Pathol. 49: 89-101 (1996).

[26]. Rubiales D, Niks RE, Dekens RG, Martín A: Histology of the infection of tritordeum and its parents by cereal brown rust. Plant Pathology 42: 93-99 (1993).

[27]. Rubiales D, Niks RE, Martín A: Genomic interactions in the resistance to mildew and rust fungi in hybrids and amphiploids involving the genera *Triticum*, *Hordeum* and *Secale*. Cereal Res. Comm. 21: 187-194 (1993).

[28]. Rubiales D, Ramírez MC, Martín A: Resistance to common bunt in *Hordeum chilense* x *Triticum* spp. amphiploids. Plant Breeding 115:416-418 (1996).

[29]. Rubiales D, Ramírez MC, Niks RE: The contribution of *Hordeum chilense* to Partial Resistance of Tritordeum to wheat brown rust. Euphytica 59: 129-133 (1992).

[30]. Rubiales D, Ramírez MC, Niks RE: Avoidance to leaf rust fungi in wild relatives of cultivated cereals. Euphytica, 87:1-6 (1996).

[31]. Rubiales D, Reader SM, Martín A: Chromosomal location of resistance to *Septoria tritici* in *Hordeum chilense* determined by the study of chromosomal addition and substitution lines in 'Chinese Spring' wheat. Euphytica 115: 221-224 (2000).

[32]. Vaz Patto MC: The genetics and mechanism of avoidance of rust infection in *Hordeum chilense*. PhD Thesis, Wageningen Agricultural University (2001).

[33]. Vaz Patto MC, Aardse A, Buntjer J, Rubiales D, Martín A, Niks RE: Morphology and AFLP markers suggest three in *Hordeum chilense* ecotypes that differ in avoidance to rust fungi. Can. J. Bot. 79: 204-213 (2001).

[34]. Weibull J: Screening for resistance againts *Rhopalosiphum padi* (L): *Hordeum* species and interspecific hybrids. Euphytica 36: 571-57 (1987).

A GENE FOR THE B SUBUNIT OF V-ATPASE IS DIFFERENTIALLY EXPRESSED BETWEEN TWO CLOSELY RELATED BARLEY CULTIVARS IN RESPONSE TO SALINITY

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ABSTRACT A barley (*Hordeum vulgare* L.) gene encoding vacuolar ATPase subunit B was differentially expressed between two near isogenic cultivars, Golden Promise and Maythorpe. This gene (BSVAP) was isolated by the mRNA differential display technique. BSVAP was salt inducible under long term salinity stress in the salt-sensitive cultivar Maythorpe but less so in the relative salt-tolerant Golden Promise and was more highly expressed under control conditions in Maythorpe. The physiological consequences of altered V-ATPase expression are discussed in relation to the salt sensitivity of Maythorpe.
Key Words: barley, vacuolar ATPase, salt-stress

Investigations of gene expression induced by salt-stress are an increasingly important aspect in the understanding of salt tolerance mechanisms in plants. Many molecular analyses of salt tolerance have been conducted with either suspension cultured cell lines of genetically unrelated cultivars. Hence there are limitations in understanding salt-tolerance mechanisms for individual cells when applied to the whole plant and particularly because of the differences in genetic backgrounds between cultivars and species (1).

Two near isogenic barley cultivars Golden Promise and Maythorpe were used in this study. Golden Promise was produced by gamma-ray irradiation of Maythorpe. The former cultivar possesses a unique combination of highly desirable agronomic characters such as earliness of ripening, short, stiff straw, and good malting potential. Golden Promise was also found to be salt tolerant compared to its parent Maythorpe (2, 3). It maintained significantly lower Na^+ concentration, higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in young tissues than Maythorpe (4). Golden Promise carries the mutated gene, *ari-e*.GP which confers semi-dwarf stature and which may also be involved in enhanced salt tolerance (3). These barley cultivars therefore provide an attractive model system for the investigation of salt tolerance in two genetically related cultivars of a major crop plant.

The present study investigates differential gene expression between the two near isogenic barley cultivars under salt-stress with the mRNA differential display technique.

1. MATERIALS AND METHODS

1.1. Plant material

Seeds of Golden Promise (GP) and Maythorpe (M) were germinated in Petri dishes and after three days single grains were transferred into 10 x 1.5cm diameter plastic tubes partially filled with vermiculite with a hole cut in the bottom to allow roots to grow out into the hydroponics solution. One seed was placed in each tube and four tubes were placed in a black tray containing 2.5 litres of distilled water for 5 days. Trays were arranged in a randomised block design with three replicates per treatment. Seedlings were transferred to half-strength Hoagland and Arnon nutrient solution for 5 days and then to full-strength Hoagland and Arnon nutrient solution for a further 5 days. The treatments were: 0 and 150 mM NaCl for one day, 150 mM NaCl for four weeks. For long term stress treatment salt was added in daily increments of 25 mM until the final concentration of 150 mM NaCl reached. Plants were cultured in a growth cabinet under the follo-

wing growth conditions: a temperature maintained between 15 and 25 ^{o}C, light intensity of 300 mmol m^{-2} s^{-1} and 16 hour photoperiod. The nutrient solution was continuously aerated and changed weekly. Young leaf blades were harvested and were shock frozen in liquid nitrogen prior to storage at -70°C. These samples were prepared for mRNA differential display.

1.2. mRNA Reverse Transcription and Differential Display

Plant material was ground in liquid nitrogen with a pestle and mortar and the resulting powder was extracted with S.N.A.P™ Total RNA Isolation Kit (Invitrogen), according to the manufacturer's instructions. mRNA differential display was based on Liang and Pardee (5) with random and anchored poly-T primers (5'-TGGATTGGTC/T_{12}AG). Thirty cycles of polymerase chain reactions (PCR) were applied with steps of 1 minute at 94 °C, 2 minutes at 40 °C, 2 minutes at 72 °C, followed by a final extension step at 72 °C for 10 minutes. Differentially expressed transcripts were directly visualised via autoratiography of polyacrylamide gels carrying RT-PCR products of Golden Promise and Maythorpe. The differential cDNA fragments were cloned into pGEM-T Easy vector (Promega) and transformed into *Escherichia coli* DH5a competent cells. Plasmid preparations from fifteen white colonies were screened by PCR for the differential cDNA fragment.

1.3. Southern and Northern blot analysis

The differential expression of target clones identified on polyacrylamide gels was checked both indirectly via Southern (slot) blots of reverse transcribed mRNA and directly via Northern blots. Southern (slot) blot analysis was carried out to confirm the positive cDNA fragment before sequencing. 0.4mg total RNA of each sample was reverse transcribed into cDNA in a reaction volume of 50 ml and 15 ml was used for PCR. Then 20 ml of the PCR product was separated on a 1% agarose gel and transblotted to the Hybond-N nylon filters (Amersham) by capillary transfer using paper towels. cDNA probes were ^{32}P-labelled with random primers (Promega). The hybridization procedures were conducted using the method of Sambrook *et al.* (6).

Northern blot analysis was carried out according to Sambrook *et al.* (6). Thirty micrograms of total RNA was separated on 0.7% (w/v) denaturing agarose gels containing 6% (w/v) formaldehyde and 1¥ MOPS [3(N-morpholino)-propanesulphonic acid] buffer. RNA molecules were then transblotted to the Hybond-N nylon filters (Amersham) by capillary transfer using paper towels. cDNA probes were ^{32}P-labelled with random primers (Promega) and used for hybridization with RNA blot in the 50% (v/v) formamide, 5¥SSC, 5¥ Denhardt's solution, 0.5% (w/v) SDS, and 0.1 mg ml^{-1} denatured Semon sperm DNA (S. P. DNA) for 18 h. The blot was washed in 1¥SSC and 0.1% SDS for 20 min at room temperature, three washes in 0.2¥SSC and 0.1% SDS for 20 min each at 68 ^{o}C and X-ray film was exposed for 2 days at -70 ^{o}C.

1.4. DNA Sequence analysis

Plasmid DNA (pGEM-T) containing the cloned differential cDNA fragments was purified by Wizard® Midipreps DNA purification system (Promega) and 30 mg purified plasmid DNA was sequenced by MWG-BIOTECH.

Analysis of the DNA sequences was carried out with the BLAST search facility in the GenBank database.

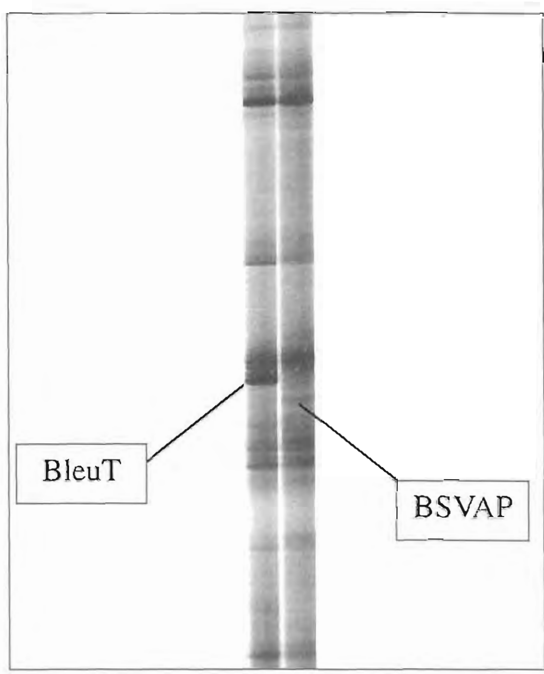
2. RESULTS

2.1. Differential band

Using the reverse transcription and differential display techniques, part of a polyacrylamide gel of Golden Promise and Maythorpe grown with different treatments is shown in Figure 1. Most of the amplified cDNA fragments were similar between the two cultivars - one of the differential bands was labelled as BSVAP (barley salt inducible vacuolar ATPase). This fragment was expressed in the young leaves of Maythorpe (M) under control (0 mM NaCl) conditions, but was not highly expressed in Golden Promise (GP) and other treatments.

Northern blot analysis showed that the BSVAP fragment was hybridised to a RNA transcript, which was around 1,900 bp in size and again varied in expression between Golden Promise and Maythorpe in the different salt treatments (Figure 2). Compared to Golden Promise, the transcript of BSVAP was over-expressed in Maythorpe in control conditions (0mM NaCl) (lanes 1 & 2), and it was also over-expressed in Maythorpe under 4 weeks salt-stress with 150 mM NaCl (lanes 3 & 4). However, there was no obvious difference in the expression of BSVAP between Golden Promise and Maythorpe under short-term salt-stress with 1 day in 150 mM NaCl. The expression of the BSVAP transcript was not affected by salinity in the salt tolerant cultivar Golden Promise both under long and short-term salt-stress when compared to its control treatment (Figure 2).

Figure 1: The result of RT-DD showing part of a polyacrylamide gel. Left lane: GP 0 mM NaCl; right lane: M 0 mM NaCl. BSVAP represents barley salt inducible V-ATPase; BleuT2 represents barley leucine zipper transcript 2, which is the subject of another paper.



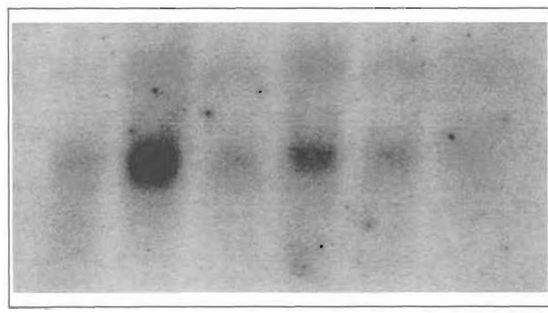


Figure 2: Autoradiograph of Northern hybridizations with the BSVAP probe on total RNA of the leaves of GP and M. Lanes 1 and 2 are 0 mM NaCl; lanes 3 and 4 are 150 mM NaCl for 4 weeks; lanes 5 and 6 are 150 mM NaCl for 1 day. The odd lanes represent Golden Promise and the even lanes represent Maythorpe.

2.2. DNA SEQUENCE ANALYSIS

The sequence of BSVAP (GenBank Acc-No: BF718809) comprising 218 bases was put into GenBank to conduct homology searches with BLAST. From the EST database, there were a large number of EST sequences with homology to BSVAP, the best four are listed in Table I. The first three ESTs (AL502302, AL501329 and AL502245) are all from barley (*Hordeum vulgare*), the identities are 188/189, 188/189 and 180/181 separately, the homology is 99%. The fourth EST was from wheat (*Triticium aestivum*) with 90% similarity i.e. 166/183 bp.

Example alignment searching results from the DNA database are presented in Table II. The differential sequence BSVAP was 98% homologous to the *Hordeum vulgare* vacuolar ATPase B subunit (L11862)(13), the identity is 196/198, the detailed alignment is shown in Figure 3. Also from Table II, the BSVAP transcript was highly homologous to the rice (*Oryza sativa*) vacuolar ATPase B gene (AF375052, AB055106), the identities being 62/71 (87%) and 41/46 (89%).

Table I. Homology of BSVAP to ESTs in GenBank

GenBank Accession Number	EST	alignment
AL502302	*Hordeum vulgare* barley roots *Hordeum vulgare* cDNA clone HW07C06u 3'	Identity=188/189 (99%)
AL501329	*Hordeum vulgare* barley roots *Hordeum vulgare* cDNA clone HW03N09u 3'	Identity=188/189 (99%)
AL502245	*Hordeum vulgare* barley roots *Hordeum vulgare* cDNA clone HW06P08u 3'	dentity=180/181 (99%)
BE517601	Wheat ABA-treated embryo cDNA library *Triticuma estivum* cDNA clone WHE0628_B12_D24.	Identity=166/183 (90%) Gaps=7/183 (3%)

Table II. Homology of BSVAP to genes in the GenBank DNA database

GenBank Accession Number	DNA	Identity
L11862	*Hordeum vulgare* vacuolar ATPase B subunit isoform mRNA	196/198 (98%)
AF375052 41/46 (89%)	*Oryza sativa* vacuolar ATPase B subunit mRNA	62/71 (87%)
AB055106	*Oryza sativa* V-ATPase B gene for vacuolar ATPase B subunit	62/71 (87%) 41/46 (89%)

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Query: 5       ttggtcatagcgtcttttgtaatattgtctcgtgtaatatcacctcctgtattgttgaac   64
Sbjct: 1699    ttggtcatggcgtcttttgtaatattgtctcgtgtaatatcacctcctgtattgttgaac   1758

Query: 65      caaagcacttgagctctttttggttccacttcccagaactgttagcaagagcatcctag   124
Sbjct: 1759    caaagcacttgagctctttttggttccacttcccagaactgttagcaagagcatcctag   1818

Query: 125     gccaagatgctcgaacacgctggttgtatctacttttgtgtatgaataaggatggggcaa   184
Sbjct: 1819    gccaagatgctcgaacacgctggttgtatctacttttgtgtatgaataaagatggggcaa   1878

Query: 185     taaaccgtgctggactgc   202
Sbjct: 1879    taaaccgtgctggactgc   1896
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Figure 3: The alignment of BSVAP (Query) to L11862 (Sbjct), *Hordeum vulgare* V-ATPase B subunit.

Because BSVAP and L11862 were both from the same plant species with 98% homology at the DNA level, there is a theoretically high probability that these two transcripts are from the same gene. Because only a partial sequence of a gene can be cloned using the RT-DD technique, the L11862 sequence was used to search in GenBank to further confirm the function of BSVAP. The best matches of the searches based on homology are presented in Table III. It is clear that the same functional gene, vacuolar ATPase B subunit was picked up although the plant species are different including *Oryza sativa* (AF375052), *Hordeum vulgare* (L11873), *Gossypium hirsutum* (U07052), *Arabidopsis thaliana* (J04185) and *Nicotiana tabacum* (AF220611).

Table III. Homology of *Hordeum vulgare* vacuolar ATPase B subunit (L11862) to the genes in the GenBank DNA database

GenBank Accession Number	DNA	alignment
AF375052	*Oryza sativa* vacuolar ATPase B subunit mRNA	Identities = 1349/1473 (91%), Gaps = 6/1473 (0%)
L11873	*Hordeum vulgare* vacuolar ATPase B subunit mRNA	Identities = 1281/1462 (87%), Gaps = 1/1462 (0%)
U07052	*Gossypium hirsutum* vacuolar H^+-ATPase subunit B mRNA	Identities = 690/831 (83%)
J04185	*Arabidopsis thaliana* nucleotide-binding subunit of vacuolar ATPase mRNA	Identities = 683/824 (82%)
AF220611	*Nicotiana tabacum* vacuolar H^+-ATPase B subunit mRNA	Identities = 661/806 (82%)

3. DISCUSSION

The RT-DD technique successfully identified differential expression of a gene transcript (BSVAP), which was overe-xpressed under control and salt treatments in Maythorpe compared to the relatively salt tolerant Golden Promise. With 98% homology (196/198 bp) to the *Hordeum vulgare* vacuolar ATPase B subunit gene at the DNA level, the result indicated that BSVAP is a part of the vacuolar ATPase B gene.

V-ATPase plays an important role in generating the electrochemical proton gradient across the tonoplast membrane, which can provide the driving force for the secondary transport of other ions and metabolites (7). In barley (*Hordeum vulgare* L.) roots this enzyme may be involved in the sequestration of Na^+ and Ca^{2+} ions into the vacuole, because the proton gradient produced by the ATPase is used by Na^+/H^+ and Ca^{2+}/H^+ antiporters to drive the uptake of Na^+ and of Ca^{2+} (8, 9). V-ATPases have three major subunits A, B, and C. The subunit B contains a nucleotide-binding site (10, 11), and this subunit is considered to have a regulatory function. From Northern blot analysis, the V-ATPase subunit B (BSVAP) was overexpressed in Maythorpe compared to Golden Promise under control conditions. The mechanism for the different expression between Maythorpe and Golden Promise under normal growth conditions was not known. A possible reason could be that Maythorpe is a tall plant with a large leaf area, which undergoes rapid cell expansion requiring large amount of water and ions to partition into the vacuole. Hence high expression of V-ATPase genes may be essential to maintain osmotic balance inside the cell. Greater transpiration flow in Maythorpe may deliver greater concentrations of potentially toxic ions to leaves which require sequestration into vacuoles indirectly via enhanced V-ATPase activity. This indicates that the BSVAP gene could play an important role in the growth, development and response to salinity of Maythorpe. It was reported that enhanced expression of the vacuolar H^+-ATPase has been associated with increased meristem activity in *Arabidopsis thaliana* (12). Under short-term salt-stress the expression of BSVAP was very low in Maythorpe. This may be caused by Na^+ ions flowing into the vacuole in a short period resulting in disturbance of the electrochemical gradient across the tonoplast membrane. As a result the disturbed membrane electrochemical gradient would cause the reduction of the expression of BSVAP. Under long-term salt stress, the expression of BSVAP in Maythorpe young leaves was stimulated by salinity, but to the same level as under control conditions. Young leaf and shoot Na^+ accumulation was significantly higher in Maythorpe than the corresponding tissues of Golden Promise (4, 13). Hence the down-regulation of BSVAP under high salinity could be an important character of salt sensitivity of Maythorpe. Down regulation of V-ATPase can also be caused by other factors, Viereck *et al.* (14) suggested that light induced down regulation of V-ATPase genes in *Zea mays*. In contrast, BSVAP was expressed to very low level in the leaves of the salt tolerant Golden Promise and was not inducible by salinity. The reduction of the expression of this gene under salinity stress could be an important mechanism contributing to increased salt sensitivity of Maythorpe when compared to Golden Promise.

4. REFERENCE

[1] Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ: Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:463-499 (2000).

[2] Forster BP, Pakniyat H, Macaulay M, Matheson W, Phillips MS, Thomas WTB, Powell W: Variation in the leaf sodium content of the *Hordeum vulgare* (barley) cultivar Maythorpe and its derived mutant cv. Golden Promise. Heredity 73:249-253 (1994)

[3] Pakniyat H, Thomas WTB, Caligari PDS, Forster BP: Comparison of salt tolerance of *GPert* and non-*GPert* barleys. Plant Breeding 116:189-191(1997).

[4] Wei WX, Bilsborrow PE, Hooley P, Fincham DA, Lombi E, Forster BP: Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise. J. Exp. Bot. In press.

[5] Liang P, Pardee AB: Differential display of eukaryotic mRNA by means of the polymerase chain reaction. Science (Washington DC) 257:967-971 (1992).

[6] Sambrook J, Fritsch EF, Maniatis TA: *Molecular cloning*, A laboratory manual. 2nd Edition. old Spring Harbor Laboratory, Cold Spring Harbor, New York, (1989).

[7] Barkla BJ, Vera-Estrella R, Maldonado-Gama M, Pantoja O: Abscisic acid induction of vacuolar H^+-ATPase activity in *Mesembryanthemum crystallinum* is developmentally regulated. Plant Physiol. 120:811-820 (1999).

[8] Taiz L: The plant vacuole. J. Exp. Biol. 172: 113-122 (1992).

[9] Garbarino J, DuPont FM: Rapid induction of Na^+/H^+ exchange activity in barley root tonoplast. Plant Physiol. 89: 1-4 (1989).

[10] DuPont FM, Bush DS, Windle JJ, Jones RL: Calcium and proton transport in vesicles from barley roots. Plant Physiol. 94: 179-188 (1990).

[11] Manolson MF, Rea PA, Poole RJ: Identification of 3-*O*-(4-benzoyl)benzoyladenosine 5'-triphosphate and *N,N'*-dicyclohexylcarbodiimide-binding subunits of higher plant H^+-translocating tonoplast ATPase. J. Biological Chemistry 260: 12273-12279 (1985).

[12] Manolson MF, Quellette BFF, Filion M, Poole RJ: cDNA sequence and homologies of the "57 kDa" nucleotide-binding subunit of the vacuolar ATPase from *Arabidopsis*. J. Biological Chemistry 263: 17987-17994 (1988).

[13] Forster BP, Pakniyat H, Macaulay M, Matheson W, Phillips MS, Thomas WTB, Powell W: Variation in the leaf sodium content of the *Hordeum vulgare* (barley) cultivar Maythorpe and its derived mutant cv. Golden Promise. Heredity 73:249-253 (1994).

[14] Viereck R, Kirsch M, Low R, Rausch T: Down-regulation of plant V-type H^+-ATPase genes after light-induced inhibition of growth. FEBS Letters 384:285-288 (1996).

CHARACTARIZATION OF RESISTANCE TO THE FUNGUS *MYCOSPHAERELLA GRAMINICOLA* CAUSING SEPTORIA TRITICI BLOTCH OF WHEAT WITH MOLECULAR MARKERS

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ABSTRACT: Resistance to the fungal wheat pathogen *Mycosphaerella graminicola* (anamorph *Septoria tritici*) was studied in a cross between the resistant cultivar Senat and the susceptible cultivar Savannah. From the F1 generation of the cross a doubled haploid population was produced, and the progeny lines and parents were tested in growth chamber and field with isolates of *M. graminicola*. In growth chamber Senat was specifically resistant to the Dutch isolate IPO323 and the Danish isolate Risø97-86, whereas Savannah was susceptible to both isolates. Segregation of resistance in the progeny population to IPO323 was in agreement to a 1:1 ratio and segregation of resistance to Risø97-86 followed a 1:3 ratio, of resistant to susceptible lines. The IPO323 resistance gene had to be present in a line for expression of resistance to Risø97-86, whereas the opposite was not the case. Using Bulked Segregant Analysis two microsatellite markers linked to the gene conferring resistance to isolate IPO323 was identified. The microsatellites, *Xwmc50* and *Xwmc60*, were linked 15.4 and 14.6 cM respectively from the resistance gene locus, on chromosome 3A. The parents and progeny lines were grown in a field experiment and inoculated with a mixture of 11 *M. graminicola* isolates, that had been shown to be virulent on Senat in growth chamber tests. A preliminary QTL analysis on the field data showed that both the resistance gene locus and the microsatellite markers had a significant effect on resistance to the inoculated *M. graminicola* population. The highest level of significance was found for the resistance gene locus, demonstrating an effect of a specific resistance gene against a virulent pathogen population in the field.

1. INTRODUCTION

Septoria tritici blotch is an important fungal disease of wheat, caused by the ascomycete *Mycosphaerella graminicola* (anamorph *Septoria tritici*). In Europe this disease has become of increasing importance over the last 20 years. As a factor contributing to this, increased susceptibility of the cultivars grown has been suggested (3). The importance of the disease combined with the increasing awareness of the potential environmentally damaging effects of fungicide use, makes resistance to *M. graminicola* an important breeding aim.

Resistance to *M. graminicola* has been reported to be controlled genetically by single dominant genes, as well as recessive genes, and additive modes of inheritance have also been found (15,18,20,21). Few examples of major single gene resistances to *M. graminicola* used in breeding exist (7). Four genes for resistance to *M. graminicola* have been described and named *Stb1*, *Stb2*, *Stb3* and *Stb4* (18,20). The chromosomal location of these genes has not been determined. In a recent study several chromosomes in two wheat cultivars, a synthetic hexaploid wheat and a *Triticum spelta* genotype were reported to carry resistance genes to two Argentinean *M. graminicola* isolates (17). The biggest effect on resistance was associated with chromosome 7D in the synthetic hexaploid, but genes on several chromosomes had an effect on resistance to the two isolates. In another study, chromosomes 3A and 7D were identified as carrying genes for resistance to two Dutch *M. graminicola* isolates (1).

In wheat breeding there is an increasing interest in using molecular markers to aid in selecting for traits, which can otherwise be difficult to select for. Resistance to *M. graminicola* is a difficult trait, because the disease is highly dependent on environmental conditions (16), and therefore selection is not likely to be possible in every year. An important part of identifying a new *M. graminicola* resistance gene is thus to establish the location of the gene on the chromosomes, and develop a molecular marker that can be used for screening of breeding lines. The objective of this study was to provide molecular markers for resistance to *M. graminicola*, for application in wheat breeding. The approach taken was mapping of resistance genes effective toward single isolates in growth chamber tests, combined with QTL analysis on field data.

2. MATERIALS AND METHODS

2.1. Plant material

Using the wheat¥maize method, a population of 76 doubled haploid lines was produced from the F1 generation of a cross between the winter wheat cultivars Senat and Savannah. The cultivar Senat possesses qualitative resistance and a high level of quantitative resistance in the field to *M. graminicola*, whereas the cultivar Savannah is highly susceptible to *M. graminicola*.

2.2. Growth chamber test

Test of parents and progeny with single spore isolates was performed in the growth chamber. Ten seeds were sown in a line next to a net. After ten days the fully developed primary leaves were placed in horizontal position by fixation to the net with a piece of string, and with the adaxial side of the leaves facing upwards. Inoculum of *M. graminicola* was produced by growing the fungus in liquid yeast glucose medium for 5 to 8 days. The seedlings were inoculated by applying a spore suspension of 10^6 spores/ml to the horizontally fixed leaves. The pots were subsequently kept at high humidity in bags for 48 hours. Assessment of disease severity as percent coverage with necroses and percent coverage with the asexual fruit bodies of the fungus, pycnidia, was performed 21-22 days after inoculation.

2.3. Field experiment

A field experiment was established with the parents and lines of the progeny population grown in two rows of 1 m. Due to lack of seed only 71 of the lines were included and replicates were only possible for half the lines of which three replicates were sown. The experiment was inoculated twice after the flag leaves were fully expanded with a mixture of 11 *M. graminicola* isolates. These isolates had previously been shown to be virulent on Senat, when tested in growth chamber. Disease severity was assessed as percent coverage of flag leaves with lesions bearing the fruit bodies of *M. graminicola*. This field experiment will be replicated on two locations in the 2001/02 season.

2.4. Molecular marker analysis

DNA of parents and progeny was extracted using the CTAB method. The molecular markers used were microsatellite markers developed by the Wheat Microsatellite Consortium (WMC). Clones containing microsatellites from a microsatellite enriched genomic library (6) were sequenced by members of the consortium and PCR primers were designed by Agrogene.

PCR reactions were performed on a Perkin Elmer 9700 thermal cycler. Cycling conditions were, 1 min. denaturation at 94°C, 1 min. annealing at 50, 55 or 60°C (depending on the microsatellite) and 1 min. extension at 72°C, for 31 cycles. The samples were initially denatured for 5 min. and the cycling was followed by an extension step of 7 min. The PCR reaction mix contained 0.25 mM of each primer, 1.5 mM $MgCl^2$, 200 mM of each deoxynucleotide, 1¥ Taq buffer, 0.5 unit Taq polymerase and 50 ng of template DNA, in a reaction volume of 10 ml. The fragment length of amplified microsatellites was determined on an ABI377 automatic DNA sequencer, using fluorescently labelled primers.

Bulked segregant analysis (BSA) (14) was performed to isolate polymorphic markers. Markers showing polymorphism in the BSA analysis were tested on the whole population, and linkage to the resistance gene was calculated using the program Mapmaker version 3.0 (13). QTL analysis was performed on field data using the nonparametric mapping procedure of the program MapQTL version 3.0 (19).

3. RESULTS

3.1. Growth chamber test

In total 16 *M. graminicola* isolates collected in Denmark and one Dutch isolate IPO323, obtained from G.H.J. Kema (Plant Research International, Wageningen, The Netherlands), were tested on Savannah and Senat in growth chamber. Five isolates were avirulent on Senat and all 17 isolates were virulent on

Savannah. Two of the avirulent isolates IPO323 and Risø97-86 gave a particularly good differentiation between Savannah and Senat. These two isolates were found to differ in their reaction on the cultivar Flame, IPO323 being avirulent and Risø97-86 being virulent (Fig. 1). IPO323 and Risø97-86 were tested on the progeny of the cross Savannah¥Senat (Fig. 2A; 2B). Lines with coverage of necroses of less than 40% in growth chamber tests were classified as resistant. However, some lines with less than 40% necroses had pycnidial coverage of more than a few percent, these lines were considered susceptible. Some lines with slightly more than 40% necroses produced no pycnidia. These lines were rated as resistant. Segregation for resistance to IPO323 was in agreement to a 1:1 ratio, and resistance to Risø97-86 did not differ significantly from a 1:3 ratio of resistant to susceptible lines (Table I). The segregation data suggested, that one gene is responsible for resistance to IPO323, and that two complementary genes are responsible for resistance to Risø97-86. Lines resistant to Risø97-86 were also resistant to IPO323, whereas lines resistant to IPO323 were not necessarily resistant to Risø97-86.

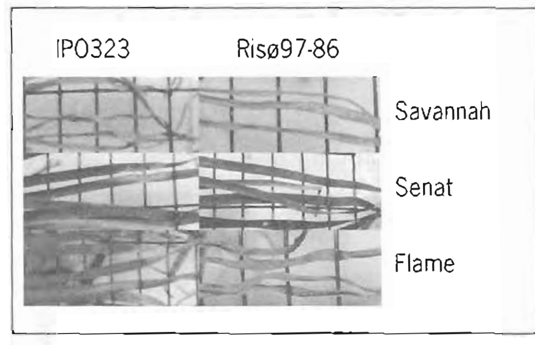


Figure 1. The cultivars Savannah, Senat and Flame inoculated with *Mycosphaerella graminicola* isolate IPO323 and Risø97-86 in growth chamber.

Table I. Segregation, based on growth chamber experiments, of specific resistance to the *Mycosphaerella graminicola* isolates IPO323 and Risø97-86 in the doubled haploid population from the cross Savannah¥Senat. Deviation from the expected segregation ratio was tested with the chi-square test (χ^2). NS = not significant.

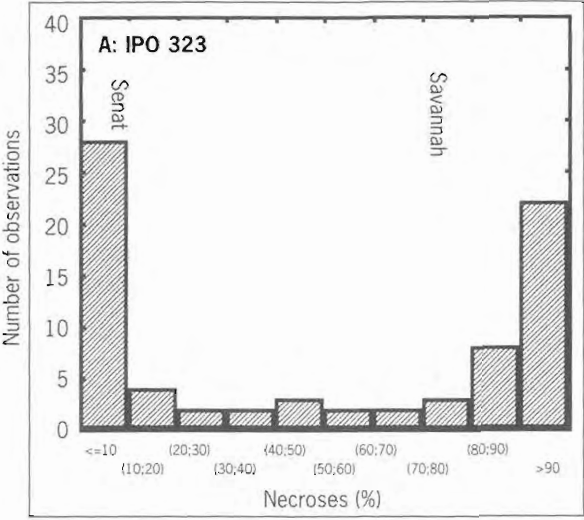
Isolate	Observed number		Expected	χ^2 (P)
	Resistant	Susceptible	ratio	
IPO323	36	40	1:1	0.64^{NS}
Risø97-86	16	59	1:3	0.46^{NS}

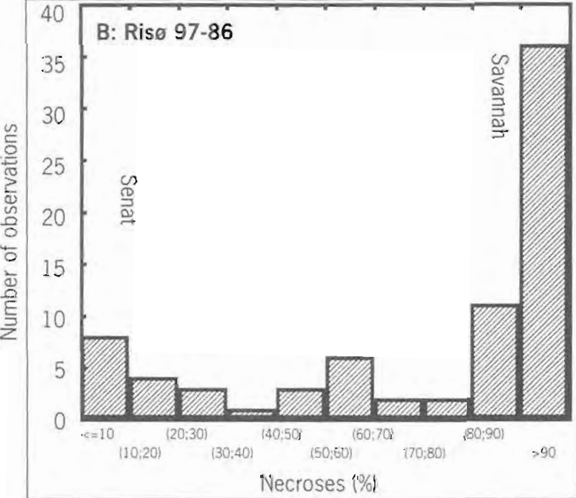
3.2. Mapping of resistance

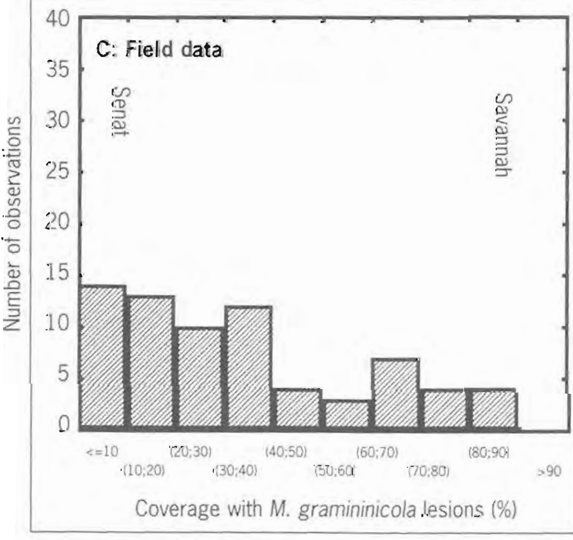
The resistance gene conferring resistance to isolate IPO323 was mapped using BSA analysis. In total, 60 WMC microsatellite markers were screened in the analysis. Two microsatellites *Xwmc50* and *Xwmc60* showed polymorphism in the BSA analysis. The microsatellite markers *Xwmc50* and *Xwmc60* were linked 15.4 and 14.6 cM respectively from the resistance gene (Table II). The marker *Xwmc50* maps to chromosome 3A in the ITMI (International Triticeae Mapping Initiative) population (M. J. Petersen, pers. comm.).

Table II. Genetic distance between two microsatellite markers and the resistance gene locus conferring resistance to *Mycosphaerella graminicola* isolate IPO323 in growth chamber, and Kruskal-Wallis statistic from MapQTL for effect of the three loci in field data.

Locus	Genetic distance (cM)	Kruskal-Wallis statistic K	Level of significance
Resistance gene	-	12.8	P < 0.0005
Xwmc60	14.6	7.4	P < 0.01
Xwmc50	15.4	6.2	P < 0.05







3.3. QTL analysis

The field experiment was assessed at growth stage 69 (BBCH scale), 27 days after the last inoculation. The data from the field experiment showed a more continues distribution than the growth chamber data with single isolates, and there was no clear segregation into susceptible and resistant lines (Fig. 2C). QTL analysis was performed with the program MapQTL. Nonparametrical Kruskal-Wallis analysis was chosen due to non-normal distribution of the data, and the few markers available making interval mapping impossible. The microsatellite markers *Xwmc50* and *Xwmc60* and the resistance gene locus for resistance to IPO323, all showed a significant effect on resistance in the field (Table II). The level of significance increased from *Xwmc50* toward the resistance gene locus. The effect in the field of possessing the IPO323 resistance gene was estimated to 23% leaf coverage, reducing the disease severity from 44.2% leaf coverage to 21.2% on average.

4. DISCUSSION

4.1. Specific resistance to M. graminicola

The specific resistance to the Dutch *M. graminicola* isolate IPO323 in the cultivar Senat, seem to be controlled by a single gene located on chromosome 3A. Several cultivars show specific resistance to IPO323 (5), and resistance to IPO323 has, in agreement with the present study, been located to chromosome 3A in the cultivar Bezostaya 1 (1). Furthermore, single gene inheritance of resistance to IPO323 has been suggested for resistance in the cultivar Flame (4). It is not known whether it is the same locus that confers resistance to IPO323 in these different cultivars.

Resistance of Senat to Risø97-86 was not independent of resistance to IPO323. The 1:3 segregation of resistant to susceptible lines, suggests that an additional gene, apart from the one located on chromosome 3A, is involved in resistance to Risø97-86. Several examples of resistance genes that require the presence of genes at additional loci for expression of resistance have been described. For example the *Rar1* and *Rar2* loci, which are necessary for *Mla12* resistance, and the *Ror1* and *Ror2* loci neces sary for the expression of *mlo* resistance (8,9). Both *Mla12* and *mlo* confer resistance to powdery mildew in barley. The *Mla12* gene is a race specific resistance gene following the gene-for-gene relationship between pathogen and host (10). Avirulence in isolate IPO323 towards a number of

Figure 2. Histograms showing the results of the disease tests on the doubled haploid progeny of the cross Savannah¥Senat. The reaction of the parents is indicated on the graphs. **A and B,** Average leaf coverage with necroses on seedlings inoculated with *Mycosphaerella graminicola* isolates IPO323 and Risø97-86, in growth chamber. **C,** Percent coverage with *M. graminicola* lesions on flag leaves in a field experiment artificially inoculated with a mixture of 11 isolates of *M. graminicola*.

cultivars is controlled at a single locus in the fungus (11), and it has been suggested that a gene-for-gene relationship exist between this isolate and the resistance gene in the cultivar Flame (4). The *Mla12* gene may thus be comparable to the gene providing resistance to IPO323. However, in the *Mla12* case *Rar1* and *Rar2* are needed for the expression of resistance against any isolate to which the *Mla12* gene provides resistance, whereas in the present case it seems that the additional loci is only necessary for the expression of resistance against Risø97-86 not IPO323.

4.2. Resistance to *M. graminicola* in the field

Among the isolates tested in growth chamber in this study, only 4 out of 16 (25%) isolates collected in Danish wheat fields, were avirulent on Senat. It would thus seem that the resistance possessed by Senat has broken down. This is in contrast to the field, where the resistance of Senat is highly effective, indicating a high level of adult plant resistance in Senat. The QTL analysis on the field data was preliminary, only data from one year and one location was available. The QTL analysis showed that there is an effect of the IPO323 resistance gene locus in the field against an artificial inoculated *M. graminicola* population, in spite of this gene being ineffective against the individual isolates of the population in growth chamber tests. QTL loci, mapping to approximately the same location as specific resistance genes have previously been reported, e.g. for powdery mildew and leaf rust of barley (2,12).

4.3. Marker assisted selection

The development of *M. graminicola* is dependent on the environmental conditions, especially rainfall (16). Consequently, in most environments optimal infection conditions cannot be insured every year, making markers for resistance to *M. graminicola* valuable. Marker assisted selection requires the identification of markers which are fairly closely linked to the gene of interest, ideally closer than the ca. 15 cM between markers and resistance gene found in this study. A search for closer linked microsatellites or AFLP markers is in progress.

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REFERENCES

[1] Arraiano L, Worland AJ, Brown JKM: Cytogenetics of resistance of wheat to septoria tritici leaf blotch. In: van Ginkel M, McNab A, Krupinsky J (eds) Septoria and Stagonospora Diseases of Cereals: A Compilation of Global Research. Mexico, D.F.: CIMMYT: 53 (1999).

[2] Backes G, Schwarz G, Wenzel G, Jahoor A: Comparison between QTL analysis on powdery mildew resistance in barley based on detached primary leaves and on field data. Plant Breeding 115: 419-421 (1996).

[3] Bayles RA: Research note: Varietal resistance as a factor contributing to the increased importance of *Septoria tritici* Rob. and Desm. in the UK wheat crop. Plant Varieties and Seeds 4: 177-183 (1991).

[4] Brading PA, Kema GHJ, Brown JKM: A possible gene-for-gene relationship for septoria tritici leaf blotch resistance in wheat. In: van Ginkel M, Krupinsky J (eds.) Septoria and Stagonospora Diseases of Cereals: A Compilation of Global Research. Mexico, D.F.: CIMMYT: 54-55 (1999).

[5] Brown JKM, Kema GHJ, Forrer HR, Verstappen, ECP, Arraiano LS, Brading PA, Foster EM, Fried PM, and Jenny E: Resistance of wheat cultivars and breeding lines to septoria tritici blotch caused by isolates of Mycosphaerella graminicola in field trials. Plant Pathology 50: 325-338 (2001).

[6] Edwards KJ, Barker JHA, Daly A, Jones C, Karp A: Microsatellite libraries enriched for several microsatellite sequences in plants. BioTechniques 20: 758-760 (1996).

[7] Eyal Z: The septoria tritici and stagonospora nodorum blotch diseases of wheat. European Journal of Plant Pathology 105: 629-641 (1999).

[8] Freialdenhoven A, Scherag B, Hollricher K, Collinge DB, Thordal-Christensen H, Schulze-Lefert P: Nar-1 and Nar-2, 2 loci required for Mla(12)-specified race-specific resistance to powdery mildew in barley. Plant Cell 6: 983-994 (1994).

[9] Freialdenhoven A, Peterhänsel C, Kurth J, Kreuzaler F, Sculze-Lefert P: Identification of genes required for the function of non-race-specific *mlo* resistance to powdery mildew in barley. Plant Cell 8: 5-14 (1996).
[10] Jørgensen JH: Genetics of powdery mildew resistance in barley. Critical Reviews in Plant Sciences. 13: 97-119 (1994).
[11] Kema GHJ, Verstappen ECP, Waalwijk G: Avirulence in the wheat septoria tritici leaf blotch fungus *Mycosphaerella graminicola* is controlled by a single locus. Molecular Plant-Microbe Interactions 13: 1375-1379 (2000).
[12] Kicherer S, Backes G, Walther U, Jahoor A: Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics 100: 881-888 (2000).
[13] Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L: MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181 (1987).
[14] Michelmore RW, Paran I, Kesseli RV: Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proceedings of the National Academy of Sciences USA 88: 9828-9832 (1991).
[15] Rosielle AA, Brown AGP: Inheritance, heritability and breeding behaviour of three sources of resistance to Septoria tritici in wheat. Euphytica 28: 385-392 (1979).
[16] Shaw MW, Royle DJ: Factors determining the severity of epidemics of *Mycosphaerella graminicola* (*Septoria tritici*) on winter wheat in the UK. Plant Pathology 42: 882-899 (1993).
[17] Simon MR, Worland AJ, Cordo CA, Struik PC: Chromosomal location of resistance to *Septoria tritici* in seedlings of a synthetic hexaploid wheat, *Triticum spelta* and two cultivars of *Triticum aestivum*. Euphytica 119: 149-153 (2001).
[18] Somasco OA, Qualset CO, Gilchrist DG: Single-gene resistance to Septoria tritici blotch in the spring wheat cultivar 'Tadinia'. Plant Breeding 115: 261-267 (1996).
[19] Van Ooijen, JW, Maliepaard C: MapQTL (tm) version 3.0: Software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen (1996).
[20] Wilson RE: Inheritance of resistance to *Septoria tritici* in wheat. In: Scharen AL (ed) *Septoria* of Cereals: Proceedings of a Workshop held at Montana State University (1985).
[21] Zhang X, Haley SD, Jin Y: Inheritance of Septoria tritici blotch resistance in winter wheat. Crop Science 41: 323-326 (2001).

ASSOCIATIONS OF SIMPLE SEQUENCE REPEATS WITH POWDERY MILDEW RESISTANCE IN WILD BARLEY, *HORDEUM SPONTANEUM*

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ABSTRACT: Wild barley, *Hordeum spontaneum* C. Koch, is the progenitor of cultivated barley, *Hordeum vulgare*. Since the wild and cultivated forms of barley are cross-compatible, wild barley germplasm can be exploited as a source of novel variation for resistance to biotic and abiotic stresses. In this study, susceptibility to powdery mildew (PMS), *Erysiphe graminis* f. sp. *hordei*, was determined in a wild barley population collected from Tabigha, Israel. Genotypic data were collated for the 52 wild barley genotypes by analysing the allelic variation at 33 genetically mapped simple sequence repeats (SSRs). Associations between SSR variation and PMS were investigated by linear regression analyses based on both repeat length variation and allele class differences. Several SSRs were associated with PMS using this association mapping approach suggesting that multiple genetic factors may control resistance in the wild barley genotypes tested. The largest percentage of the variation in PMS (41.6%) was explained by allele class differences at the SSR Bmac316 on chromosome 6HS. Most of the associations observed could be accounted for by close linkage to genes known to confer resistance to powdery mildew. However, novel genomic regions involved in the control of PMS were also detected, including regions on chromosome 3H, on which genes for powdery mildew resistance have not been mapped previously.

1. INTRODUCTION

Barley is not only an economically important crop, it is also considered a model species for genetic and physiological studies [1] because it is a diploid and inbreeding species with only seven pairs of chromosomes (2n = 2x = 14). Wild barley, *Hordeum spontaneum* C. Koch, the progenitor of cultivated barley, *H. vulgare* L. [2], and cultivated barley share a common genome (HH) and are cross-compatible. Consequently, novel variation revealed in the wild form and associated genetic markers may be of interest to barley breeders for crop improvement.

The wild barley genotypes analysed in this study were originally collected along a 100 m transect at Tabigha, Israel, from two different soil types, Terra rossa (TR) and Basalt (B) [3]. *In situ*, plants growing on TR experience more intense drought than plants growing on B due to different soil water-holding capacities [4]. Phenotypic responses to experimentally imposed water stress were examined in previous work [5], but biotic stress tolerance has not yet been studied in this material. Genetic diversity in the Tabigha wild barley population was analysed using various marker systems and genetic variation was found to be associated with adaptation in several studies (e.g. [3],[6]). In recent years, SSRs (or microsatellites), which are hypervariable DNA elements consisting of tandemly repeated motifs (e.g. $(AC)_n$; [7]) have been frequently used in studies on plant molecular genetics (for review see [8]), including work on cultivated barley (e.g. [9]). In barley, several hundred SSRs have been developed and genetically mapped (e.g. [10]).

In this study, 33 SSRs evenly dispersed over the barley genome were selected to analyse 52 wild barley genotypes from Tabigha. Information on susceptibility to powdery mildew, *Erysiphe (= Blumeria) graminis* f. sp. *hordei,* of the Tabigha genotypes was collected in a glasshouse experiment. Associations bet-

ween SSRs and susceptibility scores were investigated using two statistical methods: regression analysis based on differences between SSR allele classes (i.e. discrete groupings of specific alleles) and regression analysis based on SSR length variation (i.e. continuous decrease or increase of repeat length). The results emanating from these genotype/phenotype associations were compared with published information from more conventional mapping studies.

2. MATERIAL AND METHODS

2. 1. Plant material and site information

Wild barley genotypes were collected, where present, every metre along a 100 m transect at the Tabigha site in the Upper Galilee Mountains, Israel [3]. The transect was equally divided into two soil types, Terra rossa (TR) derived from Eocene limestones (first 50 m) and Basalt (B) derived from Pleistocene volcanic eruptions (second 50 m). Twenty-four genotypes from TR and 28 genotypes from B were analysed in this study and are henceforth referred to as TR and B sub-populations. The original collection has been maintained at the Institute of Evolution (Haifa, Israel) and, subsequently, a sub-sample has been maintained at SCRI.

2. 2. Collection and analysis of data on powdery mildew susceptibility

Information on susceptibility of the 52 Tabigha wild barley genotypes to powdery mildew was obtained by scoring the symptoms caused by natural infection during multiplication of the material at SCRI (Invergowrie, Dundee, Scotland, UK). Seeds were germinated in Petri-dishes in February 1998 and plants (2 replicates per genotype, one per block) were grown in pots in non-randomised blocks under cold Cambridge glasshouse conditions. Disease intensity (percentage of leaf area infected with powdery mildew) was recorded on 6th July 1998 using a 0-100% scale [11] when most of the plants were at the flowering stage. Analysis of variance (ANOVA) was performed on the data to investigate the effects of blocks, genotype, soil type and first order interactions. The means were calculated for the sub-populations from the two soil types TR (n = 24) and B (n = 28), and all 52 wild barley genotypes.

2. 3. Analysis of simple sequence repeats

Thirty-one SSR primer pairs, which detected 33 loci evenly covering the barley genome were selected for the genetic characterisation of the plant material. Information about primer sequences, SSR repeat motifs and PCR amplification conditions was available and the chromo-somal location of 31 SSRs was known [10]. However, in this study, one primer pair amplified three polymorphic fragments: Bmac273b maps to chromosome 7H, but Bmac273a and Bmac273c are as yet un-mapped. DNA was isolated from leaf material (sampled from one individual/genotype) and SSRs were amplified using standard PCR techniques. Out of the 33 SSR loci, 21 were analysed using radioactively labelled primers (Bmac399, Bmac213, HvHVA1, Bmac134, HVM54, HvLTTPB, Bmac209, Bmag13, Bmac29, HvOLE, HVM3, Bmac181, Bmac96, Bmag5, Bmac18, Bmag9, Bmac40, HvCMA, Bmac273a, b, c). Alleles were sized by comparison with the sequencing reaction of M13 vector DNA [12]. The other twelve SSR loci (Bmag211, WMC1E8, Bmag378, Bmag125, EBmac701, HVM67, EBmac970, Bmag223, Bmag222, Bmac316, Bmag120, Bmac156) were analysed using reverse primer labelling with one of the three fluorescent dyes, 'HEX', 'TET' or 'FAM'. Fragment lengths were determined by polyacrylamide electrophoresis using an ABI377 automated sequencer (Perkin Elmer). PCR products were run together with the internal lane size standard TAMRA 350 according to the supplier's instructions. The files resulting from each gel run were processed using the software 'GeneScan' (Perkin Elmer) and sample files were further interpreted using the programme 'GenoTyper' (Perkin Elmer). Using MS Excel software, alleles were sorted such that the range from upper to lower limit of every allele class did not exceed one basepair. While most of the allelic differences corresponded to variation in the repeat motif of the SSRs, a very small proportion of the alleles observed represented single base pair differences presumably caused by insertion and/or deletion of nucleotides.

2. 4. Analysis of associations between genotypic and phenotypic data

Associations between SSRs and phenotypic data were investigated by ANOVA and linear regression analysis using the GENSTAT for MS-Windows PC software Version 4.1. To examine associations that are more likely to be based on repeat length variation of SSRs, ANOVA was first used to screen allelic groupings at each SSR locus for significant associations and non-significant deviation from the regression line by fitting a linear polynomial contrast for repeat length. Linear regression analyses were then performed using SSR data as explanatory variates and phenotypic data as response variates. In order to detect associations based on differences in allele classes or clusters of SSR alleles, regression analyses were perfor-

med using SSR data as treatment factors. In the case of heterozygous SSR loci, the adjusted R^2 values obtained for the two pertinent SSR alleles were slightly different and were averaged (this assumes that the two alleles have an equal and additive effect).

3. RESULTS

3. 1. Evaluation of powdery mildew susceptibility

Susceptibility to a natural infection of powdery mildew was scored for all 52 wild barley genotypes from the Tabigha population. The results of the ANOVA showed that there were no significant block (1 df) or soil type (1 df) effects, but highly significant genotype effects ($p < 0.001$; 51 df). In spite of the low number of replicates (2) reliable scores for powdery mildew susceptibility (PMS) could be obtained (data not shown). Means of PMS (+/- standard deviations, SD) of the TR sub-population, B sub-population and all 52 wild barley genotypes were 44.4% (+/-30.9%), 34.3% (+/-22.9%) and 38.9% (+/-27.1%), respectively. It is apparent from both the ANOVA and the distribution of the means (with large SDs) that the phenotypic diversity for this trait was very high. No significant effects were observed for soil type indicating that, overall, PMS was not significantly different between genotypes from B and TR.

3. 2. Associations of SSRs with powdery mildew susceptibility

Two different statistical approaches were chosen to detect associations between SSR variation and susceptibility to powdery mildew. The results obtained by regression analyses using SSR data as continuous variates (<</>>; Table I) reflect associations with variation in repeat length. The results of the regression analyses using SSR data as discrete factors (++/-; Table I) indicate associations with differences in allele classes or groups of alleles. Associations of SSRs with powdery mildew susceptibility were found on the short and long arms of chromosomes 1H, 2H and 3H, on the long arm of 5H and on the short arm of 6H (Table I). Bmac273c was also associated with PMS, but the map location of this SSR is unknown.

Allele class differences at Bmac316 (6HS) accounted for the largest proportion (41.6%, $p < 0.001$) of the variation in PMS (Table I). Shorter alleles at this SSR (129, 136 and 138bp) were primarily found in genotypes with low PMS (0-25%), with the 138bp allele being the most frequent allele in the population (allele frequency: 0.32). Alleles 140, 154, 156 and 158bp were present in genotypes with intermediate PMS (25-55%) and alleles 141, 143, 146 and 182bp were associated with high PMS (55-80%; Figure 1).

Table I. SSRs showing associations with powdery mildew susceptibility (PMS) in wild barley are listed together with closely linked resistance genes. Gene symbols are given in accordance with [18] or references listed. Regression analyses (generating adjusted R^2 values) based on SSR length (<</>>) and allele class (++/-) were performed using genotypic information of 52 wild barley genotypes. F-probabilities: * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Genetic factors known to control PMS in barley			Closely linked SSRs showing associations with PMS in wild barley		
Symbol	Ref.	Chr.	SSR	adj. R2 [%]	length/class
Mlat	e.g. [19]	1HS	-	-	-
Mlra	e.g. [20]	1HS	-	-	-
Mla	e.g. [21]	1HS	Bmac399	7.9 *	<</>>
Mlk	e.g. [22]	1HS	(Bmac399)	(7.9 *)	(<</>>)
Mlnn	e.g. [23]	1HS	-	-	-
Mlp	e.g. [24]	1HS	-	-	-
MlGa	e.g. [25]	1HL	HvHVA1	10.9 **; 10.9 **	<</>>; ++/-
Mli	[26]	1HL	-	-	-
MlHb	[16]	2HS	Bmac134	5.8 *	<</>>
MlLa	e.g. [27]	2HL	-	-	-
Mlg	e.g. [28]	4HL	-	-	-
mlo	e.g. [28]	4HL	-	-	-
Mlj	[29]	5HL	Bmag222	14.6 *	++/-
MlTR	[30]	5HL	-	-	-
Mlh	e.g. [17]	6HS	Bmac316	41.6 ***	++/--
mlt	[29]	7HS	-	-	-
Mlf	[29]	7HL	-	-	-
Novel loci controlling PMS in wild barley		2HL	Bmag125	21.8 **	++/--
		3HS	HvLTPPB	18.3 *	++/-
		3HL	Bmac29	17.8 *	++/-
		-	Bmac273c	12.9 *	++/--

4. DISCUSSION

4. 1. Sources of resistance to powdery mildew

Powdery mildew, *Erysiphe (= Blumeria) graminis* f. sp. *hordei*, represents an important biotic stress of barley. Considerable selection pressure is likely to operate in wild

barley. In agriculture, the severity of the disease can be controlled chemically, by application of fungicides, and/or genetically, by growing resistant cultivars. A large number of genetic factors that confer resistance to powdery mildew in barley has been identified and many major genes have been genetically mapped (Table I, for review see [13]). Genetic resistance, especially if conditioned by race-specific genes, is however gradually overcome due to evolution of resistant races of the pathogen and strong selection if a particular gene is widely deployed. New sources of resistance, especially those at new loci, are sought in the primary barley gene pool including barley landraces (e.g. [14]) and wild barley (e.g. [15]), and the secondary gene pool (*Hordeum bulbosum*; e.g. [16]) in order to widen the options for deployment of resistance.

4. 2. SSR variation in relation to powdery mildew susceptibility

In this study, a total of nine SSRs (including one SSR of unknown map location, Bmac273c) were associated with PMS (Table I). Many of these were not highly significant and did not account for large portions of variation and may therefore represent sources of quantitative resistance. However, allele class differences of Bmac316 on 6HS accounted for a very large percentage of the variation in PMS (Table I), suggesting the presence of an effective major gene. The *Mlh* locus has been assigned to this chromosome and an allele at this locus has been widely used in barley cultivars [17]. It is, however, not clear whether Bmac316 detected the same or a different allele at the *Mlh* locus or a novel gene in this chromosomal region. Bmag125 on 2H was also associated with a large effect on mildew susceptibility (Table I). A major gene for PMS has not been reported in this area of the genome and the result may therefore indicate the presence of a novel gene. Interestingly, effects on PMS were also detected by two SSRs located on the long and short arm of chromosome 3H, which has not been known, to date, to be involved in the genetic control of the disease.

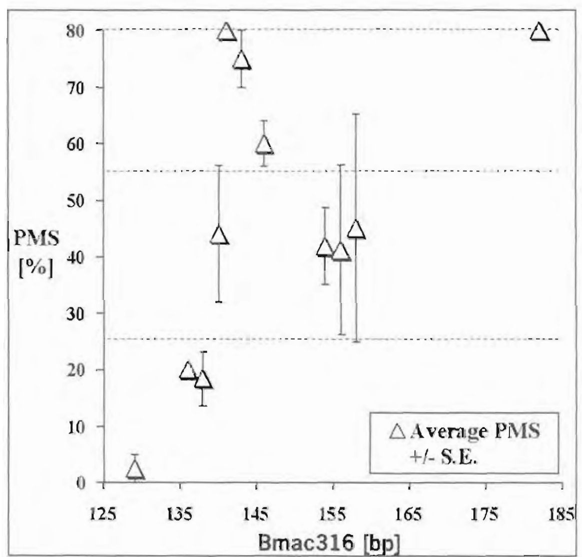

Figure 1. Association of allele class differences at the SSR Bmac316 with powdery mildew susceptibility (average PMS +/- S.E.) in 52 wild barley genotypes collected from Tabigha, Israel.

4. 2. SSRs as new tools for association genetics

Traditionally, the process of identifying and tagging disease resistance genes is lengthy. In this study using genetically mapped SSRs, simple regression analysis yielded valuable information about the map location of a putative effective major gene. Moreover, using the association mapping approach, chromosomal regions of possible value for deployment in quantitative resistance have been identified for further evaluation. The allelic information gathered can be of immediate value for molecular breeding, e.g. specific alleles at Bmac316 associated with a high degree of powdery mildew susceptibility (Figure 1) may have value in selection. The simultaneous identification of resistance donor genotypes, map location of the underlying resistance gene(s) and molecular tags in the form of specific SSR alleles, has potential in expediting the transfer of the respective gene(s) from unadapted germplasm into a breeding line via marker assisted selection.

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REFERENCES

[1] Koornneef M, Alonso-Blanco C, Peeters AJM: Genetic approaches in plant physiology. New Phytol 137: 1-8 (1997).

[2] Harlan JR: Barley. In: Smartt J, Simmonds NW (eds) Evolution of Crop Plants. 2nd edition, pp. 140-146. Longmann, London (1995).

[3] Nevo E, Brown AHD, Zohary D, Storch N, Beiles A: Microgeographic edaphic differentiation in allozyme polymorphisms of wild barley (*Hordeum spontaneum*, Poaceae). Plant Syst Evol 138: 287-292 (1981).

[4] Nevo E, Krugman T, Storch N: Edaphic natural selection for allozyme polymorphisms in *Aegilops peregrina* at a Galilee microsite in Israel. Heredity 72: 109-112 (1994).

[5] Ivandic V, Hackett CA, Zhang ZJ, Staub JE, Nevo E, Thomas WTB, Forster BP: Phenotypic responses of wild barley to experimentally imposed water stress. J Exp Bot 353: 2021-2029 (2000).

[6] Nevo E, Beiles A, Storch N, Doll H, Andersen B: Microgeographic differentiation of hordein polymorphisms of wild barley. Theor Appl Genet 64: 123-132 (1983).

[7] Tautz D, Renz M: Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucl Acids Res 12: 4127-4138 (1984).

[8] Powell W, Machray GC, Provan J: Polymorphism revealed by simple sequence repeats. Trends Pl Sci 1: 215-222 (1996).

[9] Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW: Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations and population dynamics. Proc Natl Acad Sci 91: 5466-5470 (1994).

[10] Ramsay L, Macaulay M, degli Ivanissevich S, MacLean K, Cardle L, Fuller J, Edwards K, Tuvesson S, Morgante M, Massari A, Maesti E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R: A simple sequence repeat-based map of barley. Genetics 156: 1997-2005 (2000).

[11] Peterson RF, Campbell AB, Hannah AE: A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can J Res 26: 496-500 (1948).

[12] Russell J, Fuller J, Young G, Thomas WTB, Taramino G, Macaulay M, Waugh R, Powell W: Discriminating between barley genotypes using microsatellite markers. Genome 40: 442-450 (1997).

[13] Jørgensen JH: Genetics of powdery mildew resistance in barley. Crit Rev Plant Sci 13: 97-119 (1994).

[14] Jørgensen JH, Jensen HP: Powdery mildew resistance in barley landrace material. I. Screening for resistance. Euphytica 97: 227-233 (1997).

[15] Kintzios S, Fischbeck G: Identification of new sources for resistance to powdery mildew in *H. spontaneum*-derived winter barley lines. Genet Res Crop Evol 43: 25-31 (1996).

[16] Pickering RA, Hill AM, Michel M, Timmerman Vaughan GM: The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L to barley (*H-vulgare* L) chromosome 2(2I). Theor Appl Genet 91: 1288-1292 (1995).

[17] Hayashi J, Heta H: Association of a mildew resistance gene JML_h in Hanna 906 with chromosome 6. Barl Genet Newsl 15: 46-47 (1985).

[18] Jørgensen JH: I. Genes for powdery mildew reaction; II. Loci for disease and pest reaction. Coordinator's report: Disease and pest resistance genes. Barl Genet Newsl 22: 110-134 (1992).

[19] Nilan RA: The cytology and genetics of barley 1951-1962. Monographic supplement No. 3 vol. 32, No. 1. Washington State University Press, pp 278 (1964).

[20] Saghai Maroof MA, Zhang Q, Biyashev RM: Molecular marker analyses of powdery mildew resistance in barley. Theor Appl Genet 88: 733-340 (1994).

[21] Schuller C, Backes G, Fischbeck G, Jahoor A: RFLP markers to identify the alleles on the *mla* locus conferring powdery mildew resistance in barley. Theor Appl Genet 84: 330-338 (1992).
[22] Giese H, Jørgensen JH, Jensen HP, Jensen J: Linkage relationships of ten powdery mildew resistance genes on barley chromosome 5. Hereditas 95: 43-50 (1981).
[23] Jensen J, Jørgensen JH: The barley chromosome 5 linkage map. II. Extension of the map with four loci. Hereditas 80: 17-26 (1975).
[24] Jensen J: Coordinator's report: Chromosome 5. Barl Genet Newsl 10: 88-90 (1980).
[25] Hossain MA, Sparrow DHB: Resistance to powdery mildew (*Erysiphe graminis* f. sp. *hordei*) in the barley cultivar Galleon. II. Chromosomal location and linkage with hordein protein genes. Euphytica 52: 11-17 (1991).
[26] Jahoor A, Fischbeck G: Genetical studies of resistance to powdery mildew in barley lines derived from *Hordeum spontaneum* collected from Israel. Pl Breed 99: 265-273 (1987).
[27] Hilbers S, Fischbeck G, Jahoor A: Localization of the *Laevigatum* resistance gene *MlLa* against powdery mildew in the barley genome by the use of RFLP markers. Pl Breed 109: 335-338 (1992).
[28] Wiberg A: Genetical studies of spontaneous sources of resistance to powdery mildew in barley. Hereditas 77: 89-148 (1974).
[29] Schönfeld M, Ragni A, Fischbeck G, Jahoor A: RFLP mapping of three new loci for resistance genes to powdery mildew (*Erysiphe graminis* f. *hordei*) in barley. Theor Appl Genet 93: 48-56 (1996).
[30] Falak I, Falk DE, Tinker NA, Mather DE: Resistance to powdery mildew in a doubled haploid barley population and its association with marker loci. Euphytica 107: 185-192 (1999).

THE EFFECTS OF HOMOEOLOGOUS GROUP 3 CHROMOSOMES ON RESISTANCE TO FUSARIUM HEAD BLIGHT IN TETRAPLOID WHEAT

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ABSTRACT: Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most destructive diseases of wheat in areas where the weather is warm and humid after the heading. Previous studies indicate that level of resistance to FHB varies not only among wheat cultivars but also among some of their wild relatives. No accession, however, has yet been identified to be completely immune to FHB among the Gramineae. It is know that durum wheats (*Triticum turgidum* L. conv. *durum*) are consistently more susceptible to FHB than common wheat (*T. aestivum* L.). The importance of D genome in conferring resistance to FHB has been emphasized. Meanwhile, recent studies using molecular markers report effective QTLs on chromosome 3BS in hexaploid population and on 3A in tetraploid recombinant inbred chromosome lines. In this study, we performed to evaluate the effects of homoeologous group 3 chromosomes of *T. turgidum* ssp. *dicoccoides* on resistance to FHB using a set of chromosome substitution lines of a durum wheat cultivar 'Langdon'. The accession of *T. turgidum* ssp. *dicoccoides* examined in this study was higher susceptible for Type II resistance (resistance to spread of FHB in the head) than Langdon. Both of the chromosome substitution lines of 3A and 3B showed same level of resistance with Langdon, but bleaching of the heads was completely prevented in the substitution lines of chromosome 3A without relationship to rachis fragility. It is concluded that the chromosome 3A of *T. turgidum* ssp. *dicoccoides* carries resistance gene(s) to head bleaching caused by FHB.

INTRODUCTION

Fusarium head blight is a widespread and destructive disease of wheat. The best way to prevent wheat from being affected by this disease is to develop resistant cultivars. Previous studies have shown that the resistance to FHB varies among wheat, barley and their wild relatives, but no accession has yet been identified to be completely immune (1, 2, 3). It is know that durum wheat (*Triticum turgidum* L. conv. *durum*) are consistently more susceptible to FHB than common wheat (*T. aestivum* L.) and good source of resistance have not been available (4, 5). The wild species may offer some hope to improve resistance to FHB in durum wheat. The wild emmer wheat, *T. turgidum* ssp. *dicoccoides*, have been put our hopes as a resistance source to FHB in adapted germplasm (6). The importance of D genome in conferring resistance to FHB has been emphasized (7). Meanwhile, recent studies using molecular markers report effective QTL associated with Type II resistance to FHB (resistance to spread of pathogen within heads) on chromosome 3BS in hexaploid population (8). In this study, we performed to evaluate the effects of homoeologous group 3 chromosomes of *T. turgidum* ssp. *dicoccoides* on Type II resistance to FHB using a set of chromosome substitution lines of a durum wheat cultivar 'Langdon'.

MATERIALS AND METHODS

Plant materials

A durum wheat cultivar 'Langdon' (LDN) and *T. turgidum* ssp. *dicoccoides* 'Israel A line' (DIC) were grown in pots under natural conditions in JIRCAS. The disomic substitution lines of 'Langdon', LDN(DIC3A) for chromosome 3A and LDN(DIC3B) for 3B, were also tested for Type II resistance to FHB. In the LDN durum chromosome substitution lines, a pair of chromosomes 3A or 3B were replaced with equivalent ones from DIC. LDN-DIC substitution lines were provided by Dr. L. R. Joppa, USDA-ARS, Fargo, ND, USA. We also assessed the synthetic hexaploid wheat lines derived from crosses of the tetraploid wheat and *T. tauschii* G3489 carrying non brittle rachis character, abbreviated as LDN/ G3489, LDN(DIC3A)/ G3489 and LDN(DIC3B)/ G3489, for the effect of DIC chromosomes at the hexaploid level.

Inoculation methods and disease assessment

One strain of *Fusarium graminearum* Schwabe, strain G87-36B, was used to assess Type II resistance to FHB. It was derived from a single spore that had been isolated from an FHB-affected wheat spike in the field (9). One to five individual plants of each accession were transferred to a humid chamber at their flowering stage, which was kept at 25-30°C and 100% humidity by using an ultrasonic humidifier, for infection with the fungus. On the first day, the single spikelet of the center part in plant head (two to 14 heads/ plant) was inoculated by micro pipette with a conidial suspension of G87-36B obtained from the mung bean medium that had been adjusted to approximately 100 conidia per 200-fold magnified microscopic field. After three days under humid conditions, each plant was transferred to another chamber where the temperature was maintained at 25-30°C, with 70 to 80% to favour development of the disease. The diseased parts of the florets and rachis became brownish or whitish in color. Consequently, premature death or bleaching of upper spikelets from inoculated point was observed as severe symptoms. Two aspects of Type II resistance were evaluated three weeks after the inoculation: 1) degree of FHB spreading within head (*i. e.* the frequency of diseased spikelets/ total number of spikelets); and 2) degree of top bleaching by FHB (*i. e.* the number of top bleaching heads/ total number of inoculated heads). Both parameters, calculated for each plant, were transformed by angular transformation and averaged for each accession to give indices of Type II resistance. Analysis of variance was performed for these indices, then the statistically significant difference was evaluated by the least significant difference (LSD) at 5%.

RESULTS AND DISCUSSION

The indices of Type II resistance to FHB in each accession are shown in Table I and II. One of the parameters for FHB spreading within head (FSH) varied among accessions (F=26.3**, Table III), and no accession was completely resistance. The wild emmer wheat, *T. turgidum* ssp. *dicoccoides*, 'Israel A line' (DIC) examined in this study was higher susceptible to FSH than LDN (Table I), although LDN was consistently more susceptible than representative common wheat cultivars (data not presented here). FSH reached easily to the neck of spike in DIC, then the spikes resulted in premature death without seed setting. Two disomic substitution lines for homoeologous group 3, LDN(DIC3A) and LDN(DIC3B), showed same level of resistance to FHS as LDN, indicating chromosomes 3A and 3B of DIC had not remarkable effect on it. Three accessions of the synthetic hexaploid wheat, LDN/ G3489, LDN(DIC3A)/ G3489 and LDN(DIC3B)/ G3489, did not differ in the resistance to FSH, and they were more susceptible than tetraploid accessions of LDN and disomic substitution lines of chromosome 3A and 3B. It is supposed that D genome donor *T. tauschii* G3489 had a susceptible effect to FSH resistance and gene(s) for susceptibility were harbored on D genome chromosomes.

Top bleaching of head (TBH) caused by FHB should be one of the symptoms to assess Type II resistance in wheat. Fusarium fungi infest the vessel of rachis and result in consequent death of head from lack of water at the upper parts from infected points (Fig. 1). It is recognized as a qualitative character, however expressed quantitatively in a plant. We assessed degree of TBH counting the number of bleaching heads/ total number of inoculated heads. The level of resistance to TBH was also different among accessions (F=8.51**, Table III). DIC was completely susceptible to TBH as for FSH without exception (Table II). LDN showed higher level of resistance to TBH than DIC, and the same level with LDN(DIC3B). One of the substitution lines LDN(DIC3A) expressed highest performance in resistance to TBH. All spikes inoculated evaded top breaching, although FHB spread from inoculating point. This observation indicated that resistance gene(s) to TBH caused by FHB should be located on chromosome 3A of DIC, and DIC was carrying susceptible gene(s) with epistatic effect on the resistance gene on chromosome 3A. Otto et al. reported that LDN(DIC3A) has the greatest potential as a source of Type II resistance to FHB (11). They indicated a QTL for Type II resistance on chromosome 3A using a population of 83 recombinant inbread chromosome lines derived from a cross of LDN/ LDN(DIC3A) (12). The SSR marker Xgwm2 was associated this QTL and explained 31% of the phenotypic variation over multiple seasons. The two aspects of Type II resistance (FSH

and TBH) were classified in this study. Our results of the effect on the aspects of resistance revealed that chromosome 3A of DIC harbored loci only for resistance to TBH. Other points of our observation consent to previous reports.

The resistance level to TBH at the hexaploid situation explained existence of the powerful susceptible factor(s) on D genome chromosomes from *T. tauschii* G3489. LDN/ G3489, LDN(DIC3A)/ G3489 and LDN(DIC3B)/ G3489 showed susceptibility compare with tetraploid accessions LDN and disomic substitution lines LDN(DIC3A) and LDN(DIC3B). The locus or loci controlling the susceptibility to TBH should carry epistatic effect on the resistance locus located on chromosome 3A of DIC expressing resistance in LDN(DIC3A). LDN/ G3489 showed highest susceptibility to TBH such as DIC. Because only one plant of this accession was tested in this study, further examination should be required to confirm their performance.

Table I. Indices of resistance to Fusarium head blight spreading within head (FSH) for accessions of LDN disomic substitution lines, obtained after the single spikelet inoculation with Fusarium graminearum G87-36B.

Accession	No. of plants	Averaged Arcsin (FSH%)	SD	LSD (5%)=5.9	Te rachis character
LDN	5	28.9	3.1	a	Tough
LDN(DIC3B)	5	32.1	4.0	a	Brittle (*Br*$_2$)
LDN(DIC3A)	5	33.3	3.5	a	Brittle (*Br*$_3$)
LDN/G3489	1	44.7	-	b	Tough
LDN(DIC3A)/G3489	2	42.7	0.8	b	Brittle (*Br*$_2$)
LDN(DIC3B)/G3489	2	47.6	3.8	b	Brittle (*Br*$_3$)
DIC	3	68.7	10.2	c	Brittle (*Br*$_2$+*Br*$_3$)

FSH: % of diseased spikelets/ total number of spikelets.Brittle rachis character: refer to Watanabe & Ikebata (10).

Table II. Indices of resistance to top bleaching of head (TBH) caused by Fusarium head blight for accessions of LDN disomic substitution lines, obtained after the single spikelet inoculation with Fusarium graminearum G87-36B.

Accession	No. of plants	Ave. no. of Arcsin (FSH%)	Averaged Arcsin (TBH%)	SD	LSD (5%)=5.9	Brittle rachis character
LDN(DIC3A)	5	8.6	0.0	0.0	a	Brittle (*Br*$_2$)
LDN	5	4.4	27.3	17.6	b	Tough
LDN(DIC3B)	5	5.4	34.0	37.2	b	Brittle (*Br*$_3$)
LDN(DIC3B)/G3489	2	5.4	63.9	5.5	c	Brittle (*Br*$_3$)
LDN(DIC3A)/G3489	2	4.0	76.7	18.8	cd	Brittle (*Br*$_2$)
DIC	3	3.3	90.0	0.0	d	Brittle (*Br*$_2$+*Br*$_3$)
LDN/G3489	1	7.0	90.0	-	d	Tough

TBH: % of top bleaching heads/ total number of inoculated heads.Brittle rachis character: refer to Watanabe & Ikebata (10).

Figure 1: Symptoms of Fusarium head blight spreading within head and top bleaching caused by *Fusarium graminearum* in accessions of tetraploid and synthetic hexaploid wheat.
Left to right: *Triticum turgidum* ssp. *dicoccoides* 'Israel A line' (DIC), Langdon (LDN), LDN(DIC3A), LDN(DIC3B), LDN/ G3489, LDN(DIC3A)/ G3489, and LDN(DIC3B)/ G3489.

Table III. Results of ANOVA for indices of Type II resistance to Fusarium head blight in accessions of LDN disomic substitution lines after the single spikelet inoculation with *F. graminearum* G87-36B.

Source of variance	df	SS	F	
Averaged Arcsin (FSH%)				
Accessions	6	3768.82	26.93	P< 0.01
Error	16	373.26		
Total	22	4142.08		
Averaged Arcsin (TBH%)				
Accessions	6	22791.32	8.51	P< 0.1
Error	16	7140.26		
Total	22	29931.58		

Based on the results of different effect of chromosome 3A of DIC on FSH and TBH, it is proposed that Type II resistance to FHB consists of two kind of protection against lateral and vertical spreading of fungus. Degree of TBH is may be controlled by resistance to lateral spreading of FHB. Watanabe and Ikebata reported that the fragile rachis of DIC was controlled by two dominant genes (Br2 and Br3) which were located on chromosome 3AS and 3BS, and the map distance of Br2 was 21 cM and that of Br3 was 20 cM from the centromere (10, 13). Therefore, we speculated that brittle rachis character for rachis fragility might be associated with a specific structure in wheat rachis such as an abscission layer to prevent lateral spreading of FHB. The results, however, indicated no relationship between Type II resistance to FHB and brittle rachis character (Table I and II).

In this study, it is concluded that the chromosome 3A of T. turgidum ssp. dicoccoides 'Israel A line' carries resistance gene(s) to head bleaching caused by FHB without relationship to rachis fragility. It controls Type II resistance to FHB through complete prevention of top bleaching of heads. The evidence of resistance gene located on chromosome 3A will brig out the wild emmer wheat as an attractive genetic resources both for durum and bread wheat breeders. However, ssp. dicoccoides is carrying powerful susceptible gene(s) to FHB, too. Therefore, mapping of loci associated with FHB in ssp. dicoccoides should be conducted to tag them by using DNA markers.

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REFERENCES

[1] Mesterházy Á: Selection of head blight resistant wheats through improved seedling resistance. Plant Breeding 98:25-36 (1987).

[2] Takeda K, Kanatani R, Zhang CL: Varietal variation of the scab disease resistance in wheat (*Triticum aestivum*) evaluated by the 'cut-spike' inoculation method. Japan J Breed 42:649-656 (1992).

[3] Ban T: Evaluation of resistance to *Fusarium* head blight in indigenous Japanese species of *Agropyron* (*Elymus*). Euphytica 97: 39-44 (1997).

[4] Parry DW, Jenkinson P, McLeod L: *Fusarium* ear blight (scab) in small grain cereals - a review. Plant Pathology 44: 207-238 (1995).

[5] Miedaner T: Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breeding 116: 201-220 (1997).

[6] Miller JD, Stack RW, Joppa LR: Evaluation of *Triticum turgidum* L. var. *dicoccoides* for resistance to fusarium head blight and atem rust. In: Slinkard AE (ed) Proceedings of the 9th Intl Wheat Genet Symp (Saskatoon, Canada), vol. 3: 292-293. University Extension Press, Univ. of Saskatchewan, Sascatoon (1998).

[7] Gilbert J, Aung T, Procunier JD: Influence of the D genome in Fusarium head blight resistance. Cereal Research Communications 25: 647-649 (1997).

[8] Kolb FL, Bai G-H, Muehlbauer GJ, Anderson JA, Smith KP, Fedak G: Host plant resistance genes for Fusarium head blight: Mapping and manuipulation with molecular markers. Crop Sci. 41:611-619 (2001).

[9] Ban T: Efficiency of 'cut-spike' inoculation method for evaluation of scab resistance in wheat. Japan J Breed 41 (Suppl. 2): 404 (1991).

[10] Watanabe N, Ikebata N: The effects of homoeologous group 3 chromosomes on grain colour dependent seed dormancy and brittle rachis in tetraploid wheat. Euphytica 115: 215-220 (2000).

[11] Otto CD, Kianian SF, Elias RW, Stack RW, Joppa LR: Molecular mapping for Fusarium head blight resistance in a RICL population of tetraploid wheat. p. 159. Agron. Abstr. ASA, Madison, WI (1999).

[12] Otto CD, Kianian SF, Elias RW, Stack RW, Joppa LR, Doehler ET: Molecular mapping for Fusarium head blight in a RICL population of tetraploid wheat. Agron. Abstr. ASA, (2000).

[13] Watanabe N, Sugiyama K, Yamagishi Y, Sakata Y: Comparative telosomic mapping of homoeologous genes for brittle rachis in tetraploid and hexaploid wheat. Euphytica (Submitted to).

MAPPING A QTL INVOLVED WITH GREENBUG AND RWA RESISTANCE IN WHEAT

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ABSTRACT: Greenbug (*Schizaphis graminum*, Rondani), and RWA (*Diuraphis noxia*, Mordv.) are the most dangerous aphid pests in cereal producing areas in America. Several genes of resistance have been reported, that control one or the other pest in wheat (*Triticum aestivum*,L.). These genes have been successfully introduced from other Triticeae in bread wheat. Wheat substitution lines between an aphid susceptible cultivar (Chinese Spring) and a new hexaploid wheat (Synthetic) (*T. turgidum x Aegilops squarrosa*) (AABB x DD) were used to analyse the different types of aphid resistance. Our group reported (2001) that several chromosomes of the D genome contributed to different mechanisms of resistance against both aphid pests. The 7D chromosome substitution line explained most of the antixenosis against greenbug and RWA. The aim of this work was to determine the location of genes for antixenosis type of resistance against both aphid pests The analysis of the double haploid recombinant lines for 7D chromosome permitted to locate a QTL that map very close to the centromere in both arms significantly linked with molecular markers. This QTL explained 80% of the antixenotic resistance against both aphid species. The finding that the D genome could improve aphid resistance in wheat is of a great importance in breeding resistance against biotic stresses.

1. INTRODUCTION

Several genes conferring resistance to greenbug or to RWA have been previously located on 7D chromosome in wheat. The greenbug resistance gene *Gb3*, derived from *T. tauschii*, gives resistance against biotypes C and E in 'Largo' wheat [12] was mapped on the chromosome 7D [11].

Genetic resistance to RWA in wheat was reported by Du toit [8, 9]. The resistance of wheat lines SA1684 and SA2199 were conferred by single dominant genes (*Dn1* and *Dn2*, respectively). The *Dn1* gene was located on wheat chromosome 7D [18]. Another two RWA resistance genes were reported in *T. tauschii*, a recessive one that was designed *dn3* and a dominant one, *Dn4*, [17]. Marais and Du Toit [16] used a rating of plant damage in wheat monosomic plants and identified a dominant resistant gene, named *Dn5* in chromosome 7D, that they suggested no to be allelic to the *Dn1*, *Dn2* or *dn3* and probably linked to *Dn1*; that gene was located in the 7DL arm [10]. Another dominant resistance gene, *Dn6*, was identify in 1995 [6]. A second monosomic analysis was performed [19] with the 'Chinese Spring' series of monosomics developed by Sears [19], it was reported that chromosome 7B carried a minor or complementary gene for resistance to RWA, suggesting that there would be some conserved resistance common to each of the three ancestors of bread wheat. Studying the previous mentioned genes in order to determine the allelic relationship and inheritance [7], it was found that in all the possible F2s tested the resistance against RWA was controlled by qualitative and non additive genes. In the previous works the different types of resistance against greenbug and RWA were not identified. Nonetheless, RAPD and SCAR markers linked to RWA resistance gene *Dn2* were developed in wheat. Recently, microsatellite markers (*Xgwm111*) linked to six dominant RWA resistance genes (*Dn1*, *Dn2*, *Dn5* and at least another three resistance genes) showed that they are tightly linked to each other, and provide new information about their location, being 7DS, near the

centromere, instead of as previously reported on 7DL *Xgwm635* (near the distal end of 7DS) [14]. This new results clearly marked the location of the previously suggested resistance gene in line PI 294994, now designated as *Dn8*. The previously mentioned reports take into account infested plant responses, alike the genetic studies done with greenbug, a constant number of aphids was used to infest and the plant damage was recorded and scored after two to four weeks since infestation onset, none of the different resistance mechanism was separately studied. Our group identified in *T. aestivum/ Hordeum chilense* disomic addition lines, four chromosomes of the wild species involved with antixenosis (1H*ch)*, antibiosis (4Hch, 5Hch, 7Hch) and tolerance (7Hch) to greenbug [2] that also were expressed in Tritordeum amphiploids [3]..

'Synthetic', a new hexaploid hybrid (*T. turgidum* x *T. tauschii*) [13] was reported the most tolerant cultivar to greenbug and RWA [4]. The chromosomes involved with antixenosis, antibiosis and tolerance were identified in two sets of intervarietal chromosome substitution lines between recipient 'Chinese Spring' and 'Synthetic' and 'Hope' donors [5]. Different sets of genes determined resistance to both aphid species. Nonetheless, the group of chromosomes 7 of the substitutions CS/Syn were associated with the highest levels of antixenosis against RWA and a remarkable level of this mechanism against greenbug. The 7D has also been reported to carry a resistance gene against *Mycosphaerella graminis* [1, 21]. The use of dihaploid recombinant lines allowed mapping that gene in the short arm of the 7D chromosome between *Rc3* (red coleoptile) and the wheat microsatellite WMS912 [1].

The 7D chromosome of Syn results of high interest for mapping resistance genes to pathogens and aphids. The aim of this work was to study antixenotic resistance in a group of dihaploid recombinant lines of single 7D chromosome of the CS/Syn set and to link the gene/s of this mechanism of resistance to molecular markers.

2.MATERIALS AND METHODS

2.1. Plant Material

The 7D substitution line of a set of intervarietal chromosome substitution wheat lines has Chinese Spring' (CS) as the recipient line for donor 'Synthetic' [CS(Syn)] chromosomes [13]. One hundred and three dihaploid recombinant (DHR) lines were obtained by crossing the recipient parent (CS) with the 7D substitution line. These precise genetic stocks, that have proved to be useful in mapping resistance genes [1] obtained from Dr. A. Worland (John Innes Centre, UK), were tested for their resistance against the greenbug and the Russian wheat aphid (RWA).

2.2. Aphids.

Greenbugs were collected on wheat in the vicinity of Tres Arroyos (38° 20'S.L., 60° 15' W.L.), and reared on susceptible barley and wheat cultivars ('Bordenave Ranquelina' and 'Buck Ombú', respectively) under natural conditions in La Plata (36° 36' S.L.). The clone used has been characterised as biotype C and is the most aggressive and widespread throughout Argentina.

The RWAs were collected on *Triticum turgidum* conv. *durum*, in the same locality (Tres Arroyos) and reared on the susceptible wheat cultivar 'Buck Ombú' under the same conditions as were used to culture the greenbugs. One clone was isolated from the field collected aphids for use in the experiments reported here.

Aphids were reared on the test plants for three generations to avoid the effect of the previous host [20]. Two groups of trials (one for each aphid species) were performed in two different plant growth cabinets maintained at the same temperature, humidity and photoperiod conditions (20°C, 50% RH, 16:8 L:D, respectively).

2.3. Procedures

Antixenosis, when present in host plants, results in its avoidance by the insects. It was necessary to determine the level of antixenosis in recombinant lines before studying antibiosis or tolerance. Since tolerance tests require a constant level of aphid infestation, if antixenosis is known to occurs its effect can be compensated for adding an extra number of aphids, and antixenosis effects can influence aphid fitness in antibiosis tests.

Antixenosis was assessed by allowing aphids a free-choice among plants of similar growth stage (2nd fully expanded leaf). Seedlings were planted in pots (5 cm diameter x 10 cm height), following the method of Castro et al., [5] two trials were carried out simultaneously, one for each of the two aphid species. One plant of each parental variety (CS and Syn), of the 7D substitution line and of every recombinant line were planted singly in pots (106 pots). The pots were randomly placed in a circle, with the leaves of each plant

directed towards the centre of the circle. The amount of adult aphids was equivalent to ten aphids per plant (1060 adults); they were placed in a glass dish (50 cm diameter x 2 cm height) inverted over the leaves in the centre of the circle. To avoid the direction of the light influencing plant selection by the aphids, the assay was carried out in the dark. The parents, 7D substitution line and 103 recombinants were randomly placed in ten circles for each aphid species. Every assay consisted of these 10 replicates. The number of adult aphids on each plant was recorded 4 h and 30 h after infestation. The result for each genotype was the average of the two recordings in the ten replicates.

2.4. Map construction and QTL analysis

DNA was extracted from fresh young leaves using CTAB method. The following molecular markers were identified in 7D chromosome of Syn in the DHR lines by hybridisation with cDNA clones used as probes: *Xpsr604*, *Xpsr687*, *Xpsr490*, *Wms44*, *Wms111*, *Wms121*, *Wms295*, and *Wms4*. The markers Dms67, *rc3* (red coleoptile), *srb3*, (a resistance gene to *Stagnospora nodorum*) [16], and *St* (a resistance gene to *Septoria tritici*) [1] were also identified in the DHR as polymorphic and also used to map the genes involved with the mechanisms of aphid resistance. The presence or absence of every marker was identified by 1 or 0. The analysis of linkage for aphid resistance genes was determined using MAPMAKER/QTL 1.1

3. RESULTS AND DISCUSSION

The antixenosis against greenbug was not significantly different between CS, Syn and 7D (Table I), nonetheless, there were highly significant differences between the DHR lines. Compared to the parents and the substitution line, 65 recombinant lines showed significantly less number of aphids per plant, consequently higher antixenosis. The antixenosis against RWA was significantly higher in 7D compared to CS and Syn. Only 18 DHR showed as higher antixenosis as 7D.

Castro et al., [5] have discussed that the 7D chromosome should have some type of regulator gene, since it appears to have pleiotropic effects over other genes; 'Hope'and 'Syn' 7D chromosomes changed the lack of antixenosis in CS background when they were present. It is clear that only few DHR lines were more antixenotic than the substitution line, recombinant regions would enhance the expression of other genes in CS.

The first analysis of linkage with molecular markers showed that antixenosis to any of both aphids were not based on Mendelian genes. The analysis of quantitative distribution showed that antixenosis to greenbug was significantly linked to *Rc3* gene and to *Wms437* marker (Table I). The antixenosis to RWA was also significantly associated to *Wms437*. This QTL located in both arms of 7D chromosome, very close to the centromere, associated to antixenosis against both aphid species, seems to include the genes that have been reported to confer resistance to RWA in other lines [14], although in our plant material they are not Mendelian genes as was reported by Liu et al. [14].

More than one chromosome would be involved in antixenotic resistance to greenbug. It has been reported that the 7A and 7B chromosomes have a significant effect on that type of resistance against greenbug [5], and RWA [19].

The identification of a QTL that provide a broad resistance, at least against two aphid species, could be useful in breeding wheat resistance against aphids by antixenosis. Further analysis of other mechanisms of resistance would aid in understanding the underlying genetic bases that relate the different types of resistance for different biotic stresses.

Table 1: Linkage analysis of antixenosis type of resistance against greenbug (*Schizaphis graminum*) and RWA (*Diuraphis noxia*) determined in a set of 103 DHR lines for the 7D chromosome, parental wheats (CS and Syn) and the substitution line. Values flagged points out significant differences (*** P= 0.001; ** P= 0.01; * P= 0.05)

Marker	Position	Antixenosis		
		greenbug t- value	rwa t-value	RWA t-value
Xpsr604	0.0	-0.074	-0.247	-0.247
Xpsr687	67.0	-0.841	-1.651	-1.651
Wms295	121.3	1.344	0.408	0.408
Xpsr490	152.0	1.693	-0.359	-0.359
Rc3	167.3	**2.797*** **	-0.427	0.427
Wms111	176.4	-0.080	0.004	0.004
Wms44	182.9	0.357	-0.626	-0.626
Wms437	201.0	**2.270** **	**2.004** **	**2.004** *
Wms121	211.5	0.598	0.333	0.333
Srb	401.5	-1.238	-0.748	-0.748
D67	435.5	-0.723	-0.148	-0.148

4. REFERENCES

[1] Arriano L.S. Worland A. & Brown J.K., 2000. Location of *Septoria tritici* resistance gene in wheat. 6Th International Wheat Conference, Budapest, Hungary, p: 43.

[2] Castro A.M., Martín A. & Martín L.M., 1996. Location of genes controlling resistance against greenbug (*Schizaphis graminum*, Rond.) in *Hordeum chilense*. Plant Breeding 115, 335-338.

[3] Castro A.M., Vasicek A., Ramos S., Martín A., Martín L.M., & Dixon A.F.G., 1998. Resistance against greenbug (*Schizaphis graminum*, Rondani) and the Russian wheat aphid (*Diuraphis noxia*, Mordv.) in the Tritordeum amphiploids. Plant Breeding 117, 515-522.

[4] Castro A.M., Vasicek A., Ramos S., Worland A., Suarez E., Muñoz M., Giménez D.O., Clúa A.A., & Frangi P., 1999. Different types of resistance against greenbug (*Schizaphis graminum*, Rondani) and the Russian wheat aphid (*Diuraphis noxia*, Mordv.) in wheat. Plant Breeding, 118, 131-137.

[5] Castro A.M., Ramos S., Vasicek A, Worland A., Gimenez D.O., Clua, A.A. & E. Suarez, 2001: Identification of wheat chromosomes involved with different types of resistance against greenbug (*Schizaphis graminum*, Rond.) and the Russian wheat aphid (*Diuraphis noxia*, Mordvilko). Euphytica 118, 321-330.

[6] Dong H. & Quick J.S., 1995. Inheritance and allelism of Russian wheat aphid resistance in seven wheat lines. Euphytica 81, 299-303.

[7] Dong, H., Quick J.S. & Zhang J., 1997. Inheritance and allelism of Russian wheat aphid resistance in several wheat lines. Plant Breeding 116, 449-453.

[8] Du Toit F., 1987. Resistance in wheat (*Triticum aestivum*) to *Diuraphis noxia* (Homoptera: Aphididae). Cereal Res. Comm. 15, 175-179.

[9] Du Toit, F., 1989. Inheritance of resistance in two *Triticum aestivum* lines to Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 82, 1251-1253.

[10] Du Toit, F., Wessels W. &. Marais G.F., 1995. The chromosome arm location of the Russian wheat aphid resistance gene *Dn5*. Cereal Res. Comm. 23, 15-17.

[11] Hollenhorst, M.M. & L.R. Joppa, 1983. Chromosomal location of genes for resistance to greenbug in 'Largo'and 'Amigo'wheats. Crop Sci. 23, 91-93.

[12] Joppa, L.R., Timian r.G. & Williams N.D., 1980. Inheritance of resistance to greenbug toxicity in an amphiploid of *Triticum turgidum/ Triticum tauschii*. Crop Sci. 20, 343-344.

[13] Law C.N. & A.J. Worland, 1996. Intervarietal chromosome substitution lines in wheat- revisited. Euphytica 89, 1-10.

[14] Liu X. M., C. M. Smith, B. S. Gill, V. Tolmay 2001. Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. TAG 102 (4): 504-510.

[15] MARAIS, G.F. & F. DU TOIT, 1993. A monosomic analysis of Russian wheat aphid resistance in the common wheat PI 294994. Plant Breeding 111, 246-248.

[16] Nicholson P., Rezanoor H.N. & Worland A., 1993. Chromosomal location of resistance to *Septoria nodorum* in a synthetic hexaploid wheat determined by the study of chromosomes substitution lines in ´Chinese Spring' . Plant Breeding 110, 177-184.

[17] Nkongolo, K.K., J.S. Quick, W.L. Meyer, & F.B. Peairs, 1991. Sources and inheritance of Russian wheat aphid (*Diuraphis noxia*, Mordv.) resistance in *Triticum* species, amphiploids and *Triticum tauschii*. Can. J. Plant Sci. 71, 703-708.

[18] Schroeder-Teeter S., Zemetra R.S., Smith C.M. & Schotzko D.J., 1990. Monosomic analysis of Russian wheat aphid resistance in wheat. Agronomy Abstract Annual meetings of the ASA, CSSA and SSSA, Oct 21-26, p. 108.

[19] Schroeder-Teeter S., Zemetra R.S., Schotzko D.J., Smith C.M. & Rafi M., 1994. Monosomic analysis of Russian wheat aphid (*Diuraphis noxia*) resistance in *Triticum aestivum* line PI137739. Euphytica 74, 117-120.

[19] Sears E.R., 1958. The aneuploids of common wheat. Proc. 1st Int. Wheat Genet. Symp. Winnipeg, pp. 221-227.

[20] Shufran, K.A., Margolies D.C & Black IV W.C., 1992. Variation between biotype E clones of *Schizaphis graminum* (Homoptera: Aphididae). Bull. Entomol. Res. 92, 407-416.

[21] Simon M.R. Worland A., Cordo C.A. & Struik P.C., 2001. Chromosomal location of resistance to *Septoria tritici* in seedlings of a synthetic hexaploid wheat, *Triticum spelta* and two cultivars of *Triticum aestivum*. Euphytica 118, 127-132.

by Sullivan [7]. Leaf water potential was determined by pressure chamber while osmotic potential by micro-osmo-meter as described by Slavik [8]. Leaf water loss was determined on leaves sampled from one plant growing on each moisture level by weighing them immediately after sampling and re-weighing after 24 hours desiccation and oven drying. Relative water contents were determined by the method of Weatherly [9]. Na+ and K+ contents were determined by flame photometer using dry leaves and cell sap. The cell sap was extracted by centrifugation of frozen samples. The experiment was conducted in randomized complete block design using three replications.

RESULTS

Injury percentage (IP) induced by PEG decreased with decreasing moisture contents with considerable difference in *Aegilops* species and wheat cultivars. *Ae. cylindrica* showed minimum injury at 100 % moisture, while *Ae. tauschii* and wheat cultivar Chakwal the maximum (Fig. la). At 25% moisture, IP was the lowest with no significant difference either between *Aegilops* species or wheat cultivars. The injury percentage showed negative correlation (r = -0.88, -0.56 and -0.89) with relative water content (RWC), osmotic potential (OP) and plant dry weight (DW) respectively (Table-1). The RWC decreased progressively with decrease in moisture contents (Fig 1b). *Ae.cylindrica* exhibited minimum reduction in relative water content and showed positive correlation with osmotic potential (0.87, 0.85 and 0.71) and plant dry weight (0.87, 0.89, and 0.85) respectively at al the three moisture levels.

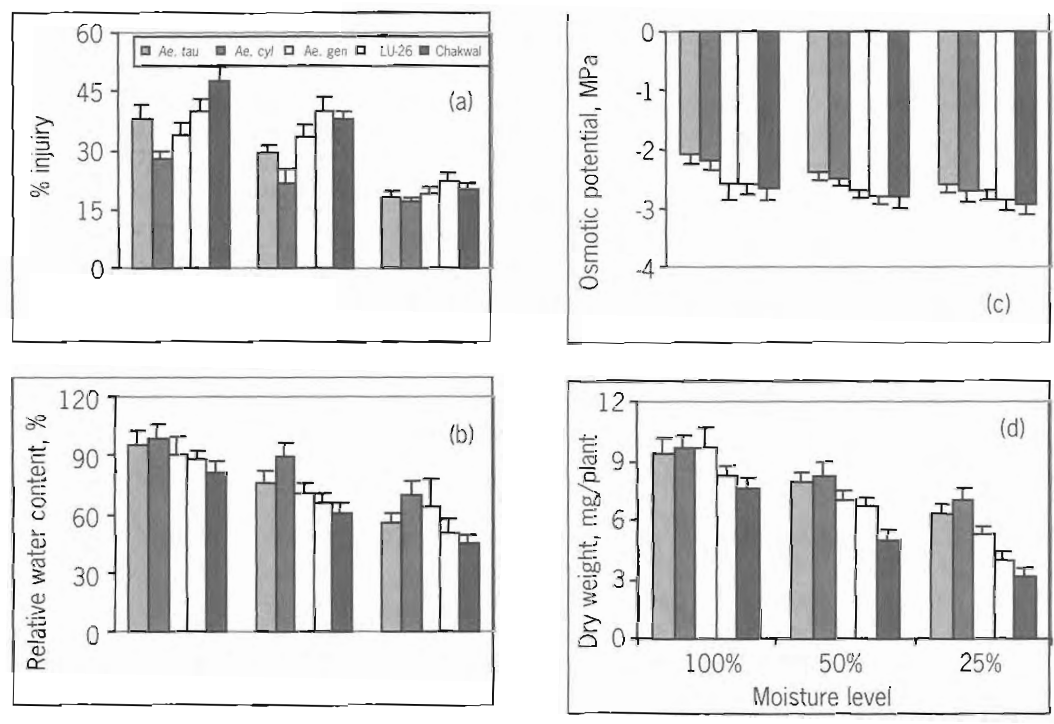

Figure 1. Percent injury (a), relative water content(b), osmotic potential (c), and dry weight of 3 Aegilops speices and 2 wheat cultivars as affected by 3 moisture levels.

Table 1. Correlation matrix between different parameters studied in wheat and *Aegilops* species growing at 3 Moisture levels; values >0.9 are significant at 0.05.

	ML	RWC	LWL	OP	DW
Injury	100	-0.88	0.89	-0.56	-0.89
	50	-0.96	0.88	-0.86	-0.80
	25	-0.73	0.98	-0.77	-0.84
RWC	100		-0.98	0.87	0.87
	50		-0.85	0.82	0.89
	25		-0.71	0.67	0.85
LWL	100			-0.84	-0.93
	50			-0.53	-0.83
	25			-0.83	-0.90
OP	100				0.64
	50				0.86
	25				0.90

Osmotic potential decreased with decrease in moisture levels (Fig. 1c). It was significantly correlated (r =0.90) with plant dry weight especially at 25% moisture level. Plant dry weight also decreased with decrease in moisture level with considerable difference between *Aegilops* species and wheat cultivars (Fig. 1d).

Wide variability was observed in Na+ and K+ contents (Fig. 2) between and within *Aegilops* species and wheat cultivars. Drought tolerant wheat cultivar Chakwal-86 exhibited comparatively higher K+ contents compared to the salt tolerant wheat cultivar LU-26. *Ae. cylindrica* exhibited the highest K+/Na+ ratio. Contents of K+ were also very high in cell sap of *Ae. cylindrica* and remained high in the leachate as well. The percent leakage of K+ was the lowest in drought tolerant wheat cultivar Chakwal-86 followed by *Ae. tauschii* and *Ae. cylindrica* (Table 2). The contents of Na+ in the sap of both the wheat cultivars were similar and significantly higher than *Ae. cylindrica* exhibiting the lowest. The contents of Na+ in the leachate also remained very low.

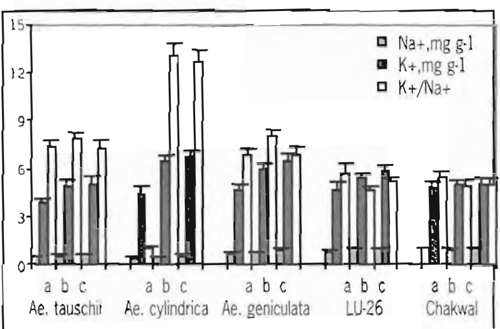

Figure 2. Content of Na+ and K+ (mg g-1 dry weight) And K+/Na+ ratio in 3 *Aegilops* species and 2 wheat cultivars growing at 3 moisture levels

DISCUSSION

The cell membrane stability (CMS) is a measurement of resistance against injury caused to plants by desiccation induced artificially with polyethylene glycol [7]. It has been used successfully for drought tolerance screening in soybean [10], sorghum [11], maize [12] and wheat [13]. In the present study we used CMS technique to screen for drought tolerance, salt tolerant accessions of different *Aegilops* species and two wheat cultivars. We have observed maximum injury in wheat cultivar Chakwal-86 at 100 % moisture level. Since Chakwal has been developed for drought prone areas therefore, as such, it is unfit for cultivation in areas with high water potential which enhances injury percentage particularly in stress tolerant wheat lines (unpublished data). Chakwal-86 is therefore, tolerant to a type of drought induced by low water potential rather than low osmotic potential. Conversely, minimum injury was observed in salt tolerant *Ae. cylindrica* which is an indication of its drought tolerance as well. However, comparatively low magnitude of injury indicated that *Ae. cylindrica* may become tolerant to a type of drought that is induced by low osmotic potential. Since plants growing in both salinity and drought suffer from non availability of water therefore, in both the cases osmotic adjustments are made by accumulating different kinds of compatible solutes in the plant

cells [14, 15]. In drought situation, the osmo-protectant accumulated in wheat includes sugars and potassium [16] while under saline conditions, it is mainly the glycinebetaine [17] and selective up take of potassium [18]. We have determined only the contents of potassium that appeared maximum in *Ae. cylindrica*. K+ contents, K+/Na+ ratio and its relation with salt tolerance in *Ae. cylindrica*, and in its hybrid derivatives has been established [5, 19]. Drought tolerant wheat cultivar Chakwal also possessed higher K+ content than salt tolerant wheat cultivar LU-26. Thus, high K+ contents in *Ae. cylindrica* followed by *Ae. tauschii* and *Ae. geniculata* particularly at 50 and 25% moisture levels also indicated that *Aegilops* species may also prove drought tolerant and confirmed the earlier observation made by Rekika et al, [20]. It could be inferred from this study, that salt tolerant *Aegilops* species could prove drought tolerant in the saline areas where low osmotic potential prevails due either to the evaporation of the water from the soil or due to successive irrigation with saline water.

Table 2. Contents of Na+ and K+ in cell sap and post-PEG test leachate in three *Aegilops* species and two wheat cultivars grown at three moisture regimes

ML*	Na+, mmol/L		K+, mmol/L		% in leachate	
	S	L	S	L	Na+	K+
			Ae. tauschii			
100	17.9	9.8	127.8	90.5	54.3	74.7
50	21.3	12.2	166.5	116.5	59.1	69.9
25	24.7	14.1	181.6	124.6	57.0	68.6
			Ae. cylindrical			
100	16.8	9.4	154.8	109.4	55.7	70.6
50	18.9	10.9	213.5	155.1	57.5	75.6
25	20.0	11.4	218.5	157.9	58.8	72.3
			Ae. geniculata			
100	27.2	15.7	119.4	113.4	57.6	95.0
50	30.8	17.8	140.6	112.1	57.7	79.7
25	36.7	19.8	153.4	124.8	54.1	81.4
			LU-26			
100	33.2	18.6	114.3	109.6	56.1	95.9
50	41.7	23.6	121.3	107.7	56.5	88.8
25	42.5	23.0	131.7	116.8	54.2	88.7
			Chakwal			
100	33.3	18.1	171.5	118.9	48.2	69.3
50	42.0	21.2	175.1	122.9	50.4	70.2
25	45.3	22.0	179.8	129.9	48.7	72.3
LSD	4.7	1.2	1.8	12.2	4.3	6.4

C, cell sap; L, cell leachate, ML, moisture level in %,

REFERENCES

[1] Gassemi F, Jakman AJ, and Nix AG: Pakistan. In: Salinization of land and water resources; human causes, extent, management and case studies. University of New South Wales Press Ltd., Sydney. 369-395 (1995)

[2] Farooq S, Asghar M, Iqbal N, Askari E, Arif M, and Shah TM: Production and evaluation of salt tolerant wheat germplasm produced through crossing wheat (*Tricticum aestivum* L.) with *Ae. cylindrica*-II. Field Evaluation of salt tolerant germplasm. Cereal Res Commun23: 275-282 (1995).

[3] Farooq S: Salt tolerance in *Aegilops* species: a success story from research and production to large scale utilization of salt tolerant wheat. In Proc. International Symposium on Prospect of Saline Agriculture in GCC countries, Dubai, UAE, (in press), March 2001.
[4] Bartel D, Nelson D: Approaches to improve stress tolerance using molecular genetics. Plant Cell and Environment 17: 659-667 (1994).
[5] Farooq S, Niazi MLK, Iqbal N, and Shah TM: Salt tolerance potential of wild resources of the tribe *Triticeae*-11. Screening of species of the genus *Aegilops*. Plant & Soil 119: 255-260 (1989).
[6] Hoagland DR, Arnon DI: The water culture method for growing plants without soil. Calif Agric Exp Sta. Univ Calf., circular No 347 (1950).
[7] Sullivan CY: Techniques for measuring plant drought stress. In: KL Larson and JD Eastin (eds) Drought injury and resistance in crops. CSSA spec. Pub.2 CSSA, Madison, WI. Pp. 1-8 (1971).
[8] Slavik B: Methods of studying water relations. Pub. Springer Verlag, Berlin. (1974).
[9] Weatherly PE: Studies in the water relations of cotton plants I. The field measurement of water deficit in leaves. New Plants 49: 81-97 (1950).
[10] Martineau JR, Williams JH, Speght JE: Temperature tolerance in soybeans-II. Evaluation of segregating population for membrane thermo-stability. Crop Sci 19: 79-81 (1979).
[11] Sullivan CY and Ross WM: Selection for drought and heat tolerance in grain sorghum. In: H Hussel and R Staples (eds) Stress Physiology in crop plants. Wiley-Interscience, New York, pp 263-281 (1979).
[12] Premachandra GS, Saneoka H, Ogta S: Nutrio phsiological evaluation of the polyethylene glycol test of cell membrane stability in maize. Crop Sci 29: 1292-1297 (1989).
[13] Blum A, Ebrecon A: Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci 21: 43-47 (1981).
[14] Arakawa K, Katayama M, Takabe T: Levels of betaine and betaine aldehyde dehydrogenase activity in the green leaves and etiolated leaves and roots of barley. Plant Cell Physiology31: 796-803 (1990).
[15] Acevedo E, Fereres E: Resistance to abiotic stresses. In: MD Hayward, NO Bosemark and I Romagosa (eds) Chapman and Hall, London. pp. 406-421 (1993).
[16] Bartel D, Engelhardt K, Roncarati R, Schneider K, Rotter M, Salamini F: An ABA and GA modulated gene expressed in the barley encodes an aldase reductase related protein. EMBO Journal 10: 1037-1043 (1991).
[17] Grumet R, Hanson AD: Genetic Evidence for an osmo-regulatory function of glycinebetaine accumulation in barley. Australian Journal of Plant Physiology 13: 353-364 (1986).
[18] Maathuis FJM, Amtmann A: K+ Nutrition and Na+ Toxicity: The basis of cellular K+/Na+ ratios. Annals of Botany 84: 123-133 (1999).
[19] Farooq S, Asghar M, Askari E and Shah TM: Production and evaluation of salt tolerant wheat germplasm produced through crossing between wheat (*Triticum aestivum* L.) and *Aegilops cylindrica*.I. Production of salt tolerant germplasm. Pak J Bot 26: 283- 292 (1994).
[20] Rekika D, Havaux M, Araus JL, Monneveux P: Variation for physiological traits related to abiotic stress tolerance in *Aegilops* species. In: AA Jaradat (eds) Triticeae III. Science Publisher Inc. USA. pp. 293-304 (1998).

BARLEY ADAPTATION PATTERNS IN NORTHERN SPAIN

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ABSTRACT: To succeed, a crop needs to match its growth cycle to the available resources. This is especially true under dryland Mediterranean conditions. Thus, knowledge of the relationship between patterns of development and yield is critical to establish sensible goals in breeding programmes. A set of 32 cultivars is being assayed in autumn-sown yield trials across Northern Spain since 1995. The purpose of this experiment is to test a reference set of genotypes to create a representative database of barley responses to local conditions. The cultivars were chosen to represent the genetic diversity of barleys currently grown in Spain: two- and six-row, winter and spring, feed and malting types, and diverse geographic origins (non-Spanish European, Spanish, ICARDA). Cultivar responses to photoperiod and temperature were characterized under contrasting environments in artificial environments. The study was completed with RFLP characterization of the materials, with 54 probes, 25% of which were reportedly close to loci controlling heading date. Genotype-by-environment interaction for grain yield was large, and dominated by genotype-by-location-by-year interaction. Only a longer series of data will settle whether geographic patterns are present. There was no single cultivar performing well at all trials. There was no simple relationship of either vernalization requirement or photoperiod sensitivity with grain yield. Highest yielding cultivars presented a variety of growth patterns. Early spring or winter cultivars, and winter types with a strong vernalization need were clearly the least favourable. Additive-multiplicative models were used to assist in the definition of environment types and groups of genotypes with specific adaptations.

1. INTRODUCTION

Genetic improvement has continuously increased yields of cereals over the last century. However, in few places is this activity as challenged as in the Mediterranean area. Diversity and unpredictability of growing conditions, and the presence of large genotype-by-environment interaction complicate the already complex task of plant breeders. To succeed in maintaining genetic gains, an understanding of genetic and environmental causes of genotype-by-environment interaction is crucial. Though there are other studies that pertain to this area, the singularities of barley cultivation in the Iberian peninsula advise local investigation of barley adaptation patterns.

The objective of this study is to provide a progress report on the analysis of genotype effects and genotype-by-environment interaction of a reference set of barley genotypes in Northern Spain. We will try to shed some light on the nature of the patterns observed crossing the data with information on molecular markers, and results from experiments on development of plants under controlled conditions. This experiment is becoming a long-term series of trials, and will continue in the foreseeable future.

2. MATERIALS AND METHODS

2.1. Materials

Thirty two cultivars or breeding lines representing the diversity of barley types grown in Spain (2- and 6-row; spring, winter, and facultative types; diverse geographic origins and breeding histories; malt and feed types) were chosen as a reference set to test the reactions of barley against Spanish barley growing conditions.

The provinces chosen for the field experiments (Lleida, Navarra, Valladolid, Zaragoza) are scattered throughout the Northen half of Spain. They represent the major barley growing areas of this part of the country.

2.2. Methods

Field trials were carried out at locations near local experimental stations at the four provinces since 1995. Autumn and winter sowings were done, though only the results for the autumn ones are reported here. Some trials failed for a variety of reasons, and thus years and locations appear unbalanced.The experimental design was an alpha-lattice, with 3 replicates. Plot size was 1.5 by 7 m (6 rows). Plot grain yield and heading date were recorded.

The responses of genotypes to photoperiod and temperature (vernalization requirement) were evaluated at the SIDTA (Valladolid) under controlled conditions in greenhouses. Genotypes were subjected to four treatments, combination of presence or absence of vernalization with long or short photoperiods. Details on the methodology and results for these experiment were provided in Igartua et al. [1]. The accumulated thermal time until heading and the number of leaves on the main stem were recorded.

Finally, the genotypes were genetically characterized with a set of 54 RFLP probes, 4 of them (Bmy1, CDO504, MWG858, MWG518) reportedly close to four major genes controlling temperature and photoperiod responses (*Sh, Sh2, PpdH1, and PpdH2*, respectively). These four probes showed association with heading dates in a subset of this series oif experiments [1]. Details on RFLP methodology are provided also provided in Casas et al. [2].

Patterns of Genotype-by-environment interaction were investigated with the use of the GGE biplot [3], and the stability variance [4]. All analysis were performed with SAS [5]. Environments were considered random, and genotypes fixed.

3.RESULTS AND DISCUSSION

Location-by-genotype component was 50% larger than year-by-genotype, suggesting that genotype-by-environment variance may present geeographic structure (Table I). Triple interaction year-by-location-by-genotype, however, was dominant in this series of trials, suggesting a high degree of unpredictability.

Table I. Grain yield variance components for the combined analysis of variance across 14 Northern Spain environments

Variance components (REML)	Estimate
Year	2.567
Location	0.723
Year x Location	0.341
Rep (Year x Location)	0.030
Year x Genotype	0.030
Location x Genotype	0.048
Year x Location x Genotype	0.088
Residual	0.305

Genotype fixed effect was highly significant. Considered as a random factor, it would have contributed half as much variance as genotype-by-environment interaction (not shown).

Thus, genotype-by-environment interaction was paramount in this dataset, but there was some sizable geographic pattern.

Table II. Description of genotypes used in the experiments. Mean grain yield and Shukla's stability variance across field trials.

Genotype Spring types	Rows	Origin	Grain yield (kg/ha)	Stab.var.
Albaicín	2	Spain	3493	2.39
Alexis	2	Germany	3648	2.83
Beka	2	France	3395	2.04
Camelot	2	France	3919	3.09
Cameo	2	UK	3807	3.00
Graphic	2	UK	4285	2.92
Hassan	2	Netherlands	3533	2.29
Kym	2	UK	3589	2.77
Nevada	2	France	4075	2.40
Pallas	2	Sweden	3521	2.62
PC-4	2	Spain	3490	2.45
S-36	6	ICARDA	3283	2.64
S-7	2	ICARDA	3491	2.58
Tremois	2	France	3553	2.33
Volga	2	France	3995	3.15
Zaida	2	Spain	3633	2.08
Average spring			**3454**	**2.45**
Winter and facultative				
Albacete	6	Spain	3242	1.82
Alpha	2	France	3721	2.50
Angora	2	Germany	3289	2.02
Barberrousse	6	France	4082	3.15
Candela	6	Spain	3942	2.98
Clarine	2	France	3337	2.09
Dobla	6	France	3629	3.09
Gaelic	2	France	3983	3.31
H-206	6	Spain	3311	1.70
Hispanic	2	France	4320	3.54
Labea	2	France	3521	3.06
Monlón	6	France	3603	2.34
Orria	6	CIMMYT, Spain	4390	3.77
Plaisant	6	France	3543	2.97
S-45	6	ICARDA	3567	2.11
Tipper	2	UK	3745	3.06
Average winter/fac.			**3484**	**2.56**

High yielding genotypes were scattered across cultivar types (Table II). Cultivars with strong winter habit presented poor adaptation (*Angora, Clarine, Labea, Tipper, Alpha*, and *Plaisant*) except *Barberrousse*. This is a high-yielding 6-row cultivar that consistently presented good adaptation in Valladolid, and bad results in Lleida (Fig. 1).

Table III. Description of environments and mean grain yield across genotypes.

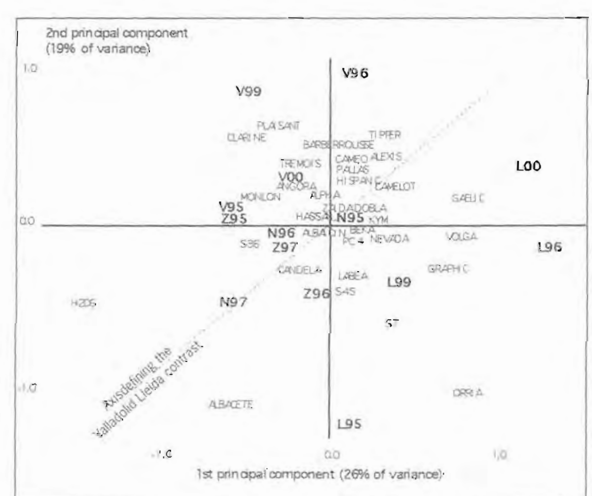
Location	Year	Mean grain yield (kg/ha)
Zaragoza	1995	701
Zaragoza	1997	1326
Valladolid	1995	1388
Navarra	1997	2142
Navarra	1995	2384
Zaragoza	1996	2622
Lleida	1995	2890
Valladolid	1996	4653
Lleida	1999	4784
Lleida	1996	5191
Valladolid	1999	5396
Navarra	1996	5580
Lleida	2000	5858
Valladolid	2000	6681

High overall yields were associated with large stability variance, suggesting poor general adaptation of the genotypes (Table II). One notable exception was cultivar *Nevada*, with high mean yield and low stability variance. *Orria* was the most productive genotype overall, but with the largest stability variance, due to its remarkable good adaptation at Lleida's trials.

Spring types presented lower mean stability variance than winter types, especially if not considering the two cultivars (Albacete and H-206) derived entirely from local germplasm.

Among the top 25% genotypes there were three relatively late heading spring genotypes -*Graphic, Nevada, Volga* -, three facultative - *Gaelic, Orria, Candela* - the last two locally bred, one winter geno type with low vernalization requirement - *Hispanic* -, and one fully winter genotype - *Barberrousse*-.

Genotype and genotype-by-environment effects were further investigated by means of the GGE biplot [x]. This combined representation of genotypes and environments just removes the variation due to differences among environments (Figure 1). The first two principal components accounted for 45% of the GGE. A third component (not shown) accounted for 14% of the variance. A first look at the biplot suggests that a contrast between Valladolid and Lleida dominated the environment distribution for the first two components. These were locations with relatively high yields (Table III). Valladolid consistently presents the best growing conditions, and trials at Lleida received some supplemental irrigations.



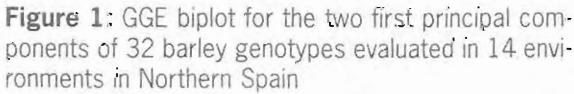


Figure 1: GGE biplot for the two first principal components of 32 barley genotypes evaluated in 14 environments in Northern Spain

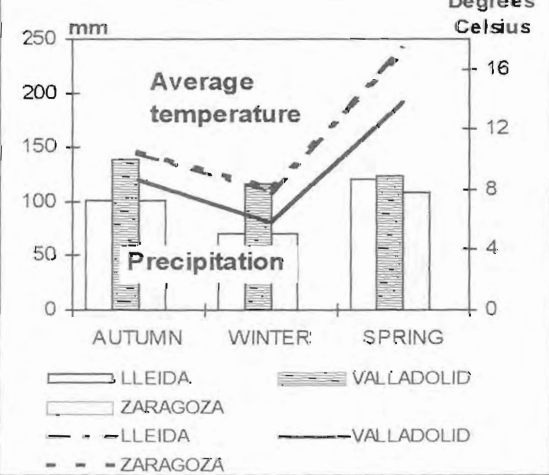


Figure 2. Long-term seasonal averages for mean temperature and rainfall (province capitals). Navarra was not included because of lack of representative data.

This contrast was mainly the result of winter cultivars yielding relatively better at Valladolid (coldest location), and late spring cultivars (*Volga*, *Graphic*) and *Orria* yielding better at Lleida. The climatic differences between these two locations may shed some light on this contrast (Figure 2). Valladolid (representative of North Plateau, main region for barley cultivation in Spain) is colder and more humid overall. Accordingly, winter types would be favoured.

The locations with lowest mean yields tended to concentrate on the lower left side of the graph (Table III, Figure 1). The two entirely Spanish cultivars, with low grain yield overall, presented specific adaptation for these environments. A genetic diversity analysis [2] revealed the large divergence between the Spanish cultivars and the rest of the genotypes, suggesting the possible existence of local adaptation traits.

This situation is consistent with findings by Muñoz et al. [6], which revealed that genetic improvement in the last decades had an impact only on relatively high yielding areas, whereas the gain for low-productive areas was negligible, even negative. All the European (and Spanish-bred from foreign parents) cultivars, were more adapted to either Valladolid or Lleida, the high yielding locations. The two Spanish cultivars (*Albacete* and *H-206*), and even some ICARDA genotypes (*S-7* and *S-45*) appeared closer to low-yielding environments (Figure 1).

Nevada was the most stable-high-yielding genotype overall (even in further principal components, not shown). Thus, specific adaptations seem to dominate the picture, but the possibility to get overall adaptation cannot be ruled out.

Table IV. Relationship of grain yield and GGE principal components (PC1, PC2, PC3) with other phenotypic and genetic (RFLP probes) variables.

Variable	Grain yield	PC1	PC2	PC3
a) Phenotypic				
	Linear correlation coefficients			
PC1	0.62*			
PC2	0.00			
PC3	0.37*			
Heading date	-0.14	-0.28	0.14	0.09
Photoperiod sensitivity	-0.08	-0.52*	-0.21	0.42*
Vernalization response	-0.05	-0.34*	0.19	0.22
b) Genetic				
	Significance in ANOVA			
Bmy1, HindIII	0.07	0.02	<0.01	0.32
CDO504, EcoRI	0.02	0.03	0.94	0.02
MWG858, EcoRI	<0.01	<0.01	0.58	0.48
MWG518, HindIII	0.70	0.09	0.91	0.06
R2	46	67	50	41

The possible relationship of genotype-by-environment interaction with phenological characters was investigated. Table IV, section (a), summarizes a preliminary analysis based on linear correlations. It is remarkable that the coefficient between grain yield and heading date was close to 0. This was also true in most cases when correlations were calculated individually for each trial (not shown). Thus, earliness is not a desirable trait for autumn sowings in this geographic area, though early genotypes are considered by dryland farmers as a 'lesser evil', or a safe option. Grain yield appeared associated with the first and third principal components from the GGE analysis. Interestingly the second principal component, unrelated to mean yield, was certainly related to both photoperiod and temperature response. Probably, these last traits are more related to specific adaptations. Photoperiod appears as more relevant in this context than vernalization, as its correlation coefficients with grain yield principal components were overall larger.

On the other hand, a model to explain grain yield and the principal components with the four RFLP probes associated to major phenology genes gave different results. Vernalization genes seemed to be more involved than photoperiod ones in the explanation of GGE patterns. The models analysed included all four

probes at the same time, without interactions. However, these results should be taken cautiously. Close linkage between these probes and genes needs further proof in some cases. On the other hand, similar analyses should be carried out with a large set of 'neutral' markers, to be able to really test the significance of the results just presented (or similar analyses). Currently, several studies addressing these aissues are ongoing.

REFERENCES

[1] Igartua E, Casas SM, Ciudad FJ, Montoya JL, Romagosa I: RFLP markers associated with major genes controlling heading date evaluated in a barley germ plasm pool. Heredity 83: 551-555 (1999).

[2] Casas AM, Igartua E, Vallés MP, Molina-Cano JL: Genetic diversity of barley cultivars grown in Spain estimated by RFLP similarity and coancestry coefficients. Plant Breeding 117:429-435 (1998).

[3] Yan W, Hunt LA, Sheng Q, Szlavnics Z: Cultivar evaluation and mega-environments investigation based on the GGE biplot. Crop Science 40:597-605 (2000).

[4] Shukla,Martín GK: Some statistical aspects of partitioning genotype-environmental components of variability. Heredity 29: 237-245 (1972).

[5] SAS Institute: SAS/STAT Guide for Personal Computers, Version 6. SAS Institute Oinc., Cary, NC.

[6] Muñoz P, Voltas J, Araus JL, Igartua E, Romagosa I: Changes over time in the adaptation of barley releases in north-eastern Spain. Plant Breeding 117:531-535 (1998).

BEKA/MOGADOR, A NEW BARLEY DOUBLED HAPLOID POPULATION FOR ADAPTATION STUDIES IN THE MEDITERRANEAN REGION

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ABSTRACT: Heading time in barley is controlled by three constituent characters, namely vernalization requirement, photoperiod sensitivity and earliness per se, or earliness in a narrow sense. Three genes, *Sh*, *Sh2 and Sh3*, located on chromosomes 4 (4H), 7 (5H) and 5 (1H) respectively, are responsible for winter/spring growth habit and vernalization response. Two more genes, *Ppd-H1 and Ppd-H2* on chromosomes 2 (2H) and 5 (1H), respectively, control photoperiod response. We present a progress report on the study of genetic control of flowering, and its relationship with grain yield, for a new doubled haploid population: Beka/Mogador. Beka is an old spring cultivar; Mogador is a winter cultivar. One hundred and twenty F1-derived doubled haploids were obtained via anther culture. Field heading date was recorded at several trials across Northern Spain. Grain yield was recorded at two contrasting field sites in 2001. Vernalization and photoperiod responses were assessed under controlled conditions. They were also assayed for a total of 156 molecular markers, including SSRs, AFLPs, RAPDs, and RFLPs. The resulting map was used to map QTL for phenological traits. Only four loci with significant effects were consistently identified both under field and controlled conditions, explaining 57-85% of measured variables. Three were coincident with previously described locations. Another one was placed near Ppd-H1, but in a region about 20 cM apart. Its pattern of effects on several traits, however, suggests it may well be *Ppd-H1*. Simple and interaction effects of the four loci are described, and preliminary data on their possible complex effect on grain yield, through a three-way epistatic interaction, are presented.

1. INTRODUCTION

The well-known wide adaptability of barley depends on a combination of many traits. One of the most important is the adjustment of crop phenology to resources and constraints of the production environment, especially under water limiting conditions. During the last years there has been an increasing number of studies aimed at revealing the genetic control of the development of the barley plant. A good review in this subject can be found in Laurie [1]. Three genes, Sh, Sh2 and Sh3, located on chromosomes 4 (4H), 7 (5H) and 5 (1H) respectively, are responsible for winter/spring growth habit and vernalization response, though only the first two segregate in temperate regions germplasm. Two more genes, Ppd-H1 and Ppd-H2 on chromosomes 2 (2H) and 5 (1H), respectively, control photoperiod response [2].

The objectives of this study were to analyze the genetic control of heading date in a spring-by-winter cross, which would provide ample opportunity for segregation. Previous information on the parents suggested that they differ at least at three loci linked to three of the major genes cited above [3].

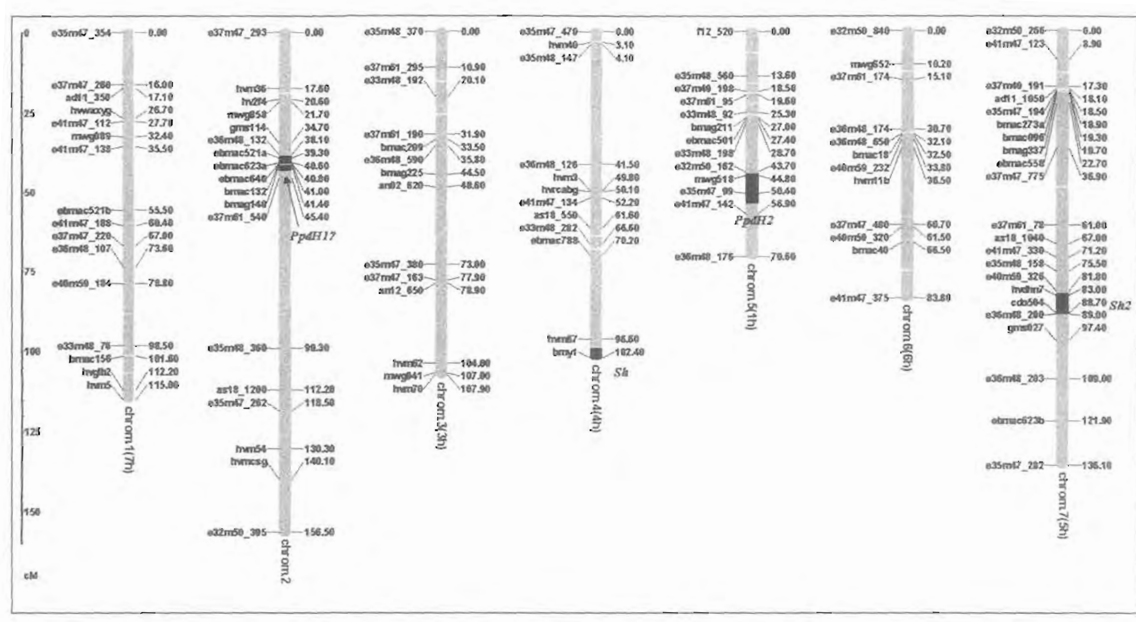


Figure 1. Linkage map of Beka/Mogador. Only 107 markers, out of 156, are represented. Locations of QTL responsible for heading date control, and candidate linked genes, are highlighted.

2. MATERIALS AND METHODS

2.1. Materials

A population of 120 doubled haploids was developed via anther culture from the F1 of the cross Beka/Mogador. Beka is an old spring cultivar; Mogador is a winter cultivar.

These entries were tested for a variety of molecular markers, which provided the following polymorphisms: 48 SSR, 86 AFLP, 15 RAPD, and 7 RFLP. Four of the RFLP probes are placed close to Sh, SH2, Ppd-H1 and Ppd-H2, respectively.

2.2. Methods

Field trials were carried out at locations near local experimental stations at the three provinces since 1998. The provinces chosen for the field experiments (Lleida, Valladolid, Zaragoza) are representative of the major barley growing areas of the Northern half of Spain. Autumn sowings were done at every location almost every year, and occasionally some winter sowings were done. A total of five autumn sowings and a winter one offered good quality data. Entries were sown unreplicated, on 2-row plots, 1.5 m long. In 2000, two yield trials were sown at Lleida and Zaragoza The experimental design was an alpha-lattice, with 3 replicates. Plot size was 1.5 by 7 m (6 rows). Plot grain yield and heading date were recorded.

Greenhouse trials consisted of four treatments that combined two levels of temperature and photoperiod, namely: vernalization, followed by long photoperiod (V-L); vernalization, followed by short photoperiod (V-S); no vernalization, short photoperiod (NV-S); and no vernalization, long photoperiod (NV-S). The treatments were similar to those described in [3]. Final number of leaves on the main stem, as well as number of rosette and cauline leaves were recorded, though only the results for total number of leaves are reported here. This experiment was carried out at the SIDTA (Valladolid).

3. RESULTS AND DISCUSSION

3.1. Phenological traits

A map was constructed, and the software MQTL [4] was used for QTL mapping. Only four regions clearly affected heading date in field experiments, and leaf number variation in the greenhouse (not shown).

Table I. Means for the two alleles for greenhouse and field experiments.

Treatment	Locus	Allele	
		Beka	Mogador
Greenhouse		*number of leaves*	
V-L	CDO504*	8.4	8.91
V-L	BMY1	8.62	8.69
V-L	MWG518*	8.5	8.8
V-L	BMAC132*	9.07	8.23
V-S	CDO504*	9.81	10.27
V-S	BMY1*	10.44	9.64
V-S	MWG518*	9.22	10.86
V-S	BMAC132*	10.92	9.16
NV-L	CDO504*	7.94	10.42
NV-L	BMY1*	8.3	10.06
NV-L	MWG518	9.06	9.3
NV-L	BMAC132*	9.7	8.66
NV-S	CDO504*	10.84	11.75
NV-S	BMY1	11.18	11.42
NV-S	MWG518*	10.32	12.28
NV-S	BMAC132*	11.78	10.81
Field		*days to heading*	
WINTER	CDO504*	66.3	81
WINTER	BMY1*	70.6	76.7
WINTER	MWG518	72.9	74.4
WINTER	BMAC132*	77.2	70.1
AUTUMN	CDO504	156.8	157.4
AUTUMN	BMY1*	156.5	157.7
AUTUMN	MWG518*	155	159.2
AUTUMN	BMAC132*	160.3	153.9

*significant $P<0.05$

Models with these four major QTL and their interactions accounted for a large proportion of the variation in field heading date (78-85%) and number of leaves produced at greenhouse treatments (57-84%). Three of these regions were coincident with expected locations for major heading date loci: the HVM67-Bmy1 interval on chromosome 4 was close to Sh; the HVDHN7-CDO504 interval on chromosome 7(5H) was close to Sh2; and MWG518-E35M48-98, on 5(1H) was close to Ppd-H2. On the other hand, the QTL detected on 2HS was about 20 cM proximal compared to previously described positions for PpdH1.

No grain yield QTL were detected at either of the two 2001 trials using MQTL.

The effects of the four major QTL detected were calculated in SAS [5] are summarized in Table I:

- Bmac132, in the PpdH1 region had the largest effect across treatments and experiments, but its effect was not merely earliness per se, as it was enhanced under vernalization and short photoperiod combined. Besides, it presented significant interactions with other photoperiod and vernalization QTL at several treatments;
- CDO504 (Sh2) effect was most evident without vernalization;
- MWG518 (PpdH2) effect was most evident under short photoperiod,
- Bmy1 presented the most complex behaviour, with effects on both photoperiod and vernalizations responses.
- QTl from regions with vernalization loci affected field heading date at the winter sowing. Regions with photoperiod response loci mostly affected heading date at autumn-sown trials.

Several significant interactions were detected at some treatments (Bmy1 x CDO504, Bmy1 x MWG518, Bmac132 x CDO594, Bmac132 x Bmy1, Bmac132 x MWG518, and the three-way interaction Bmac132 x MWG518 x Bmy1. These interactions suggest non independence of the vernalization and the photoperiod flowering-promotion pathways.

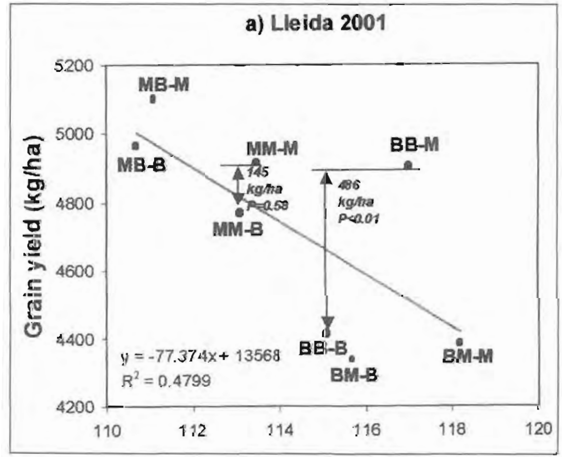
3.2. Yield-phenology QTL relationship

No single QTL was significant for grain yield at either location (not shown). When running an analysis of variance with just the four QTL on the combined locations, however, single QTL explained a significant proportion of grain yield variation (Table II).The explanation relies on the different growing conditions encountered at the two sites. Water stress affected differentially to the field trials, favouring intermediate heading in Zaragoza, and early heading in Lleida. The significant effects of Bmac132 and MWG518 were a carry over effect of the advantage of early type in Lleida. And we saw before (Table I) that the QTL nearby these two loci mostly affected earliness at autumn sown trials. The significant location-by-photoperiod QTL interactions were a result of the different relationship between earliness and grain yield.

Table II. Summary of the analysis of variance for grain yield at two environments, using as sources of variation the four major heading QTL.

Source of variation	DF	SS. type III	MS
Location	1	15865	15865
Genotype	119	3556	30 •
Bmac132	1	225	225 •
MWG518	1	83	83 •
Bmy1	1	101	101 •
Bmac132 x MWG518 x Bmy1	1	91	91 •
Location x Genotype	119	1986	17
Location x Bmac132	1	250	250 •
Location x MWG518	1	75	75 •

• significant P<0.05



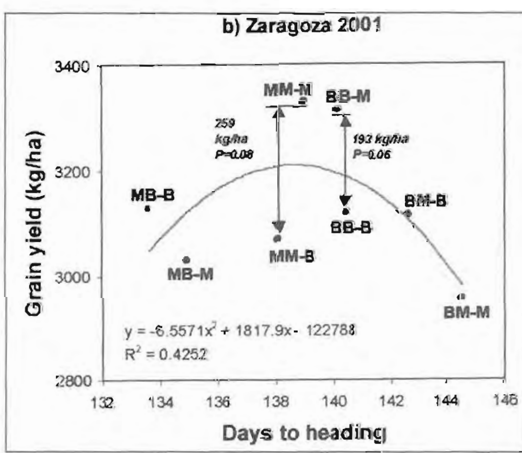


Figure 2. Grain yield represented against heading date at two locations in 2001. Points represent averages for groups of genotypes according to their allelic constitution at Bmac132, MWG518 and Bmy1 (B stands for Beka, M for Mogador). The contrasts causing the significant three-way interaction are also represented.

The most remarkable result was the presence of a significant three-way interaction (Bmac132 x MWG518 x Bmy1). This interaction was also evident for field heading date, and at three of the four greenhouse treatments. At this point, one can only speculate about the causes of this interaction. Karsai et al. [6] found that Ppd-H1 and Sh2 caused variations in several yield components, even through epistatic action. Only further analyses and, especially, more field trials can help to clarify this issue. At this point we can only conclude that the yield advantage of some haplotypes for the three loci was consistent across trials (Figure 2).

REFERENCES

[1] Laurie DA: Comparative genetics of flowering time. Plant Molecular Biology, 35:167-177 (1997).

[2] Laurie, DA, Pratchett N., Bezant JH and Snape JW: RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter x spring barley (Hordeum vulgare L) cross. Genome, 38, 575-585 (1995).

a. Igartua E, Casas SM, Ciudad FJ, Montoya JL, Romagosa I: RFLP markers associated with major genes controlling heading date evaluated in a barley germ plasm pool. Heredity 83: 551-555 (1999).
b. Tinker, N and Mather, DE: MQTL: software for simplified composite interval mapping of QTL in multiple environments 1995. J Agric Genomics 1:1-3 (1995).
c. SAS Institute: SAS/STAT Guide for Personal Computers, Version 6. SAS Institute Oinc., Cary, NC. (1987)
d. Karsai I, Mészáros K, Szücs P, Hayes PM and Z Bedö: Effects of loci determining photoperiod sensitivity (Ppd-H1) and vernalization response (Sh2) on agronomic traits in the 'Dicktoo'('Morex' barley mapping population. Plant Breeding 118:399-403 (1999).

ASSOCIATION BETWEEN A 24-KDA DEHYDRIN AND DROUGHT STRESS TOLERANCE IN WHEAT: A POSSIBLE SCREENING TECHNIQUE

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ABSTRACT: The Pacific Northwest (PNW) is an important region for winter wheat production in the USA, where water deficits are often present at sowing and during grain filling. Consequently, improving the genetic adaptation of wheat cultivars to drought stress represents an important objective. An observed response to dehydrative stresses is the accumulation of proteins called dehydrins, which are believed to protect membranes and macromolecules against denaturation. Seven wheat cultivars, 'Connie', 'Gene', 'TAM105', 'Rod', 'Hiller', 'Rhode', and 'Stephens', where evaluated under drought stress to characterize the accumulation of dehydrins in seedlings and their association to stress tolerance at adult plant. A 24-kDa dehydrin was observed in seedlings under stress, but they were not present in the irrigated controls. Connie, TAM105, and Gene started to accumulate dehydrins at the fourth day of stress, while the other cultivars showed dehydrins at the twelfth day of stress. The presence of this dehydrin observed in seedlings was related to stress tolerance characterized by a lower reduction in shoot dry matter production in Connie, TAM105, and Gene. This differential accumulation was also associated to stress tolerance at adult plant, characterized by a lower reduction in yield and in the rate of decrease in leaf water potential in Connie, TAM105, and Gene, that together with Rod where the most tolerant cultivars. The results indicate that this 24-kDa dehydrin might be used to develop a rapid screening technique at seedling stage. Selection experiments using a population segregating for stress tolerance are needed to test more conclusively whether or not this dehydrin can be used to select for cultivars with tolerance to drought stress.

1. INTRODUCTION

Most wheat-producing regions of the world are subjected to water deficits during some part of the growing season (1). Although wheat breeders have been highly effective in increasing yields under productive cultivated conditions, genetic gains have been more difficult to achieve in production regions where environmental stresses such as low water availability and high temperature are present (2, 3, 4).

The Pacific Northwest of the USA (PNW), is an important region for winter wheat production in the USA that is characterized by a Mediterranean climate with water deficits rising in spring and increasing in severity at anthesis and throughout grain filling (5). PNW winter wheat relies on water stored in the soil during the fallow period plus additional water received in fall and winter. As a consequence, improving the genetic adaptation of wheat to drought stress is one of the main objectives for wheat breeding programs in the region.

Plant breeding efforts to improve drought tolerance would be aided by the identification of biochemical markers associated with improved field performance under drought conditions. Dehydrins, also known as late embryogenesis abundant (LEA) D11 (6) proteins represent potential markers. They are members of a family of proteins that are expressed after plants are exposed to stresses with a dehydrative component. This family of proteins is characterized by the presence of a consensus amino acid sequence (EKKGIMDKIKELPG) near the carboxy terminus (7). Dehydrins can be detected using antibodies prepared against this

consensus sequence (7) and have been identified in at least 30 diverse plant species including wheat (8).

Dehydrins are expressed in different plant tissues including roots, leaves, coleoptiles seeds and crowns (7, 9) and some are associated with dehydrative stress tolerance. The expression of a specific dehydrin (WSC120) was associated with freezing tolerance in eight species of gramineae (9). Increased expression of dehydrin genes during the development of freezing tolerance was found in a more tolerant barley (*Hordeum vulgare* L.) cultivar (Dicktoo) relative to that in the less tolerant (Morex) (10). Using two sunflower (*Helianthus annus* L.) inbred lines one tolerant and one susceptible to drought stress, a higher accumulation of mRNA transcripts corresponding to HaDhn1, and HaDhn2 genes was observed in the tolerant line that was associated with cellular turgor maintenance under drought stress (11).

In a related study, a group of winter wheats adapted to the PNW showed differential accumulation of a 24 kDa dehydrin at seedling stage as a response to an impossed drought stress. The purpose of the present study is to test if this differential accumulation can be used as a screening technique to select cultivars with stress tolerance at adult plant stage.

2. MATERIALS AND METHODS

2.1. Seedling Experiments

Seven winter wheats including 'Stephens', 'Gene', 'Rod', 'Hiller', 'Rohde', 'Connie', and 'TAM 105', one barley cultivar, 'Strider', and 'Celia', a triticale, were evaluated in two greenhouse experiments under stressed (drought) and non-stressed conditions. Seeds of each cultivar were germinated at 20 °C for 48 h and, seedlings were selected for size and vigor. A row of ten seedlings of each cultivar were planted in pots containing sterilized sand. Each pot contained three cultivars. A system similar to the one developed by (12) was used to impose drought stress. Pots were placed on top of cylinders containing florist foam blocks as a hydraulic conducting medium. Roots were prevented from growing down the florist foam blocks by using a 5-micron nylon mesh at the bottom of the pot. The cylinders were connected to a tank (one for the stress treatment and one for the non-stressed control) containing a complete nutrient solution. The pots were placed in containers with a complete nutrient solution for 15 days (when the seedlings had approximately three leaves) and then transferred to the cylinders where the experiment started (first day). By using a floating valve in each tank, the water table was maintained at 4 cm from the bottom of the pots in the non-stressed treatment and at 12 cm in the stressed treatment. To increase the stress intensity, a ceramic disk (Soil Moisture Equipment Corp., Santa Barbara, CA) with an air exclusion of 0.5 MPa was inserted between the base of the pot and the florist foam in the stress treatment. The experimental design was a split-plot with four replications. Stress levels (drought and well-watered conditions) were the main plots, and sub-plot treatments (cultivars) were arranged in a randomized complete block design. From the first to the fifth day of treatment, leaf water potential (Ψ_l) was measured using a pressure chamber. All Ψ_l measurements were made between 12 PM and 2 PM. Immediately after Ψ_l was recorded, one seedling of each plot was cut and placed on dry ice. When all the determinations in the experiment were finished, the samples were stored at -80°C.

2.2. Adult Plant Experiments

After vernalization, four seedlings per cultivar were transferred to 5.6 liter pots containing a mixture of 25 % peat moss, 25 % silt loam, and 50 % pummice and finally thinned to two plants per pot. The soil was fertilized at a rate of 1.66 g of NPK fertilizer (16-16-16) and 1.45 grams of a slow release fertilizer (15-9-12) per liter of soil mixture. Plants were watered daily at 9 AM and once a week a complete nutrient solution was used instead of water. The experimental design was a split-plot with three replications. Stress levels (drought and well-watered conditions) were the main plots, and sub-plot treatments (cultivars) were arranged in a randomized complete block design. Each sub-plot consisted of six pots (12 plants). When plants reached flowering, progressive drought was imposed to stress plots by withholding water, while non-stressed plots continued receiving daily irrigation. Water withholding started independently for each cultivar as they reached flowering over a period of ten days. At the tenth day, plants were irrigated to saturation with the complete nutrient solution (same amount for each pot and cultivar), to start a second ten days cycle of progressive drought. Every other day from flowering (first sampling date) to the end of the second cycle (12th sampling date), leaf water potential (Ψ_l) was measured using a pressure chamber. Measurements were made on one flag leaf from a main tiller of each cultivar in all three replications. All Ψ_l measurements were made between 12 PM and 2 PM. At maturity all plants were hand harvested and yield recorded.

2.3. Western Blots

Only wheat cultivars were analized for the precence of dehydrin proteins. All sampled seedlings were lyophilized. Samples corresponding to the four replications in each treatment (stress and non-stress) within a sampling date were bulked to make one sample, so that 14 samples were analyzed per sampling date. Protein was extracted by grinding the samples in the presence of E buffer (125 mM Tris-HCL pH 8.8, 1 % (w/v) SDS, 10 % (v/v) glycerol, 50 mM Na2S2O2) according to (13) until a homogeneous mixture was obtained. The extract was transferred to a 1.5 ml microfuge tube and centrifuged at 14000 g for six minutes. An aliquot of the supernatant was used for protein concentration determinations and the rest was diluted (1/10 of the volume) with Z buffer (125 mM Tris-HCL pH 6.8, 12 % (w/v) SDS, 10 % (v/v) glycerol, 22 % (v/v) ß-mercaptoethanol, 0.001 % (w/v) bromophenol blue) (13). Samples containing 10 µg of total protein extracted from the seven cultivars (stressed and non-stressed) plus a pre-stained molecular weight marker (BenchMark, GIBCOBRL, Grand Island, NY) were electrophoresed in SDS-PAGE gels (14 % acrylamide) using Mini Protean II cells (Bio-Rad), and then transferred to PVDF membranes using Mini Trans-Blot cells (Bio-Rad). The membranes were then blocked in 5 % non-fat dried milk in phosphate buffered saline overnight at 4 °C. Transferred proteins were probed with a primary dehydrin antibody (StressGen Biotechnologies Corp, Victoria, Canada) prepared against a synthetic peptide containing the conserved sequence EKKGIMDKIKELPG (7). Reactive bands were detected with an anti-rabbit IgG secondary antibody conjugated to horse radish peroxidase (Immunopure, Pierce, Rockford,Il) using a chemiluminescent substrate (SuperSignal West Pico for HRP, Pierce, Rocford, Il) and clear blue x-ray films (CL-XPosure, Pierce, Rocford, Il).

2.4 .Statistical Analysis

Data analysis was performed by analysis of variance using GLM procedure (SAS Institute, Cary, NC) on dry weights. To assess the level of drought tolerance of each cultivar on the studied traits, drought susceptibility index (S) (14) was calculated as:

$$S=(1-Y_D / Y)/(1- Y_{MD}/ Y_{MI}) \quad [1]$$

where Y_D is the plot value for a genotype under stress, Y_I is the plot value for the same genotype under non-stress, and Y_{MD} and Y_{MI} are the mean value of the experiment under stress and non-stress conditions, respectively. The rate of decrease in leaf water potential per day of stress was estimated as the slope of the linear regression of Ψ_l on days of stress.

3. RESULTS AND DISCUSSION

Significant differences were observed between treatments (drought and well-watered plots) for shoot dry matter accumulation in the first (P< 0.01) and in the second (P< 0.01) seddling experiments (anova table not shown), indicating the effectiveness of the treatment to impose stress.

In the well-watered treatment, average Ψ_l (over all cultivarsin experiments 1 and 2) remained high during the experiment (from -0.66 MPa to -0.71Mpa). In contrast, the stress treatments showed a progressive decrease in the average Ψ_l from -0.66 MPa in the first sampling date to - 1.96 MPa in the sixth sampling date (figure I).

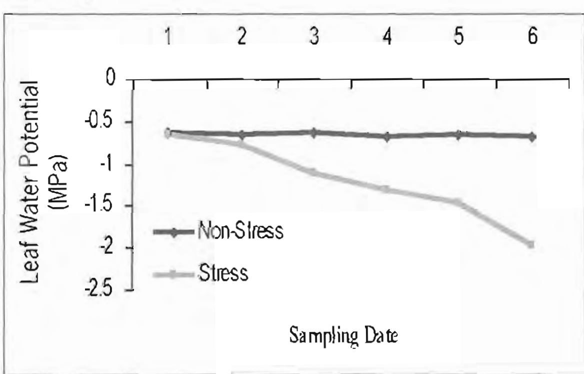

Figure 1: Observed LWP (MPa) for non-stress and stress treatments averaged over the seven cultivars studied in the first and second seedling experiments.

The seven cultivars were examined under progressive drought stress for the accumulation of dehydrin proteins. Dehydrins are usually expressed in cereal seedlings during gradual exposure to dehydrative stress (15). By the first sampling date (zero days of stress, Fig.2A) the Ψ_l in stress and non-stress treatments were similar (-0.66 MPa vs -0.64 MPa, respectively), as expected, and no dehydrins were detected. Subsequently, drought stress progressed as indicated by the Ψ_l value of -1.10 MPa at the third sampling date, however, no dehydrin bands were observed in any cultivar at this point (western not shown).

On the fourth sampling date (four days of stress, Fig. 2), when average Ψ_l in the stressed plants was measured at -1.31 MPa, a dehydrin of 24

kDa was detected only in cultivars Connie, TAM105, and Gene under stress. The latter genotype also showed a minor expression of a 19 kDa protein that reacted with the anti-dehydrin antibody.

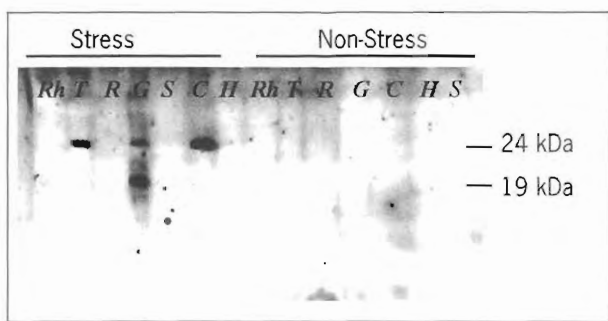

Figure 2: Western blot of dehydrins from leaves collected on the fourth sampling date in cultivars Connie (C), Rhode (Rh), TAM105 (T), Hiller (H), Gene (G), Stephens (S), and Rod (R), under stress and non-stressed treatments.

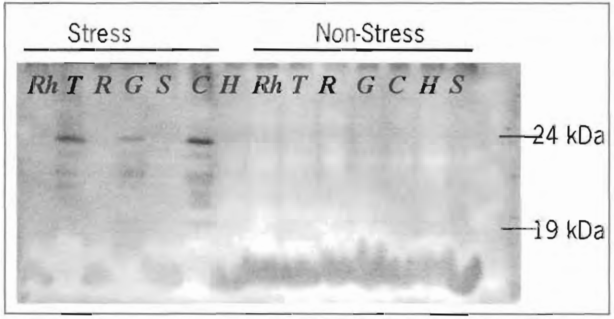

Figure 3: Western blot of dehydrins from leaves collected on the fifth sampling date in cultivars Connie (C), Rhode (Rh), TAM105 (T), Hiller (H), Gene (G), Stephens (S), and Rod (R), under stress and non-stressed treatments.

At the fifth sampling date (Ψ_1 = -1.47 Mpa in stress treatment) the 24 kDA dehydrin cultivars and faint bands between 14 kDA and 19 kDA were present (Fig. 3) in the same three cultivars. No dehydrins were observed in any of the well-watered plants or other cultivar subjected to drought.

Close and Chandler (1990) also detected a 25 kDa dehydrin in stressed wheat and barley seedlings along with faint bands between 18 and 21 kDa and no dehydrin proteins in well watered plants. Following (7), this faint bands may be intact proteins or degradation products.

On the sixth sampling date, after seedlings had been subject to 12 days of progressive stress and the average Ψ_1 was reduced to -1.96 MPa in stressed plants, dehydrin proteins were detected in Hiller (24 kDa), Stephens (19 kDa), and Rohde (19 kDa) (not shown). Since no samples were collected between days 6 and 11, it can not be precisely established in which day the production of dehydrins was induced in those cultivars. Nevertheless, Connie, TAM105, and Gene produced dehydrins at least 2 days earlier. No significant differences among the seven cultivars were observed in RDΨ_1 indicating that the imposed water stress was similar along the experiments for all the cultivars studied. In spite of the common stress conditions, Connie, TAM105, and Gene accumulated dehydrins at a higher water potential (around -1.31 MPa) than the rest of the cultivars. Differential dehydrin accumulation can result from differences in gene regulation or in genome organization such us a higher number of dehydrin gene copies (16).

3.1. Association Between the Presence of Dehydrin at Seedlings and Stress Tolerance at Adult Plant Stage

In the adult plant experiments, significant differences were observed between drought and well-watered plots in experiments 1 and 2 for yield ($P< 0.01$ and $P< 0.05$, respectively), showing that the treatment was effective in imposing stress. The average reduction (first and second experiments) in the mean of the of the drought plots compared to the well-watered plots was 73.6%. The average Ψ_1 of the well watered plants remained high during the entire experiment (between -0.64 MPa and -0.70 MPa). Conversely, a progressive decrease in average Ψ_1 was associated with the stress treatment, declining from -0.64 MPa at the first sampling date to - 2.32 MPa at the sixth sampling date (Fig.4).

A contrast between the mean drought susceptibility index for grain yield (*SY*) of cultivars that showed early (Connie, TAM105, Gene) and late (Stephens, Rod, Hiller, and Rohde) expression of the 24 kDa dehydrin in seedlings, revealed significant differences ($P< 0.01$) with a lower mean for the early group (Table I). Cultivars showing lower *S* values are more tolerant to stress, since they have a lower reduction in the value of a trait from non-stress to stress conditions in comparison to the overall reduction observed for all cultivars (see Eq. [1]).

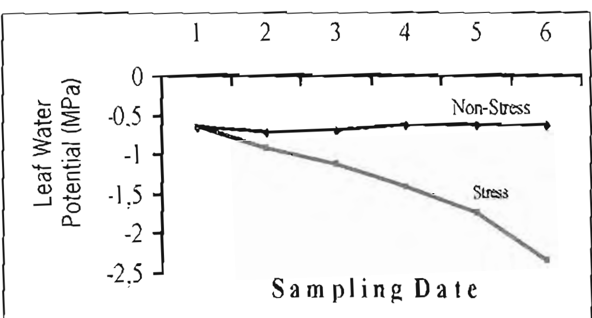

Figure 4: Observed LWP (MPa) for non-stress and stress treatments averaged over the seven cultivars studied in the first and second adult plant experiments.

Table I: Combined analysis of variance (first and second experiments) for the drought susceptibility index of grain yield (*SY*), and for the rate of decrease in leaf water potential (RDÿ*l*).

Source	D.F.	Mean Square	
		S_y	$RD\Psi_1$
Experiment (Exp.)	1	ns	**
Rep(Exp.)	4	ns	Ns
Cultivar (C)	6	**	**
Early vs Late	1	**	**
C x Exp.	6	ns	**
Residual	24		

** Significant at 0.01 probability level; ns = non-significant.

The presence of the 24 kDa dehydrin also was associated with the leaf water status of the plants. The analysis of variance for the rate of decrease in Ψ_1 per day of stress (RDÿ*l*) in adult plants during the first drying cycle in the stressed plots, revealed significant differences (P< 0.01) for the contrast between early and late groups (Table I). The mean RDÿ*l* in the earli group was higher than the mean in the later group (-0.27 Mpa day^{-1} vs -0.37 Mpa day^{-1}), indicating that TAM Connie and Gene maintained a higher Ψ_1 during the cycle. Since Ψ_1 is an estimation of the stress intensity experienced by leaves (11), the results show a physiological relation between dehydrin expression and drought tolerance.

Our results indicate that the accumulation of a 24 kDa dehydrin protein in seedlings was associated with the relative drought tolerance in adult plants that was characterized by a lower reduction in yield and in the rate of decrease in leaf water potential in Connie, TAM105, Gene. Hence, the 24 kDa dehydrin might be used to develop a rapid screening technique at seedling stage. However, selection experiments using a population segregating for stress tolerance are needed to test more conclusively whether or not this dehydrin can be used to select genotypes with tolerance to drought stress.

REFERENCES

[1] Moustafa, M.A., Boersma, L., and Kronstad, W.E. 1996. Response of four spring wheat cultivars to drought stress. Crop Sci. 36: 982-986.
[2] Rajaram, S, Braun, H.J. and M. van Ginkel. 1996. CIMMYT's approach to breed for drought tolerance. Euphytica 92: 147-153.
[3] Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. Plant Growth Regulation 20:135-148.
[4] Sadiq, M., Siddiqui, K.A. Arain, C.R. and A.R. Azmi. 1994. Wheat breeding in a water-stressed environment. I. Delineation of drought tolerance and susceptibility. Plant Breeding 113, 36-46.

[5] Donaldson, E. 1996. Crop traits for water stress tolerance. American Journal of Alternative Agriculture. 11 (2-3): 89-94.

[6] Dure III, L. 1993. A repeating 11mer amino acid motif and plant dessication. Plant Journal 3: 363-369.

[7] Close, T.J., Fenton, R.D. and F. Moonan. 1993. A view of plant dehydrins using antibodies specific to the carboxy terminal peptide. Plant Molecular Biology 23: 279-286.

[8] Campbell, S.A. and T.J. Close. 1997. Dehydrins: genes, proteins, and association with phenotypic traits. New Phytology 137, 61-74

[9] Houde, M., Dhindsa, R.S. and F. Sarhan. 1992. A molecular marker to select for freezing tolerance in Gramineae. Mol. Gen. Genet. 234:43-48

[10] Zhu, B., Choi, D.W., Fenton, R., and T.J. Close. 2000. Expression of the barley dehydrin multigene family and the development of freezing tolerance. Mol. Gen. Genet. 264: 145-153.

[11] Cellier, F, Conéjéro, G., Breitler, J.C. and F. Casse. 1998. Molecular and physiological responses to water deficit in drought-tolerant and drought-sensitive lines of sunflower. Plant physiology 116: 319-328.

[12] Snow, M.D., and D.T. Tingey. 1985. Evaluation of a system for the imposition of plant water stress. Plant Physiol. 77:602-607.

[13] Martínez-García, J.F., Monte, E., and P.H. Quail. 1999. A simple, rapid and quantitative method for preparing *Arabidopsis* protein extracts for immunoblot analysis. The Plant Journal 20 (2): 251-257.

[14] Fischer, R.A. and R. Maurer. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res., 29,897-912.

[15] Close, T.J. and P.M. Chandler. 1990. Cereal dehydrins: serology, gene mapping and potential roles. Aust. J. Plant Physiol. 17, 333-344.

[16] Labhilili, M, Joudrier, P and M.F. Gautier. 1995. Characterization of cDNA encoding *Triticum durum* dehydrins and their expression patterns in cultivar that differ in drought tolerance. Plant Science 112, 219-230.

PARTIAL RESISTANCE TO LEAF RUST IN ANCIENT SPANISH WHEATS

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ABSTRACT: A collection of 918 accessions of Spanish durum and bread wheats was screened for resistance to leaf rust (*Puccinia triticina*) in the field at several locations. Resistance levels ranged from very low to very high, being most frequent high susceptibility. Relative AUDPC (referred to susceptible check=100 %) was lower than 20 % in about 9 % of the accessions in each location. In the collection most of the lines (84 %) displayed a susceptible infection. A final selection of seven accessions (one of them durum) displaying low severity level in the field and high infection type in chamber was chosen for further studies. High levels of partial resistant with longer latency period and high percentage of early aborted colonies without necrosis were found. They might be used in breeding programmes.

1. INTRODUCTION

Wheat breeding for leaf rust resistance in modern agriculture has traditionally been based on *Lr* genes for hypersensitive resistance. This approach yielded quick and effective results to control the disease, but pathogen populations commonly were able to overcome the resistance genes by new virulent races. There is an increasing concern about the durability of the resistance [5]. Several strategies can be adopted to prolong the durability of the *Lr*-ephemeral resistance, such as gene pyramiding, diversification and application of cultivar-mixtures [10].

Another approach is the search for different types of resistance that are not based on hypersensitivity. Partial resistance has been defined as a resistance resulting in a reduced epidemic development despite a susceptible infection type [12]. An incomplete hypersensitivity resistance may also result in a reduced epidemic development, being rather quantitative, but is still recognizable by the association with some necrosis of the infected tissue. Partial resistance has been described in many pathosystems including wheat-wheat leaf rust. It is generally polygenic, but not necessarily so [3, 4].

A collection of old cultivars might be a good reservoir for genes for quantitative resistance, as breeding was mainly based on selection for field resistance [16]. The purpose of the present study was to identify sources of quantitative non-hypersensitive resistance in a collection of ancient Spanish wheat cultivars.

2. MATERIAL AND METHODS

918 landraces of bread and durum wheats from different parts of Spain kindly provided by the Centro de Recursos Fitogenéticos, INIA, Spain, were grown at 3 different locations in Southern Spain during the season 96/97. These were Córdoba, Jerez and Granada. Each line was represented by a 1 m long single row. Susceptible and resistant checks were included for comparison. No artificial inoculation was performed as leaf rust infections occur commonly in the area [7]. Disease severity (%) was assessed at 15 days interval from April till June. AUDPC (area under disease progress curve) was calculated and referred to a susceptible check T59 (=100%)

In addition infection of all the accessions was studied in seedling state in a growth chamber. Five plants per accession were sown in 1 l pots and inoculated in the second-leaf stage with a local isolate of *Puccinia triticina* (virulent on *Lr2b*, *Lr2c*, *Lr10*, *Lr11*, *Lr12*, *Lr14a*, *Lr14b*, *Lr18*, *Lr20*. *Lr21*, *Lr22*, *Lr23*, *Lr30*, *Lr32*, *Lr33*; *Lr35*, *Lr37*, *Lr44* and *LrB*). Plants were incubated 24 h in darkness with saturated RH and moved to a compartment at 20°C and 14 h photoperiod. Infection Type [6] was scored 14 days after inoculation.

Thirty-three accessions out of the 918, bearing high IT in the seedling test and low progress of the disease in the field in the 3 locations were selected for further studies. Their reaction to leaf rust was studied at 3 locations in the field in 1997-98, and their components of resistance measured in seedlings. The plants were grown in soil in plant boxes (35 x 35 x 10cm). Three consecutive replications were performed. We tested four leaves per line including in each box a susceptible (Little Club) and partial resistant check (Akabozu). Eleven days after sowing first leaves of each seedling were fixed in a horizontal position with the adaxial side upward. Per plant box, 4 mg of urediospores of the local isolate was mixed with talcum powder (1:9, vol/vol) and applied using a settling tower. The inoculum density was about 130 spores/cm^2.

After inoculation the plant boxes were incubated 24 hours in darkness at 100 % relative humidity, and then transferred to a compartment at 20°C.

Infection type, latency period, and infection frequency were determined. Infection type was recorded 12 days after inoculation. Latency period was determined by counting daily the number of uredia visible in a marked area on the leaves till the number of uredia no longer increased. The latency period was taken as the time period from the beginning of incubation to the time at which 50 % of the uredia had appeared. Infection frequency was determined on the marked areas of the leaves. The final number of uredia was used to calculate the number of uredia per cm^2.

From these experiments, the seven lines in which fungus has developed longest latency period and lowest infection frequency were selected for further studies at the macroscopic level with different isolates and for microscopic observations. These tests were performed in seedling and adult plant. Little Club (susceptible), Akabozu (partially resistant) were included as references.

For the adult plant experiments plants were grown individually in 12x12 cm pots in a greenhouse. All plants were sown at several dates in order to obtain plants at the same development stage. Experiments were done in flag leaf (DC 48-59[15], ears just emerged, young but fully expanded flag leaves) at the time of inoculation. Three series were performed, of four pots each. Inoculation was performed by dusting urediospores mixed with talcum powder over the plants. One milligram of urediospores was used per pot.

Components of resistance were again measured as described above both in seedlings and adult plant stage after inoculation with isolate *Puccinia triticina* B9414-1CA3 (virulent on *Lr1*, *2c*, *3*, *3bg*, *11*, *12*, *13*, *14a*, *14b*, *16*, *18* , *21*, *22*, *26*, *33*, *34*, *37*, *44(I)* and *LrB(I)*).

Microscopic observations:

Five days after inoculation central segments of 1 to 3 cm^2 were collected from first and flag leaves respectively. Three leaves were sampled per series. Segments were prepared as whole mounts for fluorescence microscopy [14], but instead of Calcofluor we used Uvitex 2B (Ciba-Geigy). The preparations were examined at 200x with a Leica epifluorescence equipment (DM LB, 330 to 380 nm wave length transmission). At least 100 sporelings per leaf segments were scored and classified according to their stage of development [8]. Sporelings that developed a germ tube but not an appressorium over a stoma were ignored. We defined early aborted sporelings as individuals that formed a primary infection hypha and not more than six haustorial mother cells [8]. Sporelings that had developed more haustorial mother cells were classified as established. Filter with 420 to 490 nm transmission was used to observe necrosis of host cells, which display a golden yellow autofluorescence. The length (L), and width (W) of ten arbitrarily chosen established colonies per leaf were measured with an eyepiece micrometer. Colony size (CS) was calculated as the geometric mean of L and W, CS=SQRT(1/4xLxW). The statistical analysis for percentage of sporelings aborted or associated with necrosis was performed on arc sin-transformed data if appropiate.

3. RESULTS

Relative AUDPC in the field was lower than 20 % of the susceptible check in about 8 % of the lines. High susceptibility was very frequent. 64 % of the lines displayed an AUDPC higher than 40 %. In seedling tests the most of the lines (87 %) displayed a susceptible IT. Seven lines displaying low AUDPC in the field and high IT and latency period in seedling were selected to study macroscopic and microscopic components of the resistance. The AUDPC was particularly low in lines BG12036, BG12609 and BG13781 (data not shown).

Latency period was longer in adult plant than in seedling (198 h vs. 166h). In seedling the latency period on lines BG12036 and BG12609 was longer than on the susceptible.

check Little Club and similar to Akabozu. In adult plant stage the latency period of all lines were longer than on susceptible Little Club and similar to Akabozu except lines BG12036 and BG13781. On these lines the latency period was longer than on Akabozu.

Infection type was high on the majority of lines in the seedling stage (84 %). In flag leaf the IT in the line BG11932 was reduced to 2 (only necrotic flecks). The IT in the line BG12609 was also lower in this stage (IT6).

Table 1. Macroscopic components of the resistance in selected lines to *Puccinia triticina*.

	Latency Period		Infection Type	
Lines	**Seedling**	**Flag leaf**	**Seedling**	**Flag leaf**
Little Club	100 c* (143 h)	100 c* (198 h)	9	9
BG4788	102 c	109 ab	9	9
BG11932	101 c	-	9	2
BG12036	109 ab	143 a	9	9
BG12609	113 a	110 ab	8	6
BG13781	101 c	139 a	9	9
BG13804	105 bc	113 a	9	9
BG18644	99 c	115 b	9	9
Akabozu	110 a	117 b	9	9

* Data with the same letter per column are not statistically different (Duncan, $P<0.05$)

The results of the microscopic observations are shown in the table 2. Early abortion without necrosis was higher in flag leaves than in seedling. Lines BG12036, BG13781 and Akabozu had a higher percentage of early aborted colonies without necrosis than the susceptible check in seedling stage. In flag leaf stage lines BG4788, BG11932, BG12036, BG13781 and Akabozu had a higher percentage of early aborted colonies than Little Club. It's remarkable the very high percentage of early aborted colonies in flag leaves of the lines BG13781 and, specially, in line BG12036 is remarkable.

Colonies were bigger in seedlings than in adult plant. In seedling stage, lines BG12036, BG12609, BG13804 and Akabozu supported smaller colonies than the susceptible check. In flag leaf stage smaller colonies were developed on line BG4788 than on the susceptible Little Club and similar to the partially resistant check Akabozu.

Table 2. Microscopic components of the resistance to *Puccinia triticina* in selected lines.

	% Early Abortion without necrosis		Colony size (mm^2)	
Lines	**Seedling**	**Flag leaf**	**Seedling**	**Flag leaf**
Little Club	2 cd*	6 d*	100 a*	
(0.334)	100 a*			
(0.031)				
BG4788	2 cd	24 bc	83 abcd	68 ab
BG11932	1 e	30 b	85 abcd	27 cde
BG12036	7 a	63 a	70 cd	17 de
BG12609	2 bcde	11 cd	76 bcd	38 bcde
BG13781	5 ab	51 a	88 abc	15 e
BG13804	4 abcd	16 bcd	64 d	53 bcd
BG18644	2 bcde	13 bcd	94 ab	58 bc
Akabozu	5 abc	25 bc	33 e	23 cde

* Data with the same letter per column are not statistically different (Duncan, $P<0.05$).

4. DISCUSSION

Very high levels of partial resistance were identified by longer latency period and high percentage of early aborted colonies that were not associated with plant cell necrosis. In the collection levels of latency period and early abortion were higher than ever reported in wheat before. Broers [2] reported a positive relationship between the level of partial resistance in landraces and the severity of the wheat leaf rust in several European countries. Montes [7] pointed out that wheat leaf rust is a common disease in Andalusia. The ancient landraces cultivars, having a long growing season, would allow more spore generation of the pathogen, and hence, higher disease severities to be reached. Therefore, in those landraces there may have been a greater selection pressure for quantitative types of resistance.

The selected lines in this study could be classified into three groups:

1. Lines with high level of partial resistance. Lines BG4788, BG13804 and BG18644 (the latter being the only *Triticum durum*) showed high levels of partial resistance only slightly lower than Akabozu. A long latency period were developed in this line that is correlated very well with partial resistance, specially in adult plant [1]. At microscopic level they display high levels of abortion without necrosis and small colonies specially in adult plant. These parameters are also associated to partial resistance [3, 4]. BG4788 and BG18644 express their partial resistance in adult plants only while partial resistance of BG13804 is expressed in both development stages.
2. Lines with an extraordinary level of partial resistance. Lines BG12036 and BG13781have a level of partial resistance higher even than Akabozu. The latency period is very long on this line, the abortion not associated with plant cell necrosis is very low and the colony size is very small specially in line BG12036.
3. Lines with genes for hypersensitive resistance in adult plant. There is evidence for the presence of adult plant resistance genes in lines BG11932 and BG12609. In line BG11932 the IT decreased to 2 in flag leaf. Line BG12609 showed an IT 6 in flag leaf. In the literature there are many examples of hypersensitive adult plant resistance to Puccinia triticina in wheat [11]. Line BG11932 might carry the gene Lr13 or Lr37 that act at earlier stage and give a more complete response. Line BG12609 might carry the genes Lr12, Lr22a or Lr35 that act at a more advanced stage and give a moderate infection type. It is difficult to know the level of partial resistance in lines with adult plant resistance genes because hypersensitive resistance is usually stronger than partial resistance. However there are different ways to observe it. Firstly we could see the level of partial resistance in seedling stage where the adult plant genes are inactives. Secondly histological screening might help to identify levels of partial resistance hidden behind the effects of hypersensitive resistance. High levels of early aborted colonies will indicate partial resistance in the presence of posthaustorial resistance. The percentage of infection units arrested early without any association of necrosis could be a good indicator of partial resistance even in lines with hypersensitive resistance [9]. A long latency period was observed on line BG12609 in seedling stage that doesn't correlate with the percentage of early abortion in adult plant. This line may have partial resistance only expressed in seedling stage. The presence of components of partial resistance or QTLs in the seedling stage has recently been reported in the pathosystem barley-Puccinia hordei. [13] Line BG11932 doesn't show high levels of partial resistance in seedling but in adult plant might have higher levels of partial resistance because it has a high level of early aborted colonies not associated with plant cell necrosis (similar to Akabozu).

ACKNOWLEDGEMENTS

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REFERENCES

[1] Broers LHM:. Influence of development stage and host genotype on three components of partial resistance to leaf rust in spring wheat. Euphytica 44:187-196 (1989).

[2] Broers LHM, Haan: AA: Relationship between the origin of European landraces and the level of partial resistance to wheat leaf rust. Plant Breeding 113, 75-78 (1994).

[3] Jacobs Th: Abortion of infection structures of wheat leaf rust in susceptible and partially resistant wheat genotypes. Euphytica 45:81-86 (1990).

[4] Jacobs Th, Buurlage MB: Growth of wheat leaf rust colonies in susceptible and partially resistant spring wheats. Euphytica 45:71-80 (1990).

[5] Johnson R:. Reflection of a plant pathologist on breeding for disease resistance, with emphasis on yellow rust and eyespot of wheat. Plant Pathology 41:230-254 (1992).

[6] McNeal FH, Konzak CF, Smith EP, Tate WS, Russell TS: A uniform system for recording and processing cereal research data. USDA, Agricultural Research Service, Washington, D.C. ARS 34-121 (1971).

[7] Montes F, Gimeno V, Juan J, Marinetto J: Utilización de localidades de Andalucía con mayor riesgo de incidencia de enfermedades de trigo en los programas de mejora genética. Investigación Agraria 3:219-231 (1988).

[8] Niks RE: Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Can. J. Bot. 60:714-723 (1982).

[9] Niks RE, Kuiper, HJ: Histology of the relation between minor and major genes for resistance of barley to leaf rust. Phytopathology 73:55-59 (1983).

[10] Niks RE, Lindhout WH: Breeding for resistance against diseases and pests. Wageningen Agricultural University. Department of Plant Breeding. 157 p (1998).

[11] Park RF, Mcintosh, RA.. Adult plant resistance to *Puccinia recondita* f. sp. *tritici* in wheat. New Zealand Journal of Crop and Horticultural Science 22: 151-158 (1994).

[12] Parlevliet, JE. and Van Ommeren, A. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, microplot test and latent period. Euphytica 24:293-303.

[13] Qi X, Niks RE, Stam P, Lindhout P:. Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. Theor. Appl. Genet. 96:1205-1215 (1998).

[14] Rohringer R, Kim WK, Samborski DJ, Howes NK;. Calcofluor: An optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology 67: 808-810 (1977).

[15] Zadocks JC, Chang TT, Konzak CF:. A decimal code for the growth stages of cereals. Weed Res. 14:415-421 (1974).

[16] Zhang Zhong-Jun:. Evidence of durable resistance in nine Chinese land races and one Italian cultivar of *Triticum aestivum* to *Puccinia striiformis*. European Journal of Plant Pathology 101:405-409 (1995).

SEARCH FOR PARTIAL RESISTANCE TO LEAF RUST IN A COLLECTION OF ANCIENT SPANISH BARLEYS

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ABSTRACT: A collection of 569 accessions of Spanish barleys was screened for resistance to leaf rust (*Puccinia hordei*) in the field at Córdoba during the 2000-2001 season. Level of resistance ranged from very low to very high. Relative AUDPC (L94=100%) was lower than 10 % in 14 % of the accessions.

The accessions that were most resistant in the field were tested in seedling stage under controlled conditions. Macroscopic components of resistance indicate that seven lines had a high level of partial resistance equal or higher than the partially resistant check Vada.

1. INTRODUCTION

Barley leaf rust, caused by *Puccinia hordei* Otth, is an important disease of barley (*Hordeum vulgare* L.) worldwide. Genetic resistance in the host is the best way to control this disease. Breeders usually rely on hypersensitive resistance that is governed by major genes and is race-specific. This resistance is associated with plant cell necrosis around the infection site (low infection type). This type of resistance has been widely exploited by breeders because of its monogenic nature but the pathogen is able to overcome this kind of resistance in a short period of time.

Thus, there is an increasing awareness for the need to search for more durable types of resistance such as partial resistance. It is characterised by a reduced rate of epidemic development in spite of a susceptible (high) infection type [6]. One of the most interesting aspects of partial resistance is its high stability in different classes of environments [5] and its durability.

Landraces may have fair levels of partial resistance. Farmers had made an unconscious selection against extreme susceptibility generation after generation. The long time of coexistence between pathogen and plant suggest that the landraces may carry some kind of durable resistance.

In this paper a collection of 569 ancient Spanish barley lines were screened for resistance to leaf rust in order to determine the level and type of resistance in the collection and study the mechanisms of the most partially resistant line.

2. MATERIAL AND METHODS

2.1. Field experiment

A collection of 569 lines of spring barleys kindly provided by the Centro de Recursos Fitogenéticos, INIA, Spain, was grown in the field at Córdoba during the 2000-2001 season. Each line was represented by a 1-meter long single row. Susceptible line, L94, was placed adjacent to each line and used as spreader. Hypersensitive resistant (L94+*Pa7*) and several partially resistant (Vada, 116-5, C123 and 17.5.16) checks were repeated across the plots. Artificial inoculation was done using a monopustule-derived isolate collected at Córdoba (avirulence/virulence: *Pa3, Pa5, Pa7, PaC, PaD/Pa1, Pa2, Pa4, Pa6, Pa8, Pa9, Pa12*). Inoculation was done in two ways: Transferring sporulating L94 plants inoculated in the greenhouse

to the field one month after sowing and dusting over the spreader rows a mixture of urediospores diluted in talcum powder.

Disease severity (%) was assessed thee times. AUDPC (area under disease progress curve) was calculated and referred to the susceptible check (L94=100%).

2.2. Seedling tests

The infection type (IT) of the most lines most resistant in the field (those with AUDPC lower than 20%) was studied in seedling stage in a growth chamber. Five plants per line were grown in pots and inoculated with the same isolate as before mixed with talcum powder (1:10 vol/vol). Plants were incubated 24 hours in darkness with high HR. The next day plants were transferred to a compartment at 20°C and 14 hours photoperiod. Infection type [3] was scored 12 days after inoculation. Forty-seven lines with a high infection type in seedling and low AUDPC in the field were selected in order to measure their latency period in seedling stage. Plants were grown in soil in plant boxes (35¥35¥10 cm). Ten lines with five plants each were included in each box. Susceptible, L94, and partially resistant check, Vada, were also included in each box. About eleven days after sowing, the first leaves were fixed in a horizontal position and inoculated in a settling tower. Each box received four mg of spores mixed with talcum powder (1:10 vol/vol), which resulted in a spore deposition of about 180 spores per cm2.

3. RESULTS

3.1. Resistance levels in the field

Susceptible check L94 raised a disease severity of 60%. Resistant lines were found. About 24 % of the lines displayed an AUDPC (referred to L94=100%) lower than 20%. Twenty six lines (4.5%) showed an AUDPC lower than 2%. L94+*Pa7* had 1.1%, Vada 1.6%, 116-5, 3.9% and 17.5.16 0.3%. High susceptibility was also frequent with 28% of the lines showing an AUDPC higher than 60 %. Actually there was a continuous variation in the AUDPC.

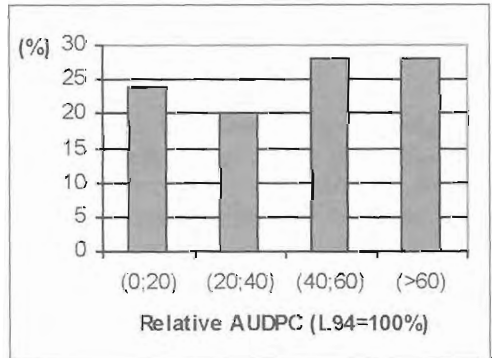

Figure 1. Distribution of the 569 barley lines according to the AUDPC of *Puccinia hordei*.

3.1. Evaluation for race-specific resistance

The seventy lines with lowest AUDPC in the field were screened for IT in seedling stage. Only 20 % of the lines showed a low infection type, suggesting that the high levels of resistance identified in the remaining 80 % of the lines is not due to hypersensitivity in the seedling stage.

3.2. Levels of partial resistance

Forty-five lines were selected on basis of their high IT and low AUDPC to study the latency period in seedling stage. The latency period on seven lines was similar or longer than Vada.

4. DISCUSSION

Field resistance was common in the collection and was not associated with hypersensitivity in 80 % of them, suggesting that levels of partial resistance delay the epidemic in spite of a susceptible IT [3].

It is reported that landraces usually build up high levels of non-hypersensitive resistance. Andenov et al. [2] screened a collection of tetraploid wheat for resistance against leaf rust and found a high IT in almost all lines and moderate levels of partial resistance. Alemayehu et al. [1] found a near-absence of race-specific, major resistance genes and a relatively high frequency of moderate levels of partial resistance to leaf rust in a collection of Ethiopian barley landraces.

Table 1. Macroscopic components of resistance to *Puccinia hordei* in selected lines of Spanish barleys.

Lines	Rel. Latency Period (seedling stage)[a]	Infection Type	AUDPC (seedling st.)
L94	100 a[1]		
	(152 h)	9	100
BG7851	114 bc	9	8.7
BG7967	115 bc	9	3.9
BG8927	111 b	9	0.7
BG8943	116 bc	9	1.8
BG9139	120 cd	9	1.8
BG9359	116 bc	9	1.7
BG11188	126 d	9	1.7
Vada	111 b	9	1.6
L94+*Pa7*	-	2	1.1

[1] Data with the same letter per column are not statistically differents (Duncan, $P<0.05$).
[a] Latency period and AUDPC are expressed as values relatives to L94 (=100%). The actual average values for L94 of latency period are presented in brackets.

2. DISCUSSION

Field resistance was common in the collection and was not associated with hypersensitivity in 80 % of them, suggesting that levels of partial resistance delay the epidemic in spite of a susceptible IT [3].

It is reported that landraces usually build up high levels of non-hypersensitive resistance. Andenov et al. [2] screened a collection of tetraploid wheat for resistance against leaf rust and found a high IT in almost all lines and moderate levels of partial resistance. Alemayehu et al. [1] found a near-absence of race-specific, major resistance genes and a relatively high frequency of moderate levels of partial resistance to leaf rust in a collection of Ethiopian barley landraces.

The non-hypersensitive resistance of landraces is intrinsically durable. Zhang [7] reported the existence of nine Chinese landraces with durable resistance to stripe rust.

It is remarkable that a recent study on modern West-European spring barleys [4] to leaf rust showed that it was common to find hypersensitive and partial resistance occurring in combination in single genotypes. It seems that breeders have incorporated major genes in their programs but in partially resistant background.

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REFERENCES

[1] Alemayehu F, Parlevliet J E: Variation for resistance to *Puccinia hordei* in Ethiopian barley landraces. Euphytica 90:365-370 (1996).
[2] Andenow Y, Hulluka M, Belay G, Tesemma T.: Resistance and tolerance to leaf rust in Ethiopian tetraploid wheat landraces. Plant Breeding 116(6):533-536 (1997).
[3] McNeal F H, Konzak C F, Smith E P, Tate W S, Russell T S: A uniform system for recording and processing cereal research data. USDA, Agricultural Research Service, Washington, D.C. ARS 34-121 (1971).
[4] Niks RE, Walther U, Jaiser H, Martínez F, Rubiales D, Andersen O, Flath K, Gymer P, Heinrichs F, Jonsson R, Kuntze L, Rasmussen M, Richter E: Resistance against barley leaf rust (*Puccinia hordei*) in West-European spring barley germplasm. Agronomie 20.769:782 (2000).
[5] Parlevliet JE,: Partial resistance of barley to leaf rust, *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24:21-27 (1975).

[6] Parlevliet, JE. and Van Ommeren, A. 1975: Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, microplot test and latent period. Euphytica 24:293-303.

[7] Zhang ZJ: Evidence of durable resistance in nine Chinese land races and one Italian cultivar of *Triticum aestivum* to *Puccinia striiformis*. European Journal of Plant Pathology 101:405-409 (1995).

COMPARATIVE STUDY OF THE PHENOTYPIC EXPRESSION OF GENES LR34 AND LR46 FOR RESISTANCE TO WHEAT LEAF RUST

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ABSTRACT: Components of resistance of *Lr46* gene, reported as conferring 'slow rusting' resistance to leaf rust in wheat, were studied and compared with *Lr34*. *Lr34* is a major gene with a non hypersensitivity-type resistance. Effect of *Lr46* resembles to that of *Lr34* and other wheats reported with partial resistance. At macroscopic level *Lr46* produced a longer latency period and a reduction of the infection frequency not associated with plant cell necrosis. Microscopically *Lr46* increased the percentage of early aborted infection units not associated with host cell necrosis and decreased the colony size. The effect of *Lr46* is comparable with that of *Lr34* in adult plant but in seedling stage its effect is rather weak.

1. INTRODUCTION

Leaf rust, caused by *Puccinia triticina* is an important disease in most wheat growing areas. To combat this disease there are several strategies. One of them is the use of fungicides but the environmental damage of these chemicals can be high. The use of genetic resistance that plants have is the most economical and environmentally friendly way to reduce crop losses. Breeders commonly have relied on major genes for hypersensitive resistance (HR), designated as *Lr* genes, which are very effective in reducing the epidemic build up in breeding programmes because of their monogenic nature. But new races of the rust fungus frequently defeat HR. There is a great interest to improve the durability of intrinsically 'non durable' types of resistance by gene pyramiding or by use of multilines. An other posibility would be to identify and introduce resistance types that are intrinsically durable like partial resistance (PR). PR is characterized by a slow epidemic build-up despite a high infection type (nonhypersensitive type resistance) indicating a compatible host-pathogen interaction [5]. PR is inherited poligenically [6,8] and hence difficult to manage by breeders. However there are some exceptions. For example, *Lr34*, located on chromosome 7D of wheat is a major gene that confers partial resistance to wheat leaf rust *sensu* Parlevliet [9].

Pavón 76 is a Mexican bread wheat carrying slow rusting to leaf rust since its release in 1976. This slow rusting is based on two genes with additive effects. One of these genes has been identified and named as *Lr46*. It is located on chromosome 1B of Pavón 76 [10].

Our aim in this paper is to characterise the phenotypic expression of *Lr46* and to compare it with *Lr34*.

2. MATERIAL AND METHODS

2.1. Plant material

The studies were performed on the susceptible Lalbahadur (Lr1) and the lines Lalbahadur+*Lr34* (Lalbahadur 'Parula 7D', chromosome substitution line) and Lalbahadur+*Lr46* (Lalbahadur 'Pavón 76 1B' chromosome substitution line) [11]. These seeds have been provided by R. P. Singh from CIMMYT.

Field and growth chamber studies were performed.

Inoculations

The isolate of *Puccinia triticina* used in the experiments was 'B9414-1CA3' (French origin). It is virulent on *Lr1, 2c, 3, 3bg, 11, 12, 13, 14a, 14b, 16, 18, 21, 22, 26, 33, 34, 37, 44(l), B(l).*

For the seedling tests, plants were grown in soil in plant boxes (37¥39¥5 cm). Seven replications of 4-6 plants each were performed.

Eleven days after sowing, first leaves were fixed adaxial side upwards in the box with metallic clips. Leaves were inoculated in a settling tower with 4 mg of rust urediospores mixed with *Lycopodium* spores (1:20 vol./vol.) resulting in a deposition of about 120 spores/cm2. The plants were incubated overnight in a mist chamber at 20° C with 100 % relative humidity. Then they were transferred to a climate room (21° C and 70 % RH) during the latency of rust and finally when the first uredia appeared these plants were transferred to a greenhouse compartment (18°-23°C day-night range).

For the adult plant test, plants were grown in 12¥12 cm pots in a greenhouse. Adult plants were inoculated when they had just expanded the flag leaf. Three replications were carried out of four plants each.

Inoculation was performed on flag leaves fixed to a surface with their adaxial side up with 1 mg of isolate per pot mixed with Lycopodium spores (1:20 vol./vol.). This mixture was evenly dusted over the plants.

Incubation was as described before.

We used four to six leaves for macroscopic observations and three for microscopic observations.

The experimental units for the statistical analysis (Duncan 0.05 test) were the average over all leaves of a genotype within a replication.

2.2. Macroscopic observations.

Latency period, infection frequency and infection type were recorded. Latency period was taken as the time period from the beginning of incubation to the time at which the 50 % of the uredia had appeared [5]. Latency period was determined by daily counting the number of uredia visible in a marked area till the number no longer increased. Infection type was determined on the marked area of the leaves. The final number of uredia was used to calculate the number of uredia per cm2. Infection type was recorded 12 days after inoculation according to a 0-9 scale [3].

The means of the observed values of latency period and infection frequency were converted into relatives values per replication, setting the observed values of Lalbahadur at 100%.

2.3. Microscopic observations

Central segments of about 3 cm2 of inoculated leaves were collected five days after inoculation. Three leaves per accession were harvested from each replication. The segments were processed for fluorescence microscopy [8] but instead of Calcofluor we used UVITEX 2B (Ciba-Geigy). The preparations were examined at 200x with a Leica epifluorescence microscope (DM LB, 330 to 380 nm wave length transmission). Sporelings that had not formed any haustorial mother cell were excluded. At least 100 sporelings per leaf segment were scored and classified according to their stage of development [4]. Infection units that formed a primary infection hypha and no more than six haustorial mother cells were considered as early aborted. Sporelings with more than six haustorial mother cells were classified as established. Necrotic of host cells was visible by use of a filter with a range between 420 to 490 nm transmission, which display a golden yellow autofluorescence. The length (L) and width (W) of ten arbitrarily chosen established colonies per leaf were measured with an eyepiece micrometer. The shape of the colony was considered as an ellipse where L and W are major and minor axis. Colony size was calculated as the geometric mean of L and W.

CS=SQRT(1/4xLxW)

2.5. Field experiment

Each line was represented by a 1-meter long single row. Three randomly replications were carried out.

3. RESULTS

3.1. Macroscopic observations

All lines displayed a high infection type both in seedlings (table 1) and adult plants (table 2). Latency period in seedlings was increased in Lalbahadur-Lr34 but not significantly in Lalbahadur-*Lr46* (table 1). Latency period on both Lalbahadur-*Lr34* and Lalbahadur-*Lr46* was significantly increased in adult plant (table 2). Lines did not differ significantly in infection frequency in seedling nor in adult plant.

Disease severity was highly reduced in Lalbahadur-*Lr34* and Lalbahadur-*Lr46* in field studies.

Table 1. Macroscopic components of resistance to *Puccinia triticina* induced by *Lr34* and *Lr46* in seedling stage.

Lines	IT[1]	RLP1	RIF1
Lalbahadur	9	100 a[2] (137 h)	100 a[2] (84 p/cm^2)
Lalb.-*Lr34*	9	107 b	86 a
Lalb.-*Lr46*	9	103 ab	94 a

1Latency period (RLP) and infection frequency (RIF) are expressed as values relatives to Lalbahadur (=100%). The actual average values for Lalbahadur are presented in brackets. Infection type (IT) is indicated as well.

2Data with the same letter per column are statistically different (Duncan, P<0.05).

Table 2. Macroscopic components of resistance to *Puccinia triticina* induced by *Lr34* and *Lr46* in adult plant stage.

	Growth chamber			Field
Lines	IT[1]	RLP1	RIF1	DS1
Lalbahadur	9	100 c[2] (175 h)	100 a[2] (28 p/cm^2)	63 a[2]
Lalb.-*Lr34*	9	116 a	84 a	15 b
Lalb.-*Lr46*	9	109 b	104 a	23 b

[1] Data with the same letter per column are statistically different (Duncan, P<0.05).

[2] Latency period (RLP) and infection frequency (RIF) are expressed as values relatives to Lalbahadur (=100%). The actual average values for Lalbahadur are presented in brackets. Infection type (IT) and disease severity (DS) in the field are indicated as well.

3.2. Microscopic observations

There were no differences in the percentage of early aborted colonies without plant cell necrosis in seedlings but in adult plant this percentage is higher on the lines Lalbahadur-*Lr34* and Lalbahadur-*Lr46*. The colonies of Lalbahadur-*Lr34* were smaller than those of Lalbahadur but those of Lalbahadur-*Lr46* did not differ from Lalbahadur. In adult plants the size of colonies supported by Lalbahadur-*Lr34* and Lalbahadur-*Lr46* was smaller than Lalbahadur. In the level of necrotic cells no differences were found between the lines neither in seedling nor adult plant stage although in general the level of necrosis was higher in adult plant.

Table 3. Microscopic components of resistance to *Puccinia triticina* induced by *Lr34* and *Lr46* in seedling (S) and adult plant stage (AP).

Lines	% EA[-1]		CS (mm^2)		% Necrosis[3]	
	S	AP	S	AP	S	AP
Lalbahadur	2 a[4]	1 a	300 a	70 a	2 a	17 a
Lalb.-*Lr34*	3 ab	16 b	200 b	25 b	2 a	12 a
Lalb.-*Lr46*	6 ab	16 b	250 ab	32 b	1 a	19 a

[1] Indicated are the percentage of early aborted colonies not associated with plant cell necrosis (%EA), the mean colony size (CS) and the percentage of colonies associated with plant cell necrosis (% Necrosis).

[4] Data with the same letter per column are statistically different (Duncan, P<0.05).

4. DISCUSSION

Lr34 gene that induces partial resistance. Rubiales & Niks [10] found a consistent increase in latency period, in percentage of early aborted colonies not associated with cell necrosis and a decrease of colony size. They also reported that differences were more pronounced in adult plants.

The effect of *Lr46* resembled that of the *Lr34* in adult plant. Both prolong the latency period and cause a higher percentage of abortion, a reduced colony size and a lower disease severity relative to the check Lalbahadur. These parameters are typical to partial resistance [1] [2].

However in seedling stage the effect of *Lr46* was too small to be detected.

Jacobs [3] in a screening of several wheat lines for partial resistance to leaf rust divided partial resistance genes in two groups: one where partial resistance are better expressed in the adult plant stage than in seedling and other group where partial resistance is only expressed in the adult plant stage. We could place *Lr34* is the first group and *Lr46* in the second one.

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REFERENCES

[1] acobs Th: Abortion of infection structures of wheat leaf rust in susceptible and partially resistant wheat genotypes. Euphytica 45:81-86 (1990).

[2] Jacobs Th, Buurlage MB: Growth of wheat leaf rust colonies in susceptible and partially resistant spring wheats. Euphytica 45:71-80 (1990).

[3] McNeal FH, Konzak CF, Smith EP, Tate WS, Russell TS: A uniform system for recording and processing cereal research data. USDA, Agricultural Research Service, Washington, D.C. ARS 34-121 (1971).

[4] Niks RE. Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Can. J. Bot. 60:714-723 (1982).

[5] Parlevliet JE, Van Ommeren A: Partial resistance of barley to leaf rust, *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24:21-27 (1975).

[6] Parlevliet JE.: Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. Euphytica 27:369-379 (1978).

[7] Parlevliet JE, Kuiper HJ:. Accumulating polygenes for partial resistance in barley to barley leaf rust, *Puccinia hordei*. I. Selection for increased latent periods. Euphytica 34:7-13 (1985).

[8] Rohringer R, Kim WK., Samborski DJ, Howes, N K: Calcofluor: An optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology 67: 808-810 (1977).

[9] Rubiales D, Niks RE: Characterisation of *Lr34*, a major gene conferring nonhypersensitive resistance to wheat leaf rust. Plant Disease. 79:1208-1212 (1995).

[10] Singh RP, Mujeeb-Kazi A, Huerta-Espino J: *Lr46*, a gene conferring slow rusting resistance to leaf rust in wheat. Phytopathology 88:890-894 (1998).

PRE-APPRESSORIAL AVOIDANCE MECHANISM TO RUST AND MILDEW FUNGI IN ECERIFERUM MUTANTS OF HORDEUM VULGARE

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ABSTRACT: High levels of avoidance to leaf rust fungi based on a poor recognition of the stomata have been described in accessions of some wild *Hordeum* species. This avoidance is due to a prominent wax layer on the stomata that inhibit triggering of the appressorium differentiation. This feature has not been found thus far in accessions of *H. vulgare*. In this study we searched for the existence of avoidance to the barley leaf rust fungus in a collection of *eceriferum* mutants of barley selected for abnormalities on features of epidermal cells and stomata number and morphology. In none of the mutants a reduction on appressorium formation was found that was as substantial as had been found in *H. chilense*. The highest decrease in appressorium formation was 30%. Three of the *eceriferum* mutants were selected for their slightly reduced induction of rust appressorium formation. They were studied to characterise the levels of avoidance. The avoidance on the cer-zh654 mutant was based on a combination of a poor orientation and poor stomata recognition.

In contrast, recognition and appressorium formation by the mildew fungus was not significantly hampered in any of the mutants, nor in the *H. chilense* check line H17.

1. INTRODUCTION

The rust and powdery mildew fungi of barley incited by *Pucinia hordei* and *Blumeria graminis* f.sp. *hordei*, respectively, are most economically important. Economic losses are attributable to the asexual stage of these fungi. During this stage, germ tubes differentiate appressoria over the stoma in the case of rust, or directly over the epidermal cells in the case of mildew. The formation of an appressorium is a critical phase of the infection because it provides the site for penetration of the host leaf. The inductive signals that trigger appressorium formation have been the focus of many studies.

In the case of rust fungi, urediniospore germlings must find leaf stomata, where appressorium formation should be triggered. Subsequently, they penetrate the stoma and may complete the infection cycle provided the plant belongs to a compatible species and genotype.

B. graminis conidia form first a single short functional germ tube (primary germ tube) soon after contacting the leaf surface. Then a second germ tube elongates and differentiates an appressorium. Formation of multiple short germ tubes is abnormal and is caused by the primary germ tube either failing to make contact with the leaf surface or failing to recognise the contact (1). On some non-hosts a large proportion of germlings develop abnormally (5). On the abaxial surface of *Lolium* spp., where epicuticular waxes form amorphous sheets, a very high proportion of *B. graminis* conidia form only multiple short germ tubes and are therefore unable to infect (2).

Preappressorial avoidance of rust fungi has only been reported occasionally in cereals, but quantitative data are not available. In *Hordeum chilense*, however, it is common (11, 14). Low appressorium formation has also been found in accessions of some other wild *Hordeum* species such as *H. brachyantherum*, *H. marinum*, *H. parodii* and *H. secalinum*, but unfortunately not in accessions of *H. vulgare* (12). Such an avoidance would be most desirable in barley breeding for rust resistance. Thus, in the present study we stu-

[6] Hoch HC, Staples RC, Whitehead B, Comeau J, Wolf ED: Signaling for growth orientation and cell differentiation by surface topography in *Uromyces*. Science 235: 1659-1662 (1987).
[7] Lewis BG, Day JR: Behaviour of urediospore germ-tubes of *Puccinia graminis tritici* in relation to the fine structure of wheat leaf surfaces. Trans.Br. Mycol.Soc. 58: 139-145 (1972).
[8] Lundqvist U, von Wettstein-Knowles P, von Wettstein D: Induction of eceriferum mutants in barley by ionising radiations and chemical mutagens. II. Hereditas 59: 473-504 (1968).
[9] Rubiales D, Carver TWL: Cellular responses of *Hordeum chilense* genotypes to inappropriate formae specialis of the cereals powdery mildew fungus. Can. J. Bot. 78: 1561-1570 (2000).
[10] Rubiales D, Niks RE: Low appressorium formation by rust fungi on *Hordeum chilense* lines. Phytopathol. 82: 1007-1012 (1992).
[11] Rubiales D, Niks RE: Avoidance of rust infection by some genotypes of *Hordeum chilense* due to their relative inability to induce the formation of appressoria. Physiol. Mol. Pl. Pathol. 49: 89-101 (1996).
[12] Rubiales D, Ramírez MC, Niks RE: Avoidance of leaf rust fungi in wild relatives of cultivated cereals. Euphytica 87: 1-6 (1996).
[13] Vaz Patto MC, Niks RE: Orientation of germ tubes of *Puccinia hordei* on the *Hordeum chilense* leaf surface. Acta Phytopath. Entom. Hungarica 35: 213-220 (2000).
[14] Vaz Patto MC, Aardse A, Buntjer J, Rubiales D, Martín A, Niks RE: Morphology and AFLP markers suggest three in *Hordeum chilense* ecotypes that differ in avoidance to rust fungi. Can. J. Bot. 79: 204-213 (2001).
[15] Yang SL, Ellingboe AH: Cuticle layer as a determining factor for the formation of mature appressoria of *Erysiphe graminis* on wheat and barley. Phytopathology 62: 708-714.

Table 1. Quantification of orientation and stomata recognition by *P. hordei* on selected *eceriferum* mutants of *H. vulgare*.

	% lost[1]	**% overgr**[2]	**% mispl**[3]	**% appr**[4]
Foma	10 b	6 b	6 a	78 a
Cer-ye[267]	18 ab	7 b	5 a	69 ab
Cer-zh[54]	20 ab	9 ab	11 a	60 bc
Cer-zh6[54]	23 a	13 a	9 a	54 c

[1]% germ tubes not reaching any stoma
[2]% germ tubes overgrowing at least one stoma
[3]% germ tubes forming an appressorium not on a stoma
[4]% germ tubes forming an appressorium over a stoma

TRITICUM TURGIDUM VAR. DICOCCOIDES AS SOURCE OF GENES FOR POWDERY MILDEW RESISTANCE

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ABSTRACT: The var. *dicoccoides* accession MG29896, very resistant to powdery mildew, was crossed to the durum susceptible cultivar Latino and a backcross inbred line population (BIL) was produced. This population was essayed for powdery mildew reactions to single strain infection in greenhouse as well as to natural population in field conditions. Two or three loci should confer greenhouse resistance, one for high resistance level and one or two for intermediate resistance. Three loci should be involved in field resistance due to the different resistance levels observed. One of field resistance loci was characterized by segregation analysis and it appears to confer dominant resistance. Microsatellite marker screening was carried out to individuate markers potentially associated to the powdery mildew resistance loci.

1. NTRODUCTION

Blumeria graminis f.sp. *tritici* is one of the most severe wheat disease in temperate climates. Due to the pathogen evolution, numerous resistance genes from cultivated varieties became ineffective and new resistance sources need to improve modern cultivars (1). Wild species offer a source of resistance genes useful to wheat breeding. *Triticum turgidum* var. *dicoccoides* is a wild tetraploid wheat (AABB) easily crossable with cultivated durum wheat. A *T.t.* var. *dicoccoides* accession resistant to powdery mildew was individuated and crossed to a *T.t.* var. *durum* cultivar. The inheritance of resistance resulted complex and at least two loci seemed involved in the control of this trait (2). Backcross inbreed lines (BILs) contain low percentage of donor genome on recurrent genetic background and can be useful to study complex traits (3; 4). In this paper it is presented the characterization of *T.t.* var. *dicoccoides* factors effective against single strain or natural population of powdery mildew using a set of 92 BILs.

2. MATERIAL AND METHODS

2.1 Plant material

A backcross inbred population was produced following the method described by Bliss (3). The recurrent parent, the durum wheat cv. Latino, is a powdery mildew susceptible commercial cultivar. The donor parent was the wild tetraploid wheat *Triticum turgidum* var. *dicoccoides*, accession MG29896, highly resistant to powdery mildew, from a collection of the Institute of Germplasm, C.N.R., Bari (Italy). One hundred and ten F_1 plants were backcrossed to the recurrent parent 3 times prior to advancement of each backcross line by self-fertilization and single-seed descent for 6 generations, resulting in one population of backcross inbred lines (BILs). No intentional selection was imposed during population development, but some lines were lost during backcrossing and self-fertilization program. The BC_3F_6 generation contained 92 BILs, which gave sufficient seed for use in replicated trials. The lines were indicated by 3BIL- followed by an order number. 5BIL-29 is a BC_5F_5 line derived from the same BC_3F_1 that originated 3BIL-29. F_2 population was originated by crossing Latino and 5BIL-29. About 600 of F_2 seeds were sown in the field.

2.2 Powdery mildew essays

For seedling reaction test (below indicated as 'greenhouse'), a powdery mildew isolate (BA10) was collected from cultivated fields of durum wheat located near Bari. A diseased leaf was dropped onto seedlings of common wheat (cv. Fortunato) growing in a test tube. The seedlings were mantained at 22°C with 12h photoperiod. After a week, a few conidia from a single colony were removed with a disposable pipette and the conidia were inoculated onto new Fortunato seedlings. Seedling resistance test was performed in a greenhouse which was mantained at 22°C and on 12h day length light regime. Seedlings were grown in trays containing vermiculite and artificially inoculated at one-leaf stage. Ten days after inoculation the seedlings were scored and classified on a 0 to 9 scale, based on Moseman et al. (5) with modifications, where 0=immune (no visible sign or symptoms), 1-3=resistant (increasing from no necrosis to large necrotic areas and no mycelium to little mycelium and sometimes with traces of individual conidia), 4-6=intermediate (necrotic areas changing to chlorotic areas, increasing in amount of mycelium and conidiospore production) and 7-9=susceptible (decreasing from chlorotic areas to no chlorosis, increasing in amount of mycelium and conidiospore production to a completely compatible relationship with the pathogen). About 10 seedlings for each BIL were essayed: if the average score resulted lower than 3 the BILs were considered resistant, if higher than 6 susceptible and between 3 and 6 the reaction was considered intermediate.

Adult plant resistance essays were performed on field conditions (below indicated as 'field'). Resistance was scored at the end of stem extention. For BILs progenies, tests were performed using the 0-9 scale of Saari and Prescott [6]. In the case of F_2 the level of infection reflects the percentage of plant surface infected (0=0-9%; 1=10-19%; 2=20-29%;...9=90-100%). F_3 progenies were essayed on randomized blocks with 3 replications; individual plants were considered susceptible if clear symptoms were observed.

2.3 Microsatellites analysis

DNA extractions were performed as described by Sharp et al. (7). Two hundred fifty-five primers pairs of wheat microsatellites, isolated and mapped by Röder et al. (8) and by Stephenson et al. [9] were essayed on Latino and MG29896 and only pairs giving polymorphic products were essayed on 3BIL-29 and 5BIL-29. The reaction conditions were set according to the authors (8; 9).

3. RESULTS

3.1. Greenhouse resistance of backcross inbred lines

The seedling of 92 3BILs showed different reactions to the single strain inoculation (Table I). Most of the lines was completely susceptible (score average between 6 and 8) as the recurrent parent (Latino), whereas 3BIL-16, -29, -38, -40, -41, -54, -81, and -87 showed an intermediate resistance (3-6) and 3BIL-57, -73, -74, and -91 showed a very reduced infection level (average score lower than 3), similar to the donor parent (MG29896). Since 3BILs are BC_3F_6 lines and the expected contribution of the donor parent is 6.125%, the high resistance observed in 4 lines, should be conferred by a single locus, whereas the intermediate resistance, found in 8 lines, is compatible with the effect of 1 or 2 loci.

Table I. Reaction to powdery mildew of BILs tested in greenhouse conditions and probability for 1, 2 or 3 resistance loci involved.

Infection reaction	BIL no.	Probability 1 locus	2 loci	3 loci
Resistant (0-3)	4	0.48	0.02	<0.01
Intermediate (3-6)	8	0.30	0.30	0.02
Susceptible (6-9)	80			
Total	92			

3.2. Field adult plant resistance

Latino, MG29896 and the 92 3BILs were cultivated in open field and the level of natural infection was scored at the end of stem extention (Table II). MG 29896 and 3BIL-29, -54, -74, -81, and -87 showed no symptoms (score=0); 3BIL-72, -79 and -83 showed very faint symptoms (score=1); 3BIL-73 and -75 showed intermediate resistance (score=3); Latino and all the remaining 3BILs showed complete susceptibility to the natural population (score=9). Three loci should be involved in the field resistance: one for the 5 hig-

hest resistant lines, one for the group of 3 lines showing very faint symptoms and one for the 2 lines showing intermediate resistance.

Table II. Reaction to powdery mildew of BILs tested in field conditions and probability for 1, 2 or 3 resistance loci involved.

Infection reaction	BIL no.	Probabilty 1 locus	2 loci	3 loci
Resistant	5	0.78	0.05	<0.01
Some symptoms	3	0.25	0.01	<0.01
Intermediate	2	0.11	<0.01	<0.01
Susceptible	82			
Total	*92*			

3.3. Segregation analysis of field resistance of line 5BIL-29

A BC$_5$F$_5$ line, derived from the BC$_3$F$_1$ that originated 3BIL-29, showed complete adult resistance to powdery mildew in field conditions. This line (called 5BIL-29) should have the same locus conferring complete field resistance present in 3BIL-29 due to the common ancestor (BC$_3$F$_1$) and to similar powdery mildew reaction (unpublished data). 5BIL-29 was crossed to Latino and analysis of adult plant resistance in field conditions was performed in F$_2$ and F$_3$ generations. About 600 seeds were sown by hand directly in the field. Only 404 plants survived and were scored for resistance (Table III). Infection score showed a bimodal distribution; the number of resistant (0-3) and susceptible (4-9) plants was 327 and 77, respectively, and the ratio was significantly different from 3:1, expected for the segregation of a single dominant resistance locus (P<0.01).

Table III. Infection severity distribution on F$_2$ population from the cross between 5BIL-29 and cv. Latino.

Score (0-9)	0	1	2	3	4	5	6 7	8	9	total	
Plants no.	310	7	6	4	9	22	16	16	9	5	404
	327				77						

Progenies from 120 randomly chosen F$_2$ plants were sown in randomized blocks in 3 replications. All F$_3$ progenies from 27 F$_2$ plants were resistant, 70 progenies were segregating (about 3:1 ratio between resistant and susceptible) and 23 were completely susceptible. This data fitted in the 1:2:1 segregation ratio expected for a single dominant resistance locus. All progenies completely susceptible derived from F$_2$ plants showing infection score higher than 4, the remaining progenies (both homozygous resistant and segregating) derived from F$_2$ plants showing infection score lower than 2.

3.4. Microsatellite analysis

To find molecular markers linked to the resistance loci, 255 microsatellite primer pairs have been essayed and 117 (46%) showed polymorphism between Latino and MG29896. All the polymorphic markers were tested on 3BIL-29 and only 12 markers presented the MG29896 allele. None of 100 polymorfic markers tested on 5BIL-29 showed the MG29896 allele.

DISCUSSION

Backcross inbred lines have been useful to individuate powdery mildew resistance factors effective on seedling single strain infection as well as on adult plant on field conditions. Resistance level induced by MG29896 loci in BILs was complete as well as partial, showing a complex of genes useful for breeding. Interestingly, 3BIL-74 showed high level of resistance in both the experimental conditions and 3BIL-73 showed high level of resistance on seedling greenhouse infection and partial resistance on field conditions; combination of two different resistance loci in these two BILs could explain this high resistance. In fact, the hypothesis of a single locus represented only in one BIL has a probability lower than 0.05, whereas the accidental combination of two indipendent loci in one of the BILs fitted better (0.25<P<0.50). On the other

hand, 4 BILs (namely 3BIL-29, -54, -81, and -87), showing complete resistance to powdery mildew on field conditions, resulted partially resistant even in the greenhouse essay. Due to the number of BILs, combination of two unlinked loci could not explain the resistance observed on both the experimental conditions ($P<0.001$). The other 4 BILs (3BIL-16, -38, -40 and -41) showing partial resistance at the seedling stage, resulted completely susceptible in field conditions. Two explainations are possible: a) seedling partial resistance is conferred by two indipendent loci, one of them very effective even in field conditions, present on 3BIL-29, -54, -81, and -87; b) the locus conferring seedling partial resistance is linked to another one conferring complete field resistance; in 4 of 8 BILs (3BIL-16, -38, -40, and -41) the linkage was broken during the development of BIL population. The first hypothesis should be more likely because no BIL completely resistant in field conditions and susceptible in greenhouse was observed. Considering all data, it is supposeded that 5 loci from MG29896 are involved in powdery mildew resistance as shown in Table 4. Locus 'a' confers complete resistance in greenhouse conditions and is ineffective on field; locus 'b' confers partial greenhouse resistance and is ineffective on field; locus 'c' confers partial resistance on greenhouse and complete resistance on field conditions; locus 'd' confers strong but incomplete resistance in field conditions and is ineffective in greenhouse; locus 'e' confers partial resistance on field conditions and is ineffective in greenhouse.

The locus c is the most promising for breeding exploitation. The 5BIL-29 (BC_5F_5) is derived from the same BC_3F_1 plant originating the 3BIL-29 (BC_3F_6). Due to the very similar powdery mildew resistance observed in field conditions, 5BIL-29 is considered to carry the same MG29896 locus 'c' conferring field complete resistance. To understand the genetic basis of this powdery mildew resistance, a cross between 5BIL-29 and Latino was carryed out. The ratio between resistant and susceptible F_2 plants is significantly higher than 3:1 ratio expected for segregation of a single dominant resistance locus. Moreover the F_3 progenies, randomly sampled, showed similar excess of resistant plants: 97 F_2 plants produced F_3 progenies homozygous resistant or segregating and only 23 F_3 progenies resulted homozygous for susceptibility. However, segregation analysis of 120 F_3 progenies showed a ratio between resistant: segregating: susceptible not significantly different from 1:2:1 expected for a single dominant locus. The excess of resistant plants in the F_2 population could be explained considering a strong selection during the field cultivation. In fact, only 2/3 of the seeds sown reached the adult stage and the advantage conferred by the resistance to powdery mildew could increase the fitness. In conclusion, this powdery mildew resistance should be determined by a single dominant locus from MG29896.

Table IV. List of BILs showing powdery mildew reactions different from cv. Latino (recurrent parent) and supposed loci controlling resistance.

Line	P.m. reaction		loci				
	greenhouse	field	a	b	c	d	e
3BIL-16	I			+			
3BIL-29	I	R			+		
3BIL-38	I			+			
3BIL-40	I			+			
3BIL-41	I			+			
3BIL-54	I	R			+		
3BIL-57	R		+				
3BIL-72		R*				+	
3BIL-73	R	I	+				+
3BIL-74	R	R	+		+		
3BIL-75		I					+
3BIL-79		R*				+	
3BIL-81	I	R			+		
3BIL-83		R*				+	
3BIL-87	I	R			+		
3BIL-91	R		+				
Total			4	4	5	3	2

R = resistant.
R* = some symptoms.
I = intermediate.

About 50% of the microsatellite markers was polymorphic between *T. turgidum durum* cv. Latino and *T. t. dicoccoides* accession MG29896. These data are similar to other comparisons between different *T. turgidum* subspecies (unpublished data) and confirm the high level of polymorphism revealable by this kind of PCR markers. Twelve on 117 microsatellite alleles from MG29896 have been found on 3BIL-29 (10.26%) higher than the expected percentage for a BC_3F_6 line (6.125%) but not significantly different ($0.05<P<0.10$). None of 100 microsatellites essayed on 5BIL-29 revealed the MG29896 allele and this result was not significantly different from the expected 1.5% for a BC_5F_5 line ($0.10<P<0.25$). The number of polymorphic markers essayed was insufficient to individuate portions of MG29896 present on 5BIL-29. To find molecular markers linked to the powdery mildew locus wider scanning of the 5BIL-29 genome has to be performed and techniques analyzing multilpe loci (e.g. AFLP) seems to be the most appropriate.

Our next goal will be to find molecular markers linked to the powdery mildew resistance loci and to understand if they are new loci or are allelic to known loci. Moreover, molecular markers linked to interesting genes are useful for marker assisted selection in breeding programs as well as starting points to isolate the genes by positional cloning strategies.

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REFERENCES

[1] Bennet FGA: Resistance to powdery mildew in wheat and review of its use in agriculture and breeding programmes. Plant Pathol 33: 279-300 (1984).

[2] Simeone R, Galasso I, Pignone D, Blanco A: Introgression of powdery mildew resistance from wild species to wheat. In: Proc 11th EWAC Conference, Novosibirsk, 24-28 July 2000, pp. 55-59 (2001).

[3] Bliss FA: The inbred backcross line method for improving quantitative traits of self-pollinated crops. Hort Sci 17: 503 (1992).

[4] Monforte AJ, Tanksley SD: Development of set of near isogenic and backcross recombinant inbred lines containing most of the *Lycopersycon hirsutum* genome in a *L. esculentum* genetic background: A tool for gene mapping and gene discovery. Genome 43: 803-813 (2000).

[5] Moseman JG, Nevo E, El Morshidy MA, Zohary D: Resistance of *Triticum dicoccoides* to infection with *Erysiphe graminis tritici*. Euphytica 33: 41-47 (1984).

[6] Saari EE, Prescott JM: A scale for appraising the foliar intensity of wheat diseases. Plant Disease Reeporter 59: 377-380 (1975).

[7] Sharp PJ, Kreis M, Shewry PR, Gale MD: Location of amylase sequences in wheat and its relatives. Theor Appl Genet 75:286-290 (1988).

[8] Röder MS, Korzum V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW: A microsatellite map of wheat. Genetics 149: 2007-2023 (1998).

[9] Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C, Gale M: Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 97: 946-949 (1998).

DIFFERENTIAL EXPRESSION OF PROTECTIVE ANTIOXIDANT COMPONENTS TO NaCl STRESS IN FOXTAIL MILLET GENOTYPES

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ABSTRACT: The modulation of antioxidant components was analysed in sedlings of a salt-tolerant (Cv. Prasad) and a salt-sensitive (Cv. Lepakshi) cultivar of foxtail millet with and without salt stress. Specifically, the regulation of superoxide dismutases (SOD) and ascorbate peroxidase (APX) were examined at the level of their transcripts, proteins and enzyme activities. Upon treatment with 200 mM NaCl the tolerant cultivar responded with an increase of the cytosolic Cu/Zn-SOD and the Mn-SOD isoform at the protein and activity level, whereas the plastid localized Cu/Zn-SOD did not show any change. Using a cDNA array containing 711 unique barley genes, we confirmed the observed changes in transcript levels for APX and found an additional transcript coding for a glutathione peroxidase-like protein (GPX) being induced by salt stress in the tolerant cultivar. This result was confirmed by Northern blotting and correlates with the induction of a 25 kDa protein. A partial amino acid sequence of this protein was obtained and found to be identical with part of the translated sequence of the foxtail millet GPX cDNA. Investigation of a barley cultivar revealed that the GPX gene is also induced by salt treatment, suggesting that its gene product may play a key role in defense against salt-induced oxidative damage in seedlings of various monocot species.

1. INTRODUCTION

Saline soil is one of the most serious problems in agriculture, affecting about 7% of the world's land area [930 million hectars (1)]. The analysis of processes induced under salt stress in salt-tolerant varieties will contribute to our understanding of gene regulation and signal transduction processes as well as ion transport and mineral nutrition in plants at high salt concentrations. Active oxygen species, including superoxide radicals, singlet oxygen and hydrogen peroxide are toxic compounds generated in the cells during salt stress as a result of interrupted electron transport chains. To detoxify such active oxygen species, a highly efficient antioxidant defense system has evolved in plants. It can be divided into two classes (a) nonenzymatic constituents including lipid-soluble membrane-associated tocopherols, water-soluble ascorbic acid and glutathione and (b) enzymatic constitutents including superoxide dismutase (SOD), peroxidases, as, for instance, ascorbate peroxidase (APX) and glutathione peroxidase (GPX). The primary scavengers in the detoxification of active oxygen species in plants are SODs, a group of metalloenzymes converting superoxide to hydrogen peroxide and oxygen. The increased production of hydrogen peroxide is subsequently counteracted by APX- or catalase-mediated detoxification of these molecules (2). Our studies indicate that the degree of oxidative cellular damage in seedlings of a tolerant foxtail millet cultivar is controlled by the capacity of the antioxidative system (3). The high activities of APX and catalase in plants have led to the conclusion that glutathione peroxidase should be either absent from plants or - if present - should not be a key actor in the detoxification of hydrogen peroxide. Recently, a GPX gene was cloned from citrus (10). In our present studies, glutathione peroxidase-like sequences have been identified in salts tolerant foxtail millet seedlings under high-salt conditions and were compared to sequences known from barley. The presented results lead to the conclusion that the salt-induced expression of GPX is part of the scavenging system developed in plants against oxidative stress.

2. MATERIALS AND METHODS

2.1. Growth measurements

Seeds of foxtail millet (*Setaria italica* L.) cultivar Prasad (salt-tolerant) and Lepakshi (salt-sensitive) were surface sterilized with 0.1% (w/v) sodium hypochlorite for 5 min and thoroughly rinsed with distilled water. Sterilized seeds were germinated in petri dishes lined with filter papers. Hoagland medium was used for control and hoagland medium with 200 mM NaCl added to impose salt stress. The petri dishes were maintained at 25°C under aseptic conditions for 5 days in the dark, and fresh weight was measured as an average of 100 seedlings. Measurements were repeated 3 times with independent experiments.

2.2. Estimation of activity patterns of SOD isoforms

Five-day-old seedlings (100 mg) of both cultivars were ground to a fine powder in liquid nitrogen, suspended in 50 mM potassium phosphate, pH 7.8, 5 mM sodium ascorbate, 7 mM ß-mercaptoethanol and 0.2% (v/v) Triton X-100 and centrifuged at 9,000 g for 10 min at 4°C. For the separation of SOD isozymes, an aliquot of the supernatant containing 25 µg protein was applied to a native polyacrylamide gel electrophoresis (PAGE) using a 5% stacking and a 13% separating gel. After electrophoresis, the gel was stained according to the method of Beauchamp and Fridovich (4) to identify each SOD isoform. After inhibition experiments, the cytosolic, mitochondrial and plastidic SOD isoforms were quantified by using the software package Bio 1D of an inmaging system (Vilber Lourmat, France).

2.3. Western blot analysis

Five-day-old seedlings of both cultivars were homogenized in 50 mM Tris-HCl, pH 7.4, at 4°C. The homogenate was centrifuged at 9,000 g for 20 min at 4°C. Aliquots of the supernatant containing 25 µg protein were resolved on a 12% SDS-PAGE. Western blot analysis was peformed as described by Kruse et al. (6). Membranes were blocked with 3% (w/v) BSA and treated with spinach cytosolic Cu/Zn-SOD antibody, tobacco Mn-SOD antibody or an antiserum against spinach cytosolic ascorbate peroxidase. The bound antibodies were detected with an anti-rabbit IgG-peroxidase conjuate using the ECL reaction according to the manufacturer's protocol (Amersham Pharmacia Biotech, Little Chalfont, UK).

2.4. Northen blot analysis

RNA was extracted from frozen seedlings as described by Heim et al. (7). Total RNA (15 µg per lane) was separated on 1% agarose-formaldehyde gels and blotted on Hybond N^+ membranes (Amesham Pharmacia Biotech, Little Chalfont, UK) according to the manufacturer's protocol. Filters were hybridized at 55°C according to the method of Church and Gilbert (8) with ^{32}P-labelled fragments specific for SODs and APX.

2.5. Construction, hybridization and evaluation of arrays

Arrays have been constructed from PCR-amplified inserts of barley cDNA clones from seeds, etiolated seedlings and roots (9). These arrays were hybridized with ^{33}P-labelled cDNA produced from RNA of foxtail millet seedlings grown under control conditions and NaCl treatment. Hybridizations were carried out in Church buffer (0.5 M sodium phosphate, pH 7.2, 7% SDS, 1% BSA, 100 µg/ml salmon sperm DNA, 1 mM EDTA) at 55°C overnight. After washing, autoradiographs were taken using a Fuji BAS 2000 Phosphoimager. For spot detection and determination of the signal intensities the program package Array Vision (Imaging Research) was used in conjunction with a common spredsheet program for further evaluation of the data.

3. RESULTS AND DISCUSSION

3.1. Growth attributes and oxidative stress conditions

The growth of the salt-sensitive cultivar of foxtail millet is completely inhibited by 200 mM NaCl, whereas the salt-tolerant cultivar will grow normally even at 250 mM NaCl (Fig. 1). Under salt stress, reactive oxygen species (ROS) accumulate as a consequence of disrupted electron transport systems in organelles with high oxidizing activities. During evolution plants have developed the capacity to eliminate these toxic ROS *via* efective scavenging systems and salt tolerance seems to be conferred by an increased antioxidative capacity.

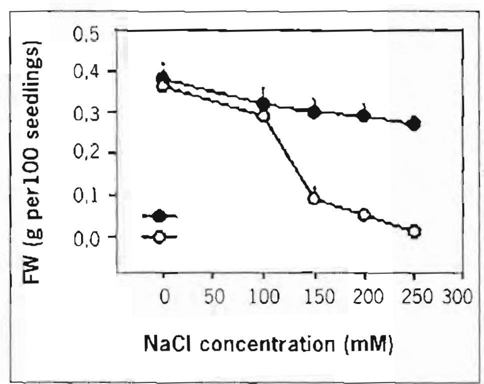
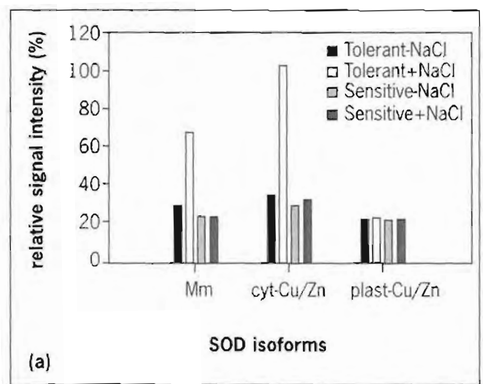
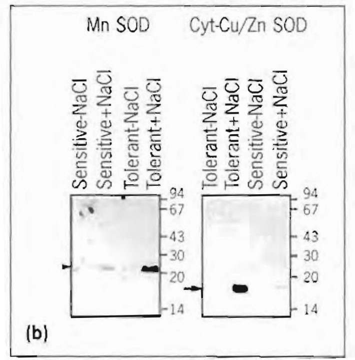


Fig. 1: Growth response of seedlings of the salt-tolerant and the salt-sensitive cultivar to increasing salt concentrations.

3.2. Compartment-specific expression of SOD isoforms in the salt-stressed tolerant variety

The steady state transcript levels of the cytosolic and plastidic Cu/Zn-SOD and of the mitochondrial Mn-SOD isoform were determined in seedlings of the salt-tolerant and the salt-sensitive cultivar under control conditions and upon salt stress (200 mM NaCl). Under salt stress the steady-state transcript and protein levels of the cytosolic Cu/Zn-SOD (17 kDa) and the Mn-SOD (22 kDa) increased strongly in the tolerant cultivar but only slightly in the sensitive cultivar (Fig. 2c and 2b, respectively). A similar pattern of activity was confirmed by inhibition experiments (see Fig. 2a). No changes were observed at the activity level for the plastid localized Cu/Zn-SOD (Fig. 2a). In response to salt stress, it seems to be important that SOD isoforms are compartement-specific induced. SOD is considered as one of the key enzymes within the antioxidant defense system of the tolerant foxtail millet.





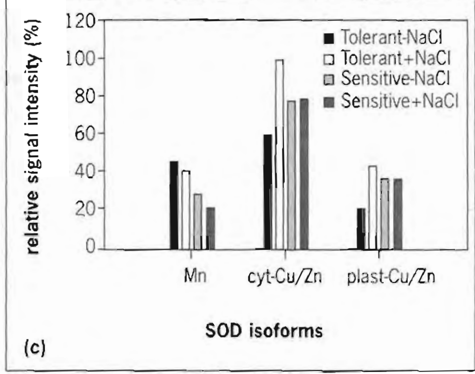
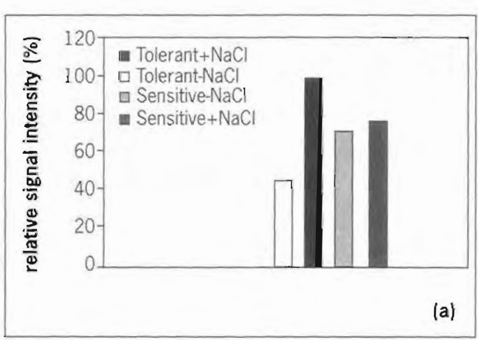


Fig. 2: Steady-state enzyme activities (a), protein levels (b) and transcript levels (c) of different SOD isoforms.

3.3. The possible role of hydrogen peroxide scavenging enzymes in tolerance against salt-mediated oxidative stress

An increase in the hydrogen peroxide-scavenging capacity is required to enable rapid removal of hydrogen peroxide (2). Following dismutation of superoxide radicals into hydrogen peroxide and oxygen by SOD, APX reduces hydrogen peroxide into water by converting ascorbate into dehydroascorbate. Therefore, the increased production of hydrogen peroxide is subsequently counteracted by ascorbate peroxidase. During NaCl treatment, the salt-tolerant foxtail millet cultivar expresse higher amounts of the cytosolic ascorbate peroxidase (APX) at both the transcript and protein level (Fig. 3a and 3b). The parallel increase of both cytosolic Cu/Zn-SOD (see above) and cytoplasmic APX activity in the tolerant cultivar under high concentration of NaCl indicates that the cytoplasm may be the site of ROS accumulation. The cellular concentration of superoxide radicals and hydrogen peroxide should be controlled by the parallel expression of both cytosolic SOD and APX.



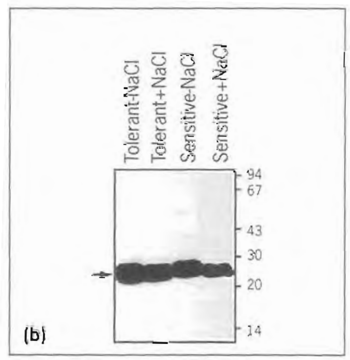


Fig. 3: Transcript (a) and protein level (b) of the cytosolic ascorbate peroxidase (APX).

In animals, glutathione peroxidase (GPX) has been demonstrated to play the key role as a major scavenger against hydrogen peroxide and lipid peroxidation. Recently, an indication for glutathione peroxidase activity in plants has been reported (10). In our study, a partial amino acid sequence of a salt-induced protein (25 kDa) isolated from salt-treated tolerant seedlings revealed significant similarity to an animal glutathione peroxidase. This finding was further supported by expression analyses using the cDNA macro array techinique where we have found the same glutathione peroxidase gene specifically expressed in seedlings of the tolerant cultivar exposed to 250 mM NaCl. We conclude, that the glutathione peroxidase gene is expressed in the tolerant cultivar to scavenge hydroperoxides and so, in turn, to prevent lipid peroxidation and to maintain the membrane integrity of plant cells under salt stress.

3.4. Coordinated expression of hydrogen peroxide producing and scavenging enzymes at the subcellular level – the most effective protection mechanism?

Under salt stress, living cells must maintain a delicate balance between the rates of superoxide radical generation and removal. The hydrogen peroxide radicals produced by SODs are highly toxic and damage the cell components immediately after production. Therefore, a parallel up-regulation of an hydrogen peroxide scavenging activity, for instance APX and GPX is necessary to protect the cells against hydrogen peroxide radical-induced damage. At 200 mM NaCl, sublethal to seedlings of the tolerant cultivar and lethal to seedlings of the sensitive one, a cytosol-specific upregulation was found for there antioxidant enzymes, Cu/Zn-SOD as well as APX and GPX. We speculate that under high-salt conditions the compartment-specific co-regulation of the hydrogen peroxide radical producing as well as scavenging activity might be the most effective way to protect plant cells against salt-induced damage.

3.5. Conclusions

In summary, the present work shows subtantial differences in the cellular reactivity between the salt-tolerant and salt-sensitive foxtail millet cultivar in response to salt stress. Upon high concentration of NaCl, the seedlings of the salt tolerant cultivar exhibit an increase in the activity of SOD, APX and GPX, which are located in cytoplasm. In view of the current data, it seems reasonable to conclude that the increased capacity of ROS scavenging system could explain the ability of the tolerant foxtail millet cultivar to grow at higher NaCl concentration than the sensitive one.

REFERENCES

[1] Szaboles I: In 'Handbook of plant and crop stress' (Ed. M. Pessarakali). Marcel Dekker, New York: 3-11 (1994).

[2] Foyer CH, Descourrieres P, Knuert KJ: Protection against oxygen radicals: An important defence mechanism studied in transgenic plants. Plant Cell and Environment 17: 507-523 (1994).

[3] Sreenivasulu N, Ramanjulu S, Kini KR, Prakash HS, Shetty HS, Savithri HS, Sudhakar C: Peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of foxtail millet. Plant Science 141: 1-9 (1999).

[4] Beauchamp C, Fridovich I: Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 44: 276-287 (1971).

[5] Bradford M: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72: 248-254 (1976).

[6] Kruse E, Mock HP, Grimm B: Reduction of coproporhyrinogen oxidase level by antisense RNA synthesis leads to deregulated gene expression of plastid proteins and affects the oxidative stress defense system. EMBO J. 14: 3712-3720 (1995).

[7] Heim U, Weber H, Bäumelin H, Wobus U: A sucrose synthase gene of Vicia faba L.: Expression pattern in developing seeds in relation to starch synthesis and metabolic regulation. Planta 191: 394-401 (1993).

[8] Church GW and Gilbert W: Genomic sequencing. Proc. Natl. Acad. Sci. USA, 81: 1991-1995 (1984).

[9] Sreenivasulu N, Siefken M, Hähnel U, Weschke W, Wobus U and Altschmied L: Multiparallel expression analysis using EST-clones from barley. Barley Genetics VIII: 196-198 (2000).

[10] Avsian-Kretchmer O, Eshdat Y, Gueta-Dahan Y, Ben-Hayyim G: Regulation of stress induced phospholipid hydroperoxide glutathione peroxidase expression in citrus. Planta 209: 469-477 (1999).

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SESSION E:

QUALITY AND UTILISATION

PROTEIN MARKERS FOR INCREASING EFFICIENCY OF TRITICEAE DUM. GENETIC RESOURCES UTILISATION IN BREEDING

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To serve as an effective basis for improvement of cultivated plants, genetic diversity stored in gene banks, should be carefully and comprehensively evaluated and characterised (investigated). Collections should be rationally organised. Each accession should be identified and registered. Preservation of the genetic composition of accessions is also included into the category of basic problems. The objective of a gene bank is to maintain an accession unchanged as regards its genetic composition. It means preserving not only the sample as such, but together with its valuable properties, in particular adaptive ones, etc. Understanding the genetic structure of biodiversity (relationships inside a gene pool or between structural elements of genetic diversity) is an important goal of genebank activities. All above-mentioned directions should be developed to facilitate the use of germplasm for improvement of cultivars. Protein markers (PM) are successfully used in VIR since 1969 for increasing utilisation efficiency of *Triticeae* Dum. genetic resources in plant breeding. Genetic resources of *Triticeae* are studied in the following aspects: a) structure of biodiversity (intraspecific relations and interspecific relationships, genome analysis); b) **identification and registration of genetic diversity and preparation of catalogues and data bases based on protein formulae;** c) identification of duplicates, development of core collections; d) **genetic integrity control; e) authorship rights control (for gene banks).** Serological markers have been successfully used in genome analysis of *Triticum* L., *Aegilops* L., *Elytrigia* Desf., *Elymus* L., *Agropyron* Gaertn. Genetic differentiation of wheat, oat and rye biodiversity based on prolamin polymorphism was carried out. Genetic diversity of the most economically important *Triticeae* species was registered on prolamin patterns, catalogues and a data base of protein (prolamin) formulae were composed.

1. INTRODUCTION

Global plant genetic resources are considered all over the world as the basic source for improvement of agricultural crops in the coming decades. The creation of sources and donors of important properties, i.e. organization of prebreeding work, is in most cases based on world genetic resources or collections of cultivated plants and their wild relatives.

Disclosure of the potential of genetic resources for the basic biological and selection properties provides a genetic base for realisation of selection programs in various directions. As a whole, the pre-breeding work includes all stages of work with plant germplasm from collecting, maintenance and study up to the legal aspects of authorship over the donors and sources of valuable characters.

Traditional approaches are founded on morphological characters which have some limitations. Proteins as primary products of gene expression reveal small changes (mutations and so on) inaccessible to visual analysis. Protein markers (PM) are, as a rule, inherited codominantly and analysis of a genotype is possible immediately by the protein phenotype. PM are successfully used in VIR for solving theoretical and applied problems of introduction, study, storage, reproduction, identification and registration of Triticeae genetic resources. Special attention is given to the development of effective tools for breeding, variety testing and seed production.

was confirmed by immunological (5), morphological (5) and molecular (8) methods. The proteins of wheat species from the *turgidum-aestivum* group revealed antigens typical for the genome of *Ae.longissima*, while the proteins of wheat with genome G revealed antigens typical for *Ae.speltoides* (1,2). It seemed likely, therefore, that *Ae.speltoides* (genome Bsp) could be the source of genome G, whereas *Ae.longissima* could be source of genome B (1,2,6).

5.3. Like *Trriticum* and *Aegilops*, the genera *Elytrigia, Elymus* and *Agropyron* include species of different ploidy levels: 2n=14,28,42,56,80. Serological markers (GSP) have helped to make a successful analysis of the interrelation between genomes belonging to genera *Triticum, Aegilops, Elytrigia, Elymus* and *Agropyron* (1,6). We used monospecific immune sera against following genome specific protein antigens: Antigen Ab (*T.boeoticum* genome), antigen Au (*T.urartu*), antigen Bl (Ae.longissima), antigen D (Ae.taushii), antigen Albumin 0,19 (*Triticum, Aegilops* species except *T.boeoticum* and *T.monococcum*) and other antigens (1). It was shown that some *Elytrygia* species, including *E.elongata* (2n=56,70), *E.intermedia* (2n=42), *E.trichophora* (2n=42) and *E.iuncea* (2n=42) possess antigens common for all three genomes of *T.aestivum*. Antigen 0,19 was also typical for them. Other *Elytrigia* and *Agropyron* species have common antigens only as far as one of these genomes is concerned. A series of *Elytrigia* and *Agropyron* species do not have wheat genome antigens or have the most common antigens. A representative of these species appear to be incompatible of poorly compatible in crosses with wheat species. Small degree of homology between the genomes of T.aestivum and Ag.yezoense was demonstrated. More than 80 species belonging to Elytrigia, Elymus and Agropyron were proved to be immunochemically distinctive from wheat genomes. It was shown that genetic compatibility between these species and wheat ones lies in their correspondence with the presence of protein antigens marking wheat genomes (1,6).

5.4. Own genome-specific antigens have been identified in proteins of the following diploid *Elytrigia* species: *E.elongata* (antigen E), *E.stipifolia, E.ferganensis* (antigen S), *E.juncea* (antigen J). Antigens marking genome E have been found in proteins of 28-56 and 70 chromosome *E.elongata* races and some of *Elytrigia* and *Elymus* species (1,6). Antigens marking genome S have been found in most of *Elytrigia* species, almost always in the absence of antigens of genome E. Antigens of genome S are present in the proteins of all *Agropyron* and many *Elymus* species. It should be noted that S-antigens distinctly differentiate *Elymus* species into two groups. *Elymus* species with genome S stand closer to *Elytrigia* species possessing genome S than to the species of their own genus (*Elymus*) which do not have this genome. *Elymus* species with S-antigens are well compatible with diploid *Elytrigia* species carrying genome S. All this agrees with the results of cytogenetic analysis and supports the opinion of those researches (9, 10) who suggest that only the species with genome S should be attributed to the *Elymus* genus. It has been suggested that only the forms that can be crossed with wheat should remain in the *Elytrigia* genus. Similar classification based on cytogenetic genome analysis was proposed (10), whereby *Elytrigia* species which could be crossed with wheat were separated from *Agropyron* genus. Antigens of genome J have been identified as traces in 28- and 42- chromosome races of *E.juncea* as well as in polyploid forms of *E.elongata* (2n=56,70), *E.intermedia and E.trichophora* (2n=42). Genome J unites the *Elytrigia* species closely related by their antigenic composition to wheat species, providing for easy crossability (6).

5.5. Thus, comparative analysis of *Elytigia, Elymus and Agropyron* showed that their diploid species carried genomes E, S and J which had specific antigens-markers. Judging by protein antigens, in most cases these genomes showed similarity with wheat genomes either by einkorns (Au,Ab) or by *Aegilops* (Bl, D). Polyploid species may involve, together with above mentioned genomes E, S and J, the genetic material from "wheat" genomes. Thus, a comparative analysis of *Triticum, Elytrigia, Elymus* and *Agropyron* species by grain proteins-antigens gave a possibility to correct the genome composition and also to elucidate genomic interrelation between the species. The problem of relationship between *Elytrigia* and wheat species is of principal interest because *Elytrigia* is often involved in hybridisation to produce *Triticum* x *Elytrigia* hybrids. However, a set of *Elytrigia* species used in distant hybridisation is very limited. Most of *Elytrigia* and *Elymus* were never involved in crossing with wheat because of the lack of information on genetic proximity of the genomes. Our results (1) partially compensate for this deficiency.

5.6. Above-mentioned data on the genomic origin have been obtained by serological methods which have advantages and also several limitations. It is impossible to make an objective evaluation of quantitative content and component composition of GSP with these methods. We have proved that these parametrs are the ones of fundamental importance in determining the degree of relationships between cereal species and genomes by antigen markers. In the recent years we have mana-

ged to develop a method of applying ELISA and immunoblotting techniques to GSP of *Triticeae* (12). The data derived from ELISA corresponded well with the results of double immunodiffusion. All this enhances the possibility of a more efficient genome analysis by GSP antigens and of solving the problems faced in the search for markers of valuable qualities and characters of cereals.

REFERENCES

[1] Molecular biological aspects of applied botany, genetics and plant breeding. Theoretical basis of plant breeding. V.Konarev and A.Konarev (Eds). Vol. I. St.-Petersburg, VIR. 228 p. (1996).

[2] Konarev V, Gavriljuk I, Gubareva N and Peneva T. Seed proteins in genome analysis, cuktivar identification and documentetion of cereal genetic resources: a review. Cereal Chem. 56: 272-278 (1979).

[3] Vvedenskaja I, Alpatyeva , Gubareva N and Konarev A. Use of storage protein electrophoresis in the analysis of genetic resources of some cereals. Erhaltung and nutzung pflanzengenetischer ressourcen - eine internationale aufgabe fur naturschutzer, genbanken und pflanzenzuchter. Vortrage fur pflanzenzuchtung. 25: 187-201 (1993).

[4] Zhukovskii P. Cultivated Plants and Their Relatives. "Kolos" .Leningrad, Russia. (1971).

[5] Flora of Cultivated Plants. I. Wheat. V.Dorofeev and O.Korovina (Eds). "Kolos". Leningrad, Russia (1979).

[6] Konarev A. The genome specific grain proteins anf phylogenetic interrelation between *Triticum* L., *Elytrigia* Desf., *Elymus* L and, *Agropyron* Gaertn. Theor. Appl. Gen. 59: 117-121 (1981).

[7] Konarev A, Gavrikjuk I and Migushova E. Differentiation of diploid wheats as indicated by immunochemical analysis. Proceeding of Soviet Agr. Sci. (USSR) 6:12 (1974)

[8] Dvorak J, McGuire P and Cassidy R. Apparent sources of the A genome of wheat inferred from polymorphism in abundance and and restriction fragment length of repeated nucleotide sequences. Genome, 1988, 30: 680-689.

[9] Tzvelev N. Poaceae of USSR. "Nauka". Leningrad, Russia (1976).

[10] Dewey D. Cytogenetics of *Elymus sibiricus* and its hybrids with Agr.tauri, E.canadensis and Agr.caninum. Bot. Gazette.135: 17-28 (1974).

[11] Sakamoto S. Patterns of phylogenetic differentiation in the tribe *Triticeae*. Seiken Ziho, 24:11-31.

[12] Chmelev V, Sinjutina N and Konarev A. Nature genome specific proteins (GSP) - serorogical markers of the genomes B and D of wheats. Agricultural Biology (Russia). 1:83-88 (1991).

CHARACTERIZATION AND TRANSFER OF A RECOMBINANT CHROMOSOME 1AS.1AL-1D WITH THE *GLU-D1* GENE FROM HEXAPLOID TRITICALE TO DURUM WHEAT

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ABSTRACT: Breadmaking quality in wheat appears to be mainly associated with high molecular weight (HMW) glutenin subunits encoded by the *Glu-1* loci on the long arm of group-1 chromosomes. The *Glu-D1d* allele (encoding the 5+10 subunits) accounts for a large part of the total variation of gluten strength. In an attempt to improve breadmaking quality of hexaploid triticale, a fragment of the long arm of wheat chromosome 1D containing the *Glu-D1d* allele was translocated to the long arms of chromosome 1A by induced homoeologous recombination (1). The goal of the present study was to transfer the 1AS.1AL-1D translocation chromosome to tetraploid wheat, thereby making the *Glu-D1* locus available for the improvement of cultivated durum wheat. Two translocation tetraploid plants expressing the 5+10 HMW glutenin subunits were recovered in the BC_1F_1 generation obtained by crossing the translocated triticale Rhino-6 with the cv. Creso of durum wheat. Plant vigour and fertility of BC_3F_2 translocation plants were similar to normal durum wheat plants, and only a slight reduction of grain yield per spike was observed. Gluten strength was affected by the presence of the 5+10 glutenin subunits as the SDS sedimentation value was significantly higher than the durum parent. Cytogenetics and molecular analyses indicated that the translocation plants have a 1A.1D chromosome containing a terminal 1DL segment about 35-40% of the recombinant arm length (11). Further chromosome engineering is required to shorten the 1D chromosome segment in this translocation for a successful exploitation in durum wheat breeding.

1. INTRODUCTION

It is generally accepted that gluten quality of dough of both common and durum wheats depends directly on grain storage protein content and protein composition (2). Environmental factors and management practices influence protein quantity, whereas protein composition is only determined by the cultivar genotype. The high molecular weight (HMW) and the low molecular weight (LMW) glutenin subunits, which constitute about 45% of the total endosperm proteins, play an important role in the physico-chemical characteristics of gluten and the rheological properties of dough with respect to breadmaking and pastamaking quality of common and durum wheats. The HMW glutenin subunits are encoded by complex loci designated *Glu-A1*, *Glu-B1* and *Glu-D1*, located on long arm of chromosomes 1A, 1B and 1D of hexaploid wheat (3). Each *Glu-1* locus contains two closely linked genes encoding a high x-type subunit and a low y-type subunit (4). Subunits coded by the *Glu-A1* locus belong to x-type, since the y-type subunit is not expressed at this locus at both tetraploid and hexaploid wheat cultivars. The x-type subunit at *Glu-A1* locus is seldom expressed in most of the durum wheat cultivars, so variation is present only at *Glu-B1* locus with only a few alleles. Several alleles of these three loci have been found in cultivars and numerous other alleles have been found in landraces and in species related to wheats. The introduction of these alleles from landraces and wild progenitor species into wheat through conventional breeding methods is usually laborious and requires the assessment of the effect of different gluten components on technological properties in the cultivated background. An approach to improve grain quality of durum wheat could be the exploitation of the useful allelic variation for storage proteins existing at *Glu-1* loci of cultivated hexaploid wheat, which effects on

gluten quality have been studied in detail. The chromosomes of tetraploid wheat show regular pairing with the A and B genomes of hexaploid wheat, therefore each allelic variant of the *Glu-A1* and *Glu-B1* loci of bread wheat can easily be transferred in durum wheat. The exploitation of allelic variation at *Glu-D1* locus could be also possible, even though the backcross program to durum wheat is usually more complicated and the use of aneuploids and of ph mutant lines is essential to induce homoeologous pairing and recombination between the chromosome 1D of bread wheat and the chromosomes 1A or 1B of durum wheat.

In a attempt to improve breadmaking quality of hexaploid triticale, a fragment of the long arm of wheat chromosome 1D was translocated to the long arm of chromosome 1R (5) and to the long arm of chromosome 1A (1) by using various aneuploids and induced homoeologous recombination The translocated fragment contained the *Glu-D1* locus encoding the HMW glutenin subunits 5+10, which accounts for the largest part of the total variation in gluten quality of bread wheat. Disomic 1D(1A), 1D(1B), 1D(1R) substitutions with the *Glu-D1a* allele were recently obtained in hexaploid triticale by Lafferty and Lelley (12). With the aim to transfer both the *Glu-D1* encoding HMW and the *Glu-D3* encoding LMW glutenin subunits into durum wheat, Ceoloni et al. (6,7) were able to isolate tetraploid translocated lines with a *Gli-D1/Glu-D3* and *Glu-D1* protein phenotypes. We carried out a similar work by using the translocated hexaploid triticale obtained by Lukaszewsky and Curtis (1) with the objective to transfer the *Glu-D1* locus to tetraploid wheat, thereby making available a different source of this gene for the improvement of cultivated durum wheat. In the present study we describe the transfer of the 1DL fragment and we report preliminary observations on some grain yield and quality components of the durum wheat translocated line.

2. MATERIALS AND METHOD

Plant material. The hexaploid triticale Rhino-4 and Rhino-6, which were kindly provided by Dr. A. J. Lukaszewsky, Department of Botany and Plant Science, University of California, Riverside, USA, was the source of the *Glu-D1* locus present on the translocation 1AS.1AL-1DL. The recipient tetraploid durum parent was the Italian semi-dwarf cultivar Creso. Rhino-4 and Rhino-6 were crossed to durum wheat and the resulting 35-chromosome pentaploid hybrids were backcrossed to Creso. The selected 28-chromosome translocation heterozygotes were backcrossed again to recover the Creso genetic background and then followed by selfing.

Ninety-nine ((Rhino-6 x Creso) x Creso) BC_3F_2 plants were grown and evaluated for grain yield and quality components. Field experiment was conducted at Valenzano, near Bari, in 2000-01, using plots consisting of one metre rows, with 10 plants at 10 cm spacing, spaced 30 cm apart. Single plants were harvested and yield components (spikelets per spike, kernels per spike, kernels per spikelet, grain yield per spike) were evaluated on three spikes per plant. A 10 g seed sample per plant was used to determine 1000 kernel weight. Protein percentage was measured on a 2-g sample of whole-meal flour by near-infrared reflectance spectroscopy and expressed on a dry weight basis. SDS-sedimentation value was determined according to Dick and Quick (8) using 1-g of whole grain meal obtained by grinding the sample in a Udi mill with a 1.0 mm sieve and expressed as height (mm) of sediment.

Mitotic analysis. Seeds were germinated on moist filter paper for a few days at 25°C in the dark. To increase the proportion of metaphases, root-tips were treated for 4 h with an aqueous solution of 0.05% colchicine or, alternatively, immersed in ice water for 24 h; they were subsequently fixed in ethanol:acetic acid (3:1, v/v). Standard Feulgen-staining and squashing procedures were used to obtain preparation for cytological analysis.

Microsatellite marker. Small-scale DNA extractions from leaves for microsatellite analysis were carried out using the Dneasy Plant Mini Kit (Qiagen, Germany). The microsatellite locus *Xgwm135-1A* was amplified using the primer pair GWM135 (9). Polimerase chain reaction and fragment detection was performed as described by Roder et al. (9).

Electrophoretic analysis. High molecular weight glutenin components of grain storage proteins were identified. Total seed protein extraction from crushed single grain halves and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels were carried out according to Payne et al. (10).

3. RESULTS AND DISCUSSION

The crosses Rhino-4 x Creso and Rhino-6 x Creso produced 4 and 9 viable seeds, respectively, all having 2n=35 chromosomes. The plants were grown in the field and were backcrossed as females with Creso. Only nine BC_1F_1 seeds were obtained from the cross (Rhino-6 x Creso) x Creso, which had 2n=28 (2), 2n=29 (1), 2n=30 (5) and 2n=31 (1) chromosomes (Table I).

Table I. Pedigree, chromosome numbers and status of the translocation chromosome 1A.1AL-1DL in F_1 and BC_1F_1 plants derived from crossing the durum wheat line Creso with the translocated triticale lines Rhino-4 and Rhino-6, the source of *Glu-D1* locus.

Pedigree	Number of plants	Chrom. number (2n)	Translocation chromosome[1]
(Rhino 4 x Creso) F_1	4	35	+ / -
(Rhino 6 x Creso) F_1	9	35	+ / -
[(Rhino 6 x Creso) x	2	28	+ / -
Creso] BC_1F_1	1	29	+ / -
	3	30	+ / -
	2	30	- / -
	1	31	+ / -

[1] Presence (+) or absence (-) of the translocation chromosome based on the expression of the 5 + 10 glutenin components coded by the *Glu-D1* locus.

The nongerm portions of each half seed were screened by SDS -PAGE for the storage proteins and the two seeds with 28 chromosomes had the 5+10 HMW glutenin subunits indicating the presence of the *Glu-D1* locus. These two heterozygous plants were vigorous and fertile and were backcrossed to durum wheat to recover the Creso genetic background. Samples of BC progeny were screened by SDS-PAGE and plants with the glutenin subunits 5+10 were always used as female and Creso as male parent. Proportions of back-cross progeny with and without the *Glu-D1* gene ranged from 9:11 to 6:8. Among the 46 $BC_{2\text{-}4}F_1$ progeny screened there were 21 plants with the glutenin subunits 5+10 and 25 without fitting the expected 1:1 ratio (Table II) and indicating a normal female transmission of the translocated chromosome. Two hundreds and three $BC_{1\text{-}3}F_2$ seeds were also screened for the presence of *Glu-D1* locus and 136 were found with the 5+10 glutenin bands and 67 without, a proportion significantly different at 0.05P from the expected ratio of 3:1 and indicating a reduced transmission of the translocated chromosome, very likely via male gametes.

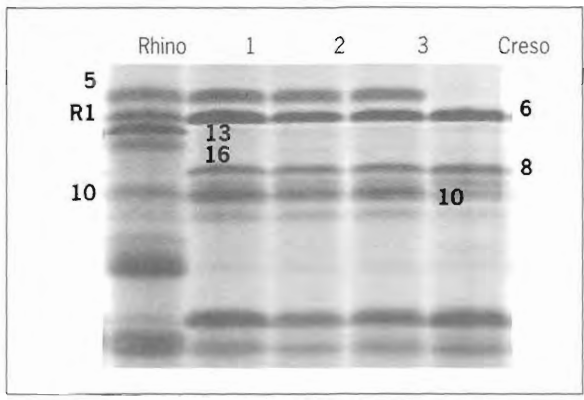
Out of 99 BC_3F_2 seeds screened for the presence of *Glu-D1* locus, 67 were found with the 5+10 glutenin bands and 32 without (Fig.1). The BC_3F_2 plants were grown in the field and a different survival of the plants with and without the 5+10 glutenin subunits was observed (64.7% and 80.6%, respectively). As Creso has a null allele at *Glu-A1* locus, a microsatellite marker (*Xgwm135-1A*), located at 8.2 cM from *Glu-A1* on 1A chromosome (9 and Röder, personal communication), was analysed on 69 BC_3F_2 fully-grown plants in order to distinguish homozygous from heterozygous plants for the 1AS.1AL-1DL translocated chromosome. DNA from 12 BC_3F_2 plants with 5+10 glutenin subunits did not generate PCR products and were therefore considered homozygous for the translocated chromosome (Fig. 2). The proportion of 25:32:12 (disomic for 1AS.1AL chromosome, heterozygous 1AS.1AL/1AS.1AL-1DL and homozygous for 1AS.1AL-1DL, respectively), even if fitted the expected 1:2:1 ratio, indicated a reduced transmission of the gametes with the translocated chromosome and/or a reduced vitality of the translocated plants.

Table II. Transmission of the translocated chromosome 1AS.1AL-1DL in backcross derivative plants with 2n=28 chromosomes of the durum wheat line Creso (recurrent parent) with the translocated triticale Rhino-6, the source of *Glu-D1* locus.

Generation	Number of plants	Translocatio chromosome[1]			$X_{1:1}$ or
		+/+	+/-	-/-	$X_{3:1}$
BC_2F_1	14		6	8	
BC_3F_1	20		9	11	
BC_4F_1	12		6	6	
Total	*46*		*21*	*25*	*ns*
BC_1F_2	80	53		27	
BC_2F_2	24	16		8	
BC_3F_2	99	67		32	
Total	*203*	*136*		*67*	**

[1] Presence (+) or absence (-) of the translocation chromosome based on the expression of the 5 + 10 glutenin components coded by the *Glu-D1* locus.

ns, * = not significant and significant at 0.01P, respectively.



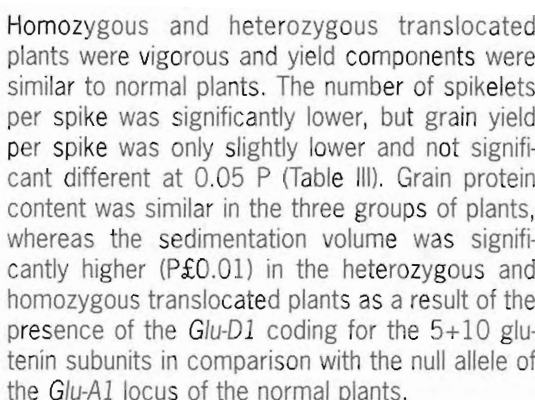

Homozygous and heterozygous translocated plants were vigorous and yield components were similar to normal plants. The number of spikelets per spike was significantly lower, but grain yield per spike was only slightly lower and not significant different at 0.05 P (Table III). Grain protein content was similar in the three groups of plants, whereas the sedimentation volume was significantly higher (P£0.01) in the heterozygous and homozygous translocated plants as a result of the presence of the *Glu-D1* coding for the 5+10 glutenin subunits in comparison with the null allele of the *Glu-A1* locus of the normal plants.

Figure 1. SDS-PAGE of the high molecular weight (HMW) glutenin subunits of three translocated durum wheat plants (lanes 1-3) and their parents, the translocated hexaploid triticale Rhino-6 (lane R) and the durum wheat cultivar Creso (lane C).

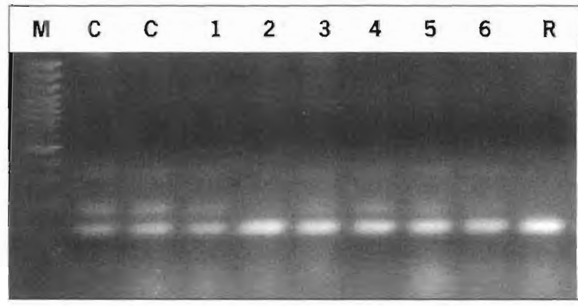


Figure 2. Amplification of the microsatellite locus *Xgwm135-1A* on acylamide gel. Durum wheat cv. Creso (C), hexaploid triticale Rhino-6 (R), homozygous translocated durum wheat plant (2), heterozygous translocated durum wheat plant or normal plant (1,3,4,5,6). M = marker

Table III . Preliminary observations of the effects of the *Glu-D1* locus from 1D chromosome on some yield and quality components in BC_3F_2 plants of the cross (Rhino-6 x Creso) x Creso.

Traits		Translocation chromosome[1] -/-	+/-	+/+
Protein genotype:	*Glu-A1*	null	null	-
	Glu-B1	6+8	6+8	6+8
	Glu-D1	-	5+10	5+10
Plant height (cm)		69.0	70.0	73.0
Spikelets per spike (n.)**		23.1 b	21.1 a	21.4 a
Kernels per spike (n.)	45.8	42.8	44.5	
Kernels per spikelets (n.)		2.0	2.0	2.1
Grain yield per spike (g)	2.0	1.8	1.8	
1000 kernel weight (g)	43.9	40.5	39.5	
Grain protein content (%)		14.9	14.8	15.6
Sedimentation value (mm)**		36.2 a	45.6 b	49.5 b

[1] Presence (+) or absence (-) of the translocation chromosome based on the expression of the 5 + 10 glutenin components coded by the *Glu-D1* locus and on the amplification of the microsatellite locus *Xgwm135-1A*.

ns,** = not significant and significant at P£0.01. a, b: values with the same letter do not differ significantly (Tukey test).

The translocated 1D segment with the *Glu-D1* gene on 1A chromosomes of hexaploid triticale Rhino-6 was analysed by C- banding by Lukaszewsky and Curtis (1) who demonstrated that the introgressed 1D segment involved the 1.5 C-band from chromosome 1DL and, consequently, was identifiable by C-banding. The amount of chromatin introgressed in the durum translocated line was evaluated by cytogenetics and molecular approach (11). Fluorescence *in situ* hybridisation with total genomic DNA (GISH) of *Aegilops squarrosa* L. indicated that translocation plants have a 1AS.1AL-1DL chromosome containing a terminal 1DL segment about 35-40% of the recombinant arm length. Comparison between GISH and microsatellite marker analyses revealed differences between physical and genetic map position of 1AL-1DL breakpoint.

The effect on grain quality as measured by SDS sedimentation value makes the 1AS.1AL-1DL a very interesting chromosome translocation in the efforts to widen the genetic variation of durum wheat. Further chromosome engineering is however required to shorten the 1DL chromosome segment in the durum wheat translocated line in order to exploit the beneficial effect of *Glu-D1* locus for durum wheat improvement.

REFERENCES

[1] Lukaszewski AJ, Curtis CA: Transfer of the *Glu-D1* gene from chromosome 1D to chromosome 1A in hexaploid triticale. Plant Breeding 112: 177-182 (1994).

[2] Bushuk W: Aspects of chemical and physical structure of wheat proteins that determine breadmaking quality. In: Borghi B (ed) Hard wheat: agronomic, technological, biochemical and genetic aspects. Commission of the European Communities, Luxemburg: 7-17 (1987).

[3] Payne PI: Genetics of wheat storage proteins and the effect of allelic variation on breadmaking quality. Ann Rev Plant Physiol 38: 141-153 (1987).

[4] Harberd NP, Bartels D and Thompson RD: DNA restriction-fragment-variation in the gene family encoding high molecular weight (HMW) glutenin subunits of wheat. Biochem Genet 24: 579-595 (1986).

[5] Lukaszewski AJ, Curtis CA: Transfer of the Glu-D1 gene from chromosome 1D of bread wheat to chromosome 1R in hexaploid triticale. Plant Breeding 109: 203-210 (1992).

[6] Ceoloni C, Ciaffi M, Lafiandra D, Giorgi B: Chro-mosome engineering as a means of transferring 1D storage protein genes from common to durum wheat. In: ZS Li, ZY Xin (eds) Proc. 8th Int Wheat Genet Symp, Beijing, China: 159-163 (1995).

[7] Ceoloni C, Biagetti M, Ciaffi M, Forte P, Pasquini M: Wheat chromosome engineering at the 4x level: the potential of different alien gene transfers into durum wheat. Euphytica 89: 87-97 (1996).

[8] Dick J and Quick JS: A modified screening test for rapid estimation of gluten strength in early-generation durum wheat breeding lines. Cereal Chem 60: 315-318 (1983).

[9] Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy Ph, Ganal MW: A microsatellite map of wheat. Genetics 149: 2007-2023 (1998).

[10] Payne PI, Holt LM and Law CN: Structural and gene-tical studies of the high-molecular-weight subunits of wheat glutenin. 1. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor Appl Genet 60: 229-236 (1981).

[11] Blanco A, Cenci A, Simeone R, Pignone D, Galasso I: Cytogenetics and molecular characterization of a translocated chromosome 1AS.1AL-1DL with the *Glu-D1* locus in durum wheat (submitted) (2001).

[12] Lafferty J and Lelley T: Introduction of high molecular weight glutenin subunits 5 + 10 for the improvement of the bread-making quality of hexaploid triticale. Plant Breeding 120: 33-37 (2001).

MOLECULAR CHARACTERISATION OF THE *GLU-1R* GENE SUBUNITS OF THE GENUS *SECALE*

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ABSTRACT: The visco-elastic properties of bread flour are associated with the presence or absence of certain HMW subunits coded by the *Glu-1* genes. Identifying allelic specific molecular markers (AS-PCR) associated to the presence of *Glu-1* genes can serve as a valuable tool for the selection of convenient genotypes. We report the use of primers designed from nucleotide sequences of the *Glu-D1* gene of wheat (AS-PCR for *Glu-D1y10*) that recognise and amplify homologous sequences of the *Glu-R1* gene subunits of rye. The primers amplify the complete coding regions and provided two products of different size in rye and in rye-wheat aneuploid lines carrying the long arm of chromosome 1R. The amplification products were cloned, subcloned, sequenced and characterised, and sequences compared with the main glutenin subunits of wheat and related species. Further, an RT-PCR experiment was performed to demonstrate that both sequences are expressed in endosperm during grain ripening. The results of these analyses suggest that both gene subunits correspond to x- and y-type genes of the *Glu-R1* locus of rye.

1. INTRODUCTION

The genes *Glu-A1*, *Glu-B1* and *Glu-D1*, located on the long arms of homoeologous group-1 chromosomes code for the high molecular weight (HMW) subunits of glutenin. Molecular studies have shown that each locus contains two tightly linked genes (x and y) coding for protein subunits of different molecular weight. These genes of wheat have different alleles from which it has been demonstrated that breadmaking quality is firmly associated. The orthologous structural gene for HMW secalins (designated *Glu-R1*) is located on the long arm of chromosome 1R in rye.

The *Glu-1* loci from genomic clones of different cultivars has been molecularly characterised in wheat by different authors (1, 2). The sequences of each orthologous locus of hexaploid wheat were analysed comparatively and it was showed them to have similar structures (3). The usefulness of PCR-based analysis for distinguishing between wheats with different HMW glutenin subunits has been also demonstrated (4, 5). Identifying allelic specific molecular markers (AS-PCR) associated to the presence of *Glu-1* genes can serve as a valuable tool for the selection of useful genotypes (5). In a previous paper the authors reported the use of primers designed to amplify the complete coding regions of a series of different alleles (AS-PCR) of the x and y subunits of genes *Glu-A1* and *Glu-D1* (5).

The primers have been applied to amplify genomic DNA of new plant materials: *Triticum aestivum* L. '7841' [2n=20" +1R(1D)"] and *Secale cereale* L. cv 'Imperial' and 'Petkus'. The wheat '7841' is a homogeneous selected line that belongs to a working collection of wheats derived from crosses involving the cultivar 'Anza/S-149" [2n=20" +1R(1D)"], kindly supplied by Dr J.P. Gustafson. The cultivars 'Chinese Spring', 'Pané-247', 'Hope', 'Ablaca' and 'Bezostaya' and the addition lines of 'Imperial' rye chromosomes into 'Chinese Spring' were also used as testers in PCR experiments.

2. HETEROLOGOUS AMPLIFICATION OF THE HMW GLUTENINS OF RYE

A set of markers that use primers designed from nucleotide sequences of *Glu-D1y10* allele amplified two products when using genomic DNA from the line '7841' [2n=20" +1R(1D)"] (which lacks chromosome 1D) (Fig. 1) . The sequences of the forward and reverse primers are as follows:

Forward: 5' CTAACTCGCCGTGCACA 3';
Reverse: 5' AGCTAAGGTGCATGCATG 3'.

Products of the same size as '7841-1' and '7841-2' were generated in rye cultivars 'Imperial' and 'Petkus'. However, '7841-1' in different rye cultivars showed size polymorphism, and polymorphism for the presence-absence of '7841-2' was seen in 'Petkus'. As the line '7841' has chromosome 1D substituted by 1R, and the primers were designed to amplify the y subunit of gene *Glu-D1* located in the long arm of 1D, it was presumed that '7841-1' and '7841-2' might represent sequences located in 1R, and that these sequences might be related to the *Glu-1* gene family. This encouraged the undertaking of experiments that might confirm this idea.

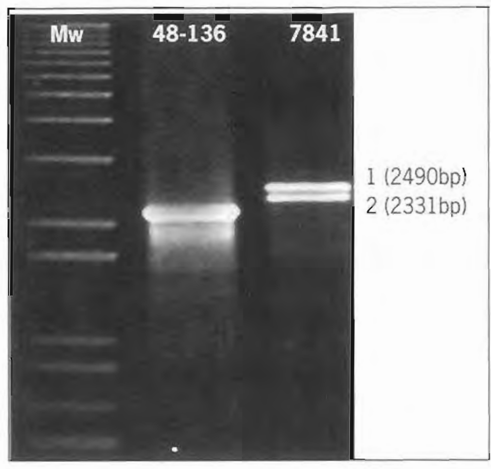

Figure 1.- Amplification products on agarose gels using the designed *Glu-Dy10* primers. From left to right: the molecular weight marker, wheat sample with 1D (GluD1y10) and the line '7841' (1R).

Direct sequencing of about 350 bases from the ends of each fragment gave a sequence highly homologous to that previously reported for HMW glutenin subunits coded for by *Glu-1* of wheat. This seems to confirm that the amplified products are members of the *Glu-1* family.

The amplified products 7841-1 and 7841-2 were inserted into adequate plasmids for cloning and using in Southern experiments and sequencing.

In order to assign the products to specific chromosomes, genomic DNA from 'Chinese Spring', an aneuploid stock of addition lines of 'Imperial' in a 'Chinese Spring' background, as well as the rye testers were amplified using the designed *Glu-D-y10* primers. The products of amplification were transferred to a membrane and alternatively probed by Southern with the plasmids carrying the inserts '7841-1' and '7841-2'. Hybridisation of the labelled probes identified both amplified products of the rye cultivars and the aneuploid lines of 'Chinese Spring' with the chromosome arm 1RL, but was absent in Southern profiles of 'Chinese Spring' and all the other aneuploid lines.

The fragments were also used as probes in Southern experiments to test homology with the PCR products obtained using specific primers performed as AS-PCR that amplify different alleles of *Glu-A1* and *Glu-D1*. The long sequence '7841-1' hybridised preferentially with x-type wheat sequences, while the short sequence '7841-2' was associated preferentially with y-type sequences.

3. SEQUENCING THE HMW GENE SUBUNITS OF RYE

The bands '7841-1' and '7841-2' were purified from the agarose gels. Three clones per fragment were selected for subcloning and sequencing. The complete nucleotide sequence and the deduced amino acid sequence of the coding region of each subunit have been deposited in the EMBL GeneBank and DDBJ under accession numbers AF216868 and AF216869. Comparison with amino acid sequences in GeneBank confirmed that the proteins coded by '7841-1' and '7841-2' are homologous to the group of glutenin genes of wheat and related species.

The coding region of both subunits lacks introns and shows the same general structure as other *Glu-1* sequences previously investigated. Both sequences contain TATAAAA between 85 and 91 bases upstream from the ATG starting codon. This agrees, both in position and sequence, with the expectations of a 'TATA' box in prolamin genes. The nucleotide sequence of the '7841-1' fragment is 2490 bp long. It contains an open reading frame of 2304 bp that starts at nucleotide 139 and encodes a foreseeable polypeptide of 766 amino acids. The fragment '7841-2' is 2331 bp long and contains an open reading frame of 2145 bp that starts at nucleotide 139, and encodes a peptide of 713 amino acids.

The coding sequence of the mature polypeptide is similar to that of other HMW glutenins of wheat in that it has a tripartite structure, consisting of a non-repetitive N-terminal region, an extensive repetitive central region, and a non-repetitive C-terminal region. The coding regions of '7841-1' and '7841-2' have an N-terminal non-repetitive domain of 87 and 105 residues respectively. Both sequences start with a 21-residue signal peptide that is almost identical to the signal peptide found in all the corresponding gene subunits encoding glutenins in wheat. The mature protein consists of 745 and 692 residues for '7841-1' and '7841-2' respectively. The internal repetitive domain reveals the presence of tandem and interspersed repeats similar to those found in the proteins encoded by the *Glu-1* genes of wheat. Finally, the non-repetitive domain at the C-terminal end has 45 residues in '7841-1' and 51 in '7841-2'. The C-terminal sequence ASQ, found in both rye subunits, is the same as the C-terminal regions found in almost all reported HMW glutenins.

The amino acid sequences encoded by the '7841-1' and '7841-2' rye subunits were aligned with those of nine previously published HMW glutenin genes of wheat, *Aegilops squarrosa* and *Hordeum vulgare*. Phylogenetic trees were built using distance method (Fig. 2). The Phylip program (version 3.5) was used to estimate the distance matrices of the DNADIST program using the two-parameter method of Kimura (6). The alignment of the sequences '7841-1' and '7841-2' with the other *Glu-1* sequences revealed inter-sequence relationships.

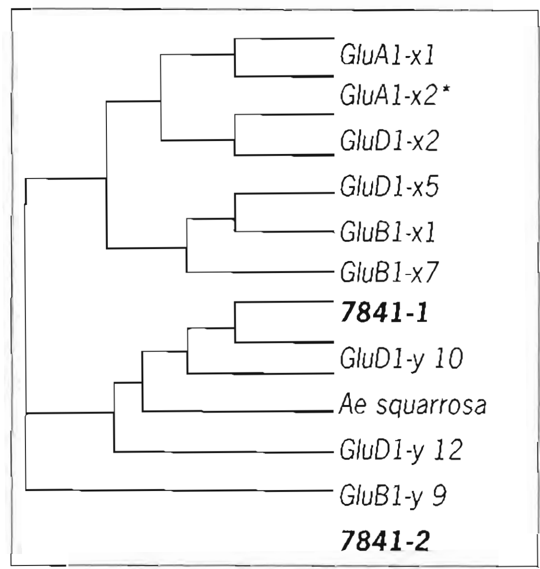

Figure 2.- Phylogenetic tree of amino acid sequences obtained from comparisons and alignment of the eleven *Glu-1* sequences

The result of this analysis is presented in the consensus tree showing that '7841-1' and '7841-2' are respectively related to those of the *x* and *y* of other HMW genes. The results suggest that '7841-1' belongs to the x-type group, whereas '7841-2' belongs to the y-type. Accordingly, the designations *Glu-R1x* and *Glu-R1y* for '7841-1' and '7841-2' are proposed.

4. RT-PCR TO ASSESS THE EXPRESION OF mRNA FROM 7841-1 AND 7841-2 IN THE ENDOSPERM

A final experiment was performed to assess the expression of the x- and y-type glutenin subunits encoded by '7841-1' and '7841-2' during grain ripening in the line '7841'. Crude extracts from immature seeds harvested at 10 days after anthesis were prepared. Poly(A)+ RNA was purified by the use of DNase and was used to obtain cDNA in an amplification reaction mixture. As a control, additional reactions were performed using Taq polymerase to detect the presence of contaminant DNA. Specific primers were designed to amplify selected regions of 330 bp and 220 bp of the presumptive mRNAs encoded by '7841-1' and '7841-2' respectively. and the PCR products were separated on 1.8% agarose gels.

Electrophoretic analyses of the RT-PCR reactions from the common wheat line '7841' and testers gave the expected results. The RT-PCR including the extracts of m-RNA of '7841' in the presence of reverse transcriptase and Taq polymerase showed bands of cDNA in agarose gels that corresponded in size to those of the selected regions of the '7841-1' and 7841-2' nucleotide sequences. No amplification products were observed in control PCR reactions after treating the endosperm extracts with DNase in the absence of reverse transcriptase and the presence of Taq polymerase. A control reaction was also performed using genomic DNA from line '7841' in the presence of Taq polymerase. This reaction gave amplification products

of the same size as obtained from cDNA. Other RT-PCRs using m-RNA extracts from lines lacking the 1RL chromosome arm showed no amplification products of the expected sizes in the corresponding reactions.

5. DISCUSION

DNA sequences of the *Glu-1* genes have indicated that the non-coding 5'- and 3'-flanks of glutenin genes are highly conserved up to 280 bp upstream of the TATA box and 140 bp downstream from the polyadenylation signal (1, 7). The primers that amplify the allele *Glu-D1y10* in wheat, and also in the rye locus described, here corresponded to these regions. The forward primer designed to amplify the wheat gene shows complete homology with the corresponding region of both -x and -y rye glutenin subunits, but has some minor variations with respect to all the remaining genes and alleles of the complex *Glu-1* family of wheat. The main differences are deletions, insertions and/or substitutions of at least two bases.

It has been demonstrated that the HMW glutenins have an important role in determining the visco-elastic properties of wheat gluten. Two structural features are responsible for the differences in the contribution of glutenins to breadmaking quality: secondary structure and the number of terminally located cysteine residues. The secondary structure predicted for the mature proteins encoded by the x- and y- subunits of rye are notably similar to those of the main glutenins of wheat. In fact, the predicted structure shows the turns of the helix to depend on specific, short sequences of amino acids that appear conserved in both species.

The cysteines are involved in intra- and intermolecular bonds, explaining the ability of HMW-glutenins to form mixed polymers involving different types of HMW and LMW glutenins. The comparative analysis of the x- and y-type subunits of the *Glu-R1* gene here described demonstrates there to be the same number of cysteine residues in *Glu-R1x* as in the homologous gene subunits of wheat (4 residues in *Glu-R1x*, *Glu-A1x1*, *Glu-A1x2**, *Glu-B1x7*, *Glu-B1x17* and *Glu-D1x2*). However, up to three extra cysteine residues have been observed in *Glu-R1y* with respect to the related alleles of wheat (4 residues in *Glu-D1y12;* 7 residues in *Glu-D1y10*, and 8 residues in *Glu-R1y*). This property, plus the increased charge density shown by both of the sequences could be of interest in the formation of a complex glutenin matrix. Reconstitution studies, in which purified HMW rye glutenins were added to wheat dough, have demonstrated a negative effect of these proteins on dough strength and this effect has been considered as due to a higher frequency of cysteine residues (8,9). This could interrupt the correct formation of the large glutenin polymers necessary for strong dough.

Characterisation of quality effects of HMW rye glutenins in different genotypic wheat backgrounds is now approachable. The more glutenin genes there are available to be used in experiments of transformation, the more precisely it will be possible to improve dough quality in transgenic forms, depending on the interactions of the other families of storage proteins.

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REFERENCES

[1] Forde J, Malpica JM, Halford NG, Shewry PR, Anderson OD, Greene FC, and Miflin BJ: The nucleotide sequence of a HMW glutenin subunit gene located on chromosome 1A of wheat (*Triticum aestivum* L.). Nucleic Acids Res. 13 (19): 6817-6832 (1985)

[2] Halford NG, Field JM, Blair H, Urwin P, Moore K, Robert L, Thompson R, Flavell RB, Tatham, AR, and Shewry PR: Analysis of HMW glutenin subunit encoded by chromosome 1A of bread wheat: (*Triticum aestivum* L.) indicates quantitative effects on grain quality. Theor Appl Genet 83: 373-378 (1992).

[3] Anderson OD, Greene FC, Yip RE, Halford NG, Shewry PR, and Malpica-Romero JM: Nucleotide sequences of the two high-molecular-weight glutenin genes from the D-genome of a hexaploid bread wheat, *Triticum aestivum* L. cv Cheyenne. Nucleic Acids Res. 17 (1): 461-462 (1989)

[4] D'Ovidio R, Masci S, and Porcedu D. Development of a set of oligonucleotides primers specific for genes at the Glu-1 complex of wheat. Theor Appl Genet 91:189-194 (1995)

[5] De Bustos A, Rubio P, and Jouve N: Molecular characterisation of the inactive allele of the gene *Glu-A1* and the development of a set of AS-PCR markers for HMW glutenins of wheat. Theor App Genet 100: 1085-1094 (2000).

[6] Kimura M: A simple method for estimating evolutionary rates of nucleotide substitutions through comparative studies of nucleotide sequence. J. Mol. Evol. 16: 111-120 (1980).
[7] Sugiyama T, Rafalski A, Peterson D, and Soll D: A wheat HMW glutenin subunit gene reveals a highly repeated structure. Nucleic Acid Res 13:8729-8737 (1985).
[8] Kipp B, Belitz HD, Seilmeier W, and Wieser H: Comparative studies of high M, subunits of rye and wheat. I Isolation and biochemical characterisation and effects on gluten extensibility. J Cereal Science 23: 227-234 (1996).
[9] Graybosch RA, Peterson CJ, and Chung OK: Quality effects of rye (*Secale cereale* L.) chromosome arm 1RL transferred to wheat (*Triticum aestivum* l.). Cereal Science 29: 211-216 (1999).

VARIABILITY FOR THE LOW-MOLECULAR-WEIGHT GLUTENIN SUBUNITS IN *Hordeum chilense*

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ABSTRACT: Eighty-one different lines of *Hordeum chilense* Roem. et Schult., representative of its distribution area, were analysed. A homocygotic genotype was obtained by at least two generations of self-pollination. The homogeneity of the progeny was tested by using nine seeds from three different plants. The glutenin subunits were studied using the SDS-PAGE technique. Jaccard's coefficient of similarity was calculated, and the species were grouped by cluster analysis using the unweighted pair group (UPGMA) method. It is concluded that *H. chilense* presents a huge variability for glutenins that could be useful in cereal breeding. Likewise these results were compared with our findings for gliadins. It is concluded that the degree of the variability for gliadins and glutenins is very similar.

1. INTRODUCTION

Hordeum chilense Roem. et Schult. is a native South American diploid wild barley included in the section *Anisolepis* Nevski which occurs exclusively in Chile and Argentina. This species is very much appreciated by cattle being a component of natural pastures (1). *H. chilense* offers the possibility of obtaining novel genomic combinations (2). In fact, tritordeum (¥*Tritordeum* Ascherson et Graebner) is the result of crossing *H. chilense* with both wheat types, which has shown promising characteristics as a new man-made crop (3). Hexaploid tritordeum has been evaluated for bread making (4, 5). The promising potential of this amphiploid has been associated with the endosperm storage proteins synthesised by the $\mathbf{H}^{ch}$ genome (6) which has been studied for storage protein polypeptide composition by SDS-PAGE (7) and Acid-PAGE (6). The storage prolamins synthesised by the $\mathbf{H}^{ch}$ genome have influence on the gluten strength of hexaploid tritordeum, mainly the high molecular weight (HMW) glutenin subunits coded at the *Glu-H^{ch}1* locus (6). This locus has been identified as homeologous of the *Glu-1* loci of wheat and is located on the short arm of the chromosome 1H^{ch} (8, 9).

Up to five additional loci for storage proteins synthesised by the $\mathbf{H}^{ch}$ genome have been found (6) using two crosses of hexaploid tritordeum. Afterwards, great variability in the D-LMW glutenin subunits and gliadins was found (10, 11). Recently, a new locus (*Glu-H^{ch}3*) for D-LMW glutenin subunits has been detected (12).

These studies suggest that *H. chilense* is a very polymorphic species at the storage protein level Therefore the genetic background of tritordeum and wheat could be increased using these resources. The aim of this work was to evaluate the variability of the LMW glutenin subunits in *H. chilense* using a collection representative of its distribution zone in Chile.

2. MATERIALS AND METHODS

2.1. Grain samples

Eighty-one lines of *H. chilense* collected from its complete distribution area (between 29° and 39° Latitude South) in Chile were studied. These lines were studied previously for their variability for monomeric prolamins (11). These lines are being maintained at the Germplasm Bank of the Instituto de Agricultura Sostenible-Consejo Superior de Investigaciones Científicas (IAS-CSIC, Córdoba, Spain).

2.2. Protein extraction

Due to the small size of *H. chilense* grain (3mg), three embryo-less seeds of each line were used to extract the endosperm storage proteins. Albumin and globulin fractions were removed with water and saline solution (0.5 M NaCl), respectively. The remaining sodium chloride was eliminated by washing with distilled water. Monomeric prolamins were extracted from the pellet with 1 ml of 70% ethanol.

Polymeric prolamins were extracted with 125mM Tris-HCl pH 6.8 + 2% (w/v) ditiothreitol + 20% (v/v) glycerol + 0.005% (w/v) bromophenol blue in a 1:10 ratio (w/v) at 60 C for 30 minutes. The samples were centrifugated at 14000g and the supernatant was transferred to a new tube and stored at -20 °C. 2.3Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) separation. Reduced proteins were fractionated in vertical SDS-PAGE slabs in a discontinuous Tris-HCl-SDS buffer system (pH:6.8/8.8) at a polyacrylamide concentration of 12% (w/v, C=2.67). The Tris-HCl/glycine buffer system of Laemmli (1970) was used. Electrophoresis was performed at a constant current of 25mA per gel at 10 °C for 45 min after the tracking dye migrated off the gel. Gels were stained overnight with 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250. De-staining was carried out with tap water.

2.4. Data analyses

All the lines were scored for presence or absence of glutening subunits, and the data were entered into a binary data matrix as discrete variables ("1" for presence and "0" for absence). Jaccard's coefficient of similarity was calculated, and the lines were grouped by cluster analysis using the unweighted pair group (UPGMA) method. Phenogram was produced as described by Sneath and Sokal (13).

3. RESULTS AND DISCUSSION

Diverse studies have suggested that the $\mathbf{H^{ch}}$ genome be more similar to the genomes of wheat (**D**) and barley (**I**) (14, 15). Therefore, polymeric proteins have been considered as glutenin-like proteins in this report.

The endosperm proteins constitute a useful tool in the evaluation of variability in cereals and quality improvement programmes.

The studies using gliadins (11) and HMW-glutenins (16) suggest a high degree of variability for endosperm storage proteins in *H. chilense*.

This study used a homocygotic genotype of each line. At least two self-pollination generations were performed, being randomly chosen one plant. The homogeneity of the offspring was confirmed using nine seeds from three different plants; consequently only part of the variability of the original populations have been evaluated. Nevertheless, the variability found was very high within the lines evaluated. The high level of polymorphism for these proteins is in agreement with the findings for gliadins (11). Out of 52 different bands detected in the LMW glutenin subunits zone, only one of them appeared in most lines evaluated. Sixty-seven patterns from nine to twelve bands, were identified with the combinations of bands. The most frequent pattern consisted of 10 bands. A representative sample of the variation detected by SDS-PAGE is shown in Figure 1.

A dendrogram was obtained (Figure 2).There are no clear groups in the dendrogram. On the contrary, the lines join continuously without forming groups similar to found in gliadins (11). The variability detected cannot correlate with ecological or geographical distribution of the species (data not shown). The variation of this species is suggested as distributed continuously along its distribution zone. The high degree of variability found could be used in cereal breeding for bread making quality.

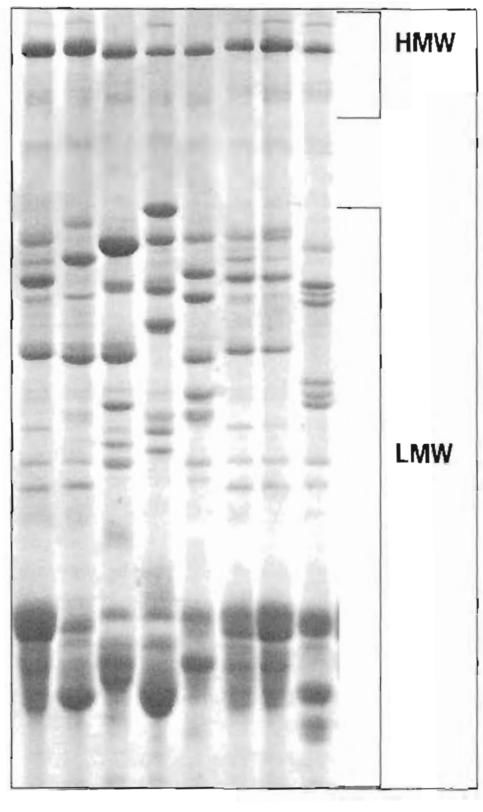

Figure 1. SDS-PAGE of LMW glutenins in *H. chilense*

The variability was compared with our findings for gliadins, (11). The indicators used were the number of bands/number of lines and the number of different patterns/number of lines. For gliadins, according to (11), these indicators were 42/82 and 68/82 while these indicators were 52/81 and 68/81 for LMW-glutenins respectively. The values for both indicators are very similar and so it may be suggested that the high degree of variation of both protein fractions in *H. chilense* is very similar.

In conclusion, *H. chilense* presents a high variability with respect to both gliadins and glutenins. Both fractions could be used to breeding for cereal quality as well as molecular markers. Likewise, the variability seems to be neutral to natural selection since it is distributed continuously in the dendrogram.

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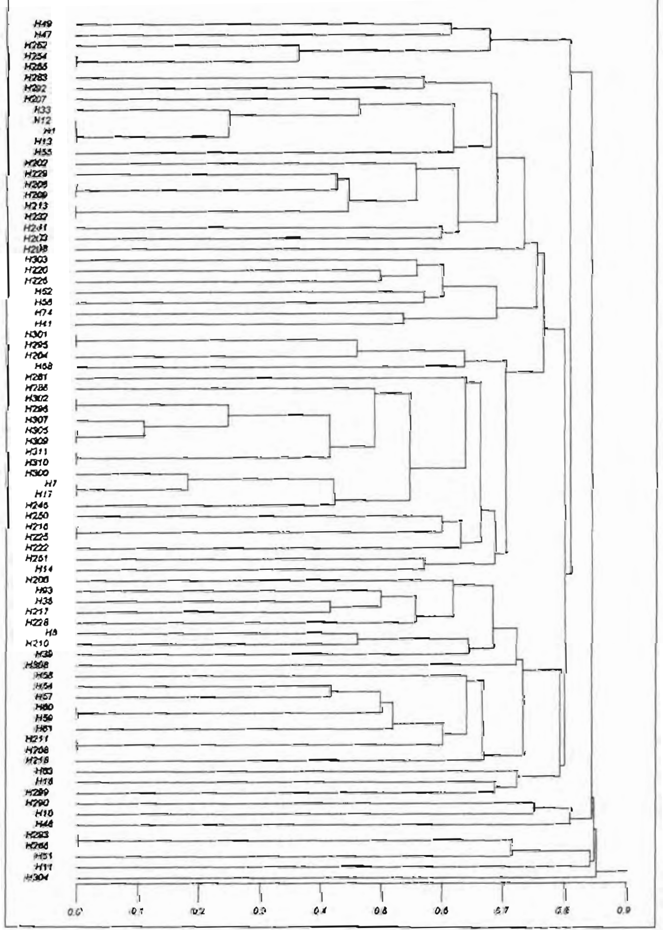

Figure 2. Dendrogram using Jaccard's coefficient of similarity.

REFERENCES

[1] Valderrama X., Cosio F., Gastó J.,Castro M.: Caracterización y reproducción de especies pratenses nativas y/o naturalizadas *Bromus unioloides* H.B.K. y *Hordeum chilense* Brogn. del dominio Seco-estival de Chile como banco de germoplasma para regenerar ecosistemas degradados. Facultad de Agronomía, Universidad Católica de Valparaiso. (1991).

[2] Martín A., Martínez C., Rubiales D.,Ballesteros J.: Tritordeum: triticale's new brother cereal. In: Güedes-Pinto H., Darvey N.,Carnide V. P. (eds) Triticale: today and tomorrow. Kluwer Academic Publishers. (1996)

[3] Martín A., Alvarez J. B., Martín L. M., Barro F.,Ballesteros J.: The development of tritordeum: a novel cereal for food processing. J Cereal Sci 30: 85-95 (1999)

[4] Alvarez J. B., Ballesteros J., Arriaga H. O.,Martin L. M.: The rheological properties and baking performances of flours from hexaploid tritordeums. J Cereal Sci 21: 291-299 (1995)

[5] Alvarez J. B.,Martin L. M.: Breagmaking quality in tritordeum: the use-possibilities of a new cereal. In: Güedes-Pinto H., Darvey N.,Carnide V. P. (eds) Triticale: today and tomorrow. Kluwer Academic Publishers. (1996).

[6] Alvarez J. B., Martín A.,Martin L. M.: Allelic variation of the D-prolamin subunits encoded at the H^{ch} genome in a collection of primary hexaploid tritordeums. Theor Appl Genet 99: 196-199 (1999)

[7] Alvarez J. B., Canalejo A. L., Ballesteros J., Rogers W. J.,Martin L. M.: Genealogical identification of hexaploid tritordeum by electrophoretic separation of endosperm storage proteins. Plant Breeding 111: 166-169 (1993)

[8] Payne P. I., Holt L. M., Reader S. M.,Miller T. E.: Chromosomal location of genes coding for endosperm proteins of *Hordeum chilense*, determined by two-dimensional electrophoresis of wheat-*H.chilense* chromosome adition lines. Biochem Genet 25: 53-65 (1987)

[9] Tercero J. A., Bernardo A.,Jouve N.: Encoding genes for endosperm proteins in *Hordeum chilense*. Theor Appl Genet 81: 127-132 (1991)

[10] Alvarez J. B., Campos L. A. C., Martín A., Sillero J. A.,Martin L. M.: Genetic analysis of prolamins synthesised by the H^{ch} genome and their effects in gluten strengh in hexaploid tritordeum. Euphytica 107: 1-8 (1999)

[11] Atienza S. G., Giménez M. J., Martín A.,Martín L. M.: Variability in monomeric prolamins in *Hordeum chilense*. Theor Appl Genet 101: 970-976 (2000)

[12] Caballero L., Alvarez J. B.,Martin L. M.: Analysis of D-prolamins synthesised by the *Hordeum chilense* genome and their effects on gluten strength in hexaploid tritordeum. Plant Breeding 120:185-187 (2001)

[13] Sneath P. H. A.,Sokal R. R.: Numerical taxonomy, the principles and practice of numerical classification. In: W. H. Freeman and Co. (1973)

[14] Fernández J. A., Sanz J.,Jouve N.: Biochemical variation to determine phylogenetic relationships between *Hordeum chilense* and other American species of the genus *Hordeum* (*Poaceae*). Pl Syst Evol 157: 105-119 (1987)

[15] Millán T.,Martín A.: Effects of *Hordeum chilense* and *Triticum* cytoplasms on agronomical traits in hexaploid tritordeum. Plant Breeding 108: 328-331 (1992)

[16] Álvarez J. B., Martín A.,Martin L. M.: Variation in the high-molecular-weight glutenin subunits coded at the *Glu-H*^{ch}*1* locus in *Hordeum chilense*. Theor Appl Genet 102: 134-137 (2001)

ALLELIC VARIATION OF THE HMW GLUTENIN SUBUNITS IN SPANISH ACCESSIONS OF SPELT WHEAT

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ABSTRACT: Variation in high molecular weight (HMW) glutenin subunit composition among 403 accessions of spelt wheat (*Triticum aestivum ssp. spelta*) of Spanish origin were analysed by SDS-PAGE. A total of 19 alleles were identified, three allelic variants were detected for *Glu-A1* locus. For the *Glu-B1* locus, two of seven alleles detected have not been found before, while four nine alleles detected for the *Glu-D1* locus are not previously described. Considering the three loci, twenty-five combinations were detected among all the evaluated accessions. Spelt wheat could be used as a source of genes for quality improvement in bread wheat.

1. INTRODUCTION

Modern agronomic practices in the last fifty years centred on high yield varieties, it has given rise to a reduction of the genetic base of the cultivated wheat. This loss of genetic diversity has promoted the search for new sources of variation that might be use in plant improvement programmes, for this reason it is need the collection, conservation and use of wild and relative species (1).

To approach this problem, it has been done many investigations on composition of the seed storage proteins, due to their relationship with the technological properties of wheat, in species such as *Triticum turgidum* ssp. *dicoccoides* (2,3), *Dasypyrum villosum* L. Candargy (4), or *Hordeum chilense* Roem. et Schult. (5).

Between the relative species with cultivated wheats are the hulled wheat such as einkorn (2n = 2x = 14; **AA**; *T. monococcum* ssp. *monococcum* A. Et D. Löve), emmer (2n = 4x = 28; **AABB**; *T. turgidum* ssp. *dicoccum* Schark) and spelt (2n = 6x = 42; **AABBDD**; *T. aestivum* ssp. *spelta* L. em. Thell). Because scarce interest of these species in the past Century, most of the genetic resources for these species are only present in germplasm banks, where the long time could have to influence on the seeding power. In Spain, spelt wheat survives in marginal farming areas of Asturias (North of Spain) and is endangered (6). This specie was widely cultivated during the first part of the 20th Century and was decreased towards the late 1960s.

The increasing demand for unconventional foods, together with the search for low-input agriculture, has led to a revival of traditional food where hulled wheats could play an important role.

In the case of the hulled wheat, these investigations on the storage proteins have been scarce. Recently, the seed storage protein composition of a collection of Spanish emmer has been analysed in our group (7), they found a high degree variation for the HMW glutenin subunits. In 1990, Rodriguez-Quijano et al, (8) analysed a collection of 118 accessions of spelt wheat and found a low variability for the HMW glutenin subunits.

The goal of the present study has been analysed the HMW glutenin subunits composition of an extensive collection of spelt wheat collected in the North of Spain in the first half of 20th Century, for evaluated their possible use in the modern Agriculture or in the wheat quality improvement.

2. MATERIALS AND METHOD

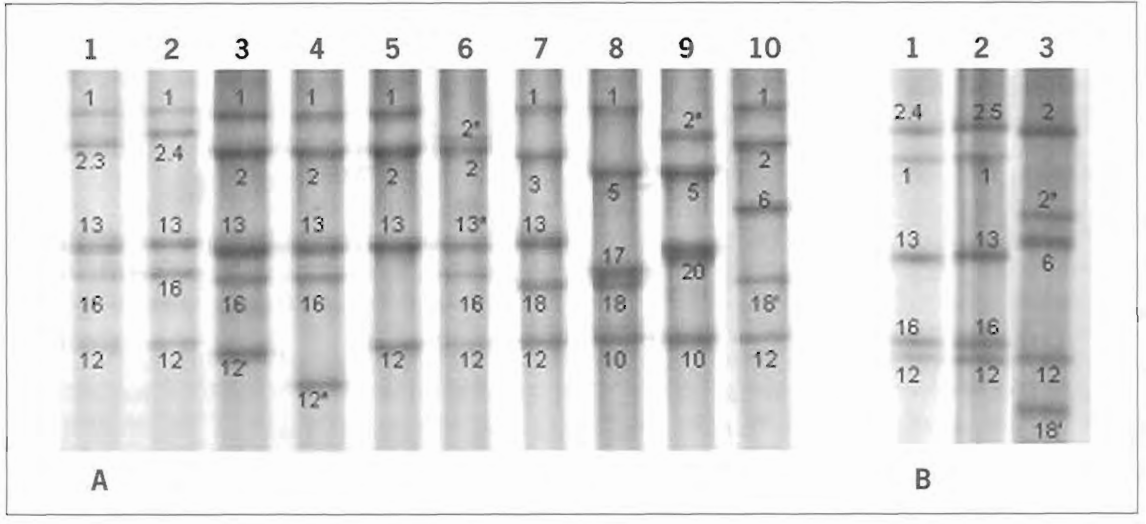
Four hundred three accessions of spelt wheat obtained from the National Small Grain Collections (Aberdeen, USA) and Centro de Recursos Fitogenéticos INIA (Alcalá de Henares, Spain), were analysed.

Proteins were extracted from crushed endosperm. Before glutenin solubilisation, the gliadins were extracted with a 1.5M dimethylformamide aqueous solution following to a double-wash with 50% (v/v) propan-1-ol at 60°C for 30 min with agitation every 10 min. Glutenin was solubilised with 250 ml of buffer containing 50% (v/v) propan-1-ol, 80mM Tris-HCl pH:8.5, 2% (w/v) dithiothreitol at 60°C for 30 min. After centrifugation, 200ml of the supernatant were transferred to a new tube, mixed with 3ml of 4-vinylpyridine, and incubated for 30 min at 60°C. The samples were precipitated with 1ml of cold acetone. The dried pellet was solubilised in buffer containing 625mM Tris-HCl pH:6.8, 2%(w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue, and 2% (w/v) dithiothreitol in a 1:5 ratio (mg/ml) to wholemeal.

Reduced and alkylated proteins were fractionated by electrophoresis in vertical SDS-PAGE slabs in a discontinuous Tris-HCl- SDS buffer system (pH: 6.8/8.8) at an 8% polyacrylamide concentration (w/v, C=1.28%) with and without 4M urea. The Tris-HCl/glycine buffer system of Laemmli was used (9). Electrophoresis was performed at a constant current of 30mA/gel at 18°C for 30min after the tracking dye migrated off the gel. Gels were stained overnight with 12% trichloroacetic acid solution containing 5% ethanol and 0.005% Coomassie Brilliant Blue. De-staining was carried out with tap water.

In this study, we have been used three different nomenclatures, the new alleles were named according the progressive Roman numeral nomenclature of Vallega and Waines (10) and Branlard et al. (11). The international nomenclature indicated by McIntosh et al. (12) has been used to name the alleles previously described. The nomenclature proposed by Payne an Lawrence (13) was used to name the subunits according to their proximity to the subunits previously described.

Figure 1. SDS-PAGE of representative sample of variation found in spelt wheat. A, without 4 M urea; B, with 4 M urea.



3. RESULTS

In the 403 evaluated accessions were found twenty different subunits. In the Figure 1, is showed a representative sample of the variation found. For the *Glu-A1* locus were detected three subunits, which had been previously described. Similar to other cultivated wheats (14) only one active component was found for this locus. These subunits were 1, 2* and *null*, where the subunit 1 was the most frequent, followed the subunit 2* (Figure 2).

For the *Glu-B1* locus, were found seven different subunits, some of them were not previously described such as the subunits 13* and 18' were catalogued with these number due to their near to the subunit 13 and 18, respectively. The subunit 13* that appears with subunit 16 was slightly faster than subunit 13 and the subunit 18' that was slightly lower than subunit 18, appears with the subunit 6. The rest of the subunits for this locus had been previously described. The subunits most frequent were 13+16 that were detected in 354 of evaluated accessions (Figure 2).

For *Glu-D1* locus, were detected ten different subunits of which four were not previously detected. These new subunits were named with numeral 2.4, 2.5, 12' and 12*. The subunits 12' and 12* appear with the subunit 2. The subunit 12' was slightly faster than the subunit 12 and the subunit 12* showed a clear difference faces to both subunits. The subunit 2.4 and 2.5 appear with the subunits 12. the subunit 2.4 was lower than the subunit 2.3 and the subunit 2.5 was slightly lower than the subunit 2.4. This last subunit was only detected when the urea was added to the gel. All these new subunits appear only in one accession each one. The subunits 2+12 were the most frequent for the evaluated accessions (89.08%), followed to the subunits 3+12 (8.68%) -Figure 2-.

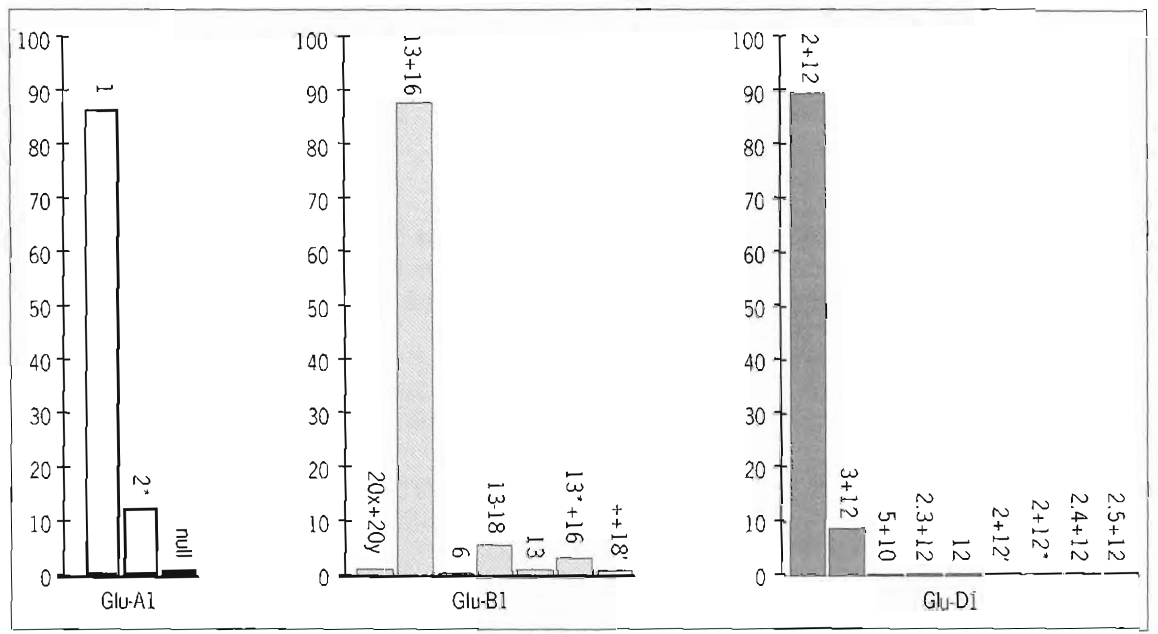


Figure 2: Allelic frequencies at three glutenin loci in 403 Spanish spelt wheat landraces.

Ninety allelic variants were found at the *Glu-1* loci into the evaluated accessions (three alleles at the *Glu-A1* locus, seven at the *Glu-B1* locus and nine at the *Glu-D1* locus). Between these allelic variants found six novel alleles were identified, two at the *Glu-B1* locus and four at the *Glu-D1* locus. These new alleles were named *Glu-B1-XVIII* (subunits 13*+16) and *Glu-B1-XIX* (6+18'), for the *Glu-B1* locus, and *Glu-D1-I* (2+12'), *Glu-D1-II* (2+12*), *Glu-D1-III* (2.4+12) and *Glu-D1-IV* (2.5+12), for the *Glu-D1* locus.

Considering the variation for the three loci, twenty-five combinations were detected. The combination dominant was 1,13+16,2+12 that appeared in 67.74% of the accessions, followed of the combinations 2*,13+16,2+12 and 1,13+16,3+12. Eleven of the combinations found appear in only one accession. Between these combinations make up 2*,20,5+10 which shows good alleles for the *Glu-A1* and *Glu-D1* loci according to indicated in bread wheat. By contract, nine combinations present the six novel alleles, whose effects on bread making quality have not been evaluated at now.

4. DISCUSSION

In this collection has been detected a high degree variation for the HMW glutenin subunits.

The results obtained in the present study showed some discrepancy with the obtained by Rodriguez-Quijano et al. (8), who analysed the HMW glutenin subunits in 118 accessions of spelt wheat, which are part of the material used in this study. They found only three allelic variants for the *Glu-B1* locus and four for the *Glu-D1* locus. All three allelic variants at the *Glu-A1* locus were also described by these authors, however the frequencies for the subunits 2* and *null* were very different in both works. In our materials, the *null* allele (*Glu-A1c*) appeared in only four accessions, while the found it in fifteen accessions by Rodriguez-Quijano et al. (8). The subunit 2* appear in 51 accessions and they detected it in four accessions.

Furthermore, we detected nine alleles (four for the *Glu-B1* locus and five for the *Glu-D1* locus) in our material that not found by Rodriguez-Quijano et al. (8). The allele *Glu-Ban* (subunit 6) was only found in one accession, it was detected in two landraces of bread wheat by these authors.

In this study we have used two types of gel, with and without 4M urea, because previous investigations carried out with other species have shown that the variability detected by normal SDS-PAGE gels could be lower than real. It seems to be due to conformational differences between the proteins (15), which causes the anomalous mobility of some subunits that appear in similar positions of other subunits described. This is eliminated by the addition of a strong denaturant such as 4M urea (15,16) used in the present work. When urea was added to gel, the mobility of all subunits showed changes that have permits to difference some subunits better and found a new allele named by us *Glu-D1-IV* (2.5+12).

We have used three different nomenclatures, in the case of the *Glu-B1* locus; we have named the new alleles continuing with the alleles indicated by Pflüger et al. (7).

The causes of the differences between the frequencies found by us and Rodriguez-Quijano et al. (8), could be due to two facts, the different number of the accessions analysed (118 faces to 403) and the use of different types of gel. In fact, we have used a low-polyacrylamide concentration gel that permits best discrimination at level of HMW glutenin subunits. Consequently, certain variants present here, might have been overlooked for these authors and classified with different number.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Jauhar PP: Alien gene transfer and genetic enrichment of bread wheat. In: Damania AB (ed) Biodiversity and Wheat Improvement. ICARDA-A Wiley Sayce Publication, Aleppo, pp 103-119. (1993).

[2] Ciaffi M, Lafiandra D, Porceddu E, Benedetelli S: Storage protein variation in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) from Jordan and Turkey. I. Electroforetic, characterization of genotypes. Theor. Appl. Genet 86: 474-480 (1993a).

[3] Ciaffi M, Lafiandra D, Porceddu E, Benedetelli S: Storage protein variation in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) from Jordan and Turkey. II. Patterns of allele distribution. Theor. Appl. Genet 86: 516-525 (1993b).

[4] Zhong CY, Quaselt CO: Allelic diversity of high-molecular-weight glutenin protein subunits in natural population of *Dasypyrum villosum* (L.) Candargy. Theor. Appl. Genet 86: 851-858 (1993).

[5] Alvarez JB, Martin LM, Martin A: Variation in the high-molecular-weight glutenin subunits coded at the Glu-H^{ch} locus in *Hordeum chilense*. Theor. Appl. Genet 102: 134-137 (2001).

[6] Peña-Chocarro L, Zapata-Peña L: Hulled wheats in Spain: history of minor cereals. In: Jaradat AA (ed) Triticeae III. Science Publihers Inc. New Delhi, pp 45-52 (1998).

[7] Pflüger LA, Martin LM, Alvarez JB: Variation in the HMW and LMW glutenin subunits from Spanish accessions of emmer wheat (*Triticum turgidum* ssp. *dicoccum* Schrank) Theor. Appl. Genet 102: 767-772 (2001).

[8] Rodriguez-Quijano M, Vazquez JF, Carrillo JM: Variation of high molecular weight glutenin subunits in Spanish landraces of *Triticum aestivum* ssp. *vulgare* and ssp. *spelta*. J. Genet & Breed. 44: 121-126 (1990).

[9] Laemmli U: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685 (1970).

[10] Vallega V, Waines JG: High molecular weight glutenin subunit variation in *Triticum turgidum* var. *dicoccum*. Theor. Appl. Genet 74: 706-710 (1987).

[11] Branlard G, Autran JC, Monneveux P: High molecular weight glutenin subunit in durum wheat (*T. turgidum*). Theor. Appl. Genet 78: 353-358 (1989).

[12] McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ: Catalogue of gene symbols for wheat. In: Proc. 9th Int. Wheat Genet. Symp., Vol 5. University Extension Press. University of Saskatchewan 235 p (1998).

[13] Payne PI, Lawrence GJ: Catalogue of alleles for the complex loci, *Glu-A1*, *Glu-B1* and *Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11: 29-35 (1983).

[14] Shewry PR, Halford NG, Tatham AS: The high molecular weight subunits of wheat glutenin. J. Cereal Sci. 15: 105-120 (1992).
[15] Goldsbrough AP, Bulleid NJ, Feedman RB, Flavell RB: Conformational differences between two wheat (*Triticum aestivum*) 'high-molecular-weight' glutenin subunits are due to ashort region containing six amino acid differences. Biochem J 263: 837-842 (1989).
[16] Lafiandra D, D'Ovidio R, Porceddu E, Margiotta B, Colaprico G: New data supporting high Mr glutenin subunit 5 as determinant of qualitative differences in pairs 5+10 vs 2+12. J Cereal Sci 18:197-205 (1993).

VARIATION OF HMW GLUTENIN SUBUNITS IN A COLLECTION OF PRIMARY TRITORDEUM.

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ABSTRACT: Tritordeum (¥*Tritordeum* Ascherson et Graebner) is the amphiploid derived from the cross between *Hordeum chilense* Roem. et. Schult. and wheat, both durum and bread wheat. This new cereal has showed potential for breadmaking industry, which has been associated with their endosperm storage proteins. The high molecular weight (HMW) glutenin subunit composition of a collection of hexa- and octoploid tritodeum has been analysed by SDS-PAGE. Although the parents were chosen according to other agronomic traits, a wide variability for these proteins has been detected.

Keywords: tritordeum - amphiploid - allelic variation - bread making quality - SDS-PAGE - glutenin.

1. INTRODUCTION

Evolutionary studies have indicated the common origin of the prolamin genes into the tribe *Triticeae* (1, 2). On this basis, the wild species belonging to this tribe have been used as a source of new prolamin genes (3, 4). In some cases, these species have been used for the synthesis of amphiploids. One of these amphiploids is tritordeum (¥*Tritordeum* Ascherson et Graebner), which was obtained from the cross between a native South American diploid wild barley (*Hordeum chilense* Roem. et Schult.) and wheat (5, 6). Some favourable agronomic traits such as biomass, number of spikelets/spike, or protein content may indicate the potential of this amphiploid as a possible new crop, mainly at hexaploid level (7).

The storage proteins composition of tritordeum studied SDS-PAGE showed that the protein pattern of the amphiploid was the result of the addition of the patterns from both parents (8, 9, 10). Further studies have suggested that hexaploid tritordeum has some potential for bread making (11), and that this trait is related with the storage proteins from *H. chilense*, mainly the high-molecular-weight glutenin subunit coded at the *Glu-H^{ch}1* locus (12). This locus has been identified as homeologous of the *Glu-1* loci of wheat and is located on short arm of the chromosome 1H^{ch} (8, 9).

In this report, the HMW glutenin subunits composition of the lines of primary tritordeum were studied by SDS-PAGE.

All the prolamins (monomerics and polymerics) of *H. vulgare* L. are usually named hordeins. For this reason some authors have named hordeins to the prolamins of *H. chilense* (8, 9); however, no evidence has been found that identified these proteins to the prolamins of *H. vulgare*. In fact, biochemical comparison between *H. chilense* and *H. vulgare* shows great differences between species (13). Some results, such as the similarity on chromosome banding pattern after *in situ* hybridisation with probe pAs1 between *H. chilense* and *Aegilops tauschii* Coss. (14) or cytoplasm compatibility (15), suggested that the *H. chilense* genome could be more similar to wheat than to barley. On basis of these results, the H^{ch} prolamins observed after the extraction procedure used in this report have been considered as glutenin-like proteins.

Previous data have indicated that *H. chilense* has one HMW glutenin subunit coded at *Glu-H^{ch}1* locus on chromosome 1H^{ch} (8, 9). These proteins are related with the gluten strength in hexaploid tritordeum (7). Probably this locus coded for two protein components, one slow-weak band and one fast-strong band. Nevertheless, the study of the first band is very difficult because of in many occasions this band can not detected in the gel. For this reason, only the fast-strong band has been studied in the present report.

2. MATERIALS AND METHODS

2.1. *Plant material*

Seeds of forty-five lines of primary tritordeum (twenty-nine hexa- and sixteen octoploid) were analysed (Table I). Two seeds were analysed individually for each tritordeum tine.

2.2. *SDS-polyacrylamide gel electrophoresis (SDS-PAGE)*

Proteins were extracted from crushed endosperm. Before glutenin solubilisation, the monomeric prolamins were extracted with a 1.5 M dimethylformamide aqueous solution following to a double-wash with 50% (v/v) propan-1-ol at 60°C for 30 min with agitation every 10 min. Glutenin was solubilised with 250 ml of buffer containing 50% (v/v) propan-1-ol, 80 mM Tris-HCl pH: 8.5, 2% (w/v) dithiothreitol at 60°C for 30 min. After centrifugation, 200 ml of the supernatant were transferred to a new tube, mixed with 3 ml of 4-vinylpyridine, and incubated for 30 min at 60°C. The samples were precipitated with 1 ml of cold-acetone. The dried pellet was solubilised in buffer containing 625 mM Tris-HCl pH: 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue, and 2% (w/v) dithiothreitol in a 1:5 ratio (mg/ml) to wholemeal.

Reduced and alkylated proteins were fractionated by electrophoresis in vertical SDS-PAGE slabs in a discontinuous Tris-HCl-SDS buffer system (pH: 6.8/8.8) at a polyacrylamide concentration of 8% (w/v, C= 1.28%). The Tris-HCl/glycine buffer system of Laemmli (16) was used. Electrophoresis was performed at a constant current of 30 mA/gel at 18°C for 45 min after the tracking dye migrated off the gel.

Gels were stained overnight with 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250. De-staining was carried out with distilled water

Table I.- Lines of primary tritordeum evaluated in the present study.

Species	Lines				Total
Hexaploid tritordeum	HT1,	HT7,	HT15,	HT19,	29
	HT22,	HT23,	HT24,	HT25,	
	HT27,	HT40,	HT41,	HT43,	
	HT44,	HT47,	HT48,	HT51,	
	HT52,	HT54,	HT55,	HT56,	
	HT57,	HT59,	HT60,	HT61,	
	HT71,	HT72,	HT75,	HT79,	
	HT80				
Octoploid tritordeum	HT4,	HT20,	HT26,	HT45,	16
	HT46,	HT50,	HT58,	HT62,	
	HT68,	HT69,	HT70,	HT73,	
	HT74,	HT76,	HT77,	HT82	

3. RESULTS AND DISCUSSION

The analysis of endosperm protein has proved to be a useful tool in the evaluation of variability in cereals and the quality improvement in the breeding programmes. In the present report, we studied the variation of the HMW glutenin subunits in forty-five lines of primary tritordeum. The electrophoretic separation by SDS-PAGE of the lines evaluated is shown in figures 1 and 2. The high molecular weight glutenin subunit composition is present in Table II.

For the *Glu-H^{ch}1* locus, three allelic variants named a, b and c (subunits 1^{Hch}, 2^{Hch}, and 3^{Hch}, respectively) were found in the lines of hexaploid tritordeum, where the subunit 1^{Hch} was the most frequent (75.86%). The lines of octoploid tritordeum showed the same three alleles for this locus, being the most frequent the subunit 3^{Hch} (56.3%).

In general, the hexaploid tritordeum showed low variability for the *Glu-A1* locus; in fact, 89.65% of the lines showed the null allele (*Glu-A1c*). At the *Glu-B1* locus, four alleles were detected, being the allele *Glu-B1d* (subunits 6+8) the most frequent (48.28%). It is possible to consequence that the parents of tritordeum were chosen according to agronomic traits, independently of the protein composition. Furthermore, these materials were synthesised at the earliest 1980s and no electrophoretic analyses were made. In octoploid tritordeum, three alleles at the *Glu-A1* locus were found. *Glu-B1* locus was the most variable, with five alleles. For the *Glu-D1* locus, only two allelic variants were present, one allele associated with good quality (subunits 5+10) and other associated with poor quality (subunits 2+12).

Although the number of lines evaluated was not large, up to sixteen lines of *H. chilense* were used in the synthesis of hexaploid tritordeums. Seven of them were also used for the octoploid tritordeums.

In total, we have detected 25 different patterns for HMW-Gs in the 45 tritordeum lines studied.

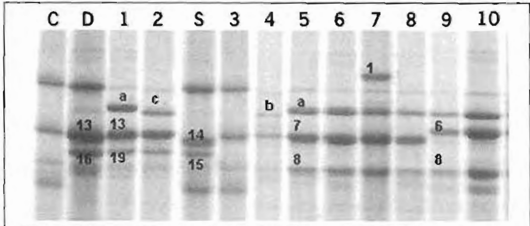

Figure 1.- One-dimensional SDS-PAGE (8%) separation of the HMW-Gs for several lines of hexaploid tritordeum, along with standard bread wheat cultivars (C: 'Chinese Spring', D: 'Duramba' and S: 'Sappo'). a, b and c: allelic variants at the Glu-Hch1

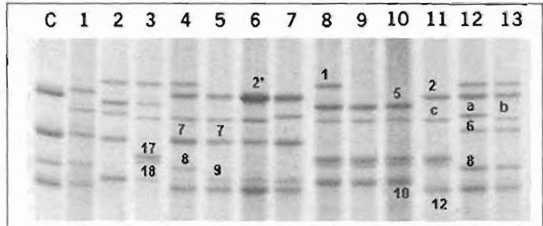

Figure 2.- One-dimensional SDS-PAGE (8%) separation of the HMW-Gs for several lines of octoploid tritordeum, along with standard bread wheat cultivar (C: 'Chinese Spring').a, b and c: allelic variants at the Glu-Hch1 locus.

4. CONCLUSIONS

Although this primary tritordeum collection shows a wide variability for the HMW-Gs composition, this variation includes only one fraction of the variability for the HMW-Gs detected at the **A**, **B**, **D**, and **H^{ch}** genomes. We think that the quality of tritordeum could be also increased by the synthesis of new tritordeums with other allelic variants at this loci.

Table II.- Allele frequencies at *Glu-1* loci in lines of primary tritordeum evaluated in this study.

	Glu-H^{ch}1	*Glu-A1*	*Glu-B1*	*Glu-D1*	Lines	
					Nº	%
HT6x	1^{Hch}	null	6+8		11	37.93
(N=29)	1^{Hch}	null	7+8		5	17.24
	3^{Hch}	null	6+8		2	6.90
	2^{Hch}	null	7+8		1	3.45
	1^{Hch}	null	20		2	6.90
	3^{Hch}	null	20		3	10.34
	1^{Hch}	null	13+19		1	3.45
	3^{Hch}	null	13+19		1	3.45
	1^{Hch}	1	7+8		1	3.45
	1^{Hch}	2*	7		1	3.45
	1^{Hch}	2*	6+8		1	3.45
HT8x						
(N=16)	1^{Hch}	1	6+8	2+12	1	5.88
	1^{Hch}	2*	7+9	2+12	1	5.88
	3^{Hch}	null	13+19	5+10	1	5.88

CONTINUACIÓN ⟶

$Glu\text{-}H^{ch}1$	*Glu-A1*	*Glu-B1*	*Glu-D1*	Lines	
				N°	%
1^{Hch}	1	7+9	5+10	2	11.76
2^{Hch}	1	6+8	2+12	1	5.88
1^{Hch}	2*	14+15	2+12	1	5.88
1^{Hch}	null	7+8	2+12	1	5.88
3^{Hch}	null	17+18	5+10	2	11.76
3^{Hch}	null	17+18	2+12	1	5.88
3^{Hch}	2*	7+9	2+12	1	5.88
3^{Hch}	1	17+18	5+10	1	5.88
3^{Hch}	1	7+8	2+12	1	5.88
3^{Hch}	1	7+8	5+10	1	5.88
3^{Hch}	null	7+9	2+12	1	5.88

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REFERENCES

[1] Kreis M, Forde BG, Rahman S, Miflin BJ, Shewry PR: Molecular evolution of the seed storage proteins of barley, rye and wheat. J. Molec. Biol. 183: 499-502 (1985).

[2] Shewry PR: Biological and evolutionary aspects of cereal seed storage proteins. In: Seed storage compounds: biosynthesis, interactions and manipulation. Shewry PR, Stobart AK (eds.). Oxford University Press, Oxford (1993).

[3] Rogers WJ, Payne PI, Miller TE, Holt LM, Law CN, Sayers EJ, Seekings JA: Introduction to hexaploid wheat and assessment for bread making quality of *Glu-A1* locus from *Triticum thaoudar* encoding two high molecular-weight subunits of glutenin. In: Proc. XII Eucarpia Congress, Part II, Gottingen, Netherlands, Wageningen (1989).

[4] Ciaffi M, Lafiandra D, Turchetta T, Ravaglia S, Bariana H, Gupta R, MacRitchie F: Breadmaking potential of durum wheat lines expressing both x and y type subunits at the *Glu-A1* locus. Cereal Chem. 72: 465-469 (1995).

[5] Martín A, Chapman V: A hybrid between *Hordeum chilense* and *Triticum aestivum.* Cereal Res. Comm. 5: 365-368 (1977).

[6] Martín A, Sanchez-Monge Laguna A: Cytology and morphology of the amphiploid *Hordeum chilense* x *Triticum turgidum* conv. *durum.* Euphytica 31: 262-267 (1982).

[7] Martín A, Alvarez JB, Martín LM, Barro F, Ballesteros J: The development of tritordeum: a novel cereal for food processing. J. Cereal Sci. 30: 85-95 (1999).

[8] Payne PI, Holt LM, Reader SM, Miller TE: Chromosomal location of genes coding for endosperm proteins of *Hordeum chilense*, determined two-dimensional electroforesis of wheat-*H.chilense* chromosome addition lines. Biochem. Genet. 25: 53-65 (1987).

[9] Tercero JA, Bernardo A, Jouve N: Encoding genes for endosperm proteins in *Hordeum chilense*. Theor. Appl. Genet. 81: 127-132 (1991).

[10] Alvarez JB, Canalejo AL, Ballesteros J, Rogers WJ, Martín LM: Genealogical identification of hexaploid tritordeum electrophoretic separation of endosperm storage proteins. Plant Breeding 111: 166-169 (1993).

[11] Alvarez JB, Ballesteros J, Arriaga HO, Martín LM: The rheological properties and baking performances of flours from hexaploid tritordeums. J. Cereal Sci. 21: 291-299 (1995).

[12] Alvarez JB, Campos LAC, Martín A, Sillero JA, Martín LM: Genetic analysis of prolamins synthesised by the H^{ch} genome and their effects on gluten strength in hexaploid tritordeum. Euphytica 107: 177-184 (1999).
[13] Fernandez JA, Sanz JC, Jouve N: Biochemical variation to determine phylogenetic relationships between *Hordeum chilense* and other american species of the genus *Hordeum* (*Poaceae*). Plant. Syst. Evol. 157: 105-119 (1987).
[14] Cabrera A, Friebe B, Jiang J, Gill BS: Characterization of *Hordeum chilense* chromosomes by C-banding and *in situ* hybridization using highly repeated DNA probes. Genome 38: 435-442 (1995).
[15] Millán T, Martín A: Effects of *Hordeum chilense* and *Triticum* cytoplasms on agronomical traits in hexaploid tritordeum. Plant Breeding 108: 328-331 (1992).
[16] Laemmli U: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685 (1970).

VARIABILITY OF LOW- MOLECULAR WEIGHT GLUTENIN SUBUNITS ASSOCIATED TO THE D GENOME OF *Aegilops tauschii* IN A COLLECTION OF SYNTHETIC HEXAPLOID WHEATS

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ABSTRACT: *Aegilops tauschii* Coss. is accepted as the **D** genome donor to cultivated bread wheats. However, the origin of hexaploid wheats has been related to an apparently small number of *Ae. tauschii* genotypes of restricted geographic origin, resulting in a narrow genetic diversity for the **D** genome of *Triticum aestivum.*

There is evidence that *Ae. tauschii* has a greater genetic variability for endosperm proteins, gliadins and glutenins, compared to *T. aestivum*. Synthetic hexaploid wheats involving genomes from *Triticum turgidum* ssp. *durum* Desf. em. M.K. and *Ae. tauschii* have been produced as means for introducing desirable characteristics into bread wheat. In this work we describe the genetic variability present at the *Glu-D^{t}3* locus, encoding low molecular weight glutenin subunits (LMW-GS) derived from *Ae. tauschii* using one- and two-dimensional electrophoresis, in a collection of synthetic hexaploid wheats, whose durum wheat parent was the cultivar 'Doy 1'.

A wide variability was present at the *Glu-D^{t}3* locus. Considering the B- and C- groups of LMW-GS contributed from *Ae. tauschii*, from 4 to 8 electrophoretic spots were assigned to this locus in the different synthetic hexaploids analysed. Similarly, for the D- group of LMW-GS, there was a variability in number and position of components as detected by two-dimensional electrophoretic separations. Accessions with a high number of subunits associated to the *Glu-D^{t}3* of *Ae. tauschii* could have significant effects on bread-making properties as these novel alleles could be used to improve technological properties of bread wheat flour.

1. INTRODUCTION

The wild progenitors of bread wheat constitute an important source of variability for usee in broadening the genetic bases of cultivated wheat. *Aegilops tauschii* Coss. is considered to be the **D** genome donor to cultivated bread wheat (*Triticum aestivum* L.) (1, 2). The origin of hexaploid wheat has been related to an apparently small number of *Ae. tauschii* genotypes of restricted geographic origin, resulting in a narrow genetic diversity for the **D** genome in *T aestivum* (3). *Ae. tauschii* may be the most suitable among the progenitor species for the introduction of new genes into cultivated wheat using standard breeding techniques (4). There is complete homology between hexaploid wheat **D** genome chromosomes and those of *Ae. tauschii* (5).

There is evidence that *Ae. tauschii* has a greater useful genetic variability than is found in the other progenitor species (6). Ae. tauschii has a greater genetic variability for endosperm proteins, gliadins and glutenins, compared to *T. aestivum*. On the short arm of the group 1 chromosomes of bread wheat, genes present at the *Glu-3* loci corresponding to the low molecular weight glutenin subunits (LMW-Gs) are present tightly linked to those encoding gliadin components (*Gli-1* loci).

Nowadays *Ae. tauschii* can be crossed directly with bread wheat, due to the development of new techniques of embryo rescues (7, 8). However, the production of synthetic hexaploid wheat is an excelent

mechanism to exploit the genetic variability of *Ae. tauschii*. (9, 10). Synthetic hexaploid wheats involving genomes from *Triticum turgidum* ssp. *durum* Desf. em. M.K. and *Ae. tauschii* have been produced at CIMMYT as means for introducing desirable characteristics into bread wheat. The high cross ability of the synthetic hexaploids with bread wheat indicates that the former could be utilised for introducing new allelic variation present at the *Glu-D^{t}1* and *Glu-D^{t}3* loci of *Ae. tauschii* into cultivated bread wheat.

In this work we describe the genetic variability at the *Glu-D^{t}3* locus, encoding LMW-Gs derived from *Ae. tauschii* using one-and two-dimensional electrophoresis, in a collection of synthetic hexaploid wheats.

2. Material and methods

A collection of 16 synthetic hexaploid wheats, derived from the cross among T. turgidum *ssp.* durum *cultivar 'Doy 1' with diverse accessions of* Ae. tauschii *produced at CIMMYT was used.*

Single seeds were used for the analyses. Each seed was cut in half and the distal part crushed with a mortar and pestle. The embryo was kept and eventually planted.

Gliadins were extracted with 1.5M dimethyl formamide and fractionated by acid-polyacrylamide gel electrophoresis according to Khan *et al.* (11). Electrophoresis was performed at a constant current of 25 mA/gel, and circulating water at a constant temperature of 20°C cooled the electrode buffer.

LMW-Gs were extracted according to Singh *et al.* (12) with the modifications of Morel (13). Glutenin subunits were analysed by one-dimensional SDS-PAGE in 7.5-13% linear acrylamide gradient gel with 1.28% cross-linker concentration. Electrophoretic separations was performed at 30 mA/gel.

Glutenin subunits were also analysed by two-dimensional A-PAGE x SDS-PAGE, according to Morel (13).

3. RESULTS AND DISCUSSION

In bread wheat, a limited variation is present for w- gliadins encoded by genes present at the *Gli-D1* locus and for D-, B- and C-group of LMW-Gs encoded by the linked genes at the *Glu-D3* locus. In fact, Masci *et al.* (14) observed that in all the bread wheats studied two main types were present according to the electrophoretic patterns of the chromosome 1D encoded w- gliadins and LMW glutenins. These resembled analogous patterns found in the bread wheat cultivars Chinese Spring (CS) and Cheyenne (CNN) and therefore indicated as CS- and CNN-type wheats. Masci *et al.* (14) also found that the D-group of LMW-Gs were present only in those bread wheats with CS-type w- gliadins, whereas they were absent in those cultivars possessing CNN-type w- gliadins.

In the synthetic hexaploids studied, a high variability for gliadin components is present at the *Glu-D^{t}3* locus (Figure 1). Some of the *Ae. tauschii* accessions used as parents of the synthetic hexaploids possess the CS-type of bands in their w- components, while a few accessions carried the CNN-type of bands.

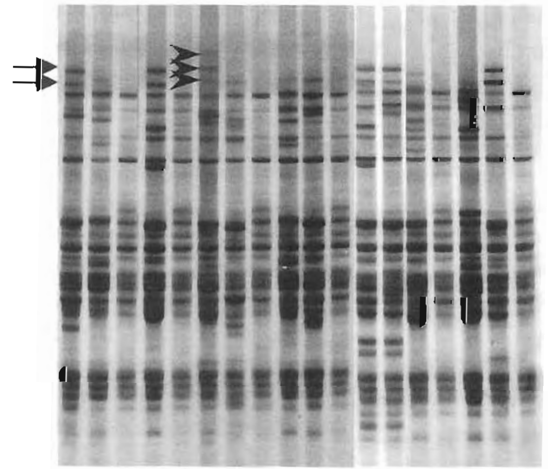

Figure 1: Gliadin electrophoretic patterns in some synthetic hexaploids. Lanes: D: durum wheat cultivar Doy 1 (progenitor of these hexaploids). 1 to 12: synthetic hexaploids. 1: Cim 395; 2: Cim 338; 3: Cim 349; 4: Cim. 401; 5: Cim 403; 6: Cim. 426; 7: Cim 398; 8: Cim 6; 9: Cim 447; 10: Cim 383; 11: Cim 373; 12: Cim 395. Arrows and arrowheads indicate 'Chinese Spring' and 'Cheyenne' types of w-gliadins.

LMW-Gs have been analysed by two dimensional electrophoretic separations (A-PAGE x SDS-PAGE), and compared with those present in the durum wheat cultivar Doy1 and the two biotypes detected in the bread wheat cultivar Newton showing gliadins and LMW-Gs associated to the *Gli-D1/Glu-D3* loci of *CS*- and CNN-type, respectively (Figure 2).

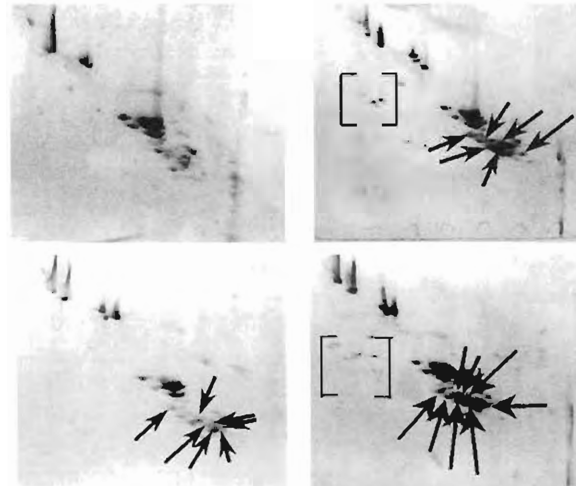

Figure 2: Two dimensional A-PAGE x SDS-PAGE of glutenin subunits from: **a**: durum wheat cultivar 'Doy 1', **b**: to **d**: synthetic hexaploids. **b**:Cim 447, **c**: Cim 403, **d**: Cim 398. Arrows indicate B- and C-LMW-Gs coded at *Glu-D^{t}3* of *Aegilops tauschi*. D-LMW-Gs typical of 'Chinese type' are shown in brackets.

Although a reduced number of genotypes was analysed, a wide variability was present at the *Glu-D^{t}3* locus. Considering the B- and C-group of LMW-Gs contributed from *Ae. tauschii*, from 4 to 8 spots were unequivocally assigned to the *Glu-D^{t}3* in the different synthetics analysed whereas a maximum of 5 subunits was found associated to the *Glu-D3* allele in bread wheat. D-group of LMW-GS were absent in synthetics having Cheyenne-type w- gliadins, similarly to what observed in bread wheat. They were also absent in a genotype whose w- gliadins were different at those found in bread wheat (synthetic hexaploid Cim 403, Figure 1 lane 5, Figure 2 c). The remaining synthetics all showed D-group of LMW-Gs, but there was a variability in number and position of their spots.

Allelic differences in LMW-Gs have been shown to be related to flour quality in durum and bread wheat. Autran *et al.* (15) have demonstrated that in durum wheat the variation in dough properties is most probably determined by the different relative amounts of LMW-Gs, these being considerably greater in the g-gliadin type 45 cultivars.

Accessions with a high number of subunits associated to the *Glu-D^{t}3* locus of *Ae. tauschii* have been detected. This could have significant effects on breadmaking properties as these novel alleles could be used to improve technological properties of bread wheat flour.

4. CONCLUSIONS

A wide variability of storage proteins present at the *Gli-D^{t}1/Glu-D^{t}3* loci was detected in the accessions of *Ae. tauschii* employed as parents of synthetic hexaploids.

More attention should be paid to the contribution of *Glu-D^{t}3* on breadmaking quality. The wide variability and the high number of subunits encoded by this locus suggests that *Ae. tauschii* accessions may be a rich source for enhancing the genetic variability of bread wheat and for improving breadmaking quality.

REFERENCES

[1] Kihara H: Discovery of the DD analyser, one of the ancestors of *Triticum vulgare*. Agric. Hortic. 19: 13-14 (1944).

[2] McFadden ES, Sears ER: The origin of *Triticum spelta* and its free treshing relatives. J. Heredity 37: 81-89, 107-116 (1946).

[3] Lagudah ES, Appels R, McNeil D: The Nor-D3 locus of Triticum tauschii: natural variation and genetic linkage to markers in chromosome 5. Genome 34 : 387-395 (1991).

[4] Appels R, Lagudah ES: Manipulation of chromosomal segments from wild wheat for the improvement of bread wheat. Aust. J. Plant Physiol. 17: 253-266 (1990).

[5] Riley R, Chapman V: The D genome of hexaploid wheat. Wheat Infor. Serv. 8: 8 (1960).

[6] Gill BS, Raupp WJ, Sharma HC, Browder LE, Hatchett JH, Harvey TL, Moseman JG, Waines JG: Resistance in Aegilops squarrosa to wheat leaf rust, wheat powdery mildew, wheat leaf rust, wheat powdery mildew, greenbug and Hessian fly. Plant Dis. 70: 553-556 (1986)

[7] Gill BS, Raupp WJ: *Direct genetic transfers from Aegilops squarrosa L. to hexaploid* wheat. Crop Sci. 27: 445-450 (1987).
[8] Cox TS, Harrell LG, Chen P, Gill BS: Reproductive behavior of hexaploid/diploid wheat hybrids. Plant Breeding 107: 105-118 (1991).
[9] Lagudah ES, MacRitchie F, Halloran GM: The influence of high-molecular-weight subunits of glutenin from *T. tauschii* on flour quality of synthetic hexaploid wheat. J. Cereal Sci. 5: 129-138 (1987).
[10] Peña RJ, Villareal RL, Mujeeb-Kazi A:. Quality characteristics and glutenin subunit composition of wheat lines derived from synthetic wheat (Triticum turgidum x Triticum tauschii) and bread wheat (T. aestivum) crosses. In: Gluten'96. Proc. of the 6th Int. Gluten Workshop. Wrigley C (ed.). Cereal Chemistry Division, Royal Australian Chemical Institute, National Library of Australia, pp. 56-59 (1996).
[11] Khan K, Hamada AS, Patek J:. Polyacrylamide gel electrophoresis for wheat variety identification: Effect of variables on gel properties. Cereal Chem. 62: 310-313 (1985).
[12] Singh NK, Shepherd KW: Linkage mapping of the genes controlling endosperm proteins in wheat. 1. Genes on the short arms of group 1 chromosomes. Theor. Appl. Genet. 75: 628-641 (1988).
[13] Morel MH:. Acid-Polyacrylamide gel Electrophoresis of wheat glutenins: A new tool for the separation of high and low molecular weight subunits. Cereal Chem. 71: 238-242 (1994).
[14] Masci S, Porceddu E, Lafiandra D: Two-dimensional electrophoresis of 1D encoded B and D glutenin subunits in common wheat with similar omega gliadins. Biochem. Genet. 29: 403-413 (1991).
[15] Autran JC, Laignelet B, Morel MH: Characterization and quantification of low molecular weight glutenins in durum wheats. Biochimie 69: 699-711 (1987).

VARIABILITY OF WAXY PROTEINS AND AMYLOSE CONTENT IN *TRITICEAE*

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ABSTRACT: A collection of 720 bread wheats, 547 durum wheats, 301 landraces of spelt wheats and 102 accessions of diploid species was analysed for waxy proteins in the grain. The electrophoresis patterns showed low polymorphism . Two alleles were detected at *Wx-A1* locus and no allelic variation was found at the *Wx-D1* locus in hexaploid and tetraploid wheats. Three and four alleles were detected at the *Wx-B1* locus in hexaploid and tetraploid wheats, respectively. In diploid species a single allele was detected in each species analysed. In hexaploid wheat no significant differences in amylose content were found between the waxy null alleles and the non-mutant alleles. In durum wheats significant differences were observed between allelic variants. In diploid species different values for amylose content were found.

1. INTRODUCTION

• The starch in the endosperm of cereals is composed of the amylose and amylopectin polysaccharides. Amylose content has been reported to affect starch gelatinisation, pasting and gelation properties in wheat. Reduced-amylose (partial-waxy) wheats have been shown to confer superior eating quality to white noodles. Amylose-free starch might find applications in both non-food and food industries. The cereal granule-bound starch synthase enzyme, named waxy protein (Wx), is known to be involved in the synthesis of amylose, and is encoded at the *Waxy* (*Wx*) locus. Bread wheat (*Triticum aestivum*) has three *waxy* loci -*Wx-A1*, *Wx-D1* and *Wx-B1*- which encode for the Wx-A1, Wx-D1 and Wx-B1 proteins, located on the short arms of chromosomes 7A and 7D and on the long arm of chromosome 4A, respectively. In durum wheat (*Triticum durum*) only *Wx-A1* and *Wx-B1* are present. It is well established the relationships between the reduction of amylose content and the presence of null alleles at the *Wx* loci.

2. MATERIAL AND METHODS

- Hexaploid bread wheat *Triticum aestivum* ssp. *vulgare* cv. 'Chinese Spring' and its nullisomic-tetrasomic lines involving chromosomes 7A, 7D, and 4A were analysed and used as standards. A collection of 720 bread wheats (cultivars and landraces mainly from Spain and Portugal), a collection of 547 durum wheats (cultivars and landraces) from Spain, 301 landraces of spelt wheats from Spain and other countries and 102 accessions of diploid species (47 Einkorn wheats, 40 of the *Sitopsis* section and 11 of *T. tauschii*) were analysed for their composition in waxy proteins.
- Starch-granule-bound protein was extracted from at least two embryo-less grain of each accession and electrophoresis was performed as described previously (1).
- Apparent amylose content was determined on 33 bread wheat landraces, 93 spelt landraces, 40 accessions of Einkorn wheats, 40 accessions of the *Sitopsis* section and 9 accessions of *T. tauschii* as described previously (2), In 11 durum wheat apparent amylose content was determined with concanavalin A (3) using the Megazyme Amylose/Amylopectin Assay Kit (Megazyme International IrelandLtd) according to the manufacture's recommendations.

3. RESULTS AND DISCUSSION

3.1. Hexaploid wheats

The electrophoretic profile of 'Chinese Spring' showed three bands with molecular masses between 58 and 61 kDa (Fig. 1). The analysis of the nulli-tetrasomic lines of 'Chinese Spring' (Fig. 1) showed that the

slowed-moving band was encoded by chromosome 7A, the intermediate mobility band by 7D and the fastest band by 4A. The alleles which correspond to these proteins were named *Wx-A1a*, *Wx.D1a* and *Wx-B1a*. The variability found among the bread wheat cultivars and landraces and spelt wheat landraces was low. Two allelic variants for the *Wx-A1* locus (named *Wx-A1a and Wx-A1b* alleles), one for the *Wx-D1* locus (*Wx-D1a* allele) and three for the *Wx-B1* locus (*Wx-B1a*, *Wx-B1b* and *Wx-B1e* alleles) were found (Fig. 1). Table 1 and Table II summarize the allelic composition found among the hexaploid collection analysed.

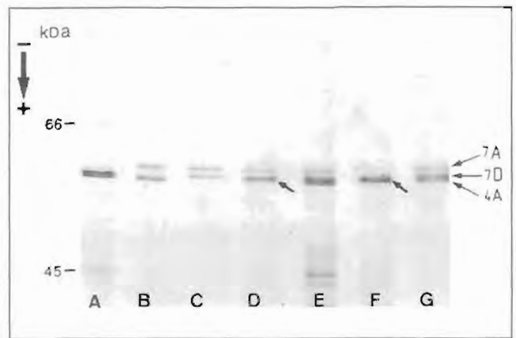


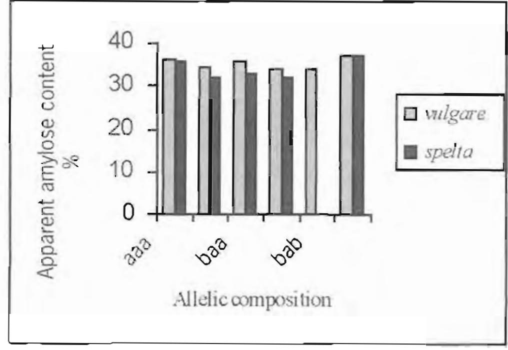
Figure 1. SDS-PAGE patterns of waxy proteins from the nuli-tetrasomic lines of bread wheat 'Chinese Spring' N7AT7B (lane A), N7DT7B (lane B) and N4AT4B (lane C), the bread wheat cultivars 'Cartaya' (*Wx-A1a, Wx-D1a, Wx-B1e)* (lane D) and 'Yécora Rojo' (*Wx-A1a, Wx-D1a, Wx-B1a)* (lane E), the bread wheat landrace 'Blanquillo de Cáceres'(*Wx-A1b, Wx-D1a, Wx-B1e)* (lane F), and 'Chinese Spring' (lane G). The small arrows (lanes D and F) indicate the waxy protein used as marker of the *Wx-B1e* allele. The chromosomal locations of the isoproteins are indicated on the right. Molecular markers are included in the left.

Table I. Allelic composition at the three waxy (*Wx*) loci of 720 bread wheas analysed

Locus and allele			Cultivars	Landraces
Wx-A1	***Wx-D1***	***Wx-B1***	n	n
a	a	a	206	257
a	a	b	68	76
a	a	e	23	64
b	a	a	0	8
b	a	e	0	13
b	a	b	0	5
Total			**297**	**423**

Table II. Allelic composition at three waxy (*Wx*) loci of 301 landraces of spelt wheat and % of total hexaploid wheat analysed

Locus and allele			Spelt wheats
Wx-A1	***Wx-D1***	***Wx-B1***	n
a	a	a	195
a	a	b	49
a	a	e	32
b	a	a	14
b	a	e	9
b	a	b	2
Total			**301**



- Amylose content, expressed as the percentage of total starch for the wheat and spelt landraces is show in Fig. 2. No significant differences in mean amylose content were found between the hexaploid wheats with the null *Wx-A1b* allele or the null *Wx-B1b* allele.

Figure 2: Mean of apparent amylose content (%) of total starch for the hexaploid wheat landraces.

3.2. Durum wheats

The variability of waxy protein detected among the durum wheat collection was also low. All the durum wheat landraces and 99% of the cultivars had the *Wx-A1a* allele. Only one durum wheat cultivars had the null *Wx-A1b* allele. The variability found at the *Wx-B1* locus was higher than in hexaploid wheats, and four alleles were detected (*Wx-B1a, Wx-B1b, Wx-B1e* and *Wx-B1f*) (Fig. 3). Table III summarizes the allelic composition found among durum wheat collection analysed.

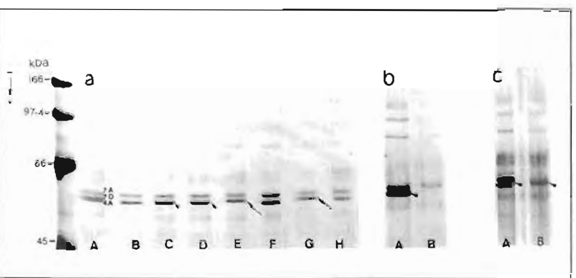


Figure 3: Electrophoresis of waxy proteins from (**a**): 'Chinese Spring' (lane A), N7DT7B (lane B) and the durum wheat landraces BG-12408 and BG-12409 (lanes C and D, respectively) (*Wx-A1a, Wx-B1e*), BG-12413 and BG-12415 (lanes E and G, respectively) (*Wx-A1a, Wx-B1f*) , BG-12414 and BG-12427 (lanes F and H, respectively) (*Wx-A1a, Wx-B1a*); (**b**): BG-18239 (*Wx-A1a, Wx-B1e*) (lane A) and 'Blanqueta' (*Wx-A1a, Wx-B1b*) (lane B); (**c**): 'Endural' (*Wx-A1a, Wx-B1e*) (lane A) and 'Astrodur' (*Wx-A1b, Wx-B1e*) (lane B). Waxy protein controlled by the *Wx-B1e* allele (arrowhead) and by *Wx-B1f* allele (arrows) are indicated. Molecular markers are included in the left.

Table III. Allelic composition at the two waxy (*Wx*) loci among a collection of 547 Spanish durum wheats.

Locus and allele		**Cultivars**	**Landraces**
Wx-A1	**Wx-B1**	*n*	*n*
a	a	64	162
a	b	0	1
a	e	35	197
a	f	3	84
b	e	1	0
Total		**103**	**444**

- Mean amylose content of durum wheats with the *Wx-B1a* allele was significantly lower ($p<0.01$) than those with *Wx-B1e* or *Wx-Bf*. Otherwise, mean amylose content of wheats with *Wx-B1e* or *Wx-B1f* was not significantly different (Fig. 4).

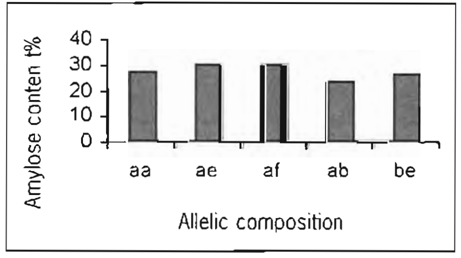


Figure 4: Amylose content(% of total starch) of durum wheat

3.3. Diploid species

- The electrophoretical analysis of the diploid species with the A genome (27 *T. monococcum*, 10 *T. boeoticum* and 10 *T. urartu*), revealed a single *Wx-A1* encoded waxy protein in all accessions and similar electrophoretical mobility to the *Wx-A1d* allele found by Yamamori et al. (1995) (4) in one accession of *T. dicoccoides* (N.KU 8937B). (Fig. 5a)
- A single protein was also observed in the diploid species of *Sitopsis* section with the S genome (22 *Ae. longissima*, 14 *Ae. sharonensis*, 4 *Ae. bicornis*, 3 *Ae. searsii* and 1 *Ae. speltoides*) and similar to that encoded by the *Wx-B1f* allele detected in durum wheat (Nieto-Taladriz et al. 1999) (5). (Fig. 5a).
- The analysis of the accessions of *T. tauschii* (D genome) showed a single waxy protein with the same mobility in all accessions, similar to that encoded by the *Wx-A1a* allele from 'Chinese Spring'(Fig. 5b.

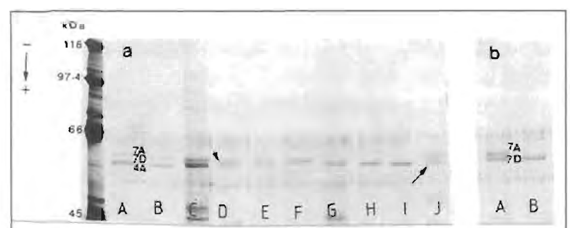


Figure 5: SDS-PAGE patterns of waxy proteins from: (**a**) 'Chinese Spring' (lane A), N7DT7B (lane B), bread wheat QT 105 (lane C), *T. dicoccum* KU 893B (lane D), *T. monococcum* (lane E), *T. urartu* (Lane F), *Ae. longissima* (lane G), *Ae. sharonensis* (lane H), *Ae. searsii* (lane I) and 'Valenciano Grano Rojo' (*Wx-A1a, Wx-B1f*) (lane J); (**b**) N4AT4B (lane A) and *T. tauschii* (lane B). Waxy protein controlled by the *Wx-A1d* allele (arrowhead) and by *Wx-B1f* allele (arrows) are indicated. Molecular markers are included in the left.

- In diploid species amylose content ranged from 22% to 35% with a mean of 29% in Einkorn wheats, ranged from 28% to 41% in *Sitopsis* section with a mean of 32% and the accessions of *T. tauschii* amylose content ranged from 28% to 33% with a mean of 31%.

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REFERENCES

[1] Rodríguez-Quijano M, Nieto-Taladriz MT, Carrillo JM: Polymorphism of waxy proteins in Iberian hexaploid wheats, Plan Breeding 117:341-344 (1998).

[2] Watanabe N, Noda Y, Goto N, Miura H: Variation for apparent amylose content of endosperm starch in *Triticum durum* and *Aegilops squarrosa*. Euphytica 101:283-286(1998).

[3] Gibson TS, Solah VA, McCleary RV: A procedure to measure amylose in cereal starches and flours with concanavalin-A. J Cereal Sci 25:111-119 (1997).

[4] Yamamori M, Nakamura T, Nagamine T: Polymorphism of two waxy proteins in the emmer group of tetraploid wheat, *Triticum dicoccoides*, *T. dicoccum* and *T. durum*. Plant Breeding 114:215-218 (1995).

[5] Nieto-Taladriz MT, Rodríguez-Quijano M, Carrillo JM: Polymorphism of waxy proteins in Spanish durum wheats. Plant Breeding 119:277-279 (2000).

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