CONGRESOS Y JORNADAS

SUNBIO 2003
Sixt European Conference on Sunflower Biotechnology

Seville, 5-9 October 2003

Consejería de Agricultura y Pesca

E ANDALUCIA

SUNBIO 2003

Sixth European Conference on Sunflower Biotechnology

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SIXTH EUROPEAN CONFERENCE ON SUNFLOWER BIOTECHNOLOGY - Seville, 5-9 October 2003

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CONFERENCE PROGRAM

Sunday October 5th

18:00-...	Arrivals
20:00-22:00	Welcome Party

Monday October 6th

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LUNCH	
16:00-19:00	Session 2 (*) INTERSPECIFIC HYBRIDIZATION AND WILD SPECIES

Tuesday October 7th

09:00-13:30	Session 3 (*) SEED, OIL AND PROTEIN QUALITY
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Gala dinner	

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09:00-13:30	Session 5 (*) GENETIC ENGINEERING, GENOMIC AND PROTEOMIC
LUNCH	
16:00-19:00	Session 6 (*) ROUND TABLE: TRENDS IN PRODUCTS BIOTECHNOLOGY CONCLUDING REMARKS

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S505 **Towards the construction of a numeric sunflower genome map with the aid of *A. thaliana* genome (ICCARE)**
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ROUND TABLE "TRENDS IN PRODUCTS BIOTECHNOLOGY"

Dr. Jose Mª Baro, REPSOL-YPF (Spain). *Biodiesels.*
Dr. Eckhard Floter, Unilever Research Vlaardingen (The Nederland). *Oil in food technology.*
Dra. Amaya Igartua, Fundación Tekniker (Spain). *Lubricants from vegetable oils.*
Dr. Julio Girón, Instituto de la Grasa, CSIC (Spain). *Functional food proteins.*

S101

Sunflower breeding for resistance to the new broomrape race

Gontcharov S.V., Antonova T.S. and Araslanova N.M.

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Broomrape (Orobanche cumana Wallr. syn. O. cernua Loefl.) is a parasitic plant, feeding on sunflower roots. The main method to control broomrape is development of resistant hybrids and (or) OP varieties. During the more than 100 years of co-evolution sunflower and broomrape in Russia biotype A was subsequently changed by B and then C. Biotype C is the predominant one on the Russia territory now. Majority of released Russian hybrids and OP varieties are resistant to that biotype, but broomrape continue to produce new races. Last years the new, more aggressive race, designated as race F (or, in Russia, biotype D) has been drastically spread in Spain.

The aims of our work were to find donors of resistance to this biotype, studying heredity of this trait and development new sunflower inbred lines combining F-race broomrape resistance with other valuable traits.

Preliminary resistance test showed that all VNIIMK released inbred lines are susceptible to the new broomrape race, so all prospective inbred lines were also tested. Majority of them proved to be susceptible also. But line VK-455 demonstrated partial resistance and VK-623 - resistance. The number of broomrape plants on the roots of sunflower plantlets varied from 0 (VK-623) to 23 (SL-1) at this experiment, and was 9.7 in average. All tested inbred lines were completely resistant to Russian biotype C.

All the breeding material, obtained with the using VK-623 as a parental line (4 hybrid combinations in the pedigree nursery), and F_1 hybrids with it were tested the same season in the greenhouse. All F_1 hybrids were susceptible to the new race with the number of germinated broomrape plants on the sunflower roots from 9.2 to 16.3, so this resistance was recessive one. It will require developing both resistant parental lines to produce resistant hybrid if this race starts to dominate in Russia.

Among the tested breeding material resistant plants were encountered with different rate. Hybrid combination 14_ _ (VK-623 _ VK-616) gave such plants with the maximum frequency. 15 resistant plants were found in the progeny of two F_3 morphologically different plants from this combination. All of them were transplanted after testing and self-pollinated. Obtained seeds were planted next season in the field and selected promising plants were self-pollinated. Their progeny proved their resistance in the next year testing.

As a result two new prospective inbred sunflower lines with resistance to both broomrape races - C and D were developed. Above that line VK-623 confirmed to be a good donor of this trait. However recessive character of obtained resistance create some difficulties in commercial sunflower hybrid breeding so we will continue to find new dominant resistance genes donors among the sunflower samples.

QTL-analysis of resistance to *Sclerotinia sclerotiorum* in sunflower

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Sclerotinia sclerotiorum (Lib.) de Bary is one of the most important sunflower pathogens. Our objective is to determine the number of quantitative trait loci (QTL) responsible for the resistance to mycelial extension of *S. sclerotiorum* in sunflower leaves and stems.

Two sunflower $F_{2/3}$ populations were developed by crossing inbred lines NDBLOS (Pop1, n = 354) and Gi3-5 (Pop2, n = 434) with CM625. In previous investigations NDBLOS and Gi3-5 showed less stem lesion length than CM625 after artificial leaf infections with *S. sclerotiorum*. Field experiments of the F_3 lines were conducted at Eckartsweier, Germany, during 1999 to 2001. The tip of one leaf of the fifth or sixth fully grown leaf pair was infected with *S. sclerotiorum* mycelium. Five plants per plot were infected. We recorded leaf length and petiole length, and the time when the first symptoms were visible at the stem, to calculate the speed of fungal growth. Furthermore leaf lesion length and stem lesion length were measured. The genotypic variances across F_3 lines were highly significant ($P < 0.01$) for all resistance traits. Variances due to genotype x environment interactions were small or not significant. Heritability was highest for stem lesion (Pop1: $h_{\_} = 0.89$; Pop2: $h_{\_} = 0,82$) and lowest for leaf lesion length (Pop1: $h_{\_} = 0.55$; Pop2: $h_{\_}=0.54$). All resistance traits showed highly significant ($P < 0.01$) but moderate phenotypic correlations with each other ($r = 0.45 - 0.80$).

More than 1100 SSR primer pairs were screened on parental lines. A genetic linkage map of 352 F_2 individuals was constructed using 114 of 117 polymorphic marker loci. For each resistance trait 6 to 9 QTL were identified using composite interval mapping method (LOD > 2.5). The QTL explained between 3.2 and 36.7 % of the phenotypic variance. In a simultaneous fit, nine QTL for leaf lesion explained 45.4 % of the genotypic variation, eight QTL for stem lesion length explained 50.4 % and five QTL for speed of fungal growth explained 40.1 % of the genotypic variation.

For Pop2 we are using the method of selective genotyping for detection of QTL. With selective genotyping, only individuals with high and low phenotypic values for the trait of interest are genotyped. At present, we are genotyping 5 % of each tail of the phenotypic distribution for the traits stem lesion length and speed of fungal growth.

Acknowledgements

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S103

Broomrape *(Orobanche cernua Loeffl.)* and herbicide resistance breeding *in* sunflower in Turkey

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Sunflower one of the important oil crops in the world and in Turkey. Trakya region, which is the European part of Turkey, has 75 % of all sunflower production in Turkey. However, 80 % of sunflower production area is infected new races of *Orobanche cernua* in the region. Broomrape made epidemic each 20 years (1960, 1980 and 2000) broke resistance of sunflower cultivars. At the last five years, there are probably three more races (F, G and H) than known (A,B,C,D,E) of *Orobanche cernua* in the region. Although some resistant and tolerant sunflower hybrids are planted, these cultivars could have susceptibility after a couple years due to high virulence attack of these new races. Besides, some important weed species such as Cocklebur (*Xanthium spp.*), Thistle (*Circium arvense*), *Convolvulus arvense*, *Sinapsis arvensis* L., *Avena spp.*, *Datura spp. Amaranthus spp.* are a big problem in sunflower production in this area. Therefore, herbicide application both controlling weeds and broomrape are so important to increase sunflower yield and to get more profit in sunflower. Orobanche resistant breeding program in sunflower has been started and conducted as national basis by Trakya Agricultural Research Institute since 1955. Based on changing races in each period, some resistant parental lines and hybrids are developing in the National Sunflower Research program. After getting resistance sources to Imidazolinone herbicide in sunflower by USDA, using IMI, Imazamox, Imazapyr post emergence herbicide research to control *O. cernua* continue widely by Trakya Agricultural Research Institute and private companies in Turkey. Intervix herbicide registered by BASF Company to control orobanche and weeds in sunflower production and one IMI resistant sunflower hybrid got production permission also in Turkey in 2003. The goals of the National Program are firstly genetic control of broomrape and then chemical control on both orobanche and weeds using IMI herbicide getting resistance genes in parental lines and hybrids.

S104

Dominance relationships for genes conferring resistance to sunflower broomrape

Pérez-Vich. B.[1*], B. Akhtouch[1], A. Mateos[2], L. Velasco[1], C.C. Jan[3], J. Fernández[2], and J.M. Fernández-Martínez[1]

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The holoparasitic angiosperm sunflower broomrape (*Orobanche cumana* Wallr.) is actually regarded as one of the most important constraints of sunflower production in many areas in Southern Europe and the Black Sea region. Breeding for resistance is considered the most effective and feasible method of controlling sunflower broomrape. Commercial hybrids cultivated in Spain carry the *Or1* to *Or5* genes that confer resistance to races A to E. However, a new race, designated F, has been identified. Germplasm with race F resistant genes derived from wild and cultivated sunflowers has been developed. Dominance reaction of these genes is an essential feature that determines the breeding method to produce race F resistant sunflower hybrids. Crosses between different sources of race F resistant lines, including those derived from wild and cultivated sunflower, and different susceptible parental lines were made. Allelic crosses between race F resistant lines, as well as crosses between race F resistant lines and race E resistant lines were also carried out. F_1 plants and their parental lines were evaluated for their disease reaction to race F or race E of broomrape. Different dominance reactions were observed. These depended on the race of broomrape, the source of race F resistance, and also the susceptible parental line used for the cross. The relevance of the different reactions observed for sunflower breeding for resistance to broomrape is discussed.

S105

Mapping and analysis of QTLs for grain oil content and agronomic traits in sunflower *(Helianthus annuus L.)*

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Crosses were made between two inbred lines of sunflower. Parents and 118 F_3 families were planted in the field in a randomized complete block design in two replications. Genetic control for some agronomical traits: grain weight by plant (GWP), 1000-grain weight (TGW), percentage of oil in grain (POG) and sowing to flowering date (STF) was investigated in F_3 families and their parents. Genetic variability was observed among the 118 F_3 families for all the traits studied. Genetic gain was obtained when the best F_3 family or the mean of 10% of the selected families were compared with the best parent for GWP, TWG and POG. Heritability was 0.23 for GWP, 0.55 for TGW, 0.57 for POG and 0.32 for STF. A set of 244 F_3 families from the same cross including the above 118 mentioned families and their two parents were screened with 276 AFLP and microsatellites markers and linkage map was constructed based on 170 markers. Two putative QTLs for GWP trait (*gmp*), one QTL for TGW (*tgw*), six QTLs for POG (*pog*) and two for STF (*stf*) were detected. The percentage of phenotypic variance explained by each QTL ranged from 2.6% to 70.9%. The percentage of total phenotypic variance explained was 50.7% for GWP, 5.4% for TGW, 90.4% for POG and 89.3% for STF. Although these regions need to be more precisely mapped and the information obtained should help in markers assisted selection.

S106

Epistatic QTL networks underlying achene domestication traits in sunflower

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Quantitative trait loci (QTL) underlying several genetically correlated achene domestication traits were mapped in cultivated sunflower (*Helianthus annuus* L.) using a 206-locus genetic linkage map constructed from 173 confectionery (RHA280) ¥ oilseed (RHA801) recombinant inbred lines (RILs) segregating for apical branching (B_1), pericarp phytomelanin pigment (*P*), and pericarp hypodermis pigment (*Hyp*) loci. RIL-mean heritabilities ranged from 0.92 to 0.98 for achene oil concentration (*aoc*), 100-achene weight (*awt*), achene length (*al*), width (*aw*), and depth (*ad*), kernel weight (*kwt*), pericarp weight (*pwt*), and kernel-to-pericarp weight ratio (*kpr*). Composite interval mapping (CIM) identified 40 QTL on 10 chromosomes for the eight traits. B_1-, *P*-, and *Hyp*-linked QTL were identified for every trait. Multilocus mixed model QTL analyses were performed on seven genetic marker loci (B_1, *P*, *Hyp*, and four SSR marker loci, ORS188, ORS371, ORS484, and ORS1068-4) and identified 219 significant intra- and interlocus effects explaining 61 to 91% of the genetic variability among RILs (44/56 additive, 74/147 additive ¥ additive, and 101/224 additive ¥ additive ¥ additive effects were signif-icant). Most of the QTL had strong pleiotropic effects. The number of significant epistatic interaction effects was lowest for achene oil concentration (9/49), next lowest for kernel-to-pericarp weight ratio (13/49), and greatest for 100-achene weight and achene width (both 31/49). The epistatic QTL networks were complex; however, three mega-trends were observed. First, the signs of net intra- and interlocus effects were in opposite directions for every trait. Net additive effects were -14.44, 3.75, 0.82, 1.75, 0.96, 0.04, 0.33, and -1.31 and net (additive ¥ additive) + (additive ¥ additive ¥ additive) effects were 4.10, -9.05, -5.05, -4.10, -2.00, -0.50, -0.35, and 0.08 for *aoc*, *awt*, *al*, *aw*, *ad*, *kwt*, *pwt*, and *kpr*, respectively. Hence, a preponderance of the epistatic interactions shifted trait means in an unfa-vorable direction. Second, net epi-static QTL effects were greater than net intralocus QTL effects for six of the eight traits (*awt*, *al*, *aw*, *ad*, *kwt*, and *pwt*). Hence, epistatic QTL effects were as important as intralocus QTL effects. Third, crossover epistatic interactions predominated. While crossover interactions are of greater consequence than non-crossover interactions for breeding, none of the recombinant genotypes produced more oil than the high oil genotype (RHA801). So, in the final analysis, alleles for increasing achene oil concentration were not identified in the confectionery parent, which strongly suggests that favorable alleles found in the oilseed parent were originally derived from exotic and wild populations used in the development of the first oilseed sunflower cultivars. Finally, selection for increased achene oil concentration in the post-1940 era of sunflower domestication reduced achene dimensions and seems to have operated on several pleiotropically acting and epistatically interacting QTL.

The creation of sunflower sterile CMS analogues on the base of different cytoplasmic backgrounds

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At present the widely used CMS source of sunflower hybrid production is the CMS PET-1. Such cytoplasmic uniformity courses a potential risk for hybrid sunflower. Numerous Research Institutes try to find the ways to solve this problem. The mobilization of different cytoplasmic backgrounds in hybrid production will improve general variability of sunflower and help to avoid the epiphytoties threat.

In this connection the aim of the performed research was to obtain sterile inbred lines on the base of different CMS sources.

Eleven CMS sources were used in the study: *Helianthus argophyllus* (ARG-1, ARG-3), *H. praecox* (PRH-1, PRR-1), *H. rigidus* (RIG-1, RIG-2), *H. fallax* (PEF-1), *H. giganteus* (GIG-1), *H. debilis* (DEB-1), including non-identified CMS sources DCS-1 and DCS-3. To obtain the sterile CMS analogues all CMS accessions were crossed with fertile line VB 1002 and then the saturated backcrossing was performed. The obtained backcrosses were estimated on the main agronomic traits (seed mass/1000 seeds, plant height, leaf area, vegetative period duration, oil content, diseases resistance). The estimation of resistance to the main sunflower pathogens was conducted under field conditions by artificial infection.

According to the estimated results all the obtained sterile CMS analogues were mainly clear-cut to the control (VB1002). But some distinctions existed. Thus, CMS RIG-2 analogue differed from the control by stem thickness and the duration of vegetative period, CMS GIG-1 analogue - by plant height, CMS PEF-1 line - by capitulum diameter, CMS DCS-3 line - by seed mass of 1000 seeds. The backcrosses carrying GIG-1, ARG-3 and DCS-3 cytoplasms were the most clear-cut to the control. These distinctions from the control probably may be caused by cytoplasmic effects of the CMS sources.

The evaluations of obtained CMS-lines for diseases resistance showed, that the majority of lines were infected by *Sclerotinia sclerotiorum, Diaporthe helianthi, Phoma macdonaldii, Verticillium dahliae*. At the same time the complex disease resistance of the following lines was indicated: CMS ARG-1 line, CMS ARG-3 line, CMS DCS-1 line and CMS RIG-2 line.

The results of investigation demonstrate that the sterile inbred lines on the base of CMS ARG-1, CMS ARG-3, CMS DCS-1 and CMS RIG-2 have the most important value for sunflower hybrid breeding and seed production purposes.

S1P1

Evaluation of genetic variability for *Sclerotinia sclerotiorum* Lib. de Bary resistance in a population from a cross between susceptible and resistant sunflower

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The inbred line R28, coming from *Helianthus argophyllus*, appears to contain most of the resistance factors to *Sclerotinia sclerotiorum*, displaying low susceptibility against both artificial and natural infection to basal stem and head (head rot) and moreover to fungus filtrate (oxalic acid). In order to obtain segregating population, a cross between this 28 R and C9304, a susceptible inbred line, was made. One C9304 plant was manually emasculated and crossed using pollen from one R 28 plant. Two of the F_1 plants obtained, were self-pollinated under tissue bags to obtain 313 F_2 descendants.

During 2002 spring, F_2, F_1 and parental lines seeds were sown under field conditions at the University of Udine Experimental Station. Fifty days after sowing, 155 F_2 plants, 10 parental plants and 10 F_1 plants, were treated placing an oat grain infected with pathogen mycelium over the basal stem (basal stem attack). The plants were weekly examined for symptoms.

At flowering time, the remaining 158 F_2 plants, 10 plants of each parent and 10 F_1 plants were artificially infected on the head (head rot attack) by ascospore test. The capitula were then covered with bags. The plants were examined for symptoms twice a week.

As result of basal stem infection, 72.4% of the F_2 plants shown symptoms and died. Symptoms appearance varied in the population, from 5 to 25 days after infection, with a population mean of 12.5 ± 6.1 days. The symptoms appearance in parental lines was 7, 10 and 16 days on C 9304, F_1 and R 28, respectively.

After the ascospore test, 60.7 % of the F_2 plants infected shown disease symptoms on the capitulum, In this case the symptoms appearance after infection ranged between 16 and 45 days with a population mean of 27.6 ± 6.4 days. The symptom appearance was 18, 25 and 35 days after infection on C 9304, F_1 and R 28, respectively.

The distribution of the F_2 population for symptom appearance in the ascospore test was unimodal and continuous and in both tests (basal stem and capitulum) some plants of the F_2 exceeded the mean value of the resistant parent (R28) and in any case the F_1 mean did not show values higher or lower than the midparent values, confirming the possibility to obtain some results from classical selection programmes utilising the symptom appearance as selection parameter. We are also considering a molecular markers assisted approach. Recently, several hundred microsatellite markers were developed for sunflower. These markers are suitable for automated and multiplexing analyses allowing to screen a big number of individuals and primers combinations. We have selected a first set of 40 primer combinations on the basis of amplicon length to facilitate multiplexing. SSR markers were screened for polymorphism using three-color multiplexes. Genotyping assays of 100 F_2 individuals were performed using pools of six markers.

S1P2

Mapping the downy mildew resistance gene PlArg in sunflower

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Resistance of sunflower (*Helianthus annuus)* to the obligate parasite *Plasmopara halstedii* is conferred by specific dominant genes, denoted *Pl*. Three genes, Pl_6, Pl_7, and Pl_8, found in wild *Helianthus* species, confer resistance to all known races of *P. halstedii*. It is not yet uncovered whether these are single resistance genes conferring some non-race-specific, complete resistance or complex loci containing several linked *Pl* genes giving resistance to individual races.

The inbred line Arg1575-2 carries the *P. halstedii* resistance locus Pl_{Arg} which has been introgressed from the wild species *H. argophyllus* and confers resistance to all known isolates of the fungus. The objective of the present study is to map the Pl_{Arg} locus within F_2 individuals of cross CmsHA342 (susceptible) x Arg1575-2 (resistant) using SSRs and resistance gene analogs (RGA) in order to elucidate the genetic fine structure of the Pl_{Arg} locus.

Phenotypic resistance evaluation was conducted with a subset of 126 $F_{2:3}$ families after immersion of the whole seedlings in a suspension of *P. halstedii* spores. 71 SSRs (Tang et al. 2002) were screened for polymorphism by bulked segregant analysis (BSA) with the susceptible inbred line CmsHA342, the resistant inbred line Arg1575-2, a resistant bulk (B_r), and a susceptible bulk (B_s). Twelve SSRs revealing polymorphisms between B_r and B_s were mapped on linkage group 1 (LG1) with the aid of 126 F_2 individuals used for phenotypic evaluation of downy mildew. However, all segregating SSRs of LG1 mapped proximal to Pl_{Arg}, with a minimum distance of 1.9 cM and a maximum of 9.3 cM.

Alternatively, three degenerated RGA primer combinations (Leister et al. 1996) were used to amplify fragments from the resistant line Arg1575-2. Several prominent bands resulting from the three NBS-LRR-like primer combinations (PC) I (s1/as1), II (s2/as2) and III (s2/as3), originating from Arg1575-2 were cloned into a plasmid vector. As expected, the obtained PCR products consist of a mixture of different sequences of similar length. Between one and 58 colonies of each cloned fragment were picked and further digested with 5 - 7 restriction enzymes. Restriction analysis was performed to identify subgroups within the pools of sequences. So far, two fragments from PC I (160 bp, and 900 bp), two fragments from PC II (310 bp, 500 bp), and one fragment from PC III (550 bp) have been analysed. Database searches revealed similarities with different kinds of sequences. For example, fragment II-500 showed up to 84% sequence similarity over 481 bp with the *Helianthus annuus* NBS-LRR resistance protein RAS4-5. Subsequent primer synthesis and mapping of interesting RGA fragments is currently done in our lab.

So far, our study has not yet provided a set of closely linked flanking markers which could replace seedling tests in breeding programs but, since Pl_{Arg} is now mapped on LG 1, it will be possible to search for additional PCR markers on this group. Varieties with multigenic resistance should provide protection against the spread of new pathotypes and pave the way to combine more than one resistance gene in an inbred line. Furthermore, mapping of

molecular markers closely linked to *Pl*$_{Arg}$ using a high-resolution mapping population will be essential to study the genetic complexity of the *Pl*$_{Arg}$ locus on LG 1.

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S1P3

Construction of a genetic map and locating major traits in sunflower *(Helianthus annuus L.)*

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We have developed a segregating mapping population of 183 F_2 individuals of the cross RHA325(cms) x HA342. RHA325 is an open American line based on the PET1 cytoplasm while HA342 is a maintainer line. The F_2 and derived populations segregate for male fertilityvssterility, downy mildew (*Plasmopara hastedii*) resistance (*Pl*$_2$) and oleic vs linoleic acid content.

A genetic map was developed covering 1751.5 cM with 202 AFLP and 19 SSR markers in 18 linkage groups. 13 linkage groups contain one or more SSR markers and are numbered according to Tang S, Yu JK, Salbaugh MB, Shintani DK & Knapp SJ 2002: Theor Appl Genet 105, 1124-1136.

A segregation ratio of 1 (male fertile, *Rf1Rf1*): 2 (male fertile, *Rf1rf1*): 1 (male sterile, *rf1rf1*) was observed in the F_2 population as expected for one restorer gene (χ^2= 2.83, P= 0.24). In F_3, 14 progeny plants of each fertile F_2 individual were evaluated for male fertility to distinguish between F_2 plants being homozygous or heterozygous for the restorer gene. The data obtained from the F_3 progenies were confirmed by segregation analyses in an F_2BC_1 population. The *Rf1* locus was shown to be located on linkage group 13, containing the SSR markers ORS388 and ORS1030.

Using a whole-seedling-immersion test (Gulya TJ, Miller JF, Viranyi F & Sackston WE 1991: Helia 14, 11-20) we found that the F_2 population segregated for Plasmopara reaction at a ratio of 1 (*Pl*$_2$*Pl*$_2$): 2 (*Pl*$_2$*pl*$_2$): 1 (*pl*$_2$*pl*$_2$); χ^2= 0.83 (P= 0.65). The *Pl*$_2$ gene was demonstrated to be located on linkage group 8 together with the SSR marker ORS599.

Furthermore, quantitative trait loci for oleic acid content (LOD > 3) could be localized on linkage group A. Future work will concentrate on marker saturation of the genetic map.

S1P4

Identification of QTLs for germination and plantlet development in sunflower *(Helianthus annuus L.)*

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A population of 84 recombinant inbred lines (RILs) developed through single-seed descent (SSD) from the cross between 'PAC-2' and 'RHA-266'were used in this study. The material was kindly provided by INRA (France). Seeds of the above-mentioned RILs and their two parents were sown in a randomized block design with three replications, and the following characters are studied: percentage of germination (PG), hypocotyle length (HL), length of roots (LR), fresh weight of plantlet (FWP), fresh weight of roots (FWR), dry weight of plantlet (DWP), dry weight of roots (DWR), percentage of normal plantlets (PNP) and abnormal plantlets (PAP).

A set of 123 RILs from the same cross, including the above 84 mentioned RILs and their two parents, were screened with 409 AFLP and SSR markers and a linkage map was constructed based on 367 markers. Four QTLs for PG (*pg*), nine QTLs for HL (*hl*), four QTLs for LR (*lr*), seven QTLs four FWP (*fwp*), eight QTLs for FWR for (*fwr*), six QTLs for DWP (*dwp*), three QTLs for DWR (*dwr*), eight QTLs for PNP (*pnp*) and Four QTLs for PAP (*pap*) were detected. The effects of each QTL are moderate ranging from 6% to 33%. but a high percentage of phenotypic variance is explained when considering all the covariants (*TR*_ ranging from 46.71% to 85.19%). Although the detected regions need to be more-precisely mapped, the information obtained should help in marker assisted selection.

Molecular breeding for resistance against major fungal pathogens of sunflower, *Plasmopara halstedii* and *Sclerotinia sclerotiorum*

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Cultivation of sunflower (*Helianthus annuus*) in humid environments is strongly affected by major pathogens like *Plasmopara halstedii* and *Sclerotinia sclerotiorum*. In an attempt to isolate the *Pl2* gene, which confers resistance to *P. halstedii* the causal agent of downy mildew, resistant sunflower lines carrying this gene were crossed to respectively susceptible lines. Mapping populations were constructed and closely linked markers for the *Pl2* locus identified. These materials were shown to be suitable for marker assisted breeding and will be used for map-based cloning of the gene aided by a BAC library constructed from one of the parental lines.

Sclerotinia sclerotiorum, causal agent of root, stem and head rot is probably the most devastating pathogen of sunflower. For that reason, our research focuses on the amelioration of sunflower for resistance against this major pathogen. In this context, asymmetric somatic hybrid (ASH) plants obtained by PEG-mediated fusions of micro-protoplasts from *H. maximiliani* and hypocotyl protoplasts of *H. annuus* (Binsfeld P.C. et al. 2000, Theor. Appl. Genet. 101:1250-1258) were evaluated for Sclerotinia reaction. In this study progenies of ASH obtained from fusion of protoplasts from *H. maximiliani*, a wild species shown to be resistant against *S. sclerotiorum*, and sunflower cv. Florom were characterized by molecular analysis (AFLP) to identify incorporated DNA-fragments from *H. maximiliani* in the sunflower genome. Wild species-specific fragments along with fragments not found in the parents were detected, and it was possible to identify AFLP-fragments which seem to be linked to *Sclerotinia* resistance (Rönicke et al., Plant Breeding 2003, accepted). Some of the interspecific progeny exhibit a stronger Sclerotinia head rot resistance than any other breeding line studied in our work so far. These results indicate that somatic hybridization can be successfully used as an efficient alternative method to overcome sexual barriers for gene transfer from wild species to cultivated *H. annuus*.

The relevance of these findings for future breeding research and sunflower cultivation will be discussed.

S202

Transfer of vigor restoration gene from *Helianthus giganteus* into cultivated background and its implication on gene transfer utilizing interspecific amphiploids

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Utilization of the perennial diploids, representing half of the 50 *Helianthus* species, is largely limited by poor crossability and F_1 sterility in interspecific hybrids. Development of a 2-stage embryo rescue technique and a seedling colchicine treatment successfully solved these problems and led to the production of interspecific amphiploids. Seed set of six amphiploids of *H. atrorubens, H. cusickii, H. grossesserratus, H. maximiliani, H. mollis, and H. pumilus* crossed with P21 averaged 3.9%, and that of their backcrosses with cultivated HA89 was 2.7%. These results suggest an adequate supply of progeny for breeding selection. These amphiploids proved to be extremely valuable in our recent transfer of resistance to the new *Orobanche* race F that has appeared in Spain. Amphiploids (2n=68), their BC_1F_1 (2n=51) and BC_2F_1 (2n= 34-51) progenies, after crossing with cultivated line HA89 (2n=34), were evaluated for *Orobanche* resistance. Resistant germplasm lines with 2n=34 were selected and released within two years.

Helianthus giganteus 1934 x HA89, F_1 was colchicine-treated and backcrossed with HA89. A total of 77 seed were obtained from 10 heads of a single cms plant, and all the BC_1F_1 progeny were triploids with 2n=50-51, indicating the success of chromosome doubling and the backcrosses were equivalent to a cross with an amphiploid. The objectives of this study were to evaluate the effectiveness of identifying and transferring a new vigor restoration gene from *H. giganteus* into HA89, to study the inheritance of the vigor restoration gene, and to identify fertility restoration genes in both cultivated and wild sunflowers. Seed set of the triploid BC_1F_1 backcrossed with HA89 averaged 5.2%, and progeny segregated for chromosome numbers from 37 to 46. Seed set of BC_2F_1 increased to 29.5%, which continued to improve when advanced to BC_4F_1. Since HA89 does not contain vigor restoration gene for the vigor reducing perennial species cytoplasm, normal (N) plants with 2n=34 were selected as having the vigor restoration genes from *H. giganteus*, and their BC progeny segregation fit the 1 N to 1 RV (reduced vigor) ratio for the one dominant gene control of vigor restoration. Meanwhile, since our effort of identifying fertility restoration gene for this cms cytoplasm was not successful using cultivated lines, crosses using amphiploid pollen was initiated. Seed set was reduced when compared with their respective crosses when HA89 was used as the pollen source, with seed only from amphploids involving *H. maximilian, H. cusickii, and H. pumilus*. This reciprocal cross difference in seed set was primarily due to the early abortion of hybrid embryos and can be easily overcome with embryo culture. Our results further support the approach of transferring genes from perennial diploid species into cultivated background utilizing interspecific amphiploids, providing embryo culture is used in specific cross combinations.

Molecular and phenotypic characterization of sunflower lines expressing enhanced Sclerotinia resistance

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Sclerotinia sclerotiorum the causal agent of root, stem and head rot is considered the most devastating pathogen of sunflower (*Helianthus annuus*) grown in humid environments world-wide. Therefore, our work focuses on the enhancement of sunflower resistance against this major pathogen.

Sunflower inbred lines, both high- and low-oleic, were screened for their reactions against *Sclerotinia sclerotiorum* by artificial inoculation of the capitulum in three environments in Germany, i. e. Gross-Gerau, Eckartsweier, and Scherzheim. Significant differences in the reaction of lines against *S. sclerotiorum* regarding both lesion length and general head infection were observed and the results of the two assessments proved to be closely related. A line showing the best resistance reaction and one of the highly susceptible lines were used for crossings, to build up mapping populations for QTL-analysis. Meanwhile, several QTL for Sclerotinia resistance of the sunflower capitulum could be detected.

Alternatively, asymmetric somatic hybrid (ASH) plants obtained by PEG-mediated mass fusion of microprotoplasts from *H. maximiliani* and hypocotyl protoplasts of *H. annuus* (Binsfeld P.C. et al. 2000, Theor. Appl. Genet. 101:1250-1258) were tested for Sclerotinia reaction. In this study progenies of ASH between *H. maximiliani*, a wild species that has been shown to be resistant to *S. sclerotiorum*, and *H. annuus* cv. Florom were characterized by AFLP analysis to identify introgressions from *H. maximiliani* into the cultivated sunflower on the molecular level. Wild species-specific fragments as well as fragments not found in either parent were detected. Progenies tended to cluster together according to the original ASH plants in the dendrogram by use of bootstrap procedure. The progenies were evaluated for their reaction to *S. sclerotiorum* using artificial head inoculation of sunflower plants. Some of the progenies showed an enhanced level of resistance compared to resistant inbred lines. Two AFLP-fragments which seem to be linked to *Sclerotinia* resistance were identified (Rönicke S. et al. 2003, Plant Breeding, in press). The superior resistance level of the ASH progenies to the pathogenic fungus *Sclerotinia sclerotiorum* seems to be one of the most important progresses and promises for resistance breeding against *Sclerotinia* in the last years. These results indicate that asymmetric somatic hybridization can be used as an efficient alternative method to overcome sexual barriers for gene flow and the genetic improvement of *H. annuus* by introgression of economical important traits from wild *Helianthus* species.

The interspecific hybridization in sunflower breeding for the economic-value characteristics

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Wild annual and perennial forms of sunflower are widely used in breeding of this culture as reliable sources for many economic-value characteristics. Herewith revealing of new valuable traits and characteristics for selection is an actual problem.

350 samples of the *Helianthus L.*, presenting hybrid and self-pollinated generations F_1, BC_n and F_2, which were obtained as a result of interspecific crossings between cultivated sunflower and annual wild forms, were used in this work. CMS PET-1 type of high-informative lines VB 132, VB 246 and VB 4703 was used as a sterility source.

Study of perennial species revealed practically complete identity of root system in all diploid forms - it is fascicular. The root system in tetraploids usually consists of 5-9 large skeleton roots at taproot absence. Hexaploids differ by long rhizoids - 0.5 - 1.0 m, - and sometimes by tubers.

Oil content in annual wild species is not high and wavers from 25 % to 40%. However it increases easily by means of backcrosses with cultivated forms. *H. debilis* is a good source of linoleic acid (77.6 %) and *H. argophyllus* - of oleic acid (47.5 %).

Tolerance to salinization of soil is observed beside *H. paradoxus* and, probably, it is controlled by one dominant gene. The highest resistance to drought is noted beside *H. argophyllus*, because this species has high - downy silver leaves, the so-called "felted downy" or "tomentose". The results of hybridological analysis have shown, that this characteristic is also controlled by one dominant gene.

Study of phenotypic realization of Rf genes on cytoplasm PET-1 has shown, that all annual wild species are reliable sources of fertility restoration genes. It's also mentioned, that the farther these species stand from cultivated sunflower phylogenetically, the higher is the concentration of Rf genes in their genotype. Particularly, this characteristic of *H. niveus* is controlled by not less than 5 genes.

Such characteristic, as "vegetative period duration", is inherited mainly by intermediate type when crossing of genetically contrast forms. Inheritance goes on female lines mainly under crossing of closely relative species.

The estimation of combining ability has shown, that 87,4 % of original material has a high GCA mark. It's usual for the material, which stands farther from cultivated sunflower phylogenetically. At the same time the material, which stands closer to cultivated sunflower, has the highest level of autogamy.

S2P1

Applications of seed proteins polymorphism to biodiversity studies in sunflower

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Besides oil, sunflower accumulates in seeds significant amounts of reserve proteins constituting up to 40% of the meal composition. The most abundant among them belong to the two major classes of polypeptides, the 11S globulin (called helianthinin, *M*~21-40 kDa), 2S albumins (10-18 kDa) and oleosins (19 kDa and 20 kDa) all differing in size, localization, physicochemical properties, amino acid composition and nutritional quality. Studies on phenotypic variation and inheritance have revealed three Mendelian loci (*Hel*A, *Hel*B, *Hel*C) for helianthinin, and one locus for methionine-rich 2S albumin SFA8 (Anisimova et al.: Proc.15th Int.Sunflower Conf., Toulouse, France, 2000, **2**, M32-M37; Euphytica, 2003, **129**, 99-107).

Molecular studies have been initiated to establish correlations between protein and DNA polymorphisms. Pairs of 20-mer primers have been designed to amplify genomic sequences for helianthinin, 2S albumins, oleosins and lipid transfer protein (LTP) on DNA fractions isolated from 30 sunflower genotypes. The genotypes (inbred lines or individual plants of the same line) differed by the presence of polymorphic polypeptide variants in electrophoretic banding patterns. Primers to SFA8 amplified a single fragment of the same size (800 bp) in all the genotypes independently on allelic constitution of the *SFA8* locus. PCR profiles of products of amplification with two different sets of primers to LTP were polymorphic among the genotypes analyzed that indicated presence of two different alleles in one DNA locus. Monoband electrophoretic patterns were also observed among the amplicons synthesized with primers to 5¢ flanking region of a helianthinin gene and to the complete gene for a 2S albumin protein. Primers to an oleosin amplified multiple fragments in a range of sizes between 550 bp and 2500 bp. Grouping of genotypes depending on presence or absence of PCR fragments was associated with protein electrophoretic profile.

Polymorphism of seed proteins was studied in a group of interspecific hybrid progenies of 20 cross combinations between inbred lines HA232, VIR114, VIR117, VIR129, VIR151, VIR471 and 15 perennial *Helianthus* L. species of various ploidy levels. Most hybrid progenies expressed protein pattern identical to or similar with that of common sunflower line. In the progenies of 13 cross combinations the alterations in expression of the *Hel*C locus were observed. These alterations were visualized already in the F_1 hybrid seeds, i.e. before meiosis, and fixed in F_3-F_8 generations after seed reproduction. Type of alterations (expression of polypeptide variant 11 controlled by the *Hel*C locus and specific for wild annual species or primitive *H.annuus* L., or weakened expression of polypeptide 12) was independent on the wild species used as the male parent but more determined by the inbred line genotype. Heterogeneity by variant polypeptides 11 and 12 was observed in the F_1 and F_2 hybrid seeds of some cross combinations examined. This heterogeneity correlated with segregation by morphological characters. In some cases different lineages derived from the same cross combination were distinguished by the presence of variants 11 or 12.

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S2P2

Wild *Helianthus* species and wild - sunflower hybridization in Argentina

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Sunflower is a very important oil crop in Argentina, at present covering two millions ha with an annual yield of 3,8 millions tons. Two wild *Helianthus* species native to North America were fortuitously introduced, probably more than 50 years ago, and have been naturalized in Argentina, *H. annuus* ssp. *annuus* and *H. petiolaris*. These species grow as adventitious in seven provinces and overlap about 50% of the crop area. Several reports account for extensive hybridization and introgression between wild and cultivated *H. annuus* in the U.S. and to a lesser extend, between cultivated sunflower and *H. petiolaris*, which chromosomes differ in a number of inversions and translocations affecting 10 of their 17 pairs. This situation has important biological and practical consequences, the former including homoploid hybrid species formation, and the latter owing to the possibility of transgene spreading from genetically modified (GM) sunflower cultivars to wild or weedy populations. Wild species distribution of populations was surveyed along 2000 to 2003 in Argentina, and isozyme polymorphisms were studied in 22 *H. petiolaris* and 13 *H. annuus* populations. Gene flow between wild-crop and among wild species seems to take place, for intermediate plants were found in several locations, but mainly wild-crop hybridization is considered in this study. Both wild species showed isozyme variation similar to that found in the origin center. Mean number of alleles, mean polymorphism and heterozigosity, and G_{ST} values were higher for *H. petiolaris*, probably due to its elder introduction and greater widespreading in the country. Three field experiments were then carried out under controlled conditions, attempting to demonstrate gene flow between cultivated sunflower and the wild species.

1. Progeny tests on a number of intermediate plants collected in the wild, based on phenotypic, phenology and reproductive traits. Variability among progeny derived from 32 single, presumed hybrid plants, was compared to variability in wild *H. annuus* and *H. petiolaris* accessions from eight different localities. Segregation of phenotypic traits, intermediate phenology and low fertility levels were found in most of the progenies, accounting for the hybrid origin of their maternal plants.
2. Screening of progenies from *H. petiolaris* populations growing up to 100 m far from sunflower crops. Bulked samples of seeds collected from wild heads exposed to pollen flow from the crop produced hybrid descendents in 10 out of 26 sampled populations. Hybrid plants were recognized by morphological traits and reduced fertility. Overall hybridization was of 1,3%.
3. Hybrid progeny was quantified on wild *H. annuus* plants growing in plots at 3, 100, 300, 500 and 1000 m far from a 500 m^2 central lot of sunflower crop. As morphological characters were not enough to assess hybrid status, achenes obtained in the wild plots were screened for an isozyme marker. The sunflower cultivar was homozygous for an acid phosphatase isozyme allele absent in the wild population. Heterozygous patterns were found up to 500 m plots with a mean frequency of 7% hybridization.

These results confirm that gene flow occurs among crop and wild *Helianthus* species, as it happens in the center of origin. Moreover it concerns to crop management and environmental impact if releasing of GM sunflower cultivars is to be authorized in Argentina.

S2P3

Wild *Helianthus annuus*, a potential source of reduced saturated palmitic and stearic fatty acids in sunflower oil

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The present trend in human diets is to decrease the consumption of the saturated palmitic and stearic fatty acids. Healthy diets restricting not only total fat, but the saturated portion of that fat, would decrease blood serum cholesterol and the risk of coronary heart diseases. Edible vegetable oils are the principal source of fats in many diets. Sunflower oil, which is fifth in production among edible vegetable oils in the world, contains 65 g kg^{-1} saturated palmitic and 45 g kg^{-1} saturated stearic acids. These levels are high compared to rapeseed oil with 40 g kg^{-1} palmitic and 20 g kg^{-1} stearic acids. A reduction of saturated fats in traditional sunflower oil would lead to a healthier edible oil. The objective of this preliminary study was to search the vast genetic diversity available from *Helianthus annuus*, the closest relative of the cultivated sunflower for a potential source of reduced saturated fatty acids, less than 70 g kg^{-1} combined palmitic and stearic fatty acids. Achenes of eighty-six populations of *H. annuus* were collected from the central Great Plains of the USA. Composited 20-achene samples from each population were analyzed for saturated fatty acids using organic base-catalyzed transesterification of fatty acid methyl esters and capillary gas chromatography. The average palmitic acid concentration ranged from 39 to 65 g kg^{-1} for the populations. Average stearic acid concentrations ranged from 19 to 37 g kg^{-1}. Achene oil of one population of wild *H. annuus* from Holmquist, South Dakota, USA had a palmitic acid level that averaged 39 g kg^{-1}, while stearic acid averaged 19 g kg^{-1}. The combined 58 g kg^{-1} palmitic and stearic acids is almost 50% lower than the present level of these fatty acids in sunflower oil. The level of saturated fatty acids observed in the population remained low when plants were grown in the greenhouse under uniform conditions. In the greenhouse, palmitic acid of this population averaged 40 g kg^{-1}, while stearic acid averaged 19 g kg^{-1}. Crossing this population with an inbred cultivated line produced F_1 plants with an achene oil that averaged 39 g kg^{-1} palmitic and 21 g kg^{-1} stearic acid. In comparison, the inbred cultivated parent averaged 61 g kg^{-1} palmitic, and 51 g kg^{-1} stearic acid. F_2 plants produced an achene oil that averaged 45 g kg^{-1} palmitic and 23 g kg^{-1} stearic acid, for a total of 68 g kg^{-1}. When F_1 plants were backcrossed to the cultivated inbred, BC_1F_1 plants produced an achene oil that averaged 45 g kg^{-1} palmitic and 26 g kg^{-1} stearic acid for a total of 71 g kg^{-1}. The inbred cultivated parent averaged 65 g kg^{-1} palmitic and 42 g kg^{-1} stearic acid, for a total of 107 g kg^{-1}. Preliminary information indicates that palmitic and stearic fatty acids in sunflower oil can be reduced by introducing genes from a wild annual species population into cultivated sunflower. Further research will be needed to determine the inheritance of these fatty acids. Other agronomic traits will also have to be monitored during the introgression of the fatty acids.

S2P4

Somatic hybrids between cultivated sunflower and *H. molis* - RAPD and morphological analysis

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Hypocotyl protoplasts of sunflower inbred line Ha-74A and leaf mesophyll protoplasts of *Helianthus molis* were fused using three 30-ms electrical pulses, with the intensity of 1250 Vcm^{-1}. Fusion products were cultured in agarose droplets according to the protocol of Trabace et al (1995). Resulting micro calluses were transferred onto solid regeneration media as described by Henn et al (1998). After several subcultures on modified D and MSE medium (Henn et al 1998), shoot formation was observed. Shoots were then transferred onto MS medium (Murashige and Skoog 1962), where some of them produced plants.

DNA for RAPD analysis was isolated from leaves of both parents and their somatic hybrid, according to the protocol of Gentzbittel et al (1994). RAPD analysis was done using five 10-base primers (Operon Technologies). PCR was carried out in a 25-ml reaction volume as described by Sossey-Alaoui et al (1998). The band patterns obtained by four primers showed bands characteristic for Ha-74A in somatic hybrids. Only in band patterns obtained with primer C15 the presence of bands characteristic for *H. molis* was observed, indicating the presence of a part of its genome in hybrid plant.

Beside the RAPD analysis, morphological comparisons between parents and hybrid were done, as well as some back-crossing.

Acknowledgments

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S2P5

Evaluation of wild sunflower species for tocopherol content and composition

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Wild *Helianthus* species are an important reservoir of useful genes for sunflower breeding. The objective of the present research was to evaluate the existing variability for tocopherol content and composition in a germplasm collection of wild sunflower species. The germplasm evaluated consisted of 262 accessions from 40 *Helianthus* species, including both annual and perennial species. Eight seeds per accession were randomly picked and bulked for tocopherol analysis, conducted by HPLC with fluorescence detection. Tocopherol content averaged 323 mg kg^{-1} seed in the analysed accessions, with an average profile of 99.0% alpha-tocopherol, 0.7% beta-tocopherol, and 0.3% gamma-tocopherol. In cultivated material, an average tocopherol content of 669.1 mg kg^{-1} seed have been reported, made up of 92.4% alpha-tocopherol, 5.6% beta-tocopherol, and 2.0% gamma-tocopherol. The maximum total tocopherol content in wild sunflower germplasm corresponded to an accession of *H. maximilianii*, with 673 mg kg^{-1} seed. Increased levels of beta-tocopherol were identified in one accession of *H. praecox* (11.2% of the total tocopherols) and one accession of *H. debilis* (11.8%). All other accessions contained less than 6.5% beta-tocopherol. Increased gamma-tocopherol levels were identified in one accession of *H. exilis* (7.4%) and two accessions of *H. nutalii* (11.0 and 14.6%, respectively). All other accessions contained less than 2% gamma-tocopherol. Although further research at an intrapopulation level is needed to confirm and isolate variants, the results of the present research indicated that wild *Helianthus* germplasm contains useful variability for tocopherol content and composition.

S2P6

Nuclear and chloroplastic microsatellite markers for introgression analysis in wild sunflower species, *H. argophyllus* and *H. debilis*

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Sunflower is a plant of the American Continent, but now many wild species are spread in different part of the world. Along the southeastern coast of Africa, two sunflower species, *H. argophyllus* and *H. debilis*, possibly both of Texas origin, grow far apart. However in two sites on sandy soil they grow together and many plants shared morphological traits typical of the two species (Olivieri *et al.*,1999). A previous study, carried out by AFLP markers and morphological observations, demonstrated a introgression of *H.debilis* germplasm into *H.argophyllus*. We hypothesise that seeds of the two species were accidentally introduced in Africa by man from the area of origin of sunflowers.

To obtain further evidences of this introgression process and to investigate the origin of these populations, we compared African populations with a set of American populations of the two species from Texas, using 20 nuclear *H. annuus* SSRs (Paniego *et al.*, 2002). To facilitated the study on interpopulational gene flow and introgressive hybridization events uniparental inherited markers were also considered and ten universal chloroplast SSRs (Weising and Gardner, 1999) were tested. The analyses were carried out on 68 and 137 plants of *H. argophyllus* sampled in Texas and Africa, respectively; 17 and 36 plants of *H. debilis* collected in Texas and Africa, respectively, and 26 hybrids isolated in Inhambane area, Mozambique. Six chloroplast SSRs and 13 nuclear SSRs worked, and 6 and 4, respectively, were polymorphic.

A preliminary test on one cpSSR locus shows a putative bottleneck in the African population of *H. argophyllus*.

The two species and the hybrids share the same alleles at all cpSSR loci, indicating a very recent divergence and/or a sustained gene flow. Nuclear loci show a high level of variability. In some cases allele size range of African populations of the two species are more similar to each other than to their respective American counterparts, also suggesting a high level of gene flow. To ascertain the introgression process, genetic and morphological data need to be directly compared in natural populations and controlled crosses. For this purpose interspecific bi-parental crosses are in progress between *H.argophyllus* and *H. debilis*.

Genetic identification of tocopherol mutations and a suppressor for high oleic mutation in sunflower

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Two new inbred lines T589 (high b- tocopherol content) and T2100 (high g- tocopherol content) recently developed in CSIC, Cordoba, Spain have been crossed to known *tph1* and *tph2* mutations obtained in VNIIMK, Krasnodar, Russia to make an allelic test. The data on genetic identification of the tocopherol mutations with corresponding TLC profiles will be presented.

Another international program includes research of a suppressor for high oleic mutation with recombinant inbred lines (RILs) developed in INRA, Montpellier, France. The results on high oleic acid character inheritance in a special set of crosses carried out in VNIIMK will help us to clarify the incomplete penetrance of *Ol* mutation influenced by a genotypic factor of reversion.

S302

Temperature and oxygen regulation of oleate desaturation in sunflower and safflower seeds

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The environmental temperature during embryo development modifies the linoleate content in oilseed oils, indicating a temperature regulation of the oleate desaturation and, particularly, of the microsomal oleate desaturase (FAD2), the enzyme responsible for the desaturation of oleic into linoleic acid. In sunflower seeds the linoleate content is greatly dependent on growth temperature, whereas in safflower seeds it is higher and much less temperature dependent. We have compared the temperature and oxygen regulation of the FAD2 activity in seeds of both plants.

The thermal stability of the FAD2 enzyme, both in vivo (peeled seeds) and in vitro (isolated microsomes), was higher in safflower than in sunflower. Notably, while hull removing provoked a strong increase of the FAD2 activity in sunflower seeds, there was no effect in safflower as it was equally high both in intact and dehulled seeds. Reducing oxygen concentrations in vivo brought about a concomitant reversible decrease of the FAD2 activity both in sunflower and safflower seeds. However, while anoxia gave rise to a rapid decrease of FAD2 activity in the seeds of both species, it was followed by a long-term increase only in sunflower seeds.

These data suggest that temperature regulates the FAD2 activity in oilseeds by two different and independent mechanisms, i) a direct non-reversible effect, mainly related to the thermal stability of the enzyme and ii) an indirect effect by which temperature determines the availability of oxygen, which, in turn, regulates the reversible modification of the enzyme activity level. The results indicate that sunflower and safflower represents two models for the temperature and oxygen regulation of oleate desaturation in oilseeds, and explain the differences in the seed linoleate content between both species.

S303

Identification of fatty acid biosynthesis precursors in developing sunflower *(Helianthus annuus L.)* seeds.

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In plants, de novo fatty acid biosynthesis occurs in the plastids. The ability of plastids to utilize exogenous substrates to support their biosynthetic and catabolic pathways is dependent on the activities of plastidial enzymes and, more importantly, on the presence of specific transporters on the plastid envelope. Previous studies have shown that a range of substrates is used for fatty acid biosynthesis by plastids isolated from different plant species. These substrates include glucose 6-phosphate, phosphoenolpyruvate, malate, pyruvate and acetate, and their relative rates of utilization depend upon the plant species, the organ studied and also development. To date nothing is known about which substrates can be utilized by plastids isolated from developing sunflower seeds. Previous studies have shown that both the rate of synthesis of fatty acyl precursors and the rate of oil synthesis itself can contribute to the control of carbon flux into oil. Understanding how the import of metabolites for fatty acid biosynthesis into plastid occurs is one step that can inform a biotechnological approach towards controlling the storage oil quantity in seeds.

In this study of plastids isolated from developing sunflower seeds we (i) describe the metabolism of a range of substrates towards three plastid sinks: starch synthesis, the oxidative pentose phosphate pathway (OPPP) and fatty acid synthesis and (ii) provide evidence for the presence of specific carbon transporters on the plastid envelope.

S304

Diversified composition of sunflower *(Helianthus annuus L.)* seeds within cultural practices and genotypes (hybrids and populations)

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Environmental factors, mainly temperature and water availability, play a major role with genotype factor in oil quality of sunflower. To manage seed composition through cultural practices and genotype choice under scarce water resources is one way to provide an added-value to crop yield and to diversify uses for edible oil or for non food applications.

The study of interactions between crop management and genotypes for yield and also for major seed components (oil, protein and fatty acids) is conducted in the Rotation-Quality device in Toulouse-Auzeville (France) during three experimental years.

Two standard populations and four hybrids (two oleic and two standard) well adapted to cropping systems in Morocco and France respectively, are investigated in conventional and late sowing dates associated with non-irrigated and irrigated treatments.

For all genotypes, delay in sowing improves oil, protein contents and seed weight but decreases yield stronger for populations compared to hybrids.

Globally, in irrigated crop, seed protein content decreases and oil content is also depressed in the one case of populations cv.

Oleic and linoleic acids contents in hybrid seeds are sensitive to sowing date compared to populations cv. contents which are more stable. Moreover, oleic acid content in standard hybrid seeds increases with a concomitant reduction of linoleic acid in late sowing date compared to oleic hybrids. By irrigation management, these acids contents are not affected whatever the genotype.

Except for standard hybrids, the variation of palmitic acid content is non significant. However, under late sowing stearic acid content is lowered in oleic hybrids and populations cv. This behaviour is enhanced in irrigated conditions.

Close negative correlation between oleic and linoleic, and between oleic and palmitic acids are pointed out in all genotypes and treatments.

The study suggests that it exists different genotype behaviours for fatty acids elaboration and composition in response to crop management.

This work is a part of a PRAD project in co-operation with the INRA team of Meknès in Morocco.

Key words: sunflower, genotype, fatty acids, oil quality, water regime, sowing date.

S305

Unusual monoenes in common oil crops: characterization of a high palmitoleic sunflower mutant.

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Palmitoleic acid ($16:1^{\Delta 9}$) is found in minor amounts in most vegetable oils, being abundant in the oils of some tropical species like macadamias or cat's claw. The production of this unusual fatty acid in oil crops is of a clear interest for industry provided it could be used a s a feedstock for the fabrication of biolubricants and nylon polymers. A newer sunflower line cumulating up to 20 % of palmitoleic acid and derivatives (palmitolinoleic acid due to the oleate desaturase activity over palmitoleic acid and asclepic acid produced through elongation by the FASII complex) have been developed by techniques of breeding and mutagenesis. In the present work a comparative biochemical study of the biosynthesis of this fatty acid was carried out among different sunflower lines. These studies implied determination of the activities of the enzymes involved in the lasts steps of the fatty acid biosynthesis: fatty acid synthase II, acyl-ACP thioesterases and stearoyl-ACP desaturase and a study of the arrangement of this fatty acid in the glycerol backbone of triacylglycerides. On the basis of these results future perspectives for the overproduction of n-7 monoenes in sunflower were discussed.

S306

Original material of sunflower for breeding on the characteristics of seeds, oil and protein quality in the conditions of central-Chernozem region

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When creating the original material for breeding on quality of the seeds, oil and protein, there were analysed some of the most significant correlations, which were obtained at research of the collections of constant lines VIS, VIR, VNIIMK, Kazakhstan, Ukraine, USA, Canada, France and Bulgaria breeding. It's also mentioned, that correlation coefficients were calculated on average means of characteristics (N > 20) and characterize accordance of their variation in genotypic variability of the cultivated sunflower.

The results that we received have shown that the achene structure changes weakly (coefficient of oil content variation is 5,6%, protein - 6,5%, husk - 11,4%), but there are rather big correlations between it and other factors. Increase of the achene oil occurs parallel with reduction of the protein content (r=-0,55) and huskness degree (r=-0,43). Obviously, there is a feedback connection between oil and protein content in achene, but the dependence of the achene content and of huskness degree is the result of selection. Oil content correlates respectively with the same traits, but a little weaker. Moreover, it depends on shoot growing speed (r=+0,26). The head size correlates positively with the achene yield (r=+0,32), oil yield (r=+0,32) and protein yield (r=+0,22). Tall and quickly growing samples are more productive because the correlation is from +0,22 to +0,39. They have higher oil content (r=+0,25).

The general analysis of the variability character and correlation structures of these characteristics allows to separate them in the following groups:

- Oil percentage in the achene, husk percent in the achenes, length of the vegetation period and height of the plants - these are the "elementary", strongly genotypicaly determined characteristics. The level of relation between oil content and huskness, as well as the plant height and vegetation period is determined in hybrid offspring mainly, by means of parental forms particularities. Herewith the average level of the characteristic itself, as well as the degree of consensus of its changes with the changes of other traits depends on environmental factors, influencing upon its realization.
- Yield and oil contents per hectare are the complex characteristics, derived and comparatively determined, with a very variable system of the dependences. Particularly, it is important to note high variability of the system of harvest correlations under more or less stable average level of its general determinancy and variation.

Development of sunflower germplasm with high delta-tocopherol content

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Tocopherols are the main compounds with antioxidant activity in oilseeds. Sunflower seeds contain predominantly alpha-tocopherol, which accounts for more than 90% of the total tocopherols in the seeds. This tocopherol derivative possesses a maximum vitamin E or *in vivo* antioxidant activity, but it exerts a minimum *in vitro* protective action in oils and food containing them. Other antioxidant derivatives such as beta-, gamma-, and delta-tocopherol are more powerful antioxidants than alpha-tocopherol. Accordingly, a partial replacement of alpha-tocopherol in sunflower seeds is an important breeding objective. So far, variants with high gamma tocopherol content (>85%), medium beta-tocopherol content (30 to 50%), and medium delta-tocopherol content (<25%) have been developed. The objective of the present research was to develop sunflower germplasm with novel tocopherol profiles. Seed of four 'Peredovik' accessions of different origins from our germplasm collection were used for chemical mutagenesis with EMS. Single-seed screening in the M_2 generation resulted in the identification of an M^2 seed with 19% gamma-tocopherol. M_3 seeds exhibited wide continuous segregation for gamma-tocopherol content, from zero to about 85% gamma-tocopherol. Such a wide segregation has not been observed in previously developed high gamma-tocopherol germplasm, which segregate for low and high but not for intermediate levels of gamma-tocopherol. Selection for high gamma-tocopherol content within this mutant produced an $M_{4:5}$ line with stable high concentration of gamma-tocopherol, above 95% of the total tocopherols. The line was designated IAST-1. Crosses between IAST-1 and the line T589, with medium beta-tocopherol content, produced segregants with increased levels of up to 68% delta-tocopherol.

S3P1

Evaluation of ornamental sunflower cultivars for disease resistance and oil characteristics

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Commercial ornamental sunflower (*Helianthus annuus*) cultivars and USDA sunflower accessions with ornamental characteristics were evaluated for resistance to several diseases and for fatty acid composition to determine if ornamental types might serve as an untapped source of genes for oilseed improvement. All eighty commercial cultivars were susceptible in greenhouse tests to the most prevalent races (300, 700 and 733) of downy mildew (*Plasmopara halstedii*), with only three cultivars displaying resistance to race 100, the least virulent race. When tested with multiple races of rust (*Puccinia helianthi*), three commercial cultivars were uniformly resistant to races 700 and 733 in greenhouse trials. Ten of the 51 USDA sunflower accessions with ornamental characteristics (ray petal color, petal number) had resistance to one or more rust or downy mildew races, but all accessions were segregating for resistance. In field tests in South Africa, one USDA ornamental accession was immune to white rust (*Albugo tragopogonis*) out of 1100 Plant Introductions tested. None of the ornamental-type cultivars showed any usable level of resistance to Sclerotinia stalk rot or Phomopsis stem canker. Twenty-seven of the commercial cultivars and 51USDA Plant Introductions were also analyzed for fatty acid composition, with most of the commercial cultivars having been grown in California, and the USDA accessions grown in Ames, IA. The total saturated fatty acid content of the entries ranged from 8.2% in PI 507906 to 18.7% in the commercial cultivar Lemon Aura, suggesting that there is sufficient genetic diversity among the cultivars to select for high or reduced saturated fatty acid content. In summary, commercial ornamental cultivars do not possess significant resistance to diseases such as rust, downy mildew, Sclerotinia wilt and Phomopsis stem canker, and are unlikely to help improve oilseed germplasm. However, the ornamentals should be considered as potential sources of germplasm for alteration of saturated fatty acid composition.

S3P2

The PERVENETS high oleic mutation: structure and functioning

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The Pervenets mutation raises the oleic acid content (OAC) (LO) from about 25 % (linoleic sunflower) to over 75 % (HO sunflower) in sunflower seed oil whatever the genetic backgrounds of the genotypes: fixed mutant lines, hybrid between fixed mutant lines and surprisingly both direction cross hybrids between high oleic x linoleic and linoleic x high oleic lines. In a series of sunflower genotypes developing embryos between 12 to 24 days after pollination without and carrying the Pervenets mutation were used to compare oleate-desaturase transcript accumulation. This, revealed the presence and absence of the transcript, in normal and Pervenets embryos, respectively, whereas stearate-desaturase transcript accumulation was equivalent in both types. In comparison to the linoleic sunflower, those carrying Pervenets mutation display besides a common 5.8kb *Eco*RI a RFLP due to an extra 8 kb *Eco*RI fragment and the shift from 8kb to 16kb of a *Hind*III fragment, revealed with an oleate-desaturase cDNA as a probe. The common 5.8kb *Eco*RI fragment to Pervenets and normal sunflower carries an oleate-desaturase gene and the extra *Eco*RI fragment was found adjacent to it.

The insertion unique to the sunflower carrying Pervenets mutation carries oleate-desaturase sequences as expected. Part of coding sequences and intron were present. This enables a PCR diagnostic of the insertion specific to genotypes carrying the Pervenets mutation.

However, we pointed out previously that the presence of the mutation is not sufficient to induce the HO phenotype in the presence of suppressor of the mutation "supole". The supole allele is revealed in half of the HO RI lines, and consequently its accurate mapping is difficult.

Functioning of the mutation was modelled since duplication of oleate-desaturase sequences, the lack of oleate-desaturase transcript, and the dominance of the mutation over the linoleic genotype suggest that the extra copy makes silent the normal gene.

These findings explain already reported features of this mutation in different genetic backgrounds and most of the abnormal behaviour of the high oleic acid trait faced by breeders.

S3P3

Generation of hypocholesterolemic peptides by hydrolysis of sunflower proteins with pepsin

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Sunflower protein isolates were hydrolyzed *in vitro* with pepsin during 150 minutes to study the possible production of hypocholesterolemic peptides during sunflower protein digestion in the organism. Cholesterol is absorbed mainly in the duodenum in the form of micelles made of bile salts. It has been proposed that peptides may compete with cholesterol for the micelles reducing micellar solubility of cholesterol and therefore cholesterol absorption. Several sunflower protein hydrolysates obtained with pepsin significatively decreased micellar cholesterol content. These results suggest that hypocholesterolemic peptides may be readily generated *in vivo* by digestion of sunflower seed proteins. These results may increase the functional value and application of sunflower proteins.

S3P4

Acyl-CoA glycerol 3-phosphate acyltransferase: characterization, solubilisation, and partial purification from developing sunflower *(Helianthus annuus L.)* seeds microsomes.

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The major storage lipids of plant tissues are the triacylglycerols. These molecules are produced by the Kennedy pathway. The formation of triacylglycerols and plant membrane lipids is likely to be controlled by a number of factors including the size and composition of the endogenous pool of acyl-CoAs, the developmental stage of the tissue and the activities and selectivities of key enzymes in the pathway.

Microsomal membrane preparations from the developing seeds of sunflower (*Helianthus annuus* L.) catalyzed the assembly of triacylglycerols from *sn*-glycerol 3-phosphate and acyl-CoA. *Sn*-glycerol-3-phosphate acyltransferase (GPAT) activity associated with the endoplasmic reticulum membranes was studied in a 40.000 g particulate fraction from cotyledons of developing sunflower seeds. The fractions were incubated with [^{14}C]glycerol 3-phosphate and incorporation of radioactivity into Kennedy pathway intermediates studied. Microsomal GPAT activity was characterised: pH optimum, time course, protein dependence, substrate kinetic constants, etc. Tween80 solubilisation (0.75%, w/v) of microsomal GPAT activity was used as an initial step in purification of this enzyme. Ion-exchange chromatography was carried out to partial purification of microsomal GPAT from sunflower seeds.

S3P5

Half-seed analysis for comparing linoleic acid synthesis between high & low oleic acid sunflower inbred lines

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During the first phase of growth after seeding, storage lipids, mainly triacylglycerols (TAG) by the action of desaturases such as linoleic acid desaturase which are present in the green tissues of cotyledon in sunflower, are catabolized and new synthesis of polar lipids (PL), like linolenic acid takes place. In this study half-seed analysis, was used to determine how fatty acid composition would change during the first stage of the cotyledon development in controlled conditions in high oleic and low oleic sunflower lines.

From two inbred lines, HA89 and R978, low oleic acid and high oleic acid respectively, ten seeds of external regions of five capitula for each line, were sampled. Then seeds were cut horizontally in two parts: the portion containing the embryo was planted for germination in MS solid medium and the fatty acid profile of cotyledon at 7-10-13-16 days after seeding (DAS) was detected by gas chromatography, the other part was analysed for identifying the fatty acid composition of seed.

Data were subjected to analysis of variance and it was concluded that the increase of linolenic acid in the cotyledon in both lines was significant. Linolenic acid synthesis under controlled conditions in both lines was similar, but significant increasing of that acid in normal line was higher than in mutant line, moreover positive and negative correlation between linolenic acid with palmitic acid and oleic acid respectively, were observed.

Key words: Linolenic acid, Half-seed analysis, Desaturase, Sunflower.

S3P6

Initial characterization of the glycolytic pathway in developing sunflower *(Helianthus annuus L.)* seeds.

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During oil deposition in developing oilseeds, photosynthate is imported in the form of carbohydrates, mainly sucrose, into the embryo and converted to triacylglycerols. However, the conversion of carbohydrates provided by photosynthesis into precursors of fatty acid biosynthesis in developing oilseeds is poorly understood.

In *Arabidopsis*, low-seed-oil mutant, wrinkled1, presents a reduction on several glycolytic enzymes, in particular hexokinase and pyrophosphate-dependent phosphofructokinase, suggesting that the primary metabolic defect is in the conversion of carbohydrates into precursors of fatty acid biosynthesis.

To establish the importance of the different glycolytic activities on the sunflower seed total oil content we are characterizing this pathway in developing sunflower seeds. The apparent kinetic parameters from the following enzymes have been determined: invertase (EC 3.2.1.26) hexokinase (EC 2.7.1.1), phosphoglucoisomerase (EC 5.3.1.9), fructokinase (EC 2.7.1.4), ATP-dependent 6-phosphofructokinase (EC 2.7.1.11), pyrophosphatedependent 6-phosphofructokinase (EC 2.7.1.90), fructose-1,6-bisphosphate aldolase (EC 4.1.2.13), triose phosphate isomerase (EC 5.3.1.1), glyceraldehyde-3-P dehydrogenase (EC 1.2.1.12), phosphoglycerate kinase (EC 2.7.2.3), phosphoglycerate mutase (EC 5.4.2.1), enolase (EC 4.2.1.11), and pyruvate kinase (EC 2.7.1.40).

S401

In vitro experiments for the resistance of sunflower to broomrape biotypes of different geographical origin

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Five *Orobanche* sp. populations were studied, one from Turkey, one from Spain, one from Yugoslavia and two from Romania (Călărasi and Constanta).

For the characterization of the virulence of these broomrape populations were used two sunflower genotypes, belonging to the host differential screening: one inbred line with Or5 gene and an other one, with Or6 gene. The experiments were developed *in vivo* and *in vitro* conditions, *In vitro* experiment has used the Hidroponic co-culture of sunflower and broomrape.

Orobanche sp. seeds were surface sterilized for 5 min in sodium hypoclorite (3,61%) and rinsed five times with distilled water. They were preconditioned at 21°C for a week on glass fibre filtre paper moistened with 5 ml sterile distilled water in a Petri dish. *Helianthus* seeds were surface sterilized in the same way, before being sown in vials containing balls of glass (2 mm diameter) moistened with sterile distilled water. Seedlings were transferred to Petri dishes, 1 week later, the roots being covered with a piece of glass fibre filter paper and 1 cm thick layer of rook wool. The bottom of this system was soaked in a sterile solution of Coïc neutrophyle nutrient solution and the whole was covered with aluminium foil and maintained at 25°C (300 µmol m-2 s-1 PAR under a 16 h photoperiod).

Preconditioned broomrape seeds were stimulated for germination with GR24, after that being placed on the roots of 10 days old, sunflower seedlings. After infestation, broomrape development was observed weekly, under a binocular microscope. Was noted the presence of necrotic broomrape and their number. Interactions between broomrape and sunflower genotypes have bean investigated on haustoria collected on the sunflower genotypes.

The results obtained were given by the mean values of the fixation of *Orobanche* germination on host root (stage 1), formation of *Orobanche* tubercle (stage 2), tubercle with adventive roots (stage 3) and *Orobanche* with stem (stage 4).

Twenty one days after infestation, only 1 attachment (at stage 1) of broomrape was observed per sunflower plant of the differential for the race 6 (race F). This result is valid for the populations of broomrape coming from Turkey and Constanta Romania. Number of the attachments is biger for the broomrape population from Spain, in this case having and the formations described for the stage 2 and stage 3, but these are in a small number. The behaviour of the differential for the race 5 (race E) shows that there are some differences, even between the races of broomrape in Turkey and Constanta (Romania). The race from Constanta - Romania is different of the race in Calarasi - Romania.

The races in Yugoslavia are totally different of races in Spain, Romania and Turkey (less virulent than those). Nectrotic fixations were observed in all cases, mainly at stage 1 and 2.

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S402

The results of immunological estimation of the original and breeding material of sunflower VIS

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The majority of sunflower phytopathogens are fungi imperfecti, i.e. facultative parasites or semi-saprophytes - white and grey rots, phomopsis, fusarial wilt and other ones. So they infect everything contractly, usually without clear varietal differentiation on resistance-susceptibility, as this is observed at obligate parasites - rust, downy mildew, broomrape. In the first case resistance is horizontal, polygenic, i.e. quantitative, and it's better to speak of tolerance. In the second - resistance is vertical, it's defined by a small number of the main genes and is a qualitative trait.

Original and breeding material of sunflower for immunological estimation was presented with varieties, with female and male lines, different sources of CMS and Rf-genes, annual and perennial wild species, intra- and interspecific hybrids of VIS breeding, collectional nurseries INRA (France) and other institutions and countries. During 1999-2002 years more than 3 thousand samples were estimated. The material on downy mildew, sclerotinia, phomopsis and broomrape was studied under artificial inoculation in laboratory and field conditions. There were used the common methods of the inoculation and estimation.

The results have shown that 15 samples were immune to downy mildew: lines RHA 373, RHA 397, RHA 398, VIR 655, VIS 2002 and other ones. The breeding material of VIS is generally resistant to *Plasmopara halstedii*.

On the background on broomrape the mixture of the races C and D was used. 12 samples from the female lines were not infected by *Orobanche cumana*: VB 2, VB 110, VKU 61, VKU 102 and others. Male line VB 27 V was also immune. Generally breeding material of VIS is resistant to this plant-parasite.

At the same time the majority of samples of the original and breeding material was to considerable extent susceptible to white rot. The usage of some interspecific hybrids, the particularities of head structure, erect location of leaves and pear-shaped radical root collar of stem provides the creation of varieties and hybrids of sunflower, tolerant to *Sclerotinia sclerotiorum*.

INRA lines, received by VIS on GRESO-program, were infected by phomopsis within 10 - 40 %. Amongst this material the sufficient number of immune and tolerant to *Diaporthe helianthi* forms is selected.

Female lines VB 2, VB 171, VIR 130, NA 371, male lines VB 166, VB 1617, VB 1618 and the other 11 lines showed complex resistance or tolerance on all four backgrounds of infection. Among the CMS-forms of no-PET-1 type lines BC_3(VB 1002 x DCS 1), BC_1(VB 1002 x RIG 1) and BC (VB 1002 x ARG 3) had complex resistance. There were also chosen such forms, that are not infected with grey rot, verticilliose, rust, alternarial blight and phomosis in natural conditions.

S403

In vitro and In vivo water stress in sunflower *(Helianthus annuus L.)*

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In this research, response of sunflower cultivars to drought stress under both *in vitro* and *in vivo* conditions was investigated. Murashige and Skoog basal medium supplemented with a range of polyethylene glycol (PEG-1000) concentrations (0, 3, 6, 9, 12 % w/v) was used for *in vitro* drought screening. Results from both *in vitro* and *in vitro* showed that plant growth decreased with increasing PEG concentrations. In addition, there were differences between the cultivars in terms of their response to drought. According to relative tolerance, the most affected traits *in vitro* were shoot length and shoot fresh weight whereas, *in vivo*, shoot and root weight was the most affected characters.

The analyses of the characters measured in this study indicated clearly that, drought has a highly significant and generally detrimental effect on sunflower cultivars grown under both *in vitro* and *in vivo* conditions. *In vitro* experiment showed that PEG could be successfully used as a drought simulator. In addition, the significant correlations between *in vitro* (except number of roots) and *in vivo* characters indicate that an *in vitro* approach could be useful in screening and selection for responses to drought prior to field trial. As a result, all *in vitro* characters measured (except number of roots) could give clues for performance of sunflower genotypes against drought *in vivo*.

S4P1

Sunflower black stem: determinism of host tolerance to disease

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Phoma macdonaldii (*Leptosphaeria lindquistii*) is responsible for the black stem disease of sunflower which is preponderant in European countries, Americas and Australia. Black stem disease induces decreased seed oil content, decreased thousand seed weight and 10 to 30 % total yield loss reaching 70% if the pathogen leads to early plant senescence. Chemical control exists, but application of the fungicides is limited to early stages of development, its efficiency is incomplete, and it is ecologically unfriendly.

So far, no resistant genotype to this disease has been identified. Tolerant genotypes exist and their tolerance was shown to be highly variable.

The aim of our work is to unravel the genetic mechanisms which underlie this tolerance. Our research is concentrated on the plant defence systems, following an *a priori* approach. We developed three main axes:

1. an ultrastructural investigation of sensitive and tolerant genotypes: for example, it was previously shown that cuticle or cellular wall can be considered as a physical barrier able to repel the invader or to limit its progression in the plant tissues;
2. an expression investigation of characterised genes : we made a comparison of PAL, chitinase and glucanase transcript accumulation in compatible interactions between *Helianthus annuus* and *Phoma macdonaldii* using tolerant and sensitive sunflower with an aggressive race of *P.macdonalldii*;
3. a physiological investigation : scopoletine, a sunflower phytoalexin was tested on germination and growth of *Phoma*. Quantification of this compound was realised in planta;

This work will help us to set up a "without *a priori*" approach and will hopefully allow to identify genes or molecular markers leading to the selection of highly tolerant genotypes.

S4P2

The response of five Romanian sunflower genotypes *(Helianthus annuus)* to hydric stress

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Understanding the mechanisms involved in the response of sunflower to hydric stress is a prerequisite for choosing a suitable genotype in a given environment. Present study was carried out to identify morphological and physiological parameters that might be involved in adaptation of sunflower to drought conditions.

Five Romanian sunflower hybrids were submitted, under greenhouse conditions, to two watering regimes: 70 % from TSWC (total soil water capacity)] and 40 % from TSWC.

The results showed that hydric stress significantly reduced leaf area, shoot size, biomass accumulation, root volume, chlorophyll content and increased the peroxidase content. Leaf area was more affected (57.6 % for Justin), followed by the height of plants (64.2% for Justin). Biomass accumulation and root volume were also affected by hydric stress. Thus, under hydric stress conditions increased the root/shoot ratio for young plants. This suggested that the main sink is the survive. In late stage of vegetation the root/shoot ratio decrease under drought stress in some hybrids but increased in the other hybrids, this suggesting that for mature plants the main sink is the yield.

Chlorophyll content declined under stress conditions except for Favorit and Justin. At the opposite the peroxidase content of stressed plants increased and some modification in isoenzime pattern were registered.

Under stress conditions, all genotypes accumulated more linoleic acid, in addition with the reduction in oleic acid concentration.

Root/shoot ratio and yield were positively correlated ($r = 0.95^{***}$), root/soot ratio and diameter of head were negatively correlated ($r = -0.58^{*}$) and weren't correlation between drought susceptibility index (SI) and yield ($r = 0.42$).

It was concluded that leaf area, biomass, plant height or root volume could be a suitable criteria for screening plants for drought tolerance.

The lack of correlation between drought susceptibility index and yield may be combined in improved sunflower cultivars.

Key words: sunflower, *Helianthus annuus L.*, hydric stress, physiological parameters, fatty acid composition.

S4P3

Genetic variability in some Romanian sunflower genotypes under in vitro stress induced by *Phomopsis helianthi* filtrate

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During 2002-2003 at ARDI Fundulea, a lot of experiments for *in vitro* testing and selection of some Romanian sunflower genotypes with tolerance to *Phomopsis helianthi* have been performed. 14 out of the 30 tested genotypes were selected for their good pretability to the *in vitro* culture. As follows of the treatment applied on MS culture medium supplemented with 150 ml/l filtrate and on the basis of the results obtained regarding the leaf index, chlorophyll content, TKW, seed oil percentage and its composition determination, seven genotypes with increased resistance vs. this pathogen have been selected. The determinations have been performed by the Minolta Chlorophyll meter (SPAD units) for chlorophyll content, RMN method for oil content and gas-chromatography method (Shimadzu-GC-14B) for fatty acid content from oil.

ANOVA for the leaf index emphasized a very different behaviour of the tested lines, with positive or negative differences between genotypes, statistically ensured depending on both disease tolerance and the degree of genotypes to be suited to the *in vitro* culture. Eight genotypes in which the leaf area was not diminished by the treatment as compared with control, have been identified.

As regards the chlorophyll content, it ascertained that for all tested genotypes, at the treatment variant, the average/variant was diminished with 5.2 SPAD units vs. the control.

In variant treated with filtrate, TKW was drastically diminished, statistically ensured at six genotypes and the oil content strongly diminished at seven out of the 14 genotypes. The oleic acid content was more decreased in comparison with the control in all lines excepting the LC 4010 line.

These seven lines were included, from this year, as part of Sunflower breeding Programme for the resistance to the diseases with to obtain new cultivars with increased resistance to *Phomopsis helianthi*.

Key words: *Sunflower, Phomopsis helianthi, in vitro stress, physiological indices.*

S501

Transcription factors involved in gene regulation during embryo desiccation in sunflower: a new tool for improving seed quality and stress-tolerance?

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Our lab is interested in characterizing the factors that are specifically involved in the developmental induction of sHSP (small Heat Shock Protein) genes during zygotic embryogenesis. We recently identified one such factor in sunflower: HaHSFA9 (1). We report that HaHSFA9, functionally, and physically interacts with a second factor: HaDREB2. HaDREB2 belongs to the AP2 (Apetala2-like) family. We cloned HaDREB2 by one-hybrid interaction in yeast. As bait, we used defined *cis*-elements that were necessary for the transcriptional activation, of the *Hahsp17.6G1* promoter, during embryo desiccation in transgenic tobacco. The two factors synergistically *trans*-activated this promoter in sunflower embryos. The transient activation by HaHSFA9 and HaDREB2 did not necessarily require the presence of the DNA-binding sites for HaDREB2, thus indicating that the two factors could physically interact with each other. This was supported by two-hybrid assays, in yeast, and by *in vitro* GST pull-down. We have mapped protein domains involved in the physical and functional interactions.

We conclude that HaDREB2 would mediate the developmental activation of sHSP genes during embryo desiccation in sunflower. HaDREB2 would function trough two non-excluding mechanisms: 1. - Direct (protein-DNA) interactions with *cis*-elements similar to those present in *Hahsp17.6G1*. 2. -Indirect (protein-protein) contacts through HaHSFA9. HaDREB2 could thus function as an HSF-specific activating co-regulator during embryo desiccation. The mRNA accumulation patterns, of HaDREB2 and HaHSFA9, in sunflower do not exclude additional functions connected with water stress response in vegetative organs.

In addition, we will present recent results that indicate the contribution of additional transcription factors to the developmental regulation of sHSPs. We will discuss a predominant role for HaHSFA9, and how we are exploring the potential uses of HaHSFA9 for improving seed-quality and stress-tolerance in transgenic tobacco.

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S502

Genomics tools and resources for domesticated and wild sunflowers

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Our laboratory has been developing genomics tools and resources for cultivated sunflower (*Helianthus annuus* L.) through various independent and collaborative research programs. The most significant of the latter has been the Compositae Genome Program (CGP). The CGP has produced 69,188 sunflower and 68,179 lettuce (*Lactuca sativa* L.) expressed sequence tags (ESTs) from cDNA libraries constructed from multiple genotypes and species (*H. annuus*, *H. argophyllus*, *H. paradoxus*, *L. sativa*, and *L. serriola*), tissues and developmental stages, and biotic and abiotic stress treatments. The ESTs can be accessed through GenBank and TIGR and a versatile searchable database developed by the CGP (http://cgpdb.ucdavis.edu). The CGP identified 18,031 unigenes in sunflower, 19,523 unigenes in lettuce, and 2,185 conserved orthologous sequences (COS). The EST database is a powerful tool for gene discovery and has been mined for single nucleotide polymorphisms (SNPs), insertion-deletion polymorphisms (INDELs), and simple sequence repeat (SSRs). One of the goals of our laboratory has been to increase the density and utility of the public genetic linkage map for sunflower by developing and mapping SNP, INDEL, or SSR markers for ESTs and candidate genes. DNA markers have been developed for 941 ESTs or candidate genes so far, and 540 EST or candidate gene loci have been mapped, increasing the number of SNP, INDEL, or SSR marker loci on the public genetic linkage map to 1,250. The cross-taxa utility of DNA markers for resistance gene candidates (RGCs), other candidate genes, and ESTs have been tested by screening several wild *Helianthus* species, safflower (*Carthamus tinctorius*), and cultivated and wild lettuce. Numerous conserved DNA markers have been identified for syntenic and comparative genomic analyses in the Compositae. Various comparative maps have been or are being constructed. Finally, the genomics tools and resources described herein increase the prospects for routinely applying marker-assisted selection in hybrid sunflower breeding programs, greatly facilitate candidate gene and quantitative trait locus (QTL) analyses of biologically or economically important traits in sunflower, and create a vehicle, unbounded by ownership, for advancing the genetics of domesticated and wild sunflowers worldwide.

Map-based cloning strategy for isolating the restorer gene Rf1 of the PET1 cytoplasm in sunflower *(Helianthus annuus L.)*

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Up to now a single cytoplasmic male sterility (CMS) source, PET1, is used for hybrid breeding in sunflower worldwide. Cloning of the restorer gene *Rf1*, responsible for fertility restoration, requires tightly linked markers to the gene of interest. Screening 1200 decamer primers by bulked segregant analyses identified seven RAPD markers mapping on the same linkage group as the restorer gene *Rf1*. In the F_2 population of the cross RHA325 (cms) x HA342 (183 individuals) one of the RAPD markers, OPK13_454, mapped 0.9 cM from *Rf1*, followed by OPY10_740 with 2.2 cM. Bulked segregant analyses using 1024 AFLP primer combinations identified 282 polymorphisms in 203 primer combinations. To integrate additional markers into the map, selected single plants from the bulks and recombinant plants were screened to further reduce the number of primer combinations to be mapped in the F_2-population. The map for the linkage group carrying the restorer gene now consists of 43 markers (7 RAPD-, 1 SSR-, and 35 AFLP-markers) and covers 191.9 cM. E32M36_155, and E44M70_275 were mapped 0.1 cM and 0.7 cM from the restorer gene, respectively. Two of the RAPD markers, OPK13_454 and OPY10_740, were successfully converted into SCAR markers, HRG01 and HRG02, which are now available for marker-assisted selection. Investigating a set of 11 restorer and nine maintainer lines of PET1, the markers OPK13_454/HRG01 and HRG02 were absent in all maintainer lines but present in all restorer lines apart from the high oleic line RHA348 and the dwarf line Gio55 including restorer lines developed from interspecific hybrids. For cloning the restorer gene *Rf1*, colony hybridizations against high density BAC filters of our sunflower BAC library and 3D-PCR pooling strategies were used to identify positive BACs. BAC fingerprinting using different restriction enzymes in combination with hybridizations was performed to develop a contig around the restorer locus *Rf1*.

S504

Gene structures within the sf21-gene family in sunflower *(Helianthus annuus)*

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In plants, the growth of the pollen tubes towards the ovary is influenced by compounds present in the pollen and pistil, and by products synthesized through interactions between the pistil and the growing pollen tubes. The pistil- and pollen-expressed gene *sf21* encoding a 352 amino acid polypeptide was isolated in sunflower by differential screening of a floral cDNA library and characterized. The deduced polypeptide is structurally related to the animal proteins *RTP / Ndrl* and the vertebrate inositol 1,4,5 triphosphate (IP3) receptor. In sunflower pistils, the *Sf21* protein is localized in the nucleus of the stigma cells, and in the cytoplasm and nucleus of the stylar transmitting tissue cells. In an effort to elucidate the possible role of *sf21* in sexual reproduction of sunflower, we initiated the identification of the *sf21* homologous genes and their promoter regions using a sunflower BAC library constructed from the restorer line *RHA325*. Screening of the BAC library with radiolabelled sunflower *sf21* probe identified eight positive clones. Positive BAC clones were digested with *Hind*III und subcloned into *pUC18*. Of these eight clones, seven have already been subcloned, obtaining positive subclones with insert sizes in the range of 1.7 kb to 14.0 kb. We identified the partial structure of *sf21* gene in four positive subclones by sequencing. In order to determine whether the *sf21* genes identified in the BAC clones are expressed and to identify the exon-intron borders we carried out transcript analysis using RT-PCR with RNA from different organs.

Subsequently, promoter deletion constructs using GUS as reporter gene will be produced to identify promoter elements responsible for the tissue-specific expression of the gene(s). Transformations of tobacco with these constructs are planned. GUS tests will evaluate the tissue specificity of each construct. Functional analysis of the *sf21* gene will be performed using RNA interference (RNAi) technology.

Towards the construction of a numeric sunflower genome map with the aid of A. thaliana genome (ICCARE)

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Unravelling the plant genome organisation is necessary to understand its function. Usually, genome complexity increases with its size (duplication events, repeated regions, transposons, multigenic families, etc...). Sunflower (*Helianthus annuus* L.) is considered to be a "big genome" plant: 3 billions bases (equivalent to the size of the human genome) with a physical organisation still unknown.

Over the past few years, model plant genomes were sequenced: *Arabidopsis thaliana*, and rice (*Oryza sativa*). Knowledge acquired could help us to decipher the sunflower genome organisation. With the aim to use the A. thaliana genome sequence as a predictor of sunflower genome organization, we created a web site (/index.htm" http://genopole.toulouse.inra.fr/~cmuller /index.htm) called "ICCARE for PLANTS" for "Interface de Cartographie Comparée pour l'Agronomie et la Recherche sur l'Evolution". Derived and adapted from ICCARE for mammals (Faraut T. ICCARE, a comparative genomic approach for ESTs clustering. (2nd European Conference on Computational Biology), Paris September 2003. Submitting), ICCARE for plants allows us to construct a numeric sunflower genome map by using the similarities and the location informations existing between the *Arabidopsis thaliana* cDNAs and the sunflower ESTs.

The features of ICCARE are: 1. to rapidly analyse a high number of sequences and to display 'on line' informations, 2. to estimate the splicing sites of sunflower EST sequences, making inferences from the *A. thaliana* genome annotation 3. to construct a virtual sunflower genome map and 4. to design primers or oligo probes on conserved regions.

Until now, 56,000 EST sequences are present in the database. We have found about 17,500 EST sequences presenting similarities to *Arabidopsis* cDNA (30% of EST similitude). The *A. thaliana* cDNA presenting similarity with sunflower ESTs represent about 12% of the *Arabidopsis* genes. *A. thaliana* cDNAs have, on average, similarities with 4 to 5 sunflower ESTs, but some of them, like the ferredoxin-NADP+ reductase, could exhibit similarities to 147 sunflower ESTs. The number of sunflower EST similar to Arabidopsis cDNA is equally distributed among the five Arabidopsis chromosomes.

In the order to test the feasibility of using *A. thaliana* information to construct a minimal physical map of sunflower genome, a first set of 20 overgo probes have been designed using sunflower ESTs similar to *A. thaliana* cDNAs located on chromosome 5 (Rubisco and nearby genes). A BAC library (4- to 5-fold genome coverage) had been screened with these probes. Until now, about 400 BAC clones exhibit positive signals. These BAC clones are currently processed in order to identify contigs.

In conclusion, ICCARE has shown to be extremely useful in order to have a global view of the sunflower genome and to select ESTs of interest to design probes or PCR primers.

S5P1

Improved in vitro shoot regeneration, rooting and ex vitro development from proximal mature seed explant of ten sunflower genotypes

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Sunflower recalcitrance to *in vitro* culture is well known, but the progress made in the last years is significant. Numerous methods for *in vitro* sunflower regeneration were established for different genotypes. In spite of this progress sunflower regeneration and efficient transformation are still restricted to few responsive genotypes. The goal of our study was to establish a genotype-independent system for *in vitro* sunflower regeneration of Romanian sunflower genotypes. Ten sunflower genotypes were used: Florom 328, Select, Turbo, Alcazar, Rapid, HS2411, Santiaga, Felix, Coril (hybrids produced by the Research Institute for Cereal and Industrial Crops Fundulea, Romania), and the private French line 47320bcd. As initial explants proximal fragment of mature seed has been used. A great advantage of using seed as explant source avoiding germination step is that juvenile tissue is more responsive to *in vitro* culture. A three stage protocol for *in vitro* regeneration was developed consisting of: (1) culture in the dark, at 28-32^{0}C, for 7-14 days on RMG1 medium; (2) culture in the light until shoot stage on RMG2 medium; (3) rooting of regenerated shoots on media with different pH, activated charcoal or AIB. Well developed plants were successfully transferred *ex vitro* and grown in the laboratory up to mature seeds. The best protocol for *in vitro* efficient rooting and acclimatization was refined for cv. Turbo. The regeneration protocol allowed a very efficient in vitro regeneration for all genotypes tested so far, up to 80% of the initial explants regenerating a large number of shoots (10 up to 40 plantlets per initial explant depending on the genotype). The presence of AIB or activated charcoal in the rooting medium was essential for root development. The efficiency of acclimatization for the shoots with well-developed roots was good (up to 61%) and ex vitro plants did flower and set seeds.

S5P2

Analysis of expressed-sequence-tag databases of sunflower embryos

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In higher plants, embryogenesis is a process of main importance which corresponds to the establishment of the correct embryo pattern and to the accumulation of storage reserves. Thus, the knowledge of the molecular and physiological events of this process represents a major interest for agronomy to improve grains quality and yields.

However, the analysis of the early stages of development is often difficult because the embryo is small and embedded inside the maternal tissue. Moreover, one can speculate that mutants at critical stages of development as embryogenesis would be lethal or severely disorganised, thus the mutant analysis only give rise to incomplete information.

Sunflower (*Helianthus annuus L.*), which presents an inflorescence with numerous and relatively big embryos whose development is synchronized, has been proposed as a complementary model for the study of zygotic embryogenesis in dicotyledonous. Moreover, the study of sunflower embryogenesis is of particular interest as, in this exalbuminous plant, reserves (oil and proteins) are stored directly in the embryo.

In order to analyse the transcriptional program induced during the embryo patterning, reference and subtracted libraries from heart and cotyledonar-shaped embryos have been constructed and a reference library from globular embryos is on the point to be realized. Several hundreds of ESTs from each library have been sequenced and are being annotated. ESTs analysis allowed us to identify the major cell functions implicated at each stage of development and some interesting genes with potential substantial roles in plant and animal embryogenesis were already detected as Argonaute, Shaggy Kinase or Mago Nashi like genes.

Libraries will be used to construct micro-arrays in order to classify cDNA in expression groups during embryo development. This classification will be performed using several controls identified by cDNA-AFLP from all the studied embryogenesis stages. This functional genomic study should allow us to elaborate a putative model for the different molecular and physiological processes involved in the embryo patterning of sunflower.

S5P3

Establishment and transformation of callus cultures of sunflower *(Helianthus annuus L.)* using *Agrobacterium tumefaciens*

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Agrobacterium-mediated gene transfer has been proved to be a suitable method for generating transgenic callus cultures as protein expression systems. Here, we present this technique for the high-oleic sunflower hybrid line proleic-204.

Friable callus cultures were initiated from cotyledons and hypocotyls of the sunflower hybrid line. Formation and growth of callus material was measured on callus induction medium (CIM) containing an auxin:cytokinin ratio of 1:1. Transformation was achieved by cocultivation of calli with *Agrobacterium tumefaciens*. In order to increase the transformation efficiency, the following parameters were modified: different bacterial strains (LBA4404, C58, GV3101, EHA101 and EHA105), bacterial concentration (OD_{600} 0.4, 1.0, and 2.5), co-cultivation media (MS and YEB), virulence inducers (acetosyringone and coniferyl alcohol) and antibiotics (kanamycin and geneticin). Expression of *gus* was detected histochemically and fluorimetrically in calli. Since the bacterial strain GV3101 has been proved to be the most effective one, it was used for all the subsequent cocultivation experiments. The strain harboured the plasmid pBI121, which carried *gus* under the control of the 35S-promoter and the selectable marker *nptII* under control of the NOS-promoter. An OD 1.0 using MS as a cocultivation medium were found to be a compromise between cell vitality and transformation efficiency for both cotyledon and hypocotyl-derived calli. Addition of virulence inducers increased the fluorimetric gus activity 3.6 (acetosyringone) and 2.4 fold (coniferyl alcohol) for cotyledon-derived callus, in compare to calli transformed without the inducer substances. For hypocotyl-derived callus only acetosyringone increased the expression of *gus* 1.7 fold. Thus, acetosyringone turned out to be more effective on both callus types. A preliminary transformation experiment combining all optimized parameters revealed an average of 1 to 2 transgenic lines per g FW of callus. The presence of *gus* in the sunflower calli was confirmed by PCR analysis. The results give rise to establish transgenic callus suspension cultures of sunflower as expression systems for establishing pharmaceutically relevant proteins.

S5P4

Assessment and optimization of parameters enhancing *Agrobacterium*-mediated transformation of high oleic sunflower *(H. annuus L.)* genotypes.

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Agrobacterium-mediated gene transfer is one of the most important techniques used in sunflower genetic engineering experiments. The transformation efficiency was increased by varying parameters for optimizing transformation conditions of two high oleic sunflower (*H. annuus L.*), cv.capella and SWSR2 inbred line .The parameters include four bacterial strains (GV3101, LBA4404, C58, and EHA101), bacterial concentration (OD_{600} 0.5,1.0, 1,5 and 2.0), split and intact shoot apices explants, cocultivation media (MS and YEB), virulence inducers (acetosyringone and coniferyl alconol),and cocultivation duration (2 and 3 days). On the basis of the ß-glucuronidase (*gus*) reporter gene expression and the vitality of the regenerated shoots, the bacterial strain GV3101 was evaluated to be more effective for all the cocultivation experiments in SWSR2 inbred line in contrast to LBA4404, which was more effective in cv.capella. Both strains harbour the binary vector pBI121 and the *gus* reporter gene under the control of the CaMV 35S-promoter and the selectable marker *nptII* (neomycin phosphotransferase II) under the control of NOS-promoter. The best OD_{600} was 1.0 using MS medium with split shoot apices and three days cocultivation . Addition of acetosyringone increased the fluorimetric GUS activity two fold comparing with the treatment without virulence inducers. Molecular analysis was performed to confirm the integration of *gus* gene into sunflower *(H. annuus L.)* genome. Therefore, the investigation of parameters which enhance T-DNA transfer is an important step aiming efficient *Agrobacterium*-mediated transformation of shoot apices of high oleic sunflower genotypes.

S5P5

In vitro floret differentiation in the sunflower capitulum

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In vitro culture has become a useful tool for studies concerning with the assessment of physiological and biochemical factors that influence floral morphogenesis. For the sunflower, *in vitro* techniques starting from different explants yield good results on culture initiation and plant regeneration. Nevertheless, even though inflorescence generation on cultured shoots is sometimes achieved, there are no reports about *in vitro* florets developed from undifferentiated capitula.

The present work describes a protocol for the *in vitro* culture of sunflower reproductive meristems where floral organogenesis can be expressed.

Experiments were carried out using sunflower capitula at floral stage 4-5**(a)**, excised from plants grown in greenhouse conditions. White and Linsmaier and Skoog (LS) culture media were compared. LS medium was chosen for further experiments, based on comparatively large capitula receptacle area expansion, reduction of vitrification and low percentage of calli formation.

The presence of casein hidrolisate in the culture medium was also tested, resulting appropriate in reducing the surgical stress and in the later recovery of the explants. The addition of kinetin to LS medium plus casein hidrolisate proved to induce differentiation of new primordia at the capitulum generative front**(b)**. Explants cultured with 0.1 ppm of kinetin only differentiated mother bract primordia. Explants cultured with 1 ppm of kinetin showed larger expansion of the receptacle area and new floret primordia did not differentiate their mother bract. Intermediate levels of kinetin (0.5 and 0.8 ppm) were tested in addition with two levels of AIA (1.3 and 2.5 ppm), resulting 0.8 ppm kinetin and 2.5 ppm AIA the combination capable of differentiating floret primordia that closely resemble those developed in normal capitula.

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S5P6

Cloning, expression and characterization of a novel thioesterase from developing seeds of sunflower *(Helianthus annuus L.)*

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The substrate specificity of acyl-acyl carrier protein (ACP) thioesterases (EC 3.1.2.14) plays a mayor role in determining which acyl chains are cleaved and released from the acyl-ACP pool, and thus, directly determine free fatty acids available to be exported to extraplastidic compartments. These enzymes terminate acyl-chain elongation during fatty acid biosynthesis by hydrolysing the thioester bond of acyl-ACP. Using different degenerated oligos and 15 DAF sunflower seed cDNA, we isolated, by PCR, fragments that indicate the presence of two thioesterase activities, a FatA-like thioesterase, with high affinity for oleoyl-ACP (18:0-ACP), and a FatB-like enzyme, with preference for long-chain saturated fatty acids. With the aim of elucidating the mechanisms involved in the sunflower fatty acids biosynthesis, a cDNA clone of a novel acyl-ACP thioesterase, was isolated from developing seeds, cloned and sequenced. This cDNA was functionally expressed in *Escherichia coli* as a recombinant protein (6xHIS-tagged), and purificated using Ni^{2+} affinity chromatography matrices. Kinetic parameters for acyl-ACPs of different lengths and unsaturations can provide evidence about the interaction of this enzyme with their substrates. Here, we describe the cloning, expression and characterization of this thioesterase and the alterations that its expression produces in *E.coli* fatty acids profile.

S5P7

Cloning and expression of HMGR and SMT2 c-DNA in sunflower *(Helianthus annuus L.)* immature embryos

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Sterols are isoprenoid-derived lipids that play diverse roles in all eucaryotes. Bulk sterols regulate membrane fluidity and permeability in conjunction with phospholipids. In plants, in addition to their structural role, sterols are precursors of brassinosteroids, known to regulate cell division, differentiation and to modulate reproduction and development. Plant sterols (phytosterols) are synthesised via the mevalonate pathway of the isoprenoid metabolism. They are derived from cycloartenol via a series reactions to produce sitosterol and stigmasterol on one hand, and campesterol on the other. The most abundant sterol in plant cells is sitosterol followed by campesterol, which is the precursor of brassinosteroids. Key enzymes of the sterol biosynthesis pathway are HMGR (3-hydroxy-3-methyl glutaryl coenzyme A reductase) and SMT 1 and 2 (sterol-C-methyltransferase). SMT2 plays a central role in balancing the ratio of sitosterol to campesterol.

Phytosterols, minor constituents of edible oils, are known for their plasma-cholesterol lowering properties and for their hydrating properties of the derma. They are thus valued in the by the pharmaceutical industry. To date, phytosterols are extracted from steam distillates from rapeseed oil. Biodiesel production from vegetable oils is one of the priorities of the European Commission. According to the amendment of Directive 92/81/EEC, the Member States are under the obligation of using 2% of biofuels out of their global consumption as from 2005, to reach at least 5.75% in 2010. Methyl esters incorporated into fuel are derived from lipids of oil seed rape. Considering the objectives of the European Commision, oil seed rape culture exclusively will not be able to meet the demands. Sunflower oil has been proposed to complement oilseed rape.

The major objective of this project is centred on the enrichment of phytosterols in sunflower seeds A pre-requisite to tailoring phytosterol enrichment in sunflower varieties relies on the knowledge of the expression and regulation of key genes controlling phytosterol synthesis. To date, little data is available in sunflower.

We report here on the expression of HMGR and SMT in sunflower leaves and in developing immature embryos by RT-PCR. Using specific oligonucleotides designed in conserved regions of both c-DNA corresponding to different plant species, we could amplify a 500pb c-DNA with SMT primers and three bands of 900,700 and 400pb respectively with HMGR primers. The 500pb c-DNA was cloned, sequenced and found to share 85% homology with *Nicotiana tabacum* SMT2. No HMGR c-DNA was found using this technique. Screening a sunflower EST bank using specific "overgo" probes to HMGR, has allowed us to isolate a 580pb c-DNA, which shows 92% homology to HMGR from *Arthemisa annua*.

These tools will now allow us to view transcriptional profiles in sunflower embryos and make eventual correlations with phytosterol levels. Furthermore, we will attempt to clone the complete c-DNA sequence of both genes and to envisage a transgenic approach in sunflower.

S5P8

Ferredoxin: The forgotten link between photosynthesis and fatty acids desaturation

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The sensitivity of higher plants to chilling is closely correlated with the degree of unsaturation of the fatty acids in the thylakoid membranes of their chloroplasts (Murata et al. 1982). On the other hand, the effects of the low temperature impeding the photosynthesis are known to be related with an irreversible damage to the D1 protein (Yong Moon et al. 1995). This phenomenon is known as low-temperature photoinhibition. From our point of view a deficient desaturation on thylakoid membrane is the consequence of this photoinhibition. As ferredoxin is a common cofactor for chloroplastic fatty acid desaturases, we start studying ferredoxin as the link between the unsaturation of thylakoid membrane lipids and the low-temperature photoinhibition in higher plants. In this work we clone and characterize the ferredoxin from sunflower (*Helianthus annuus* L.).

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