

CULTIVOS  
HERBACEOS

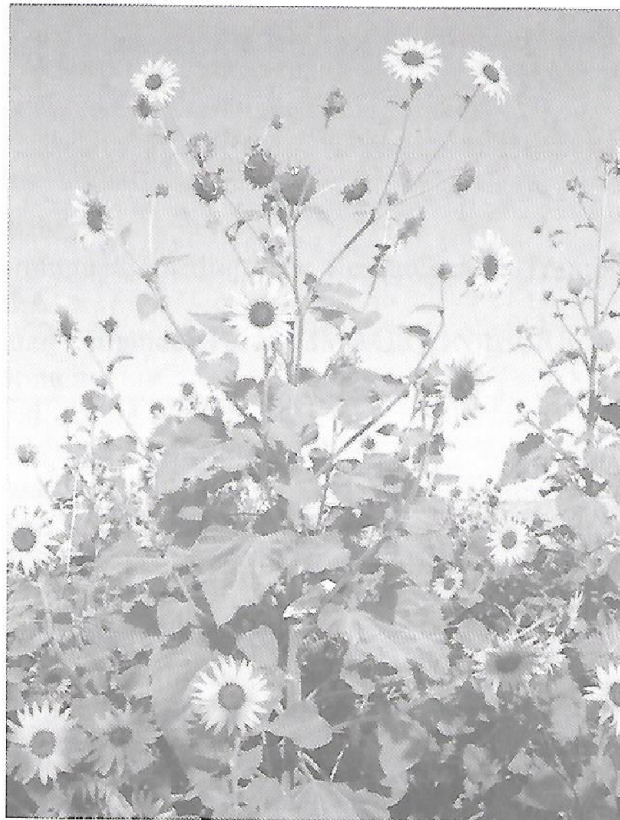
# 17<sup>th</sup> INTERNATIONAL SUNFLOWER CONFERENCE

Vol. 2



Proceedings of the  
**17th International  
Sunflower Conference**

**Vol. 2**



**Córdoba, Spain**  
June 8-12, 2008

Sponsored by The International Sunflower Association, Paris, France



Instituto de Investigación y Formación Agraria y Pesquera  
CONSEJERÍA DE AGRICULTURA Y PESCA



International Sunflower  
Association

*Proceedings of the 17<sup>th</sup> International Sunflower Conference*  
Córdoba, Spain. June 8-12, 2008

**Editor:** Leonardo Velasco

**Editorial Committee:**

José M. Fernández-Martínez  
Luís García-Torres  
Pedro González  
José M. Melero-Vara  
Francisco Orgaz  
Begoña Pérez-Vich  
Francisco Villalobos

**Organizing Committee:**

Chairman: Juan Domínguez: Institute for Research and Training in Agriculture and Fisheries (IFAPA).  
Deputy Chairman: Juan Fernández Pérez LIMAGRAIN IBERICA  
ISA Secretariat: Laurecine Lot  
ISA Secretary General: André Pouzet  
Luis Carlos Alonso Arnedo. KOIPESOL S.A.  
José M. Fernández Martínez. Institute for Sustainable Agriculture (CSIC)  
José Antonio García de Tejada. ARLESA Semillas  
José Rafael García Ruiz. Institute for Research and Training in Agriculture and Fisheries (IFAPA)  
Luis López Bellido. University of Cordoba  
José M. Melero Vara. Institute for Sustainable Agriculture (CSIC)  
Leonardo Velasco. Institute for Sustainable Agriculture (CSIC)

Photography: *Feral sunflowers growing in a sunflower field in Santa Cruz, Córdoba, Spain.* Courtesy of Marie-Hélène Muller.

## Foreword

The proceedings of the 17<sup>th</sup> *International Sunflower Conference* contain 142 contributions from scientists of 24 countries. They include plenary lectures in several disciplines and regular communications presented in posters during the conference and discussed in the corresponding workshops. The manuscripts are classified by disciplines. They offer a good picture of the current state of the art of sunflower research and cultivation around the world.

The manuscripts in the *Proceedings* have been reviewed by an editorial committee with the main objective of helping the authors to improve their manuscripts through a critical reading. The authors received the edited manuscripts together with the comments of the reviewers and then went on to draft their final version. All the manuscripts received have been published in the *Proceedings*. The contents of the manuscripts are the responsibility of the authors. They should be considered as being privileged communications that require the express consent of the authors to be reprinted in part or as a whole. We wish to thank both the members of the Editorial Committee for their dedication to the task of editing such a large number of manuscripts, as well as all the authors for their collaboration throughout the whole edition process.

The Organizing Committee would also like to thank Diana Badder and José A. Palacios for their excellent editorial assistance in the preparation of these *Proceedings*. We are indebted to the Spanish Association of Sunflower Breeders (Asociación Española de Mejoradores de Girasol), which collaborated actively in the organization of the conference, and, very especially, to Juan Parejo, who was in charge of the financial side.

Finally, we would like to thank all the participants in the conference, who have contributed to its success by a careful preparation and revision of manuscripts and posters, presentation of their research in the workshops, and stimulating discussions throughout the conference on the scientific and technical aspects of sunflower research and cultivation in the world.

The Organizing Committee  
17<sup>th</sup> International Sunflower Conference  
Córdoba, Spain. June 8-12, 2008

## Volume 2

### Table of Contents

#### CROP PRODUCTION – PHYSIOLOGY

<b>Early responses to high crop population density in sunflower: Controls and effects of the crop self-organization process</b>	
Mónica López Pereira, Nora Trápani, Jorge Casal, Antonio Hall.....	417
<b>Early sowing as a means of drought escape in sunflower: effects on vegetative and reproductive stages</b>	
Clémentine Allinne, Nedjoua Ghoribi, Pierre Maury, Rhym Maougal, Ahmad Sarrafi, Nadia Ykhlef, Philippe Grieu .....	423
<b>SUNFLO: A joint phenotyping and modelling approach to analyse and predict the differences in yield potential of sunflower genotypes</b>	
Jérémie Lecoeur, Richard Poiré-Lassus, Angélique Christophe, Lydie Guilioni.....	429
<b>Root system and water extraction variability for sunflower hybrids</b>	
Lydie Guilioni, Ando M. Radanielson, Angélique Christophe, Jérémie Lecoeur.....	435
<b>Effects of high water table conditions on sunflower growth and quality</b>	
Satoko Yasumoto, Yukari Terakado, Morio Matsuzaki, Kensuke Okada.....	441

#### CROP PRODUCTION – FERTILIZER

<b>Optimizing of potassium and magnesium fertilizers in sunflower production</b>	
E. Sepehr, F. Nourgolipour, M.J. Malakouti.....	447
<b>Sunflower response to mineral nitrogen, organic and bio-fertilizers under two different levels of salinity</b>	
M.M. Keshta, T.Y. Rizk, E.T. Abdou.....	451
<b>The effect of different amounts of animal manure on qualitative and quantitative traits of sunflower hybrid varieties</b>	
A. Faramarzi, B. Mirshekari, H. Mohammadi, A. Ahmadi.....	455
<b>Nitrogen fertilization of high oleic sunflower in wet climate</b>	
Gian Paolo Vannozzi, Maurizio Turi, Sattar Tahmasebi Enferadi, Zohreh Rabiei.....	459

#### CROP PRODUCTION – WEEDS

<b>Efficiency of modeling sunflower and <i>Amaranthus retroflexus</i> L. competition</b>	
Bahram Mirshekari, Ali Faramarzi, Mahmood Poor Yousef.....	463
<b>Sunflower protection from negative effects of 2,4-D</b>	
Vladimir Strelkov, Ludmila Fyadyuchenko, Lidia Isakova.....	469
<b>Análisis del crecimiento de genotipos de girasol resistentes y susceptibles a herbicidas imidazolinonas</b>	
Fernando S. Adegas, Marcelo F. Oliveira, Alexandre M. Brighenti.....	473

- Assessment of sunflower yield maps and discrimination of late-season weed patches by using field spectroradiometry and remote sensing: the case of *Ridolfia segetum* Moris  
F. López-Granados, J.M. Peña-Barragán, M. Jurado-Exposito, L. García-Torres..... 477
- Control of *Cirsium* and *Xanthium* in sunflower hybrids resistant to express 50 SX  
Alexandrina Popescu..... 483
- Development of CLHA-Plus: a novel herbicide tolerance trait in sunflower conferring superior imidazolinone tolerance and ease of breeding  
Carlos Sala, Mariano Bulos, Mariel Echarte, Sherry Whit, Gregory Budziszewski, William Howie, Bijay Singh, Brigitte Weston..... 489
- BREEDING AND GENETICS**
- Selection of sunflower hybrids for Banja Luka area in Bosnia and Herzegovina  
Jovan Kondić, Krsto Mijanović..... 495
- Principal component analysis as a reflector of combining abilities  
Mehdi Ghaffari, Ebrahim Farrokhi..... 499
- New sunflower hybrids tolerant of Tribenuron-Methyl  
Sinisa Jocić, Vladimir Miklić, Goran Malidza, Nada Hladni, Sandra Gvozdenovic..... 505
- Genetic improvement of oil quality in sunflower mutants under water stressed conditions  
A. Ebrahimi, P. Maury, M. Berger, F. Shariati, P. Giteu, A. Sarrafi..... 509
- Estimation of genetic diversity of sunflower single cross hybrids using principal component analysis  
Hossein Zainalzadeh Tabrizi, Hassan Monirfar, Varahram Rashedi, Mehdi Ghaffari..... 513
- Relationship between genetic distance and heterosis based on quantitative traits and SSR markers in sunflower  
Sandra Gvozdenovic, Dejana Satic-Pankovic, Sinisa Jocić, Dragan Skoric..... 519
- The Perenets mutation in sunflower knocks out the wild microsomal oleate desaturase gene and leads to high oleic acid content in the seed oil  
Severine Lacombe, Irène Souyris, André Berville..... 525
- Vegetation period and hybrid sunflower productivity in breeding for earliness  
Sergey Gontcharov, Maria Zaharova..... 531
- Homo- and heterozygous longitudinal gradient of oleic acid content in sunflower seeds  
Yakov Demurin, Oxana Borisenko, Nikolay Bochkarev..... 535
- White rot resistance, seed weight and seed oil content in sunflower test crosses  
M<sup>a</sup> Antonela Giussani, Fernando Castaño, Raúl Rodríguez, Facundo Quiroz..... 539
- Gene effects and combining abilities of sunflower morphophysiological traits  
Nada Hladni, Sinisa Jocić, Vladimir Miklić, Marija Kraljajević-Balalić, Dragan Skoric..... 545

<b>Ichraq: Première variété de tournesol d'automne au Maroc</b>	
Abdelghani Nabloussi, Bassou Akhtouch, Mohamed Boujghagh, Mohamed El Asri, Mohamed El Fechtali.....	551
<b>Estimation of breeding potential for tocopherols and phytosterols in sunflower</b>	
Alicia Ayerdi Gotor, Monique Berger, Françoise Labalette, Sylvie Centis, Jean Daydé, Anne Calmon.....	555
<b>Studies on general and specific combining abilities in sunflower</b>	
Ebrahim Farrokhi, Abolghasem Khodabandeh, Mehdi Ghaffari.....	561
<b>Heredabilidad de componentes de rendimiento en dos poblaciones de girasol de la EEA Pergamino</b>	
Julio González, Nora Mancuso, Pedro Ludueña.....	567
<b>General combining ability analysis in sunflower maintainer lines using line x tester crosses</b>	
Ebrahim Farrokhi, Bahram Alizadeh, Mehdi Ghaffari .....	571
<b>Selection of sunflower genotypes for Central Brazil</b>	
Claudio Guilherme Portela de Carvalho, Ana Cláudia Barneche de Oliveira, Anna Karolina Grunvald, Francielle Pereira da Silva.....	575
<b>Selection of sunflower genotypes for sowing dates in August/September in Southern region of Brazil</b>	
Ana Cláudia Barneche de Oliveira, Cláudio Guilherme Portela de Carvalho, Anna Karolina Grunvald, Francielle Pereira da Silva.....	579
<b>Identification of a new CMS cytoplasm and localization of its fertility restoration gene in sunflower</b>	
Jiuhuan Feng, C.C. Jan.....	583
<b>Germoplasma mejorado de girasol de la EEA Pergamino</b>	
Julio González, Nora Mancuso, Pedro Ludueña.....	589
<b>Heterosis for yield and oil content of sunflower lines developed from bi-parental populations</b>	
G. Chigeza, P. Shanahan, M.J. Savage, K. Mashingaidze.....	595
<b>A modifying gene affecting gamma-tocopherol content in sunflower</b>	
María J. García-Moreno, José M. Fernández-Martínez, Begoña Pérez-Vich, Leonardo Velasco.....	601
<b>MAPPING AND MARKER ASSISTED SELECTION</b>	
<b>QTL for capitulum resistance to <i>Sclerotinia sclerotiorum</i> in sunflower</b>	
Felicity Vear, Isabelle Jouan-Dufournel, Pierre-François Bert, Frédéric Serre, Florence Cambon, Caroline Pont, Pascal Walser, Sylvie Roche, Denis Tourvieille de Labrouhe, Patrick Vincourt.....	605
<b>HeliaGene, a bioinformatics portal for <i>Helianthus</i> sp. genomics</b>	
Sébastien Carrere, Jérôme Gouzy, Nicolas Langlade, Pascal Gamas, Patrick Vincourt.....	611
<b>Mapping a novel fertility restoration gene in sunflower</b>	
Gustavo Abratti, María Eugenia Bazzalo, Alberto León.....	617
<b>Verification of positive BAC clones near the <i>Rf1</i> gene restoring pollen fertility in the presence of the PET1 cytoplasm in sunflower (<i>Helianthus annuus</i> L.) and direct isolation of BAC ends</b>	
Sonia Hamrit, Barbara Kusterer, Wolfgang Friedt, Renate Horn.....	623

- 629 The efficiency of different molecular indices in sunflower breeding  
 Maria Duca, Ana Capatana.....
- 635 Differential gene expression in SuCMoV-tolerant and susceptible  
 sunflower lines  
 Daniel Mallo, Monica Poverene, Fabian Giolitti, Sergio Lenardon.....
- 641 Molecular mapping of a new induced gene for nuclear male sterility in  
 sunflower (*Helianthus annuus* L.)  
 Ana Capatana, Jihuan Feng, Brady A. Vick, Maria Duca, C.C. Jan.....
- 645 Fine mapping of the downy mildew resistance locus *PlARG* in sunflower  
 Silke Wieckhorst, Volker Hahn, Christina M. Duble, Steven J. Knapp, Chris Carolin  
 Schön, Eva Bauer.....
- 651 Identification of molecular markers linked to a new nuclear male-sterility  
 gene *ms7* in sunflower (*Helianthus annuus* L.)  
 Caifeng Li, Jihuan Feng, Fengming Ma, Brady A. Vick, C.C. Jan.....
- 655 Construction of a linkage map with TRAP markers and identification of  
 QTL for four morphological traits in sunflower (*Helianthus annuus* L.)  
 Bing Yue, Brady A. Vick, Jerry F. Miller, Xiwen Cai, Jinguo Hu.....
- 661 Candidate gene analysis and identification of TRAP and SSR markers  
 linked to the *Or5* gene, which confers sunflower resistance to race E of  
 broomrape (*Orobanchaceae cumana* Wallr.)  
 Angelustas Marquez-Lema, Philippe Delavault, Patricia Letouzey, Jinguo Hu, Begoña  
 Pérez-Vich.....
- 667 Tribenuron-methyl resistance in accessions of annual wild sunflower  
 species from the Novi Sad germplasm collection  
 Stren Terzic, Jovanika Atlagic.....
- 673 Hybridization between cultivated sunflower *Helianthus annuus* L. and  
 wild perennial species *Helianthus pumilus* Nuttall  
 Miroslava M. Hristova-Cherbadzi, Michail Christov.....
- 679 Studies on some morphological characters of wild *Helianthus annuus* L.  
 accessions with different origin  
 Daniela Valkova, Miroslava Hristova-Cherbadzi, Michail Christov, Emil Penchev.....
- 685 Weedy sunflowers in France: Prevalence and first inferences on their  
 origin  
 Marie-Hélène Muller, Vincent Lecomte, Bernard Garric, Pierre Jouffret, Martine Letlon,  
 Florent Pourgeaux, Richard Ségura.....
- 691 Characterization of hybrids from crosses between cultivated *Helianthus  
 annuus* L. and subspecies *rydbergii* (Britton) Long of perennial diploid  
*Helianthus nuttallii*  
 Miroslava M. Hristova-Cherbadzi, Michail Christov.....
- 697 Preventing botanical contamination risk of sunflower hybrid seed in the  
 Valle Bonaerense del Río Colorado, Argentina  
 Miguel Cantamutto, Alejandro Presotto, Juan Pablo Renzi, Mónica Poverene.....



**Seed morphology and oil composition of wild *Helianthus ann.***

**Argentina**

Miguel Cantamutto, Daniel Alvarez, Alejandro Presotto, Ivana Fernandez-Moron, Seiler, Mónica Poverene.....

***Helianthus* species in breeding research on sunflower**

Michail Christov.....

**Wild sunflower species from the southeastern United States as potential sources for improving oil content and quality in cultivated sunflower**

Gerald J. Seiler, Tom J. Gulya, Gary Kong.....

**Cytogenetic study of an F<sub>1</sub> sunflower interspecific hybrid (*Helianthus annuus* x *Helianthus praecox*)**

Jovanka Atlagić, Sreten Terzić..... 721

**Sunflower nested core collections for association studies and phenomics**

Marie Coque, Sébastien Mesnildrey, Michel Romestant, Bruno Grezes-Beset, Félicity Vear, Nicolas B. Langlade, Patrick Vincourt..... 725

**Using interspecific hybrids with *Helianthus tuberosus* L. to transfer genes for quantitative traits into cultivated sunflower, *H. annuus* L.**

Brent S. Hulke, Donald L. Wyse..... 729

**2008 update: The USDA sunflower collection at the north central regional plant introduction station, Ames, IA, USA**

Laura Fredrick Marek, Charles C. Block, Candice C.A. Gardner..... 735

***Helianthus annuus* natural populations to increase the whole genetic diversity of domesticated sunflower: the concept of neodomestication**

Hervé Serieys, Hamidreza Nooryazdan, François Kaan, Roberto Bacilieri, Jacques David, Marie-France Ostrowski, Marie-Hélène Muller, André Bervillé..... 741

**VARIATION IN OIL AND MEAL QUALITY**

**Effect of the environment on the chemical composition and some other parameters of sunflower seed quality**

Velimir Radić, Siniša Jocić, Jelena Mrda ..... 747

**Variability and genetic analysis of sterols content in sunflower seeds**

Marion Alignan, Jane Roche, Felicity Vear, Patrick Vincourt, Andrée Bouniols, Muriel Cerny, Zephirin Mouloungui, Othmane Merah..... 751

**Caractérisation par infra-rouge des teneurs en acides gras de la graine entière décortiquée de tournesol**

Alicia Ayerdi Gotor, Philippe Moreau, Antoine Gaillard, Anne Calmon..... 757

**Near infrared spectrometry (NIRS) prediction of minor components in sunflower seeds**

Alicia Ayerdi Gotor, Monique Berger, Françoise Labalette, Sylvie Centis, Jean Daydé, Anne Calmon..... 763

**CURRENT STATUS AND NEW USES OF THE CROP**

**Expansion of sunflower crop production in Brazil: a survey of future trends**

Nilza Patrícia Ramos, Cláudio César de A. Buschinelli, Ariovaldo Luchiani Junior, Adriana M. Moreno Pires..... 769

775	Breeding of sunflower as a biogas substrate Volker Hahn, Martin Ganssmann.....
779	Veinte años de ensayos de girasol en Andalucía: evolución del rendimiento de semilla y riqueza grasa J. R. García Ruiz, J. Domínguez Giménez, J. García López.....
785	The situation and future directions of sunflower production in the Black Sea Region Yalcin Kaya, Mukadder Ustun Kaya, Veyzel Kaya, Ibrahim Sahin.....
791	The future potential of oleic type sunflower in Turkey Yalcin Kaya, Veyzel Kaya, Ibrahim Sahin, Mukadder Ustun Kaya, Nesrin Citak.....
797	Oil type sunflower production in Turkey Yalcin Kaya, Veyzel Kaya, Goksel Evci, Ibrahim Sahin, Mukadder Ustun Kaya.....
803	Oilcake as a fuel alternative to wood pellets Yuichi Kobayashi, Hitoshi Kato, Genta Kanai.....

## Relationship between genetic distance and heterosis based on quantitative traits and SSR markers in sunflower

Sandra Gvozdenovic<sup>1</sup>, Dejana Saftic-Pankovic<sup>1</sup>, Sinisa Jocić<sup>1</sup>, Dragan Škorić<sup>2</sup>

<sup>1</sup>Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia,

E-mail: sandra@ifvcns.ns.ac.yu

<sup>2</sup>Serbian Academy of Science and Arts, Branch in Novi Sad, Nikole Pasica 6, 21000 Novi Sad, Serbia

### ABSTRACT

The objective of this study was to determine the relationship between SSR based genetic distance (GD) of new NS sunflower inbred lines for most important agronomic traits and heterosis. Twenty three sunflower inbred lines (twenty restorer lines and three female lines used as testers) were selected based on their diverse genetic background for plant height, head diameter, thousand seed mass, oil content, seed yield per plant and oil yield per plant. Significant heterosis was observed in hybrid combinations for all examined traits except oil content. Genetic distance between pairs of tested sunflower inbred lines ranged from 0.13 to 0.8. There was no significant positive correlation between genetic distance and mid- and better-parent heterosis, specific combining ability and mean value in any of the examined traits for all 60 hybrids. A highly significant negative correlation was found between GD and mean oil percentage ( $r=-0.33$   $p<0.01$ ). However significant correlations between GD and better-parent heterosis for thousand seed weight were found for hybrids of the tester line HA-19 ( $r=0.43$   $p<0.05$ ) and between GD and mid-parent heterosis for plant height for hybrids of the tester line HA-26 ( $r=0.47$   $p<0.05$ ). Although GD was generally a poor predictor of heterosis, better results are obtained if hybrid combinations for each tester and each trait are analyzed separately.

**Key words:** correlations sulfonylurea – genetic distance sulfonylurea – heterosis sulfonylurea – hybrid performance – sunflower.

### INTRODUCTION

Identification of parental combinations that produce hybrids of superior yield is the most important step in the breeding program of sunflower (*Helianthus annuus* L.). However, developing hybrids is a costly and long term process, as it is necessary to cross a lot of inbred lines and evaluate hybrids in field trials. Therefore, only a limited number of hybrids among all possible crosses can be tested. Utilisation of genetic distance for predicting hybrid heterosis has been of great interest to breeders. The efficiency of hybrid breeding programs could be increased if the inbred lines *per se* could be screened and the superior crosses predicted before field evaluation (Melchinger et al., 1990).

Studies of genetic diversity in relation to hybrid performance have been undertaken in several crops. Investigations in corn, *Zea mays* L. have shown that the genetic diversity of parents was significantly correlated with hybrid performance and that yield heterosis could be predicted using molecular markers (Smith et al., 1990; Betran et al., 2003; Rief et al., 2003; Schrag et al., 2006). Conversely, weak correlations have been reported between genetic distance and hybrid performance and heterosis in oilseed rape, *Brassica napus* L. (Diers et al., 1996), pepper, *Capsicum annuum* L. (Geleta et al., 2004), faba bean, *Vicia faba* L. (Zeid et al., 2004), and alfalfa, *Medicago sativa* L. (Riday et al., 2003).

Different sunflower gene pools have been studied for their genetic diversity with different marker systems (Tersac et al., 1993; Gentzbittel et al., 1994; Berry et al., 1994; Zhang, 1995; Hongtrakul, 1997; Cheres and Knapp, 1998; Yu et al., 2002; Tang and Knapp, 2003; Pankovic et al., 2004; Solodenko et al., 2005). However, the literature data on the predication of sunflower heterosis and hybrid performance by marker based genetic distance of the parental lines is scarce (Tersac et al., 1994; Cheres et al., 2000). Cheres et al. (2000) used AFLP markers and found a significant correlation between GD and seed yield, but genetic distance was generally a poor predictor of hybrid performance. The objective of this study was to determine the association between SSR based genetic distance of new NS sunflower inbred lines for most important agronomic traits and heterosis.

## MATERIALS AND METHODS

Twenty three sunflower inbred lines (20 restorer lines and three female lines used as testers) were selected based on their diverse genetic background for examined agronomic traits. The selected restorer lines (labeled R-1 through R-20) are new inbred lines developed in the breeding program of the Oil Crops Department, of the Institute of Field and Vegetable Crops, in Novi Sad, Serbia. Female lines used as testers (HA-48, HA-26 and HA-19) are commercial lines with good combining abilities.

Female lines were crossed with restorer lines to produce all possible combinations of F<sub>1</sub> hybrids using the line x tester method (Singh and Choudhary, 1976). Seeds of the 60 F<sub>1</sub> hybrids produced and their parents were sown in a breeding nursery of the Oil Crops Department, of the Institute of Field and Vegetable Crops. The experimental design was a randomized block system with four replications.

Plant height (PH), head diameter (HD), thousand seed weight (TSW), oil content (OC), seed yield per plant (SY) and oil yield per plant (OY) were used for quantitative characterization of 23 parental lines and their 60 F<sub>1</sub> hybrids. Plant height and head diameter were measured at the end of flowering. Seed yield was measured by harvesting the middle row of each plot by hand. Seed samples from each plot were analyzed for oil content by nuclear magnetic resonance.

Analysis of variance and specific combining abilities (SCA) for quantitative traits were performed using the line x tester method (Singh and Choudhary, 1976). Heterosis was determined as follows:

$$\text{Mid-parent heterosis (MPH) (\%)} = ((F_1 - MP) / MP) * 100$$

$$\text{Better-parent heterosis (BPH) (\%)} = ((F_1 - BP) / BP) * 100$$

where, F<sub>1</sub> is the F<sub>1</sub> performance, MP = (P<sub>1</sub>+P<sub>2</sub>)/2 in which P<sub>1</sub> and P<sub>2</sub> are the performances of inbred parents and BP is the betterparent value (Geleta et al., 2004). Significance of heterosis was determined by the t-test (Kraljevic-Balalic et al., 1991).

Genomic DNA of 23 parental lines was extracted following the modified method of Dellaporta et al. (1983). The 15 SSR sunflower primers used in the study were: ORS 1, ORS 5, ORS 7, ORS 8, ORS 10, ORS 12, ORS 14, ORS 16, ORS 31, ORS 37, ORS 47, ORS 66, ORS 78, ORS 509 and ORS 595 (Tang et al., 2002). The selected primers have previously revealed DNA polymorphism of sunflower NS breeding material (Pankovic et al., 2004; Terzic et al., 2006). Fragments were separated using 2% agarose and 6% denaturing polyacrylamide gels. DNA polymorphism between two inbred lines was estimated by comparison of amplified fragments. Jaccard coefficient (J) of similarity was calculated according to Staub et al. (2000). Genetic distances (GD) among the 23 parental lines were estimated according to Spooner et al. (1996) as GD = 1-J.

Values of genetic distance as measured by SSR markers were correlated with MPH and BPH to estimate their relationship. Correlations were done for F<sub>1</sub> combination from each tester line separately and all tester lines.

## RESULTS AND DISCUSSION

Parental lines and 60 F<sub>1</sub> hybrids were evaluated in field trials for plant height, head diameter, thousand seed weight, oil content, seed yield per plant and oil yield per plant. There was a great variation among inbred lines and hybrids, respectively (Table 1). The mean values of the hybrids were significantly higher than the parental lines for plant height, head diameter, thousand seed mass, seed and oil yield per plant.

**Table 1.** Mean values, standard error of the means and coefficient of variation (V) for the sunflower parental lines and their F<sub>1</sub> hybrids

Trait	Female line		F <sub>1</sub> hybrid		Restorer	
	Mean	V	Mean	V	Mean	V
Plant height (cm)	157.77±0.87	20.10	201.88±0.45	45.19	141.48±0.36	51.43
Head diameter (cm)	18.69±0.01	19.47	22.48±0.02	36.98	14.21±0.01	66.04
Tousand seed weight (g)	50.66±0.21	9.49	54.33±0.07	8.62	34.49±0.25	22.07
Oil content (%)	46.77±0.10	6.11	47.36±0.09	5.42	47.90±0.13	6.12
Seed yield (g per plant)	35.38±0.65	10.15	57.05±0.58	14.60	12.24±0.20	38.69
Oil yield (g per plant)	16.46±0.33	4.66	26.99±0.26	14.83	5.91±0.05	42.46

The heterotic effect was observed in all examined traits, except oil content (Table 2). The mean values of hybrids were between parental means for oil content and both parental lines were selected for high oil quantity. The highest effect of heterosis (MPH) was observed for oil yield per plant (143.77%) followed by seed yield per plant (142.04%).

**Table 2.** Mean values and range of heterosis (%) for six quantitative traits of the 60 F<sub>1</sub> sunflower hybrids (PH=plant height, HD=head diameter, TSW=thousand seed weight, OC=oil content, SY=seed yield per plant and OY=oil yield per plant)

Heterosis		PH	HD	TSW	OC	SY	OY
MPH	Mean	35.36**	37.17**	21.37**	0.06	142.04**	143.77**
	Range	15.32-66.86	17.24-66.56	0.20-65.22	-7.12-9.72	60.17-249.44	55.77-247-24
BPH	Mean	21.28**	19.00**	3.45*	-0.46	62.04**	64.10**
	Range	-4.01-42.70	0.74-47.81	-18.27-34.85	-9.98-6.98	29.14-130.92	34.72-125.32

\*\*significant at P=0.05 , \*significant at P=0.01

Analysis of fifteen SSR markers detected 44 alleles, with an average polymorphism PIC= 45.3%. The number of alleles per locus ranged between 2 and 5, with a mean of 2.93. Genetic distance between pairs of tested sunflower inbred lines ranged from 0.13 (HA-19 vs. HA-48 and R-12 vs. R-18) to 0.8 (HA-19 vs. R-18) (data not presented).

The relationship between genetic diversity based on SSR markers of all inbred lines and their hybrid performance depended on the trait examined. Correlation coefficients between GD and parental means, SCA and heterosis were not significant for the most examined traits (Table 3). The only significant correlation was a negative one, between GD and mean oil content ( $r=-0.33$   $p<0.01$ ). For plant height, correlation between GD and heterosis was positive but not significant ( $r=0.232$  and  $0.172$ ). Similar results were obtained for thousand seed weight (0.226 and 0.245).

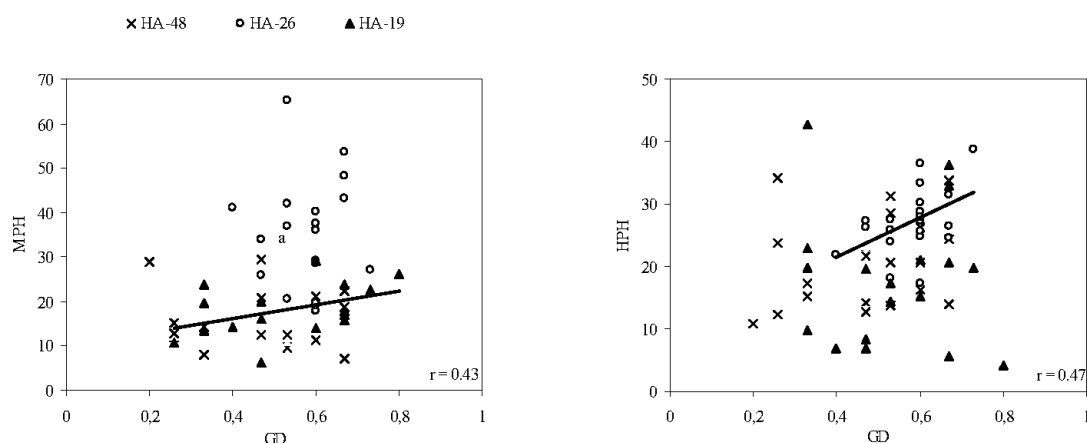
**Table 3.** Correlation between genetic distance (GD) and mid- (MPH) and better-parent heterosis (BPH), specific combining ability (SCA) and mean values (MV) for each trait in sunflower hybrids (PH=plant height, HD=head diameter, TSW=thousand seed weight, OC=oil content, SY=seed yield per plant and OY=oil yield per plant).

	PH	HD	TSW	SY	OC	OY
GD vs. MPH	0.232	0.096	0.226	-0.213	-	-0.202
GD vs. BPH	0.172	0.101	0.245	-0.067	-	-0.071
GD vs. SCA	0.020	0.099	0.090	-0.159	-0.154	-0.178
GD vs. MV	-0.115	-0.102	0.071	0.021	-0.330**	-0.103

$r_{(0.05)}=0.25$ ,  $r_{(0.01)}=0.325$

Correlation between genetic distance and heterosis was not significant for most of the examined traits. The poor correlation might be due to several causes. SSR markers used in this study were chosen solely for their high PIC values. Charcosset et al. (1991) and Bernardo et al. (1992) suggested that genetic distance cannot accurately predict hybrid performance unless the DNA markers used in the analysis were linked to the genes affecting the trait. Therefore, the 60 F<sub>1</sub> hybrids were divided into three groups according to the parental tester line and correlation of the GD with hybrid performance, and heterosis within the groups was examined for all six traits. Only significant correlations were found between GD and better-parent heterosis for thousand seed mass for hybrids with the tester line HA-19 ( $r=0.43$   $p<0.05$ ) and between GD and mid-parent heterosis for plant height for hybrids with the tester line HA-26 ( $r=0.47$   $p<0.05$ ) (Fig. 1). In these two cases hybrid heterosis increased linearly with increased GD between parental lines. However, the correlations obtained were too low to be of any predictive value.

Tersac et al. (1994) described relationships between heterosis and enzymatic polymorphism of 39 sunflower populations. The correlation coefficients for all enzyme systems were too low to be used as predictors of the general combining ability, but when enzyme systems were analyzed separately, four of them turned out to be useful markers for breeding purposes. Cheres et al. (2000) have used 360 AFLP markers and found that although genetic distances were significantly correlated with hybrid seed yield and percent of heterosis for seed yield ( $r=0.79$  and  $0.76$ ), hybrid performance varied greatly among hybrids of inbreds with similar genetic distance (GD). Zeid et al. (2004) pointed out that the lack of association between heterosis and genetic dissimilarities for inter group hybrids might be explained by absence of crosses between related parents i.e. by the absence of variation for parental relatedness: all crosses have unrelated parents.



**Fig. 1.** Plots of genetic distance vs. mid-(MPH) and better-parent heterosis (BPH) for plant thousand seed weight (left) and plant height (right) of sunflower hybrid combinations ( $r_{(0,05)}=0.42$ ,  $r_{(0,01)}=0.54$ ).

The results of this study confirm that GD generally correlates poorly with heterosis and specific combining abilities. Previous studies in various crop species such as corn, pepper, alfalfa, wheat, and rapeseed also showed low correlations of GD with heterosis (Melchinger et al., 1990; Diers et al., 1996; Geleta et al., 2004; Zeid et al., 2004; Riday et al., 2003). Although genetic distance is a poor predictor of hybrid performance, our results indicate that better results are obtained if hybrid combinations for each tester and each trait are analyzed separately. Our further field trials for identification of sunflower heterotic performance will be planned on prior information on genetic distance of inbreds, obtained by more molecular markers, involving the ones associated with QTLs for examined traits.

#### ACKNOWLEDGEMENTS

This work was supported by Ministry of Science of Republic of Serbia.

#### REFERENCES

- Bernardo, R. 1992. Relationship between single-cross performance and molecular marker heterozygosity. *Theor. Appl. Genet.* 83:628-634.
- Berry, S. T., R. J. Allen, S. R. Barnes, and P. D. S. Caligari. 1994. Molecular marker analysis of *Helianthus annuus* L. 1. Restriction fragment length polymorphism between inbred lines of cultivated sunflower. *Theor. Appl. Genet.* 89:435-441.
- Betran, F. J., J. M. Ribaut, D. Beck, and D. Gonzales de Leon. 2003. Genetic diversity, specific combining ability and heterosis in tropical maize under stress and nonstress environments. *Crop Sci.* 43:797-806.
- Charcosset, A. M., M. Lefort-Buson, and A. Gallais. 1991. Relationship between heterosis and heterozygosity at marker loci: a theoretical computation. *Theor. Appl. Genet.* 81: 571-575.
- Cheres, M.T., and S. J. Knapp. 1998. Ancestral origins and genetic diversity of cultivated sunflower: analysis of the pedigrees of public germplasm. *Crop Sci.* 38: 1476-1482.
- Cheres, M.T., J.F. Miller, J.M. Crane, and S.J. Knapp. 2000. Genetic distance as a predictor of heterosis and hybrid performance within and between heterotic groups in sunflower. *Theor. Appl. Genet.* 100: 889-894.
- Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA mini-preparation: version 2. *Plant Mol. Biol. Reporter* 1:19-21.
- Diers, B.W., B.E. McVetty, and T.C. Osborn. 1996. Relationship between heterosis and genetic distance based on RFLP markers in oilseed rape (*Brassica napus* L.). *Crop Sci.* 36:76-83.
- Geleta, L. F., M. T. Labuschagne, and C.D. Viljoen. 2004. Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. *Plant Breed.* 123:467-473.
- Gentzmittel, L., X. Z. Zhang, F. Vear, B. Griveau, and P. Nicolas. 1994. RFLP studies of genetic relationship among inbred lines of the cultivated sunflower (*Helianthus annuus* L.): evidence for distinct restorer and maintainer germplasm pools. *Theor. Appl. Genet.* 89:419-425.

- Hongtrakul, V., G.M. Huestis, and S.J. Knapp. 1997. Amplified length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. *Theor. Appl. Genet.* 95: 400-407.
- Kraljevic-Balalic, M., S. Petrovic and Lj. Vapa. 1991. *Genetika: teorijske osnove sa zadacima. Poljoprivredni i Prirodno-matematički fakultet, Univerzitet u Novom Sadu.*
- Melchinger, A.E., M. Lee, K.R. Lamkey, and W.W. Woodman. 1990. Genetic diversity for restriction fragment length polymorphisms: relation to genetic effects in maize inbreds. *Crop Sci.* 30:1033-1040.
- Pankovic, D., S. Jocić, N. Lacok, Z. Sakac, and D. Škorić. 2004. The use of PCR-based markers in the evaluation of resistance to downy mildew in NS-breeding material. *Helia* 27(40):149-158.
- Riday, H., E.C. Brummer, T.A. Cambell, and D. Luth. 2003. Comparison of genetic and morphological distance with heterosis between *Medicago sativa* and subsp. *falcata*. *Euphytica* 131:37-45.
- Rief, J.C., A.E. Melchinger, X.C. Xia, M.L. Warburton, D.A. Hoisington, S.K. Vasal, G. Srinivasan, M. Bohn, and M. Frisch. 2003. Genetic distance based on simple sequence repeats and heterosis in tropical maize population. *Crop Sci.* 43:1275-1282.
- Schrag, T.A., A. E. Melchinger, A. P. Sørensen, and M. Frisch. 2006. Prediction of single-cross hybrid performance for grain yield and grain dry matter content in maize using AFLP markers associated with QTL. *Theor. Appl. Genet.* 113:1037-1047.
- Singh, R. K., and B.D. Choudhary. 1976. *Biometrical Techniques in Genetics and Breeding.* International Bioscience Publishers. Hisar. India.
- Smith, O.S., J.S.C. Smith, S.L. Bowen, R.A. Tegborg, and S.J. Wall. 1990. Similarities among a group of elite maize inbreds as measured by pedigree, F1 heterosis and RFLPs. *Theor. Appl. Genet.* 80:833-840.
- Solodenko, A., and Y. Sivolap. 2005. Genotyping of *Helianthus* based on microsatellite sequences. *Helia* 28:19-26.
- Spooner, D.M., J. Tivang, J. Nienhis, J.T. Miller, D.S. Douches, and M.A. Contreras. 1996. Comparison of four molecular markers measuring relationship among the wild potato relatives *Solanum* section *Etuberosum* (subgenus Potato). *Theor. Appl. Genet.* 92:532-540.
- Staub, J.E., Y. Danin-Poleg, G. Fazio, T. Horejsi, N. Reis, and N. Katzir. 2000. Comparative analysis of cultivated melon groups (*Cucumis melo* L.) using random amplified polymorphic DNA and simple sequence repeat markers. *Euphytica* 115: 225-241.
- Tang, S., J.K. Yu, M.B. Slabaugh, D.K. Shintani, and S.J. Knapp. 2002. Simple sequence repeat map of the sunflower genome. *Theor. Appl. Genet.* 105:1124-1136.
- Tang, S., and S.J. Knapp. 2003. Microsatellites uncover extraordinary diversity in native American land races and wild populations of cultivated sunflower. *Theor. Appl. Genet.* 106:990-1003.
- Tersac, M., D. Vares, and P. Vincourt. 1993. Combining groups in cultivated sunflower populations (*Helianthus annuus* L.) and their relationship with country of origin. *Theor. Appl. Genet.* 87:603-608.
- Tersac, M., P. Blanchard, D. Brunel, and P. Vincourt. 1994. Relationship between heterosis and enzymatic polymorphisms in populations of cultivated sunflower (*Helianthus annuus* L.). *Theor. Appl. Genet.* 88:49-55.
- Terzic, S., J. Atlagic, and D. Pankovic. 2006. Characterization of F1 interspecific hybrids between wild *Helianthus annuus* L. populations and cultivated sunflower. *Genetika* 38(2):159-168.
- Yu, J.K., J. Mangor, L. Thompson, K.J. Edwards, M.B. Slabaugh, and S.J. Knapp. 2002. Allelic diversity of simple sequence repeat markers among elite inbred lines in cultivated sunflower. *Genome* 45:652-665.
- Zeid, M.M., C.C. Schon, and W. Link. 2004. Hybrid performance and AFLP- based genetic similarity in faba bean. *Euphytica* 139:3, 207
- Zhang, Y. X., L. Gentzbittel, F. Vear, and P. Nicholas. 1995. Assessment of inter- and intra- inbred line variability in sunflower (*Helianthus annuus* L.) by RFLP. *Genome* 38:1040-1048.