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Foreword

The International Sunflower Association (ISA) and the Argentine Sunflower Association (ASAGIR) are pleased to present this guide to the 18th International Sunflower Conference.

At the time the main objectives for the meeting were defined, organizers aimed to provide a forum for the international sunflower research community with interest in any aspect of science and technology relating to the crop (in its oil-seed and confectionery variants) that would allow all involved to:

- Update knowledge in all fields of sunflower research since the previous conference held at Córdoba, Spain, June 2008;
- Review recent technological advances in sunflower production and identify knowledge gaps that require attention;
- Analyze the status and expectations for current and prospective demands for sunflower products;
- Provide a venue for workshops and special-interest meetings focusing on unresolved research, market, and production issues;
- Provide new generations with an opportunity to interact with global leaders in sunflower research.

The local Program Committee, with the help of the International Steering Committee, has developed a program covering the whole spectrum of relevant topics from genes and genomics through to field agronomy, crop protection, and industry and market issues. The program comprises 14 plenary and 13 invited presentations, 14 short oral presentations, an exhibition of 160 posters that can be visited during each of the first three days of the meeting. In addition, there will be three associated workshops (Bird Damage, Breeding, International Sunflower Genome Initiative), a special-interest presentation of the Global Crop Diversity Trust, and facilities will be available on request for small groups who wish to discuss business or scientific topics.

On the last day of the meeting, the Conference Field Day will be held at the joint INTA-Universidad de Mar del Plata facility in Balcarce. This time the traditional Conference demonstration plots of hybrids from International Sunflower Association member countries and from the host country will be complemented by a broad range of demonstrations of production and management techniques, as well as demonstrations of research techniques in current use by Argentine sunflower research teams.

This Conference has been made possible by the work of many people, by the support of sponsors from both the public and the private sector (sponsors are recognized on the back covers of this guide) and last, but certainly by no means least, those responsible for the lectures, short oral presentations, posters, associated workshops and special interest meetings, and field and laboratory demonstrations that make up the rich and varied bill of fare for this Conference, as reflected in this guide. The Organizing Committee extends their heartfelt thanks to all these individuals and organizations.

ISA and ASAGIR trust that this guide will enable all attendees to have an interesting and fruitful 18th International Sunflower Conference.

Welcome

It has been 27 years since the 11th International Sunflower Conference was held in Mar del Plata, Argentina, March 10-13, 1985. Since then, very many things have changed in the world of sunflower science, technology, and crop production and management. As the global sunflower community reconvenes once again in the same city, its members will have the opportunity to review progress in the last four years, which has been substantial in many areas.

Mar del Plata, a vibrant city located by the sea, with a fishing port, good restaurants, an unusually good choice of golf courses, and kilometers of sandy beaches, together with Balcarce, provide excellent venues for the Conference lectures and Field Day, and will allow attendees to appreciate a unique combination of seas, hills and Pampas. It is a great pleasure for the Organizing Committee to be able to host attendees to this meeting, which we hope will be both enjoyable and fruitful.

Welcome to Argentina, to Mar del Plata and Balcarce, and to the 18th International Sunflower Conference.

| 05-VC-13 |

Estimation of combining abilities in sunflower (*Helianthus annuus L.*)

Sinisa Jocic*, Sandra Cvejic, Mihajlo Ciric, Nada Hladni, Dragana Miladinovic, Vladimir Miklic, Ilija Radeka
Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21 000 Novi Sad, Serbia
*Corresponding author: sinisa.jocic@ifvcns.ns.ac.rs

ABSTRACT

- In commercial production, sunflower hybrids are more represented than open pollinated varieties. The basic advantage of hybrids over varieties is that the hybrids allow the use of heterosis. Sunflower hybrids are more stable, highly self-fertile, with high yield performance, and more uniform at maturity in comparison to open pollinated varieties. Also, it is easier to incorporate disease resistance in hybrids, as it was confirmed in the development of hybrids which are genetically resistant to rust, downy mildew, broomrape and some other disease. The heterotic performance of a hybrid combination depends on the combining abilities of its parents. Combining ability analysis is an important tool for the selection of desirable parents as well as for getting the information regarding nature and magnitude of gene effects controlling quantitative traits. The aim of study was to determine general and specific combining ability of 20 newly developed restorer inbred lines resistant to *Orobanche cumana* Wallr.
- The line x tester analysis method has been used to breed both self and cross-pollinated plants and to estimate favorable parents and crosses, and their general (GCA) and specific (SCA) combining abilities. Three cytoplasmic male sterile lines and twenty fertility restorer lines were crossed in all possible combinations, under cages to secure genetic purity of experimental hybrids. Male parents were selected on the basis of resistance to broomrape (*Orobanche cumana*) and high tolerance to *Phomopsis*. Female parents were inbred lines of known good combining abilities that were also the parents of some of the best commercial hybrid. All sixty experimental hybrids were planted in randomized complete block design with three replications in two locations. Three traits were studied after harvesting: seed yield (kg/ha), seed oil content (%) and oil yield (kg/ha).
- The main results were: i) general and specific combining abilities for main productivity traits – (seed yield, seed oil content and oil yield) for 20 new restorer lines and 3 tester female lines were estimated; ii) the components of genetic variance and the ratio of GCA-indicators of additive genetic variance to SCA-indicators of nonadditive genetic variance were determined; and iii) the percentage of the contribution of the restorer lines, testers and their interactions in the expression of the studied traits were estimated.
- Significant differences among the tested sunflower genotypes with regard to the mean values of all of the investigated traits were determined. The analysis of variance of combining abilities and the analysis of genetic variance components confirmed that the nonadditive component of genetic variance played the main role in the inheritance of the traits concerned. This conclusion is also supported by the fact that values of the GCA/SCA ratio for all traits were lower than one. For each of the traits, highly significant positive and negative GCA and SCA values were recorded.
- The success of any hybridization program depends on selection of suitable parental lines. Information regarding types of gene action, relative magnitude of genetic variance and combining ability for important parameters are essential for the improvement of sunflower crop. This information could become an essential tool to the sunflower breeders in the making of better parental combinations for further cultivated sunflower improvement.

Key words: combining ability - oil yield - seed yield – sunflower

INTRODUCTION

The main objective of sunflower breeding is to develop productive F_1 hybrid cultivars with stabile and high yield. The basic goal of this sunflower breeding method is utilization of heterosis, i.e. increased vigor of F_1 generation relative to parents, which results in higher yields (Kaya et al., 2012). Heterosis in sunflower is mostly utilized through two-way (single-cross) hybrids developed by crossing female inbred lines, that are cytoplasmatic male sterile, and male inbred lines possessing fertility-restoring genes (Hladni, 2010). The basic advantages of two-way hybrids over open pollinated varieties are their uniformity and higher yields (Miler, 1987; Skoric, 1988). Hybrids achieve seed yields 25–30% higher than cultivars. Besides higher genetic potential for seed yield, hybrids also have other advantages over cultivars. They are genetically homogenous and uniform in plant height and growing period, resulting in decreased harvest losses and seed of the same moisture appropriate for storing. Another important advantage of hybrids over cultivars is easier insertion of genes for resistance to the most important sunflower diseases. From the genetic point of view, heterosis is a result of intra-allelic (dominance and superdominance) and inter-allelic interaction (epistasis). Heterosis is successfully attained by crossing genetically divergent self-pollinated homozygous lines (inbred lines). Nonetheless, crossing any two lines does not necessarily cause heterosis, since lines can be genetically related. Due to this, it is needed to test combining abilities of the newly-created lines. Final assessment of the value of even most carefully selected inbred lines is performed based on their results in hybrid combinations. Good combining ability means the ability of one inbred line to provide superior progeny when combined with another line. Combining abilities can be general and specific. General combining ability (GCA) is a mean value of an inbred line based on its performance when crossed with any other line. Specific combining ability (SCA) is a value of an inbred line when crossed with a specific other line.

Combining abilities estimation of new lines is performed based on testers. These can be cultivars or lines of known good combining abilities, but inbred lines which serve as parents for the best commercial hybrids are most often used as testers. Miller et al. (1980) and Dominquez and Fernandez (1987) determined that lines of the best combining abilities can be successfully identified in this way. Since parental inbred lines of the best hybrids are used as testers most often, sterile forms of such lines have been developed, so these can be used as testers.

The main goal of plant breeding is to increase sunflower yield. All of the other goals in breeding for individual traits are ultimately subordinated to this objective. Yield is not a simple trait; rather, it is a complex one, an aggregate of traits, or a super trait (Skoric et al., 2007) that is highly influenced by the environment. Since seed yield is the main focus of attention of all breeders, there are a large number of papers dealing with the mode of inheritance of this trait in sunflower. However, the results of these studies are not in complete agreement with each other. All of them agree that both the additive and nonadditive components of genetic variance play a role in the inheritance of seed yield. One group of authors gives more significance to the additive component (Sheriff and Appadurai, 1985; Sigh et al., 1989; Petakov, 1992; Karasu et al., 2010), whereas another argues that the nonadditive one is more important (Goksoy et al. 2000; Skoric et al., 2000; Cecconi et al., 2000; Gvozdenovic et al., 2008a; Hladni et al., 2011).

As seed oil content definitely has a direct effect on the formation of oil yield per hectare, it is important that sunflower breeders develop inbred lines with a seed oil content of over 50%. The manifestation of heterosis for this trait as well as its mode of inheritance is highly important, too. Since the increased oil yield per area unit is the ultimate goal of sunflower growing, along with studying the aforementioned direct oil yield components, the mode of inheritance of this complex character must be understood as well. Skoric (1976), Marinkovic (1984) and Bedov (1985) all came to the conclusion that the role of the additive component of genetic variance was more important than the nonadditive one in the inheritance of this character in the F_1 generation. On contrary, Skoric et al. (2000), Laureti and Gatto (2001), Ortis et al. (2005) and Gvozdenovic et al. (2008b), found a larger role of the nonadditive component in sunflower oil yield inheritance.

The aims of this study were to: i) estimate general and specific combining abilities of 20 new restorer lines and 3 know tester female lines for main productivity traits – seed yield, seed oil content and oil yield; ii) determine the components of genetic variance and the ratio of GCA-indicators of additive genetic variance to SCA-indicators of nonadditive genetic variance; and iii) estimate the percentage of contribution of the restorer lines, testers and their interactions to the expression of the studied traits.

MATERIALS AND METHODS

Twenty newly developed restorer inbred lines: RH-49, PC-252, RH-1, RH-SP, PC-18, PC-19, PC-20, PC-21, PC-22, PC-26, PC-29, PC-30, PC-32, PC-33, PC-38, PC-46, PC-49, PC-55, PC-56 and PC-61, were crossed with each of the three testers: L-26, L-95 and L-48. Male parents were selected on the basis of resistance to broomrape (*Orobanche cumana*) and high tolerance to *Phomopsis*. Female parents are inbred lines of know good combining abilities and they are the parents of the best commercial hybrids. Crossing was done by hand under cages to secure genetic purity of the experimental hybrids. All of the obtained sixty experimental hybrids were examined in randomized complete block design, in three replications.

The experiment was conducted at the experimental field of the Institute of Field and Vegetable Crops, in Rimski Sancevi - region of Backa (RS) and in Banatska Topola - region of Banat (BT). Sowing was done at optimal sowing time, with net plot of 14 m^2 and with plant distance 70 x 25 cm. Three traits were studied after harvesting: seed yield (kg/ha), seed oil content (%) and oil yield (kg/ha). All traits were determined on 40 plants per replication. Seed yield was determined from the two inside rows and was corrected by humidity percent. Seed oil content was measured by nuclear magnetic resonance (NMR) in all replications. Oil yield was calculated from seed yield and seed oil content.

Analysis of the combining abilities were performed by using GEN software package (program for quantitative genetic analysis) for the line x tester method by Singh and Choudhary (1976) modification by Sharma (2006) when parents are not included in comparative trails. The results of the SCA will not be shown in tables due to their volume; instead, they will be only commented.

RESULTS AND DISCUSION

The analysis of variance for seed yield, seed oil content and oil yield showed highly significant differences among genotypes in both locations. The analysis of variance for line x tester for seed yield indicated highly significant differences in line x tester interaction in both locations. For oil content significant differences existed between the lines in one location, and highly significant differences between testers and interaction line x test in both locations. Line x tester interaction was highly significant for oil yield in both locations (Table 1). The significance of the line x tester interaction suggested that testers were able to separate within this set of inbred lines.

Table 1. ANOVA line x tester for seed yield, seed oil content and oil yield (mean squares)

Source of variation	df	Seed yield RS	Seed yield BT	Oil content RS	Oil content BT	Oil yield RS	Oil yield BT
Replication	2	123916	52116	4.28	2.94	44133	7240
Treatments	59	326985**	357576**	25.00**	21.60**	134407**	110353**
Lines	19	62232	77195	4.21*	3.16	26644	17516
Testers	2	47844	50642	31.52**	24.83**	50087	80418
Lines x testers	38	474053**	513921**	35.05**	30.65**	192726**	158347**

*significant at p=0.05 probability level; **significant at p=0.01 probability level

The estimation of the genetic components of variation, as well as the ratio of GCA/SCA showed that additive component was lower than the dominance component which suggests that, at both locations, seed yield, seed oil content and oil yield were predominately controlled by nonadditive gene action (Table 2). The preponderance of nonadditive gene action for these traits was supported in the results of Skoric et al. (2000), Laureti and Gatto (2001), Ortis et al. (2005), Gvozdenovic et al. (2008b), Hakim et al. (2008) Gvozdenovic et al. (2008a), Karasu et al. (2010) and Hladni et al. (2011).

Table 2. Components of genetic variance for seed yield, seed oil content and oil yield

Components	Seed yield (kg/ha) RS	Seed yield (kg/ha) BT	Oil content (%) RS	Oil content (%) BT	Oil yield (kg/ha) RS	Oil yield (kg/ha) BT
GCA	-48581.43	-52174.17	-1.99	-1.93	-17896.82	-12681.69
SCA	223008.65	161917.81	21.93	18.38	102016.86	67682.32
GCA/SCA	0.22	0.32	0.09	0.11	0.18	0.19

Comparative analysis of the GCA effects of the parents and data of the means of related experimental hybrids for all observed traits are given in Table 3. Variation in GCA effects was estimated among inbred lines for indicated plant traits to identify the best parent for subsequent hybrid development program. In general, significance of the GCA effect was not stable for the seed yield regarding locations. The estimations of general combining ability pointed out that the best general combiner for the seed yield concerning both locations was restorer line RH-SP. Also the highest average seed yield in both locations had a group of experimental hybrids with common parent RH-SP (5718 and 4743 kg/ha). This line seemed to possess favorable alleles for seed yield as the deduction from the highly significant positive GCA effects it exhibited. Positive significant GCA effects for seed yield were obtained from the restorer lines RH-49 and PC-61, but only in one location. Significant and negative GCA effects for seed yield were recorded for PC-19, PC-29, PC-30, PC-32, PC-61 and L-26.

Table 3. Estimates of general combining ability (GCA) effects and mean values (M) of lines and testers for seed yield, oil content and oil yield characters.

Inbred Lines	Seed yield (kg/ha)				Oil content (%)				Oil yield (kg/ha)			
	RS		BT		RS		BT		RS		BT	
	M	GCA	M	GCA	M	GCA	M	GCA	M	GCA	M	GCA
RH-49	5585	277*	3817	-191	47.35	1.27**	42.86	-2.32**	2647	202**	1643	-168*
PC-252	5500	192	4280	272	49.59	3.52**	47.02	1.85**	2731	285**	2015	204**
RH-1	5217	-89	3991	-17	49.21	3.14**	47.08	1.91**	2566	121	1882	71
RH-SP	5718	410**	4743	735**	44.42	-1.66**	42.30	-2.87**	2539	93	2008	197*
PC-18	5544	236	3935	-73	46.67	0.59	46.60	1.43*	2585	140*	1833	22
PC-19	4947	-360*	4001	-8	42.51	-3.57**	41.86	-3.31**	2102	-343**	1679	-132
PC-20	5416	108	4151	143	44.74	-1.34**	44.93	-0.24	2424	-22	1862	51
PC-21	5470	162	4153	145	43.31	-2.77**	44.40	-0.77	2365	-81	1840	29
PC-22	5178	-128	4225	216	43.74	-2.34**	44.22	-0.96	2261	-184**	1868	57
PC-26	5455	147	4065	56	45.37	-0.71	46.14	0.97	2478	32	1879	68
PC-29	4960	-347**	3817	-191	45.48	-0.60	45.01	-0.16	2250	-196**	1719	-91
PC-30	5050	-256*	3691	-317	45.25	-0.83	44.82	-0.36	2289	-156*	1654	-157*
PC-32	5037	-270*	3633	-375*	47.65	1.58**	47.13	1.96**	2402	-43	1723	-88
PC-33	5461	153	3887	-121	44.37	-1.71**	43.76	-1.41*	2426	-19	1705	-106
PC-38	5543	236	3776	-232	44.69	-1.38**	42.67	-2.50**	2478	32	1603	-208**
PC-46	5217	-90	3894	-114	48.84	2.77**	47.08	1.90**	2552	107	1834	23
PC-49	5276	-31	4058	49	46.02	-0.06	45.17	0.00	2428	-17	1834	23
PC-55	5457	149	3709	-299	48.86	2.79**	46.85	1.68**	2667	222**	1735	-76
PC-56	5313	6	3840	-168	47.16	1.09*	46.72	1.54**	2501	56	1794	-17
PC-61	4799	-508**	4497	489**	46.29	0.22	46.82	1.65**	2219	-227**	2107	296**
L-26	5212	-95*	4001	-7	47.83	1.75**	46.43	1.26**	2496	51	1857	46
L-95	5375	68	3925	-83	43.64	-2.43**	42.95	-2.22**	2345	-100	1685	-125**
L-48	5335	27	4099	91	46.76	0.68**	46.13	0.96**	2495	49	1890	79**
Mean	5307		4008		46.08		45.17		2445		1810	

*significant at p=0.05 probability level; **s significant at p=0.01 probability level

Contrary to seed yield, significance of the GCA effect was very stabile for the seed oil content according to the locations. Restorer lines PC-252 and RH-1 had the highest highly significant positive GCA effects, whereas line PC-19 showed the highest highly significant negative GCA effects in both locations. According to GCA effects for seed oil content, we can divide all of the tested lines into three groups: lines with significant positive GCA effects (PC-18, PC-32, PC-46, PC-55, PC-56, PC-61, L-26 and L-48), lines with significant negative GCA effects (RH-49, RH-SP, PC-19, PC-20, PC-21, PC-22, PC-33, PC-38 and L-95) and lines without significant GCA effects (PC-26, PC-29, PC-30 and PC-49).

The average oil yield of the experimental hybrids ranged from 2445 kg/ha in the location Rimski Sancevi to 1810 kg/ha in the location Banatska Topola. Experimental hybrids with common parents PC-252 had the highest mean of oil yield (2731 and 2015 kg/ha) in both locations. Considering results from both locations, the best combiner for oil yield was restorer line PC-252. Significant and positive GCA effects were recorded for RH-49, RH-SP, PC-18, PC-55, PC-61 and L-48 in one location in terms of oil yield, while lines RH-49, PC-19, PC-22, PC-29, PC-30, PC-38, PC-61 and L-95 had negative and significant GCA effect for the same character. Restorer lines with high positive GCA effects for seed yield, seed oil content and oil yield are good candidates to be used as parents in a population improvement program.

The hybrids which showed significant positive SCA for seed yield on location Rimski Sancevi were PC-30 x L-26, PC-26 x L-48 and PC-33 x L-48, while on the second location there was no significant SCA. These hybrid combinations involved low x low combining parents indicating over dominance and epistatic interaction. In case of involvement of both poor general combiners in some hybrids or one of the parents as poor general combiner, these hybrids expressed significant SCA effects in the desirable direction which might be due to concentrations and interaction between favorable genes contributed by parents. For seed oil content, the best specific combiner was experimental hybrid RH-1 x L-48 because it had significant positive SCA effects in both locations. Both parents had high GCA effects suggesting additive x additive type of gene action, what indicating that selection could be effective in progenies and utilized in transgressive breeding. Average seed oil content of that hybrid combination was 51.08 %. The best specific combiners for oil content had high x high and high x low GCA parental combination, indicating a genetic interaction of the additive and nonadditive types. Similar results were obtained by Chandra (2001). The analysis of the SCA effects in the 60 evaluated experimental hybrids showed that the best specific combiners for oil yield were PC-30 x L-26 in the first location and PC-56 x L-95 in the second location. Only seven hybrids expressed significant positive SCA effects, while only seven showed significant negative effects. Based on high significant SCA three hybrid combinations PC-30 x L-26, PC-26 x L-48 and PC-33 x L-48 were identified as promising for seed and oil yield.

Table 4. Average contributions (%) of the lines, testers, and their interactions to the expression of seed yield, seed oil content and oil yield

Average Contribution	Seed yield		Oil content		Oil yield	
	RS	BT	RS	BT	RS	BT
Lines	6.13	6.95	5.43	4.71	6.38	5.11
Testers	0.50	0.48	4.27	3.90	1.26	2.47
Lines/ testers	93.37	92.57	90.30	91.40	92.35	92.42

A line x tester analysis of sunflower with three cytoplasmic male sterile lines as testers and twenty restorer lines were carried out to obtain the average contribution of lines, testers and lines x testers to the total variance for seed yield, seed oil content and oil yield (Table 4). The average contribution of lines x tester interaction was very high for all of the investigated traits. It revealed preponderance of male and female interaction influenced for all these traits, indicating the importance of nonadditive effects that are involve in the control of these traits. These results were in agreement with Khan et al. (2008) and partially in agreement with Gvozdenovic et al (2008b).

Nonadditive gene effects were predominant for the expression of seed yield, seed oil content and oil yield. Among the newly developed lines that were tested, restorer RH-SP was best general combiner for seed yield, PC-252 and RH-1 for seed oil content and PC-252 for oil yield. Based on a high significant SCA three hybrid combinations PC-30 x L-26, PC-26 x L-48 and PC-33 x L-48 were identified as promising for seed and oil yield. The performance of these experimental hybrids needs to be critically evaluate over different years and locations to confirm their superiority and stability. The average contribution of lines x tester interaction was very high for all of the investigated traits, indicating the importance of the nonadditive effects involved in their inheritance.

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