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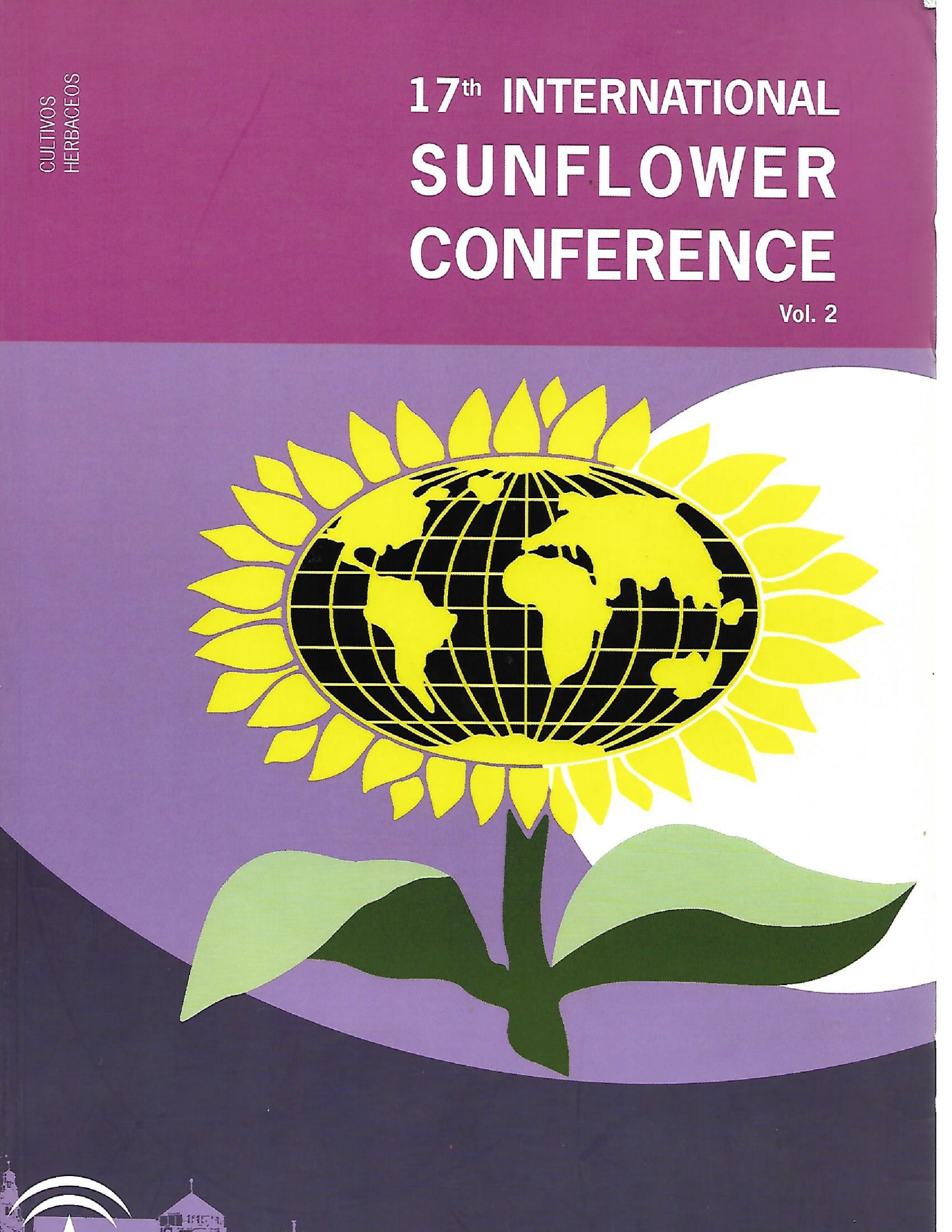

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Photography: *Feral sunflowers growing in a sunflower field in Santa Cruz, Córdoba, Spain.* Courtesy of Marie-Hélène Muller.

Foreword

The proceedings of the *17th 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
17th International Sunflower Conference
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WILD SPECIES AND GENETIC RESOURCES

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

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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_1 hybrids using the line x tester method (Singh and Choudhary, 1976). Seeds of the 60 F_1 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_1 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:

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

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

where, F_1 is the F_1 performance, MP = $(P_1+P_2)/2$ in which P_1 and P_2 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_1 combination from each tester line separately and all tester lines.

RESULTS AND DISCUSSION

Parental lines and 60 F_1 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 coeficient of variation (V) for the sunflower parental lines and their F_1 hybrids

Trait	Female line		F_1 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_1 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_1 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.

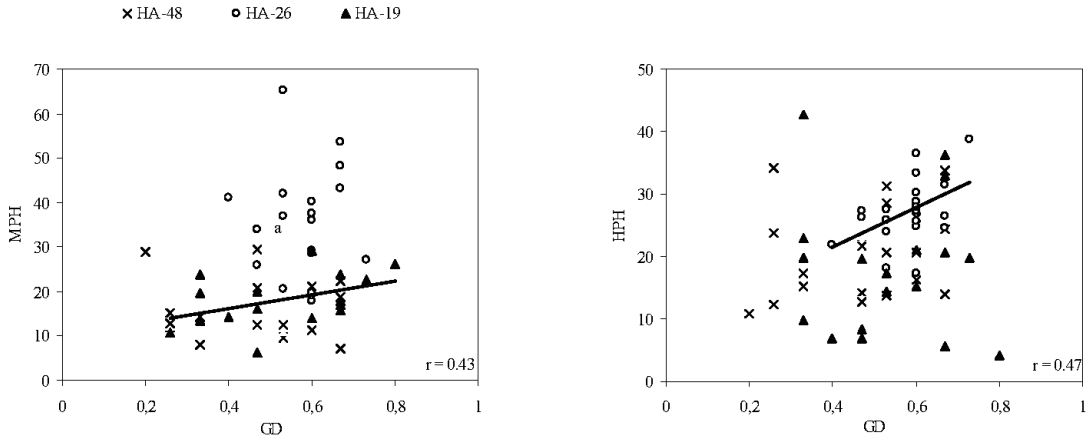

Fig. 1. Plots of genetic distance vs. mid-(MPH) and better-parent heterosis (BPH) for plant thousand seed weight (left) and plant height (right) of sunflower hybrid combinations ($r_{(0,05)}$=0.42, $r_{(0,01)}$=0.54).

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

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