

CORRELATION BETWEEN HETEROSIS AND GENETIC DISTANCE BASED ON SSR MARKERS IN SUNFLOWER (*Helianthus annuus* L.)

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Abstract: The objective of this study was to determine the correlation between SSR based genetic distance (GD) of new NS sunflower inbred lines and heterosis for six agronomic traits. Twenty three sunflower inbred lines (20 restorer lines and three female lines used as testers) and their hybrids were examined 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 ($r=-0.33$ $p<0.01$). 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: sunflower, heterosis, genetic distance, correlations.

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 costly and long

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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. Utilization 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 (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 its genetic diversity with enzymes (Tersac et al. 1993), RFLP markers (Gentzbittel et al. 1994, Berry et al. 1994, Zhang 1995, Hongtrakul 1997) and SSR markers (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 were 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 mass (TSM), 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:

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

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

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 better parent 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 were used in the study (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 first separated on 2% agarose (voltage 220 V) and then on 6% denaturing polyacrylamide gels (power voltage is constant 50W), for the fine separation. 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 using Pearson's coefficient

of correlation. Correlations were done for F₁ combination from each tester line separately and all tester lines. Significance of correlation was determined using table of Snedecor (1959).

Results and Discussion

Parental lines and 60 F₁ hybrids were evaluated in field trials for plant height, head diameter, thousand seed mass, oil content, seed yield per plant and oil yield per plant. There were 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.

Tab. 1. - Mean values, standard error of the means and coefficient of variation (V) for the sunflower parental lines and their F₁ 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 mass (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 trait, 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%).

Tab. 2. - Mean values and range of heterosis (%) for six quantitative traits of the 60 F₁ sunflower hybrids (PH=plant height, HD=head diameter, TSM=thousand seed mass, OC=oil content, SY=seed yield per plant and OY=oil yield per plant)

	Heterosis	PH	HD	TSM	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 mass (0.226 and 0.245).

Tab. 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, TSM=thousand seed mass, OC=oil content, SY=seed yield per plant and OY=oil yield per plant)

	PH	HD	TSM	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 the most examined traits. The poor correlation might be due to the 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 can not accurately predict hybrid performance unless the DNA markers used in the analysis were linked to the genes affecting the trait. Therefore, the 60 F₁ 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, but not highly, 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 predictive value.

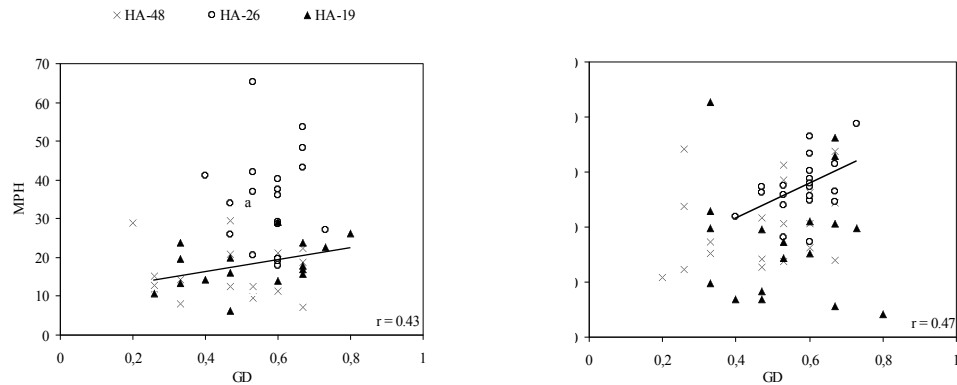


Fig. 1. - Plots of genetic distance vs. mid-(MPH) and better-parent heterosis (BPH) for plant thousand seed weight (left) and plant height (right) of sunflower hybrid combinations (table values Snedecor (1959), $r_{(0,05)}=0.42$, $r_{(0,01)}=0.54$).

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 enzymatic 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.

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 correlation of GD with heterosis (Melchinger et al. 1990, Diers et al. 1996, Geleta et al. 2004, Zeid et al. 2004, Riday et al. 2003).

Conclusion

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.

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ODNOS IZMEĐU HETEROZISA I GENETSKE UDALJENOSTI
ODREĐENA SSR MARKERIMA KOD SUNCOKRETA
(*Helianthus annuus* L.)

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Re z i m e

Cilj rada je utvrđivanje odnosa između genetske udaljenosti (GD) koja je određena SSR markerima (mikrosatelitima) kod novih inbred linija suncokreta i heterozisa za šest agronomskih svojstava. Ispitano je dvadeset tri inbred linije (dvadeset restorer linija i tri tester linije majki) i njihovi F1 hibridi za sledeća svojstva: visina biljke, prečnik glave, masa 1000 semena, sadržaj ulja u semenu, prinos semena i ulja po biljci. Zapažen je značajan heterozis kod hibridnih kombinacija za sva ispitivana svojstva sem za sadržaj ulja. Genetska udaljenost između parova ispitivanih inbred linija je bila od 0,13 do 0,8. Između genetske udaljenosti i heterozisa, posebnih kombinacionih sposobnosti i srednjih vrednosti svih ispitivanih svojstava, nije bilo značajne pozitivne korelacije. Jedina značajna vrednost, ali negativna, bila je u odnosu srednje vrednosti ulja i genetske udaljenosti ($r=-0.33$ $p<0.01$). Iako se genetskom udaljenosti slabo može predvideti heterozis, bolji će se rezultati dobiti ako se analiziraju hibridne kombinacije za svaki tester i svako svojstvo pojedinačno.

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