

THE RELATIONSHIP BETWEEN HETEROSIS AND GENETIC DISTANCES BASED ON SSR MARKERS IN *HELIANTHUS ANNUUS*

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ABSTRACT

Identifying the best inbred combinations for the development of commercial hybrid of sunflower remains the main challenge to sunflower breeders. In the present research the level of heterosis of F₁ hybrids, genetic diversity of parental lines based on SSR markers, as well as its connection with specific combining ability of sunflower were studied. Ten sunflower elite inbred lines (3 restorer lines and 7 cytoplasmic male sterility lines) and their hybrids were examined for plant height, seed yield, thousand seed mass, oil content and husk content. Field tests were carried out in 5-6 seasons. The level of heterosis was calculated using measurement of midparent heterosis. Genetic distance between pairs of tested sunflower inbred lines ranged from 0.45 to 0.74. Significant positive correlation was found between genetic distances among lines, measured using SSR markers and midparent heterosis for seed yield of hybrids ($r = 0.79$ $p < 0.05$). The correlation between genetic distances and the level of midparent heterosis for other studied agronomic traits was not reliable. The dependence of seed yield of hybrids on genetic distances among parental lines may be used for planning of effective crossbreeding of sunflower. Further research is needed to determine the best inbred combinations for the development of commercial hybrid of sunflower.

Keywords: *Helianthus Annuus*, Inbred Line, Hybrid, Heterosis, SSRs, Genetic Distance

1. INTRODUCTION

Development of heterotic hybrids is currently the main focus of sunflower breeding (*Helianthus annuus*). At the same time, open-pollinated varieties are being substituted in agricultural industry for hybrids. The advantages of heterosis hybrids of F₁ generation compared to varieties are higher productivity, rate of development and resistance to biotic and abiotic environmental factors. Moreover, exceptional equalization of hybrids by seedling height and period of ripening is also considered to be a virtue (Collard and Mackill, 2008).

Presently obtained data suggest that heterotic organisms demonstrate increased plasticity, more effective

homeostasis and well-balanced metabolism based on non-allelic and intergenomic interactions (Milborrow, 1998).

Although there are a number of hypotheses concerning the nature of heterosis none of them fully explains its molecular biological mechanisms (Lamkey and Edwards, 1999; Manousos, 2013; Wongchai, 2014).

Most of scientists suggest that formation of heterosis is controlled by both nuclear and cytoplasmic determinants and realization of their complementary interactions depends on environmental conditions (Lippman and Zamir, 2006; Aubaidan *et al.*, 2014; Saedi *et al.*, 2014). Highly productive heterotic hybrids can be obtained only from parents with genetically predetermined combining ability. Introduction of DNA-

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marker analyses into biological studies opened new opportunities for investigation of heterosis role in selective process; particularly for development of parental inbred lines and analysis of their combining ability (Lariepe *et al.*, 2012; Daisy and Selvi, 2014; Karthikeyan and Rengarajan, 2014).

Use of molecular markers for prediction of heterosis on the basis of genetic diversity of parental lines was demonstrated on rice (Zhang *et al.*, 1996), wheat (El-Maghraby *et al.*, 2005), rape (Riaz *et al.*, 2001), soybean (Woody *et al.*, 2011) and maize cultures (Shieh and Thseng, 2002; Kiula *et al.*, 2008).

However, the results of different studies are often controversial with respect to connection between genetic distances and heterosis (Riday *et al.*, 2003; Geleta *et al.*, 2004; Teklewold and Becker, 2006; Zeid *et al.*, 2003; Ghaly and Al-Sowayan, 2014; Malini and Vasanthanayaki, 2014; SenthilSingh and Manikandan, 2014).

In the present research the level of heterosis of F₁ hybrids, genetic diversity of parental lines based on SSR markers, as well as its connection with specific combining ability of sunflower were studied.

2. MATERIALS AND METHODS

2.1. Plant Materials and Field Evaluation

Highly productive heterotic hybrids and elite parental sunflower lines, which were kindly provided by V.S. Pustovoyt Don Experimental Station of the Research Institute for Oil Cultures were studied for selectively valuable traits under field conditions (**Table 1**).

The study was carried out on the basis of don experimental station for oil cultures (azov district, Rostov region) located on common carbonate chernozem.

Field trials were conducted on 25-50 m² allotments in six repeats using dotted sowing. Distance between rows was 70 cm. Each allotment consisted of 4-6 rows. Edge rows of each allotment were excluded from analysis.

Several important agronomic traits of plants were tested. These were plant height (cm), seed yield (centner per hectare), 1000-seed weight (g), oil content and husk content (%). Field tests were carried out in 5-6 seasons (2007-2013).

2.2. DNA Isolation and PCR

Genomic DNA was isolated from young sunflower leaves as described in (Boom *et al.*, 1990).

Twelve SSR primers were used in PCR analysis: Ha 432, Ha 514, Ha 1442, Ha 1608, Ha1287, IUB6, ORS6, ORS 509, IUB4, HNCA1, OSU-1, HNCA2 (Zhang *et al.*, 2005; Antonova *et al.*, 2006).

Table 1. Hybrids zoned in Rostov region

Hybrid name	Origin (parental lines)
Donskoy 151	♀ VD151 × ♂ VD541
Partner	♀ ED1443 × ♂ VD541
Donskoy 22	♀ VD22 × ♂ VD541
Garant	♀ ED931 × ♂ VD62
Prestige	♀ ED169 × ♂ VD62
Donskoy 1448	♀ VD1448 × ♂ VD62
Signal	♀ ED236 × ♂ VD110

Amplification was carried out in palm cyclor corbett research amplifier (Australia). Temperature regime was adjusted for each pair of primers on the basis of their sequence. For most reactions, optimal regime was initial denaturation at 96°C for 2 min followed with 30 cycles of primer annealing at 55-60°C for 40 sec, 1 min elongation at 70°C, 30 sec denaturation at 94°C and 2 min final elongation.

2.3. Data Analysis

The level of midparent heterosis (MPH) of hybrids was calculated for each of the traits as follows:

$$MPH = ((V_h - (V_p + V_m)/2) / (V_p + V_m)/2) * 100\%$$

where, V_h-hybrid trait, V_p-paternal trait, V_m-maternal trait.

In order to perform quantitative assessment of polymorphism between the sunflower lines studied, data of SSR analysis were presented as a matrix of binary trait conditions, in which presence or absence of similar size amplicons in SSR spectra was indicated as 1 or 0. Only those amplicons, which are clearly visible on electrophoregram were assigned as 1.

Genetic Distances (GD) (Jaccard's coefficient of community) between parental lines were calculated with WinBoot (Yap and Nelson, 1996) program on the basis of the matrix of binary trait conditions.

Polymorphic Information Content (PIC) of SSR markers was calculated as follows:

$$PIC_i = 1 - \sum_{j=1}^n P_j^2$$

where, P is frequency of j pattern for locus i and sum is distributed on n patterns.

To perform correlation analysis spearman's rank correlation coefficient was calculated (Online Handbook of Biological Statistics <http://udel.edu/~mcdonald/statspearman.html>).

3. RESULTS

3.1. Heterosis of F₁ Hybrids

Heterosis coefficient values (MPH, %) of six hybrids with respect to mean values of parental form traits are shown in **Table 2**.

To obtain first six hybrids shown in the table only two paternal lines (VD 541 Rf, first group-hybrids Donskoy 151, Partner and Donskoy 22; VD 62 Rf, second group-hybrids Garant, Prestige and Donskoy 1448) were used, whereas maternal line was unique for each hybrid. Therefore, these combinations of hybrids may be considered as a unique genetic model that allows analyzing of contribution of maternal organism into heterosis. In this case, the hybrid Signal may be related to the third, so called “outer control group”.

It was shown that crossbreeding of variable maternal lines with the same paternal line led to different level of heterosis by selective agronomic traits in F₁ (**Table 2**). In the first group of hybrid combinations the major effect was observed for seed yield of the hybrid Partner (283% increase with respect to mean value of parental lines), while the minor effect was observed for the hybrid Donskoy 22 (207% increase with respect to mean value of parental lines). In the second group of hybrid combinations the minor heterotic effect was observed for yield of the hybrid Donskoy 1448 (120% increase with respect to mean value of parental lines), while hybrids Garant and Prestige demonstrated higher values (150-160% increase with respect to mean value of parental lines).

Therefore, it can be concluded that that genome of maternal line affects yield of sunflower hybrids. The effect of cytoplasm on heterosis expression with respect to this trait was also demonstrated.

Similar results were obtained for parameters of plant height and weight of 1000 seeds. Similarly to the parameter of yield, the major effects on the parameters of plant height and weight of 1000 seeds in the first and second group was observed in hybrids partner and garant respectively. On the whole, hybrids of the first group demonstrated stronger heterotic effect by all selective traits studied except plant height. The latter was most affected in hybrids of the second group. This phenomenon can be explained by the same genetic basis of hybrids within a group determined by a paternal line. The hybrid Signal, which belonged to the third group, demonstrated intermediate position with respect to the level of heterosis.

3.2. SSR-Polymorphism

Highly productive sunflower hybrids cultured in Rostov Region were genotyped using SSR markers. Electrophoretic spectra of the parental lines of these hybrids were analyzed (**Table 3**). Reliable individual SSR spectra, which differed by the number of amplicons and electrophoretic mobility were estimated for each line.

Analysis of 12 microsatellite markers of these sunflower genotypes revealed 42 alleles. Number of alleles varied from 2 to 8 for each locus (**Table 3**). Size of detected DNA fragments varied from 110 to 900 b.p. In order to analyze amplification of microsatellite DNA, polymorphism information content index (PIC) was estimated. It was expected to mirror informativeness of the studied markers and varied from 0.1 (for IUB 4 primer) to 0.9 (for Ha 1287 primer). Mean PIC value for the group of genes studied was 0.48 (**Table 3**). Primer HNCA1 appeared to be monomorphic in the set of genotypes studied.

3.3. Genetic Distances Between Parental Lines

Genetic distances that show genetic difference of parental genotypes of highly productive sunflower hybrids were calculated on the basis of SSR analysis (**Table 4**).

It is quite obvious that this system of markers allowed us to reveal high genetic diversity between parental cytoplasmic male sterile and fertility restorer inbred lines of sunflower. Highest genetic distances were found between parental lines of the hybrid Signal, which is related to the outer group. On the whole, hybrids of the first group demonstrated higher genetic diversity of parental lines compared to hybrids of the second group, similarly to heterosis.

3.4. Correlation Between Genetic Distances and Heterosis

Data of correlation between genetic distances and heterosis by the selection traits studied are shown in **Table 5**.

The correlation between genetic distances and the level of midparent heterosis was not reliable in almost all studied agronomic traits. Negative insignificant correlation was revealed between GD and parameters of plant height, as well as GD and oil content. Positive low correlation was found between GD and weight of 1000 seeds, as well as between GD and husk content. The analysis showed significant positive correlations of GDs assessed by SSR with midparent heterosis for seed yield of hybrids (**Table 5**).

Table 2. Heterosis effect of hybrids with respect to mean values of parental form traits

Hybrid	Seed yield (%)	Oil content (%)	Husk content (%)	1000 seed weight (%)	Height plant (%)
Donskoy151	249,7±5,1	2,5±0,6	3,0±0,1	24,4±0,3	27,1±2,6
Partner	283,3±3,9	4,7±1,1	-0,9±0,2	31,6±1,2	35,0±1,6
Donskoy22	207,6±8,7	-1,9±0,2	7,3±0,9	25,2±3,8	26,4±2,3
Garant	159,0±6,2	11,4±0,8	-7,2±0,9	20,3±1,7	42,3±1,7
Prestige	151,5±7,0	25,6±4,2	-18,3±0,7	7,9±0,9	36,5±2,6
Donskoy1448	120,5±4,4	11,2±1,2	-11,7±0,5	11,7±1,4	35,5±2,3
Signal	210,8±6,7	7,2±0,3	-1,1±0,2	25,7±2,6	28,5±0,9

Table 3. Primers used for SSR analysis of sunflower breeds and hybrids cultivated in Rostov region

Locus	Number of alleles	PIC	PCR product size, b.p.
<i>Ha 432</i>	8	0,72	180-900
<i>Ha 1442</i>	6	0,63	170-240
<i>Ha 1608</i>	4	0,67	220-270
<i>IUB 4</i>	2	0,10	130-190
<i>ORS 509</i>	3	0,48	190-210
<i>Ha514</i>	3	0,60	180-210
<i>IUB6</i>	2	0,50	350-370
<i>ORS6</i>	2	0,38	250-260
<i>Ha1287</i>	6	0,90	210-400
<i>HNCA1</i>	1	0,0	110
<i>HNCA2</i>	3	0,42	210-340
<i>OSU-1</i>	3	0,34	150-160
Mean value	3,6	0,48	

Table 4. Genetic distances between parental sunflower lines calculated on the basis of SSR analysis

Paternal lines	Maternal lines	Genetic distances	Hybrids
VD54	VD151	0,72	Donskoy151
	ED1443	0,64	Partner
	VD22	0,57	Donskoy22
VD62	ED931	0,55	Garant
	ED169	0,57	Prestige
	VD1448	0,45	Donskoy1448
VD11	ED236	0,74	Signal

Table 5. Spearman's rank correlation coefficient between genetic distances and midparent heterosis by the studied agronomic traits

	Genetic distances
Genetic distances	1
Seed yield	0,79*
Oil content	0,43
Husk content	0,65
1000-seed weight	0,65
Plant height	-0,56

*p<0.05

4. DISCUSSION

We studied polymorphisms of nucleic genome microsatellite loci of seven sunflower hybrids and their parental lines cultivated in Rostov Region. The material for selection was obtained from don experimental station

for oil cultures (azov district, Rostov region). Several-year field studies have shown that hybrids of this selective breeding demonstrate high level of heterosis by the majority of valuable agronomic traits analyzed.

In the first generation, heterosis can occur either by one or several traits. Sometimes, F₁ hybrid yields the parental lines by some of the traits, though excels both of them by combinations of traits (including multiple ones) in genotype. This type of heterosis is especially important in case of combination in one hybrid such traits as seed size, high productivity, early maturation, etc., which typically correlate negatively with one another. It is also significant for overcoming of either negative effects of undesirable recessive alleles of one of crossbreeding participants or their pleiotropic effects. Recently, new evidences that advantageous properties of F₁ hybrids are based on a positive effect of hybridization of the whole system of genetic interactions in

ontogenesis rather than its single components were published (Lariepe *et al.*, 2012). Nevertheless, it is widely assumed that the genetic components of heterosis are still obscure (Lippman and Zamir, 2006).

Eleven out of twelve SSR markers were found to be polymorphic and suitable for genotyping of lines and hybrids of Russian selection. These SSRs may be also informative for analysis of polymorphism and specific combining ability of parental sunflower lines in order to develop new crossbreeding schemes for obtainment of highly productive hybrids. In total, 42 allelic variants were found. Most of the alleles were detected for Ha 432 and Ha 1442 primers. Primer HNCA1 appeared to be monomorphic within the set of sunflower genotypes studied. Assessment of genetic distances by the studied loci revealed significant differences between maternal and paternal lines of sunflower heterotic hybrids.

Presently, the problem of connection of genetic distances between parental lines and heterosis of F_1 hybrids is still a matter of discussion. Early studies on maize demonstrated that genetic distances estimated on the basis of RFLP markers were well corresponding to the data of pedigree for distributing lines to heterotic groups (Stuber, 1995; Stuber *et al.*, 1992; Williams *et al.*, 1995). Similar data were obtained by RAPD (Shieh and Tseng, 2002). On the other hand, studies on soya showed no association between genetic distances and heterosis by RFLPs (Cerna *et al.*, 1997), whereas association between isozyme loci was revealed in oilseed rape.

Later studies with hybrid performance in maize showed that significant correlation between genetic distances and heterosis occurred in intragroup crossbreeding of inbred lines rather than in intergroup crossbreeding (Menkir *et al.*, 2004; Kiula *et al.*, 2008). Significant correlation between genetic distances (RAPD and AFLP) among inbred lines and heterosis by yield was shown in 34 hybrid combinations of carrot. However, no correlation between genetic distances and qualitative characteristics of carrot was found (Jagosz, 2011). A possibility to use genetic distances between parental lines to predict heterosis in F_1 hybrids of wheat was demonstrated as well (Krystkowiak *et al.*, 2009).

On the other hand, studies with hybridization performances conducted in mustard and alfalfa showed significant correlation between heterosis and morphological distances; whereas genetic distances calculated on the basis of DNA polymorphism (SSR, RAPD, AFLP markers) did not demonstrate such correlation (Riday *et al.*, 2003; Teklewold and Becker, 2006). No reliable correlation was found using genetic distances by SSR markers to predict specific combining ability of sunflower parental lines (Gvodenovic *et al.*,

2009). Low correlation between GD and hybrid performance was also shown in studies conducted in agricultural plants, such as maize, pepper, soya, alfalfa, wheat, rape and cotton. (Barbosa-Neto *et al.*, 1996; Cerna *et al.*, 1997; Riday *et al.*, 2003; Geleta *et al.*, 2004; Landi *et al.*, 2012; Livini *et al.*, 1992; Zeid *et al.*, 2003).

Studies carried out on 30 hybrid combinations of oilseed rape (*Brassica napus* L) revealed different levels of association between genetic distances and hybrid performance by several agriculturally significant traits, but not by seed yield (Yu *et al.*, 2005). Despite restrictions in application of this approach for prediction of heterosis, the parameters of seed yield and hybrid plant height demonstrated significant correlation with genetic distances calculated via AFLP fingerprinting in sunflower (Cheres *et al.*, 2000). The authors also showed no such correlation with genetic distances calculated on the basis of the coancestry matrix (Cheres *et al.*, 2000).

Such contradiction between the data of different studies may be explained by using of different molecular markers that deal with different parts of genome and different breeding material. Our study carried out with highly heterotic hybrids (long-term study) using SSR markers revealed reliable correlation between genetic distances and seed yield heterosis. However, no correlation with other agronomic traits studied was observed.

5. CONCLUSION

It is obvious that the data obtained in the present study cannot be used directly for prognosis of heterotic effect in F_1 sunflower hybrids. Nevertheless, this information may be important for understanding of nature of heterosis and development of new approaches for selective breeding of agriculturally important plants.

It may be concluded that the studied system of markers demonstrates high discrimination potential for identification of cultivated sunflower lines. The dependence of genetic distances on crop-producing power may be used for planning of effective crossbreeding of sunflower. Further research is needed to determine the best inbred combinations for the development of commercial hybrid of sunflower.

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