

# The estimation of heterosis effect based on some molecular indexes (RAPD) at sunflower

## 2. The genetic distance – breeding factor in obtaining of productive hybrids

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### Abstract

*It was studied the heterosis effect based on RAPD polymorphism at sunflower. The polymorphism was shown using eleven homo- and heterozygous sunflowers' genotypes. The objectives of our study were to estimate the effect of hybrid vigor using molecular markers, to classify the breeding material, to appreciate the genetic similarity and genetic distance between parental lines and hybrids. With the aim of revealing the molecular polymorphism were used five polymorphic RAPD primers. As result of agarose gel electrophoresis molecular polymorphism was revealed. Based on genetic similarity and genetic distance of 11 homo- and heterozygous sunflower genotypes was shown that big genetic distance among parental lines is associated with hybrid performance.*

Key words: heterosis, sunflower, genetic similarity, genetic distance

### Introduction

Programs of sunflower breeding are based on the employment of the heterosis phenomenon, which requires the selection of crossing lines that could have the biggest effect in F<sub>1</sub> generation. The economic importance of hybrid vigor leads to many fundamental and applicative studies that refer to evaluation of parental line selection criteria and prognosis of hybrid performance [8, 10].

Many morpho-physiological and physiologo-biochemical studies were done, which revealed the peculiarities of photosynthesis [12], respiration [2], activities of different enzymes [7], accumulation of mineral substances and metabolites [5, 9] in homozygous lines and F<sub>1</sub> hybrids. This way was conjectured that the probably of heterosis manifestation in F<sub>1</sub> depends on genetic distance between parental lines used in breeding [11]. As rule, on purpose of obtaining high productive hybrids, the parental lines should be characterized by essential differences in conformity with the specter of components of protein markers [1].

The appreciation of combinative capacity by traditional breeding methods is expensive and of long-term, that is why it was attempted to use isoenzymes [16], RFLP analysis [6] and other molecular markers for prognosis of hybrid performance [17, 18].

The molecular organization of DNA molecule reflect the functional parameters of genome, and respectively, of cell and of plant in integrity. Hence could be solved several problems concerning revealing the nature of hybrid vigor and its prognosis by different techniques of molecular biology [14].

The aim of our research was to establish the classification mode of breeding material depending on genetic diversity based on RAPD amplicons and to estimate the existing associations between genetic distance and hybrid performance.

### Materials and Methods

As object of study were used 4 hybrids of sunflower (Xenia, Oxana, Performer, Valentino) and their parental lines, yield of 2002 – breeding material was offered by Science and Production Association MAGROSELECT, Soroca.

Total genomic DNA was isolated from fresh leaf material with extraction buffer (133 mM Tris-HCl, pH 7.8; 6.7 mM Na<sub>2</sub>EDTA; 0.95 M NaCl; 1.33% Na sarcosyl; 1.33% mercaptoetanol), following the purification with mixture CH<sub>3</sub>Cl/isoamyl alcohol 24:1, ulterior it was precipitated with isopropanol, following the washing

with 76% ethanol with 0.2 M Na acetate and with 70% ethanol. At the end DNA was dissolve in 100-300  $\mu$ l bidistilled water.

### RAPD analysis

For RAPD (Random Amplified Polymorphic DNA) amplification were used 5 arbitrary primers - P2, P6, P8, P37, P39, designate and selected World Nucleotide Banc and selection programs.

**Table 1.** Genetic polymorphism within RAPD analysis

Primer	Nucleotide sequence	GC composition	Loci
P2	gAc AgA cAg AcA	50	20
P6	gAg cAA gTT cAg ccT g	56	13
P8	cAg gAA AcA gcT gAc	53	9
P37	cTg Acc Agg Agc	67	12
P39	ccA ggT cgc c	80	15
Total			69

PCR reaction was realized in a volume of 30 $\mu$ l in following conditions: 0.1 mg/ml DNA, buffer 10x (670 mM Tris-HCl, pH 8.8; 67 mM MgCl<sub>2</sub>; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 0.1% Tween 20), dNTP mixture (2 mM of each), 0.1  $\mu$ M primer, 1u/ $\mu$ l Taq-polymerase. Amplifications were performed in DNA Thermo-cycler programmed for the following conditions: 5 min at 95°C; for 45 consecutive cycles: 1 min at 95°C, 1-2 min at 37-42°C, 2 min at 72°C; and finally 7 min at 72°C.

Following amplification, the samples were subjected to electrophoresis in 1.5% agarose gel. The electrophoresis was realized in TAE (0.004 M Tris-acetat; 0.001 M EDTA; pH 8.0).

Approximate sizes of amplification products were determined by reference to electrophoretic mobilities of markers with known sizes: "Smart", "Eurogentec" 200-3000 pb.

This analysis has as initial point the presence of a band at a given level designated as "1," and its absence, "0." Based on scored data were calculated the following indices [11, 15, 19]: the similarity coefficient

$GS = \frac{2 \times N_{ij}}{N_i + N_j}$ , where  $N_{ij}$  – the number of common bands for two samples,  $N_i$  and  $N_j$  - the number of bands in samples  $I$  and  $J$  and genetic distance (GD) – [6]  $GD = -\ln(GS)$ , where GS – similarity coefficient.

The matrices of genetic distances were constructed. Further, these matrices were subjected to cluster analysis (unweighted pairwise method with arithmetic mean (UPGMA)) [13].

## Results and Discussion

RAPD analysis was performed using random primers (P2, P6, P8, P37 and P39). These primers have produced a total number of 396 bands, 124 (31.3 %) from which were polymorph. There were stated among 3-10 bands to each genotype, with length of 90-2950 bp [4].

Cluster analysis, based on the appreciation of the genetic similarity and distance of different sunflower's genotypes, was carried out on the obtained RAPD sequences. According to data, related literature [19] there were analyzed the genetic polymorphisms of genotypes.

The dendrogram based on RAPD loci revealed the presence of five main clusters: A, B, C, D and E (fig. 1)

*The group A* included three of 12 sunflower's genotypes - Xenia ♀, Xenia F<sub>1</sub> and Oxana ♀, with genetic similarity of 0.57-0.81 and with an average of 0.69. First generation hybrid Xenia showed high resemblance of 0.77 with its maternal line.

Valentino family genotypes and male sterile line Performer represented *the cluster B*. The genotypes from this group were characterized by a similarity of among 0.71. Valentino family was of special interest, because all representatives of this family were localized in the same cluster. There was displayed that the hybrid genotype Valentino was similar, as well to paternal form (0.78), as to maternal lines (0.77). The Valentino parental genotypes was less analogous (0.66) between them. In this cluster was grouped Performer maternal line, which showed a resemblance of 0.65 with all genotypes from the group B.

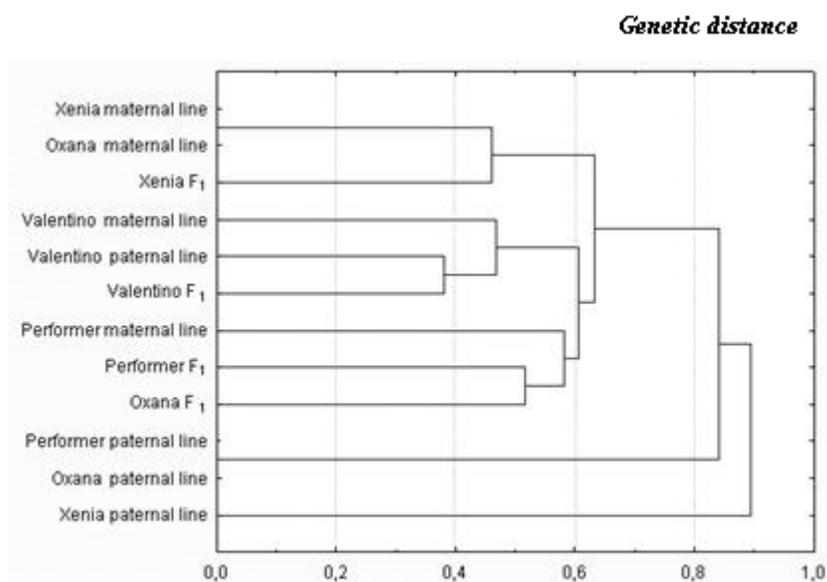
The genotypes Performer ♀, Performer F<sub>1</sub> and Oxana F<sub>1</sub> were grouped in *C cluster*, with similarity of 0.64-0.74. Performer first generation hybrid was 64% maternal line like.

The restorer fertility line of Performer group represented *cluster D*. This genotype presented significant difference from other genotypes placed in clusters A and B, genetic similarity between them was about 0.43-

0.75. The biggest genetic similarity was revealed between Performer paternal line and Performer F<sub>1</sub> (0.75), while the biggest genetic distance was revealed between this line and Valentino maternal genotype.

*Cluster E* was separated from the other clusters and it was represented by the Xenia paternal genotype. At the same time, this genotype differs from the rest genotypes proving a maximum similarity coefficient of 0.66 with Xenia F<sub>1</sub> and minimum of 0.41 with the Valentino maternal genotype.

### Genotype



**Figure 1.** Repartition dendrogram of sunflower's genotypes based on amplification products with arbitrary primers

The comparative analysis of genetic distance (GD) at homozygous genotypes of four studied families disclosed values in the range GD = 0-1.50, and varied subject to primer. Parental forms of the Valentino hybrids were more similar from the genetic point of view, while the more significant differences were revealed in the Oxana homozygous lines. The Xenia and Performer parental genotypes were characterized by intermediary values of genetic distances (tab. 2).

**Table 2.** Genetic distance of sunflower's homozygous genotypes

Primer	Genotypes	Genetic distance			
		Performer	Xenia	Oxana	Valentino
P2	♀-♂	0.85	0.61	0.87	1.25
P6	♀-♂	0.63	0.69	0.53	0.49
P8	♀-♂	0.41	0.69	0.15	0.29

<b>P37</b>	♀-♂	0.00	0.19	0.06	0.06
<b>P39</b>	♀-♂	0.69	0.61	1.50	0.32
<b>Average</b>		<i>0.52</i>	<i>0.56</i>	<i>0.62</i>	<i>0.48</i>

Genetic similarity between homo- and heterozygous genotypes displayed values over the range 0.29-1.00. RAPD sequences amplified with P2 primer shown low GS's values and it was stated that genome regions corresponding to P37 primer were inherited in the most part in F<sub>1</sub> (tab. 3).

**Table 3.** Genetic similarity of sunflower's homo- and heterozygous genotypes

Primer	Genotypes	Genetic similarity			
		Performer	Xenia	Oxana	Valentino
<b>P2</b>	♀-F <sub>1</sub>	0.46	0.54	0.60	0.33
	♂-F <sub>1</sub>	0.95	0.50	0.57	0.55
<b>P6</b>	♀-F <sub>1</sub>	0.62	0.50	0.84	1.00
	♂-F <sub>1</sub>	0.94	0.67	0.56	0.61
<b>P8</b>	♀-F <sub>1</sub>	0.29	1.00	0.89	0.67
	♂-F <sub>1</sub>	0.57	0.50	0.75	0.91
<b>P37</b>	♀-F <sub>1</sub>	0.94	0.95	0.95	0.94
	♂-F <sub>1</sub>	0.94	0.89	0.89	1.00
<b>P39</b>	♀-F <sub>1</sub>	0.92	0.83	0.40	0.89
	♂-F <sub>1</sub>	0.36	0.77	0.37	0.83

Valentino hybrid genotype showed the biggest GS to parental genotypes, being more similar to paternal line. The Oxana hybrid was characterized by the smallest values GS in comparison with homozygous lines and this hybrid inherited the most part of genetical information from maternal line. Xenia and Performer first generation hybrids were characterized by intermediary values of genetic similarity to homozygous genotypes, in one case the hybrid was more similar to maternal line (Xenia), while in other case to the paternal one (Performer).

Generalizing the obtained results (tab.3) we can conclude that the small genetic distance (0.48) within the Valentino parental homozygous lines obtained as result of RAPD specters analysis explains the reduced productivity (25 c/ha) in comparison with the productivity of Oxana hybrid (29 c/ha). The last hybrid was characterized by the biggest mitotic activity [3], while the RAPD study revealed that the Oxana hybrid was more polymorphic (0.1-0.75) in comparison with the other hybrids (0-62.5). The amplicon specters clusterization separated the heterozygous genotype from the both parental genotypes, results which show a big genetic distance between them ( $GD_{♀-F_1}=0.30$  in comparison with  $GD_{♂-F_1}=0.46$ ).

## Conclusion

The performed cluster analysis on studied genotypes allowed the determination of sunflower genotype similarity, which can be helpful in work material classification for breeding and selection the pairs of parental lines for obtaining heterozygous hybrids.

The analysis of sunflower genotype dendrogram by RAPD loci revealed the presence of five main clusters: A, B, C, D and E, where the genotypes were grouped depending on existing similarities and differences between RAPD amplicons patterns.

The comparative analysis of genetic distance of homozygous genotypes revealed that the most similar were the parental lines of Valentino hybrid, while the biggest difference were signed at homozygous genotypes Oxana, whereas Xenia and Performer parental lines were characterized by intermediary values of genetic distance.

The study of genetic similarity established that Valentino hybrid genotype was more resembling to paternal line, and Oxana hybrid was characterized by the smallest values of similarity coefficient with homozygous lines.

Thus the biggest genetic distance between parental lines was revealed for Oxana hybrid, which was characterized by the biggest productivity (2900 kg/ha).

The results relate the applicability of RAPD analysis in sunflower breeding programs, while the information about genotypes' similarity and filiations can be useful for classification of work material in order to select pair lines with the purpose of obtaining high quality hybrids.

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