GENETIC DISTANCE EVALUATION, A USEFUL TOOL IN HETEROSIS EFFECT PREDICTION OF SUNFLOWER GENOTIPES

EVALUAREA DISTANȚEI GENETICE ÎN SCOPUL PRONOSTICĂRII EFECTULUI DE HETEROZIS LA DIVERSE GENOTIPURI DE FLOAREA-SOARELUI

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Abstract. *The goal of the research was to assess the RAPD banding pattern among different cytoplasmic male sterile, fertility restorer lines and hybrid sunflower genotypes to be associated with restorer of fertility and heterotic traits. It is discussed the efficiency of parental selection based on genetic distance by RAPD polymorphisms clustering. Also, screening of three Operon primers, previously reported as polymorphic, revealed four amplification products ($OPG10_{510}$; $OPG10_{680}$; $OPI16_{450}$ and $OPI16_{550}$) specific only for studied Rf lines, suggesting on their potential use for indirect selection of fertility restorer trait.*

Key words: sunflower, CMS lines, Rf lines, heterosis effect, hybrids

Rezumat. *Scopul cercetării a constat în evaluarea polimorfismului genetic la diverse genotipuri ASC, Rf şi F_1 pentru evidențierea gradului de heterozis şi identificării unor potențiali markeri asociați cu capacitatea de restaurare a fertilității polenului. Este discutată eficiența de selecție a liniilor parentale utilizând distanța genetică în baza profilelor RAPD. Totodată, au fost identificați ampliconi RAPD ($OPG\ 10_{510}$, $OPG\ 10_{680}$, $OPI\ 16_{450}$ şi $OPI\ 16_{550}$) specifici liniilor restauratoare care ulterior, pot fi utilizați în elaborarea markerilor moleculari linckați cu acest caracter.*

Cuvinte cheie: floarea-soarelui, linii ASC, linii Rf, vigoare hibridă, hibrizi

INTRODUCTION

The RAPD (Random Amplification of Polymorphic DNA) technique is successfully used from various laboratories to investigate the genetic polymorphism, especially due to the experiment low cost. Although, other marcher technology are considered more reliable and reproducible, the good experimental optimization could assure the effective parent selection for hybrid breeding related various important qualitative and quantitative traits (productivity, high resistance to different stress factors).

The objective of this study was to assess the genetic distance based on RAPD polymorphism with OPG10, OPI16 and OPH15 primers among different cytoplasmic male sterile, fertility restorer lines and hybrid sunflower genotypes.

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MATERIAL AND METHOD

Six fertility restorer lines (*Xenia; Drofa; Valentino; LC Raus; LC 4; LC Cium)*, seven cytoplasmic male sterility lines (*Xenia; Drofa; Valentino; LC40; SW38; VIR101PET1; VIR116PET1*), commercial (*Drofa, Xenia, Valentino*) and experimental *(LC40 CMS x LCRaus Rf; Drofa CMS x LC43 Rf; Drofa CMS x VIR 681 Rf)* F_1 hybrids were investigated in a three-year field experiment carried out in a randomized complete block design with 3 replications.

Total genomic DNA for RAPD analysis was isolated from young leaves using CTAB (Doyle J.J., 1990). The purity and yield was analyzed by 1% agarose gel electrophoresis and UV absorbance (A260/A280) using PG instruments UV.VIS NC 60 spectrophotometer.

PCR amplification was performed in the following mixture: 25 ng DNA, dNTP 200μM of each type, 1,25 U per reaction of GoTaq ADN-polymerase (Promega), 2,5 mM $MgCl_2$ and 0,1 μM of decamere primers (Nandini R., 2005): OPG10 (5 `-AGGGCCGTCT-3 `); OPI16 (5`-TCTCCGCCTT-3 `) and OPH15 (5`-AATGGCGCAG-3 `) in the Corbett Research thermocycler, programmed with the cycling profile: initial denaturation at 95°C for 5 min followed by 40 cycles: 95°C - 1 min, 36°C – 1,5 min, 72°C - 2 min. and a final extension at 72° C for 3 min. Amplification products were displayed through electrophoresis in 1,5% agarose gel with 0,5 μg/ml ethidium bromide in presence of 50 bp DNA ladder (Sigma, Saint Louis, Missouri, USA). The electronic images of ethidium bromide stained gels were documented using Photo-Capt Analysis System. The experiments described above were repeated 2-3 times with the same DNA extract and the negative control samples contain all components of the PCR except genomic DNA. No amplification products were seen for any primer. Obtained amplification products were marked according primer designation and band size, for example: $OPG10_{510}$.

Amplified fragments were scored as the presence (+) or absence (—) of a fragment. Genetic distance (GD) between the genotypes were estimated according to the Nei and Li cited by [PVIGILA D., 2005: $GD = 1-[2Nxy / (Nx + Ny)]$, where N*x* is the number of bands in individual X, N*y* is the number of bands in individual Y, and N*xy* is the number of RAPD bands present in both X and Y. Dendrogram was constructed with STATISTICA 7 program (using Euclidian distance for clustering).

RESULTS AND DISCUSSIONS

The analyze of three primers profiles (OPG10, OPI16, OPH15) to select new combination between different Moldavian parental lines and especially to identify potential RAPD markers associated with fertility restoration trait, revealed 15 - 27 amplified fragments per primer depends of genotype.

The OPG10 generated a series of specific amplification products in terms of their molecular masses and amounts and was more informative to other studied primers. Twenty bands were polymorphic – table 1.

Significant differences in OPG10 profiles was ascertained among Rf lines especially respecting the banding pattern that comprise primarily of high molecular weight bands. The maternal lines are characterized by more homology between comigrating bands in contrast with F_1 hybrids and paternal forms, their profile including only 4 polymorphic fragments: $OPG10_{350}$, $OPG10_{950}$, $OPG10_{1200}$ and $OPG10_{1400}$.

Table 1

RAPD-OPG10 profiles at *Rf*, *CMS* and F_1 genotypes

OPG10	Amplification products																			
	1900	1800	1700	1600	1550	1400	1200	1050	950	800	750	700	680	510	450	440	400	380	350	150
Fertility restorer lines																				
Xenia				+		+			+	+		+	+	+						
Drofa						+			+			+	+	+			+	+	+	+
Valentino			+	+	+	+			+		+		+	+			+	+	+	+
Raus				+			+		+		+		+	+		+		+		+
LC 4				+		+					+		+	+		+		+		
Cium						+	+			+			+	+						+
Cytoplasmic male sterility lines																				
Xenia			+		+	+	+		+							+		+		
Drofa			+			+	+		+							+		+		+
Valentino			+			+	+	+								+		+	+	+
LC 40			+					+								+		+	+	+
SW 38			+					+								+		+		
VIR 101			+					+								+		+	+	+
VIR 116	+		+					+								+		+		+
Hybrids genotypes (*CMS x Rf*)																				
Xenia	+	+						+							+		+		+	
Drofa								+							+			+		+
Valentino						+		+				+			+				+	+
LC 40 x Raus		+						+				+			+	+		+	+	+
Drofa x LC43						+		+				+			+			+	+	
Drofa xVIR 681		+				+		+				+			+			+	+	

The most male specific fragments amplified by the investigated primer were inherited by their respective hybrids, substantiating the hybridizing level. Only two from all ascertained polymorphic fragment: 510 bp and 680 bp have shown a constant presence in all restorer forms (three replicates) denoting an association with fertility restorer trait. These were considerate as potential RAPD markers linked to the *Rf* genes - $OPG10_{510}$ and $OPG10_{680}$. The densitometry analysis of these bands at paternal lines showed a variable fluorescence intensity (especially for 680 bp) that could be presented in decreasing order as higher intensity at *LC Cium, Xenia, Valentino* followed by *LC 4, Drofa* and *LC Raus* (figure 1).

The OPI16 RAPD profile as those generated by OPG10 revealed polymorphisms among the amplification products of different homo- and heterozygote genotypes. About 70 percentage of fragments showed absence/presence polymorphism, which could be particularly useful for discriminating most of the hybrids along with their parents. It should be emphasized that these RAPD profiles have differentiated between sunflower lines carrying the dominant (*Rf* lines) and homozygous recessive allele of fertility restorer genes (*CMS* lines) through two weak $OPI16_{450}$ and $OPI16_{550}$ bands, that are more intensive at Xenia *Rf* and Valentino *Rf*. Also, the $OPI16_{590}$ there is present at all parental lines and absent for hybrids – table 2.

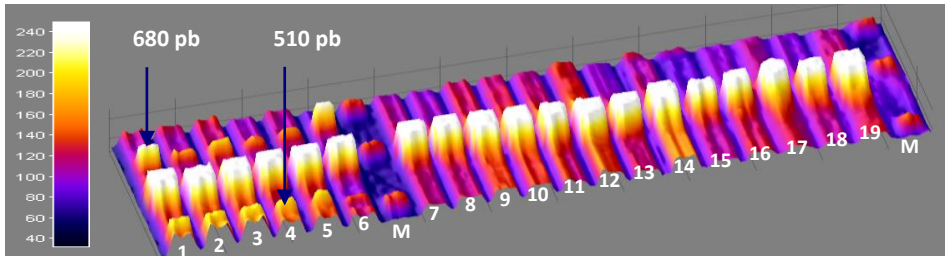

Fig. 1 – Densitometry analysis of $OPG10_{510}$ and $OPG10_{680}$; *1-Xenia; 2-Drofa; 3-Valentino; 4-LC Raus; 5-LC 4; 6-LC Cium; CMS: 7-Xenia; 8-Drofa; 9-Valentino; 10-LC 40; 11-SW 38; 12-VIR 101 PET 1; 13- VIR 116 PET 1; F_1: 14- Xenia; 15- Drofa; 16- Valentino; 17-LC Raus Rf x LC 40 CMS; 18-Drofa ASC x LC 43 Rf; 19-Drofa ASC x VIR 681 Rf.*

Table 2

RAPD-OPI 16 profiles at *Rf*, *CMS* and F_1 genotypes

OPI 16	Amplification products																		
	2000	1800	1700	1600	1500	1450	1250	1100	1000	950	880	790	600	590	550	500	450	350	320
Fertility restorer lines																			
Xenia				+	+	+			+	+	+	+		+	+	+	+	+	+
Drofa				+		+				+	+	+		+	+	+	+	+	+
Valentino				+					+	+	+	+		+	+	+	+	+	
Raus				+		+			+	+	+	+		+	+	+	+	+	
LC 4				+		+				+	+	+		+	+	+	+	+	
Cium				+				+	+		+	+		+	+	+	+	+	
Cytoplasmic male sterility lines																			
Xenia				+				+			+	+		+		+		+	
Drofa				+								+		+		+		+	
Valentino				+				+			+	+		+		+		+	
LC 40				+		+		+			+	+		+		+		+	
SW 38		+		+		+		+			+	+		+		+		+	
VIR 101				+				+				+		+		+		+	
VIR 116				+				+	+		+	+				+		+	
Hybrids genotypes (*CMS x Rf*)																			
Xenia	+		+	+	+		+		+		+	+	+			+		+	+
Drofa							+					+	+			+		+	
Valentino					+			+			+	+	+			+		+	
Raus x LC 40				+	+	+					+	+	+			+		+	
Drofa x LC 43				+	+						+	+	+			+		+	
Drofa x VIR 681				+	+	+					+	+	+			+		+	+

The analysis of OPH 15 primer's RAPD profiles revealed a total number of 15 amplicons. The more intensive fragments have a length between 350 and 1000

bp. As compared to the previous primers screening that showed a possibility to distinguish one variety from other no relevant heterogeneity can be detected among the amplification products of investigated parental and hybrid forms. Several polymorphisms (absence/ presence type) observed - $OPH15_{1700}$ and $OPH15_{1900}$ appear ambiguous and with specificity more of genotype than fertility restorer trait, thus not being useful as genetic markers associated with trait of interest.

In addition to linkage with target traits, the developing of molecular markers is determined by its applicability in efficient parental selection based on genetic distance assaying. Parental selection is the first step in any plant breeding program. It is known that the probability of recovering a superior progeny genotype is greater if genetic distance among parents is greater. Thus, the greatest value of genetic distances among parental genotypes can be seen between: *Xenia ASC* and *Xenia Rf*; *Valentino CMS* and *Cium Rf*; *VIR116 CMS* and *Cium Rf*; *LC40 CMS* and *Xenia Rf; LC40 CMS* and *LC Raus Rf*. Such information could be used for the prediction of heterosis and combining ability effects to design of new hybrid combinations (figure 2).

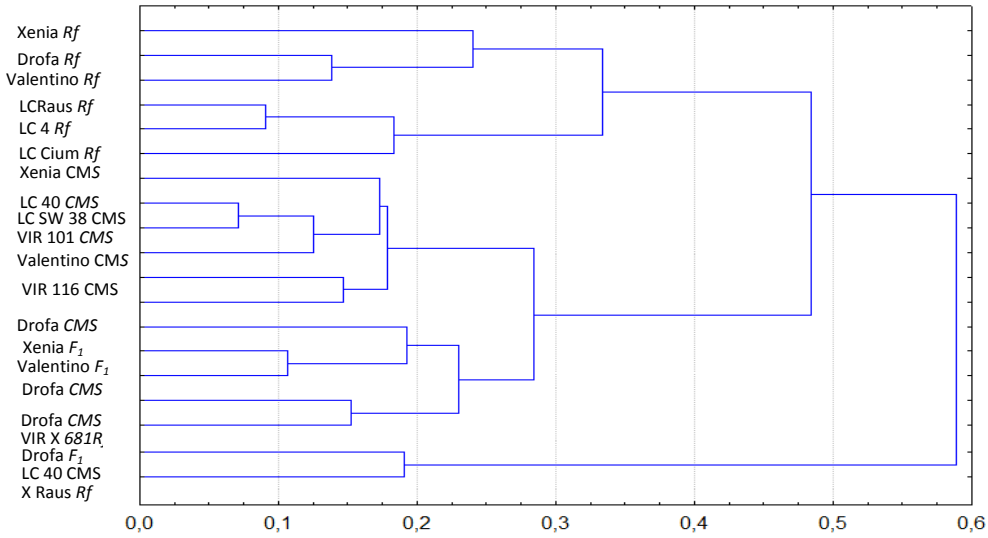

Fig. 2 - Clustering dendrogram of sunflower parental lines and hybrids based on RAPD patterns.

These findings have been verified by phenotype data (Midoni A., 2010) that indicated high values of the experimental hybrids productivity to average of parents and commercial hybrids.

CONCLUSIONS

1. Screening of three Operon primers, revealed four amplification products ($OPG10_{510}$; $OPG10_{680}$; $OPI16_{450}$ and $OPI16_{550}$) specific only for studied *Rf* lines, suggesting on their potential use for indirect selection of fertility restorer trait.

2. The discriminating RAPD polymorphisms revealed the greatest value of genetic distances among following parental genotypes: *Xenia ASC* and *Xenia Rf*; *Valentino CMS* and *Cium Rf*; *VIR116 CMS* and *Cium Rf*; *LC40 CMS* and *Xenia Rf; LC40 CMS* and *LC Raus Rf*. Such information could be used for the prediction of heterosis and combining ability effects.

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