CLUSTERING ANALYSIS OF SUNFLOWER GENOTYPES CULTIVATED IN MOLDOVA ON THE BASIS OF MICROSATELLITE SEQUENCES

ANALIZA CLUSTERIANĂ A GENOTIPURILOR DE FLOAREA-SOARELUI CULTIVATE ÎN REPUBLICA MOLDOVA ÎN BAZA SECVENȚELOR MICROSATELITE

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Abstract. *A number of 21 homo- and heterozygous sunflower genotypes were studied by applying 13 pairs of SSR primers. Data revealed a relatively high ability of distinction of genotypes based on SSR loci as shown by the general grouping of all analyzed genotypes as well as the separate clusters of parental and hybrid genotypes. Analysis of distribution of genotypes based on microsatellite sequences gives the possibility of usage of selected markers in fingerprinting and hybridization degree estimation.*
Key words: clustering analysis , SSR markers, sunflower

Rezumat. *A fost studiat un număr de 21 genotipuri homo- şi heterozifote de floarea-soarelui prin analiza SSR cu 13 perechi de primeri. Datele obținute au pus în evidență capacitatea relativ înaltă de distincție a genotipurilor în baza locilor SSR, fapt demonstrat prin gruparea generală a tuturor genotipurilor analizate, dar şi clusterizarea separat a genotipurilor parentale sau hibride. Analiza de repartiție a genotipurilor în baza secvențelor microsatelite demonstrează posibilitatea aplicării markerilor selectați în amprentarea genotipurilor şi estimarea gradului de hibridare a formelor hibride.*
Cuvinte cheie: analiza clusteriană, markeri SSR, floarea-soarelui

INTRODUCTION

SSR markers have a very high level of polymorphism, allowing broad use for genomic fingerprinting and mapping, study of genetic and phylogenetic relationships, marker assisted selection and population genetics.

Development of SSR markers for cultivated sunflower (Tang et al., 2002, Yu, 2002) offers solutions to scarcity of DNA markers and obtaining of reference maps by the combination of several genetic linkage maps elaborated independently by different researchers. Tang and coauthors developed the first comprehensive genetic map of sunflower based on SSR makers in 2002. From the set of 1089 SSR markers described by Tang and coauthors (2002) and Yu et al. (2002), 717 showed polymorphism in elite inbred lines and 408 in RHA280 x RHA801.

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Actually, SSR markers are considered the most effective, but their use remains limited, due to the difficulty and time-consuming stages of their development. There are two general strategies in the development of SSR markers:

- identification of microsatellite sequences in available databases;
- obtaining and screening of genomic libraries with corresponding microsatellite probes.

SSR markers selection strategy based on the search of sequences stored in databases - EMBL, GenBank, etc., is relatively simple and quick. It is important to mention that the exploration of data on expressed sequences, contributes to loss of a quantity number of sequences with potential for generating sequence polymorphism, because microsatellites are generally present in non-coding regions of the genome. Most genetic maps based on sunflower SSR markers can be accessed from Sunflower CMap Database http://sunflower.uga.edu/cmap/.

SSR profile can be used for estimation of genetic variability, serving as a criterion for selection of parental forms for obtaining of highly productive hybrids, since it is known that genetic distance of various genotypes is positively correlated with hybrid performance (Dua and Yadava, 1985, Miller et al., 1980).

The aim of the research consisted in the SSR analysis performed for 21 homo- and heterozygous genotypes and clustering based on their profiles for determination of the genetic diversity both within the parental lines as well as in relation to hybrid forms.

MATERIAL AND METHOD

As a study material have served 21 sunflower genotypes, which include Rf lines (Drofa, LC Raus, LC 637, LC 7 and LC 39), CMS lines (Drofa, Xenia, LC 40, LC 38 and LC SW 391A) and first generation hybrids (Drofa, Xenia, LC 40 ASC x LC Raus Rf, Drofa ASC x LC Raus Rf, Drofa ASC x LC 637 Rf, Drofa ASC x LC 7 Rf, LC SW 38 ASC x LC 637 Rf, LC SW 38 ASC x LC 4 Rf, Drofa ASC x LC 39 Rf, Xenia ASC x LC 39 Rf şi LC 40 ASC x Xenia Rf). Extraction of DNA was performed from etiolate seedlings using CTAB standard protocol (Doyle and Doyle, 1990) with some modifications.

SSR reaction was performed in a final volume of 10 µl: 1 µl DNA, 1 µl 10X buffer with $(NH_4)_2SO_4$, 0.8 µl 25 mM $MgCl_2$, 2,5 mM dNTP 1 µl, 6 pmol primer and 1,25 units of Taq DNA polymerase (Dial, Russia). Touchdown PCR was runed at MyCycler™ Thermal cycler (Bio-Rad) using the following conditions: 94°C for 2 min, 1 cycle at 94°C - 30 s, 63°C - 30 s and 72°C - 45 s, 5 cycles at 94°C - 30 s, 62°C - 30 s - 1°C/cycle and 72°C - 45 s, followed by 30 cycles at 94°C - 30 s, 56°C - 30 s and 72°C - 45s, with final extension at 72°C - 5 min. Were selected primers from ORS series labeled with Cy5 fluorescent dye: 78, 237, 243, 349, 366, 432, 509, 546, 595, 656, 811, 815 and 836. Primers were chosen on the base of information from literature, by the level of polymorphism (Solodenko and Sivolap, 2005).

Amplification profile was analyzed on ALFexpress ™ II DNA sequencer Analysis System (Amersham Biosciences) using denaturant gel electrophoresis in 6,0% polyacrylamide gel at a voltage of 400 V for 2 h.

Estimation of genetic distance was done using the software Treecon (http://bioinformatics.psb.ugent.be/software/details/3) according to Nei and Li (1979) and clustering was performed using UPGMA method.

RESULTS AND DISCUSSIONS

From 13 pairs of SSR primers used for genotyping of sunflower lines three primer pairs (ORS432, ORS546, ORS595) have not generated a clear profile and thus were excluded from the analysis. Other primers: ORS78, ORS237, ORS243, ORS349, ORS366, ORS509, ORS656, ORS811, ORS815, ORS836 showed different levels of polymorphism. To appreciate the differences between markers, PIC (Polymorphic Information Content) was used, calculated according to Anderson et al. (1993). Thus, the average value of the parameter PIC was 0,589, the highest of 0,85 for the lowest for ORS815 and ORS237 0,59. SSR loci included in the research according to the number of repetitions represented four di-, four three-, and one hexanucleotidic, and one complex repetition.

According to the linkage groups (LG) described for sunflower (Tang et al., 2002) there were identified one locus on LG1, LG2, LG4, LG5, LG6, LG8, LG16 and LG17, and two loci on LG10.

Table 1

Inherintance of SSR markers in hybrids and their parental forms

Genotype	*ORS78*				*ORS237*			*ORS243*					*ORS349*		
bp	156	161	165	167	195	198	201	143	147	164	167	171	253	255	263
Drofa ASC															
Drofa Rf															
Drofa F1															
Drofa ASC															
LC Raus Rf															
Drofa ♀ x LC Raus Rf															
Drofa ASC															
LC 637 Rf															
Drofa ♀ x LC 637 Rf															
Drofa ASC															
LC 7 Rf															
Drofa ♀ x LC 7 Rf															
Drofa ASC															
LC 39 Rf															
Drofa ♀ x LC 39 Rf															
LC 40 ASC															
LC Raus Rf															
LC 40 ASC x LC Raus Rf															
LC SW 38 ASC															
LC 637 Rf															
LC SW 38 ASC x LC 637 Rf															
Xenia ASC															
LC 39 Rf															
Xenia ♀ x LC 39 Rf															

Table 1 (continuation)

Inherintance of SSR markers in hybrids and their parental forms

Genotype	*ORS366*				*ORS509*			*ORS811*			*ORS815*					*ORS836*		
bp	187	209	211	213	179	193	199	109	113	156	173	178	181	186	190	198	201	207
Drofa ASC	■						■	■	■		■	■						■
Drofa Rf			■			■			■	■	■	■				■		
Drofa F1	■		■			■	■	■	■	■	■	■				■		■
Drofa ASC	■						■	■	■		■	■						■
LC Raus Rf	■	■				■			■	■			■	■	■			■
Drofa ♀ x LC Raus Rf	■	■				■	■	■	■	■	■	■			■			■
Drofa ASC	■						■	■	■		■	■						■
LC 637 Rf	■				■				■	■				■	■			■
Drofa ♀ x LC 637 Rf	■				■		■	■	■		■	■			■			■
Drofa ASC	■						■	■	■		■	■						■
LC 7 Rf	■				■				■	■	■	■						■
Drofa ♀ x LC 7 Rf	■				■		■	■	■	■	■	■						■
Drofa ASC	■						■	■	■		■	■						■
LC 39 Rf	■	■					■		■	■		■	■		■		■	■
Drofa ♀ x LC 39 Rf	■	■				■	■		■	■	■	■	■		■		■	■
LC 40 ASC	■					■			■			■	■			■		
LC Raus Rf	■	■				■			■	■			■	■	■			■
LC 40 ASC x LC Raus Rf	■	■				■			■	■		■	■		■	■		■
LC SW 38 ASC	■			■		■			■			■	■			■		
LC 637 Rf	■				■				■	■				■	■			■
LC SW 38 ASC x LC 637 Rf	■			■	■	■			■		■		■		■	■		■
Xenia ASC		■					■		■	■		■	■				■	
LC 39 Rf	■	■					■		■	■		■	■		■		■	■
Xenia ♀ x LC 39 Rf	■	■				■	■		■	■		■	■		■		■	

*Grey color indicates the presence of amplicons

SSR loci analysis allows estimation of the hybridization degree of hybrid forms based on the codominant nature of their inheritance. Thus, for F_1 hybrid Drofa ORS237, ORS366, ORS509, ORS811 and ORS836 markers showed co-dominance (table 1).

In case of experimental hybrid Drofa ♀ x LC Rf Raus, codominance was demonstrated for markers ORS509, ORS811, ORS815 and ORS836. Markers ORS78, ORS243, ORS509, ORS811 and ORS815 showed codominance in the same maternal line in combination with LC 637 Rf.

For hybrid Drofa 7 ♀ x LC Rf ORS78 codominance was revealed for following markers, ORS237, ORS243, ORS509 and ORS811. Hybrid combination of the same maternal line, but with a different paternal line LC 39, showed only two codominant markers: ORS78 and ORS815. Markers ORS349,

ORS815 and ORS836 were inherited codominant by the hybrid LC 40 ASC x LC Raus Rf. Also was highlighted the hybrid LC SW 38 ASC x LC 637 Rf for which were revealed five codominant markers from 10 analyzed (ORS78, ORS243, ORS349, ORS509 and ORS836).

Thus, codominant nature of inheritance was demonstrated for 2-5 loci, depending on the hybrid genotype. However, parental genotypes can be clearly distinguished based on SSR profiles.

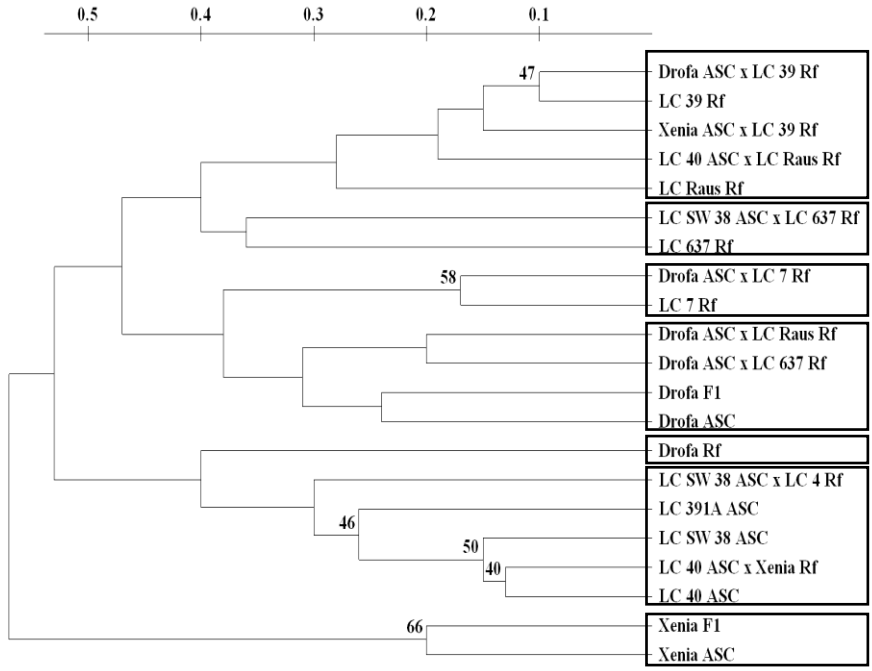

Fig. 1 - Dendrogram of the distribution of sunflower genotypes based on microsatellite sequences

Clustering analysis revealed seven distinct groups (fig. 1). The first group consists of paternal genotypes LC Raus Rf, LC 39 Rf and their hybrids: Drofa ASC x LC 39 Rf, Xenia ASC x LC 39 Rf, LC 40 ASC x LC Raus Rf. Although in hybrid combinations were used different maternal genotypes, the genetic profile is strongly influenced by the paternal form.

The second and third groups include paternal lines and hybrid forms properly: paternal genotype LC 637 Rf and it's hybrid LC SW38 ASC x LC 637 and LC 7 Rf and Drofa ASC x LC 7 Rf hybrid, respectively.

Cluster four contains maternal genotype and most of it's hybrids: Drofa F_1 şi Drofa ASC x LC Raus Rf, Drofa ASC x LC 637 Rf. Only one hybrid genotype obtained using LC 39 Rf parental form was classified in first group.

Drofa Rf genotype was positioned in cluster number five, while the sixth group includes three maternal genotypes LC 391A ASC, LC SW 38 ASC, LC 40 ASC and two hybrids LC 40 ASC x Xenia Rf, LC SW 38 ASC x LC 4 Rf.

The last group consists of genotype Xenia ASC and Xenia F_1 hybrid, which are characterized by high similarity. Also observed that another hybrid, which served as the Xenia ASC maternal form, is classified in the first cluster, which shows comparatively strong influence of the paternal genotype LC 39 Rf in the formation of this hybrid.

Thus, clustering showed substantial similarity between hybrids and maternal or paternal lines, characterizing a particular recombination capacity of parental genotypes. Profile similarity shows the nature of maternal or paternal genotype influence and inheritance of these profiles in hybrids.

CONCLUSIONS

1. 21 homo- and heterozygous genotypes were studied using SSR analysis with 13 primer pairs of which ORS78, ORS237, ORS243, ORS349, ORS366, ORS509, ORS656, ORS811, ORS815, ORS836 showed different levels of polymorphism.

2. Distribution analysis of sunflower genotypes based on microsatellite sequences shows possibility of aplication of the selected markers in genome fingerprinting and hybridization degree estimation.

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