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GENETIC DIVERSITY ANALYSIS USING RAPD MARKER IN INBRED LINES OF SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

RAPD marker was used to evaluate genetic relationships in a set of 16 inbred lines of sunflower representing the genetic stock, including restorers and maintainer lines, of the classical cytoplasmic male sterility. The genotypes were grouped into eight cluster at 0.83 coefficient level. A total of 164 bands were detected, of which 69.51% were polymorphic among the genotypes tested. The similarity coefficient was maximum between TNAU7 6/8 and EC 68414/1 (0.90) indicating less divergence between them. Lower similarity indices were observed between 62B and GP324 (0.67) and between 852B and GP 324 (0.68), indicating more divergence. Crossing between the genotypes with low similarity coefficient will manifest high heterosis.

Key words: sunflower, RAPD, similarity index

INTRODUCTION

Sunflower is a rich source of edible oil and is considered good from health point of view due to high concentration of polyunsaturated fatty acids. The importance of sunflower as an oilseed crop in India is of recent origin and dates back three decades only. The success of sunflower breeding depends on the ability to further exploit the vast amount of genes and gene combinations in the genepools that would produce higher heterosis. The magnitude of heterosis depends on the choice of appropriate parental lines. The genetic diversity studies using D^2 statistics of Mahalanobis (1936) is being used for selection of prospective parents for hybrid production. Nowadays, molecular genetic polymorphism is used to analyze the variability, becoming one of the most efficient approaches in plant genetics. Randomly amplified polymorphic DNA (RAPD) analysis is widely used for studying taxonomy of various genera (Devas and Gale, 1992), species (Igbal *et al.*, 1995; Faroog *et al.*,

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1995), for differentiation of intraspecies (Sweeny and Danneberger, 1995; Mackil, 1995) and to study the genetic diversity of various cultivars and lines. The recently developed RAPD (Williams *et al*., 1990) based on polymerase chain reaction (PCR) is widely adapted in genetic diversity analysis. A RAPD assay is rapid and easy to perform and also requires only limited amount of DNA. Recently RAPDs have also been extensively used for assessment of genetic variation. Earlier, inbred lines were discriminated only on the basis of morphological characters. Currently DNA markers such as restriction fragment length polymorphism (RFLP) have been applied to interspecific studies of the genus *Helianthus* (Choumane and Heizmann, 1988; Riesberg *et al*., 1988, 1990; Gentzbittel *et al*., 1992). Wagh and Powell (1992) described the use of genetic diversities among the grape cultivars *Vitis vinifera* (Grando *et al*., 1994). The aim of the present study was to estimate genetic similarities in a set of sunflower inbred lines.

MATERIAL AND METHODS

Sixteen genotypes *viz*., GP 225, GP 161, GP 270, GP 324, TNHAP 2, TNHAP 75, ARM 242, ARM 248, TNAUSUF 6/8, EC 68414/1, AC 18871-8, 2 B, 852 B, 336 B, 62 B and 7-1B, were used for RAPD marker analysis. The sixteen genotypes were grown in small-sized mud pots in the green-house (CPBG, TNAU). A total of five leaves were collected from each entry.

DNA extraction

The DNA was extracted following the protocol described by Gentzbittel *et al*. (1992) with modification, from frozen leaves stored at -80°C. The leaves cut into pieces were completely homogenized in liquid nitrogen. Extraction buffer (100 mM Tris HCl (pH 8.0), 20 mM EDTA, 1.4 mM NaCl, 2% CTAB per liter) was added in 50 ml tubes filled with leaf powder to a volume of 15 ml and mixed well. The tubes were incubated at 65°C for 30 minutes with repeated shaking. Equal volume of chloroform : isoamylalcohol mix (24:1) was added and mixed thoroughly for 15 minutes, followed by centrifugation at 4000 rpm for 30 minutes. Equal volume of isopropanol was added to the supernatant. DNA was hooked out after half an hour and washed in 70% ethanol and suspended in 500 μl of TE buffer (pH 8.0). The DNA was incubated with 10-15 μl (10 μg/μl concentration) of RNAse for 30 minutes. To this, equal volume of chloroform : isoamyl alcohol was added and centrifuged at 12,500 rpm for 10 minutes. Twice the volume of absolute ethanol and $1/10^{th}$ volume of 3 M sodium acetate were added to the aqueous layer and incubated overnight. The content was centrifuged at 12,500 rpm for 15 minutes and supernatant was discarded. The pellet was washed with 70% ethanol and air dried. Then, the pellet was dissolved in 500 μl of TE and stored at -20°C. The quality and quantity were checked through 0.8% agarose gel by electrophoresis. DNA concentration for PCR reaction was estimated, by comparing the band intensity produced by the known dilution that gave good amplification.

RAPD analysis

Genomic DNA was used as template for PCR amplification as described by Williams *et al*. (1990). A set of 20 arbitrary primers (OPERON Technologies, Inc. California, USA) was used to produce distinct marker profiles for 16 parental lines (Table 1). Amplification reactions were in the volumes of 20 μl containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, dATP, dCTP, dGTP and dTTPs each at 0.1 mM, 0.2 mM primer, 25-30 ng of genomic DNA mol 0.5 unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore). Amplification was performed with Thermal Controller (MJ Research Inc.) programmed for 40 cycles. After initial denaturation for two minutes at 94°C, each cycle consisted of one minute at 94°C, one minute at 36°C and two minutes at 72°C. The 40 cycles were followed by seven minutes of final extension at 72°C.

Table 1: List of random primers used in genetic diversity analysis

Nomber	Name of the primer	Sequence 5'to 3'
1	OPAB 02	5'-GGAAACCCCT-3'
2	OPAB 07	5'-GTAAACCGCC-3'
3	OPAB 08	5'-GTTACGGACC-3'
4	OPAB 09	5'-GGGCGACTAC-3'
5	OPAB 13	5'-CCTACCGTGG-3'
6	OPAB 18	5'-CTGGCGTGTC-3'
7	OPAB 19	5'-ACACCGATGG-3'
8	OPAK 04	5'-AGGGTCGGTC-3'
9	OPAK 05	5'-GATGGCAGTC-3'
10	OPAK 09	5'-AGGTCGGCGT-3'
11	OPAK 10	5'-CAAGCGTCAC-3'
12	OPAK 17	5'-CAGCGGTCAC-3'
13	OPAK 19	5'-TCGCAGCGAG-3'
14	OPAK 20	5'-TGATGGCGTC-3'
15	OPAL 15	5'-AGGGGACACC-3'
16	OPAL 17	5'-CCGCAAGTGT-3'
17	OPAM 02	5'-ACTTGACGGG-3'
18	OPAW 06	5'-TTTGGGCCCC-3'
19	OPAW 17	5'-TGCTGCTGCC-3'
20	OPAW 18	5'-GGCGAACTG-3'

PCR amplified products were subjected to electrophoresis on 1.5% agarose gel in 1 × TBE buffer at 120 V for 4 hours using Hoefer Super Submarine electrophoresis unit (Pharmacia Biotec, USA).

The electronic images of ethidium bromide stained gels were captured using Kodak Digital Science DC 120 Digital Camera (Eastern Kodak Company, Rochester, USA) and gels were documented using Electrophoresis Documentation and Analysis System (EDAS 120).

Data analysis

Data generated from the polymorphic fragments was scored as present (1) or absent (0) for each of the 16 genotypes. The diversity among the lines was worked out by subjecting the RAPD scores to cluster analysis. Sequential Agglomerative Hierarchic Non-overlapping (SAHN) clustering was performed on simple matching similarity matrix by Unweighted Pair Group Method with Arithmetic Averages (UPGMA). The data analysis was done using the software $NTSYS_{PC}$ version 2.02 (Rohlf, 1994).

RESULTS AND DISCUSSION

A set of 20 random primers was used in PCR reaction to amplify DNA fragments from 16 genotypes. A total of 164 bands were detected, of which 30.48% (50 bands) was monomorphic across all genotypes. The remaining 114 bands (69.51%) were polymorphic among the varieties tested. This accounted to an average of 8.22 polymorphic bands per primer indicating the high level of polymorphism expressed by arbitrary primers.

The primers OPAW 18 and OPAK 04 produced maximum number of fragments (12) followed by OPAM 02, OPAB 19 and OPAW 17 each with 11 fragments. The primer OPAW 06 recorded minimum number of fragment (3). The RAPD profiles for three primers *viz*., OPAB 13, OPAK 19 and OPAW 18, which produced diagnostic markers are given in plates 1 to 3. The simple matching similarity was calculated using RAPD score and dendrogram was constructed using unweighted pair group method with arithmetic averages (UPGMA) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). The genotypes were grouped into eight clusters at 0.83 coefficient level (Table 2).

Cluster I consisted of two subclusters, which included most of the testers. The subcluster A of cluster I consisted of GP 255 and ARM 242, while subcluster B comprised GP 161, ARM 248, TNASUF 6/8, EC 68414/1, TNHAP 2 and one maintainer, 336 B. Cluster II included AC 18871-B and 2B. Clusters III and VIII had one genotype each *viz.*, TNHAP 75, 852 B, 62 B, 7-1B, GP 270 and GP 324, respectively. The similarity coefficient was maximum between TNASUF 6/8 and EC 68414/1 (0.90) while, minimum was observed between 62 B and GP 324 (0.67).

Molecular markers analysis of the sixteen genotypes using 20 random RAPD primers produced polymorphism for most of the studied loci. As per the similarity index, the genotypes were grouped into eight clusters. The highest similarity index between the genotypes TNASUF 6/8 and EC 68414/1 (0.90) indicated less divergence between them. Low similarity indices were observed between 62 B and GP 324 (0.67) and between 852 B and GP 324 (0.68) which indicated more divergence. Crossing between the genotypes with low similarity coefficient will manifest high heterosis.

Table 2: Similarity coefficient among 16 sunflower genotypes based on RAPD analysis

	GP 255	GP 161	GP 270	GP 324	TNHAP 2	TNHAP 75	ARM 242	ARM 248	TNAUSUF 6/8	EC 6814/1	AC1887 1-8	2B	852 B	336 B	62 B	7-1B
GP 255	1.00															
GP 161	0.84	1.00														
GP 270	0.76	0.82	1.00													
GP 324	0.69	0.73	0.74	1.00												
TNHAP 2	0.84	0.83	0.80	0.72	1.00											
TNHAP 75	0.82	0.80	0.72	0.71	0.78	1.00										
ARM 242	0.85	0.82	0.74	0.73	0.79	0.83	1.00									
ARM 248	0.85	0.86	0.80	0.71	0.87	0.80	0.85	1.00								
TNAUSUF 6/8	0.85	0.88	0.77	0.70	0.86	0.86	0.87	0.89	1.00							
EC 68414/1	0.80	0.84	0.76	0.72	0.84	0.83	0.84	0.87	0.90	1.00						
AC 1887 1-8	0.80	0.82	0.74	0.74	0.79	0.83	0.83	0.82	0.83	0.86	1.00					
2B	0.80	0.80	0.73	0.76	0.79	0.78	0.82	0.79	0.82	0.84	0.85	1.00				
852 B	0.77	0.83	0.73	0.68	0.76	0.80	0.79	0.80	0.81	0.83	0.83	0.78	1.00			
336 B	0.83	0.80	0.72	0.67	0.79	0.78	0.76	0.76	0.82	0.79	0.79	0.79	0.79	1.00		
62 B	0.83	0.84	0.79	0.73	0.83	0.80	0.80	0.83	0.86	0.84	0.84	0.78	0.80	0.83	1.00	
7-1B	0.77	0.80	0.76	0.75	0.79	0.79	0.76	0.82	0.79	0.79	0.80	0.77	0.77	0.75	0.83	1.00

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ANÁLISIS DE LA DIVERGENCIA GENÉTICA MEDIANTE MARCADORES RAPD EN LAS LÍNEAS INBRED DE GIRASOL (*Helianthus annuus* L.)

RESUMEN

En el trabajo, mediante los marcadores RAPD, se evaluaron las relaciones genéticas dentro del grupo de 16 líneas inbred de girasol, que incluía las líneas restauradoras y las líneas mantenedoras de esterilidad citoplasmática masculina. Los genotipos eran clasificados en ocho grupos en el nivel de coeficiente de 0.83. En total se detectaron 164 fajas, de las cuales 69,51% eran polimorfas entre los genotipos investigados. El coeficiente de similitud era el mayor entre las líneas TNAU7 6/8 y EC 68414/1 (0.90), lo que indica un grado inferior de divergencia entre ellos. El menor índice de similitud, fue observado entre las líneas 62B y GP324 (0.67) y 852B y GP324 (0.68), lo que indica una mayor divergencia. El cruzamiento entre los genotipos de menor coeficiente de similitud, demostrará mayor heterosis.

ANALYSE DE LA DIVERGENSE GÉNÉTIQUE À L'AIDE DE MARQUEURS RAPD DANS LES LIGNES INBRED DE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

Les rapports génétiques à l'intérieur d'un groupe de 16 lignes inbred de tournesol incluant des lignes de restauration et des lignes de maintien de la stérilité mâle cytoplasmique ont été évalués à l'aide de marqueurs RAPD. Les génotypes ont été distribués en huit groupes à un niveau de coefficient de 0,83. Au total, 164 bandes ont été détectées parmi lesquelles 69,51% étaient polymorphes parmi les génotypes testés. Le coefficient de similarité était le plus grand entre les lignes TNAU7 6/8 et EC 68414/1 (0.90), ce qui indique un degré moindre de divergence. Un index inférieur de similarité a été constaté entre les lignes 62B et GP324 (0.67) et 852B et GP324 (0.68) ce qui indique une plus grande divergence. Le croisement entre les génotypes à moindre coefficient de similarité fera apparaître un plus grand hétérosis.