APPLICATION OF RAPD MARKERS FOR GENETIC DISTANCE ANALYSIS OF SUNFLOWER (*HELIANTHUS ANNUUS*) INBREDS

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Abstract

The genetic similarities of 24 sunflower inbreds were estimated using RAPD markers. Molecular genetic distances derived from Dice's similarity coefficient were based on 151 RAPD markers. Cluster dendrograms were generated for molecular genetic distances and genotypes were clustered into six groups. Genetic variability among male sterile counterparts, (maintainer lines) was lower than among the varieties and the germplasm accessions of sunflower. The maximum genetic distance distinguished by 20 decamer primers was 0.19 (81% similarity). The cluster analysis based on these markers revealed that the inbreds studied were not related to geographic distribution and the absence of such extreme groups may facilitate free gene flow among them. Thus population improvement and hybrid breeding programs can be effected with these inbreds.

Introduction

Since sunflower (*Helianthus annuus* L.) is a cross pollinated crop, the wide genetic variation is being exploited to produce commercial varieties. But the realization of potential performance of the genotypes is perturbed predominantly by soil factors, principally sodic soils. Exploring populations in sodic soil may warrant breeding for salt tolerant hybrids/varieties. Knowledge of the relative genetic distance among individuals or populations is useful in a breeding program because it permits organization of germplasm and provides more efficient sampling of genotypes (Nienhuis et al., 1993). At the inception of a breeding program, knowledge of the genetic relationships among genotypes could be used to complement phenotypic information in the development of breeding populations. Ultimately, knowledge of the genetic similarity between genotypes may facilitate the choice of individuals to cross in hybrid combinations to optimize expression of heterosis (Melchinger et al., 1990).

Classifying germplasm and breeding materials exclusively based on a few discrete morphological traits may not provide an accurate indication of genetic similarity since it is highly influenced by the environment (Menkir et al., 1997) and also time intense. DNA based markers are now widely used owing to their virtues (Mignouna et al., 1998; Rajora and Rahman, 2003; Paris et al., 2003; Lu et al., 2003). Among the DNA markers, usage of RFLP markers is limited because of their low level of polymorphisms and higher cost compared to

RAPDs (Zhang et al., 1996). Most notably the application of RAPDs compared to RFLP does not necessitate any prior knowledge of genomic nucleotide sequences (Williams et al., 1990). Williams et al. (1993) and Welsh and McClelland (1990) demonstrated the utility of single short oligonucleotide primers of arbitrary sequence for the amplification of DNA segments distributed randomly throughout the genome, and further the amplified bands were utilized for the determination of their relatedness (Tinker et al., 1993; Sun et al., 2001). Previously, the reality of RAPD has been well exploited to determine the genetic similarity of sunflower genotypes (Lawson et al., 1994; Arias and Reisberg, 1995; Sivalop and Soledenko, 1998).

In the present effort, the genetic relatedness and evolutionary relationships of genotypes originated and maintained at different geographic locations were studied to understand the crossability nature with the aim to breed for salt-tolerant genotypes.

Materials and Methods

Plant Materials. Twenty-four sunflower genotypes were used, including six maintainer lines, five high yielding popular cultivars and 13 germplasm accessions, including eight exotic genotypes received from Ames, Iowa, USA (Table 1). These genotypes possessed

Table 1. List of sunflower inbreds utilized for the study.

Sl. No.	Genotype	Particulars
1	CO 2	Derivative of seven single-cross hybrids of Russian origin
2	CO 3	Mutant from CO 2 (5 KR of gamma rays)
3	CO 4	Dwarf x Surya derivative
4	MORDEN	EC 101495 – Cernianka 66 (Introduction)
5	SURYA	From Maharashtra
6	5B	Maintainer lines maintained at Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore.
7	6B	
8	302B	
9	336B	
10	400B	
11	86B3	
12	GP 324	Germplasm accessions maintained at Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore.
13	GP 93	
14	GP 86	
15	GP 336	
16	GP 161	
17	GP 255	
18	SF 7	Acc. No. Ames 3300 Origin Germany
19	SF 30	Acc. No. Ames 20080 Origin Bulgaria
20	SF 45	Acc. No. PI 243074 Origin Jordan
21	SF 60	Acc. No. PI 243174 Origin Argentina
22	SF 83	Acc. No. PI 331176 Origin Romania
23	SF 91	Acc. No. PI 431516 Origin Egypt
24	SF 54	Acc. No. PI 289626 Origin France

some desirable features, viz., high oil content, head diameter, bold seeds etc., and are subjected for sodicity tolerance.

DNA Extraction and RAPD Analysis. For each genotype, one gram of young seedling leaves was taken for DNA extraction, and the method described by McCouch et al. (1998) was adopted. DNA of 24 inbreds was amplified using a set of 20 arbitrary oligonucleotide decamer primers (Operon Technologies, Alameda, California, USA) (Table 2).

Table 2. Twenty decamer primers used and number of RAPD marker detected by them.

Primers	Sequence (5' to 3')	Total no. of RAPD markers	Polymorphic marker	
			No.	(%)
OPD 12	GGTCTACACC	5	2	40.00
OPD 16	TTGGCACGGG	5	2	40.00
OPD 02	GGACCCAACC	7	5	71.43
OPD 20	GGAAGCTTGG	7	0	0.00
OPF 15	CCAAGCTTCC	6	0	0.00
OPT 10	GGCAGGCAGA	6	4	66.67
OPT 12	GAAGAGACTC	11	5	45.45
OPT 15	GGGTTTGGCA	7	4	57.14
OPT 17	GGCAGGCTGT	8	4	50.00
OPT 07	GGCAGGCTGT	5	0	0.00
OPF 06	GGGAATTCGG	10	5	50.00
OPR 06	GTCTACGGCA	7	4	57.14
OPH 16	GTAATTCGCA	7	3	42.86
OPS 11	GTCCACTGTG	8	4	50.00
OPS 02	CCAAGTTCGC	8	5	62.50
OPA 01	CAGGCCCTTC	9	5	55.56
OPF 05	AGGGGTCTTG	10	6	60.00
OPM 13	GGTGGTCAAG	7	5	71.43
OPT 09	CACCCCTGAG	10	4	40.00
OPW 12	TGGGCAGAAG	8	5	62.50
TOTAL		151	72	47.68

Amplification reactions were in volumes of 20 µl containing 10mM Tris HCl (pH 9), 50mM KCl, 5mM MgCl2, 0.001 percent gelatin, dNTPs (each at 0.1 mM), 0.2 mM primer, 10 ng of template DNA and 0.5 unit of Taq DNA polymerase (Genei, Bangalore, India). Amplifications were performed in 92-well thin-wall polycarbonate microtitre plates (Corning, Inc.) in a thermal cycler (Perkin Elmer) programmed for 40 cycles of 1 min. at 94C, 1 min. at 36C, 2 min. at 72C, preceded and followed by 2 minutes at 94C and 10 min. at 72C respectively.

PCR amplified products were subjected to electrophoresis in 1.5 percent agarose gels in 1X TBE buffer at 140 V for 3 hours using a Hoefer® super electrophoresis unit. (Pharmacia Biotech.). RAPDs were scored for presence (1) or absence (0) of bands. The data matrix obtained from 24 sunflower genotypes using 20 decamer primers was used for computing genetic distance.

Sequential Agglomerative Hierarchial Non-Overlapping (SAHN) clustering was performed on squared Euclidean distance matrix and similarity matrix using Dice's coefficient for the binary data utilizing the Unweighted Pair Grouping Method (UPGMA) (Sneath and Sokal, 1973). Data analysis was done using NTSYS-pc version 2.02 (Rohlf, 1994).

Results and Discussion

RAPD Analysis. Twenty randomly selected decamer primers detected 151 RAPD markers among the 24 inbreds (Table 2). Out of these, 47.68% bands were polymorphic. The number of bands ranged between 5 and 11 with an average of 7.55 per primer. Of these 20 primers, the decamer sequence of OPD 20, OPF 15, OPT 07 could not produce any polymorphic markers, however the remaining primers exhibited distinctness. The primers OPD 02 and OPM 13 differentiated the genotypes to a tune of 71.43% polymorphic markers with a distinct banding pattern (Figure 1). The minimum level of polymorphism i.e., 40% was affected by primers OPD 12, OPD 16 and OPT 09. Thus these results show that the RAPD technique is technically simpler, quicker, relatively inexpensive, and nonradioactive, and can detect sufficient polymorphisms for germplasm characterization and genetic distance studies.

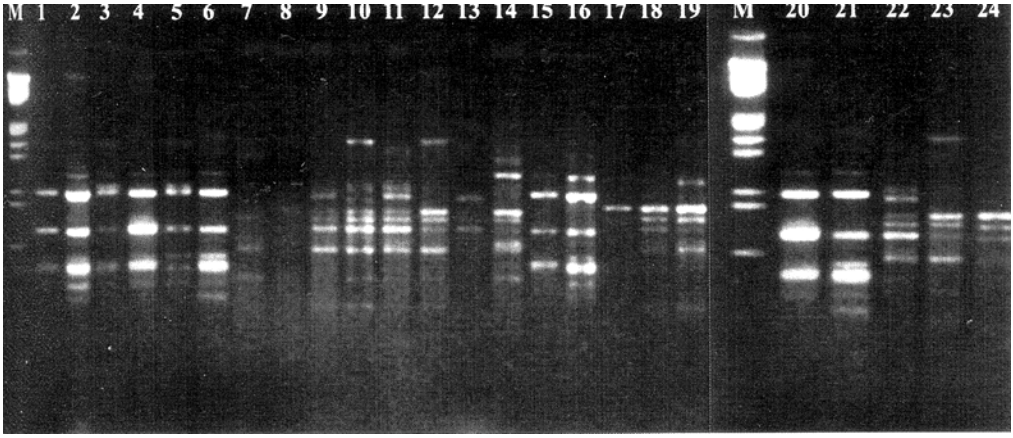

Figure1. RAPD marker profiles of 24 sunflower inbreds produced by random primer OPM 13. (1-SF30, 2-Morden ,3-302B, 4-GP86, 5-CO3, 6-GP93, 7-GP336, 8-SF60, 9-Surya ,10-SF45, 11-400B 12-5B, 13- GP324, 14-GP255, 15-86B3, 16-SF54, 17-SF91, 18-GP161 ,19-CO2, 20-CO4 ,21-336B ,22-6B, 23-SF7, and 24-SF83).

Cluster Analysis Based on RAPD. Analysis of the relationship based on 151 RAPD markers revealed that the genetic distances among 24 genotypes ranged from 0.089 (91% similarity) to 0.19 (81% similarity) (Data not shown). The germplasm accessions had shown greater variability manifesting an extreme genetic distance of 0.19. While the maintainer lines, which may have originated from a single source, exhibited a very close similarity except for the genotype 6B that was derived from other sterile counterparts and shows a similarity coefficient of 89%. The dendrogram constructed using the UPGMA method classified the genotypes into six clusters. Genotype GP161 is most distantly separated from other accessions with a similarity of 0.81 (Figure 2).

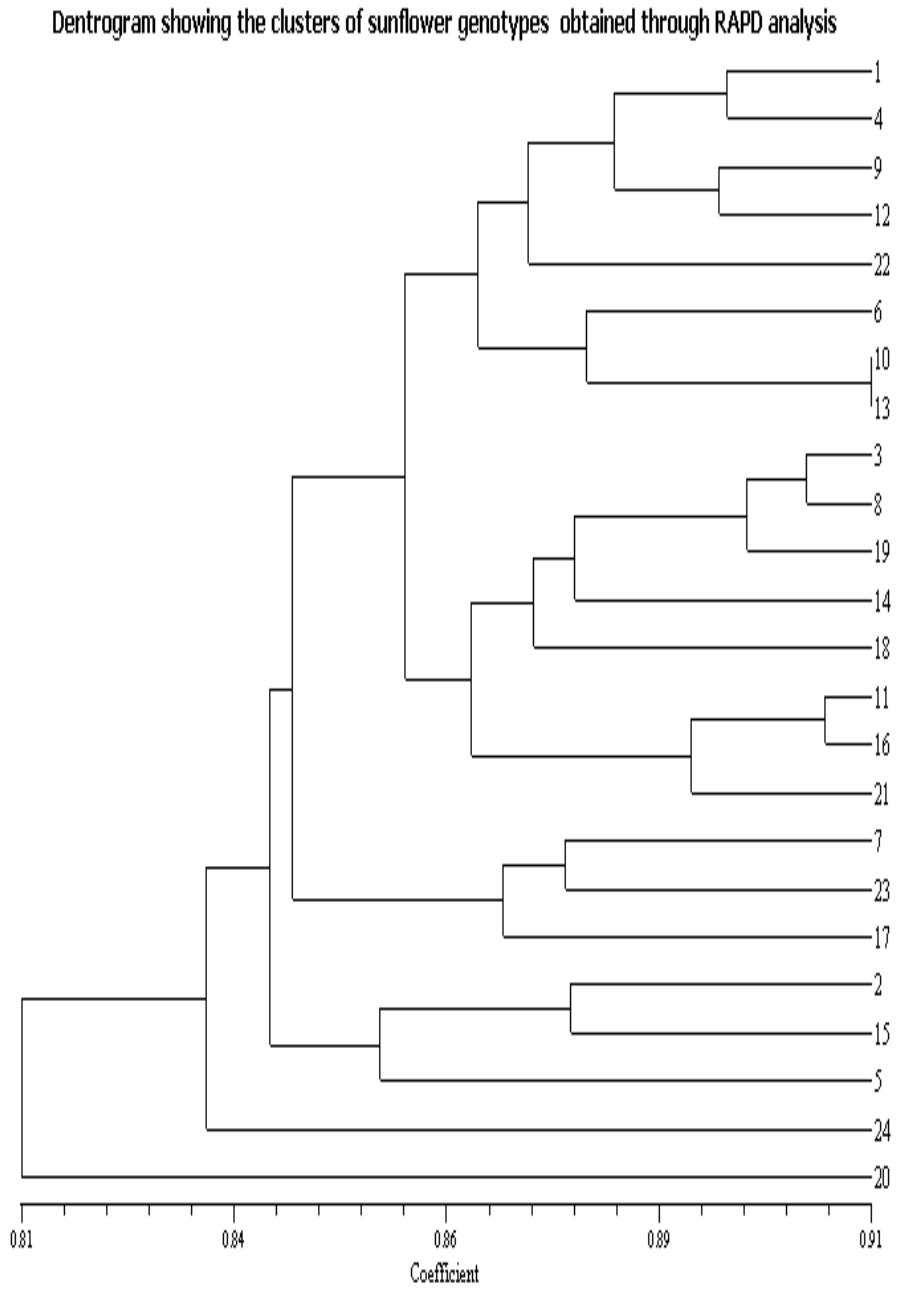

Figure 2. Dendrogram showing the clusters of sunflower genotypes obtained through RAPD analysis.

The variety Morden does not group with any other genotype and is separated at a similarity of 0.84. This dwarf, early-maturing variety with a large head diameter is an early introduction from Russia and could not match the other inbreds in terms of morphological traits. The genotypes SF 45 and SF 30 with different origins *viz*. Jordan and Bulgaria respectively, were found to have no genetic difference and were accommodated in the first cluster. Thus the results prove that molecular markers such as RAPD epitomize genetic variation at the DNA level irrespective of origin, providing more accurate measures of relationships between individuals without the influence of environmental variation (Naghia et al., 2002).

This study is a preliminary work for the breeding program intended to identify sodic-tolerant lines and crafting a sodicity-enduring hybrid. In this work, the sunflower genotypes studied had shown little genetic polymorphism and the separation of these individuals into six groups was not related to geographic distribution. The absence of such distinct groups among the inbreds studied may result in free gene flow. Crossing between the genotypes at different clusters will mix useful segregates for population improvement. Similarly the crossing between the male-sterile lines studied with the variety Morden, and the salt tolerant inbred GP 255 could maximize the opportunities to obtain superior hybrids because unrelated parents would be expected to contribute unique desirable alleles at different loci (Tatineni et al., 1996).

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