GMR
The Original

RAPD AND ISSR MARKERS: MOLECULAR APPROACH TO SELECT DIVERSE PARENTS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) FOR NON-TRADITIONAL AREAS OF INDIA

R. R. Dhutmal[1], S. Maloo[2], K. S. Baig[3], H. V. Kalpande[4], S. G. Shinde[5*], D. H. Sarang [6], S. P. Pole[7], V. M. Panchal[8]

[1, 7]Associate Professor (GPB),
[2] Ex Dean, Rajasthan College of Agriculture, Udaipur, Rajasthan,
[3] Director of Research, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani 431 402, Maharashtra State, India.
[4]Head, Department of Genetics & Plant Breeding, College of Agriculture, Parbhani,
[5, 6]Assistant Professor (GPB),
[8]Senior Research Assistant, Safflower Research Station, VNMKV, Parbhani
*Corresponding Author: S. G. Shinde,

ABSTRACT
Considering the significance of sunflower cultivation under various agro-climatic conditions, an examination was done to recognize assorted parental lines utilizing RAPD and ISSR markers to lessen environmental effects or test blunders. The estimation of similarity coefficient went from 0.41 to 0.87 for example 41-87% with genetic diversity went from 13-59%. The normal closeness across all genotypes was discovered to be 0.64, demonstrating that the genotypes were tolerably comparable genetically. The most extreme closeness estimation of 0.87 was seen between genotypes 234B and 2-7-1B whereas, same trend was also observed between CMS 234B and 89-1B. While EC 623023 and 6D 1R were discovered to be hereditarily assorted with least similitude estimation of 0.41. Genotype EC 623023 demonstrated low likeness esteems with the vast majority of the genotypes and subsequently was generally assorted from rest of the genotypes. RAPD and ISSR group tree examination of 14 sunflower genotypes indicated that they were predominantly separated into two fundamental clusters with a similitude coefficient of 0.61. The genotype EC 623023 was prohibited from any remaining genotypes. In view of RAPD and ISSR investigates, the genotypes EC 623023, R 16, (tester) and CMS 234B (line) were discovered to be genetically diverse. Most cross breeds of these parental lines showed huge SCA impacts, heterosis and high in per se performance.

KEYWORDS: Sunflower, RAPD, ISSR, Diversity

INTRODUCTION

The significance of sunflower as an oilseed crop in India is exceptionally recent and goes back three decades. In any case, its commitment to self-sufficiency in consumable oil as well as to the "yellow revolution" in the country is remarkable (Rai, 2002). In India, Seetharama (1981) developed the first sunflower hybrid by the use of well-defined CMS system has generated a vast interest in the crop. Hybrids are favored over open pollinated cultivars on account of their high efficiency regarding seed and oil yield. With the expanding interest and demand for consumable oils, there is a need to develop new sunflower hybrids with an extensive level of heterosis for economic characteristics, which are likewise reasonable for various agro-climatic zones of India.

There is a scope to bring the area under cultivation of sunflower in Rajasthan since agriculture in Rajasthan is primarily rainfed, receives much less annual rainfall and frequency of periodic drought is high. Under such situations, sunflower by virtue of its photo-insensitivity, short duration, suitability for contingency cropping, high water use efficiency, high yield, good quality oil, low cost of cultivation, fits ideally in the varied agro-climatic situations of Rajasthan.

Hybrids developed from genetically diverse parental lines are more heterotic and stable, diversity based on phenotypic values may not be the perfect representation of natural grouping of cultivars. Therefore, precisely diverse parental lines can be identified by employing suitable molecular markers thereby reducing environmental effects or experimental errors.

As of late, impressive advancement has been made in the use of molecular markers to the characterization and assessment of plant genetic resources. Among a few productive techniques for uncovering genetic distinctness within and among plant populations, the absolute most ordinarily utilized strategies are RAPD (Wolff and Paters-van Rijn, 1993; Washira *et al*., 1995; Swoboda and Bhalla, 1997) and ISSR (Guo *et al*., 2006; Heikal *et al*., 2008). In many studies, DNA-based markers were found better than isozymes in the recognition of genetic distinctness.

Specifically, RAPD and ISSR are simpler to use than the other molecular marker systems, in light of the fact that in both of these techniques earlier information on the target sequences is not needed (Reddy *et al*., 2002).

Looking to the importance of sunflower crop in uplifting the economy of non-traditional farming community of the Rajasthan State specially under rainfed/famine situations, a study was carried-out by crossing four diverse

CMS lines with ten testers.

MATERIAL AND METHODS

Fourteen phenotypically diverse parental lines for seed yield, oil content and maturity comprised 4 maintainers and 10 restores of this species having indigenous and exotic origin were selected for the present study.

Description of parents

Parents	Origin	Important traits
CMS 234A	UAS, Bangalore	Early maturity and high oil content
CMS PET 89-1A	IIOR, Hyderabad	High seed yield.
CMS PET 2-7-1A	IIOR, Hyderabad	High seed yield.
ARM 249A	IIOR, Hyderabad	High seed yield.
R-16	IIOR, Hyderabad	Early maturity and High seed yield.
RHA 1-1	ORS, Latur	Downy mildew resistant.
RHA 138-2	ORS, Latur	High seed yield and black color seed.
R-271-1	ORS, Latur	Early maturity and High seed yield.
IC-294064	NBPGR, New Delhi	Early maturity.
EC-623023	IIOR, Hyderabad	High seed yield.
EC-623016	IIOR, Hyderabad	High seed yield.
EC-601957	IIOR, Hyderabad	High seed yield.
EC-601924	IIOR, Hyderabad	High oil content and Downy mildew resistant.
6-D-1R	UAS, Bangalore	High oil content.

A set of 20 random 10-mer RAPD and 15 ISSR primers were used for detecting the polymorphism among 14 genotypes of *H. annus* L.

Details of RAPD primers used in molecular analysis of sunflower genotypes

S. No.	Primers	Sequence (5'-3')	G:C Content (%)
1	OPD-2	GGACCCAACC	70
2	OPM-13	GGTGGTCAAG	60
3	OPA-04	AAT CGG GCT G	60
4	OPA-05	AGG GGT CTT G	60
5	OPA-06	GGT CCC TGA C	70
6	OPA-09	GGG TAACGC C	70
7	OPT-10	GGCAGGCAGA	70
8	OPS-02	CCAAGTTCGC	60
9	OPW-04	CAGAAGCGGA	60
10	OPW-06	AGGCCCGATG	70
11	OPW-09	GTGACCGAGT	60
12	OPW-10	TCGCATCCCT	60
13	OPW-15	ACACCGGAAC	60
14	OPQ-09	GAACGGACTC	60
15	OPG-02	GGCACTGAGG	70
16	OPG-04	AGCGTGTCTG	60
17	OPG-09	CTGACGTCAC	60
18	OPH-20	GGGAGACATC	60
19	OPJ-06	TCGTTCCGCA	60
20	OPR-04	CCCGTAGCAC	80

Details of ISSR primers used in molecular analysis of sunflower genotypes

S. No.	Primers	Sequences (5'-3')	No. of nucleotides (bases)
1	UBC-820	GTGTGTGTGTGTGTGTC	17
2	UBC-810	GAGAGAGAGAGAGAGAT	17
3	UBC-807	AGAGAGAGAGAGAGAGT	17
4	UBC-836	AGAGAGAGAGAGAGAGYA	18
5	UBC-849	GTGTGTGTGTGTGTGTYA	18
6	UBC-873	GACAGACAGACAGACA	16
7	UBC-880	GGAGAGGAGAGGAGA	15
8	UBC-808	AGAGAGAGAGAGAGAGC	17
9	UBC-881	GGGTGGBGGTGGGGTG	16
10	UBC-845	CTCTCTCTCTCTCTCTRC	18

11	UBC-840	GAGAGAGAGAGAGAGAYC	18
12	IS-07	CACACACACACACAGT	16
13	HB-08	GAGAGAGAGAGAGG	14
14	UBC-827	TGTGTGTGTGTGTGTGA	17
15	HB-09	GTGTGTGTGTGTGG	14

DNA amplification conditions and gel electrophoresis

For RAPD and ISSR markers, the amplification reaction was carried out in 25µL reaction volume containing 1xPCR buffer, 1.5mM $MgCl_2$, 0.25 mM dNTPs, 25 ng primer (Operon Technologies Inc. USA), 1.5 unit of *Taq* DNA polymerase and 20 ng template DNA. PCR amplification was performed in a T-gradient thermal cycler (Bio-Rad; T Gradient). It was programmed to fulfill 40 cycles (for RAPD analysis) or 35 cycles (for ISSR analysis) after an initial denaturation cycle for 5 min at 94 ºC. The annealing temperature of 37^0C was found to be optimum for generating clear and reproducible bands for RAPD primers. For ISSR primers annealing temperature varied from 27.0º to 47.7ºC for PCR amplification. Therefore, the thermocycler was programmed for an initial denaturation step of 4 min at 94^0C, followed by 40 and 30 cycles of denaturation (94^0C, 1 min.) for RAPD and ISSR respectively, annealing (37^0C, 1 min. for RAPD and 27.0^0C-47.4^0C, 1 min for ISSR primers) and extension (72^0C, 2 min.) followed by a final extension of 72^0C for 5 min. with a hold temperature of 4^0C at the end that resulted in clear and reproducible bands. For ISSR primers the annealing temperature varied from 27.0^0C to 47.4^0C for PCR amplification because ISSR primers, designed to anneal to a micro-satellite sequence, are longer than RAPD primers and due to this reason ISSR markers are more reproducible than RAPD markers (Goulao and Oliveira, 2001).

RAPD and ISSR data analysis

Photographs of the gel were taken with a Gel Documentation System, under UV transilluminator, to evaluate and obtain banding patterns. DNA ladders were loaded along with the PCR product for the one sight estimation of the molecular size of the PCR products. Scoring of bands was done as "1" and "0" for presence and absence of band, respectively. Faintly noticeable bands were not scored, yet a significant band relating to faint bands was considered for scoring.

Genetic analysis and cluster tree analysis

A matrix, through unweighted Pair Group Mean Arithmetic Average (UPGMA)was generated percentage using disagreement values (PDVs). For this, the statistical programme- NTSYSpc version 2.02, suggested by Rolf (1998) was used.

RESULTS AND DISCUSSION

Polymorphism using RAPD primers

The amplification products in terms of the percentage of PCR products, as generated by the RAPD primers are listed in Table 1. OPD 05 primer produced the majority of the markers in the genotypes studied. Ten out of twenty selected Random Amplified Polymorphic DNA primers were found polymorphic thus produced large number of bands in 14 sunflower genotypes under evaluation. A total of 587 distinct, scorable bands were produced by 10 polymorphic RAPD primers with an average of 58.7 amplicons/primers (Figure 1). However, 334 out of a total of 587 bands were polymorphic. The highest number of fragments (98 amplicons) were generated by the primer OPD 05, whereas, OPW 15 produced lowest number (38 amplicons). A high variation in the number of fragments could be attributed to the distinctions in the binding sites all through the genome of the included genotypes. While evaluating Indian bitter gourd (*Momordica charantia* L.) Behera *et al* (2008) also reported such a high variation. Table 1 also revealed that, in all the 14 genotypes average polymorphism was found to be 56.88 percent with 33.4 polymorphic amplicons for each of the 10 primers. Figure 1 shows the RAPD profile for the 14 genotypes derived from OPA -5, OPA -9, OPD-5, OPG-9, OPQ-9, OPW-6, OPW-9, OPW-4, OPW-15and OPA -4.

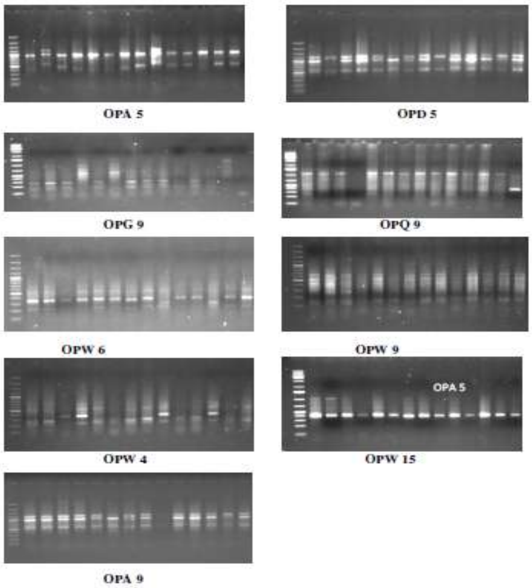
Table 1 DNA amplification profile and polymorphism generated in sunflower using RAPD markers

S.N.	RAPD Marker	Size range (bp)	Total no. of Bands	Monomorphic bands	Polymorphic bands	% polymorphism
1	OPA-5	300-1500	84	19	65	77.38
2	OPA-9	300-1500	70	45	25	35.71
3	OPD-5	300-1500	98	41	57	58.16
4	OPG-9	300-1500	45	9	36	80.00
5	OPQ-9	300-1500	70	24	46	65.71
6	OPW-6	300-1500	42	19	23	54.76
7	OPW-9	300-1500	42	20	22	52.38

8	OPW-4	300-1500	42	10	32	76.19
9	OPW-15	300-1500	38	16	22	57.89
10	OPA-4	300-1500	56	50	6	10.71
Mean	**Total**		**587**	**253**	**334**	**56.88**

Polymorphism using ISSR primers

In the present study, a total of 15 ISSR primers were investigated out of which 6 ISSR primers namely UBC -820, UBC -810, UBC -807, UBC -836, UBC -849 and UBC -873 showed positive results. A total of 160 polymorphic bands out of 300 amplified bands were obtained from 6 ISSR primers used. DNA amplification profile and polymorphism generated in sunflower using ISSR markers is given table 2 revealed that UBC 873 (57) yielded highest number of polymorphic bands whereas UBC 807 (37) produced least. Percent polymorphism for calculated using total number of amplified bands and total number of polymorphic bands for each ISSR primer, separately which ranges from 24.32 percent for UBC 807 to 84.78 for UBC 836 with an average polymorphism of 52.24 percent across 14 genotypes. The total size of PCR amplified products ranged from 300bp to 1500bp. Mahmoud and Abdel-Fatah (2012) and Garayalde (2011) additionally noticed comparative outcomes for (ISSR) markers in sunflower.



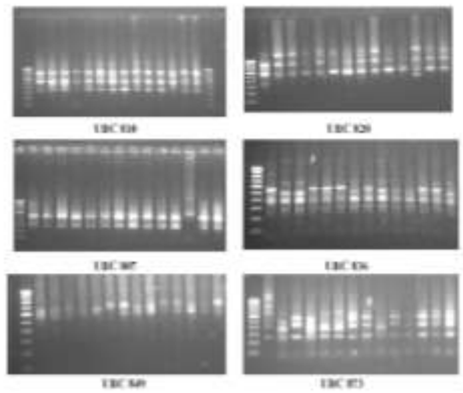


Figure 1 Polymorphism resultant from the use RAPD primers for *H. annuus* genotype

Fig. 2. Polymorphism resultant from the use of of ISSR primers for *H. annuus* genotype

Table 2. DNA amplification profile and polymorphism generated in sunflower using ISSR markers

S.N.	ISSR Marker	Size range (bp)	Total no. of Band s	Monomorphi c bands	Polymorph ic bands	% polymor phism
1	UBC-820 di with 1	300-1500	55	28	27	49.09
2	UBC-810	300-1500	52	35	17	32.69
3	UBC-807	300-1500	37	28	9	24.32
4	UBC-836 di with 2	300-1500	46	7	39	84.78
5	UBC-849	300-1500	53	11	25	47.16
6	UBC-873 quadra	300-1500	57	19	43	75.43
Total			**300**	**128**	**160**	**52.245**

Similarity matrices based on RAPD data

RAPD similarity matrix data for RAPD markers given in Table 3 revealed 3 to 59 percent genetic diversity in the study material. The range of 0.41 to 0.97 was observed for similarity coefficient with the average similarity of 0.73 across all the 14 genotypes. This indicated a moderate genetic similarity among the genotypes. genotypes

234B and 2-7-1B showed Maximum similarity value of 0.97 whereas the least minimum similarity value of 0.41 between EC 623023, RHA 1-1 and R 271-1 showed that these were genetically different. Genotype EC 623023 showed low similarity values with most of the genotypes and thus was most different from the rest of the genotypes.

Isaacs *et al*., (2003) reported that crossing between genotypes with low similarity coefficient manifest high heterosis. In the present study hybrids developed from parents with low or moderate similarity coefficient viz. CMS 234B (line), EC 623023, R16 (testers) also identified as stable hybrids on the basis of high mean for seed yield along with stability of component traits (Table 6) and also manifested high magnitude of heterobeltiosis and economic heterosis (Data not shown). Earlier, Ghany (2012) and Iqbal *et al.* (2008) observed the maximum similarity of 77.78% and the lowest similarity of 51.59% in Sunflower.

Table 3 Jaccard's similarity coefficient for RAPD profile generated by Agarose gel electrophoresis

Genotypes	6D-1R	R-16	EC 623016	RHA1-1	RHA 138-2	R-271-1	CMS 234B	IC 294064	EC 623023	PET 89-1B	PET 2-7-1B	ARM 249B	EC 601957	EC 601924
6D-1R	1													
R-16	0.74	1												
EC 623016	0.82	0.71	1											
RHA1-1	0.64	0.58	0.71	1										
RHA1 38-2	0.84	0.74	0.87	0.74	1									
R-271-1	0.69	0.58	0.71	0.74	0.74	1								
CMS234B	0.82	0.61	0.89	0.71	0.82	0.71	1							
IC 294064	0.76	0.66	0.84	0.76	0.87	0.71	0.84	1						
EC 623023	0.46	0.61	0.48	0.41	0.56	0.41	0.48	0.53	1					
PET 89-1B	0.84	0.64	0.87	0.69	0.74	0.74	0.87	0.76	0.46	1				
PET 2-71B	0.84	0.64	0.92	0.69	0.84	0.74	0.97	0.87	0.51	0.89	1			
ARM249B	0.71	0.56	0.79	0.76	0.76	0.71	0.79	0.74	0.53	0.82	0.82	1		
EC 601957	0.76	0.61	0.89	0.61	0.82	0.61	0.84	0.79	0.58	0.76	0.87	0.74	1	
EC 601924	0.82	0.61	0.84	0.66	0.82	0.61	0.79	0.79	0.43	0.76	0.82	0.64	0.79	1

Similarity matrices based on ISSR data

ISSR similarity matrices of 14 *Helianthus annuus* genotypes showed that they were interrelated (Table 4). The similarity indices represent genetic relatedness among the genotypes which facilitates identification of most distantly related genotypes with a view to obtain as high as possible magnitude of Heterosis in the resultant hybrids. In the present study it ranged from 0.358 to 0.871 percent representing diversity ranged from 13 to 65 percent.

The average closeness or distinctness is also estimated which falls in the moderate range with an index of 0.72. Two groups of restores viz., R-16 and EC 623016 and RHA 138-2 and R-271 and one group of seed parent viz., CMS 234B and PET 89-1B have shown the maximum similarity index of 0.87. However, the similarity index between another two restores viz., 6D-1R and EC 623023 was found to be the least (0.35) indicated their genetic distinctness from rest of the parental lines. Such genetically districts parents when crossed may produce the hybrids with significantly higher magnitude of heterosis for economic traits.

Table 4. Jacquard's similarity coefficient for ISSR profile generated by Agarose gel electrophoresis

Genotypes	**6D-1R**	**R-16**	**EC623016**	**RHA-1-1**	**RHA138-2**	**R-271-1**	**CMS234B**	**IC294064**	**EC623023**	**PET89-1B**	**PET 2-7-1B**	**ARM249B**	**EC601957**	**EC601924**
6D-1R	1													
R-16	0.61	1												
EC 623016	0.53	0.87	1											
RHA-1-1	0.61	0.74	0.76	1										
RHA 138-2	0.53	0.82	0.74	0.76	1									
R-271-1	0.56	0.69	0.61	0.74	0.87	1								
CMS 234B	0.48	0.66	0.74	0.61	0.79	0.76	1							
IC 294064	0.51	0.79	0.82	0.74	0.82	0.69	0.82	1						
EC 623023	0.35	0.74	0.82	0.69	0.71	0.64	0.76	0.79	1					
PET 89-1B	0.41	0.69	0.71	0.53	0.71	0.74	0.87	0.79	0.79	1				
PET 2-7-1B	0.41	0.64	0.71	0.53	0.61	0.58	0.76	0.69	0.79	0.84	1			
ARM 249B	0.51	0.74	0.76	0.64	0.76	0.69	0.76	0.79	0.69	0.74	0.69	1		
EC 601957	0.53	0.71	0.69	0.61	0.69	0.61	0.79	0.82	0.71	0.82	0.76	76	1	
EC 601924	0.48	0.71	0.69	0.61	0.64	0.61	0.79	0.82	0.71	0.82	0.76	0.66	0.84	1

Similarity matrices based on RAPD and ISSR data

Combined RAPD and ISSR similarity matrix data is given in Table 5. A range of 0.41 to 0.87 was observed for similarity coefficient value equivalent to 41-87 percent of genetic relatedness or a range of 13 to 59 percent genetic distinctness. On the basis of combined RAPD and ISSR matrix data it was inferred that the parental lines used in investigation were moderately distinct with an average similarity index of 0.64 indicated a moderate scope for selection of diverse parental lines.

The average similarity across all genotypes was found to be 0.64, indicating that the genotypes were moderately similar genetically. The maximum similarity value of 0.87 was observed between genotypes 234B and 2-7-1B and 234B and 89-1B. Whereas EC 623023 & 6D 1R were found to be genetically diverse with minimum similarity value of 0.41. Genotype EC 623023 showed low similarity values with most of the genotypes and hence was most diverse from rest of the genotypes.

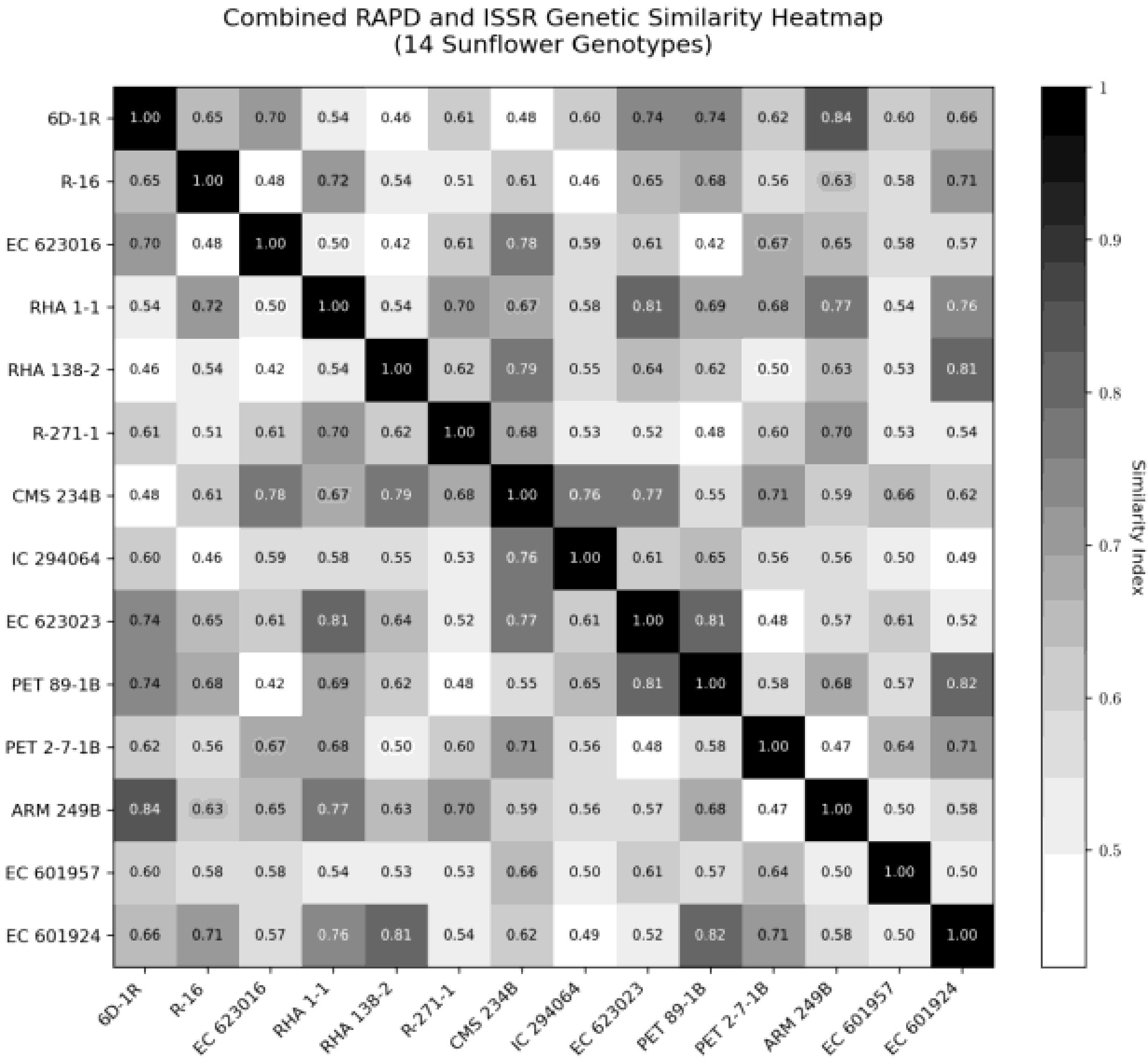


Table 5 Jaccard's similarity coefficient for RAPD and ISSR profile generated by Agarose gel electrophoresis

Genotypes	6D-1R	R-16	EC 623016	RHA1-1	RHA 138-2	R-271-1	CMS 234B	IC 294064	EC 623023	PET 89-1B	PET 2-7-1B	ARM 249B	EC 601957	EC 601924
6D-1R	1													
R-16	0.67	1												
EC 623016	0.67	0.79	1											
RHA-1-1	0.62	0.66	0.74	1										

RHA 138-2	**0.69**	**0.78**	**0.80**	**0.75**	**1**									
R-271-1	**0.62**	**0.44**	**0.66**	**0.74**	**0.80**	**1**								
CMS 234B	**0.65**	**0.64**	**0.82**	**0.66**	**0.80**	**0.74**	**1**							
IC 294064	**0.64**	**0.73**	**0.83**	**0.75**	**0.84**	**0.70**	**0.83**	**1**						
EC 623023	**0.41**	**0.60**	**0.65**	**0.55**	**0.64**	**0.52**	**0.62**	**0.66**	**1**					
PET 89-1B	**0.62**	**0.60**	**0.79**	**0.61**	**0.73**	**0.74**	**0.87**	**0.78**	**0.62**	**1**				
PET 2-7-1B	**0.62**	**0.64**	**0.82**	**0.61**	**0.73**	**0.66**	**0.87**	**0.78**	**0.65**	**0.87**	**1**			
ARM 249B	**0.61**	**0.65**	**0.78**	**0.70**	**0.76**	**0.70**	**0.78**	**0.76**	**0.61**	**0.78**	**0.75**	**1**		
EC 601957	**0.65**	**0.66**	**0.79**	**0.61**	**0.75**	**0.61**	**0.82**	**0.80**	**0.65**	**0.79**	**0.82**	**0.75**	**1**	
EC 601924	**0.65**	**0.66**	**0.76**	**0.64**	**0.73**	**0.61**	**0.79**	**0.80**	**0.57**	**0.79**	**0.79**	**0.65**	**0.82**	**1**

RAPD marker-based cluster tree analysis

RAPD cluster tree examination of 14 *Helianthus annuus* genotypes indicated that they were primarily separated into two primary groups with half closeness (Figure 3). Genotype EC 623023 was isolated from any remaining genotypes at half comparability and shaped the principal cluster. At 69% similarity was the subsequent cluster, which incorporated any remaining genotypes. Genotype PET 89-1B was again out-grouped and shaped another singular cluster with 74% likeness. The second sub-cluster of the main cluster II comprised of promising restores viz., RHA 1-1, R 16 and 6 D-1R. The sub-cluster of the main cluster II was additionally partitioned into two groups - group I and group II which includes promising seed parental lines viz., CMS 234B and ARM 249B, respectively. Group I comprised of a high yielding restorer in particular EC 601957 while group II comprised of rest of the 7 genotypes specifically EC 623016, 234B, PET 2-7-1B, EC 601957, RHA 138-2 and IC 294064. The promising seed parental lines viz., 234A, ARM 249B and restores viz., R 16, EC 623023 and RHA 138-2 falls in distinct clusters owing to their genetic dissimilarities which in term may results in the development of heterotic hybrids.

The cluster tree again indicated comparable outcomes over similarity matrices. The relationship between various genotypes has appeared as a dendrogram. The genotypes that were nearer to one another in the dendrogram were more like each other than those that were separated. The dendrogram additionally demonstrated the overall degree of likeness between the various genotypes of *Helianthus annuus* utilized in the current investigation. Mostafa and Altrmawy (2011) likewise utilized RAPD markers to survey hereditary relatedness in sunflower.

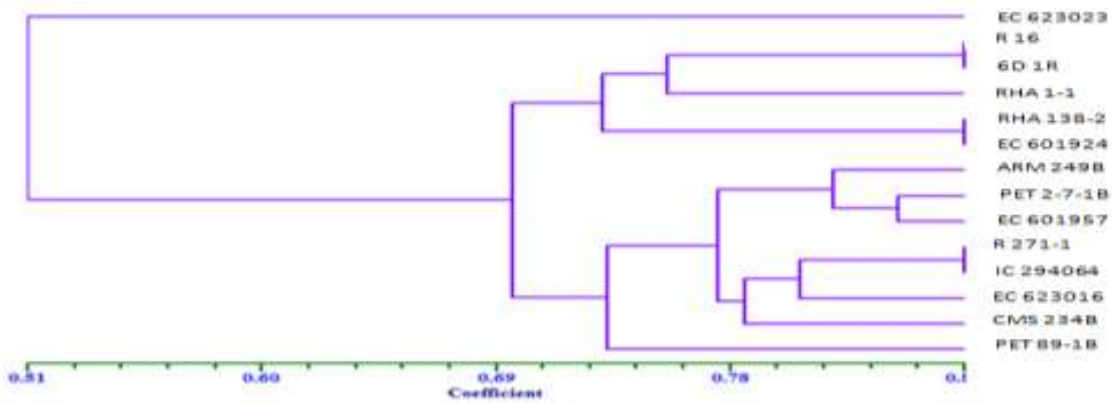


Figure 3 Dendrogram constructed with UPGMA using RAPD primers

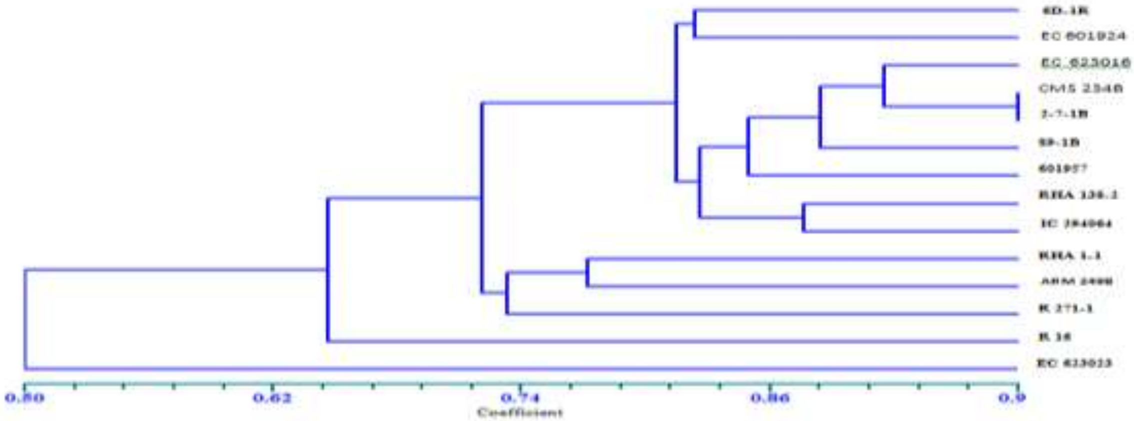


Figure 4 Dendrogram constructed with UPGMA clustering method using ISSR primers

ISSR marker-based cluster tree analysis

ISSR cluster tree examination of 14 *Helianthus annuus* parental lines demonstrated that they were primarily separated into two fundamental clusters with 51% closeness (Figure 4). Genotype EC 623023 was isolated from any remaining genotypes at 51% similitude and framed the principal cluster. At 64% similitude, the subsequent cluster was with any remaining genotypes. The subsequent sub-cluster of the main cluster II was additionally partitioned into two primary groups at 73% similitude and assembled the genotype PET 89-1B from any remaining genotypes. The sub-group of the main cluster II was additionally partitioned into two gatherings - group I and II at 78% similitude. Group I comprised of genotypes extensively utilized seed parent, 234B and rest were the pollen parents with varying seed and oil characteristics such as IC 294064 and R 271-1, while group II comprised of genotypes EC 601957, PET 2-7-1B and ARM 249B. Yang (2012) additionally detailed high hereditary distinctness in sunflower.

In view of ISSR examination, genotypes EC 623023, R 16, (testers) and CMS 234B (line) were discovered to be hereditarily assorted as they fall into various bunches. In this manner, their hereditary origin is by all accounts unique and could be proficiently utilized in superior hybrid development programs. These genotypes had high mean values for seed yield, and oil content with critical GCA impacts and consequently could be further exploited. The vast majority of the mixtures of these parental lines indicated significantly higher SCA impacts, heterosis and high mean seed yield performance (Table 7). Rest of the genotypes may have some hereditary/phylogenic relationship because of migration/inflow of genes.

RAPD and ISSR marker-based cluster tree analysis

RAPD and ISSR cluster tree analysis of 14 sunflower genotypes demonstrated that they were basically partitioned into two principal clusters with a closeness coefficient of 0.61 (Figure 5). The genotype EC 623023 was isolated from any remaining genotypes with a closeness coefficient of 0.61 and shaped the primary group. At a coefficient of 0.64, the subsequent cluster was situated with all remaining genotypes. Genotype 6D-1R was again delimited and shaped another lone group with a similarity coefficient of 0.64. The subsequent sub-group of the cluster II was additionally isolated into two principal group at a similarity coefficient of 0.68. The sub-group I of main clusters II was additionally isolated into two gatherings - isolating R 16 from different genotypes at a coefficient of 0.69. The fundamental group was additionally isolated into 2 groups with a coefficient of 0.76 and out grouped ARM 249B. The fundamental subgroup of the major cluster additionally split into 2 groups at a coefficient of 0.79. The principal cluster contained the genotypes EC 623016, RHA 138-2 and IC 294064, while the subsequent gathering comprised of CMS 234B, PET 89-1B, PET 2-7-1B, EC 601957 and EC 601924. Then again, the subsequent sub-group of the group II comprised of RHA 1-1 and R 271-1. The parental lines falling in different groups had much higher genetic diversity than those falling in same or closely spaced groups. Furthers this parental line with genetic distinctness upon crossing may produce highly heterotic hybrids of sunflower.

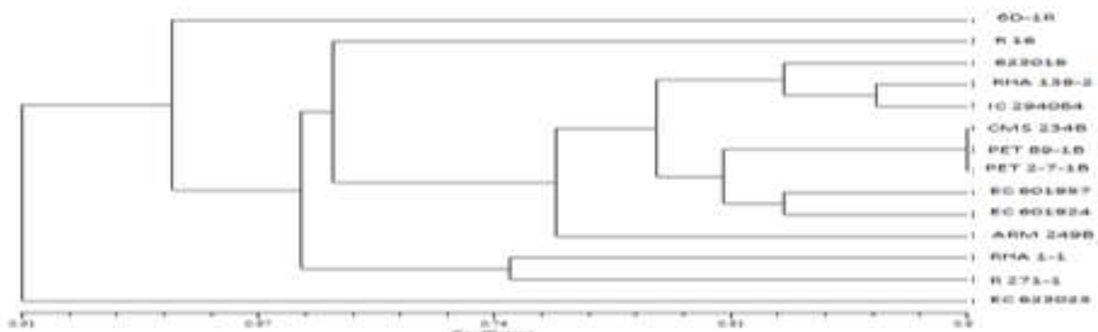


Figure 5 Dendrogram constructed with UPGMA clustering method among 14 genotypes of sunflower using RAPD and ISSR primers

CONCLUSION

Based on RAPD and ISSR analyzes, the genotypes EC 623023, R 16, (tester) and CMS 234B (line) were found to be genetically distinct and fall into different clusters. Therefore, their genetic origin seems to be different and could be efficiently used in improvement programs. In view of this, we studied the nature of gene activity and effects of combining ability, heterosis and stability parameters for seed yield, its 10 component traits and oil content (data not shown) in 3 environments (non-traditional areas in Rajasthan state of India) using 4 lines and 10 testers with different genetic background. It was observed that these genotypes per se possessed high performance for seed yield, principal component traits and oil content with significant GCA effects. Most of the hybrids of these parental lines showed significant SCA effects, heterosis and high per se performance (Table 6). Rest of the genotypes might have some ancestral/phylogenic relatedness due to migration/influence of genes.

Table 6 Stable hybrids identified on the basis of high mean for seed yield along with stability of component traits

Hybrids	Mean seed yield (g)	Stable for component traits
CMS 234A x RHA 138-2	63.55	DF^{++}, PH, HD, BY^{+}, HI^{+}, Oil, H, VW^{++}
CMS 234A x EC 601957	63.07	DF^{++}, DM^{++}, PH, HD, Oil^{+}, VW^{+}, TW^{++}, BY^{+}, HI^{++}
CMS 234A x R 16	62.67	DH^{++}, HD, SFP, Oil H^{++}, VW^{++}, TW^{++}, BY^{+}
ARM 249A x EC 623023	62.17	DF^{+}, DM^{++}, PH, HD^{++}, SFP, Oil H^{++}, BY^{+}, HI^{+}

$^{+}$ *Better for favorable environment,* $^{++}$ *Better for unfavorable environment*

DF=Days to 50% flowering, DM= Days to maturity, PH= Plant height, HD= Head diameter, SFP= Seed filling percentage, Oil= Oil content, H= Hull content, VW= Volume weight, TW= Test weight, BY= Biological yield, HI= Harvest index.

REFERENCES

1. Rai, M. (2002). Oilseeds in India. Andhra Pradesh Agricultural Research Journal, 13-15.
2. Seetharama, A. (1981). Hybrid sunflower for higher yields. Seeds and Farms, 6, 27-29.
3. Wolff, K., & Peters-Van Rijn, J. (1993). Rapid detection of genetic variability in chrysanthemum (Dendranthema grandiflora Tzvelev) using random primers. Heredity, 71, 335-341. https://doi.org/10.1038/hdy.1993.146
4. Teulat, B., et al. (1994). Characteristics of random amplified polymorphic DNA markers discriminating *Helianthus annuus* inbred lines. Agronomie, 14(8), 497-502. https://doi.org/10.1051/agro:19940802
5. Guo, D. L., et al. (2006). Genetic diversity of Vitis vinifera L. revealed by ISSR markers. Scientia Horticulturae, 107(4), 369-374. https://doi.org/10.1016/j.scienta.2005.11.009
6. Heikal, A., et al. (2008). Genetic diversity among Egyptian sunflower (*Helianthus annuus* L.) cultivars as revealed by RAPD and ISSR markers. Arab Journal of Biotechnology, 11(2), 241-252.
7. Reddy, M. P., et al. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128, 9-17. https://doi.org/10.1023/A:1020691618797
8. Goulao, L., & Oliveira, C. M. (2001). Molecular characterization of cultivars of apple using microsatellite markers. Euphytica, 122, 81-89. https://doi.org/10.1023/A:1012022230156
9. Behera, T. K., et al. (2008). Relative efficiency of DNA markers (RAPD, ISSR and AFLP) in detecting genetic diversity of bitter gourd. Journal of Science Food and Agriculture, 88, 733-737. https://doi.org/10.1002/jsfa.3138
10. Mahmoud, A. M., & Abdel-Fatah, B. E. (2012). Analysis of genetic diversity among sunflower genotypes using agro-morphological traits and molecular markers. Australian Journal of Basic and Applied Sciences, 6(13), 419-432.
11. Garayalde, A. F., et al. (2011). Wild sunflower diversity in Argentina revealed by ISSR and SSR markers: an approach for conservation and breeding programmes. Annals of Applied Biology, 158(3), 305-317. https://doi.org/10.1111/j.1744-7348.2011.00465.x
12. Isaacs, S. M., et al. (2003). Genetic diversity analysis using RAPD markers in inbred lines of sunflower. Helia, 26(39), 59-66. https://doi.org/10.2298/HEL0339059I
13. Iqbal, M. A., et al. (2008). Estimation of genetic diversity among sunflower genotypes through random amplified polymorphic DNA analysis. Genetics and Molecular Research, 7(4), 1408-1413. https://doi.org/10.4238/vol7-4gmr494
14. Ghany, A. A. (2012). Genetic diversity in sunflower (*Helianthus annuus* L.) using RAPD markers. Journal of Applied Sciences Research, 6(8), 4196-4203.
15. Hongtrakul, V., et al. (1997). Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. Theoretical and Applied Genetics, 95(5-6), 894-903. https://doi.org/10.1007/s001220050576
16. Tang, S., et al. (2002). Simple sequence repeats map of the sunflower genome. Theoretical and Applied Genetics, 105(8), 1124-1136. https://doi.org/10.1007/s00122-002-1048-4
17. Lai, Z., et al. (2005). Identification and mapping of SNPs from ESTs in sunflower. Theoretical and Applied Genetics, 111(8), 1532-1544. https://doi.org/10.1007/s00122-005-0079-z
18. Filippi, C. V., et al. (2015). Population structure and genetic diversity characterization of a sunflower association mapping population using SSR and SNP markers. BMC Plant Biology, 14, 360.

https://doi.org/10.1186/s12870-014-0360-x
19. Rauf, S., et al. (2020). Validated markers for sunflower (*Helianthus annuus* L.) breeding. OCL, 27, 67. https://doi.org/10.1051/ocl/2020067
20. Dimitrijevic, A., & Horn, R. (2018). Sunflower Hybrid Breeding: From Markers to Genomic Selection. Frontiers in Plant Science, 8, 2238. https://doi.org/10.3389/fpls.2017.02238
21. Rauf, S. (2008). Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. Communications in Biometry and Crop Science, 3(1), 29-44.
22. Hladni, N., et al. (2018). Comparison of methods for the estimation of best parent heterosis among lines developed from interspecific sunflower germplasm. Euphytica, 214(5), 107. https://doi.org/10.1007/s10681-018-2197-0
23. Parameshwarappa, K. G., et al. (2009). Heterosis in relation to genetic divergence in parental lines of sunflower, *Helianthus annuus* L. Journal of Oilseeds Research, 26(2), 1-8.
24. Ahmed, H. G. M. D., et al. (2022). Molecular characterization and validation of sunflower (*Helianthus annuus* L.) hybrids through SSR markers. PLoS ONE, 17(5), e0267383.
https://doi.org/10.1371/journal.pone.0267383
25. Darvishzadeh, R., et al. (2026). Genome-wide assessment of genetic diversity and selective signatures in sunflower (*Helianthus annuus* L.) using a 10 K SNP array. Scientific Reports. https://doi.org/10.1038/s41598-026-40372-2
26. Rohlf, F. J. (1998). NTSYSpc: Numerical Taxonomy and Multivariate Analysis System. Exeter Software, New York.