Int.J.Curr.Microbiol.App.Sci (2019) *8*(9): 1919-1927

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 09 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.809.222

Investigation on Genetic Analysis of Safflower (*Carthamus tinctorius* L.) Genotypes using Rapd Molecular Markers

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Keywords

Safflower genotypes, Genetic diversity, RAPD.

Article Info

Accepted:
20 August 2019
Available Online:
10 September 2019

A B S T R A C T

Knowledge of genetic diversity and relationship among breeding material at molecular level has significant impact on safflower improvement. Genetic diversity of eight parental genotypes of safflower (*Carthamus tinctorius* L.) was investigated at DNA level with Random Amplified Polymorphic DNA (RAPD) procedure. Forty RAPD primers generated 943 alleles, out of which 679 alleles were found polymorphic, resulting 72.00 per cent polymorphism. The similarity coefficient ranged from 0.42 to 0.76 with an average of 0.59, thus suggesting considerable genetic variation among the safflower genotypes studied. Further, the dendrogram generated by UPGMA cluster analysis based on Jaccard's similarity coefficient grouped eight parental genotypes into two clusters *viz*., cluster A and cluster B. Maximum number (7 out of 8) cultivars fell in cluster A. Cluster A was further sub divided in sub cluster A_1 and A_2. Least similarity of (0.42) demonstrated by GMU-2720 and EC-757665 followed by PBN-96 and EC-757665 as well as by NARI-6 and EC-757665. Genotypes, GMU-2720 and PBNS-12, GMU-1339 and PBNS-12 were found to be (0.76) per cent similar based on molecular analysis, but could not consider as duplicate. It may be due to narrowness of the genetic base of widely grown safflower and the results may be different if large number of RAPD primers may use in the study. Our result suggests that, RAPD molecular markers can detect high polymorphism and efficient for assuring genetic diversity and relatedness and also aid in selection of diverse outstanding lines to be used in future breeding programs of safflower.

Introduction

Safflower (*Carthamus tinctorius* L.) is one of the most remarkable oldest domesticated *Rabi* oilseed crop belongs to family Asteraceae. The genus *Carthamus* is very diverse consist of 36 species, out of which *Carthamus tinctorius* L. (2n=24) is the only cultivated species of safflower which is used as seed and oil extraction while the rest are wild species. (Dajue and Mundel, 1996). The assessment of the genetic diversity in crop species is of interest for the conservation of genetic resources, broadening of the genetic base and practical applications in breeding programs. In order to design an appropriate breeding program, it is important to know how much the phenotypic variation of a trait is heritable (Kearsey and Pooni, 1996), since the efficiency of a selection program is mainly

dependent on the magnitude of genetic variation (Falconer and Mackay 1996). Genetic diversity plays a key role in the crop improvement and needed among all correlated varieties, and required for crops evolutionary history and genetic resources (Zada *et al.,* 2013). RAPD primers are extensively used for germplasm categorization and genetic diversity evaluation, gene tagging, in genetic purity testing and also for the identification of cultivars (Shinwari *et al.,* 2013) also it has relatively low cost and high speed is an asset of RAPD markers for evaluation of genetic diversity of different crops (Williams *et al.,* 1990). Therefore, the present study was focused on the characterization of genetic diversity among the eight genotypes of safflower using RAPD markers to provide essential information for future marker facilitated breeding.

Materials and Methods

The materials included in the study consisted of eight parental lines of safflower genotypes. The seeds of these entries were collected from All India Coordinated Research Programme on Safflower, V.N.M.K.V., Parbhani. The pedigree of the chosen parental genotypes is given in (Table 1.)

Isolation of genomic DNA of safflower genotypes

The total genomic DNA was isolated from all eight safflower genotypes by CTAB method (Saghai-Maroof *et al.,* 1984).

RAPD analysis

RAPD PCR amplifications were carried in a total volume of 20µl reaction containing 50ng of genomic DNA, 1XPCR buffer with $MgCl_2$, 0.2 mM of each dNTP, 2.5pmol primer and 1 unit of Taq DNA polymerase (Aristogene, Bengaluru). PCR tubes were placed in thermal cycler (Sensoquest, Germany) for amplification of the genomic DNA as per the standardized protocol:initial denaturation of 7 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C with a final extension step at 72°C for 10 min and hold at 4°C

Data scoring and analysis

The banding patterns generated by RAPD primers were examined to determine the level of polymorphism and the genetic relatedness among the safflower parental genotypes. The presence of band at an amplicon level was scored as (1) and its absence as (0). The binary data was analyzed using standard procedure in NTSYS-PC (Version 2.1; Exeter Biological Software, Setauket, NY) software package (Rohlf, 1998). The data were subjected to the SIMQUAL option to obtain association coefficients using Jaccard's coefficient of similarity to generate a similarity matrix. Clustering analysis was performed with the unweighted pair-group method using arithmetic averages (UPGMA) in the SAHN (sequential, agglomerative, hierarchical and nested clustering method) module of NTSYS-PC.

Results and Discussion

Marker polymorphism

The amplification profiles of the eight safflower parental genotypes produced by the 40 arbitrary oligonucleotide primers revealed a total of 943 bands, 679 of them were polymorphic accounting for 72.00% polymorphism, with an average of 1.80 polymorphic band per primer pair (Table 2 and Fig. 1). The number of fragments per primer ranged from 9 (OPA-10) to 41 (RPI-6, RPI-14) with an average 23.32 bands per primer pair. Among the 40 RAPD primers, 23 primers showed polymorphism to an extent

100 per cent while 13 primers were monomorphic and primers RPI-11, RPI-20, OPA-8, OPA-12 showed no polymorphism respectively. The primer RPI-9 and OPA-10 exhibited (100%) polymorphism with a smaller number of DNA fragments while, the primer RPI 6 showed lowest (37.72) per cent polymorphism. Panahi *et al.*, (2013) detected 65.5 per cent polymorphism among 20 genotypes of safflower using 9 RAPD primers whereas; Safavi *et al.*, (2017) also reported 81.08 polymorphism in 20 safflower genotypes using 13 RAPD primers. Thus, these findings support the results obtained in present research programme.

Cluster analysis

Determining true genetic dissimilarity between individuals using molecular markers is an important and decisive point for clustering which provides visual idea about variability presented in studied genotypes in addition to assuring the continued genetic improvement. Jaccard's pair wise similarity coefficient values (Table 4.) generated using pooled data of 40 RAPD primers for eight safflower genotypes obvious that parents, *viz*., GMU-2720 and PBNS-12, PBNS-12 and GMU-1339 showed highest similarity (0.76) and could not be distinguished from each other and fell in nearby sub-cluster. On the other hand, least similarity of (0.42) were revealed by GMU-2720 and EC-757665 followed by PBN-96 and EC-757665 as well as by NARI-6 and EC-757665 indicating more divergent. Such a trend of least similarity between genotypes of *C. Carthamus* L. was also reported by Safavi *et al.*, (2010), Soregaon *et al.*, (2007), Amini *et al.*, (2007). This authors also reported maximum similarities between genotypes of close relationship between their characters while minimum similarities between genotypes possessing different morphological characters. Further, all the eight parental genotypes showed diversity among themselves indicating that there is a considerable amount of variation which can be exploited through appropriate breeding programme.

Table 1. Pedigree information of parental genotypes of safflower used in the study

Sr. No.	Parents	Sources	Pedigree
1	GMU-2720	AICRP, Parbhani	High yield, low hull content.
2	PBNS-12	AICRP, Parbhani	Resistant to wilt and tolerant to aphid.
3	JMU-1339	AICRP, Parbhani	High yield, low hull content.
4	GMU-3423	AICRP, Parbhani	High yield, Bold capitulum
5	PBN-96	AICRP, Parbhani	High yield, low hull content.
6	NARI-6	AICRP, Parbhani	Non-spiny, suitable for petal collection.
7	GMU-3431	AICRP, Parbhani	Non-spiny, suitable for petal collection.
8	EC-757665	AICRP, Parbhani	Spiny, Resistant to wilt and tolerant to aphid.

Table 2. Total number of bands, number of polymorphic bands and per cents polymorphism using RAPD primers in parental lines of safflower (*Carthamus tinctorius* L.).

Sr. No.	Name of primers	Total number of bands	Number of polymorphic bands	Per cent polymorphism
1	RPI 1	18	18	100.00
2	RPI 2	14	14	100.00
3	RPI 3	22	22	100.00
4	RPI 4	12	12	100.00
5	RPI 5	23	15	65.21
6	RPI 6	**41**	33	37.72
7	RPI 7	36	28	77.77
8	RPI 8	18	18	100.00
9	RPI 9	10	10	100.00
10	RPI 10	22	22	100.00
11	RPI 11	24	0	0.00
12	RPI 12	18	18	100.00
13	RPI 13	36	36	100.00
14	RPI 14	41	33	80.48
15	RPI 15	16	16	100.00
16	RPI 16	27	11	40.74
17	RPI 17	23	23	100.00
18	RPI 18	26	18	69.23
19	RPI 19	25	25	100.00
20	RPI 20	24	0	0.00
21	RPI 21	18	10	55.55
22	RPI 22	17	17	100.00
23	RPI 23	16	16	100.00
24	RPI 24	18	18	100.00
25	RPI 25	19	19	100.00
26	OPA 1	33	1	96.96
27	OPA 2	38	30	78.94
28	OPA 3	13	13	100.00
29	OPA 4	32	32	100.00
30	OPA 5	25	25	100.00.
31	OPA 6	18	10	55.55
32	OPA 7	26	26	100.00
33	OPA 8	32	0	0.00
34	OPA 9	39	7	82.05
35	OPA 10	**9**	9	100.00
36	OPA 11	18	10	55.55
37	OPA 12	24	0	0.00
38	OPA 13	29	29	100.00
39	OPA 14	27	19	70.37
40	OPA 15	16	16	100.00
Total		**943**	**679**	**72.00**

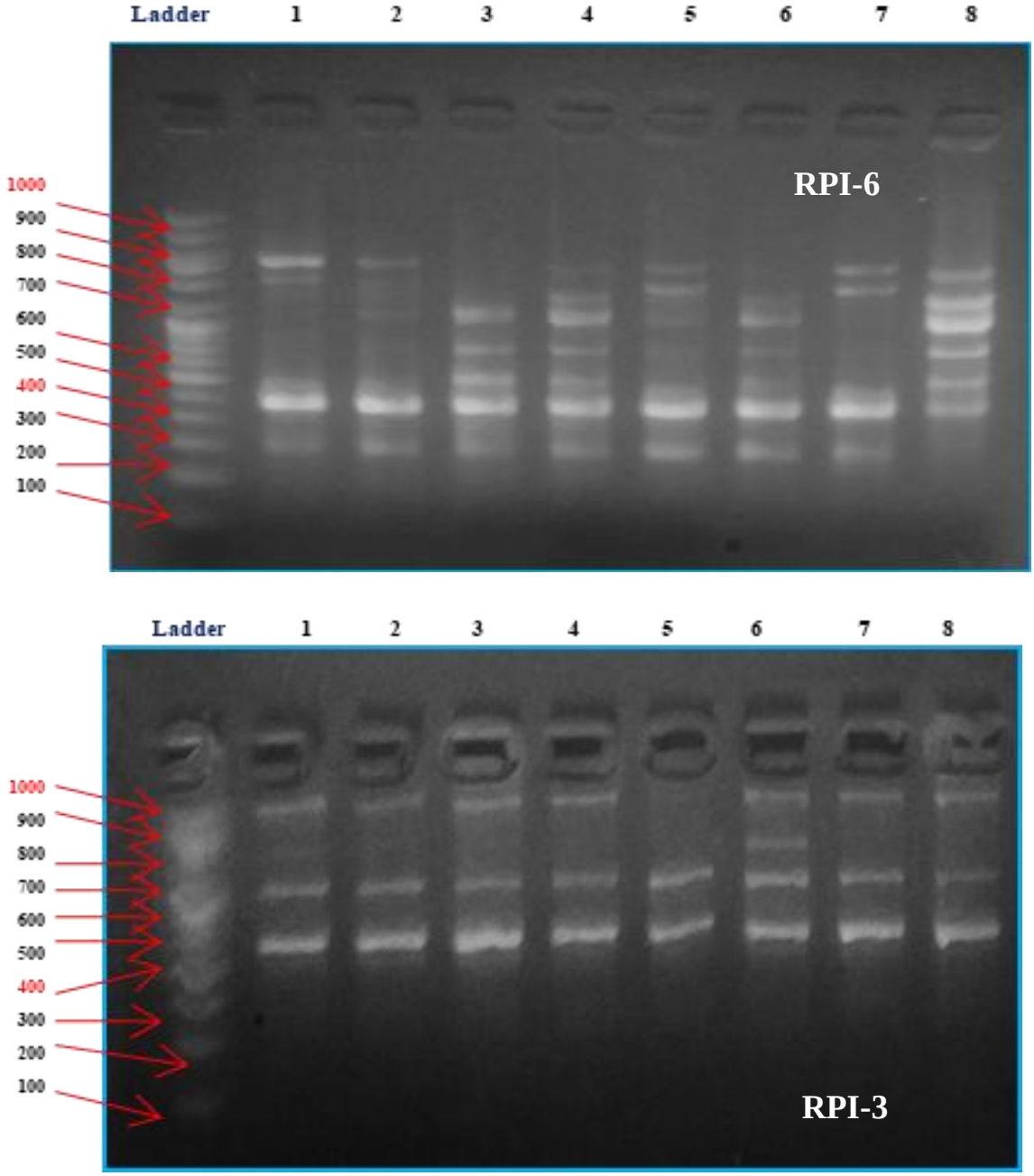
Table 3. Summary statistics of RAPD analysis in parental lines of safflower

Features	RAPD
Total number of markers used	40
Total number of bands	943
Maximum number of bands generated by marker	41
Minimum number of bands generated by marker	9
Total number of polymorphic bands	679
Per cent polymorphism	72.00
Average polymorphism	1.80
Average number of bands per marker	23.32

Table 4. Genetic similarity based on Jaccard's coefficient between parental lines of safflower according to RAPD banding pattern.

Parents	GMU-2720	PBNS-12	JMU-1339	GMU-3423	PBN-96	NARI-6	GMU-3431	EC-757665
GMU-2720	1.00							
PBNS-12	**0.76**	1.00						
JMU-1339	0.52	**0.76**	1.00					
GMU-3423	0.58	0.70	0.74	1.00				
PBN-96	0.52	0.64	0.56	0.70	1.00			
NARI-6	0.60	0.64	0.68	0.66	0.52	1.00		
GMU-3431	0.68	0.62	0.54	0.56	0.74	0.62	1.00	
EC-757665	**0.42**	0.54	0.54	0.44	0.42	**0.42**	0.44	1.00

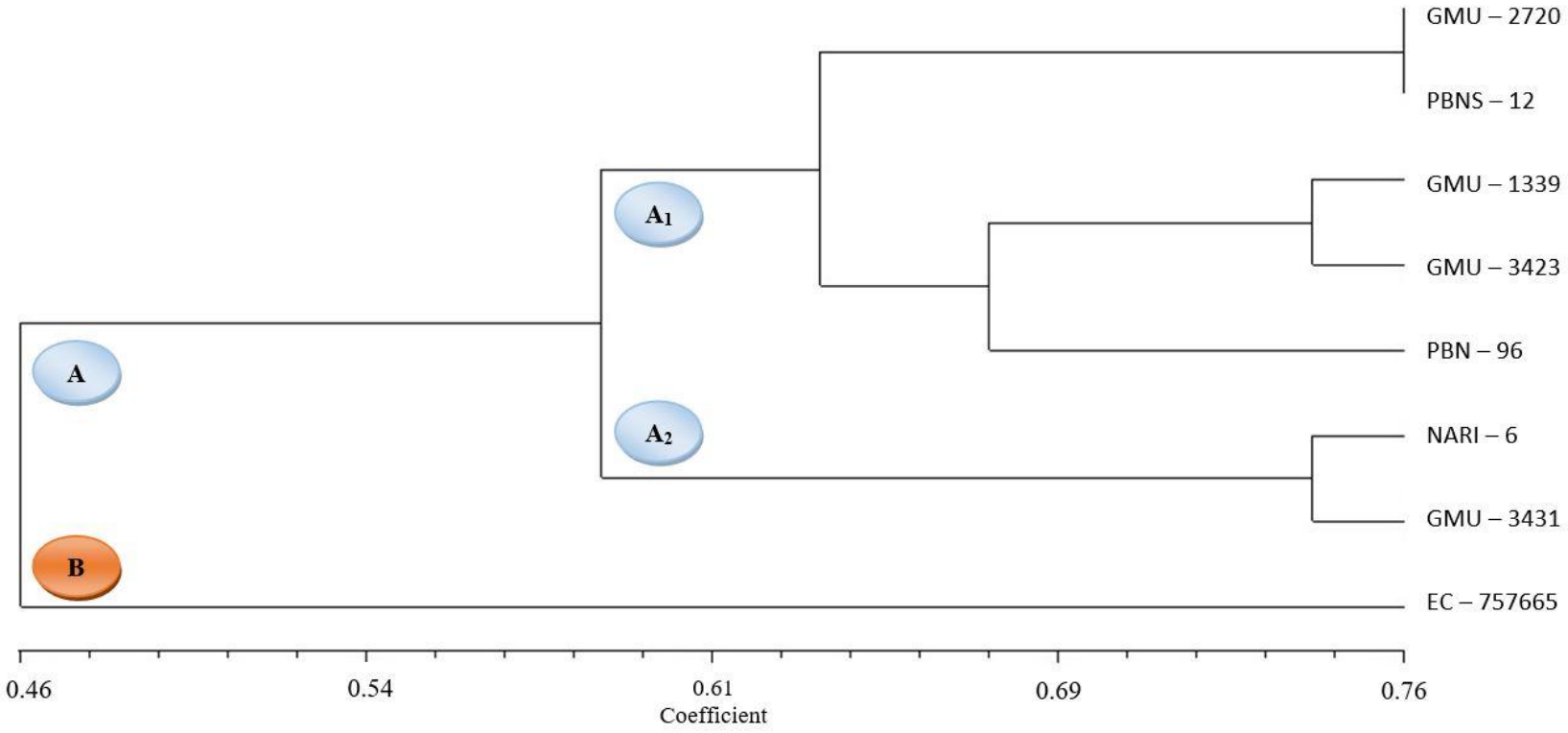
Fig. 1 DNA amplification pattern of parental genotypes using RAPD primers



Whereas,

1	=	GMU-2720	**5**	=	PBN-96
2	=	PBNS-12	**6**	=	NARI-6
3	=	GMU-1339	**7**	=	GMU-3431
4		GMU-3423	**8**	=	EC-757665

Fig. 2. Dendrogram derived from UPGMA cluster analysis using Jaccard's coefficient based on RAPD markers in parental lines of safflower.



Dendrogram generated by UPGMA clustering pattern of eight genotypes using RAPD markers (Fig. 2) ranges the similarity coefficient from 0.46 to 0.76. The dendrogram clearly revealed two clusters named as cluster A and cluster B having similarity coefficient 0.46. Maximum number (7 out of 8) of cultivars fell in cluster A. Cluster A was sub-divided into two sub-clusters, A_1 and A_2 having similarity coefficient 0.59. Sub-cluster A1 has Five genotypes *viz.,* GMU-2720, PBNS-12, GMU-1339 and GMU-3423, PBN-96 with higher similarity. Out of these, GMU-2720, PBNS-12 and GMUU-1339, PBNS-12 showed high genetic similarity (0.76) and could not be distinguished from each other and fell in nearby sub-cluster. Sub-cluster A2 have two genotypes *viz*., NARI-6 and GMU-3431 which were found to genetical similarity (0.74). Genotype, EC 757665 remained isolated forming an out group from the rest of genotypes in cluster B. Earlier, Soregaon *et al.,* (2007), Panahi *et al.,* (2013) and Wadikar *et al.,* (2017) also reported this type of similar clustering. In the present study, however genotypes GMU-2720 and PBNS-12 and GMUU-1339 and PBNS-12 were found to be 0.76 per cent similar based on molecular analysis, but could not be considered as duplicates. It may be due to narrowness of the genetic base of widely grown safflower genotypes and the results may be different if large number of RAPD primers may use in the study.

The present study suggest that, with the help of clustering pattern and genetic relationship, breeder can identify the diverse genotype with least similarity from clusters and employ them in the future breeding programmes of safflower.

The molecular characterization of these genotypes based on RAPD is faster, less expensive and more reliable.

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How to cite this article:

Dhare, S. L., P. B. Wadikar, M. R. Magar and Sonawane, S. J. 2019. Investigation on Genetic Analysis of Safflower (carthamus tinctorius l.) Genotypes using Rapd Molecular Markers.. *Int.J.Curr.Microbiol.App.Sci.* 8(09): 1919-1927. doi: https://doi.org/10.20546/ijcmas.2019.809.222