

Original Research Article

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Molecular Characterization of *Fusarium* Wilt Resistant and Susceptible Safflower (*Carthamus tinctorius* L.) Genotypes

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ABSTRACT

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Safflower (*Carthamus tinctorius* L.) is an oilseed crop that is valued as a source of high quality vegetable oil. The present study was carried out with the objective of evaluation of genetic diversity among 11 *Fusarium* wilt resistance and 5 susceptible safflower genotypes by using RAPD and SSR markers. 241 RAPD primer as well as 17 SSR polymorphic primers were used in this study. Analysis diversity showed significant variation in genotypes for the *Fusarium* wilt disease-related traits. Based on RAPD markers, a total of 1444 bands were amplified and 885 bands (6076%) of these were polymorphic and SSR yielded a total of 20 scorable bands and 19 bands (97.06%) were polymorphic. A logical similarity between the genotype clusters based on molecular data with their resistance or susceptibility to *Fusarium* wilt was observed. PIC for RAPD markers ranged between 0.50-0.92 with an average of 0.80 and for SSR markers ranged between 0.12-0.96 with an average of 0.63. Therefore, from the present study, it can be proved that RAPD and SSR markers can detect high polymorphism and are very useful in studying variation among different safflower genotypes.

Introduction

Safflower (*Carthamus tinctorius* L.) belongs to the family Asteraceae. It is one of the remarkable oldest domesticated crops (Hamdan *et al.*, 2011). It is cultivated in some regions of Europe, Australia, Asia, and the Americas to produce high quality vegetable and industrial oil. Nowadays, it is cultivated mainly for its seed, which is used as source of edible oil and as birdseed (Dajue and Mundel, 1996). In the last few years, the safflower has been used as a biofuel, pharmaceutical production (McPherson *et al.*, 2004) and particular oil types (Velasco and Fernandez-Martinez, 2004). Thereby, the interest in this

crop has been increasing by both private and public sectors. Recent and future investigations about safflower may lead to the growth of this crop as important oilseed crop (Nabloussi and Boujghagh, 2006).

Biochemical, morphological and molecular markers are exploited to evaluate plant genetic diversity. During the past decades, molecular breeding, which has involved both markers assisted selection and genetic engineering, has witnessed significant innovations and advances (Xu *et al.*, 2012). Among various genetic markers, it is very

important to identify, characterize and select the most appropriate ones for effective molecular studies. The most useful molecular markers are Inter Simple Sequence Repeats (ISSR), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) (Kumari *et al.*, 2013).

Different molecular markers including RAPD (Sehgal and Raina, 2005; Ravikumar *et al.*, 2008; Mahasi *et al.*, 2009), AFLP (Zhang *et al.*, 2006), ISSR (Yang *et al.*, 2007; Golkar *et al.*, 2011), and EST-SSR (Chapman *et al.*, 2009; Mayerhofer *et al.*, 2009; Barati and Arzani, 2012) have been used for evaluating genetic diversity in safflower. However, safflower has an enormous variability and several traits that could be genotyped *via* the available molecular marker systems (Sujatha, 2008). The markers reveal genetic relationship and distinctiveness among cultivars of a crop. The knowledge of diversity pattern will allow breeders to understand evolutionary relationship among accessions, to sample germplasm in a more systematic fashion. The genetic relationship and distinctiveness obtained for a cultivar facilitates selection of less related germplasm for intercrossing. This intercrossing ensures sustainable breeding programme in future. The knowledge of diversity also helps to develop strategies to incorporate useful diversity in breeding programmes. Considering above facts, the present investigation was carried out with the objective of assessing genetic diversity in *Fusarium* wilt resistant and susceptible genotypes of safflower by using molecular markers.

Materials and Methods

Plant material

Eleven *Fusarium* wilt resistant and five susceptible safflower genotypes were

collected from Directorate of Oilseeds Research (DOR), Hyderabad (A.P), AICRP on Safflower of Vasant Rao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani and Nimbkar Agricultural Research Station (NARI), Phaltan (M.S)(Table 1).

Isolation of genomic DNA of safflower genotypes

The total genomic DNA was isolated from all sixteen safflower genotypes by CTAB method (Saghai-Marouf *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 MgCl₂, 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

SSR analysis

SSR PCR amplifications were carried in a total volume of 20µl reaction containing 50 ng of genomic DNA, 1X PCR buffer with MgCl₂, 0.05mM of each dNTP, 0.5pmol 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 5 min at 94°C, followed by 35 cycles of 45sec at 94°C, 55sec at T* (Opt), 1 min at 72°C with a final extension step at 72°C for 8 min and hold at 4°C. Amplification products were separated by using 1.5% for RAPD and 2% for SSR

agarose gel electrophoresis run for 1 hour in 1xTAE buffer and stained with ethidium bromide. DNA bands were visualized and photographed in gel documentation system (AlphaImager HP, USA).

Data scoring and analysis

The amplified product of RAPD and SSR from agarose gel images were scored for presence (1), absence (0), missing and doubtful case was scored as 9. Band size was determined by comparison with 100bp and 1000bp DNA ladder (ThermoScientific, USA). Data analysis was performed using NTSYS-PC (Numerical Taxonomy System, Version 2.02) Rohlf, (2000). The SIMQUAL programme was used to calculate the Jaccard's coefficient. Dendrogram was constructed using unweighted pair group method for arithmetic mean (UPGMA) based on Jaccard's coefficient (Figs 2 and 4).

Results and Discussion

RAPD analysis

Total 241 RAPD primers were selected on the basis of consistency and reproducibility of bands. These primers yielded a total of 1444 scorable bands. Total 885 bands were found to be polymorphic with 60.76 percent polymorphism (Table 2). The highest number of bands (14) was obtained with primer OPC-07 (Fig. 1) and OPD-18, while the lowest number (2) was obtained with primer OPJ-08. Primers OPA-20, OPC-06, OPD-05, OPD-09, OPD-16, OPD-17, OPF-18, OPG-03, OPG-09, OPG-20, OPH-02, OPH-04, OPH-12, OPH-13, OPH-17, OPH-18, OPH-19, OPJ-08, OPO-15, OPP-10, Primer-139, UBC-229, UBC-513 and UBC-521 showed maximum polymorphism i.e. 100%, whereas primer OPD-02 showed minimum i.e. 12.50% polymorphism. Total amplified fragments 18211 with average 75 amplified fragments per primer were obtained. Minimum amplified fragments 24 by primer

OPC-12 and maximum 164 by primer OPC-07. Size of bands amplified by primers was noted, the minimum size ranged 200-1000bp and maximum size ranged 750-3500bp. The calculated PIC values of these polymorphic primers ranged from 0.50-0.92 with an average of 0.80.

Similar results were obtained by Safavi *et al.* (2010) used 13 RAPD primers generating 74 amplified fragments in which 60 were polymorphic showing related polymorphism i.e. 81.08 % and noticed 3-9 RAPD fragments generated per primer with an average of 6 bands per primer in safflower. Mahasi *et al.*, (2009) and Khan *et al.*, (2009) found out 61 and 78 amplified fragments from 14 and 15 RAPD primers and noticed 1-10 and 3-16 RAPD fragments generated per primer with an average of 5.5 and 9.5 bands in safflower, respectively. On safflower same work was reported by Amini *et al.*, (2008), and Souframani and Gopalakrishna (2004) by using 15 and 25 RAPD primers which produced total polymorphic 132 and 44 bands and calculated amplified products range from 3 to 9 through standard of 1.8 and 13 bands for each primer, respectively.

Cluster analysis based on RAPD data

The dendrogram obtained using RAPD data indicates five clusters. Cluster I contained maximum (5) genotypes *viz.*, GMU-5016, PBNS-117, NARI-38, GMU-5080 and NARI-6 all are resistant to *Fusarium* wilt with 82% similarity. Cluster II comprised of 2 genotypes *viz.* NARI-NH-1 and GMU-4965 at 84% similarity value (Fig. 3). Cluster III contains 4 resistant genotypes i.e. NARI-H-15, GMU-5153, GMU-4546 and GMU-4925 with 83% similarity. All 5 susceptible genotypes clustered separately in 2 main clusters i.e. IV and V. Cluster IV contains 3 susceptible genotypes *viz.* NIRA, BHIMA and SSF-708 sharing 83.5% similarity.

Table.1 List of safflower genotypes resistant and susceptible to Fusarium wilt

S. No.	Name of the Safflower Genotype	Resistant/ Susceptible	Source of Collection
1	GMU 5016	Resistant	Directorate of Oilseeds Research, Hyderabad
2	PBNS 117	Resistant	All India Coordinated Research Programme on Safflower, V.N.M.K.V., Parbhani
3	GMU 5080	Resistant	Directorate of Oilseeds Research, Hyderabad
4	NARI-6	Resistant	Nimbkar Agricultural Research Institute, Phalthan
5	NARI-38	Resistant	Nimbkar Agricultural Research Institute, Phalthan
6	NARI-H-15	Resistant	Nimbkar Agricultural Research Institute, Phalthan
7	NARI-NH-1	Resistant	Nimbkar Agricultural Research Institute, Phalthan
8	GMU-5153	Resistant	Directorate of Oilseeds Research, Hyderabad
9	GMU-4546	Resistant	Directorate of Oilseeds Research, Hyderabad
10	GMU-4925	Resistant	Directorate of Oilseeds Research, Hyderabad
11	GMU-4965	Resistant	Directorate of Oilseeds Research, Hyderabad
12	NIRA	Susceptible	Nimbkar Agricultural Research Institute, Phalthan
13	BHIMA	Susceptible	All India Coordinated Research Programme on Safflower, V.N.M.K.V., Parbhani
14	SSF-708	Susceptible	All India Coordinated Research Programme on Safflower, V.N.M.K.V., Parbhani
15	PBNS-40	Susceptible	All India Coordinated Research Programme on Safflower, V.N.M.K.V., Parbhani
16	PBNS-12	Susceptible	All India Coordinated Research Programme on Safflower, V.N.M.K.V., Parbhani

Table.2 Characteristics of the amplification products obtained by using RAPD primers

S. No.	Primer ID	Sequence	Total Bands	Mono morphic bands	Poly morphic bands	Poly morphic %	No. of amplified fragments	Size of amplicons (range in bp)	PIC
1	OPA-01	CAGGCCCTTC	7	1	6	85.71	100	300-2500	0.86
2	OPA-02	TGCCGAGCTG	8	3	5	62.50	112	250-1000	0.87
3	OPA-03	AGTCAGCCAC	9	4	5	55.56	113	250-2000	0.87
4	OPA-04	AATCGGGCTG	6	2	4	66.67	68	200-1500	0.81
5	OPA-05	AGGGGTCTTG	8	5	3	37.50	113	300-1500	0.87
6	OPA-06	GGTCCCTGAC	7	1	6	85.71	100	300-2500	0.85

7	OPA-07	GAAACGGGTG	8	2	6	75.00	107	240-2000	0.87
8	OPA-08	GTGACGTAGG	4	2	2	50.00	45	300-1000	0.69
9	OPA-09	GGGTAACGCC	6	5	1	16.67	85	200-2400	0.82
10	OPA-10	GTGATCGCAG	4	1	3	75.00	41	250-2000	0.68
11	OPA-11	CAATCGCCGT	4	3	1	25.00	57	250-1000	0.74
12	OPA-12	TCGGCGATAG	7	4	3	42.86	102	200-1500	0.86
13	OPA-13	CAGCACCCAC	5	2	3	60.00	67	300-1500	0.78
14	OPA-14	TCTGTGCTGG	5	1	4	80.00	70	300-2500	0.8
15	OPA-15	TTCCGAACCC	6	1	5	83.33	90	300-1000	0.83
16	OPA-16	AGCCAGCGAA	5	1	4	80.00	62	750-3000	0.78
17	OPA-17	GACCGCTTGT	9	3	6	66.67	86	250-2000	0.85
18	OPA-18	AGGTGACCGT	9	1	8	88.89	78	300-2500	0.85
19	OPA-19	CAAACGTCGG	8	3	5	62.50	109	300-1000	0.87
20	OPA-20	GTTGCGATCC	10	0	10	100.00	68	250-2000	0.88
21	OPAB-02	GGAAACCCCT	6	2	4	66.67	85	200-1500	0.83
22	OPAB-03	TGGCGCACAC	5	3	2	40.00	71	300-1500	0.79
23	OPAB-04	GGCACGCGTT	5	3	2	40.00	77	300-2500	0.8
24	OPAB-05	CCCGAAGCGA	8	3	5	62.50	103	300-1000	0.86
25	OPAB-06	GTGGCTTGGA	4	3	1	25.00	63	500-2000	0.75
26	OPAB-07	GTAAACCGCC	6	4	2	33.33	77	400-1700	0.81
27	OPAC-09	AGAGCGTACC	5	4	1	20.00	79	200-1500	0.8
28	OPAC-10	AGCAGCGAGG	13	3	10	76.92	143	300-2500	0.91
29	OPAI-11	ACGGCGATGA	5	3	2	40.00	68	600-4000	0.78
30	OPAK-05	GATGGCAGTC	7	3	4	57.14	80	400-2500	0.83
31	OPAZ-01	TCGGATCCGT	7	5	2	28.57	103	300-1000	0.85
32	OPAZ-02	CCTGAACGGA	6	4	2	33.33	78	500-2000	0.81
33	OPAZ-03	GGCTGTGTGG	5	3	2	40.00	59	400-1700	0.76
34	OPAZ-04	CCAGCCTCAG	4	2	2	50.00	46	400-2500	0.71
35	OPAZ-07	CACGAGTCTC	6	1	5	83.33	83	200-2000	0.83
36	OPB-01	GTTTCGCTCC	7	2	5	71.43	79	300-2500	0.83
37	OPB-02	TGATCCCTGG	8	6	2	25.00	124	400-2300	0.87
38	OPB-03	CATCCCCCTG	6	5	1	16.67	85	240-2500	0.82
39	OPB-04	GGACTGGAGT	4	1	3	75.00	55	750-3500	0.75
40	OPB-05	TGCGCCCTTC	5	4	1	20.00	78	250-2000	0.8
41	OPB-06	TGCTCTGCC	7	3	4	57.14	90	300-2500	0.85

42	OPB-07	GGTGACGCAG	5	2	3	60.00	64	300-1000	0.79
43	OPB-08	GTCCACACGG	9	5	4	44.44	120	240-2000	0.88
44	OPB-09	TGGGGGACTC	8	4	4	50.00	93	300-1500	0.85
45	OPB-10	CTGCTGGGAC	4	1	3	75.00	54	200-1500	0.75
46	OPB-11	GTAGACCCGT	5	2	3	60.00	71	300-1000	0.8
47	OPB-12	CCTTGACGCA	6	2	4	66.67	81	200-2400	0.83
48	OPB-13	TTCCCCGCT	6	3	3	50.00	79	250-2000	0.8
49	OPB-14	TCCGCTCTGG	8	3	5	62.50	97	300-2500	0.86
50	OPB-15	GGAGGGTGTT	6	1	5	83.33	64	300-1500	0.81
51	OPB-16	TTTGCCCGGA	5	3	2	40.00	76	200-2000	0.8
52	OPB-17	AGGGAACGAG	7	3	4	57.14	89	300-2500	0.85
53	OPB-18	CCACAGCAGT	4	2	2	50.00	51	300-1500	0.71
54	OPB-19	ACCCCGAAG	7	6	1	14.29	110	200-1500	0.86
55	OPB-20	GGACCCTTAC	9	2	7	77.78	78	300-1000	0.85
56	OPBA-18	CTCGGATGTC	5	1	4	80.00	58	200-2400	0.78
57	OPBH-02	GTAAGCCGAG	8	2	6	75.00	114	250-2000	0.87
58	OPC-01	TTCGAGCCAG	5	1	4	80.00	59	250-1000	0.78
59	OPC-02	GTGAGGCGTC	5	1	4	80.00	62	200-1500	0.78
60	OPC-03	GGGGGTCTTT	5	2	3	60.00	67	300-1500	0.78
61	OPC-04	CCGCATCTAC	6	3	3	50.00	78	300-2500	0.82
62	OPC-05	GATGACCGCC	5	1	4	80.00	37	300-1500	0.71
63	OPC-06	GAACGGACTC	7	0	7	100.00	79	300-1000	0.84
64	OPC-07	GTCCCGACGA	14	4	10	71.43	164	200-2400	0.92
65	OPC-08	TGGACCGGTG	6	3	3	50.00	82	300-1500	0.82
66	OPC-09	CTCACCGTCC	6	2	4	66.67	74	200-1500	0.82
67	OPC-10	TGTCTGGGTG	4	1	3	75.00	44	300-1000	0.72
68	OPC-11	AAAGCTGCGG	7	5	2	28.57	95	200-2400	0.84
69	OPC-12	TGTCATCCCC	4	1	3	75.00	24	250-2000	0.52
70	OPC-13	AAGCCTCGTC	5	1	4	80.00	64	250-1000	0.79
71	OPC-14	TGCGTGCTTG	5	2	3	60.00	70	200-1500	0.8
72	OPC-15	GACGGATCAG	7	1	6	85.71	53	300-1300	0.79
73	OPC-16	CACACTCCAG	5	1	4	80.00	54	250-1000	0.76
74	OPC-17	TTCCCCCAG	4	2	2	50.00	62	200-1500	0.75
75	OPC-18	TGAGTGGGTG	4	1	3	75.00	59	300-1500	0.75
76	OPC-19	GTTGCCAGCC	7	2	5	71.43	103	300-2500	0.86

77	OPC-20	ACTTCGCCAC	7	5	2	28.57	96	250-2000	0.85
78	OPD-01	ACCGCGAAGG	7	6	1	14.29	110	300-1000	0.86
79	OPD-02	GGACCCAACC	8	7	1	12.50	127	300-1500	0.87
80	OPD-03	GTCGCCGTCA	6	3	3	50.00	72	200-1500	0.81
81	OPD-04	TCTGGTGAGG	5	2	3	60.00	48	300-1000	0.74
82	OPD-05	TGAGCGGACA	8	0	8	100.00	79	200-2400	0.84
83	OPD-06	ACCTGAACGG	8	1	7	87.50	59	250-2000	0.82
84	OPD-07	TTGGCACGGG	4	3	1	25.00	63	250-1000	0.75
85	OPD-08	GTGTGCCCCA	5	2	3	60.00	44	200-1500	0.71
86	OPD-09	CTCTGGAGAC	5	0	5	100.00	51	300-1500	0.77
87	OPD-10	GGTCTACACC	6	1	5	83.33	63	500-2500	0.78
88	OPD-11	AGCGCCATTG	3	2	1	33.33	47	200-2000	0.67
89	OPD-12	CACCGTATCC	6	4	2	33.33	94	300-2500	0.83
90	OPD-13	GGGGTGACGA	7	4	3	42.86	106	400-2300	0.86
91	OPD-14	CTTCCCAAG	5	1	4	80.00	42	200-1200	0.73
92	OPD-15	CATCCGTGCT	4	2	2	50.00	61	200-1500	0.75
93	OPD-16	AGGGCGTAAG	4	0	4	100.00	31	300-1500	0.65
94	OPD-17	TTTCCCACGG	6	0	6	100.00	53	300-2500	0.82
95	OPD-18	GAGAGCCAAC	14	5	9	64.29	130	250-2000	0.91
96	OPD-19	CTGGGGACTT	7	4	3	42.86	108	300-1000	0.86
97	OPD-20	ACCCGGTCAC	6	3	3	50.00	79	200-2400	0.82
98	OPE-15	ACGCACAACC	5	3	2	40.00	61	250-2000	0.77
99	OPF-01	ACGGATCCTG	3	1	2	66.67	46	250-1000	0.67
100	OPF-02	GAGGATCCCT	4	3	1	25.00	53	200-1500	0.72
101	OPF-03	CCTGATCACC	6	5	1	16.67	94	300-1500	0.83
102	OPF-04	GGTGATCAGG	4	3	1	25.00	53	300-2500	0.72
103	OPF-05	CCGAATTCCC	6	5	1	16.67	94	300-1000	0.83
104	OPF-06	GGGAATTCGG	6	4	2	33.33	70	500-2000	0.81
105	OPF-07	CCGATATCCC	4	1	3	75.00	56	400-1700	0.75
106	OPF-08	GGGATATCGG	5	3	2	40.00	63	400-2500	0.76
107	OPF-09	CCAAGCTTCC	6	3	3	50.00	64	200-2000	0.79
108	OPF-10	GGAAGCTTGG	4	3	1	25.00	51	300-2500	0.7
109	OPF-11	TTGGTACCCC	4	1	3	75.00	33	400-2300	0.66
110	OPF-12	ACGGTACCAG	6	4	2	33.33	77	240-2500	0.81
111	OPF-13	GGCTGCAGAA	6	4	2	33.33	76	750-3500	0.81

112	OPF-14	TGCTGCAGGT	6	3	3	50.00	70	250-2000	0.79
113	OPF-15	CCAGTACTCC	4	1	3	75.00	47	300-2500	0.73
114	OPF-16	GGAGTACTGG	9	2	7	77.78	92	200-1500	0.86
115	OPF-17	AACCCGGGAA	7	4	3	42.86	102	250-1000	0.86
116	OPF-18	TTCCCGGGTT	4	0	4	100.00	59	250-1500	0.75
117	OPF-19	CCTCTAGACC	6	4	2	33.33	77	240-2000	0.81
118	OPF-20	GGTCTAGAGG	7	1	6	85.71	74	300-1500	0.82
119	OPG-01	CTACGGAGGA	6	2	4	66.67	71	300-1500	0.81
120	OPG-02	GGCACTGAGG	9	1	8	88.89	97	200-1500	0.88
121	OPG-03	GAGCCCTCCA	4	0	4	100.00	52	300-1000	0.74
122	OPG-04	AGCGTGTCTG	5	2	3	60.00	41	200-2400	0.68
123	OPG-05	CTGAGACGGA	4	1	3	75.00	44	250-2000	0.72
124	OPG-06	GTGCCTAACC	5	2	3	60.00	68	200-1500	0.78
125	OPG-07	GAACCTGCGG	8	3	5	62.50	108	300-1500	0.87
126	OPG-08	TCACGTCCAC	5	3	2	40.00	68	300-2500	0.79
127	OPG-09	CTGACGTCAC	6	0	6	100.00	64	300-1000	0.81
128	OPG-10	AGGGCCGTCT	6	3	3	50.00	82	500-2000	0.82
129	OPG-11	TGCCCGTCGT	6	2	4	66.67	74	400-1700	0.82
130	OPG-12	CAGCTCACGA	3	1	2	66.67	38	400-2500	0.66
131	OPG-13	CTCTCCGCCA	5	1	4	80.00	40	200-2000	0.73
132	OPG-14	GGATGAGACC	5	3	2	28.57	91	300-2500	0.84
133	OPG-15	ACTGGGACTC	5	1	4	80.00	41	400-2300	0.69
134	OPG-16	AGCGTCCTCC	6	2	4	66.67	75	240-2500	0.82
135	OPG-17	ACGACCGACA	6	1	5	83.33	63	750-3500	0.78
136	OPG-18	GGCTCATGTG	8	2	6	75.00	88	250-2000	0.86
137	OPG-19	GTCAGGGCAA	7	6	1	14.29	111	300-2500	0.86
138	OPG-20	TCTCCCTCAG	5	0	5	100.00	36	300-1000	0.71
139	OPH-01	GGTCGGAGAA	5	2	3	60.00	52	400-2000	0.76
140	OPH-02	TCGGACGTGA	8	0	8	100.00	57	300-1000	0.81
141	OPH-03	AGACGTCCAC	5	3	2	40.00	69	300-2500	0.78
142	OPH-04	GGAAGTCGCC	5	0	5	100.00	44	400-2300	0.73
143	OPH-05	AGTCGTCCCC	4	3	1	25.00	62	240-2500	0.75
144	OPH-06	ACGCATCGCA	5	2	3	60.00	57	750-3500	0.76
145	OPH-07	CTGCATCGTG	8	1	7	87.50	84	250-1500	0.87
146	OPH-08	GAAACACCCC	6	1	5	83.33	71	240-2000	0.81

147	OPH-09	TGTAGCTGGG	8	3	5	62.50	121	300-1500	0.87
148	OPH-10	CCTACGTCAG	6	2	4	66.67	88	200-1500	0.83
149	OPH-11	CTTCCGCAGT	7	3	4	57.14	94	300-1000	0.85
150	OPH-12	ACGCGCATGT	5	0	5	100.00	68	200-2400	0.8
151	OPH-13	GACGCCACAC	6	0	6	100.00	80	250-2000	0.86
152	OPH-14	ACCAGGTTGG	4	2	2	50.00	62	250-1000	0.75
153	OPH-15	AATGGCGCAG	6	2	4	66.67	87	200-1500	0.83
154	OPH-16	TCTCAGCTGG	5	1	4	80.00	52	300-1500	0.73
155	OPH-17	CACTCTCCTC	6	0	6	100.00	80	300-2500	0.83
156	OPH-18	GAATCGGCCA	5	0	5	100.00	62	300-1000	0.78
157	OPH-19	CTGACCAGCC	5	0	5	100.00	72	500-2000	0.8
158	OPH-20	GGGAGACATC	6	1	5	83.33	63	240-2000	0.79
159	OPI-16	TCTCCGCCCT	8	1	7	87.50	70	200-1500	0.83
160	OPJ-01	CCCGGCATAA	7	4	3	42.86	77	250-1000	0.82
161	OPJ-02	CCCGTTGGGA	6	2	4	66.67	75	200-2400	0.81
162	OPJ-03	TCTCCGCTTG	6	1	5	83.33	64	250-2000	0.8
163	OPJ-04	CCGAACACGG	4	2	2	50.00	56	250-1000	0.74
164	OPJ-05	CTCCATGGGG	7	2	5	71.43	94	200-1500	0.85
165	OPJ-06	TCGTTCCGCA	5	3	2	40.00	78	300-1500	0.8
166	OPJ-07	CCTCTCGACA	5	1	4	80.00	41	750-2000	0.68
167	OPJ-08	CATACCGTGG	2	0	2	100.00	28	250-1500	0.5
168	OPJ-09	TGAGCCTCAC	4	1	3	75.00	56	240-2000	0.74
169	OPJ-10	AAGCCCGAGG	4	2	2	50.00	61	300-1500	0.75
170	OPJ-11	ACTCCTGCGA	7	1	6	85.71	86	250-1300	0.84
171	OPJ-12	GTCCCGTGGT	6	2	4	66.67	74	400-2500	0.82
172	OPJ-13	CCACACTACC	5	3	2	40.00	65	200-2000	0.78
173	OPJ-14	CACCCGGATG	9	5	4	44.44	127	300-2500	0.88
174	OPJ-15	TGTAGCAGGG	5	2	3	60.00	71	400-2300	0.8
175	OPJ-16	CTGCTTAGGG	6	2	4	66.67	76	240-2500	0.82
176	OPJ-17	ACGCCAGTTC	6	3	3	50.00	56	750-3500	0.75
177	OPJ-18	TGGTCGCAGA	4	2	2	50.00	56	250-2000	0.73
178	OPJ-19	GGACACCACT	4	2	2	50.00	53	300-2500	0.72
179	OPJ-20	AAGCGGCCTC	4	2	2	50.00	50	300-1000	0.7
180	OPK-10	GTGCAACGTG	5	2	3	60.00	44	250-1000	0.71
181	OPK-13	GGTTGTACCC	7	2	5	71.43	84	240-2000	0.84

182	OPK-14	CCCGCTACAC	4	1	3	75.00	46	400-2500	0.73
183	OPK-15	CTCCTGCCAA	11	4	7	63.64	145	300-1700	0.9
184	OPK-16	GAGCGTCGAA	5	2	3	60.00	77	300-1000	0.8
185	OPK-17	CCCAGCTGTG	4	1	3	75.00	57	200-2400	0.75
186	OPK-18	CCTAGTCGAG	4	2	2	50.00	62	250-2000	0.75
187	OPK-19	CACAGGCGGA	6	1	5	83.33	72	250-1000	0.8
188	OPK-20	GTGTCGCGAG	7	3	4	57.14	97	200-1500	0.85
189	OPM-20	AGGTCTTGGG	6	2	4	66.67	90	300-1500	0.81
190	OPN-04	GACCGACCCA	6	3	3	50.00	82	300-2500	0.82
191	OPO-09	TCCCACGCAA	6	1	5	83.33	76	300-1000	0.82
192	OPO-15	TGGCGTCCTT	5	0	5	100.00	69	500-2000	0.8
193	OPO-19	GGTGCACGTT	9	4	5	55.56	119	240-2500	0.9
194	OPP-07	GTCCATGCCA	6	4	2	33.33	86	750-3500	0.83
195	OPP-08	ACATCGCCCA	9	4	5	55.56	131	250-2000	0.89
196	OPP-10	TCCCGCCTAC	8	0	8	100.00	107	300-2500	0.87
197	OPP-11	AACGCGTCGG	7	4	3	42.86	86	300-1000	0.83
198	OPP-13	GGAGTGCCTC	4	1	3	75.00	52	240-2000	0.73
199	OPP-14	CCAGCCGAAC	6	4	2	33.33	81	240-2500	0.82
200	OPQ-05	CCGCGTCTTG	7	3	4	57.14	86	750-3500	0.83
201	OPT-08	AACGGCGACA	5	4	1	20.00	79	250-2000	0.8
202	OPW-09	GTGACCGAGT	6	2	4	66.67	76	300-2500	0.82
203	OPX-01	CTGGGCACGA	6	4	2	33.33	94	300-1000	0.83
204	OPX-05	CCTTCCCTC	7	3	4	57.14	97	240-2000	0.85
205	OPX-09	GGTCTGGTTG	4	2	2	50.00	54	240-2500	0.73
206	OPX-16	CTCTGTTCGG	8	5	3	37.50	113	750-3500	0.87
207	OPZ-10	CCGACAAACC	8	2	6	75.00	90	300-2000	0.84
208	Primer-139	GTTCGCTCC	5	0	5	100.00	56	300-2500	0.76
209	Primer-142	AACGCGAAC	6	4	2	33.33	88	400-2300	0.83
210	RC-11	TGACACCTC	4	2	2	50.00	61	240-2500	0.75
211	RC-14	CTGCTGAAG	6	1	5	83.33	63	750-3500	0.79
212	RC-15	CGGCTCGGT	9	3	6	66.67	119	250-2000	0.88
213	RC-16	GAGCGTTGT	7	2	5	71.43	76	300-2500	0.83
214	RC-20	ACCCGGACA	6	2	4	66.67	79	300-1000	0.82
215	RKAT-01	CAGTGGTTCC	6	3	3	50.00	93	300-2500	0.83
216	RKAT-03	GACTGGGAGG	9	3	6	66.67	93	400-2300	0.86

217	RKAT-04	TTGCCTCGCC	7	1	6	85.71	99	250-1000	0.85
218	RKAT-09	CCGTTAGCGT	7	5	2	28.57	105	250-1500	0.86
219	RKAT-13	CTGGTGGAAAG	4	2	2	50.00	50	240-2000	0.73
220	UBC-030	CCGGCCTTAG	6	5	1	16.67	93	300-1500	0.83
221	UBC-120	GAATTTCCCC	9	6	3	33.33	138	200-1500	0.89
222	UBC-125	GCGGTTGAGG	5	2	3	60.00	70	300-1000	0.79
223	UBC-194	AGGACGTGCC	4	1	3	75.00	52	200-2400	0.74
224	UBC-229	CCACCCAGAG	7	0	7	100.00	83	350-1700	0.84
225	UBC-302	CGGCCACGT	8	4	4	50.00	110	250-1000	0.87
226	UBC-324	ACAGGGAACG	4	2	2	50.00	42	250-1500	0.68
227	UBC-359	AGGCAGACCT	5	3	2	40.00	51	240-2000	0.70
228	UBC-410	CGTCACAGAG	5	4	1	20.00	72	300-1500	0.79
229	UBC-414	AAGGCACCAG	6	3	3	50.00	73	200-1500	0.81
230	UBC-440	CTGTCAACC	3	2	1	16.67	62	300-1000	0.79
231	UBC-459	GCGTCGAGGG	6	2	4	80.00	74	200-2400	0.8
232	UBC-497	GCATAGTGCG	6	2	4	66.67	70	250-2000	0.81
233	UBC-513	TCGGACGTAA	6	0	6	100.00	69	200-2000	0.8
234	UBC-521	CTGAGACGCA	4	0	4	100.00	46	250-1000	0.72
235	UBC-841	GACACGACAC GACACGACAC	8	2	6	75.00	83	250-1000	0.85
236	UBC-880	GGAGAGGAGA GGAGAGGAGA	6	2	4	66.67	64	200-1500	0.79
237	Em12	GACTGCGTAC GAATTCT	5	3	2	40.00	73	300-1500	0.79
238	Em5	GACTGCGTAC GAATTAAC	6	5	1	16.67	95	300-2500	0.83
239	IR-74	CTCCTCCTCCT CCTC	6	2	4	66.67	84	300-1000	0.83
240	IS-31	GCACACACAC ACACACA	5	3	2	40.00	77	500-2000	0.8
241	Me8SCAR2	TGAGTCCAAA CCGGACTACA AG	9	2	7	77.78	102	250-2500	0.87
Total			1444	559	885	---	18211	---	---
Average			6	2	3	60.76	75	---	0.80
Minimum			2	0	1	12.50	24	200-1000	0.50
Maximum			14	7	10	100	164	750-3500	0.92

Table.3 Similarity matrix among 16 safflower genotypes using RAPD and SSR data

	GMU-5016	PBNS-117	GMU-5080	NARI-6	NARI-38	NARI-H-15	NARI-NH-1	GMU-5153	GMU-4546	GMU-4925	GMU-4965	NIRA	BHIMA	SSF-708	PBNS-40	PBNS-12		Average	Minimum	Maximum
GMU-5016	1	0.65	0.70	0.70	0.75	0.70	0.75	0.55	0.60	0.70	0.55	0.55	0.40	0.50	0.35	0.40		0.62	0.35	0.75
PBNS-117	0.83	1	0.75	0.55	0.70	0.65	0.70	0.60	0.65	0.65	0.80	0.70	0.55	0.55	0.50	0.35		0.65	0.35	0.80
GMU-5080	0.81	0.85	1	0.60	0.95	0.70	0.75	0.55	0.60	0.80	0.75	0.75	0.60	0.60	0.25	0.40		0.67	0.25	0.95
NARI-6	0.79	0.85	0.84	1	0.55	0.70	0.75	0.75	0.60	0.60	0.65	0.65	0.60	0.50	0.55	0.50		0.64	0.50	0.75
NARI-38	0.81	0.86	0.82	0.81	1	0.75	0.80	0.60	0.65	0.85	0.70	0.70	0.55	0.65	0.20	0.35		0.67	0.20	0.95
NARI-H-15	0.81	0.82	0.81	0.80	0.83	1	0.85	0.75	0.80	0.70	0.55	0.65	0.50	0.60	0.35	0.50		0.67	0.35	0.85
NARI-NH-1	0.75	0.74	0.75	0.74	0.74	0.81	1	0.70	0.75	0.85	0.60	0.70	0.55	0.65	0.30	0.45		0.70	0.30	0.85
GMU-5153	0.81	0.80	0.81	0.80	0.80	0.84	0.79	1	0.85	0.65	0.80	0.70	0.65	0.65	0.50	0.35		0.67	0.35	0.85
GMU-4546	0.78	0.79	0.79	0.80	0.79	0.86	0.80	0.87	1	0.70	0.65	0.65	0.50	0.60	0.45	0.30		0.65	0.30	0.85
GMU-4925	0.78	0.81	0.82	0.80	0.83	0.83	0.77	0.83	0.84	1	0.65	0.75	0.60	0.80	0.25	0.40		0.68	0.25	0.85
GMU-4965	0.77	0.79	0.79	0.77	0.78	0.84	0.83	0.81	0.84	0.83	1	0.80	0.75	0.65	0.50	0.35		0.67	0.35	0.80
NIRA	0.77	0.77	0.77	0.78	0.80	0.79	0.75	0.81	0.81	0.81	0.78	1	0.85	0.85	0.40	0.55		0.70	0.40	0.85
BHIMA	0.80	0.79	0.79	0.79	0.80	0.81	0.76	0.83	0.81	0.82	0.78	0.84		0.80	0.55	0.60		0.63	0.40	0.85
SSF-708	0.76	0.79	0.76	0.77	0.79	0.82	0.76	0.77	0.79	0.78	0.79	0.80	0.84	1	0.45	0.60		0.65	0.45	0.85
PBNS-40	0.70	0.69	0.71	0.69	0.69	0.73	0.71	0.68	0.71	0.70	0.72	0.70	0.74	0.77	1	0.55		0.45	0.20	0.55
PBNS-12	0.73	0.72	0.73	0.73	0.71	0.74	0.71	0.72	0.72	0.71	0.73	0.72	0.76	0.79	0.83	1		0.48	0.30	0.60
Average	0.79	0.81	0.80	0.80	0.80	0.82	0.78	0.81	0.81	0.81	0.80	0.79	0.81	0.80	0.74	0.75				
Minimum	0.70	0.69	0.71	0.69	0.69	0.73	0.71	0.68	0.71	0.70	0.72	0.70	0.74	0.76	0.68	0.71				
Maximum	0.83	0.86	0.85	0.85	0.86	0.86	0.83	0.87	0.87	0.84	0.84	0.84	0.84	0.84	0.83	0.83				

Below diagonal RAPD similarity matrix, above diagonal SSR similarity matrix.

Table.4 Characteristics of the amplification products obtained by using SSR primers

S. No.	Primer ID	Sequence	Annealing Temp (°C)	Total Bands	Mono morphic bands	Poly morphic bands	Poly morphic %	No. of amplified fragments	Size of amplicons (range in bp)	PIC
1	CaSTM S-21	F-CTACAGTCTTTTGTCTTCTAGCTT R-ATATTTTTTAAGAGGCTTTTGGTAG	55	1	0	1	100	11	150	0.53
2	LG-112	F- AAATGCACGCGATTTTCGCT R- CGAAGCGAGTAAAGGGTTCGT	55	1	0	1	100	10	2000	0.61
3	LG-122	F-GGAATCGGAATCGGGGACTC R-AGGTTTGACCTGCGTATCGG	58	1	0	1	100	15	200	0.12
4	LG-123	F-GTTATGTGTTTTCGCGGTGT R-AGGCAAGGAGTTTTCGCTG	58	1	0	1	100	9	250	0.68
5	LG-124	F-GTGTATGTGTTTTCGCGGT R-GTATCGGCACTTCGACCCTT	54	1	0	1	100	12	700	0.44
6	LG-125	F-TGTTATGTGTTTTCGCGGTG R-CACTTCGACCCTTCAACCCT	53	1	0	1	100	13	300	0.81
7	LG-132	F-TGAAATCCACCACTGCCCAT R-GCCCCACCACAACCTGAAACT	60	1	0	1	100	8	200	0.44
8	LG-135	F-AAATCCACCACTGCCCATCT R-CTGGCAAAAACGGTGCAGAA	58	1	0	1	100	12	300	0.75
9	LG-138	F-AAATCCACCACTGCCCATCTT R-ACAGAAGTGGATGGACGTGT	60	1	0	1	100	12	400	0.44
10	RGA-A	F- GGGGGTGGGAAGACAACCTAT R- CTCCTGGACCAAACCAATC	60	1	0	1	100	8	300	0.75
11	RGA-C	F- GGGTGGGGAAAACCACTCTA R- CTGCTTGCTCTAAGGTTGCC	52	1	0	1	100	14	100	0.23
12	RGA-D	F- GCTCCCTGAACCAAACCAT R- GGGGTCGGGAAGACAACCTAT	53	1	0	1	100	5	300	0.90
13	RGA-E	F- GGGGTCGGGAAGACAACCTAT R- TCTGCTTCCTTTACCAAACCA	57	1	0	1	100	5	250	0.90
14	RGA-F	F- AATTTGATTTGAAAGGGTGGG R- GGGGAAGCCATCACATTTT	48	2	0	2	100	14	500, 300	0.96
15	RGA-G	F- CCAATTTCTGTGTACTGCC R- GGGAGGCCATTAGCATACT	48	2	0	2	100	25	200,100	0.53

16	RGA-H	F- GAAGACAGCCATAGCCGAAG R- TTTGCTACCTGCACCAAACC	53	2	1	1	50	25	500, 300	0.68
17	TA-22	F-TCTCCAACCCTTTAGATTGA R-TCGTGTTTACTGAATGTGGA	51	1	0	1	100	14	800	0.87
Total			---	20	1	19	---	212	---	10.64
Average			54.8	1.18	0.06	1.12	97.06	12.47	435.71	0.63
Minimum			48	1	0	1	50	5	100	0.12
Maximum			60	2	1	2	100	25	2000	0.96

Fig.1 RAPD profile of 16 safflower genotypes by primer OPC-07

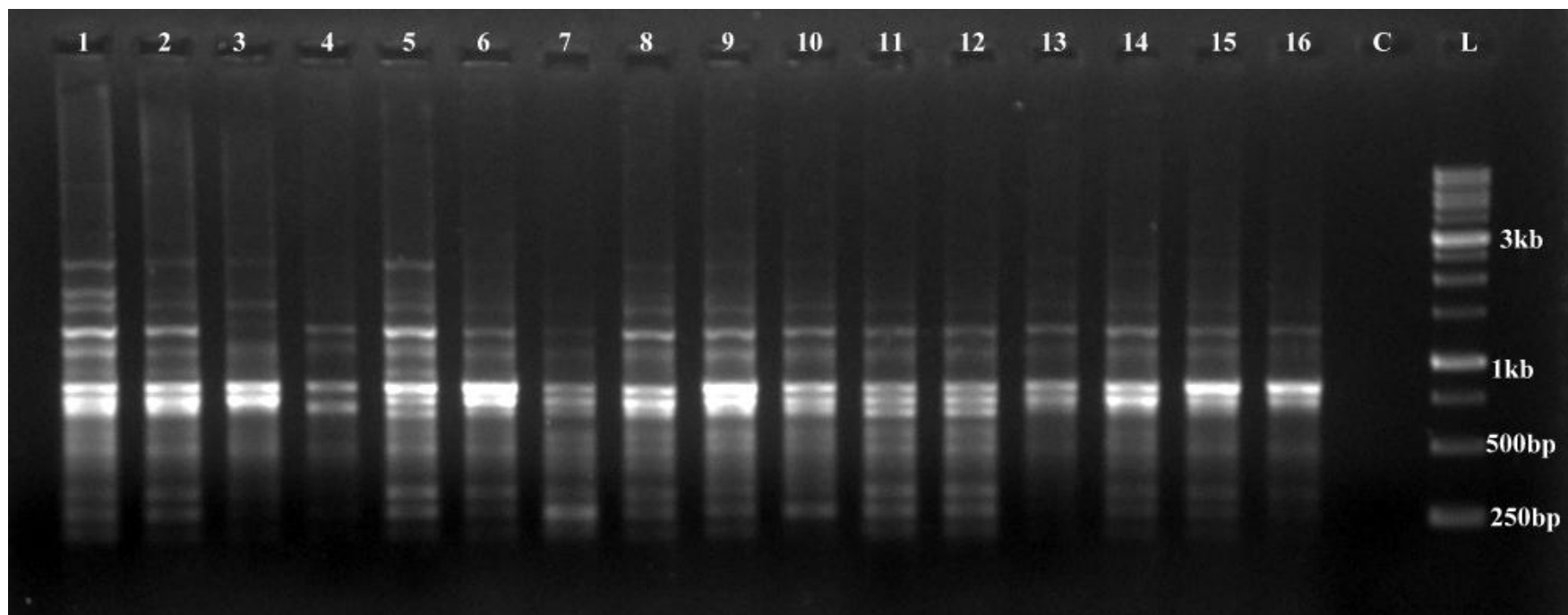


Fig.2UPGMA based dendrogram showing genetic relationship among 16 safflower genotypes based on RAPD analysis

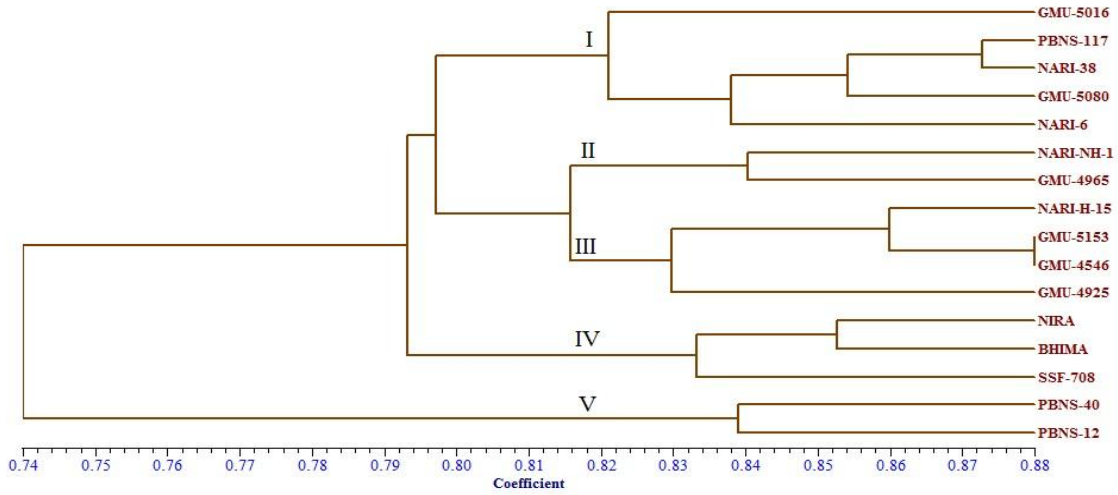


Fig.3 SSR profile of 16 safflower genotypes by primer TA-22

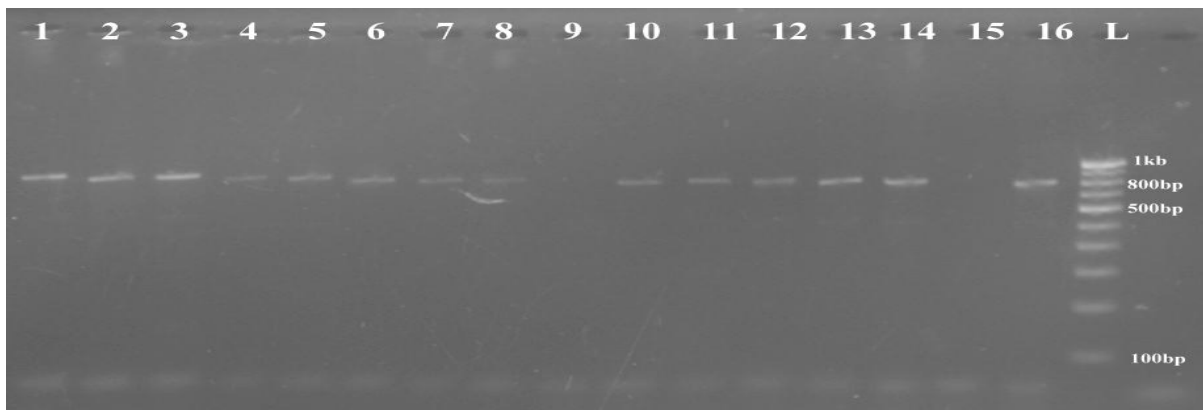
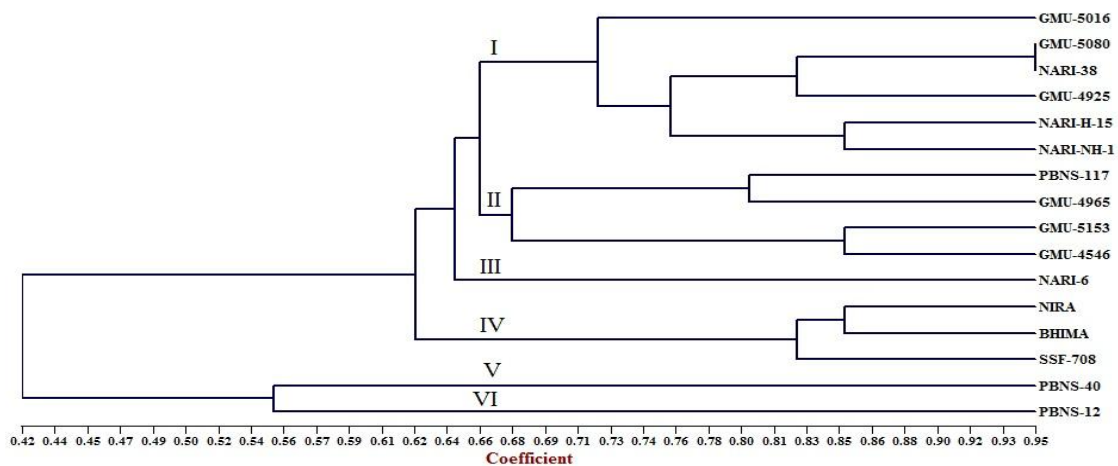


Fig.4 UPGMA based dendrogram showing genetic relationship among 16 safflower genotypes based on SSR analysis



Cluster V contains 2 susceptible genotypes PBNS-40 and PBNS-12 showing 84% similarity with each other and 74% with all other genotypes.

Similarity matrix was calculated by using the data generated by RAPD primers. Maximum similarity (0.87) was noted among GMU-5153 and GMU-4546 whereas minimum similarity 0.68 was noted among GMU-5153 and PBNS-40. Average similarity was noted 0.80 (Table 3). Safavi *et al.*, (2010), reported that 20 safflower accessions were separated into main 5 clusters with a cluster distance ranged from 0.27 to 0.85. Various others studies conducted by different investigators showed similar results such as Khan *et al.*, (2009) and Mahasi *et al.*, (2009). Safavi *et al.*, (2010) and Amini *et al.*, (2008) noticed similarity coefficient in the range of 0.26 to 0.57 and 0.30 to 0.89, respectively.

SSR analysis

Seventeen SSR primers yielded a total of 20 scorable bands. Total 19 bands were found to be polymorphic with 97.06 percent polymorphism (Table 4). All primers showed maximum 100% polymorphism except primer RGA-H which showed minimum, 50% polymorphism. Total amplified fragments were 212 with an average 12.47 amplified fragments per primer. Minimum 5 fragments were amplified by primer RGA-D and RGA-E and maximum 25 by primer RGA-G and RGA-H. Primer LG-125 and TA-22 (Fig. 3) amplified 13 and 14 fragments showing PIC 0.81 and 0.87 respectively. Size of bands amplified by primers on an average ranged between 100-2000bp. The calculated PIC values of these polymorphic primers ranged from 0.12-0.96 with an average of 0.63. Sehgal *et al.*, (2009) revealed 57.6 and 68.0% polymorphism by using RAPD and SSR primers, respectively, among 111 and 72 genetic loci amplified from the 85 accessions.

Cluster analysis based on SSR data

The dendrogram obtained using SSR data grouped safflower genotypes into six clusters. Cluster I contained maximum (6) genotypes *viz.*, GMU-5016, GMU-5080, NARI-38, GMU-4925, NARI-H-15 and NARI-NH-1 all are resistant to *Fusarium* wilt with 72% similarity. Genotypes GMU-5080 and NARI-38 showed maximum similarity, 95%. Cluster II comprised of 4 genotypes *viz.*, PBNS-117, GMU-4965, GMU-5153 and GMU-4546 at 68% similarity value (Fig. 3). Cluster III contains only 1 resistant genotype NARI-6 showing 64% similarity with genotypes clustered in cluster I and II. All 5 susceptible genotypes clustered separately in 3 main clusters IV, V and VI. Cluster IV contains 3 susceptible genotypes *viz.*, NIRA, BHIMA and SSF-708 sharing 82% similarity with each other. Cluster V and VI contains 1 susceptible genotype each PBNS-40 and PBNS-12 respectively, showing 55% similarity with each other and 42% with all other genotypes.

Similarity matrix was calculated by using the data of SSR primers. Maximum similarity 0.95 was noted among GMU-5080 and NARI-38. Minimum similarity 0.20 was noted among NARI-38 and PBNS-40. An average similarity value was 0.63 (Table 3). Our results are in accordance with Betha *et al.*, (2015) they characterized 148 safflower accessions for genetic diversity using 44 simple sequence repeat (SSR). According to them SSR allelic variation was low as indicated by average number of alleles (3.6) per locus and polymorphism information content (0.28) and cluster analysis (neighbour-joining tree) revealed five major genotypic groups. Similarly, Chapman *et al.*, (2010) reported that across 76 accessions of *Carthamus tinctorius*, the 24 EST-SSR loci amplified between 3 and 15 alleles per locus

(mean 7.5), expected heterozygosity ranged from 0.10 to 0.84 (mean 0.44) and separated in 5 main clusters.

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