Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 2723-2731
Received: 20-07-2017
Accepted: 22-08-2017

Sarita Kumari
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Ram Chandra Choudhary
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Kumara Swamy RV
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Vinod Saharan
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Arunabh Joshi
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India.

Jayshree Munot
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Correspondence
Sarita Kumari
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Assessment of genetic diversity in safflower (*Carthamus tinctorius* L.) genotypes through morphological and SSR marker

Sarita Kumari, Ram Chandra Choudhary, Kumara Swamy RV, Vinod Saharan, Arunabh Joshi and Jayshree Munot

Abstract
The genetic diversity of twenty safflower genotypes was evaluated using morphological and SSR markers. Total nine morphological traits were studied viz. plant height; number of branches per plant, test weight, harvest index, seed yield and oil content showed the significant variation. Ward's cluster analysis based on morphological traits separated the 20 safflower genotypes into two groups. Amongst the tested SSR primes, 11 primers produced amplification with 17 polymorphic bands expressing 56 per cent average polymorphism. The similarity coefficient ranged from 0.40 to 0.90 with an average of 0.65. The dendrogram based on SSR data clearly divided the 20 safflower genotypes into six main clusters. In general, there was a notable association between genotypes (SSF-748,11-12 A1, GMU-5133, Bhima and Girha) and there morphological characters (oil content, test weight, branches/plant and days to maturity) strongly linked with SSR markers. In this context, in the present investigation demonstrated that SSR markers viz SES-129,SSR-5,SES-81,SES-85 can be further used to marker assisted selection.

Keywords: safflower, *Carthamus tinctorius* L, morphological

1. Introduction
Safflower (*Carthamus tinctorius* L.), a member of *Compositae* family, mostly self-pollinating dicot with an estimated haploid genome size of 1.4 GB and has 2n=24 chromosomes (Jhajharia *et al*., 2013) [20]. It is a significant staple oilseed crop of special economic importance in an oilseed industry worldwide. The crop is grown in wide geographical zones across the world (Weiss, 2000) [37] with Kazakhstan and India currently dominating safflower production (FAOSTAT 2013) [12]. It is a multi-purpose crop betrothed for diverse uses such as dye production, edible oil extraction and for medicinal applications (Weiss, 2000) [37]. It has also been exploited for production of biofuel and industrial oil (Ilkilic *et al*., 2011) [17]. Recently, transgenic safflower has been employed as a plant factory for production of significant pharmaceuticals of human interest such as insulin and apo-lipoprotein (McPherson *et al*., 2009; Flider, 2013; Carlsson *et al*., 2014) [26, 13, 6]. Considering the enviable oil composition of safflower and its resilience to dry conditions, it can serve as an imperative source of edible oil especially in arid regions of the world with advantages of adaptability to salinity and drought (Weiss, 2000) [37]. For yield improvement, it is essential to have acquaintance on the variability of different characters such as days to 50% maturity, days to maturity, plant height (cm), number of branches/plant, number of effective capitula/plant, 1000-seed weight (g), harvest index (%), seed yield (kg/ha) and oil content. Morphological traits can be used to assess phenotypic variation in growing environments and are also used as tools for the indirect analysis of genetic variability and diversity.

The crop holds tremendous potential for improvement through concerted molecular breeding programs due to the availability of significant genetic and phenotypic diversity. Conventional breeding programs in several crop species have resulted in the development of cultivars with improved yield and augmented resistance to several diseases. Improvements can be achieved more efficiently and quicker through analysis of global genetic diversity existing in the crop for selection of elite genotypes and by molecular breeding approaches (Collard *et al*., 2005) [7]. Implication of molecular markers in crop breeding has established to be a powerful method for upgrading of several crop species (Varshney *et al*., 2007) [36]. A stipulation for successful implementation of molecular breeding in crops is the availability of sturdy molecular marker-trait association (Collard *et al*., 2005) [7]. An inclusive program to raise yield is crucial for safflower improvement (Golkar, 2014) [15]. On the other hand, safflower genetics and genomics are mainly unexplored and shortage of consistent molecular markers is a key limitation for

development of valuable molecular breeding programs in the crop (Garcia-Moreno Maria *et al*., 2010; Hamdan *et al*., 2011) [14, 16]. One of the most important components required for an efficient system for molecular breeding is the identification and characterization of suitable genetic markers. Most molecular markers in safflower are randomly amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR) and amplified fragment length polymorphism (AFLP). These are usually the markers of choice for crops with inadequate genomic resources, because they do not require prior sequence information. However, the dominant inheritance pattern of these markers does not allow detection of allelic information, which is important for crop breeding. On the other hand, co-dominant markers allow detection of allelic diversity but in safflower, the repertoire of co-dominant markers is narrow. SSRs (simple sequence repeats) or microsatellite markers consist of repeats of simple nucleotide motifs that are abundant in most eukaryotic genomes have gained importance because of their high degree of polymorphism, co-dominant, reproducibility and locus specificity (Toth *et al*. 2000; Philips and Vasil, 2001) [34, 29]. While the initial cost of developing SSRs is relatively high, once developed, these polymerase chain reaction (PCR) based marker are cost effective. Until now, few studied have been carried on the genetic diversity of safflower based on morphological makers (Ashri 1975; Pascual and Alburquerque 1996; Dwiedi *et al*., 2005; Jaradat and Shahid 2006; Amini *et al*., 2008) [3, 28, 11, 19, 2] and using SSR markers (Naresh *et al*., 2009; Ushakiran *et al*., 2015) [27, 35]. The present study was conducted to assess the genetic diversity among safflower genotypes which are least studied using both morphological and SSR markers and find out promising SSR marker(s) linked to specific characters for further use in marker assisted selection.

2. Materials and Methods

2.1 Field experiment

The present field investigations were carried out during 2012 (season, '*Kharif*') at the Research Farm, Rajasthan College of Agriculture, MPUAT, Udaipur (24°35′N, 70°42′E), Rajasthan (India). Twenty safflower genotypes (Tara, SSF- 708, SSF-748, SSF-704, 11-12 A1, SSF-807, Nira, GMU-5133, Phulekusum, Bhima, SSF-733, Girha, Naari-6, SSF-734, A-1, PBNS-12, Shaed -9, PBNS-40, SSF-682 and SSF-691) were used in this study. All the genotypes were procured from Directorate of Oilseed Research, Hyderabad. A randomized complete block design with three replications was used. Each plot consisted of four rows 50cm apart and 4m in length plant to plant distance of 10 cm. Days to 50% flowering and days to maturity were visually recorded for each plot. In addition, plant height and yield components including branches per plant, effective capitula per plant and test weight were recorded on ten plants per plot and their average were used. Each plot was harvested individually to determine the seed yield (Amini *et al*., 2008) [2]. Oil content was measured by using Soxhlet apparatus as per method of Sadasivam and Manickam (2008) [32]. Harvest index was calculated as formula given (Donald, 1962).

Harvest index (%) = Economic yield (g) X 100/ Biological yield (g)

Oil content (%) =Weight of oil (g) X 100/ Weight of sample (g)

2.2. Genomic DNA extraction and quantification

Genomic DNA was isolated from young leaves (21-28 days old seedling) of plants from each genotype following the Cetyl Tri-methyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987). The isolated DNA was assessed qualitatively and quantitatively using agarose gel electrophoresis and spectrophotometrically on a nanospectrophotometer (Implen, Germany) respectively. Further this DNA samples were used for setup SSR reactions.

2.3 SSR analysis

Total 20 SSR primers were used for PCR amplification. The sequences of these primers were selected after referring to the literature. The details of primer sequences, their GC content and melting temperature are as given in Table 1. DNA was amplified by PCR in a total volume of 22µl containing 50ng template DNA, 1X buffer (75mM Tris-HCl (pH 9.0), 200µM of each dNTP, 0.5µM SSR primers, and 1 unit DNA polymerase. PCR reactions were carried out in a Thermocycler (Eppendrof). Cycling parameters were initial denaturation step at 94 °C for 3 min, followed by 94 °C, 20 sec, 58 °C, 30 sec and 65 °C, 50 sec. This cycle was repeated 35 times, followed by 5 min extension at 65 °C for one cycle. The final extension was used to correct for non-template addition by Taq polymerase of a nucleotide, primarily adenosine, to the 3' end of amplification products. The amplified products were separated on 3 % agarose gels (Bhosle *et al*., 2015) [5]. Gels were stained with ethidium bromide (0.5µg/ml). Allele sizes were estimated in comparison with 100bp DNA ladder. DNA banding patterns were visualized using UV transilluminator and photographed using a gel documentation system (Alpha Digi Doc, Germany) (Amini *et al*., 2008) [2].

2.4. Morphological data analysis

Analysis of variance (ANOVA) of data for morphological traits was performed using General Linear Model of SAS program (SAS Institute 1997) [33]. Data on morphological characters were standardized using the YBAR option of the stand program from the NTSYS-pc 2.2 software. Clustering of genotypes based on the morphological traits was performed by NTSYS-pc and the genetic distance matrix using unweighted pair group method with arithmetic mean (UPGMA) (Rohlf, 2004) [31].

2.5 SSR data scoring and cluster analysis

The amplified DNA fragments appearing without ambiguity were scored by presence (1) and absence (0) for each safflower accession. Similarity coefficients between 20 genotypes, in a pair wise comparison, were computed using Jaccard's coefficient and the resulting similarity matrix was further analyzed using the unweighted pair-group method arithmetic average (UPGMA) clustering algorithm; the computations were carried out using NTSYS-pc version 2.2 (Rohlf, 2004) [31]. Cluster analysis was performed on both morphological and molecular data.

3. Results

3.1. Morphological analysis

In the present investigation, nine important yield-related morphological and qualitative characters have been studied to evaluate the pattern and extent of genetic variability and relatedness among 20 genotypes of safflower. The results of ANOVA showed that genotypes significantly differed for morphological traits (Table.2). The results obtained from the mean value of morphological characters (Table 3) demonstrated that plant height, number of branches of per plant, test weight, harvest index, seed yield and oil content showed the significant variation at 1% level. The average

plant height was 111.73 cm. It ranged from 97 cm to 128 cm. The maximum plant height was shown by 11-12 A1 (128 cm) followed by SSF-708 and Naari-6 (123.33 cm). The SSF-734 (97 cm) showed minimum plant height. The average numbers of branches per plant were 9.98. It ranged from 6.67 to 12.33. The genotype Tara had maximum in number of branches per plant (12.33) which was followed by SSF-807 and PBNS-40 (11.67). The SSF-682 (6.67) had minimum number of branches. The average test weight was 5.73 g. It ranged from 3.85 g to 7.41g. Maximum test weight showed by SSF-734 (7.41 gm) followed by 11-12 A1 (6.89 g) and the phulekusum (3.83 g) showed minimum test weight. The average harvest index was recorded 30.51. It ranged from 20.13 to 41.18. The Nira showed maximum value for harvest index, followed by SSF-708 (39.75). The PBNS-12 (20.13) showed minimum value for harvest index. The average seed yield was 2002.63 kg/ha. It ranged from 764.90 kg/ha to 3126.68 kg/ha. The Nira exhibited maximum seed yield (3126.68 kg/ha), followed by Naari-6 (2871.71 kg/ha). The phulekusum (764.90kg/ha) had minimum seed yield. The average oil content was 28.51 per cent. It ranged from 24.70 to 31.88 per cent. The SSF-734 (31.88%) was found to have maximum oil content, followed by GMU-5133 (30.19%). The genotype SSF-682 (24.70%) had minimum oil content. Days to 50% flowering, days to maturity and number of effective capitula per plant showed non-significant variation. The average days to 50 per cent flowering was observed 120.20. It ranged from 114 to 124 days. The minimum days were taken by shaed-9 (114) to reach to 50 per cent flowering followed by SSF-691 (115). The average number of days to maturity was 150.05. It ranged from 147-156.67 days. The minimum numbers of days were taken by Girha (147) to reach at the maturity stage followed by SSF-708 (147.33). Whereas Phulekusum took maximum number of days (124) to 50 per cent flowering and (156.67) days to maturity. Number of effective capitula per plant ranged from 25.33-35. The SSF-704 (35) showed the maximum number of effective capitula per plant followed by SSF-734 (33.67). The Bhima (25) had minimum number of effective capitula per plant.

Ward hierarchical cluster analysis was carried out on the basis of nine morphological characters studied. It was used to measure genetic distance between 20 safflower genotypes (Fig. 1). Cluster analysis grouped the genotypes into two clusters, cluster I and cluster II. Cluster I included 14 genotypes. This cluster was further divided into two sub-clusters, I-A andI-B which was 11 units apart. Sub cluster I-A was again divided into two sub-clusters I-A1 and I-A2. These two sub clusters were 4 units apart. Sub-cluster I-A1 included seven genotypes *viz.*, SSF-748, SSF-691, SSF-704, Bhima, SSF-807, PBNS-40 and 11-12A1. Sub-cluster I-A2 included five genotypes *viz*., Tara, Girha, SSF-708, SSF-734 and SSF-682. Days to 50 per cent flowering, days to maturity, number of effective capitula per plant were similar for all the genotypes. However plant height, seed weight (test weight), harvest index, seed yield and oil content were significantly different in these genotypes (CD at 1%). Number of branches per plant was significantly different in these genotypes (CD at 5%).Sub-cluster I-B included only two genotypes *viz.* Phuleksum and SSF-733. Number of branches per plant and oil content were similar for Phulekusum and SSF-733 genotypes, whereas seed yield, harvest index, test weight, number of effective capitula per plant, plant height and days to maturity were significantly different in these genotypes. Cluster II included 6 genotypes *viz.* GMU 5133, PBNS-12, A1, Shaed-9, Nira and Nari-6.ClusterII was further divided into two sub-clusters II-C and sub-cluster II-D which was 2 units apart. Sub-clusters II-C included four genotypes *viz*., GMU-5133, PBNS- 12, A1 and Shaed-9. Sub-cluster II-D included two genotypes *viz*., Nira and Nari-6. The number of branches per plant, number of effective capitula per plant, seed weight, oil content and seed yield were similar for all these genotypes whereas plant height, days to 50 per cent flowering and days to maturity were significantly different in these genotypes.

3.2. Divergence based on SSR analysis

The genetic divergence was analyzed among the 20 safflower genotypes using twenty SSR primers having 50-60% or more G+C content, only 11 primers showed amplification. Out of these 11 primers, 6 primers (SES-129, SES-139, SSR-5,SSR-9,SES-104 andSES-106) showed polymorphism and 5 primers (SES-81, SES-85, SES-91, SES-98 and SSR-16) produced only monomorphic bands. Among all the primer tested, primer SES-106 proved to be the best primer. It produced 41 amplicons and 8 scorable bands in the range of 250 to 1000 bp, with 100 per cent polymorphism. Primer SES-104 produced 4 scorable bands of which 3 were polymorphic which amount to 75 per cent polymorphism. A total of 25 amplified bands were obtained of which 17 were polymorphic. The DNA amplification and polymorphism generated among 20 genotypes of safflower using SSR primers are presented in table 4. The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently. The total number of amplified bands varied between 1 (SES-139) to 8 (SES-106) with an average of 2.2 bands per primer. The polymorphism ranged from as low as 50 per cent (primer SES-129) to as high as 100 per cent (primer SES-139, SES-106, SSR-5 and SSR-9). Average polymorphism across all the 20 genotypes of safflower was found to be 56 per cent and overall size of PCR amplified products ranged between 50 bp to 1000 bp. Amplification profiling of 20 genotypes with the SSR-9 and SES-85primer is shown in Fig. 2.

Genetic similarity

The SSR fingerprint data were used to estimate genetic similarity on the basis of number of shared amplification products which were denoted by cluster dendrogram (Fig. 3) and coefficient of similarity matrix (Table 5). Based on SSR similarity matrix data, the value of similarity coefficient ranged from 0.20 to 0.90. The average similarity across all the genotypes was found out to be 0.55 showing that there is sufficient genetic diversity. The highest similarity coefficient of 0.90 was observed between genotypes GMU-5133 and PBNS-40, Bhima and G11 SSF-733. The minimum similarity coefficient (0.20) was observed between GSSF-708 and A-1.

Clustering analysis

The genetic relationships among 20 safflower genotypes based on SSR markers were estimated by UPGMA cluster analysis of the GS matrix (Fig. 3). All the 20 genotypes could be distinguished by 20 SSR markers and categorized into six major clusters. Cluster I included three genotypes and divided into two sub-clusters, sub cluster I-A and I-B. Sub-cluster I-A included only one genotype viz. Tara. Sub- cluster I-B included two genotypes viz. SSF-748 and 11-12A1 at similarity coefficient of 0.85. Morphologically genotypes SSF-748 and 11-12A1 also showed similarity. Sub-cluster I-B joined with I-A at the similarity coefficient 0.725.Total 9 morphological characters were studied of which 4 *viz.* days to 50 per cent flowering, number of effective capitula per plant,

oil content and test weight were similar in these genotypes. Cluster II included five genotypes *viz.*,GMU-5133, Bhima, SSF-733, Girha and Naari-6.It joined cluster I at the similarity coefficient of 0.595. Cluster II was divided into two sub-clusters, II-A and II-B. Sub-cluster II-A was further sub divided into subgroups II-A1 and II-A2. IIA1was again divided into two sub-clusters II-A' and II-A''.II-A' included two genotypes GMU-5133 and Bhima. Similarity coefficient between GMU-5133 and Bhima was 0.90. These two genotypes showed the maximum similarity coefficient. Sub-clusterII-A2'' included only one genotype viz. Girha which joined the sub-cluster A1'' at the similarity coefficient 0.735. Total 9 morphological characters were studied of which 5 *viz.* oil content, days to 50 per cent flowering, days to maturity; branches per plant and seed weight were similar in these genotypes. Sub-cluster II-B included only one genotype viz. Naari-6which joined with sub-cluster II-A at the similarity coefficient 0.665. Cluster III included only one genotypes viz., SSF-807. It joined cluster II at the similarity coefficient of 0.579. Cluster IV was the largest among all the clusters. It included eight genotypes and divided into two sub-clusters, IV-A and IV-B. Sub-cluster IV-A was further divided into two subgroups IV-A1 and IV-A2.IV-A1 was further divided into IV-A1' and IV-A1''.IV-A1' included two genotypes Nira and GSSF-734 and the similarity coefficient between them was0.86. Sub-cluster IV-A1'' included only one genotype *viz.,* A-1. It joined the sub-cluster IV-A' at the similarity coefficient 0.72. Sub-clusterIV-A2 was subdivided into two sub-clusters, IV-A2' and IV-A2''. IV-A2' was again divided into cluster X and Y. Sub-cluster X included only one genotype viz. PBNS-12. While Sub-cluster Y included two genotypes viz. Shaed-9 and SSF-682 the similarity coefficient between them was 0.875. The cluster Y joined with cluster X at the similarity coefficient 0.76. Sub-cluster IV-A2'' included only one genotype *viz.,* PBNS-40which joined the sub-cluster IVA2' at the similarity coefficient 0.69. Sub-cluster IV-B included only one genotype *viz.,* SSF-691. It joined the sub-cluster IV-A at the similarity coefficient of 0.61. Cluster V included two genotypes viz. SSF-704 and Phulekusum, the similarity coefficient between them was 0.685.Cluster V joined cluster IV at the similarity coefficient of 0.55. Cluster VI included only one genotype viz. SSF-708 at the similarity coefficient 0.40 with cluster V. The dendrogram analysis indicated that the genotype SSF-708 was genetically apart from other genotypes. It was also recorded as independent genotype in clustering pattern in SSR analysis reflecting high diversity. It showed best performance for plant height and harvest index; hence it can be exploited in a synergistic way to create a wider genetic base. (Yerremsetty *et al.* 2005) [38].

Table 1: Detail of SSR primers used in molecular analysis of safflower genotypes

S. No.	Primer	Sequence (5'-3')	AT (^{0}C)	GC content (%)
1.	SES-10 (F)	ACGGGTAGATTTAAGGAAGG	58.6	45
	SES-10 (R)	ACAATCCAACAGAGATTTGC	59.2	40
2.	SES-27 (F)	CTCTTCTTGCAATTTCTTCG	59.2	40
	SES-27 (R)	GAAGCTTCTCAAACTCATCC	58	45
3.	SES-30 (F)	GAAGATGAGAGTGAAATTGAGC	64	55
	SES-30 (R)	CTGGAGGGTAATTAGTCTGG	63	55
4.	SES-33 (F)	CGTTCTAGGACGACTACTCC	63	50
	SES-33 (R)	ACTGCTTTTTGTCTCTTTCC	64	55
5.	SES-94 (F)	ACGGCGGTTTTCACTAGG	64	61
	SES-94 (R)	ACACCAATAATCACGAATCC	64	55
6.	SES- 81 (F)	GCAATACCATCATCATCCTCAC	64	52
	SES-81 (R)	AGGAGGTGAAAGGGAAGAG	63	45
7.	SES-85 (F)	GGGTTCACTTCTTTCTCTCTC	63	50
	SES-85 (R)	AGTACTCCTCCAGTGACATACAG	64	61
8.	SES-86 (F)	ACCCTAGATTCATTCATTCC	64	61
	SES-86 (R)	GATTACAGTCTGAGAAACATCG	65	61
9.	SES-91 (F)	CATTCCGTCATCTATTTTGC	64	52
	SES-91 (R)	GAAGTAATCGACTAACCAACG	64	52
10.	SES-98 (F)	ACCTCACATGGCGAAGAG	64	61
	SES-98 (R)	GATTTCCGGAATGAAACAG	65	55
11.	SES-99 (F)	TTCTCTACTCTTCACGATTTGG	59.9	47
	SES-99 (R)	CCATCTGTCTTAAGCTGTTCC	60.3	48
12.	SES-100(F)	CATCCAACAAGAACACACC	58.9	50
	SES-100(R)	CGCTATGATCCTAGTGTATCC	57.8	40
13.	SES-104(F)	TCCGTTCCTAACTGAATCC	59.1	47
	SES-104(R)	AGCTCAGATCAATCACTTTCC	59.9	43
14.	SES-106(F)	GGGGCTTTCTTTACTTCC	62	52
	SES-106(R)	TATTGCTGTTGTTGTCTAGGG	52	61
15.	SES-122(F)	GGGATGAGACTGAGATCG	61	40
	SES-122(R)	GACAGTTTGGAAGGTGTAGC	63	45
16.	SES-129(F)	CTCTTTATTTCGACTGGAACTG	63	50
	SES-129(R)	ATGCTTGTTGTTGCCTTATC	64	66
17.	SES-139(F)	TTTGCGTGTCGATAATCC	55	42
	SES-139(R)	TATCCTCATCGTAACATCATCC	64	52
18.	SSR 5 (F)	TGATGTTCTTGATGAGTCAATGC	63	50
	SSR 5 (R)	CATGTTAGCAAGCATTTGTGG	63	40
19.	SSR 9 (F)	GGGGAGGTTACTACCCACTTC	63	50
	SSR 9 (R)	CCCAGGAAACAAGCTTATCC	63	40
20.	SSR 16 (F)	CAAGCTTAAGTCTTGGTCTTCTCT	63	55
	SSR 16 (R)	GGCCTGACCCAAAACTAAGGGAAGTG	63	60

Table 2: Analysis of variance for various characters in 20 genotypes of safflower

Source of variation	DF	Mean squares								
		Days to 50% Flowering	Days to maturity	Plant Height	Branches/plant	Capitula/ plant	Test weight	Harvest index	Seed yield	Oil content
Replication	2	9.80	12.20	5.29	0.31	7.46	0.65	5.28	4172.2690	3.81
Genotype	19	25.66	20.95	333.07**	5.87*	22.75	3.64**	124.29**	1094903.36**	8.71**
Error	38	13.96	11.74	30.31	2.59	12.47	0.61	19.66	23.00	2.94

*Significant at $P < 0.01$

Table 3: Mean performance of 20 safflower genotypes for 9 different characters

S.No.	Genotype	Code No.	Days to 50% Flowering	Days to maturity	Plant Height (cm)	Branches/plant	Capitula/ plant	Test weight (g)	Harvest index (%)	Seed yield (kg/ha)	Oil content (%)
1.	Tara	G1	121.67	149.33	122.59	12.33	29.00	6.33	39.58	2160.50	29.80
2.	SSF-708	G2	119.33	147.33	123.33	11.33	29.00	6.34	39.75	2079.98	29.32
3.	SSF-748	G3	121.00	151.67	121.24	9.00	29.00	4.97	23.00	1529.79	29.10
4.	SSF-704	G4	123.67	152.33	108.00	11.33	35.00	6.76	37.33	1625.06	31.88
5.	11-12 A1	G5	119.67	147.67	128.56	9.67	28.00	6.89	30.33	1744.50	26.09
6.	SSF-807	G6	122.67	153.67	120.40	11.67	26.67	6.62	27.75	1798.18	28.49
7.	Nira	G7	120.67	153.33	118.33	9.00	33.67	4.52	41.18	3126.68	28.91
8.	GMU5133	G8	121.33	150.00	99.33	11.00	30.33	5.12	24.41	2495.97	30.19
9.	Phulekusum	G9	124.00	156.67	122.67	9.00	26.00	3.83	28.92	764.90	29.01
10.	Bhima	G10	120.67	147.33	122.00	9.67	25.33	6.40	27.23	1650.57	29.17
11.	SSF-733	G11	115.33	148.33	98.00	10.33	30.00	6.53	33.67	899.09	29.37
12.	Girha	G12	116.00	147.00	103.00	10.33	32.33	6.42	32.33	2241.01	29.83
13.	NAARI-6	G13	122.00	152.33	123.33	10.00	32.33	5.32	28.15	2871.71	28.63
14.	SSF-734	G14	123.00	147.67	97.67	9.67	33.67	7.41	20.67	2066.56	29.09
15.	A-1	G15	122.00	152.00	102.67	9.00	30.00	4.80	34.33	2589.91	27.11
16.	PBNS-12	G16	121.33	149.00	100.80	10.67	27.67	4.15	20.13	2453.49	27.90
17.	SHAED 9	G17	114.00	147.67	108.33	9.67	27.33	6.67	37.67	2651.05	28.91
18.	PBNS-40	G18	121.33	149.33	101.67	11.67	31.00	6.49	32.23	1793.29	27.49
19.	SSF-682	G19	119.33	148.67	105.33	6.67	27.33	5.28	23.82	1975.59	24.70
20.	SSSF-691	G20	115.00	149.67	107.33	7.67	27.00	3.84	27.78	1534.74	25.23
	GM		120.20	150.05	111.73	9.98	29.53	5.73	30.51	2002.63	28.51
	SE(m)		2.16	1.98	3.18	0.93	2.04	0.45	2.56	131.20	0.99
	CD@5%		6.18	5.66	9.10	2.66	5.84	1.30	7.33	375.59	2.84
	CD@1%		8.28	7.59	12.19	3.57	7.82	1.74	9.82	503.36	3.80
	CV		3.11	2.28	4.93	16.14	11.96	13.67	14.53	11.35	6.02

Table 4: Polymorphism information of SSR primers

S.No.	Primer	Total No. of bands (a)	Total No. of polymorphic bands (b)	Polymorphism% (b/a × 100)	Range of band size (bp)
1	SES-10	NA	NA	NA	-
2	SES-27	NA	NA	NA	-
3.	SES-30	NA	NA	NA	-
4.	SES-33	NA	NA	NA	-
5.	SES-94	NA	NA	NA	-
6.	SES- 81	1	0	0	-
7.	SES-85	2	0	0	50-200
8.	SES-86	NA	NA	NA	-
9.	SES-91	1	0	0	-
10.	SES-98	1	0	0	-
11.	SES-99	NA	NA	NA	-
12.	SES-100	NA	NA	NA	-
13.	SES-104	4	3	75	50-500
14.	SES-106	8	8	100	250-1000
15.	SES-122	NA	NA	NA	-
16.	SES-129	2	1	50	50-200
17.	SES-139	1	1	100	-
18.	SSR 5	2	2	100	50-200
19.	SSR 9	2	2	100	200-250
20.	SSR 16	1	0	0	-
	Total	25	14	56	

NA – Null allelic

Table 5: Jaccard similarity coefficient for 20 safflower genotypes based on SSR profiling

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20
G1	1.00																			
G2	0.57	1.00																		
G3	0.66	0.45	1.00																	
G4	0.62	0.50	0.56	1.00																
G5	0.78	0.45	0.84	0.66	1.00															
G6	0.50	0.40	0.64	0.60	0.53	1.00														
G7	0.47	0.31	0.60	0.38	0.60	0.43	1.00													
G8	0.64	0.42	0.69	0.53	0.69	0.61	0.69	1.00												
G9	0.50	0.40	0.52	0.68	0.52	0.47	0.62	0.50	1.00											
G10	0.57	0.36	0.61	0.46	0.61	0.53	0.75	0.90	0.53	1.00										
G11	0.53	0.35	0.57	0.43	0.57	0.50	0.83	0.81	0.60	0.90	1.00									
G12	0.53	0.35	0.57	0.43	0.57	0.61	0.57	0.81	0.41	0.72	0.66	1.00								
G13	0.50	0.40	0.62	0.58	0.62	0.66	0.62	0.71	0.47	0.64	0.60	0.71	1.00							
G14	0.42	0.34	0.52	0.42	0.52	0.38	0.85	0.60	0.64	0.64	0.71	0.60	0.64	1.00						
G15	0.31	0.20	0.41	0.31	0.41	0.35	0.71	0.46	0.52	0.50	0.57	0.46	0.62	0.73	1.00					
G16	0.58	0.40	0.44	0.35	0.44	0.38	0.73	0.60	0.55	0.64	0.71	0.60	0.55	0.75	0.62	1.00				
G17	0.58	0.40	0.52	0.35	0.52	0.47	0.73	0.60	0.47	0.53	0.60	0.60	0.55	0.64	0.52	0.75	1.00			
G18	0.62	0.36	0.38	0.52	0.47	0.41	0.56	0.53	0.58	0.46	0.53	0.43	0.50	0.50	0.47	0.68	0.68	1.00		
G19	0.70	0.50	0.64	0.45	0.64	0.50	0.75	0.62	0.57	0.56	0.62	0.52	0.57	0.66	0.55	0.76	0.87	0.70	1.00	
G20	0.58	0.34	0.52	0.50	0.62	0.47	0.62	0.60	0.55	0.53	0.60	0.60	0.55	0.64	0.52	0.64	0.55	0.58	0.66	1.00

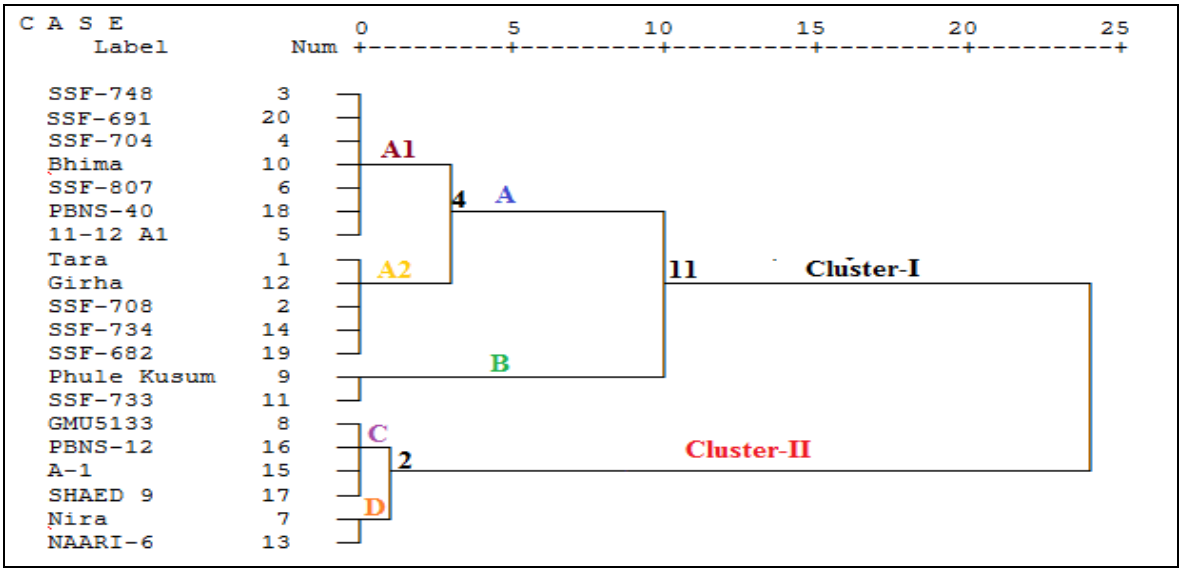

Fig 1: Dendrogram generated for 20 safflower genotypes using ward's clustering method based on morphological traits

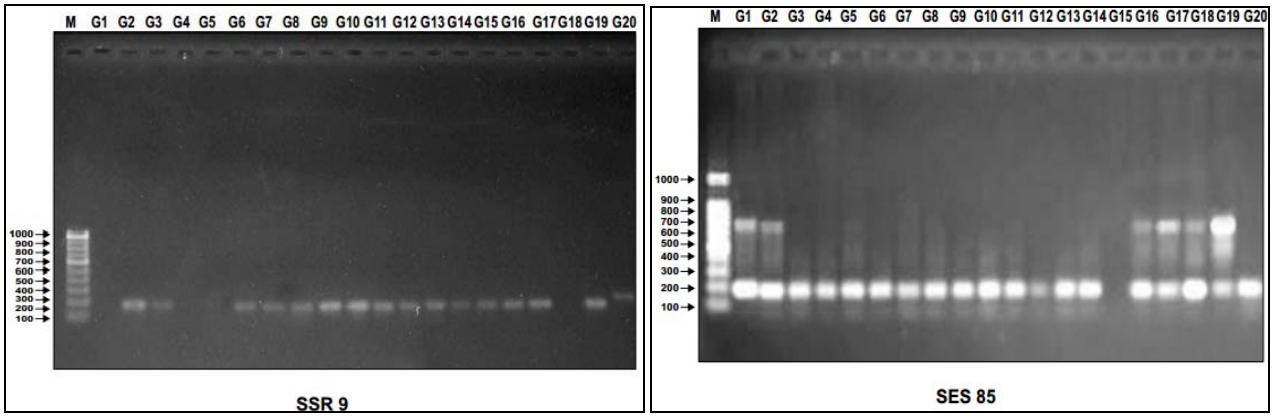

Fig 2: PCR amplification profile of SSR marker SSR-9 and SES-85 in 20 safflower genotypes

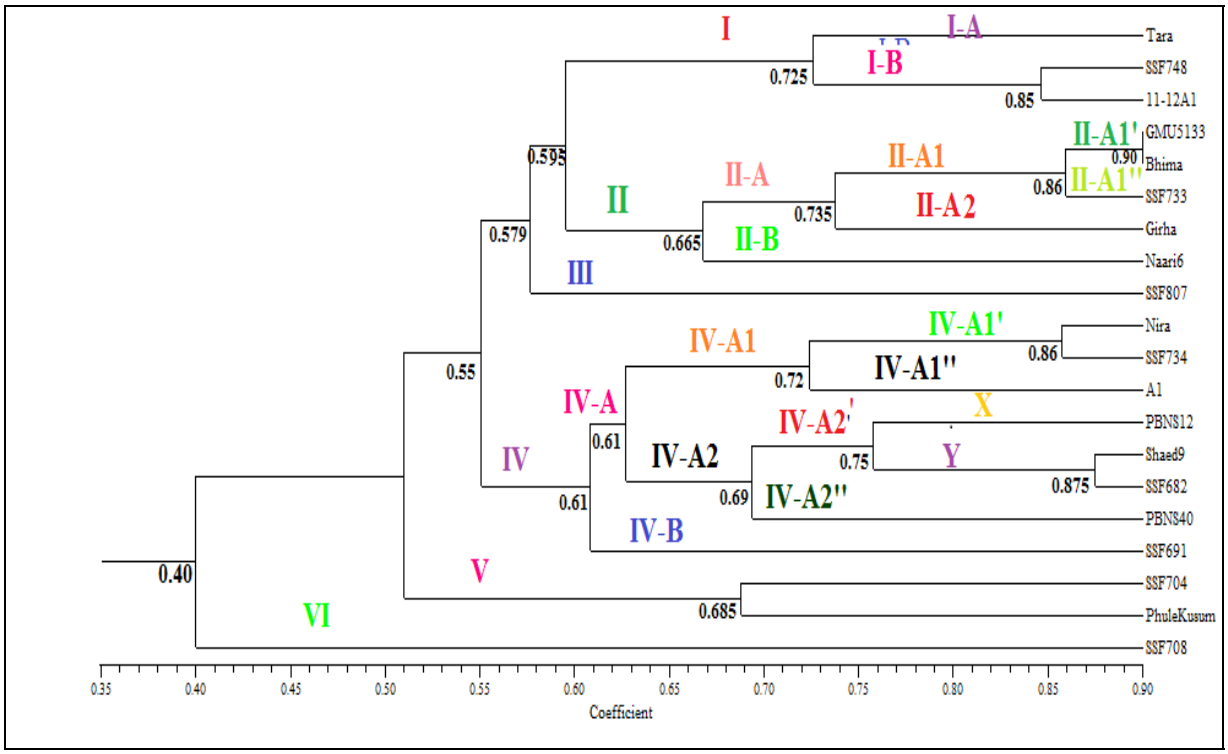

Fig 3: Dendrogram generated for 20 safflower genotypes using UPGMA cluster analysis based on jaccard similarity coefficient

4. Discussion

In the present investigation, nine important yield attribute morphological and qualitative characters have been studied to evaluate the pattern and extent of genetic variability and relatedness among 20 genotypes of safflower. Morphological characterization based on nine characters revealed significant diversity in plant height, seed weight (test weight), harvest index, seed yield and oil content. A dendrogram generated from morphological data grouped all 20 genotypes into 2 major clusters (Figure 1). SSR data generated from the 20 genotypes with 20 primers was sufficient to provide inferences on genetic divergence and relationships. The total number of amplified bands varied between 1 (SES-139) to 8 (SES-106) with an average of 2.2 bands per primer. The SSR markers polymorphism ranged from as low as 50 per cent (primer SES-129) to as high as 100 per cent (primer SES-139, SES-106, SSR-5 and SSR-9). Average polymorphism across all the 20 genotypes of safflower was found to be 56 per cent and overall size of PCR amplified products ranged between 50 bp to 1000 bp. Similar results were reported by Mace *et al.* (2006) [25] in groundnut. They analyzed 22 groundnut genotypes with different level of resistant to rust and LLS (Late leaf spot) disease using 23 SSR markers. These markers could detect 135 alleles. 12 out of 23 SSR markers showed 52 per cent level of polymorphism with PIC value ≥0.5. The molecular diversity analysis provided valuable information to the groundnut breeder to develop disease resistant varieties of groundnut. Hamdan *et al.* (2011) [16] used 108 SSR markers for characterization of 10 safflower lines. Out of 108 SSR markers, 64 SSR markers showed the polymorphism. The number of alleles per locus ranged from 2 to 8 with mean value of 3.2, whereas heterozygosity ranged from 0.18 to 0.86 with mean value of 0.52. Barati (2012) [4] estimated genetic variation of 48 genotypes belonging to three species (*C.lanatus*, *C. oxyacanthus* and *C. tinctorius*) using 109 EST-SSR primers pairs. Out of 109 EST-SSR primers 42 primers generated a total of 145 bands, size ranging from 100 bp to 500 bp with 2 to 8 alleles per locus. The similar results were also reported by Rao *et al.* (2012) [30]. They studied genetic diversity among 14 genotypes of castor using 10 EST SSR markers. Total twenty three alleles were detected and all were of polymorphic. The number of alleles detected on a single locus ranged from 2 to 3 with an average of 2.3 alleles per locus. Thus, it is opined that SSR assay can be efficiently used in identifying genetic variation. Jaccard' sgenetic similarity coefficient values of SSR ranged from 0.20 to 0.90, revealing a high level of genetic diversity through SSR markers. The average similarity across all the genotypes was found out to be 0.55 showing that there is sufficient genetic diversity. The highest similarity coefficient of 0.90 was observed between genotypes GMU-5133 and PBNS-40, Bhima and SSF-733. The minimum similarity coefficient (0.20) was observed between SSF-708 and A-1.The results obtained were in accordance with those reported by Darvishzadeh *et al.* (2010) [8]. They studied the genetic diversity in 28 genotypes of sunflower using 38 SSR markers. Mean number of alleles per locus was 2.32. Jaccard similarity coefficient ranged from 0.25 to 0.9 which indicated that there was significant genetic diversity among the genotype studied. The SSR analysis of 20 safflower genotypes were grouped divided into six clusters. The dendrogram analysis indicated that the genotype SSF-708 was genetically apart from other genotypes. The result showed that there was an association between dendrogram obtained by SSR analysis and morphological characters. Pairs of genotypes *viz.,* SSF-748 and 11-12 A1, PBNS-40 and SSF-691, SSF-734 and SSF-682 and A1, PBNS-12 and Shaed-9 were genetically as well as morphologically related with each other in number of effective capitula per plant, oil content, test weight and days to maturity. These results indicate that selection can be effectively employed for improvement these yield components. Increasing in branches and capitula per plant as the most important yield components in safflower can be used as the selection criteria to enhance the seed yield (Ashri *et al.*,

1974; Able and Driscoll 1976; Lahane *et al*.,1999) [1, 23]. However, based on the results of present study, direct selection for seed yield can also effective, since a wide range of genetic variation was observed for this trait. Improvement of seed yield is the main objective of breeding program in safflower (Amini *et al.,* 2008) [2]. Similar results were reported by Izzal *et al.* (2013) [18] studied genetic diversity and relationship among 91 *Brassica oleracea* cultivars by using 69 polymorphic microsatellite markers. Total 359 alleles were detected with an average 5.20 alleles per locus. Polymorphism information content (PIC) values ranged from 0.06 to 0.73, with an average of 0.40. Cluster analysis grouped 91 cultivars of *Brassica oleracea* into six different clusters. Kumar *et al.* (2011) [22] studied genetic diversity using morphological characters and molecular markers. It was reported by the author that morphological traits alone could not be considered as the true reflection of their genotypic characters. However morphological analysis coupled with molecular analysis proved quite effective. It was also recorded as independent genotype in clustering pattern in SSR analysis reflecting high diversity. It showed best performance for plant height and harvest index; hence it can be exploited in a synergistic way to create a wider genetic base. (Yerremsetty*et al.* 2005) [38]. Dendrogram based on morphological characters grouped 20 genotypes of safflower into two clusters, whereas dendrogram based on SSR data grouped them into six clusters. There was not absolute consistency between the clusters obtained by these two methods. This may be due to influence of environment and agronomic practices on morphological characters. Similar results were reported by Johnson *et al.* (2007) [21] for safflower and by Lei *et al.* (2008) [24] for coconut. Therefore the results of SSR marker can be precisely and effectively used in determination of genetic diversity among safflower genotypes and can assist in further breeding programs by characterizing the diverse genotype. In sum up, genotypes SSF-748, 11-12 A1, GMU-5133, and Bhima Girha showed charaters like oil content, test weight, branches/plant and days to maturity were strongly linked with SSR markers viz. SES-129, SSR-5,SES-81,SES-85, which could be futher expoited for marker assisted selection for impartment of the genotypes.

Acknowledgement
The authors are gratefully acknowledged to the Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan and Directorate of Oilseed Research, Hyderabad for their constant support during the research work.

References

1. Able GH, Driscoll MF. Sequential trait development and breeding for high yield in safflower. *Crop Sci.* 1976; 16:213-216.
2. Amini F, Saeidi G, Arzani A. Study of genetic diversity in safflower genotypes using agro-morphological traits and RAPD markers. *Euphytica*. 2008; 163:21-30.
3. Ashri A. Evaluation of the germplasm collection of safflower *Carthamus tinctorius* L. V: Distribution and regional divergence for morphological characters. *Euphytica.* 1975; 24:651-659.
4. Barati AM. Genetic diversity revealed by EST-SSR markers in cultivated and wild safflower. *Biochem. Syst. Ecol*. 2012; 44:117-123.
5. Bhosle SM, Chavhan RL, Hinge VR, Ghodke MK. Molecular fingerprinting and assessment of genetic purity of sunflower (*Helianthus annuus* L.) hybrids by using RAPD and SSR markers. *The Bioscan.* 2015; 10(2):773-779
6. Carlsson AS, Zhu LH, Andersson M, Hofvander P. Platform crops amenable to genetic engineering-a requirement for successful production of bio-industrial oils through genetic engineering. *Biocatal Agric Biotechnol*. 2014; 3(1):58-64.
7. Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*. 2005; 142:169-196.
8. Darvishzadeh R, Azizi M, Hatami-Maleki H, Bernousi I, Abdollahi Mandoulakani B, Jafari M *et al*. Molecular characterization and similarity relationships among sunflower (*Helianthus annuus* L.) inbred lines using some mapped simple sequence repeats. *Afr J Biotechnol.* 2010; 9(43):7280-7288.
9. Donald CM. In search of yield. *Journal of Australian Institutional Agricultural Science.* 1962; 28:171-178.
10. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochem Bull*. 1987; 19:11-15.
11. Dwiedi S, Upadhyaya HD, Hegde MD. Development of core collection in safflower (*Carthamus tinctorius* L.) germplasm. *Genet. Resour. Crop Evol.* 2005; 52:821-830.
12. FAOSTAT, 2013. Available: http://faostat.fao.org/site/567/default.aspx#ancor.
13. Flider FJ. Development and commercialization of GLA safflower oil. *Lipid Technol.* 2013; 25(10):27-229.
14. García-Moreno Maria J, Velasco L, Begona Perez-Vich. Transferability of non-genic, 2010.
15. Golkar P. Breeding improvements in safflower (*Carthamus tinctorius* L.): a review. Aust J Crop Sci. 2014; 8(7):1079-1085.
16. Hamdan YAS, García M, María J, Redondo NJ, Velasco L, Begona PV. Developmentand characterization of genomic microsatellite markers in safflower (*Carthamus tinctorius* L.). *Plant Breed*. 2011; 130(2):237-241.
17. Ilkılıç C, Aydın S, Behcet R, Aydin H. Biodiesel from safflower oil and its application in a diesel engine. *Fuel Process Technol*. 2011; 92(3):356-362.
18. Izzal KN, Lee J, Perumal S, Park YJ, Perumal S, Park YJ *et al*. Microsatellite based analysis of genetic diversity in 91 commercial *Brassica oleracea* L. cultivars belonging to six varietal groups. *Genet. Resour. Crop Evol.* 2013; 70:1967-1986.
19. Jaradat AA, Shahid M. Patterns of phenotypic variation in a germplasm collection of *Carthamus tinctorius* L. from the Middle East. *Euphytica*. 2006; 53:225-244.
20. Jhajharia S, Choudhary P, Jhajharia A, Meena LK, Singh D. Heterosis and combining ability in safflower (*Carthamus tinctorius*) germplasm lines. *The Bioscan.* 2013; 8(4):1453-1460.
21. Johnson RC, Kisha TJ, Evans MA. Characterizing safflower germplasm with AFLP molecular markers. *Crop Sci.* 2007; 47:1728-1736.
22. Kumar H, Anubha, Vishwakarma KM, Lal PJ. Morphological and molecular characterization of *Brassica rapa* species yellow sarso mutants. *J Oilseed Brassica.* 2011; 2(1):1-6.
23. Lahane PS, Mukewar AM, Zope JS, Kalpande HV, Kalpande VV. Genetic variability for different traits in safflower (*Carthamus tinctorius* L.). *J Soil Crops*. 1999;

9:130-132.
24. Lei XL, Hua T, Dong DL, Qian W, Qing YL, Rong Z. Genetic diversity of coconut cultivars from Hainan province based on SSR molecular markers. Acta Hortic. Sin. 2008; 35(8):1199-1204.
25. Mace SE, Phong TD, Upadhyaya DH, Chandra S, Crouch HJ. SSR analysis of cultivated groundnut (*Arachis hypogaea* L.) germplasm resistant to rust and late leaf spot diseases. *Euphytica.* 2006; 152:317-330.
26. McPherson MA, Yang RC, Good AG, Nielson RL, Hall LM. Potential for seed-mediated gene flow in agro ecosystems from transgenic safflower (*Carthamus tinctorius* L.) intended for plant molecular farming. *Transgenic Res*. 2009; 18(2):281-299.
27. Naresh V, Yamini KN, Rajendrakumar P, Kumar VD. EST-SSR marker-based assay for the genetic purity assessment of safflower hybrids. *Euphytica.* 2009; 170:347-353.
28. Pascual VMJ, Alburquerque N. Genetic variation of a safflower germplasm collection grown as a winter crop in southern Spain. *Euphytica.* 1996; 92:327-332.
29. Philips RL, Vasil KI. DNA based markers in plants, 2nd edn, Kluwer Academic Publishers, Dordrecht, 2001.
30. Rao PVR, Shankar VG, Pavani JVP, Rajesh V, Reddy AV. Geneticdiversity analysis for drought tolerance in castor using EST SSR markers. *Crops Res.* 2012; 44:152-155.
31. Rohlf FJ. NTSYS-pc. Numerical Taxonomy and MultivariateAnalysis System. Setauket, NY, USA: Exeter Software, 2004.
32. Sadasivam S, Manickam A. Biochemical methods. 3rd edn. New Age International Publishers, New Delhi, India, 2008.
33. SAS Institute SAS/STAT Software: changes and enhancements through Release 6.12. SAS Institute Inc., Cary, NC, 1997.
34. Toth GZ, Gaspari, Jurka J. Microsatellites in different eukaryotic genomes: survey and analysis. Genome Res. 2000; 10:967-981.
35. Ushakiran B, Mukta N, Kadirvel P, Alivelu K, Senthilvel S, Kishore P *et al*. Genetic diversity of safflower (*Carthamus tinctorius* L.) germplasm as revealed by SSR markers. *Plant Genet. Resour*. 2015, 1-11.
36. Varshney RK, Mahendar T, Aggarwal RK, Borner A. Genetic molecular markers in plants: development and applications. In Genomics-assisted crop improvement (pp.13-29). Springer Netherlands, 2007.
37. Weiss EA. Oil Seed Crops. Blackwell Science Ltd, Oxford, UK, 2000.
38. Yerramsetty PN, Anderson PM, Taliafero CM, Martin LD. DNA fingerprinting of seeded Bermuda grass cultivars. *Crop Science.* 2005; 45:772-777.