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Development and characterization of microsatellite markers from enriched genomic libraries in safflower (*Carthamus tinctorius* L.)

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Abstract

Microsatellite markers are the ideal genetic markers for crop improvement. In this study, we developed and characterized a set of 200 genomic SSR markers in safflower, an important oilseed crop of the world. A microsatellite enriched genomic library was constructed from an Indian cultivar, A-1. A total of 750 SSR-positive clones was generated of which 617 were identified as unique sequences and sequencing of them revealed 238 SSR motifs. The SSRs are validated in a panel of 24 genotypes and found 42 polymorphic markers. The number of alleles ranged from 2 to 4 with an average of 2.7 with polymorphic markers.

The polymorphic information content (PIC) value ranged from 0.08 to 0.61 with an average of 0.33. A dendrogram based on the polymorphic SSR loci clearly indicated the genetic relationships among genotypes. The reported SSR markers would be useful for characterization of genetic diversity and trait mapping purposes in safflower.

Keywords: Genetic diversity, genomic library, molecular markers, oilseed crop, polymorphism.

Introduction

Safflower (*Carthamus tinctorius* L.) is an important annual oilseed crop of the family Asteraceae[1]. It is one of the oldest crop grown in India primarily for high quality edible oil rich in polyunsaturated fatty acid (linoleic acid, ~75%), which is considered healthy for heart[2,3]. It is also a multi-purpose crop with the potential for production of bird seed, extraction of natural dye (carthamin) from the flowers and manufacturing of pharmaceutical products[4]. Characterization of genetic diversity is critical for improvement of safflower crop for higher productivity and quality.

Crop genetic diversity can be determined by agro-morphological, biochemical and DNA marker analysis. However, agro-morphological traits and biochemical markers have drawbacks which are limited in number and influenced by the environment[5]. The DNA markers are highly advantageous as they are unlimited in number, highly reproducible, highly polymorphic and environmentally neutral. Various DNA markers such as random amplified polymorphic DNA (RAPD)[6-9], inter-simple sequence repeats ISSR[6,10-15], amplified fragment length polymorphism (AFLP)[6,16,17], sequence related amplified polymorphism (SRAP)[18] have been used in safflower mostly for understanding genetic diversity and species relationships. Simple sequence repeats (SSRs) in particular are considered perfect genetic markers for crop improvement due to their availability, locus specificity, co-dominant and multi-allelic nature, high polymorphism and reproducibility[19].

Previously, significant efforts have been made to develop SSR markers from expressed sequence tag (EST) and genome sequences in safflower[20-25], but most of them have not yet been genetically mapped. Furthermore, in various studies, SSR markers showed very low level of polymorphism in cultivated germplasm[22-24,26,27] and wild species[21,28,29], which is a concern for development of high density SSR linkage map in safflower. However, availability of greater number of SSRs would enhance the chances of finding more polymorphic markers for linkage map construction.

In this study, we isolated and characterized new 200 microsatellite markers in safflower through a microsatellite enriched genomic library approach, which would enhance the SSR marker resources and facilitate trait mapping efforts in safflower.

Material and Methods

Plant materials and genomic DNA isolation: Genomic DNA was extracted from 150mg of young leaf tissues using a modified CTAB method with little modifications[30]. DNA from the cultivar, Annigeri-1 (A-1) was used for microsatellite enriched genomic library construction. A panel of 24 genotypes was used for microsatellite genotyping. The genotypes represented trait specific parental lines that are used for trait mapping purposes at ICAR-Indian Institute Oilseeds Research, Hyderabad, India (Table 1).

SSR-enriched library construction: The modified biotin-streptavidin capture method was used for constructing microsatellite-enriched genomic library[31]. Genomic DNA (5 µg) was digested by blunt end-generating restriction endonucleases *Rsa*I and *Xmn*I [New England Biolabs (NEB), USA]. The DNA digested products were then separated on a 1.5% TAE agarose gel and fragments of size from 300-1000 bp were removed from the gel and purified with QIA quick purification Kit (Qiagen, USA). After purification, double stranded super SNX linkers were ligated to the blunt end of digested DNA using 50 ng linker/µg of

genomic DNA using DNA ligase (NEB, USA) overnight at 14°C.

Linker ligation to the digested DNA fragments was confirmed through PCR using super SNX forward primer at 55 °C annealing temperature. The confirmed constructs are heat denatured and hybridized to biotinylated oligonucleotides. The hybridizations were carried out overnight at 60 °C using 75 µl of 6×SSC and 150 nM of each biotinylated repeat oligos CT, GA, CA, afterward bound to streptavidin - coated magnetic beads. In order to capture the target sequences, the beads were incubated at room temperature for 15 min. The unbound genomic DNA was consequently removed through a sequence of washes; twice in 6× SSC; 0.1% SDS (atv25 °C), twice in 1× SSC (at 25 °C) and finally twice in 6×SSC at 60 °C for 5 mins each.

The DNA attached to the magnetic beads was eluted in TE buffer preheated to 95 °C as single stranded fragments. The fragments were amplified by PCR using super SNX24 forward as the primer and cloned in the plasmid vector pGEM-TEasy Vector (Promega Corp., USA) by incubating overnight at 14 °C. The vector transformed to *E. coli* DH10B cells (Invitrogen, USA) by heat shock method. The cloned fragments from the libraries were evaluated by colony PCR using M13 forward and reverse primers.

Sequencing of clones, identification of SSRs and primer designing: The positive clones were picked and grown overnight in liquid ampicillin (100 µg/mL) LB media. Plasmid DNA was extracted using Micro Plasmid Prep Kit (Amersham Biosciences, USA). DNA inserts was sequenced using M13 Primer following the di-deoxynucleotide chain termination method on ABI3700 sequencer. Clone sequences were extracted from the chromatogram using chromos. The sequencing data were analyzed using the ClustalW package at the European Bioinformatics Institute (EBI: http://www.ebi.ac.uk/).

The unique sequences were compared against the GeneBank database (National Center for Biotechnology Information, NCBI)) using the BLASTN search program (http://www.ncbi.nlm.nih.goc/blast). MISA and primer3 were used for identification of SSR motifs and designation of primer pairs flanking SSR regions[32].

Amplification and visualization of microsatellite loci in the genotype panel: Microsatellites amplification was performed in 10 µl reaction mix containing 0.4 pM of each primer, 0.1 U TaqDNA polymerase (Genei, India), 0.2 mM of each dNTP (Genei, India), 1% reaction buffer and 10 ng of template DNA using a thermocycler with the following conditions: 94 °C for 5 min, 35 cycles of 94 °C for 30 sec, annealing temperature(55/59 °C) for 30 sec, 72 °C for 30 sec and final extension of 7 min at 72 °C. Each primer pair was initially screened for product polymorphism and the annealing temperature was later optimized to produce clear and robust amplification. The amplified fragments were resolved in non-denaturing 6% polyacrylamide gels with the silver staining procedure[33] and the size of the fragments was predicted by comparison to a standard marker (100bp ladder).

Statistical analysis: To evaluate the SSR allelic variation in the genotype panel, we used the following measures: number of alleles (N_a) per locus, maximum allele frequency (M_{af}), observed heterozygosity (H_o), expected heterozygosity or gene diversity (H_e) and polymorphism information content (PIC) using software POWERMARKER version 3.25[34]. The SSR allelic data were used for computing the inter-individual genetic dissimilarity based on simple matching coefficient by using DARwin 6.0.018[35]. The neighbour-joining (NJ) tree based dendrogram used dissimilarity matrix to depict genetic relationships among genotypes.

Results and Discussion

SSR-enriched library: The two blunt end restriction enzymes digested the genomic DNA into numerous fragments of sizes less than 3.0 kb observed as smeared banding pattern (Fig. 1). This genomic library was enriched for CT, GA and CA SSR repeat motifs. The PCR positive bands indicated the selective amplification of DNA fragments containing CT, GA and CA repeats. Further, the amplified DNA fragments were used to construct three clone libraries. Numerous colonies were obtained for each library.

A total of 750 SSR positive clones were identified from three libraries by colony PCR using M13 primers which showed that the cloned fragments were in the size range of 300 to 1000 bp (Fig. 2). Among these, 617 (82.2%) clones had unique sequences, which were analyzed with MISA and it was found that 238 (38.4%) of them were found to have one or more SSRs. The remaining 133 (17.7%) clones had redundant sequences which were discarded (Table 2).

The sequences obtained in this study have been deposited in the GeneBank (NCBI) viz. accession numbers KJ586129 to KJ586228 and KX914750 to KX914860. Analysis of the sequence information of these clones indicated the insert size in the clones range of 84 bp to 990 bp with an average size of 316 bp. Majority of the clones (52.6%) contained the insert of medium size (200 bp-400 bp) while 16.9% clones contained small inserts (50 bp-200 bp) and 30.5% clones contained large inserts (>400 bp). Similar to the results obtained in this study, Hamdan et al[22] reported that 35% of the colonies were found to contain SSRs in safflower.

The efficiency of the SSR enrichment procedure achieved in this study was comparable with other SSR isolation studies in crops, for instance in groundnut (10% to 30%)[36-38]. The SSR enrichment rate obtained in this study is higher because frequency of SSRs in the non-enriched genomic DNA libraries had been very low; for instance, 0.1% in Brassica, 0.1% in rye and 0.4% in Paspalum[39-41]. However, more efficient SSR enrichment has been obtained in crops namely

groundnut (68%)[42], wheat (71%)[43], pomegranate (74.4%)[44], coconut (75%)[45] and turmeric (84%)[46].

Definitely, the factors like restriction enzyme used for library construction and the SSR motifs used for enrichment etc. play a role in efficiency of SSR enrichment. The procedure used in the current study seems to be efficient SSR enrichment for isolation in safflower. The redundancy level of 21% observed in this study is substantially lower compared to the genomic SSR enrichment library study in safflower by Hamdan et al[22] who reported 84% redundancy. Comparable rate of redundant SSR-containing clones has been reported in other plant species, for example, onion (24.3%)[47], groundnut (26%)[42], pomegranate (9.3%)[44], olive tree (16.6%)[48] and castor (19.4%)[49].

Occurrence and features of SSRs: Sequence analysis of clones showed the presence of one or more SSRs in 238 (38.4%) clones. According to definition of SSRs by Weber[50], in the present study, 79.5% of SSRs were perfect, 1.5% imperfect and 19% were compound. Similar distribution of SSR classes was observed in different SSR isolation studies in safflower[21-24] and other crops like pomegranate, bittergourd, *Morus* sp., pumpkin, sugarcane, wheat and pea[44,51-56]. The 'perfect' type of repeat regions is more common in plant genome and has more mutation rates, attribute to increase in the mutation and evolutionary rates[37,57,58]. Therefore, the SSR loci containing perfect repeats are more useful for diversity analysis[51]. The compound repeat motifs are rare in plant genomes but they seem to exhibit high polymorphism, which is more desirable for genetic diversity analysis and trait mapping[42,50,59,60].

In this study, dinucleotide repeat motifs were predominant (in 152 clones; 63.5%) followed by tri-nucleotide repeats (in 76 clones; 31.7%) which is in accordance with other studies in safflower[22, 24]. It has been observed that dinucleotide repeat motifs were more frequent in genomic SSRs, but tri-nucleotide motifs were more frequent in EST-SSRs[61,62]. In safflower, Chapman et al[20] and Mayerhofer et al[21] reported higher tri-nucleotides repeat followed by di- and tetra-nucleotide-repeats through EST data mining.

However, Yamini et al[23] reported higher dinucleotide than trinucleotide repeat motifs through EST data mining. The presence of more dinucleotide repeats in safflower is in accordance with previous information of microsatellites in other crops such as castor[49], rice[63], wheat[64] and maize[65]. Among the different types of SSRs, di- nucleotide appears to be common characteristic of plant genomes on a database search[66].

Higher occurrence of dinucleotide motifs[65,67,68] coupled with higher levels of polymorphism has been reported in plants[69,70]. Among the repeat motifs, the GA/TC repeat motif was the most frequent contributing of 33.8% among all repeat motifs followed by CA/TG repeat at 29.2% and AG/CT (22.1%).

However, inference on the predominance of any microsatellite motif in the safflower genome from the results of this research needs to be done cautiously owing to the high level of redundancy introduced by enrichment of the library. In plants, most repeated dinucleotide motif differs between studies and species and thus could be due to variation of genome structure[54]. The previous results showed (AT)*n* as the most frequently occurring dinucleotide repeat motifs[71,72] in plant genomes. However, other studies have suggested that the (CT)*n* and/or (TG)*n*[66,73-75] motifs may also be highly prevalent. Lagercrantz et al[70] demonstrated that single strand of (AT)/(TA) and (CG)/(GC) repeated units more easily makes the self-complementary structure than the other kinds of repeat units of DNA sequences.

Therefore, the observed low SSR frequency of (AT)/(TA) and (CG)/(GC) in our study may be the result of the self-complementary nature of these probes. Abundance of CA/TG, GA/TC and AG/CT repeat motifs in the present study is in agreement with earlier reports on development of SSRs in safflower[22,24].These results are also consistent with the frequency of di-nucleotides reported in rice[63], maize[65], wheat[64,76], groundnut[42], barley[77], coffee[78], castor[49] and sugarcane[54]. The overall repeat motif number ranged from 4 to 32.

Primer design and SSR polymorphism: The sequences with SSRs were used for designing of primers following the standard criteria: primer length 18-25 bp; Tm 50-60 °C; GC content 40-60%; max Tm difference between forward and reverse primer 1.5°C and primer-pairs were designed for SSRs from 200 clone sequences. For 38 sequences, the primer designing could not be possible as the SSR motifs are located at start or end of the fragments. The proportion of primers designed to the number of sequenced clones (26.6%) is higher than some studies in wheat (21%)[73], groundnut (21.6%)[52], pomegranate (11.3%)[44] and lower than groundnut (43.7%)[66], bitter gourd (32.5%)[51] and sugarcane (27%)[54].

Sequence of some clones was not suitable for primer design because of short or missing flanking regions. This may be attributed to the size range of insert, the restriction enzyme used for construction of library and the method used for SSR enrichment etc.[19]

Developed SSR primer-pairs were tested on two genotypes i.e. A1 and Bhima for amplification. Out of 200, only 164 (82.2%) primer-pairs yielded scorable amplicon in the genotypes examined which are higher than the other safflower studies such as Chapman et al[20] (56.3%), Lee et al[24] (59.3%) and Yamini et al[23] (65.5%). In order to evaluate the amplification and polymorphism percentage of developed SSR primer-pairs (200) in safflower genotypes, they were tested in a panel of 24 genotypes (Supplementary Table 1). Only 42 (25.6%) primer-pairs out of 164 detected polymorphism in the genotype panel (Table 3, Fig. 3). Compared to other studies on safflower, in the present study the polymorphism was higher. For example, Lee et al[24]

reported 10% polymorphism with 100 diverse accessions of cultivated species.

In contrast, Chapmanet al[20] found that 89.4% of their EST-SSR safflower markers were polymorphic across a diverse panel of 24 safflower lines composed of three species *C. tinctorius*, *C. oxyacanthus*, *C. palestinus* and Hamdan et al[22] observed 72% polymorphism with 10 safflower lines of *C.tinctorius*. The number of polymorphic SSRs from enriched libraries of other crops was observed low as 11.8% in castor [49], 12.5% in sugarcane[54], 20% in bitter gourd[51], 15% grape vine[79], 14% sorghum[80], 23% wheat[64,81] and higher in 44.2% in groundnut[42], 44% in pomegranate[44], 43% in *Brassica* species[82]. The previous studies indicated that percentage of polymorphism increases with repeat length[37,83].

The locus mCtDOR38 with (TG)13 repeat motif had a high PIC value of 0.61 while mCtDOR30 with higher (CA)32 repeat motifs had PIC value of 0.32. Even the markers mCtDOR20 (GA17), mCtDOR43 (TC10), mCtDOR41 (AG8-AG13), mCtDOR (AC11) were equally capable of detecting polymorphism in safflower lines revealing that SSR length is not necessarily a benchmark for detecting polymorphism[84]. In some reports, no relationship or weak correlation was observed between SSR polymorphism and repeat unit length[85,86]. With the genotypes used in the present study, the numbers of SSR alleles ranged from 2 to 4 with an average of 2.7 per locus. Majority of primer-pairs produced only two alleles. The major allele frequency ranged from 0.39-1.00 with an average of 0.73. The PIC values of SSR primer-pairs ranged from 0.00-0.61 with an average of 0.32.

Table 1
Details of the safflower genotype panel used for characterization of SSR markers

Genotype	Source	Characteristics
A-1	India	Variety spiny high yielding resistant to wilt and aphid high linoleic acid
Bhima	India	Variety spiny high yielding high linoleic acid content
PBNS-12	India	Variety high yielding spiny tolerant to wilt and aphid high linoleic acid
NARI-57	India	Variety spiny high oil high linoleic acid
CO-1	India	Variety non-spiny susceptible to aphid
GMU-472-1	India	Bold capitula with high seed number
GMU-184	India	Spiny tolerant to aphid
EC-523374-p1-2-p8	India[Selection from breeding line(USA)]	High oil thin hull
EC-542438-1-1-p2-4-p6	India [Selection from breeding line(USA)]	Bold capitula thin hull type
EC-542438-1-1-p2-7-p5	India [Selection from breeding line(USA)]	Bold capitula
EC-523368-2	India [Selection from breeding line(USA)]	Sparsely spiny resistant to aphid
EC-755659-1	Mexico [Selection from Mexican variety Ciano-OL]	Spiny high linoleic acid
EC-755660 (S-334)	Mexico	Variety spinyhigh oil high oleic acid
EC-755664 (CW-99)	Mexico	Variety spiny high oil high oleic acid
EC-755673-1	Mexico [Selection from Mexican varietyHumaya-65]	Variety high oil high oleic acid
EC-755675-1	Mexico [Selection from Mexican variety Aceitera]	Variety spiny high oil high oleic acid
EC-755688 (Ciano-Lin)	Mexico	Variety spiny high oil high linoleic acid
EC-736514 (Oleic Leed)	USA	Variety spinyhigh oleic acid
EC-736515 (Montola-2000)	USA	Variety spiny high oil high oleic acid
EC-736516 (Centennial)	USA	Variety spiny high oil high linoleic acid
EC-736487	USA	Breeding line spiny high oil
EC-736498	USA	Breeding line spiny high oil
EC-736499	USA	Breeding line spiny high oil
EC-736501	USA	Breeding line spiny high oil

These results showed that the allelic diversity in the safflower lines was low.[22] In safflower, similar results were reported by Hamdan et al[22] (N_A=3.2, PIC=0.55), Barati and Arzani[28] (N_A=3.43, PIC=0.32), Lee et al[24] (N_A=2.8, PIC=0.325), Derakhshan et al[29] (N_A=3.8, PIC=0.30) and Usha Kiran et al[27] (N_A=3.6, PIC=0.28).

Genetic relationships revealed by newly developed SSR markers: Overall, pair-wise simple matching coefficients ranged from 0.0 (CW99- S-334 genotypes) to 0.74 (A1-EC736487 and NARI-57-EC736487 genotypes) with the average of 0.35. Cluster analysis based on simple matching coefficient detected three major groups (A, B, C) in the panel of 24 genotypes (Fig. 4).

Cluster A included 13 genotypes with two sub-clusters A1 and A2. The sub-cluster A1 included 7 genotypes; 6 of them were either exotic germplasm accessions from USDA or the selections made from them and CO-1, an Indian non-spiny cultivar. The sub-cluster A2 included exclusively high yielding varieties and breeding lines of India namely A-1, Bhima, PBNS-12, NARI-57, GMU472-1 and GMU-184. The cluster B included 7genotypes; of which 6 were high oleic types (S-334, CW-99, Oleic Leed, Montola-2000, EC-755673-1, EC-755675-1) and one was thin hull type (EC-736487). The cluster C consisted of 4 exotic genotypes from USDA and Mexico. EC-75559-1, EC-736516, EC-755688 and EC-5223368-2.

Genotypes originating from India grouped in first cluster A and the high oleic genotypes of Mexico and USA were distributed in other clusters. The dendrogram gives an understanding of the genetic diversity within the parental lines for constructing the mapping population(s) for mapping the seed traits and oil content and quality in safflower. Evaluation of these markers for assessment of genetic diversity among 24 safflower parental lines of mapping population indicates their potential in genetic analysis of safflower for mapping, variety protection and hybrid seed purity testing.

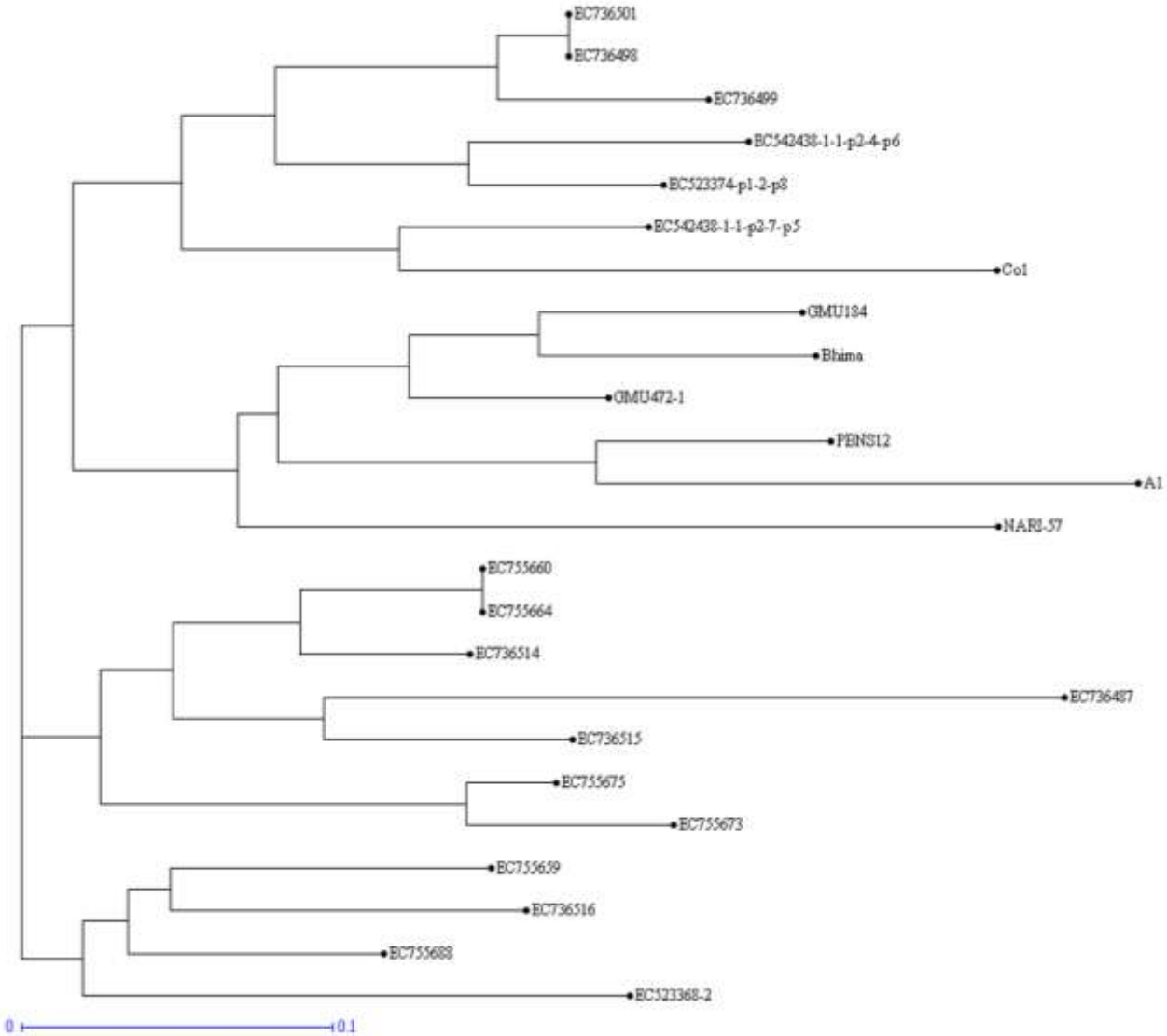

Figure 1: Dendrogram showing the genetic relationships among 24 safflower genotypes based on similarity coefficients derived from SSR polymorphism data

Table 2
Summary of the microsatellite enriched library constructed for safflower

S. N.	Characteristic	Number
1	Clones sequenced	750
2	Number of redundant clones	133
3	Unique clones	627
4	Number of sequences containing more than one SSR Repeats	238
5	Primers developed	200
6	Primers standardized/lous specific amplification	164
7	Polymorphic markers	42

Table 3
Details of polymorphic safflower microsatellite markers

Locus	Genebank accession	Primers	Repeat motif	Repeat type	Size range (bp) (approx)	N_a	M_{AF}	H_e	Gene diversity	PIC
mCtDOR3	KJ586131	F: TCCCAACCCTCTCACTTTTT	$TTAA_3$-CA_{13}	C	110-125	2	070	000	042	033
		R: GTGTCCACACACCGTCAAG								
mCtDOR4	KJ586133	F: GGTCCCAGAAGCAGTAGTGA	AC_9	P	220-230	2	096	000	008	008
		R: CCCCGATAGCAACTACAGGT								
mCtDOR8	KJ586138	F: ATCTGAAGAGAGTCTCCGGC	AT_9	P	210-230	3	087	000	023	022
		R: CCATTCGACTATATCCGCTT								
mCtDOR20	KJ586151	F: GATGTAACAAGGTGCAGGGA	GA_{17}-$GTGA_5$	C	250-285	4	061	004	056	050
		R: GATCCAACGCCATTTTCTCT								
mCtDOR23	KJ586155	F: TGGCCTATGTAGTTTTCTCG	TAA_6	P	230-240	2	091	000	017	015
		R: GACTCAAAGGGTTCGACGAT								
mCtDOR30	KJ586163	F: TGAGAGGGTAGATGCACTGG	CA_{32}	P	180-195	2	067	004	044	034
		R: CTCAGACTGGTTGTTGGTGG								
mCtDOR32	KJ586165	F: ATGTGGGAGGAATCAAGGAG	GAA_8-GT_9	C	125-140	3	091	000	016	016
		R: CCATCTTCTCACCATGAAAACC								
mCtDOR33	KJ586166	F: CGTTATGGCGGCAGATAAAT	$GATT_9$	P	190-200	2	071	000	041	033
		R: TCTAACCACGTTTTCCCACA								
mCtDOR37	KJ586171	F: AGGGATTCAAGTAATAAATC	TC_{10}	P	280-300	2	096	000	008	008
		R: GGCAAGGGTTTACGCCAAAT								
mCtDOR38	KJ586172	F: GGACCTTCAAATATCACGCC	TG_{13}	P	185-200	4	044	004	067	061
		R: GTATTCCACCGATTCCTTCG								
mCtDOR40	KJ586174	F: GGAGCAATAAGCAGGAGGAG	TC_5-TC_7	I	350-360	2	092	000	015	014
		R: CGAGATTGATAACGCCTTGA								
mCtDOR41	KJ586175	F: TGAGGACAATTGTGTGCGTA	AG_8-AG_{17}	C	235-260	3	044	004	062	054
		R: ATAGGACAAAACCAACCCCA								
mCtDOR42	KJ586176	F: GATGCCCTAAAGTGGTCCAT	AG_9	P	120-150	4	075	000	041	039
		R: AACAGATGCAAGTTTGGCAG								
mCtDOR43	KJ586177	F: CGCCACTCCTCTTCCTCCGA	TC_{10}	P	190-210	3	039	000	066	059
		R: GAGTAATCTATACCTACACTAC								
mCtDOR45	KJ586179	F: TTTTGCTCATGAACAGCCTC	TG_{10}	P	285-290	2	075	000	038	030
		R: AGGGTATCGATCTTGTTGCC								
mCtDOR52	KJ586186	F: CACACAAACCACATGAAGCA	CA_8	P	220-245	4	071	000	047	043
		R: ACATTGAAAGATGTGAGGCG								
mCtDOR53	KJ586187	F: GAGTTGTAATAAGGGATTCAAG	TC_7	P	280-300	2	096	000	008	008
		R: GGCAAGGGTTTACGCCAAAT								
mCtDOR54	KJ586188	F: AAAAGGGTAAACGGAAGGGT	AC_{11}	P	270-280	3	055	000	060	053
		R: AAAAGCACCCTAAGGTCGTG								
mCtDOR56	KJ586190	F: ACTCGCTTTCTCTCTCATGT	GA_9-GA_{10}-AG_6	C	260-280	4	077	004	038	036
		R: TATTCATACCGCTTTTCCCC								
mCtDOR57	KJ586191	F: AGCTCCATGAAGAAAGGCAT	GA_9	P	245-250	2	083	000	028	024
		R: CTCACAACCCAAAGTGGATG								
mCtDOR64	KJ586198	F: ACAAGTTCGATACACACCCG	AG_5-AG_6-AG_9	I	165-170	2	070	000	044	035
		R: GAGGGCGTTAACTCGACG								
mCtDOR74	KJ586209	F: AACTGCTTCTTACGTTCCTCTG	TC_8-AC_{13}	C	220-240	2	067	000	044	035
		R: ACGAAATGCTTGGAGAACAG								
mCtDOR75	KJ586212	F: TGTGCCTAAAGTTGTCAAAGAC	CA_5-CT_{11}	C	180-210	3	073	000	042	036

		R: GCAACTGGTGTGCTTTTAGAA								
mCtDOR100	KX914760	F: AAAATGAGAGCAAGGATGAA	TAA_6	P	300-320	3	050	002	046	052
		R: GCGTTGTTACCTTTCACAAT								
mCtDOR101	KX914761	F: CAAATATCCAGTCCAACCAT	AC_9	P	290-305	4	065	010	038	040
		R: ATGGGGTTGTTTACAAGTGA								
mCtDOR113	KX914773	F: CTACCCATATGCACCTAAGC	AAC_6-GCG_5	C	120-145	2	085	004	020	024
		R: ATGATCAACAACCTCACCAT								
mCtDOR114	KX914774	F: CCATCATCTTCACCATCTTT	TGA_6	P	134-150	2	063	000	050	042
		R: AATTTCTCAAACCCATCTCC								
mCtDOR126	KX914786	F: GTCTGACTAGGGGTGTGCT	TC_9	P	210-225	3	047	000	052	053
		R: CCCTGGCTAGTGAAATACTG								
mCtDOR128	KX914788	F: GCTACGAGCAGTAAGTCGTT	GT_{10}	P	220-250	3	053	001	047	052
		R: GCTAATTACGGAAGCAGAAA								
mCtDOR136	KX914796	F: AAACCAATTTTGCCATTAAA	CAA_6	P	110-135	2	091	000	010	009
		R: TGGTAAGTGTAGTCGGCTTT								
mCtDOR143	KX914803	F: CAAATATCCAGTCCAACCAT	AC_9	P	200-240	2	049	004	052	037
		R: ATGGGGTTGTTTACAAGTGA								
mCtDOR149	KX914809	F: TCGTCAATAAGGTCGAGAGT	CA_8	P	220-235	2	070	000	042	033
		R: GCTAAGATGGTGACGTGTCT								
mCtDOR152	KX914812	F: AAGATGAGGTCAACTCCAAA	TTA_6	P	200-220	3	064	010	037	060
		R: ATTTCCAACAACTGCATACC								
mCtDOR157	KX914817	F: GAATTCTGATTGGTGGAAAA	TA_{10}	P	220-245	3	043	000	046	055
		R: GAAGAAGCATTTGAGACCAG								
mCtDOR161	KX914821	F: GCTTCATATCATCCCCATTA	CAG_6	P	220-230	2	024	000	036	021
		R: ACACCCGATAAAAAGTAGCA								
mCtDOR164	KX914824	F: ATGAAACGAACTGATGAAGG	CTG_6	P	200-225	2	036	005	045	033
		R: ACCGATGTATGGTCACTAGG								
mCtDOR165	KX914825	F: ATAGCTCCATTCACCATCAC	CAA_6	P	190-215	4	029	000	035	027
		R: ATTTGGCTTATTTCCACTGA								
mCtDOR171	KX914831	F: AATCCCTCTTCTCTCACTCC	TGA_6	P	220-240	2	0013	002	027	0013
		R: CCGTCAAAAGACAGAGAAAC								
mCtDOR178	KX914838	F: AGGAAGATACGATACGACCTC	TCT_6	P	220-235	2	04	004	035	046
		R: GAATTAATCACCGATGGAAA								
mCtDOR182	KX914842	F: ATCTCCGATCACACACTTTC	TC_{11}	P	220-230	3	022	002	036	021
		R: GATGGAGTGAGAGAGAGCTG								
mCtDOR184	KX914844	F: GCTACGAGCAGTAAGTCGTT	GT_{10}	P	350-370	3	085	000	020	024
		R: GCTAATTACGGAAGCAGAAA								
mCtDOR193	KX914853	F: AAGAGGGAGAGGGAGGTCAA	TAA_7	P	320-345	2	036	000	040	037
		R: CCTTGCAAGCTCTTGCTTTT								

Supplementary Table 1
Details of 200 safflower microsatellite markers designed in this study

Locus	Genebank accession	Primers	Repeat motif	Repeat type	Size range (bp) (approx.)
mCtDOR1	KJ586129	F: GTCCCGAAAACTAGGACCAA	TC_6	P	400
		R: ACCCTTCGCTCAATGAGAA			
mCtDOR2	KJ586130	F: CAGCAGCAGCATCTTCAAA	AG_5	P	NA
		R: CGACAATCGGGTTATCAGTG			
mCtDOR3	KJ586131	F: TCCCAACCCTCTCACTTTTT	$TTAA_3$-CA_{13}	C	110-125
		R: GTGTCCACACACCGTCAAG			
mCtDOR4	KJ586133	F: GGTCCCAGAAGCAGTAGTGA	AC_9	P	220-230
		R: CCCCGATAGCAACTACAGGT			
mCtDOR5	KJ586134	F: GTCTGTTGCAAGAAGAGTTGTG	AGA_8	P	180
		R: TGCTGGAATTGGTTCTGTCT			
mCtDOR6	KJ586135	F: CTAGCAGAATCCCTTCCCTG	GAA_{13}	P	190
		R: TGCCTCTGTCTGCTCACTTT			
mCtDOR7	KJ586137	F: CCAAGTCCCAACATGCACAA	CA_7	P	180
		R: GTTATATCTTGTAGATAGGCAG			
mCtDOR8	KJ586138	F: ATCTGAAGAGAGTCTCCGGC	AT_9	P	210-230
		R: CCATTCGACTATATCCGCTT			
mCtDOR9	KJ586139	F: GTTTTTCCCCTAAACCTCCC	CA_7	P	130
		R: TGAAAGTGATCAAGGGTCCA			

mCtDOR10	KJ586140	F: CCTTTTTCAAATCCTGCTGC R: ACCGAGATCAATGCAGTCAA	GAA_8	P	140
mCtDOR11	KJ586141	F: GACTGTTATTCAAAAGGTCC R: GCCTCCCGGAATTTGATTGA	AT_6	P	260
mCtDOR12	KJ586142	F: TCTCTCTCTCTCTTGATTTCCCA R: AAATGGGTTGGAAATCGAAGTTT	CT_9-CT_7	C	280
mCtDOR13	KJ586143	F: TCACACTTCTTCTTGCCACA R: CTTCCCTGATTCTGAAGAGGA	CTT_{11}	P	150
mCtDOR14	KJ586144	F: GGCCGATACTCGACTCTAGC R: GAAGCCTCCATACACATACA	TC_{12}	P	130
mCtDOR15	KJ586145	F: AACTCCACCGAAAAATCACC R: TAGAGCGGCAATTGACTTGA	TG_{16}	P	135
mCtDOR16	KJ586147	F: TACAGCACCCACAACGAAAT R: TCATTGCGCTCGATCTGTAT	CA_7	P	NA
mCtDOR17	KJ586148	F: TCCAAGACCATGATTTGCAG R: CGCACATGTTACCCACAAGT	AT_9	P	NA
mCtDOR18	KJ586149	F: AACTCCACCGAAAAATCACC R: AGGCCTAAGCTTGCAGAATC	TG_6-TG_6	C	320
mCtDOR19	KJ586150	F: ATGAGGTTGTCGTTCGGGAT R: TACATGAAACATGTATAATTC	AC_7	P	200
mCtDOR20	KJ586151	F: GATGTAACAAGGTGCAGGGA R: GATCCAACGCCATTTTCTCT	GA_{17}-$GTGA_5$	C	250-285
mCtDOR21	KJ586153	F: CCCTCTTTTACCCAGATCCA R: CAAGAACCAGACCACTTCCC	TGA_7-$AAAGAA_4$	C	215
mCtDOR22	KJ586154	F: GTTCTCCTTTAAACTTTCACC R: TTCACTTGTCTTTACCGCCT	AT_5-GA_6-GA_9	C	280
mCtDOR23	KJ586155	F: TGGCCTATGTAGTTTTCTCG R: GACTCAAAGGGTTCGACGAT	TAA_6	P	230-240
mCtDOR24	KJ586156	F: CTCACGAGATCGATGCCTTA R: CCAACTTCGTGGGATTTCTT	AG_5	P	285
mCtDOR25	KJ586157	F: TAGCGGAATGTTCACAAAGC R: CTATGGGCAACCCAGATACC	TG_8	P	200
mCtDOR26	KJ586158	F: TCTTGCTATCTGTTTCCGGC R: CCCTAGATCCAAAACCGAAA	AT_8	P	190
mCtDOR27	KJ586160	F: TAGAACCCTCTCAGCCCTTC R: AGCCCATGTGTTGTGTGTGT	$TTAA_3$-CA_{10}	C	265
mCtDOR28	KJ586161	F: AGGGAAGGAATCCTAGGCCC R: GTTGATACATAAAGTTGCCT	$AAGA_8$	P	200
mCtDOR29	KJ586162	F: TACACACACTGAATACACCAAGA R: TGTAAGTCTGAGTTAGTGTGGAG	AC_6	P	110
mCtDOR30	KJ586163	F: TGAGAGGGTAGATGCACTGG R: CTCAGACTGGTTGTTGGTGG	CA_{32}	P	180-195
mCtDOR31	KJ586164	F: AAGAGAGATCGCCGGAGTAA R: AGTTACCTTCCGAGCACGTT	AG_6	P	110
mCtDOR32	KJ586165	F: ATGTGGGAGGAATCAAGGAG R: CCATCTTCTCACCATGAAAACC	GAA_8-GT_9	C	125-140
mCtDOR33	KJ586166	F: CGTTATGGCGGCAGATAAAT R: TCTAACCACGTTTTCCCACA	$GATT_9$	P	190-200
mCtDOR34	KJ586168	F: TTATCATTTCAGGGCGTGTG R: ACCCATCATCAGAGATGCAA	TA_7	P	400
mCtDOR35	KJ586169	F: ACATTGAAAGATGTGAGGCG R: CACACAAACCACATGAAGCA	GA_7	P	210
mCtDOR36	KJ586170	F: ACCGGTTGATGTGTATCCCT R: ATCGTTGGAGATGAAGTTGC	TC_{15}	P	320
mCtDOR37	KJ586171	F: AGGGATTCAAGTAATAAATC R: GGCAAGGGTTTACGCCAAAT	TC_{10}	P	280-300
mCtDOR38	KJ586172	F: GGACCTTCAAATATCACGCC R: GTATTCCACCGATTCCTTCG	TG_{13}	P	185-200

mCtDOR39	KJ586173	F: CGGCGATCTCTCCTCTTATC R: ACAACAACCCAGATGCCATA	ACT_5	P	250
mCtDOR40	KJ586174	F: GGAGCAATAAGCAGGAGGAG R: CGAGATTGATAACGCCTTGA	TC_5-TC_7	I	350-360
mCtDOR41	KJ586175	F: TGAGGACAATTGTGTGCGTA R: ATAGGACAAAACCAACCCCA	AG_8-AG_{17}	C	235-260
mCtDOR42	KJ586176	F: GATGCCCTAAAGTGGTCCAT R: AACAGATGCAAGTTTGGCAG	AG_9	P	120-150
mCtDOR43	KJ586177	F: CGCCACTCCTCTTCCTCCGA R: GAGTAATCTATACCTACACTAC	TC_{10}	P	190-210
mCtDOR44	KJ586178	F: TGTGATCTGTTGTAGCGTGG R: GATCCTGCCGTTTCCTCTAA	GA_8	P	205
mCtDOR45	KJ586179	F: TTTTGCTCATGAACAGCCTC R: AGGGTATCGATCTTGTTGCC	TG_{10}	P	285-290
mCtDOR46	KJ586180	F: TAAGCCATGGGCTTTTTACC R: CTTTCCCAAACACCCAAAGA	ATC_4	P	290
mCtDOR47	KJ586181	F: GATAGAGAATTAACTGGGCTCCC R: AGTTTTGGAGTCAGAATCCAGT	TA_5	P	180
mCtDOR48	KJ586182	F: TATACCGACGGTTATGGTGC R: TCCAGTCGGTGATACGTAGG	$GATC_4$	P	300
mCtDOR49	KJ586183	F: CACAAGGTTCCAAGCAAAGA R: AAACCGTGACAACACTCCAA	$TGTGT_3$	P	200
mCtDOR50	KJ586184	F: ACTGACAGTGACCAGACTCG R: CAATACCTTCAGTGACTTGT	$TTCAT_3$	P	330
mCtDOR51	KJ586185	F: AGGAACAAAACCACGAATCC R: CTTTGTGAGCTCATCTCGGA	CA_9	P	250
mCtDOR52	KJ586186	F: CACACAAACCACATGAAGCA R: ACATTGAAAGATGTGAGGCG	CA_8	P	220-245
mCtDOR53	KJ586187	F: GAGTTGTAATAAGGGATTCAAG R: GGCAAGGGTTTACGCCAAAT	TC_7	P	280-300
mCtDOR54	KJ586188	F: AAAAGGGTAAACGGAAGGGT R: AAAAGCACCCTAAGGTCGTG	AC_{11}	P	270-280
mCtDOR55	KJ586189	F: CGTCTTCGATCTTGCATATA R: AGGAGGATATGAAGCACTGC	$TATT_3$	P	220
mCtDOR56	KJ586190	F: ACTCGCTTTCTCTCTCATGT R: TATTCATACCGCTTTTCCCC	GA_9-GA_{10}-AG_6	C	260-280
mCtDOR57	KJ586191	F: AGCTCCATGAAGAAAGGCAT R: CTCACAACCCAAAGTGGATG	GA_9	P	245-250
mCtDOR58	KJ586192	F: CAAAGTATCGGCTCCAGTCA R: CCTTGATTAAAGTCCAAGCG	AT_6	P	NA
mCtDOR59	KJ586193	F: GTTCATGCTGTTATGAATAGG R: GTCGATCCGCCCCCCAGGAT	CT_{13}	P	NA
mCtDOR60	KJ586194	F: ATCCCTGACCTTGCTGATTC R: TTCAAACGACAACCAGGGTA	GTT_4-$TTCTGG_3$-GTT_6	C	280
mCtDOR61	KJ586195	F: AATCTTAATGCAAGGGCACC R: CCCATCCTATTGCTAGTCCC	TA_5	P	230
mCtDOR62	KJ586196	F: TGAAATGGAGAAATGAAGTG R: CCTTGTGGCCAGCCCCTACC	GA_8	P	90
mCtDOR63	KJ586197	F: CACCTTGAAAAACGTCATGC R: GCAAGGAAAGCACAAAGACA	TG_8	P	220
mCtDOR64	KJ586198	F: ACAAGTTCGATACACACCCG R: GAGGGCGTTAACTCGACG	AG_5-AG_6-AG_9	I	165-170
mCtDOR65	KJ586199	F: TCACACTCTGAGGTCACACG R: GCCTAGCCCATTTTTGGATA	CA_5	P	140
mCtDOR66	KJ586200	F: CAAAGACACTCAAGACGCAC R: CCCTTAGCAACAAGTCTAGCC	CA_5-CT_{11}	C	190
mCtDOR67	KJ586201	F: TCTGATCATGGGAAACAGGA R: GATTGGAGCTTGGTGATGTG	CAT_4	P	250

mCtDOR68	KJ586202	F: CATGATGGGCCTTACCTTTT R: GCGACAAGATCGAGTTGGTA	GT_5-TG_5	C	NA
mCtDOR69	KJ586203	F: TATGCGTTCACCGCTACTTC R: TCCTTGAGAAGCAAGCAAGA	TC_7-CT_5	C	180
mCtDOR70	KJ586204	F: TCTGGTTCTGAAGTGCTTGG R: GGTAGTGGTCCTGATTGGCT	TCC_5	P	280
mCtDOR71	KJ586205	F: CTATATGGATTAGGTTTGGTG R: TGACCACGATCCAACCCAAT	TGG_4	P	NA
mCtDOR72	KJ586206	F: GAGGTGAGAGGTGTGTGGAA R: CCCCATGGCTCTCTCTCATA	GT_5	P	260
mCtDOR73	KJ586207	F: TTGCTTAGTAACGACGCCAC R: CAATGTATGTGACGGTGCAA	AC_6-AC_5	I	130
mCtDOR74	KJ586209	F: AACTGCTTCTTACGTTCCTCTG R: ACGAAATGCTTGGAGAACAG	TC_8-AC_{13}	C	220
mCtDOR75	KJ586212	F: TGTGCCTAAAGTTGTCAAAGAC R: GCAACTGGTGTGCTTTTAGAA	CA_5-CT_{11}	C	180
mCtDOR76	KJ586213	F: ATTCTTGACCACCACCCAAT R: TTCAATGTCCTTGGTGCTGT	CA_7	P	280
mCtDOR77	KJ586214	F: CCACAAGATAGAAGCACCCA R: GGGATGCTTTGTGATGCTAA	AC_{10}	P	180
mCtDOR78	KJ586216	F: GCTGGTTGACTTGACGAAAA R: CGCCAGGATAAGGTTCAAAT	TA_6	P	100
mCtDOR79	KJ586217	F: CCACAAGATAGAAGCACCCA R: TTTCGGTTTCGAGTCAAGTG	TA_8	P	110
mCtDOR80	KJ586219	F: GCTTCACTCTAAGGCGGAAC R: CAAATCGAGGCAAACTCTGA	CT_7	P	340
mCtDOR81	KJ586220	F: CCCTTCCCTTTATCCTTTCC R: TGGTTGTGTGGTAGCCTGAT	CA_9	P	230
mCtDOR82	KJ586221	F: CCTCAAACGGTCAAATGATG R: TGGCGAACATAATGTCTGGT	AT_7	P	400
mCtDOR83	KJ586222	F: CCCTGAAAACAGTAATTGGG R: AGCTGGATCAACAATCTCCC	CAT_6	P	160
mCtDOR84	KJ586223	F: GAAACCTCATTCAGCCACAA R: GCCCAAACTAATGAAGCCAT	CTT_6	P	NA
mCtDOR85	KJ586224	F: GGAGCAAGGAAGATCAGAGG R: GGAGCAAGGAAGATCAGAGG	CTT_7	P	260
mCtDOR86	KJ586225	F: GGGTCTAAAGAAGAACAGAGAC R: TTATAGATCCATCCCCCGAA	TG_6	P	410
mCtDOR87	KJ586226	F: CGTGCATCCAGTAGGAATTG R: AAGGACCGCTACTCCAAAGA	GA_5	P	430
mCtDOR88	KJ586227	F: ACAGCATCGATAAACCCACA R: GTCGTAGTCTTTTTGCCCGT	TA_5	P	NA
mCtDOR89	KJ586228	F: ATAACGAAGGGTCTCCAACG R: CCCACTTTTGTGTTGTCTGC	GT_5-GA_5	C	300
mCtDOR90	KX914750	F: GTTTGTGGACACCGCGAAG R: CGTGTTCCAAATCCCAGGTA	TG_{12}-AT_8	C	NA
mCtDOR91	KX914751	F: GCTGTCCAATTCTCTCTCAG R: GCAGTTTCTTGACCTTCTTG	CT_9	P	NA
mCtDOR92	KX914752	F: CTCCAAGAACCCTACAGGA R: TCTGTACCACATGCATAAACA	TG_{10}	P	NA
mCtDOR93	KX914753	F: AAACGCAACCTTATGAAGAA R: GAACACGGTCATGATAATCC	CT_8-AT_{11}	C	250
mCtDOR94	KX914754	F: TGGAAGTGAAATCTGTAGAGG R: CCCATCTTCTTCTTCCTTTT	GT_9	P	NA
mCtDOR95	KX914755	F: CACCGATTTGTGAGTAAAAA R: AAGCATTTCATCAAACAGGT	GT_9	P	NA
mCtDOR96	KX914756	F: TTCCTCTGCTTCACTCTCAC R: ACAGCAATCAAAGATCCAAC	GAA_7	P	180
mCtDOR97	KX914757	F: CACACGTCCTCTTTTCTTTC	TA_8-AC_{11}	C	270

		R: AATTCAGGTTCGAGGTTGTA			
mCtDOR98	KX914758	F: CCATAGGGACCAAAAACATA	CT_9	P	NA
		R: TTGAATGTGGAGAAGAAAGC			
mCtDOR99	KX914759	F: ATGGAGGATTGTGGAAGACG	TG_8-AG_9	C	240
		R: CAAGATCCACCTCGAACACC			
mCtDOR100	KX914760	F: AAAATGAGAGCAAGGATGAA	TAA_6	P	300-320
		R: GCGTTGTTACCTTTCACAAT			
mCtDOR101	KX914761	F: CAAATATCCAGTCCAACCAT	AC_9	P	290-305
		R: ATGGGGTTGTTTACAAGTGA			
mCtDOR102	KX914762	F: ATGCTCTTCTTCACCATCAT	AAG_8	P	250
		R: AGTTTGGATATTGGGGATTT			
mCtDOR103	KX914763	F: AAAACACGATCATCATCTCC	CTT_7	P	120
		R: GGTGTCAAGAGGGTACAAGA			
mCtDOR104	KX914764	F: GAAACCACCACCATAACCTA	ACA_6	P	260
		R: GTCCTGTTTGTTGAACCACT			
mCtDOR105	KX914765	F: GAATCCCACAAAAATCTTGA	ACC_6CCA_6-CCA_7	C	NA
		R: ATATCGTTTTCCTGATGTGG			
mCtDOR106	KX914766	F: TCTTCTTCGTAATCCTCGTC	GA_9	P	NA
		R: AAGACGAAGGGTTAAATGGT			
mCtDOR107	KX914767	F: ACGAAGACTTTTGGTGTTGT	TCA_7	P	280
		R: ATCAGAAGGTGATGAAGGTG			
mCtDOR108	KX914768	F: CTTGCATGTTATGTGGATTG	TA_{10}	P	220
		R: GTCCCTTCCTCGACTCTTAG			
mCtDOR109	KX914769	F: TGTTTCAAAATTTTCGGATT	AC_9	P	NA
		R: TTTACTCTTGTTATGGGTTCC			
mCtDOR110	KX914770	F: CAGCAGACAATTGAAGTTGA	TA_{11}	P	230
		R: ATAAGAACCAAACCACCAAA			
mCtDOR111	KX914771	F: TCCTTCTTCCTCCTACTTCC	CTC_7	P	290
		R: ATCAGGGTCTAGCTCTTCCT			
mCtDOR112	KX914772	F: TTACAAAGGACTCCCAGAAA	CT_{11}	P	300
		R: CAGAAGGATCGATCAAAGAG			
mCtDOR113	KX914773	F: CTACCCATATGCACCTAAGC	AAC_6-GCG_5	C	120-145
		R: ATGATCAACAACCTCACCAT			
mCtDOR114	KX914774	F: CCATCATCTTCACCATCTTT	TGA_6	P	134-150
		R: AATTTCTCAAACCCATCTCC			
mCtDOR115	KX914775	F: CTATCCCTACACCCCACTAA	GA_8AC_9	C	210
		R: AAACTCCTCTAAGGGGGAAT			
mCtDOR116	KX914776	F: GCAGTCTTCTTGTCGGTAAA	TG_9	P	220
		R: CTTCGGTTGTTCAGTTGATT			
mCtDOR117	KX914777	F: TGATAAAAGGAAGGTTTCGT	AAT_7	P	240
		R: AGAAACAAAGCTGTTTGACA			
mCtDOR118	KX914778	F: GTTGGTTTTGGAGTTGTGTT	TG_8	P	140
		R: TTCGGTCTGAATAATCCTGT			
mCtDOR119	KX914779	F: GTTGTGCTTGAACTTTGGTT	TG_9	P	195
		R: ATCCACTCATCCCTTTACCT			
mCtDOR120	KX914780	F: GGTGGTGATTTTCAATTGTT	CT_{10}	P	NA
		R: AAGGAAGCTTGTTGAGATGA			
mCtDOR121	KX914781	F: TTTACTGTTGGGCTAGCATC	TCA_7	P	310
		R: CCAGATTTCAGGTATGTGGT			
mCtDOR122	KX914782	F: GAAATTCATGAGGTGGAAAA	TGA_6	P	320
		R: ATCGATGAAGATGATTGAGG			
mCtDOR123	KX914783	F: TGGTCTTAGAGATTGAAGCTG	GT_9-AAG_7	C	280
		R: ACGATAAATTAGCACTGTTGC			
mCtDOR124	KX914784	F: GCTTCCAGTGCTCCTAGAAT	GT_9	P	220
		R: TCTTGCAAGTTGGTAGGATT			
mCtDOR125	KX914785	F: CATACAAGCGACTCAAACAA	GGA_5	P	NA
		R: GAATGCATGGAAGCTCTATC			
mCtDOR126	KX914786	F: GTCTGACTAGGGGTGTGCT	TC_9	P	210-225
		R: CCCTGGCTAGTGAAATACTG			

mCtDOR127	KX914787	F: TTGAATGGCTTTTTCTTGAT R: AGGAGGTGGATGACGTTT	CTC_8	P	NA
mCtDOR128	KX914788	F: GCTACGAGCAGTAAGTCGTT R: GCTAATTACGGAAGCAGAAA	GT_{10}	P	220-250
mCtDOR129	KX914789	F: TAGCTTCGAAAAGCTTCCTA R: TCGGTGGGTTTATATTGTTC	AC_9	P	220
mCtDOR130	KX914790	F: ATGTACCCACCAACTAATGC R: AGTTCTGGAGGAGGATTTTC	AAC_6-AGT_7	C	220
mCtDOR131	KX914791	F: ATCGATTGCACAGATTTGAT R: AAACCAACCCATCCACTT	TG_9	P	250
mCtDOR132	KX914792	F: GGTGATGGTGGGTAAAGTAT R: AAACCATAGGGACCAAATCT	GTG_6	P	350
mCtDOR133	KX914793	F: TTCCAAGTACAACTGCATCA R: CTTGGAAAACCTTCCTACCT	GAT_6	P	240
mCtDOR134	KX914794	F: CTCTAAAATTGGGAAGCAAC R: TCGTTAATGGCAAAAAGAGT	TG_6-TC_{11}	C	290
mCtDOR135	KX914795	F: CCTTCCAACTACGTCCATAA R: GACTATTTGCAACAGCAACA	CAG_6	P	320
mCtDOR136	KX914796	F: AAACCAATTTTGCCATTAAA R: TGGTAAGTGTAGTCGGCTTT	CAA_6	P	110-135
mCtDOR137	KX914797	F: GTGTCGACTTCAGGGAACT R: AAAAATCCAATGAAAACGAA	TCG_7	P	160
mCtDOR138	KX914798	F: GAGAGGTGGAATGGTGAGTA R: CACACATGCATAGAAACCAG	GA_9	P	220
mCtDOR139	KX914799	F: TACCAGTCTCCGGCTTTTAT R: GACAGACACAGGCCAATC	GTC_7	P	190
mCtDOR140	KX914800	F: ATGTCGTGGGACAACATTAT R: GAGAGGGAGTTTGAGGAGAT	TCA_7	P	NA
mCtDOR141	KX914801	F: GGACAATAAAGATGGCAAAA R: TTTCTCTCTCCCTCATGCTA	TC_9	P	NA
mCtDOR142	KX914802	F: ACTCTTGTGTTTGTGGAAGG R: GATTGATAGCTTCGGACTTG	CGA_5-GAA_6	C	NA
mCtDOR143	KX914803	F: CAAATATCCAGTCCAACCAT R: ATGGGGTTGTTTACAAGTGA	AC_9	P	200-240
mCtDOR144	KX914804	F: CTTGCATGTTATGTGGATTG R: GTCCCTTCCTCGACTCTTAG	TA_{10}	P	NA
mCtDOR145	KX914805	F: TAACACGAAAAGGGATGTCT R: TTCTTCTTTCTTGAGCTTGG	GAT_6	P	210
mCtDOR146	KX914806	F: CAATCAATCCTCTTCTCCAA R: GGGTTTCGAGAAGTTAAGGT	CA_8-CA_7	C	230
mCtDOR147	KX914807	F: GTCTGACTAGGGGTGTGCT R: CCCTGGCTAGTGAAATACTG	TC_9	P	200
mCtDOR148	KX914808	F: CCTGTCTTAAATCGGTGTTC R: GGATTAAGCCAAAACACAAA	GC_5CA_8	C	NA
mCtDOR149	KX914809	F: TCGTCAATAAGGTCGAGAGT R: GCTAAGATGGTGACGTGTCT	CA_8	P	220
mCtDOR150	KX914810	F: CTGGAATCATCAATCACCTT R: GTTTTTCCTGAAACCAACAA	CAT_6	P	230
mCtDOR151	KX914811	F: TAGCTTCGAAAAGCTTCCTA R: TCGGTGGGTTTATATTGTTC	AC_9	P	220
mCtDOR152	KX914812	F: AAGATGAGGTCAACTCCAAA R: ATTTCCAACAACTGCATACC	TTA_6	P	200-220
mCtDOR153	KX914813	F: CCCTTTTCATCTTCCTTTTT R: TAACTTCGTGAGGAGATCGT	TCT_6	P	230
mCtDOR154	KX914814	F: GAATGGAATGGATGATGTGT R: AGGTGGTGGTGTAAGAACTG	TC_8	P	400
mCtDOR155	KX914815	F: TACTTTCCCTCCATTTCCTT R: AGCTTATAAAGGCGGAAATC	CCA_6	P	350
mCtDOR156	KX914816	F: GATTTCGGATTCGAGTTAAG	GAA_6	P	NA

		R: AATGATACAAGCCCCAAAC			
mCtDOR157	KX914817	F: GAATTCTGATTGGTGGAAAA	TA_{10}	P	220-245
		R: GAAGAAGCATTTGAGACCAG			
mCtDOR158	KX914818	F: GGGAAGAAAGGTTGAAGTTT	TG_9	P	360
		R: CTTCTCTCGATCACGATTTC			
mCtDOR159	KX914819	F: CGCATACAAATCCATTATCA	CT_8	P	NA
		R: TTGCGGTAAGATTAGGGTTA			
mCtDOR160	KX914820	F: GTGAGGAGGTGGCAGAAG	TCA_6	P	NA
		R: AGCCCTGTTTCTCTTCTCTT			
mCtDOR161	KX914821	F: GCTTCATATCATCCCCATTA	CAG_6	P	220-230
		R: ACACCCGATAAAAAGTAGCA			
mCtDOR162	KX914822	F: GCCATAAATTGTCACACAAG	TC_9	P	250
		R: TAAGGGTTTCTTTGGTTTCA			
mCtDOR163	KX914823	F: TTTCTTCTTCCCCTTTTCAT	CA_8-GCA_5	C	230
		R: CTGAGATTCGGAGGTTAATG			
mCtDOR164	KX914824	F: ATGAAACGAACTGATGAAGG	CTG_6	P	200-225
		R: ACCGATGTATGGTCACTAGG			
mCtDOR165	KX914825	F: ATAGCTCCATTCACCATCAC	CAA_6	P	190-215
		R: ATTTGGCTTATTTCCACTGA			
mCtDOR166	KX914826	F: TCCTTTCAAAGCTTCACCTA	TCA_6	P	190
		R: TTTGCCCTAGTTTTATGGAA			
mCtDOR167	KX914827	F: TTGTTGTAGCTGTGCTGTTC	CAT_6	P	380
		R: AATCCATATCCAACCCTTCT			
mCtDOR168	KX914828	F: ACCAAACTCAAAAATGGATG	GCT_6-GAA_5	C	210
		R: AGCCAATTGTGTTTTTCAAC			
mCtDOR169	KX914829	F: TGCATTTGGTCCTTGATTA	TCT_6	P	270
		R: TAAGAGACGGATTTCACGAT			
mCtDOR170	KX914830	F: CGATACCAGTGATCGAAAAT	TCT_6	P	420
		R: AAAGCATCCTGTAGAACGAA			
mCtDOR171	KX914831	F: AATCCCTCTTCTCTCACTCC	TGA_6	P	220-240
		R: CCGTCAAAAGACAGAGAAAC			
mCtDOR172	KX914832	F: TATGCTCCCCTAGTCTTTGA	ATC_6-TTA_5	C	180
		R: TAAATAAACCCCCTCCTCAT			
mCtDOR173	KX914833	F: GTTGGCATTGATCAAGAACT	CAC_6	P	350
		R: TCGTCTCACTCTTCCAACTT			
mCtDOR174	KX914834	F: GAATGCACAATCGGAGTTAT	TC_8	P	190
		R: GCATTTACCTACAAGGGTGT			
mCtDOR175	KX914835	F: CCACACATAACTTCCACCTT	TCC_6	P	200
		R: TCATAGTCCACTGTGCCATA			
mCtDOR176	KX914836	F: ATAAGCTGCAGTGAGAGAGC	AG_6-GA_9	C	NA
		R: GCTAGGCTAGGGTTTCATCT			
mCtDOR177	KX914837	F: TTACAAAGGACTCCCAGAAA	CT_{11}	P	220
		R: CAGAAGGATCGATCAAAGAG			
mCtDOR178	KX914838	F: AGGAAGATACGATACGACCTC	TCT_6	P	220-235
		R: GAATTAATCACCGATGGAAA			
mCtDOR179	KX914839	F: CAACCAAAAGAGGGTTTTT	CA_9	P	240
		R: GGAGTTCTTCGATCTCCTTT			
mCtDOR180	KX914840	F: AACAAACCACCTTCAAAAGA	TC_8	P	195
		R: TCAGAAACCCTAATCAGGAA			
mCtDOR181	KX914841	F: TCCATGCTTTCTTCTCTCTC	TC_8	P	NA
		R: AGCATTCAATTGACGATTTT			
mCtDOR182	KX914842	F: ATCTCCGATCACACACTTTC	TC_{11}	P	220-230
		R: GATGGAGTGAGAGAGAGCTG			
mCtDOR183	KX914843	F: GCGGTTGATCATCCATTA	TA_9	P	350
		R: GAGCAAGTATGGTCAAAAGG			
mCtDOR184	KX914844	F: GCTACGAGCAGTAAGTCGTT	GT_{10}	P	350-370
		R: GCTAATTACGGAAGCAGAAA			
mCtDOR185	KX914845	F: TTTCTTTTCCGTTATCCAAC	TC_9-TCT_6	C	NA
		R: CTTTCCAACTGAAATCTTGC			

mCtDOR186	KX914846	F: TGTTTCTCGTATGAATCTCCCTC R: AGCTCCTGATGATGATTCCG	TA_9	P	290
mCtDOR187	KX914847	F: ATAGTTTAAATAGTTCCATGCACAA R: GAGGAGTGACCGGAGTTTCA	TA_{10}	P	230
mCtDOR188	KX914848	F: AAGGGTCAAAGGCCTTCCT R: CATGGGAGCATTTGGAGATT	GTT_7	P	NA
mCtDOR189	KX914849	F: GTTGGGAAGACAGGGGAAAT R: GGTGAGATCCCTCATGCAAT	CAT_6	P	320
mCtDOR190	KX914850	F: TCACCCACAAGATTTTCTTTGTT R: GTTCGGTTCGGATCTTGAAA	CT_6-AG_9	C	190
mCtDOR191	KX914851	F: GGTCCTGTCCTGGCTGTATG R: CCAGAGCACTGCAAGTGAAA	CT_{11}	P	175
mCtDOR192	KX914852	F: GTGCTCATGTCGAGTTGGGT R: ACATCCCGACCATTCACAAT	GTT_6	P	250
mCtDOR193	KX914853	F: AAGAGGGAGAGGGAGGTCAA R: CCTTGCAAGCTCTTGCTTTT	TAA_7	P	320-345
mCtDOR194	KX914854	F: GCACCATTGTGGAATTAGGG R: CAAACCCCCAAATCTCTGTT	GA_{11}	P	240
mCtDOR195	KX914855	F: CAAACCCAAGGAAAGTCCAA R: TCTCGCCATTGGAAGAAACT	ACG_8	P	NA
mCtDOR196	KX914856	F: GGACGGCCTTTCTTCTTCTT R: TCCAGCAGTCGGAGTTTTCT	TC_6-CT_9	C	290
mCtDOR197	KX914857	F: GGTAAATGTGGAGGTGGTGG R: TCAGATAGCAATGGCAGACG	GTG_8	P	320
mCtDOR198	KX914858	F: CCATCTTCATTTGCATCTTCA R: GCTTTCGCTTGTTGATTCCT	AC_5-TA_8	C	NA
mCtDOR199	KX914859	F: CAGATGAATCGATCAGTGGAAA R: CGTGGAAGCCTCAAGAAGTG	GAA_7	P	260
mCtDOR200	KX914860	F: TGAAGTAAAGAGTAGTCTGTAAAG R: AATTATAAGCTTGCAATTGGTG	GT_{10}	P	NA

NA-Not amplified

Conclusion

It is essential to isolate and characterize more SSR markers in safflower for genetic analysis, linkage and trait mapping and marker assisted selection. So, in this study development of reliable and efficient microsatellites in safflower was reported. The present study also contributed 200 new SSR markers in cultivated safflower. In order to assess the potential and polymorphism, newly developed SSR markers screened in 24 genotypes showed reasonable level of polymorphism.

The SSR markers were detected on an average of 2.7 alleles per locus and average PIC value of 0.33. Finally, the newly developed safflower microsatellite markers are of immense importance as they belong to the few available polymorphic SSR markers for constructing genetic and trait mapping.

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