

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 Asteraceae¹. 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 products⁴. 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 environment⁵. 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 reproducibility¹⁹.

Previously, significant efforts have been made to develop SSR markers from expressed sequence tag (EST) and genome sequences in safflower²⁰⁻²⁵, 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 modifications³⁰. 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 library³¹. Genomic DNA (5 µg) was digested by blunt end-generating restriction endonucleases *RsaI* and *XmnI* [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 (at 25 °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.gov/blast>). MISA and primer3 were used for identification of SSR motifs and designation of primer pairs flanking SSR regions³².

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 procedure³³ 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 3.25³⁴. The SSR allelic data were used for computing the inter-individual genetic dissimilarity based on simple matching coefficient by using DARwin 6.0.018³⁵. 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 al²² 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 Paspalum³⁹⁻⁴¹. 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 al²² 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 Weber⁵⁰, 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 safflower²¹⁻²⁴ 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 analysis⁵¹. 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 al²⁰ and Mayerhofer et al²¹ reported higher tri-nucleotides repeat followed by di- and tetra-nucleotide-repeats through EST data mining.

However, Yamini et al²³ 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 castor⁴⁹, rice⁶³, wheat⁶⁴ and maize⁶⁵. Among the different types of SSRs, di- nucleotide appears to be common characteristic of plant genomes on a database search⁶⁶.

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 structure⁵⁴. 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 al⁷⁰ 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 rice⁶³, maize⁶⁵, wheat^{64,76}, groundnut⁴², barley⁷⁷, coffee⁷⁸, castor⁴⁹ and sugarcane⁵⁴. 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 al²⁰ (56.3%), Lee et al²⁴ (59.3%) and Yamini et al²³ (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 al²⁴

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

In contrast, Chapman et al²⁰ 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 al²² 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 castor⁴⁹, 12.5% in sugarcane⁵⁴, 20% in bitter melon⁵¹, 15% grape vine⁷⁹, 14% sorghum⁸⁰, 23% wheat^{64,81} and higher in 44.2% in groundnut⁴², 44% in pomegranate⁴⁴, 43% in *Brassica* species⁸². 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 polymorphism⁸⁴. 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 spiny high oil high oleic acid
EC-755664 (CW-99)	Mexico	Variety spiny high oil high oleic acid
EC-755673-1	Mexico [Selection from Mexican variety Humaya-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 spiny high 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 al²² ($N_A=3.2$, $PIC=0.55$), Barati and Arzani²⁸ ($N_A=3.43$, $PIC=0.32$), Lee et al²⁴ ($N_A=2.8$, $PIC=0.325$), Derakhshan et al²⁹ ($N_A=3.8$, $PIC=0.30$) and Usha Kiran et al²⁷ ($N_A=3.6$, $PIC=0.28$).

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 7 genotypes; 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.

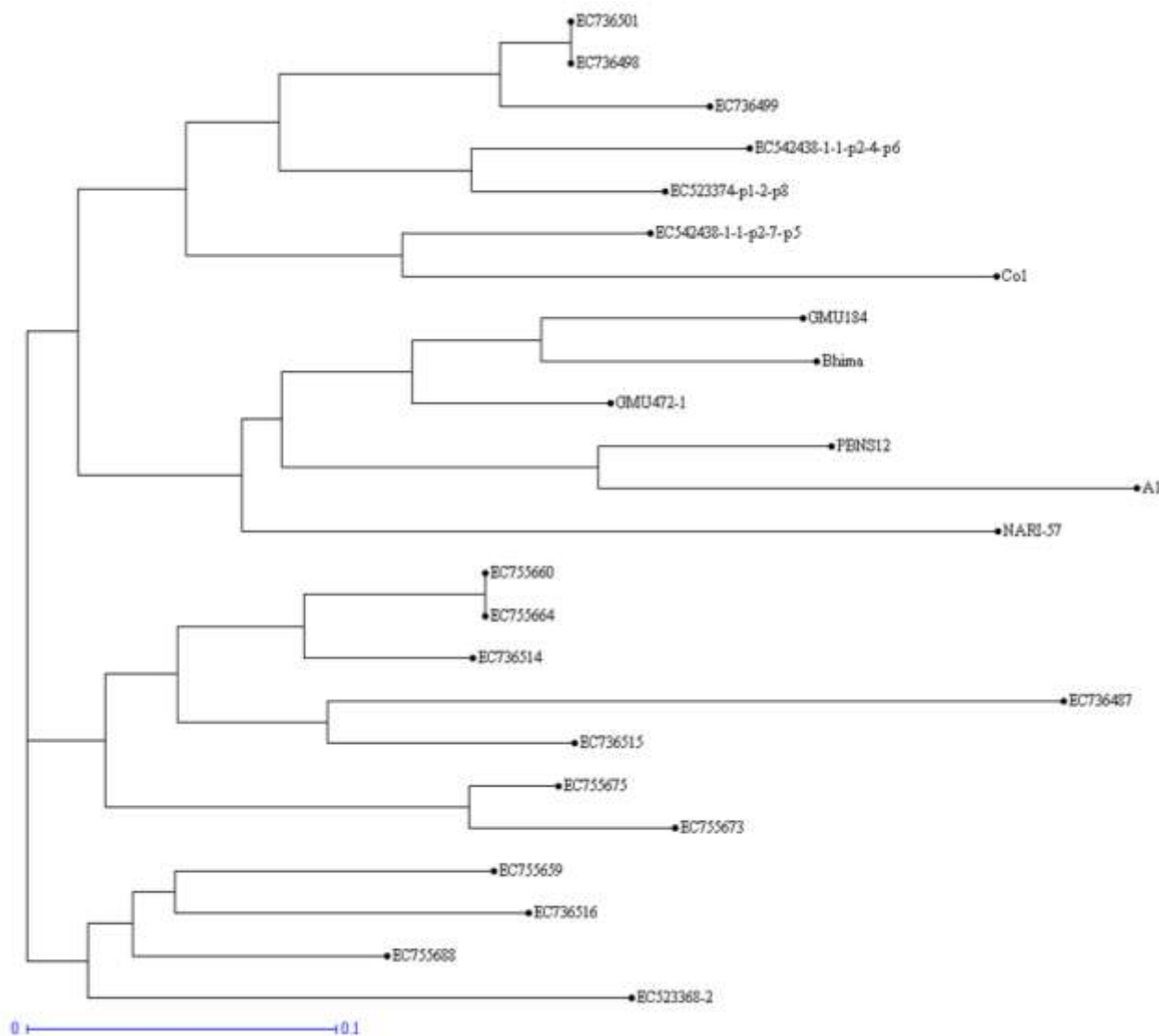


Figure 1: Dendrogram showing the genetic relationships among 24 safflower genotypes based on similarity coefficients derived from SSR polymorphism data

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: TCCCAACCCTCTCACTTTT R: GTGTCCACACACCGTCAAG	TTAA ₃ -CA ₁₃	C	110-125	2	070	000	042	033
mCtDOR4	KJ586133	F: GGTCCCAGAAGCAGTAGTGA R: CCCCAGATAGCAACTACAGGT	AC ₉	P	220-230	2	096	000	008	008
mCtDOR8	KJ586138	F: ATCTGAAGAGAGTCTCCGCGC R: CCATTCGACTATATCCGCTT	AT ₉	P	210-230	3	087	000	023	022
mCtDOR20	KJ586151	F: GATGTAACAAGGTGCAGGGA R: GATCCAACGCCATTTTCTCT	GA ₁₇ -GTGA ₅	C	250-285	4	061	004	056	050
mCtDOR23	KJ586155	F: TGGCCTATGTAGTTTTCTCG R: GACTCAAAGGGTTCGACGAT	TAA ₆	P	230-240	2	091	000	017	015
mCtDOR30	KJ586163	F: TGAGAGGGTAGATGCACTGG R: CTCAGACTGGTTGTTGGTGG	CA ₃₂	P	180-195	2	067	004	044	034
mCtDOR32	KJ586165	F: ATGTGGGAGGAATCAAGGAG R: CCATCTTCTCACCATGAAAACC	GAA ₈ -GT ₉	C	125-140	3	091	000	016	016
mCtDOR33	KJ586166	F: CGTTATGGCGGAGATAAAT R: TCTAACCACGTTTTCCCAACA	GATT ₉	P	190-200	2	071	000	041	033
mCtDOR37	KJ586171	F: AGGGATTCAAGTAATAAATC R: GGCAAGGGTTTACGCCAAAT	TC ₁₀	P	280-300	2	096	000	008	008
mCtDOR38	KJ586172	F: GGACCTTCAAATATCACGCC R: GTATTCCACCGATTCTCTCG	TG ₁₃	P	185-200	4	044	004	067	061
mCtDOR40	KJ586174	F: GGAGCAATAAGCAGGAGGAG R: CGAGATTGATAACGCCTTGA	TC ₅ -TC ₇	I	350-360	2	092	000	015	014
mCtDOR41	KJ586175	F: TGAGGACAATTGTGTGCGTA R: ATAGGACAAAACCAACCCCA	AG ₈ -AG ₁₇	C	235-260	3	044	004	062	054
mCtDOR42	KJ586176	F: GATGCCCTAAAGTGGTCCAT R: AACAGATGCAAGTTTGGCAG	AG ₉	P	120-150	4	075	000	041	039
mCtDOR43	KJ586177	F: CGCCACTCCTCTTCCTCCGA R: GAGTAATCTATACCTACACTAC	TC ₁₀	P	190-210	3	039	000	066	059
mCtDOR45	KJ586179	F: TTTTGCTCATGAACAGCCTC R: AGGTATCGATCTTGTGTC	TG ₁₀	P	285-290	2	075	000	038	030
mCtDOR52	KJ586186	F: CACACAAACCACATGAAGCA R: ACATTGAAAGATGTGAGGCG	CA ₈	P	220-245	4	071	000	047	043
mCtDOR53	KJ586187	F: GAGTTGTAATAAGGGATTCAAG R: GGCAAGGGTTTACGCCAAAT	TC ₇	P	280-300	2	096	000	008	008
mCtDOR54	KJ586188	F: AAAAGGGTAAACGGAAGGTT R: AAAAGCACCCTAAGGTCGTG	AC ₁₁	P	270-280	3	055	000	060	053
mCtDOR56	KJ586190	F: ACTCGCTTTCTCTCATGT R: TATTCATACCGCTTTTCCCC	GA ₉ -GA ₁₀ -AG ₆	C	260-280	4	077	004	038	036
mCtDOR57	KJ586191	F: AGCTCCATGAAGAAAGGCAT R: CTCACAACCCAAAGTGGATG	GA ₉	P	245-250	2	083	000	028	024
mCtDOR64	KJ586198	F: ACAAGTTCGATACACACCCG R: GAGGGCGTTAACTCGACG	AG ₅ -AG ₆ -AG ₉	I	165-170	2	070	000	044	035
mCtDOR74	KJ586209	F: AACTGCTTCTTACGTTCTCTG R: ACGAAATGCTTGGAGAACAG	TC ₈ -AC ₁₃	C	220-240	2	067	000	044	035
mCtDOR75	KJ586212	F: TGTGCCTAAAGTTGTCAAAGAC	CA ₅ -CT ₁₁	C	180-210	3	073	000	042	036

		R: GCAACTGGTGTGCTTTTAGAA									
mCtDOR10	KX914760	F: AAAATGAGAGCAAGGATGAA R: GCGTTGTTACCTTTACAAT	TAA ₆	P	300-320	3	050	002	046	052	
mCtDOR10	KX914761	F: CAAATATCCAGTCCAACCAT R: ATGGGGTTGTTTACAAGTGA	AC ₉	P	290-305	4	065	010	038	040	
mCtDOR11	KX914773	F: CTACCCATATGCACCTAAGC R: ATGATCAACAACCTCACCAT	AAC ₆ -GCG ₅	C	120-145	2	085	004	020	024	
mCtDOR11	KX914774	F: CCATCATCTTCACCATCTTT R: AATTTCTCAAACCCATCTCC	TGA ₆	P	134-150	2	063	000	050	042	
mCtDOR12	KX914786	F: GTCTGACTAGGGGTGTGCT R: CCCTGGCTAGTGAAATACTG	TC ₉	P	210-225	3	047	000	052	053	
mCtDOR12	KX914788	F: GCTACGAGCAGTAAGTCGTT R: GCTAATTACGGAAGCAGAAA	GT ₁₀	P	220-250	3	053	001	047	052	
mCtDOR13	KX914796	F: AAACCAATTTTGCCATTTAA R: TGGTAAGTGTAGTCGGCTTT	CAA ₆	P	110-135	2	091	000	010	009	
mCtDOR14	KX914803	F: CAAATATCCAGTCCAACCAT R: ATGGGGTTGTTTACAAGTGA	AC ₉	P	200-240	2	049	004	052	037	
mCtDOR14	KX914809	F: TCGTCAATAAGGTTCGAGAGT R: GCTAAGATGGTGACGTGTCT	CA ₈	P	220-235	2	070	000	042	033	
mCtDOR15	KX914812	F: AAGATGAGGTCAACTCCAAA R: ATTTCCAACAACCTGCATACC	TTA ₆	P	200-220	3	064	010	037	060	
mCtDOR15	KX914817	F: GAATTCTGATTGGTGGAAAA R: GAAGAAGCATTGAGACCAG	TA ₁₀	P	220-245	3	043	000	046	055	
mCtDOR16	KX914821	F: GCTTCATATCATCCCCATTA R: ACACCCGATAAAAAGTAGCA	CAG ₆	P	220-230	2	024	000	036	021	
mCtDOR16	KX914824	F: ATGAAACGAACTGATGAAGG R: ACCGATGTATGGTCACTAGG	CTG ₆	P	200-225	2	036	005	045	033	
mCtDOR16	KX914825	F: ATAGCTCCATTCACCATCAC R: ATTTGGCTTATTTCCACTGA	CAA ₆	P	190-215	4	029	000	035	027	
mCtDOR17	KX914831	F: AATCCCTCTTCTCTCACTCC R: CCGTCAAAAAGACAGAGAAAC	TGA ₆	P	220-240	2	0013	002	027	0013	
mCtDOR17	KX914838	F: AGGAAGATACGATACGACCTC R: GAATTAATCACCGATGGAAA	TCT ₆	P	220-235	2	04	004	035	046	
mCtDOR18	KX914842	F: ATCTCCGATCACACACTTTC R: GATGGAGTGAGAGAGAGCTG	TC ₁₁	P	220-230	3	022	002	036	021	
mCtDOR18	KX914844	F: GCTACGAGCAGTAAGTCGTT R: GCTAATTACGGAAGCAGAAA	GT ₁₀	P	350-370	3	085	000	020	024	
mCtDOR19	KX914853	F: AAGAGGGAGAGGGAGGTCAA R: CCTTGCAAGCTCTTGCTTTT	TAA ₇	P	320-345	2	036	000	040	037	

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: GTCCCGAAAACCTAGGACCAA R: ACCCTTCGCTCAATGAGAA	TC ₆	P	400
mCtDOR2	KJ586130	F: CAGCAGCAGCATCTTCAAA R: CGACAATCGGGTTATCAGTG	AG ₅	P	NA
mCtDOR3	KJ586131	F: TCCAACCCTCTCACTTTTT R: GTGTCCACACACCGTCAAG	TTAA ₃ -CA ₁₃	C	110-125
mCtDOR4	KJ586133	F: GGTCCAGAAAGCAGTAGTGA R: CCCCAGATAGCAACTACAGGT	AC ₉	P	220-230
mCtDOR5	KJ586134	F: GTCTGTTGCAAGAAGAGTTGTG R: TGCTGGAATTGGTTCTGTCT	AGA ₈	P	180
mCtDOR6	KJ586135	F: CTAGCAGAATCCCTTCCCTG R: TGCTCTGTCTGCTCACTTT	GAA ₁₃	P	190
mCtDOR7	KJ586137	F: CCAAGTCCAACATGCACAA R: GTTATATCTTGTAGATAGGCAG	CA ₇	P	180
mCtDOR8	KJ586138	F: ATCTGAAGAGAGTCTCCGGC R: CCATTCGACTATATCCGCTT	AT ₉	P	210-230
mCtDOR9	KJ586139	F: GTTTTTCCCCTAAACCTCCC R: TGAAAGTGATCAAGGGTCCA	CA ₇	P	130

mCtDOR10	KJ586140	F: CCTTTTTCAAATCCTGCTGC R: ACCGAGATCAATGCAGTCAA	GAA ₈	P	140
mCtDOR11	KJ586141	F: GACTGTTATTCAAAAGGTCC R: GCCTCCCGGAATTTGATTGA	AT ₆	P	260
mCtDOR12	KJ586142	F: TCTCTCTCTCTTGTATTCCCA 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: AGGCCTAAGCTTGCAAGATC	TG ₆ -TG ₆	C	320
mCtDOR19	KJ586150	F: ATGAGGTTGTCGTTCCGGAT 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: TAGCGGAATGTTCAAAAGC R: CTATGGGCAACCCAGATACC	TG ₈	P	200
mCtDOR26	KJ586158	F: TCTTGCTATCTGTTCCGGC R: CCCTAGATCCAAAACCGAAA	AT ₈	P	190
mCtDOR27	KJ586160	F: TAGAACCCTCTCAGCCCTTC R: AGCCCATGTGTTGTGTGTGT	TTAA ₃ -CA ₁₀	C	265
mCtDOR28	KJ586161	F: AGGGAAGGAATCCTAGGCC 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: AAGAGAGATCGCCGAGTAA R: AGTTACCTTCCGAGCACGTT	AG ₆	P	110
mCtDOR32	KJ586165	F: ATGTGGGAGGAATCAAGGAG R: CCATCTTCTCACCATGAAAACC	GAA ₈ -GT ₉	C	125-140
mCtDOR33	KJ586166	F: CGTTATGGCGGCAGATAAAT R: TCTAACCACGTTTTCCACA	GATT ₉	P	190-200
mCtDOR34	KJ586168	F: TTATCATTTTCAAGGCGTGTG 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: GGCAAGGGTTTACGCCAAT	TC ₁₀	P	280-300
mCtDOR38	KJ586172	F: GGACCTTCAAATATCACGCC R: GTATTCCACCGATTCTTCCG	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: AGGGTATCGATCTTGTGGCC	TG ₁₀	P	285-290
mCtDOR46	KJ586180	F: TAAGCCATGGGCTTTTTACC R: CTTTCCCAAACACCCAAAGA	ATC ₄	P	290
mCtDOR47	KJ586181	F: GATAGAGAATTAAGTGGGCTCCC 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: AAAAGCACCCCTAAGGTCGTG	AC ₁₁	P	270-280
mCtDOR55	KJ586189	F: CGTCTTCGATCTTGCATATA R: AGGAGGATATGAAGCACTGC	TATT ₃	P	220
mCtDOR56	KJ586190	F: ACTCGCTTTCTCTCATGT 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: CCTTGATTAAGTCCAAGCG	AT ₆	P	NA
mCtDOR59	KJ586193	F: GTTCATGCTGTTATGAATAGG R: GTCGATCCGCCCCCAGGAT	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: CACCTGAAAAACGTCAATGC 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: CCCATGGCTCTCTCTCATA	GT ₅	P	260
mCtDOR73	KJ586207	F: TTGCTTAGTAACGACGCCAC R: CAATGTATGTGACGGTGCAA	AC ₆ -AC ₅	I	130
mCtDOR74	KJ586209	F: AACTGCTTCTTACGTTCTCTG 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: CGCCAGGATAAAGTTCAAAT	TA ₆	P	100
mCtDOR79	KJ586217	F: CCACAAGATAGAAGCACCCA R: TTTCGGTTTCGAGTCAAGTG	TA ₈	P	110
mCtDOR80	KJ586219	F: GCTTCACTCTAAGGCGGAAC R: CAAATCGAGGCAAACCTCTGA	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: GAAACCTCATTACGCCACAA R: GCCCAAACATAATGAAGCCAT	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: GTCGTAGTCTTTTGCCCGT	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: CCCATCTTCTTCTTCTTTT	GT ₉	P	NA
mCtDOR95	KX914755	F: CACCGATTTGTGAGTAAAAA R: AAGCATTTTCATCAAACAGGT	GT ₉	P	NA
mCtDOR96	KX914756	F: TTCCTCTGCTTCACTCTCAC R: ACAGCAATCAAAGATCCAAC	GAA ₇	P	180
mCtDOR97	KX914757	F: CACACGTCCTCTTTCTTTC	TA ₈ -AC ₁₁	C	270

mCtDOR98	KX914758	R: AATTCAGGTTTCGAGGTTGTA F: CCATAGGGACCAAAAACATA R: TTGAATGTGGAGAAGAAAGC	CT ₉	P	NA
mCtDOR99	KX914759	F: ATGGAGGATTGTGGAAGACG R: CAAGATCCACCTCGAACACC	TG ₈ -AG ₉	C	240
mCtDOR100	KX914760	F: AAAATGAGAGCAAGGATGAA R: GCGTTGTTACCTTTCACAAT	TAA ₆	P	300-320
mCtDOR101	KX914761	F: CAAATATCCAGTCCAACCAT R: ATGGGGTTGTTTACAAGTGA	AC ₉	P	290-305
mCtDOR102	KX914762	F: ATGCTCTTCTTCACCATCAT R: AGTTTGGATATTGGGGATTT	AAG ₈	P	250
mCtDOR103	KX914763	F: AAAACACGATCATCATCTCC R: GGTGTCAAGAGGGTACAAGA	CTT ₇	P	120
mCtDOR104	KX914764	F: GAAACCACCACCATAACCTA R: GTCCTGTTTGTGAACCACT	ACA ₆	P	260
mCtDOR105	KX914765	F: GAATCCCACAAAAATCTTGA R: ATATCGTTTTCTGATGTGG	ACC ₆ CCA ₆ - CCA ₇	C	NA
mCtDOR106	KX914766	F: TCTTCTTCGTAATCCTCGTC R: AAGACGAAGGGTTAAATGGT	GA ₉	P	NA
mCtDOR107	KX914767	F: ACGAAGACTTTTGGTGTGT R: ATCAGAAGGTGATGAAGGTG	TCA ₇	P	280
mCtDOR108	KX914768	F: CTTGCATGTTATGTGGATTG R: GTCCCTTCCTCGACTCTTAG	TA ₁₀	P	220
mCtDOR109	KX914769	F: TGTTTTCAAAATTTTCGGATT R: TTTACTCTTGTTATGGGTCC	AC ₉	P	NA
mCtDOR110	KX914770	F: CAGCAGACAATTGAAGTTGA R: ATAAGAACCAAACCACAAA	TA ₁₁	P	230
mCtDOR111	KX914771	F: TCCTTCTCCTCCTACTTCC R: ATCAGGGTCTAGCTCTTCCT	CTC ₇	P	290
mCtDOR112	KX914772	F: TTACAAAGGACTCCCAGAAA R: CAGAAGGATCGATCAAAGAG	CT ₁₁	P	300
mCtDOR113	KX914773	F: CTACCCATATGCACCTAAGC R: ATGATCAACAACCTCACCAT	AAC ₆ -GCG ₅	C	120-145
mCtDOR114	KX914774	F: CCATCATCTTCACCATCTTT R: AATTTCTCAAACCCATCTCC	TGA ₆	P	134-150
mCtDOR115	KX914775	F: CTATCCCTACACCCACTAA R: AAACCTCCTAAGGGGGAAT	GA ₈ AC ₉	C	210
mCtDOR116	KX914776	F: GCAGTCTTCTTGTCGGTAAA R: CTTGCGTTGTTTCAGTTGATT	TG ₉	P	220
mCtDOR117	KX914777	F: TGATAAAAGGAAGGTTTCGT R: AGAAACAAAGCTGTTTGACA	AAT ₇	P	240
mCtDOR118	KX914778	F: GTTGGTTTTGGAGTTGTGTT R: TTCGGTCTGAATAATCCTGT	TG ₈	P	140
mCtDOR119	KX914779	F: GTTGTGCTTGAACCTTGGTT R: ATCCACTCATCCCTTACCT	TG ₉	P	195
mCtDOR120	KX914780	F: GGTGGTGAATTTCAATTGTT R: AAGGAAGCTTGTTGAGATGA	CT ₁₀	P	NA
mCtDOR121	KX914781	F: TTTACTGTTGGGCTAGCATC R: CCAGATTTTCAGGTATGTGGT	TCA ₇	P	310
mCtDOR122	KX914782	F: GAAATTCATGAGGTGGAAAA R: ATCGATGAAGATGATTGAGG	TGA ₆	P	320
mCtDOR123	KX914783	F: TGGTCTTAGAGATTGAAGCTG R: ACGATAAATTAGCACTGTTGC	GT ₉ -AAG ₇	C	280
mCtDOR124	KX914784	F: GCTTCCAGTGCTCCTAGAAT R: TCTTGCAAGTTGGTAGGATT	GT ₉	P	220
mCtDOR125	KX914785	F: CATAAAGCGACTCAAACAA R: GAATGCATGGAAGCTCTATC	GGA ₅	P	NA
mCtDOR126	KX914786	F: GTCTGACTAGGGGTGTGCT R: CCCTGGCTAGTGAAATACTG	TC ₉	P	210-225

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: TCGGTGGGTTTATATTGTTT	AC ₉	P	220
mCtDOR130	KX914790	F: ATGTACCCACCAACTAATGC R: AGTCTGGAGGAGGATTTTC	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: TTCCAAGTACAACCTGCATCA R: CTTGGAAAACCTTCCTACCT	GAT ₆	P	240
mCtDOR134	KX914794	F: CTCTAAAATTGGGAAGCAAC R: TCGTTAATGGCAAAAAGAGT	TG ₆ -TC ₁₁	C	290
mCtDOR135	KX914795	F: CCTTCCAACACTAGTCCATAA R: GACTATTTGCAACAGCAACA	CAG ₆	P	320
mCtDOR136	KX914796	F: AAACCAATTTTGCCATTAAA R: TGGTAAGTGTAGTCGGCTTT	CAA ₆	P	110-135
mCtDOR137	KX914797	F: GTGTCGACTTCAGGGAAC R: AAAAATCCAATGAAAACGAA	TCG ₇	P	160
mCtDOR138	KX914798	F: GAGAGGTGGAATGGTGAAGTA 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: CCTGTCTTAAATCGGTGTTT R: GGATTAAGCCAAAACACAAA	GC ₅ CA ₈	C	NA
mCtDOR149	KX914809	F: TCGTCAATAAGGTTCGAGAGT R: GCTAAGATGGTACGTGTCT	CA ₈	P	220
mCtDOR150	KX914810	F: CTGGAATCATCAATCACCTT R: GTTTTTCCTGAAACCAACAA	CAT ₆	P	230
mCtDOR151	KX914811	F: TAGCTTCGAAAAGCTTCCTA R: TCGGTGGGTTTATATTGTTT	AC ₉	P	220
mCtDOR152	KX914812	F: AAGATGAGGTCAACTCCAAA R: ATTTCCAACAACCTGCATACC	TTA ₆	P	200-220
mCtDOR153	KX914813	F: CCCTTTTCATCTTCTTTT R: TAACTTCGTGAGGAGATCGT	TCT ₆	P	230
mCtDOR154	KX914814	F: GAATGGAATGGATGATGTGT R: AGGTGGTGGTGTAAAGAACTG	TC ₈	P	400
mCtDOR155	KX914815	F: TACTTTCCTCCATTTCCTT R: AGCTTATAAAGGCGGAAATC	CCA ₆	P	350
mCtDOR156	KX914816	F: GATTCGGATTTCGAGTTAAG	GAA ₆	P	NA

mCtDOR157	KX914817	R: AATGATACAAGCCCCAAAC F: GAATTCTGATTGGTGGAAAA R: GAAGAAGCATTGAGACCAG	TA ₁₀	P	220-245
mCtDOR158	KX914818	F: GGAAGAAAGGTTGAAGTTT R: CTTCTCTCGATCACGATTC	TG ₉	P	360
mCtDOR159	KX914819	F: CGCATACAAATCCATTATCA R: TTGCGGTAAGATTAGGGTTA	CT ₈	P	NA
mCtDOR160	KX914820	F: GTGAGGAGGTGGCAGAAG R: AGCCCTGTTTCTTCTCTT	TCA ₆	P	NA
mCtDOR161	KX914821	F: GCTTCATATCATCCCCATTA R: ACACCCGATAAAAAGTAGCA	CAG ₆	P	220-230
mCtDOR162	KX914822	F: GCCATAAATTGTCACACAAG R: TAAGGGTTTCTTTGGTTTCA	TC ₉	P	250
mCtDOR163	KX914823	F: TTTCTTCTCCCTTTTCAT R: CTGAGATTCGGAGGTTAATG	CA ₈ -GCA ₅	C	230
mCtDOR164	KX914824	F: ATGAAACGAACTGATGAAGG R: ACCGATGTATGGTCACTAGG	CTG ₆	P	200-225
mCtDOR165	KX914825	F: ATAGCTCCATTACCATCAC R: ATTTGGCTTATTTCCACTGA	CAA ₆	P	190-215
mCtDOR166	KX914826	F: TCCTTTCAAAGCTTCACCTA R: TTTGCCCTAGTTTTATGGAA	TCA ₆	P	190
mCtDOR167	KX914827	F: TTGTTGTAGCTGTGCTGTTC R: AATCCATATCCAACCCTTCT	CAT ₆	P	380
mCtDOR168	KX914828	F: ACCAAACTCAAAAATGGATG R: AGCCAATTGTGTTTTTCAAC	GCT ₆ -GAA ₅	C	210
mCtDOR169	KX914829	F: TGCATTTGGTCTTGATTA R: TAAGAGACGGATTCACGAT	TCT ₆	P	270
mCtDOR170	KX914830	F: CGATACCAGTGATCGAAAAT R: AAAGCATCCTGTAGAACGAA	TCT ₆	P	420
mCtDOR171	KX914831	F: AATCCCTCTTCTCTCACTCC R: CCGTCAAAAGACAGAGAAAC	TGA ₆	P	220-240
mCtDOR172	KX914832	F: TATGCTCCCCTAGTCTTTGA R: TAAATAAACCCCTCCTCAT	ATC ₆ -TTA ₅	C	180
mCtDOR173	KX914833	F: GTTGGCATTGATCAAGAACT R: TCGTCTCACTCTTCCAACCTT	CAC ₆	P	350
mCtDOR174	KX914834	F: GAATGCACAATCGGAGTTAT R: GCATTTACCTACAAGGGTGT	TC ₈	P	190
mCtDOR175	KX914835	F: CCACACATAACTTCCACCTT R: TCATAGTCCACTGTGCCATA	TCC ₆	P	200
mCtDOR176	KX914836	F: ATAAGCTGCAGTGAGAGAGC R: GCTAGGCTAGGGTTTCATCT	AG ₆ -GA ₉	C	NA
mCtDOR177	KX914837	F: TTACAAAGGACTCCCAGAAA R: CAGAAGGATCGATCAAAGAG	CT ₁₁	P	220
mCtDOR178	KX914838	F: AGGAAGATACGATACGACCTC R: GAATTAATCACCGATGGAAA	TCT ₆	P	220-235
mCtDOR179	KX914839	F: CAACCAAAGAGGGTTTTT R: GGAGTTCTTCGATCTCCTTT	CA ₉	P	240
mCtDOR180	KX914840	F: AACAAACCACCTTCAAAGA R: TCAGAAACCCTAATCAGGAA	TC ₈	P	195
mCtDOR181	KX914841	F: TCCATGCTTCTTCTCTCTC R: AGCATTCAATTGACGATTTT	TC ₈	P	NA
mCtDOR182	KX914842	F: ATCTCCGATCACACACTTTC R: GATGGAGTGAGAGAGAGCTG	TC ₁₁	P	220-230
mCtDOR183	KX914843	F: GCGGTTGATCATCCATTA R: GAGCAAGTATGGTCAAAGG	TA ₉	P	350
mCtDOR184	KX914844	F: GCTACGAGCAGTAAGTCGTT R: GCTAATTACGGAAGCAGAAA	GT ₁₀	P	350-370
mCtDOR185	KX914845	F: TTTCTTTTCCGTTATCCAAC R: CTTTCCAACCTGAAATCTTGC	TC ₉ -TCT ₆	C	NA

mCtDOR186	KX914846	F: TGTTTCTCGTATGAATCTCCCTC R: AGTCCTGATGATGATTCCG	TA ₉	P	290
mCtDOR187	KX914847	F: ATAGTTTAAATAGTTCATGCACAA R: GAGGAGTGACCGGAGTTTCA	TA ₁₀	P	230
mCtDOR188	KX914848	F: AAGGGTCAAAGGCCTTCCT R: CATGGGAGCATTGGAGATT	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: GGTCTGTCTGGCTGTATG R: CCAGAGCACTGCAAGTGAAA	CT ₁₁	P	175
mCtDOR192	KX914852	F: GTGCTCATGTCGAGTTGGGT R: ACATCCCGACCATTCAAAAT	GTT ₆	P	250
mCtDOR193	KX914853	F: AAGAGGGAGAGGGAGGTCAA R: CCTTGCAAGCTCTTGCTTTT	TAA ₇	P	320-345
mCtDOR194	KX914854	F: GCACCATTGTGGAATTAGGG R: CAAACCCCAAAATCTCTGTT	GA ₁₁	P	240
mCtDOR195	KX914855	F: CAAACCCAAGGAAAGTCCAA R: TCTCGCCATTGGAAGAACT	ACG ₈	P	NA
mCtDOR196	KX914856	F: GGACGGCCTTTCTTCTTCTT R: TCCAGCAGTCGGAGTTTCT	TC ₆ -CT ₉	C	290
mCtDOR197	KX914857	F: GGTAATGTGGAGGTGGTGG R: TCAGATAGCAATGGCAGACG	GTG ₈	P	320
mCtDOR198	KX914858	F: CCATCTTCATTTGCATCTTCA R: GCTTTCGCTTGTGATTCTT	AC ₅ -TA ₈	C	NA
mCtDOR199	KX914859	F: CAGATGAATCGATCAGTGGAAA R: CGTGGAAAGCCTCAAGAAGTG	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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