Euphytica (2005) 146: 67–76
DOI: 10.1007/s10681-005-8496-2
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Genotyping safflower (*Carthamus tinctorius*) cultivars by DNA fingerprints

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Received 12 December 2004; accepted 7 June 2005

Key words: AFLP, diagnostic markers, fingerprinting, ISSR, marker informativeness, RAPD, safflower cultivars

Summary

Carthamus tinctorius ($2n = 2x = 24$) (family Asteraceae), commonly known as safflower, is widely cultivated in agricultural production systems of Asia, Europe, Australia and the Americas as a source of high-quality vegetable and industrial oil. India ranks first in the production of safflower oil. Fourteen cultivars, widely cultivated in various agro-climatic regions of India, have been fingerprinted by RAPD, ISSR, and AFLP markers utilizing 36, 21 primers, and 4 primer combinations, respectively. On an individual assay basis, AFLP has proven to be the best marker system as compared with the other two markers applied as assessed by high discriminating power (0.98), assay efficiency index (33.2), marker index (18.2), resolving power (40.62), and genotype index (0.856). Thirty-six RAPD and 21 SSR primers could differentiate a maximum of eight and four cultivars, respectively, whereas, two AFLP primer combinations could fingerprint all the 14 cultivars. To understand genetic relationships among these cultivars, Jaccard's similarity coefficient and UPGMA clustering algorithm were applied to the three marker data sets. Mean genetic similarities ranged from 0.689 (AFLP) to 0.952 (ISSR). Correlation coefficient comparisons between similarity matrices and co-phenetic matrices obtained with the three markers revealed that AFLP displayed no congruence vis-a-vis RAPD and ISSR data. However, strong correlation was observed between RAPD and ISSR marker systems. This paper reports the start of molecular biology programme targeting nuclear genome of safflower, a major world oilseed crop about whose genetics very little is known.

Introduction

Carthamus tinctorius ($2n = 2x = 24$), commonly known as safflower, is a member of the tribe Cynareae, subfamily Tubulifloreae, and family Asteraceae. The eastern part of the Mediterranean region is regarded as the centre of origin of the genus (Ashri & Knowles, 1960). Historically, safflower was grown in Egypt and Euphrates exclusively as a source of red dye 'carthamin' extracted from its florets. Around mid last century, its cultivation was extended to Asia, Europe, Australia, and the Americas due to its recognition as a source of good quality oil valued for edible and industrial purposes. Safflower edible oil cultivars have the highest quantity of polyunsaturated fatty acids vis-a-vis other established oilcrops (Ashri, 1973; Knowles, 1955; Weiss, 1971). It is also used as a feed for livestock. Inspite of safflower being one of the major oilseed crops in the world, it has received very little attention from geneticists, cytogeneticists and molecular biologists alike (Kumar, 1991).

India ranks first in the production of safflower oil (50% of total world production) with ~0.4 million hectares under cultivation. In the past few decades, many promising cultivars have been released by the All India Coordinated Research Projects on Oilseeds (AICORPO). These cultivars cover ~90% of the total acreage of cultivation in various agro-climatic regions of India.

The germplasm resources of safflower have so far been characterized entirely on the basis of morphological traits, agronomic characters, biotic and (or) abiotic stress and (or) biochemical characters (Ashri, 1971a,b, 1974, 1975; Aslam & Hazara, 1993;

Fernandez-Martinez et al., 1993; Futehally, 1982; Han & Li, 1992). These approaches have, however, not been able to accomplish the desired goals. The precise cataloguing of germplasm resources including cultivars by molecular DNA markers has lately gained lot of attention (Aggarwal et al., 2002; Araki et al., 1998; Blair et al., 1999; Fang & Roose, 1997; Guena et al., 2003; Lombard et al., 2000) for many reasons including protection of released cultivars from pilferage, protection of Intellectual Property Rights (IPR) and for molecular breeding. Before the advent of DNA fingerprinting by various molecular DNA markers, the precise cataloguing of the cultivars was an impossible task. In the present study, we assayed various regions of the genome of released safflower cultivars by RAPD, ISSR, and AFLP markers with a view to not only fingerprint the cultivars and assess their relative diversity but also to identify the primer(s) most suitable for fingerprinting safflower cultivars as well.

Materials and methods

Plant material

The details of the 14 cultivars investigated are given in Table 1. The seed samples were obtained from AICORPO, Solapur, and Nimbkar Agricultural Research Institute (NARI), Phaltan.

Ten seeds of each cultivar were sown in earthen pots. For DNA extraction, young leaves were pooled together from ten individual plants of each cultivar. Total genomic DNA was extracted by modified CTAB method (Sue Porebski et al., 1997).

RAPD fingerprinting

Thirty-six randomly selected decamers from different series (A, C, H, K, and I) (Operon Technologies, USA) were used for PCR reaction. The reaction mixture of 25 μl volume contained 2.5 μl 10× assay buffer (Bangalore Genei, India), 0.24 mM dNTPs (Amersham Pharmacia Biotech, USA), 15 ng primer, 0.5 U *Taq* DNA polymerase (Bangalore Genei), 20 ng template DNA, and 1.5 mM $MgCl_2$ (Promega, USA). DNA amplification was performed in a Perkin Elmer Cetus 480 DNA thermal cycler programmed to 1 cycle of 4 min 30 s at 94 °C (denaturation), 1 min at 34 °C (annealing), and 2 min at 72 °C (extension); followed by 44 cycles of 1 min at 94 °C, 1 min at 34 °C, and 2 min at 72 °C ending with 1 cycle of 15 min at 72 °C (final extension).

ISSR fingerprinting

Twenty-one randomly selected SSR primers (University of British Columbia Biotechnology Laboratory, Canada) were used for PCR. The 25 μl reaction volume contained 2.5 μl 10× assay buffer (Bangalore Genei), 0.24 mM dNTPs (Amersham Pharmacia Biotech), 5 μM primer, 1.1 U *Taq* DNA polymerase (Bangalore Genei), 75 ng template DNA,

Table 1. Safflower cultivars used in the present study

Cultivar	Pedigree	Research center[a]	Remarks
A-1	Hybridisation (pedigree method) (A-482-1 × A-300)	Annigeri	Suitable under scanty and assured moisture regions
Bhima	Selection from A-300	Jalgaon	
Girna	Hybridisation (pedigree method) (A-1 × G 1254)	Jalgaon	Moderate wilt tolerant
JSF-1	Selection from IC 11839	Indore	Suitable for early and late sowings
Sharda	Selection from No. 168	Latur	
HUS-305	Selection from germplasm	Varanasi	Salt and wilt tolerant
S-144	Pure line selection from local variety	Raichur	Suitable for dry areas
Nira	Hybridisation (pedigree method) (NS 1572 × EC 32012)	Phaltan	For irrigated conditions
CO-1	Selection from PI 250528	Coimbatore	Non spiny
APRR-3	Selection from EC 27250	Hyderabad	Rust resistant
Nari-6	Hybridisation (pedigree method) (CO-1 × JL-8)	Phaltan	Non spiny
Manjira	Pure line selection from SF-65	Hyderabad	Medium statured
JSI-7	Selection from JSF 1909	Indore	Non spiny
Nari-2	Selection from HUS-296-3	Phaltan	Non spiny

[a]The centre where the cultivar was bred.

2% formamide (Amersham Pharmacia Biotech), and 2.5 mM $MgCl_2$ (Promega). Initial denaturation in Perkin Elmer Cetus 480 DNA thermal cycler was done at 94 °C for 7 min, followed by 45 cycles of 30 s at 94 °C, 45 s at the particular annealing temperature and 2 min at 72 °C ending with 1 cycle of 7 min at 72 °C. The annealing temperature for different primers was calculated by Wallace rule (Thein & Wallace, 1986).

The amplification products in both cases were size separated by standard horizontal electrophoresis in 1.4% agarose (Sigma, USA) gels and stained with ethidium bromide. The reproducibility of DNA profiles were tested by repeating the PCR amplifications twice with each of primers analyzed. The robust bands were found to be repeatable, and were the products considered in this study.

AFLP fingerprinting

About 500 ng of genomic DNA was digested with *Eco*RI and *Mse*I at 37 °C for 2 h followed by heat treatment at 70 °C for 10 min to inactivate the enzymes. The digested DNA was ligated to *Eco*RI and *Mse*I adaptors for 2 h at 20 °C. The ligation mixture was then diluted 5 fold, and selectively pre-amplified (*Eco*RI primer + A, *Mse*I primer + C) during 20 PCR cycles each at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s. Twenty-five fold diluted aliquots of preamplified fragments were then selectively amplified in the presence of ^{32}P-labelled *Eco*RI + 3 and *Mse*I + 3 (primers with 3 selective nucleotides) primers. The PCR profile for this amplification reaction was one cycle at 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 60 s, followed by 12 cycles in which the annealing temperature was progressively lowered by 1 °C, and finally 20 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s. The amplified fragments were electrophoresed in 6% denaturing polyacrylamide sequencing gel on a Sequi-Gen (BioRad, USA) sequencing cell. Electrophoresis was carried out for 3 h in 1 × TAE at 50 W at 55 °C. Gel was wrapped in saran wrap and dried for 1 h at 80 °C. Autoradiogram was developed by exposing Konica X-ray film (AX) to the dried gel overnight at −80 °C with intensifying screens.

Data analysis

Amplification products were scored for the presence (1) or absence (0) of bands and binary matrices were assembled for the three markers. The binary matrices were subjected to statistical analyses using NTSYS-pc version 2.02 k (Rohlf, 1992). Jaccard's similarity coefficient was employed to compute pairwise genetic similarities. The similarity matrices were constructed for each marker type. The corresponding dendrograms were constructed by applying unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm. To check the goodness of fit of a cluster analysis to the associated similarity matrix, co-phenetic correlation was computed for all the markers employed. Degree of congruence between different marker types was ascertained by Mantel matrix correspondence test (Mantel, 1967), a randomization procedure that compares the correlation between two matrices.

For individual primer/primer combination, number of polymorphic bands (n), percentage polymorphism (p), number of banding patterns (N), confusion probability (C), and limit of discriminating power (D_L) were calculated as described by Tessier et al. (1999). Confusion probability (C), the probability that two randomly chosen individuals in a set of N individuals have identical banding patterns, was calculated as

$$C = \sum_{i=1}^{I} p_i \frac{N_{p_i} - 1}{N - 1}$$

where p_i is the frequency of ith banding pattern; N the sample size and I, total number of patterns generated by a primer/primer combination. Limit of discriminating power (D_L), representing the discriminating power of a single primer/primer combination when sample size becomes infinite, was calculated as

$$D_L = 1 - \sum_{i=1}^{I} p_i^2$$

where p_i is the frequency of ith banding pattern. This is an extension of polymorphic information content (PIC) (Anderson et al., 1993) available from frequencies of different banding patterns generated by a primer/primer combination (Table 2).

The three marker systems as a whole were characterized by effective number of patterns per assay unit (P), assay efficiency index (A_i), marker index (MI), resolving power (R_p) and genotype index (GI). Effective number of patterns per assay unit (P) determines the ability of a marker system on per assay basis to distinguish number of individuals in a population when the population size tends to be infinite (Belaj et al., 2003). Effective number of patterns per assay unit (P) was calculated as $P = 1/1 - D_L$, where D_L is average limit of discriminating power. Assay efficiency index (A_i)

Table 2. Number of polymorphic bands (n), percentage polymorphism (p), number of banding patterns (N), confusion probability (C), and limit of discriminating power (D_L) calculated for primers (RAPD and ISSR) and primer combinations (AFLP)

Primer	n	p	N	C	D_L
RAPD					
OPA02	1	25.0	2	0.84	0.14
OPH03	1	20.0	2	0.62	0.34
OPA08	3	50.0	4	0.59	0.37
OPA16	6	66.6	6	0.18	0.76
OPK07	3	25.0	4	0.42	0.53
OPK04	1	14.2	2	0.49	0.46
OPK19	2	28.5	3	0.60	0.36
OPC07	2	40.0	2	0.72	0.24
OPA01	2	50.0	3	0.60	0.36
OPC13	1	25.0	2	0.72	0.24
OPC14	2	50.0	4	0.50	0.46
OPK06	2	40.0	3	0.52	0.44
OPA20	2	50.0	3	0.71	0.25
OPH17	2	28.5	3	0.37	0.57
OPI01	2	33.3	3	0.45	0.50
OPC08	1	16.6	2	0.84	0.14
OPA17	3	75.0	5	0.40	0.55
OPI14	1	12.5	2	0.84	0.14
OPI04	3	50.0	4	0.50	0.46
OPC04	8	88.8	8	0.17	0.77
Mean	*2.4*	*24.2*	*3.35*	*0.55*	*0.40*
ISSR					
UBC 881	1	20.0	2	0.72	0.24
UBC 872	1	20.0	2	0.84	0.14
UBC 880	1	25.0	2	0.84	0.14
UBC 873	1	16.6	2	0.72	0.24
UBC 879	1	25.0	2	0.72	0.24
UBC 842	2	33.3	3	0.52	0.44
UBC 835	1	20.0	2	0.62	0.34
UBC 843	1	33.3	2	0.55	0.41
UBC 817	1	20.0	2	0.72	0.24
UBC 814	1	25.0	2	0.72	0.24
UBC 834	3	42.8	4	0.33	0.61
UBC 855	1	25.0	2	0.84	0.14
UBC 810	2	40.0	2	0.84	0.14
Mean	*1.3*	*17.8*	*2.23*	*0.69*	*0.27*
AFLP					
EACG + MCAT	16	41.0	13	0.01	0.92
EACG + MCAG	23	69.6	13	0.01	0.92
EAGG + MCAG	21	72.4	10	0.05	0.88
EAGG + MCAT	22	66.6	12	0.03	0.91
Mean	*20.5*	*61.1*	*12*	*0.02*	*0.90*

was calculated as the total number of effective alleles per assay unit (Pejic et al., 1998). Marker index (MI) (Milbourne et al., 1997) (Assay efficiency index and marker index provides an estimate of polymorphism information obtained per experiment for a given marker system) represents the product of two functions; DI (diversity index) and EMR (effective multiplex ratio), where $\mathrm{DI} = 1 - \sum p_i^2$ and $\mathrm{EMR} = n_p\beta$; p_i being the frequency of ith allele, n_p being the number of polymorphic loci analyzed by a marker system and β being the fraction of polymorphic loci. Resolving power (R_p) was calculated as $R_p = \sum I_b$, where I_b (band informativeness) was calculated as $I_b = 1 - (2 \times |0.5 - p|)$; p being the proportion of total genotypes containing the band (Prevost & Wilkinson, 1999). Genotype index (GI) was calculated as the number of genotypes with unique profiles expressed as a fraction of the total genotypes fingerprinted. It has a range of 0 to 1 (Archak et al., 2003).

Results

RAPD fingerprinting

A total of 198 amplification products were scored by 36 RAPD primers with an average frequency of 5.5 bands per primer. The molecular size of bands ranged from 496 bp with primer OPH16 to 2872 bp with primer OPA16. Twenty primers produced polymorphic products and banding patterns. Figure 1a and b shows the representative RAPD profiles of safflower cultivars with two primers. The number of polymorphic products (n) ranged from 1 to 8 with an average frequency of 2.4 per primer. The number of banding patterns (N) ranged from 2 to 8 with an average of 3.35 per primer. Confusion probabilities (C) of RAPD primers ranged from 0.17 to 0.84 with a mean of 0.55. The limit of discriminating power (D_L) ranged from 0.14 to 0.77 with a mean of 0.40 (Table 2).

ISSR fingerprinting

The total number of products amplified by 21 SSR primers was 95 with an average frequency of 4.5 bands per primer. The products were in the size range from 170 bp with primer UBC 817 to 2836 bp with primer UBC 822. Thirteen primers produced distinct polymorphic products and banding patterns. Three primers that were dinucleotide repeat motifs, produced 2–3 polymorphic bands while the remaining 10 primers

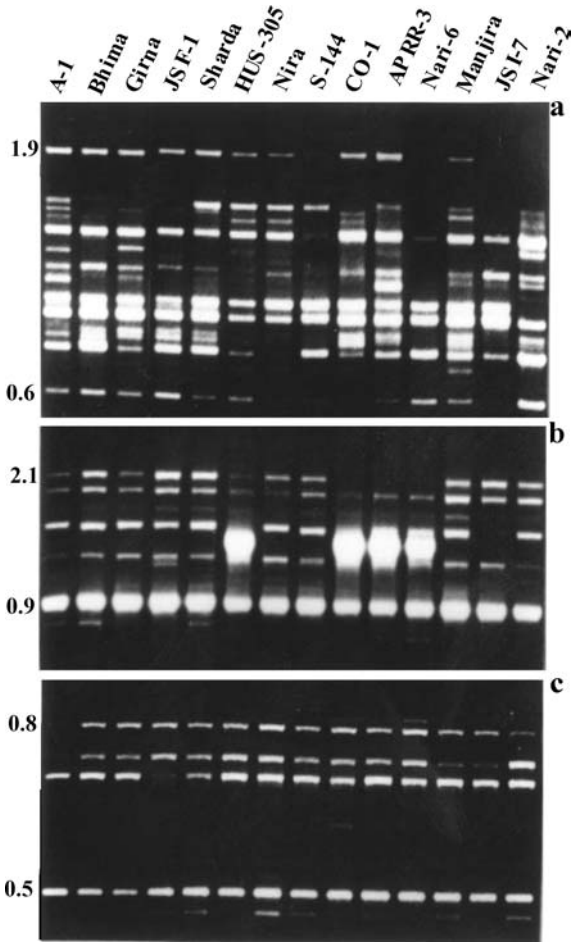

Figure 1. Gel electrophoresis of amplification products obtained with RAPD primers OPC04 (a), OPI04 (b), and ISSR primer UBC 881 (c) in safflower cultivars. The size of fragments in kilobases is indicated on the left.

produced only one polymorphic band with an overall average frequency of 1.3 polymorphic bands per primer. The number of banding patterns produced by 11 primers was two (Figure 1c). The remaining two primers (UBC 842 and UBC 834) produced three and four banding patterns, respectively. Among the three markers (RAPD, ISSR, and AFLP) presently employed, the average confusion probability was the highest for the ISSR marker (0.69) (Table 2).

AFLP fingerprinting

The four primer combinations resulted in 134 amplification products with 33.5 bands per combination. The polymorphic products in the four combinations ranged between 16 and 23 (Figure 2a and b) with an average of 20.5 per combination. Ten, 12, and 13 banding patterns were produced by EAGG + MCAG, EAGG + MCAT, and EACG + MCAT and EACG + MCAG, respectively. EACG + MCAT and EACG + MCAG had the highest limit of discriminating power (0.92) and least confusion probability (0.01). Both these primer combinations individually fingerprinted 13 out of 14 cultivars. EACG + MCAT could not discriminate between cultivars Girna and JSF-1 while EACG + MCAG could not discriminate between cultivars S-144 and APRR-3. Average confusion probability and average limit of discriminating power for AFLP markers was 0.02 and 0.90, respectively.

Marker informativeness

Based on the parameters detailed in Table 3, AFLP markers were found to be most informative in discriminating the present safflower cultivars.

Cluster analysis

Pairwise genetic similarities with regard to AFLP, RAPD, and ISSR markers ranged from 0.55 to 0.98, 0.86 to 0.99, and 0.89 to 1.00, with mean value of 0.689, 0.923, and 0.952, respectively.

The clustering pattern obtained with RAPD and ISSR data showed distinctive congruence with each other. All the three dendrograms grouped 14 cultivars into two main clusters. Cluster 1 in RAPD- and ISSR-based dendrograms had cultivars A-1, Girna, JSF-1, Sharda, Bhima, Manjira, Nira, and S-144 in common while cluster 2 was constituted by CO-1 and APRR-3 in RAPD-based dendrograms, and cultivars APRR-3 and Nari-6 in ISSR data-based dendrogram. HUS-305 was genetically the most distinct cultivar in both dendrograms. Five (A-1, Girna, Bhima, Sharda, and JSF-1) of the 14 cultivars grouped together in the entire three marker based dendrograms. With regard

Table 3. Comparison of information generated with three marker systems

Parameter	RAPD	ISSR	AFLP
Effective number of patterns per assay unit, P	1.66	1.36	10
Assay efficiency index, A_i	0.75	0.82	33.2
Marker index, MI	1.41	0.70	18.2
Resolving power, R_p	15	4.92	40.62
Genotype index, GI	0.167	0.087	0.856

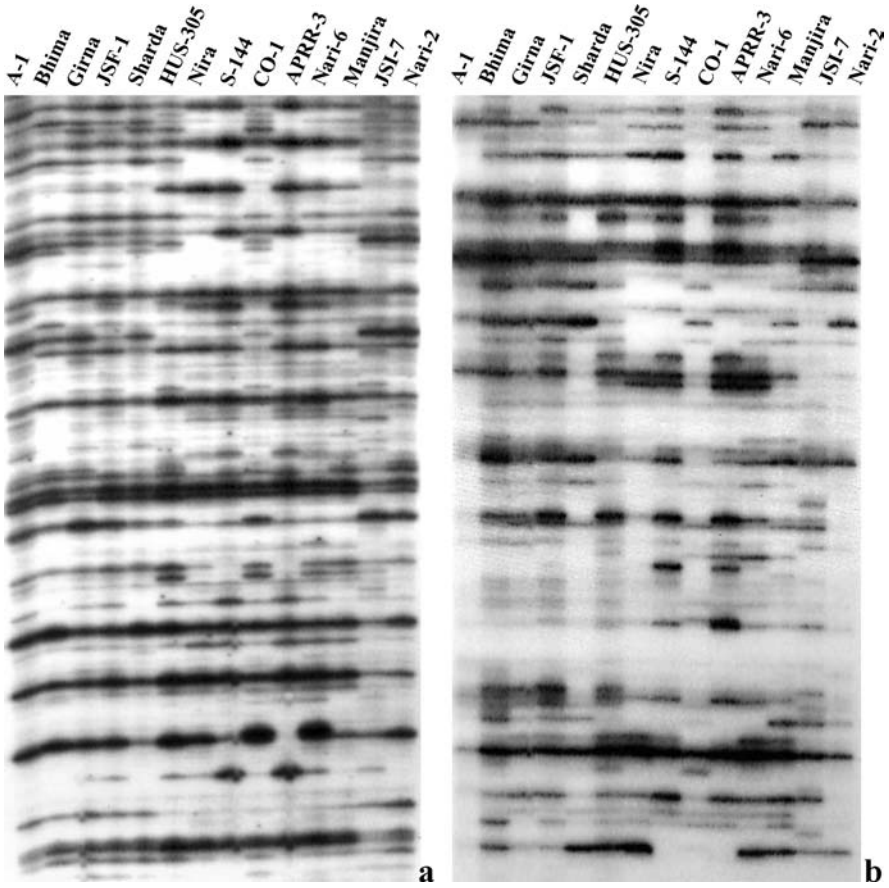

Figure 2. AFLP profiles of 14 safflower cultivars with primer combinations EACG + MCAT (a) and EAGG + MCAT (b).

to the remaining nine cultivars, the phenogram based on AFLP data was quite distinct compared to the one based on either RAPD or ISSR data (Figure 3a–3c).

The Mantel's test (Mantel, 1967) resulted in a very good fit of co-phenetic values ($0.902 \leq r \leq 0.938$) for all the three marker systems, indicating that the dendrograms obtained with the three marker systems are a proper representation of their respective similarity matrices. The correlation coefficients computed between the similarity and co-phenetic matrices generated with the three markers demonstrated that AFLP markers showed no correspondence vis-a-vis RAPD and ISSR (Table 4). Significant correlation, however, was obtained between RAPD and ISSR markers.

Table 4. Correlation coefficient comparison between similarity matrices and co-phenetic matrices derived from different data sets

	RAPD	ISSR	AFLP
RAPD	*0.931*	0.726	0.105
ISSR	0.785	*0.902*	0.131
AFLP	0.091	0.159	*0.938*

Note. Above diagonal values represent correlation coefficients between similarity matrices and below diagonal values represent correlation coefficients between co-phenetic matrices. Values on the diagonal represent co-phenetic correlation for markers. Values above 0.5 are statistically significant at 1% probability level.

Diagnostic markers

Eight RAPD (OPA08, OPA02, OPA01, OPK07, OPC08, OPI14, OPC04, and OPA17), four SSR (UBC 880, UBC 842, UBC 895, and UBC 872) primers, and one AFLP primer combination (EACG + MCAG) generated 14, 5, and 5 diagnostic markers, respectively for 11 out of 14 cultivars (Tables 5 and 6).

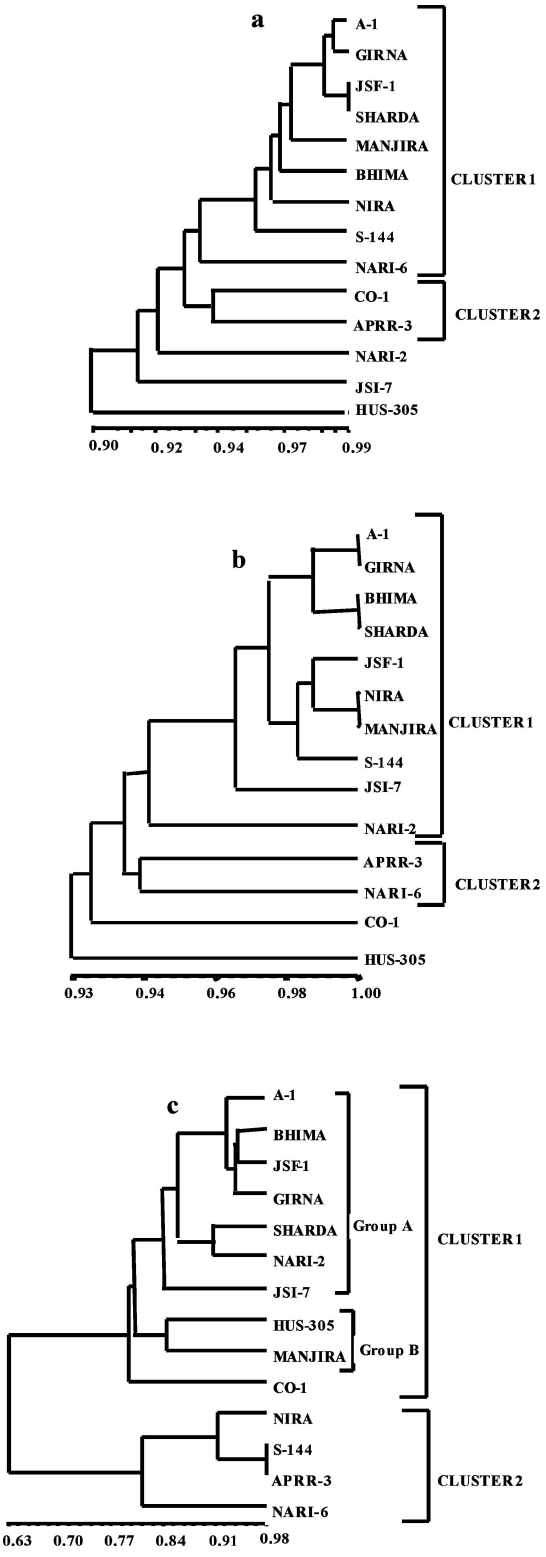

Figure 3. Phenograms, generated using UPGMA, of 14 safflower cultivars based on RAPD (a), ISSR (b), and AFLP (c) data.

Discussion

The amount of polymorphism unveiled in safflower cultivars by RAPD and ISSR markers was hardly 24.2 and 17.8%, respectively. AFLP marker system, on the other hand, was able to yield 61.1% polymorphic bands which is higher (23.1, 37.5, and 46.8%) than obtained for melon, oat, and barley cultivars, respectively but considerably lower than obtained for strawberry (84.6%), olive (82%), and Cuban rice cultivars (100%) (Belaj et al., 2003; Degani et al., 2001; Fuentes et al., 1999; Garcia-Mas et al., 2000; Paczos-Grzęda, 2004; Russell et al., 1997).

Primers/primer combinations have been rated according to confusion probabilities and limit of discriminating power. The higher the confusion probability of a primer/primer combination the lesser it becomes suitable for fingerprinting. The most useful RAPD primers were OPC04 and OPA16, generating eight and six banding patterns with their limit of discriminating power being 0.77 and 0.76, respectively. Similarly, the most effective AFLP primer combinations were EACG + MCAT and EACG + MCAG, both generating 13 banding patterns with limit of discriminating power of 0.92. None of the 21 SSR primers utilized in the present study could be, however, selected as the most useful for fingerprinting safflower cultivars. This may be due to the under-representation of presently chosen SSR primer motifs in the safflower genome. In addition to highest (0.90) value of average limit of discriminating power (D_L), the capacity of just two AFLP primer combinations vis-a-vis 36 RAPD, and 21 SSR primers to effectively fingerprint the 14 cultivars is indicative of the fact that AFLP is the marker of choice for fingerprinting safflower cultivars and vast germplasm resources. The *P* value estimates for the three markers presently employed indicate, that the ability of AFLP marker to distinguish between the safflower genotypes would be more than five times higher than for RAPD and ISSR markers when sample size will be infinite. The superiority of AFLP marker system is also reflected in other parameters evaluated for characterizing marker informativeness.

The incongruence of AFLP data-based dendrogram with both RAPD- and ISSR-based dendrograms indicates that the genetic relationships determined by AFLP marker system is different from the one determined by RAPD and ISSR marker systems. Such incongruity between various DNA marker systems is not uncommon (see Degani et al., 2001; Archak et al., 2003). The congruence between various marker

Table 5. Diagnostic RAPD and ISSR markers identified in the present investigation

	RAPD markers			ISSR markers	
Cultivar	Presence of band	Absence of band	Cultivar	Presence of band	Absence of band
Bhima		OPA02[a]-2715[b]	Nari-2	UBC880-1256	UBC895-811
		OPA08-1407		UBC842-1139	UBC895-733
		OPA08-1167	APRR-3	UBC872-2234	
CO-1		OPA08-719			
HUS-305		OPA01-1462			
		OPK07-802			
		OPC08-896			
Sharda		OPI14-762			
Nari-2	OPC04-1080	OPC04-788			
Nira		OPC04-677			
A-1	OPC04-963				
Girna	OPC04-1019				
APRR-3		OPA17-579			

[a]The primer.
[b]bp.

Table 6. Diagnostic AFLP markers for cultivar identification

Primer combination	Number of markers	Cultivar
EACG + MCAG	3	JSI-7
	1	Manjira
	1	CO-1

systems has been reported to occur in autogamous crop species and inbred lines (Archak et al., 2003). Bohn et al. (1999), however, reported little correlation between marker systems in wheat which is autogamous as safflower. Powell et al. (1996) also observed little correlation between various marker systems in soybean. Also in maize inbred lines (Pejic et al., 1998) RAPD and AFLP marker systems were incongruent with each other. According to Powell et al. (1996), the relationships may be rather dependent on genome coverage and/or the type of sequence variation recognized by each marker system. More detailed studies are needed for safflower before any conclusions can be made with regard to genome coverage of markers.

Notwithstanding the discordance between the three marker systems as above, cultivars A-1, Girna, Bhima, Sharda, and JSF-1 showed similar topography in the three dendrograms indicating thereby their genetic relatedness. The genetic relatedness between A-1, Bhima, and Girna is expected since A-300 was common ancestor in the origin of former two cultivars while A-1 was one of the ancestors for the later cultivar. On the same ground, Nari-6 should have clustered together with CO-1 since Nari-6 is a hybrid between CO-1 and JL-8, but that was not so in any of the three dendrograms. Probably either rigorous selection programme, generally practiced in pedigree method after the desired cross is made, or highly divergent genetic constitution of JL-8 might have been the cause of divergence of Nari 6 from CO-1.

Identification of cultivar specific markers is yet another important finding in the present study. These markers could be of potential use for detecting mixtures and duplicates in the germplasm. The maximum number of diagnostic markers were obtained for Nari-2 followed by HUS-305, Bhima, and JSI-7 indicating that four of the 14 cultivars had presumably added advantage in safflower breeding programme(s) as the source of new and novel alleles. Furthermore, the markers, if tagged to some gene of interest, for instance, gene for rust resistance, can serve as an addendum to the existing breeding and selection programmes in safflower.

Acknowledgments

Thanks are due to AICORPO and NARI for supplying the seed samples. This work was supported, in part, by grant-in-aid for scientific research from Department of Biotechnology and Council of Scientific and Industrial Research, Ministry of Science and Technology, Government of India.

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