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Molecular Diversity in Carthamus species and development of inter-specific mapping population toward development of the first molecular map in safflower.

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Abstract

The molecular genetic relationship between four Carthamus species and the cultivated varieties was determined using RAPD markers. The RAPD primers produced a large number of polymorphic markers. The polymorphic markers were also used to assess the inter relationships of the species and to estimate the overall genetic variation in the species. The *Carthamus oxyacantha* and *Carthamus palaestinus* were more closely related to the cultivated species supporting the previous taxonomic studies based on cytogenetics. These two species were reported to be the progenitors of cultivated *Carthamus tinctorius* L. *Carthamus glaucus* L. formed a distinct solitary cluster and showed least similarly with cultivated species. The evidence obtained through molecular markers support the conclusions obtained through cytogenetic and morphological studies. The study revealed the significant genetic differences in the genome of Carthamus species and species-specific markers with discriminating power were identified. There was no crossing barrier between different Carthamus species and cultivated varieties. The species-specific markers were used to identify true inter-specific hybrid plants. The F_2 generation of three inter-specific hybrids A 1× *C. palaestinus*, A 2 × *C. glaucus* and A 2 × *C. palaestinus* were grown during Rabi (post rainy) season of 2007-08. From each F_2 population 125 F_2 plants were randomly selected to record observations on seven quantitative traits viz., plant height, number of branches per plant, number of capitula per plant, number of seeds per capitulum, 100 seed weight, volume weight and seed yield per plant. The inter-specific F_2 populations recorded significant variability for all the seven quantitative traits studied. The F_2 population of A1× *C. palaestinus* was selected for development of molecular map in safflower. The DNA from individual F_2 plants was extracted by following CTAB method. Fifty-eight genomic SSR primers of chickpea, sorghum and sunflower were used for amplification in safflower. Efforts are being made to identify more SSR primers from other crops, which show amplification in safflower. Twenty-five out of 144 RAPD primers and four out of 58 SSR primers showed parental polymorphism. Fourteen RAPD markers have been tested on F2 plants and the linkage map has been developed. The markers formed 3 linkage groups.

Key words : Safflower - molecular map - inter-specific hybridization - genetic variability.

Introduction

Safflower (*Carthamus tinctorius L)* is an important oil seed crop of the tropical countries, which belongs to family compositae or Asteraecae. India is the largest producer of safflower in the world with an area of 363 thousand hectares; production of 229 thousand tonnes and productivity of 631 kg / ha (Anon, 2007). The area under safflower in India has been significantly decreasing from 800 thousand ha in 1986 to 363 thousand ha in 2006 (Anon, 2007). The trend is same in many safflower-growing countries. The low oil content of 28% and low yield of around 600 kg / ha make safflower a poor competitor. The success of safflower as a commercial oilseed crop will largely depend on the extent of improvement made in both its yield and oil content. However the genetic variability within the cultivated species is limited (Ramachandram 1985). In addition, the linkage between seed yield and oil content genetic components inhibits frequency of genetic recombination for production of desirable segregates (Hanson 1959). The different species of Carthamus are important sources of genetic variation for improvement of

cultivated safflower. The genus Carthamus contains more than 25 species (Anjani et al 2006). The Carthamus species were identified as sources of several productivity traits like number of capitala, test weight, early maturity, branches per plant and biotic and abiotic stress tolerance (Anjani et al 2006; Ash et al 2003). There is a need for introgression of genes from related species as reported in major crops like rice. Reproductive barriers were not found between many Carthamus *species* (Kumar and Agrawal 1989)

Molecular markers have been shown to be of immense utility in plant breeding. They have tremendous application in fingerprinting, introgression of alleles, selection of useful traits etc., in crop improvement programmes. There is a lot of progress in this area in major crops like rice, maize, sorghum and wheat. However, the dry land crops like safflower have been ignored for a long time. There is an urgent need to utilize such technological advancements in crops like safflower; which are hitherto ignored. Little has been done to develop mapping populations and molecular markers in safflower. The present study is the beginning in this direction. Efforts have been made to study the molecular diversity in selected *Carthamus species* and development of interspecific mapping population and molecular map in safflower.

Materials and methods

Molecular diversity in *Carthamus species* : Three wild species *Carthamus glaucus, Carthamus palaestinus and Carthamus oxyacantha* and five cultivated genotypes- A1, A2, NARI 6, GMU 1553, and GMU 2363 - were selected for molecular diversity analysis. The genotypes were grown in small pots and the DNA was extracted from individual samples following mini prep rapid method with little modifications (Edwards et al 1991). A total 25 random primers were used for the study (Table1).

The polymerase chain reaction was carried out (Soregaon et al 2007) and the bands were separated on agarose gel of 1.2 percent and the banding patterns were visualized on a UV-transilluminator after staining the gels with ethidium bromide. The amplification products were scored for presence or absence of band generating the '1' and '0' matrix for 25 primers and all the genotypes. Only clear and reproducible bands were scored as loci. The clustering was done using the symmetric matrix of similarly coefficient and cluster obtained based on unweighted pair group arithmetic mean (UPGMA) using Shan module of NTSys-PC version (Rohlf 1998).

Interspecific hybridization: The cultivated genotype A_1 was used as female and crossed to *Carthamus palaestinus* to produce hybrid seeds. The hybrid seeds were sown in the field and the true F_1 plants were identified using species-specific markers. The F_1 plants were selfed to produce the seeds for F_2 generation. The DNA was extracted from seventy-five F_2 plants and the plants were advanced to next generation by selfing. A further two more interspecific hybrids A 2 × *Carthamus palaestinus* and A 2 × *Carthamus glaucus* were produced. The two interspecific F_2 populations were grown in the field. Nearly one hundred twenty five plants in each F_2 were selected for recording observations on seven quantitative traits viz. plant height, number of branches per plant, number of capitulum per plant, capitulum diameter, number of seeds/capitulum, test weight and plant yield. The mean and range for each trait was determined.

Parental polymorphism: The DNA from two parents A 1 and *Carthamus palaestinus* were used to identify primers producing polymorphic markers. A total of 144 RAPD primers were used for PCR amplification. RAPD primers were analyzed as mentioned earlier**.**

SSR analysis: The SSR analysis included 28 chickpea, 20 sorghum and 10 sunflower SSR primers to check cross species utility of the primers. PCR amplifications were performed in 20µl reaction volumes .The amplification products were separated on 3% metaphor agarose gels and the banding patterns were visualized on UV transilluminator after staining the gels with ethidium

bromide. In both RAPD and SSR analysis, only clear and reproducible polymorphic bands were scored at loci.

Table 1. Primers used for diversity analysis and their sequences

Sl. No.	Primer	Sequence (5' to 3')
1	RKAT-1	CAGTGGTTCC
2	RKAT-2	CAGGTCTAGG
3	RKAT-3	GACTGGGAGG
4	RKAT-4	TTGCCTCGCC
5	RKAT-5	ACACCTGCCA
6	RKAT-6	CCGTCCCTGA
7	RKAT-7	ACTGCGACCA
8	RKAT-9	CCGTTAGCGT
9	RKAT-13	CTGGTGGAAG
10	RKAT-14	GTGCCGCACT
11	RKAT-15	TGACGCACGG
12	RKAT-18	CCAGCTGTGA
13	RKAT-19	ACCAAGGCAC
14	RKAZ-1	TCGGATCCGT
15	RKAZ-3	GGCTGTGTGG
16	RKAZ-7	CACGAGTCTC
17	RKAZ-8	TCGCTCGTAG
18	RKAZ-10	ACTCTGGGGA
19	OPK-04	CCGCCCAAAC
20	OPK-06	CACCTTTCCC
21	OPK-07	AGCGAGCAAG
22	OPAG-02	CTGAGGTCCT
23	OPAG-06	GGTGGCCAAG
24	OPAG-14	CTCTCGGCGA
25	OPB-17	AGGGAACGAG

Linkage analysis: Twenty-five RAPD markers showing polymorphism in the parents i.e., A 1 and *Carthamus palaestinus* were used for the analysis of seventy five F_2 plants. The clear and polymorphic bands were scored for the F_2 plants. The chi square test was used to assess goodness of fit to the expected monogenic 3:1 segregation ratio for each (dominant) RAPD marker. Fourteen polymorphic RAPD markers for which the analysis in F_2 plants was completed were used for linkage analysis performed using Map marker 3 (Van ooijen and Voorrips 2001). The markers were classified into linkage and groups (LGS) using minimum LOD thresh hold of 8.00 and Kosambi mapping function (Kosambi 1944) to estimate the map distance.

Results and Discussion

Three *Carthamus species* and five cultivated types were analyzed using 25 RAPD primers and all the primers produced polymorphic bands and revealed a high DNA polymorphism among cultivated genotypes and *Carthamus species.* Twenty-five primers produced a total of 217 markers. Among those 210 were polymorphic with an average of 97.41 parent polymorphism. The highest diversity coefficient was observed between *C. glaucus* and GMV-2363. The *Carthamus glaucus* also showed higher diversity coefficient values with *C. palaestinus and C. oxyacantha.* The *C. palaestinus* was more similar to *C. oxyacantha* compared to other genotypes.

The dendrogram contributed from the pooled data revealed two distinct clusters with one cluster having a solitary accession (*C. glaucus*). This species is least similar to the other species. The *Carthamus oxyacantha* and *C. palaestinus were* placed along with cultivated genotypes in the second cluster (Fig -1). However, the second cluster is further divided in to two clusters. The cultivated genotypes and wild species were placed in to two different clusters. The results indicate that the *Carthamus palaestinus* and *C. oxyacantha* are distinct from *C. glaucus* but more similar to cultivated species. Cytogenetic studies also suggested that *C. palaestinus* is a progenitor of *C. oxyacantha* (Imire and Knowles, 1970). Both species are considered as parental species of the cultivated *Carthamus tinctorius L* (Ashri and Knowles 1960). The other species *C. glaucus* is a weedy species with 10 pairs of chromosomes. The genome is also different (AA/A3A3) from other species (BB) (Anjani et al 2006). The evidence obtained through molecular markers in the present study support the conclusions obtained through cytogenetic and molecular studies. Thus revealing the applicability of molecular markers for diversity analysis in *Carthamus species.*

True hybrid plants between A 1 and *C. palaestinus were* identified using species-specific molecular markers. Twenty-five of the 144 RAPD markers, and only 4 out of 58 SSR markers were polymorphic in these parents. At the time of preparation of this manuscript, the analysis of 14 RAPD markers was completed in the mapping population. The linkage analysis was carried out using 14 markers. All the markers except one showed normal monogenic segregation of 3:1 in the F_2 generation (Fig-2). Only one marker OPC-14 showed distorted segregation. Of the 13 markers, 9 were linked to 3 linkage groups that span a total length of 124.6 cM at LOD score of 8.00. Four markers were unlinked. This represents the first skeletal molecular map in safflower (Fig- 3). The linkage group 1 was longest with four markers and the smallest linkage group 3 had only two markers. There were no previous attempts to our knowledge to develop safflower linkage map. The present map is small with only nine markers but the beginning has been made in this direction. It is important to apply novel tools in the safflower to improve the productivity before it is dropped from cropping rotations.

The genetic improvement and productivity in safflower has been stagnant for the last 50 years in all the safflower growing countries including India (Anon 2006). Consequently, there is a significant decline in the cultivated area of this crop and the trend may continue. The success of safflower as an alternative crop mainly depends on the extent of improvement made in productivity. The main limitation in safflower improvement is the narrow spectrum of variability available for all the productive traits in cultivated species and crosses (Ramachandram ,1985). The wild species of *Carthamus* are the important sources of genetic variation for important traits like capitulum number, seeds per capitulum and test weight. The F_2 of the two interspecific

crosses A 2 X *Carthamus glaucus* and A 2 X *Carthamus palaestinus* were studied for seven quantitative traits. Both the populations showed a very wide range for all the traits. Between the two populations, the F_2 of A 2 X *C. palaestinus* had significantly higher yield suggesting the potential for selection of high yielding segregants. The range observed for the most important yield traits like number of capitulum / plant ranges from 5 to 48 in A 2 X *C. glaucus* and 6 to 36 in A 2 X *C. palaestinus (*Table-2).

Table –2: Mean and range for seed yield and its component traits in interspecific F_2 population of Carthamus species

Characters	**A_2× C. *glaucus***		**A_2 × *C. palaestinus***	
	Mean	**Range**	**Mean**	**Range**
Plant Height	98.636	70.00-130.00	90.606	60.00-135.00
No. of branches/ plant	7.157	4.00-12.00	9.051	5.00-17.00
No. of capitulum/ plant	17.328	5.00-48.00	18.068	6.00-36.00
Capitulum diameter	2.887	1.50-3.90	2.725	2.00-3.60
No. of seeds/capitulum	38.228	6.00-61.00	37.444	20.00-68.00
100 seed weight	4.802	2.51-7.82	4.688	2.35-6.53
plant yield	17.524	1.58-68.82	22.494	6.02-58.31

Similarly for number of seeds per plant also the wide range was observed. The results clearly indicated that the interspecific hybridization is a potential source for generating desirable variability in Carthamus species. Ramachandram (1985) in his review reported limited variability in Intra-specific crosses. There are no reproductive barriers between different Carthamus species. We have made 24 inter specific hybrids involving eight cultivated lines and 3 Carthamus species. The study is in progress to understand the extent of genetic variability thrown in inter-specifc crosses in Safflower.

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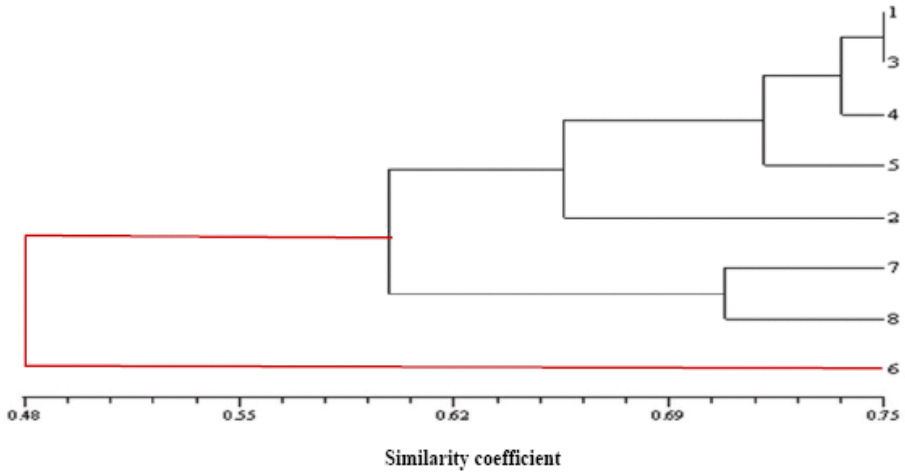

1. A – 1
2. A – 2
3. NARI – 6
4. GMU – 1553
5. GMU – 2363
6. *Carthamus glaucus*
7. *Carthamus palaestinus*
8. *Carthamus oxyacantha*

Fig. 1. Dendrogram obtained from pooled data of 25 RAPD profiles of different genotypes and species of Carthamus

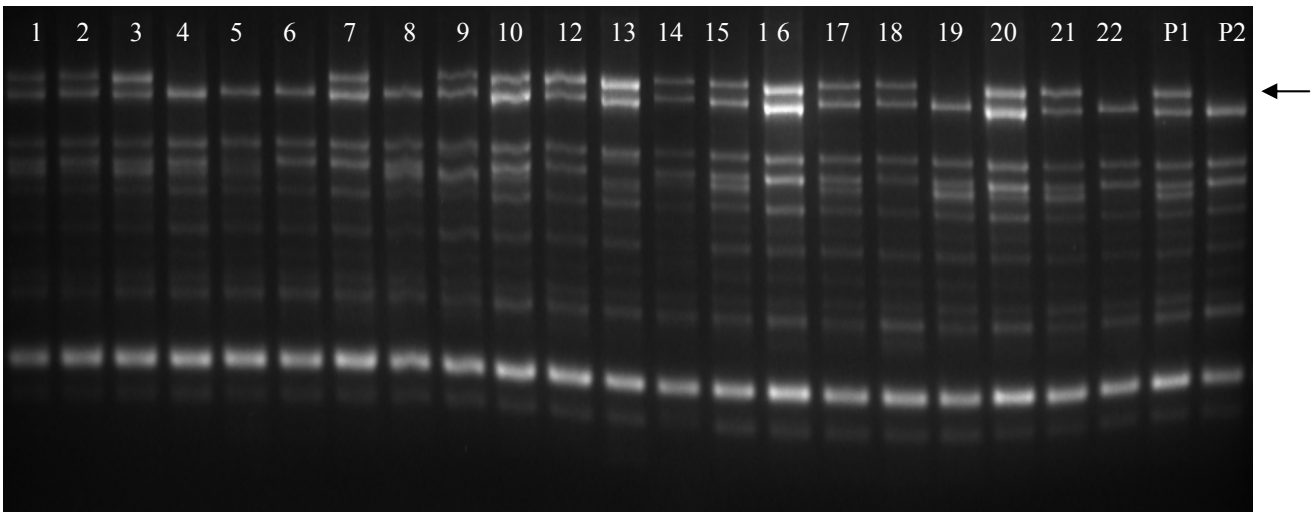

Fig-2: Parental polymorphism and segregation of RAPD marker OPI 05 in the interspecific F_2 population of the cross A1 *X C. palaestinus* (Lane 1 to 22 F_2 plants; P_1=A1 ; P_2 *C. palaestinus*)

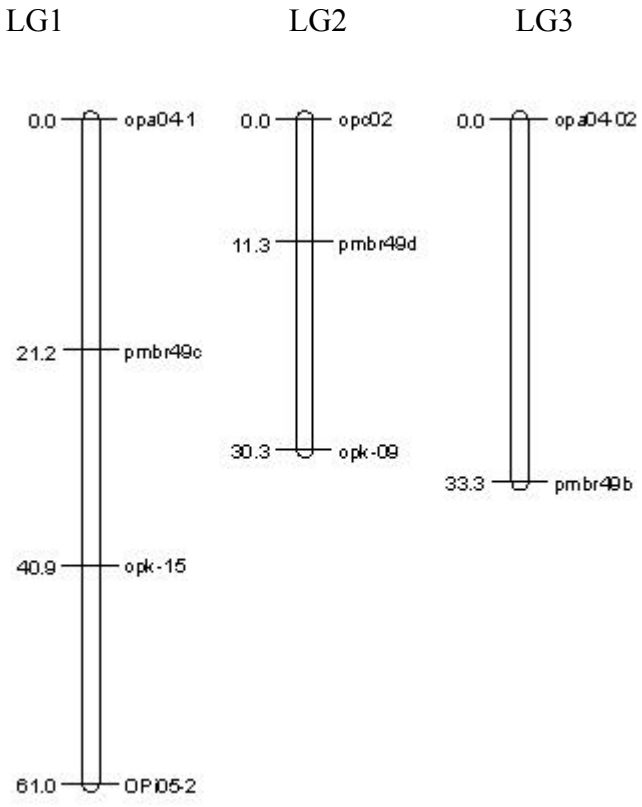

Fig 3 : Linkage map of Safflower