Indian J. Genet., 67(4): 347-351 (2007)

Molecular diversity in *Carthamus* species and species identification using RAPD markers

C. D. Soregaon, R. L. Ravikumar[1] and D. Satish

Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad 580 005

(Received: January 2007; Revised: September 2007; Accepted: November 2007)

Abstract

The molecular genetic relationship between four *Carthamus* species and the cultivated variety A-1 was determined using 44 RAPD primers. The RAPD primers produced a large number of markers. On an average 11.65 bands per primer were produced. Totally 83.75 percent of the markers were polymorphic. 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 proginators of cultivated *Carthamus tinctorius* L. The *Carthamus glaucus* L., which has a chromosome number of n = 10; as against n = 12 of the cultivated species, formed a distinct solitary cluster and showed least similarity with cultivated species and its progenitors. The *Carthamus* species (unidentified) was distinct from all the other *Carthamus* species studied and formed a solitary cluster. The study revealed the significant genetic differences in the genome of *Carthamus* species and species specific markers were identified. The cultivated varieties A-1 and A-2 were crossed to different *Carthamus* species and interspecific hybrids were produced. The species specific markers were useful in identifying true interspecific hybrid plants.

Key words: *Carthamus* species, genetic diversity, RAPD marker, interspecific hybridization, safflower

Introduction

Safflower (*Carthamus tinctorius* L.) is one of the traditional crops of India having the-highest area and production in the world. But, the area under this crop has been decreasing in India from 8 lakh hectares in 1986 to 3.80 lakh hectares [1]. The low oil content of 28-30 per cent and low yield of 600 kg /ha of Indian varieties makes safflower a poor competitor. There is a need to develop high yielding varieties/hybrids with resistance to biotic and abiotic stresses. The traditional plant breeding approaches have not resulted in the desirable variability to improve the productivity under limited moisture conditions. Most research investigating the productivity and economic potential of safflower has centered on cultivated germplasm. A few attempts have been made to broaden the genetic base of the crop and little effort has been given to evaluating and characterizing the genetic diversity in *Carthamus* species. The different species of *Carthamus* are important sources of genetic variation for the improvement of cultivated safflower. The genus *Carthamus* contains more than 25 species [2]. The *Carthamus* species were identified as sources of several productivity traits like number of capitula, test weight, early maturity, branches per plant and biotic and abiotic stresses resistance [2, 3, 4]. There is a need for introgression of disease and insect resistance in to cultivated safflower along with improvement for agronomic and seed quality characteristics. The wild and weed species - *Carthamus oxyacantha*; *C. glaucus*; *C. palaestinus* and *C. persicus* - are proven sources of resistance and productive traits. There are no reproductive barriers between these species and cultivated safflower [5, 6, 7].

The knowledge of genetic relationships among species of *Carthamus* provides a basis for effective utilization of characteristics distributed in different species of *Carthamus* in breeding programmes. An appraisal of past cytogenetic research in safflower

[1]Corresponding author

revealed gaps and deficiencies in genetic relationship of species [8]. The characterization based on morphological, agronomic and biochemical traits, however not able to accomplish goals. The precise cataloguing of species diversity by molecular markers has gained a lot of attention in many crop plants [9, 10]. The application of molecular markers has been limited in safflower [4, 11, 12]. In the present study we analyzed the cultivated and weed species of *Carthamus* by RAPD markers with a view not only to assess their relative diversity but also to identify most suitable primers and markers for differentiating the species. Such primers were used in identification of true interspecific hybrids between different species.

Materials and methods

Four *Carthamus* species *viz., C. oxyacantha, C. glaucus* and *C. palaestinus* and an unidentified *Carthamus* species (morphologically similar to *Carthamus*) were selected for the molecular analysis. These *Carthamus* species were crossed to two cultivated popular varieties A-1 and A-2 producing 3 inter specific hybrids, A-1 x C *oxyacantha*, A-2 x *C. palaestinus* and A-2 x *C. glaucus*. Four *Carthamus* species, three interspecific hybrids and the national check, A-1 were used for molecular analysis.

DNA extraction

Five selected seeds of each of the selected lines were sown in small earthen pots. Genomic DNA was extracted from 0.5 g of leaf tissue using mini prep rapid method with little modifications [13]. The quality and concentration of the DNA was confirmed by electrophoresis on 1% agarose gel.

PCR amplification and electrophoresis

A set of 44 arbitrary primers (OPERON technologies, Inc. California, USA) was used for the amplification of genomic DNA of all the eight genotypes. The PCR amplifications were carried out in a volume 20 µl consisting of 20-50 ng of template DNA, 5 pM of random decamer primer, 0.1 mM of dNTPs, 1 unit of *Taq* polymerase (Bangalore Genei, India), 1X PCR buffer (10mM Tris PH 8.0, 50 mM KCl, and 1.8mM $MgCl_2$ and 0.01 mg/ml gelatin). PCR amplification was carried out using Master Thermal Cycler 5331- Eppendorf version 2.30, 31-09, Germany. The initial denaturation was at 95°C for 5 minutes followed by 40 cycles of 1 minute at 94°C, 1 min at 36°C, 2 min at 72°C and final 8 min extension at 72°C.

The electrophoresis of PCR amplified products was carried out on agarose gel of 1.4% in 1X TAE at 80 V for 2 hours. The electronic images of ethidium bromide stained gels were captured and documented using Uvidoc (model : UVIDOC DOC-008-XD).

Data analysis

Only the bands that could be unambiguously scored across all the sampled genotypes were used in this study. The amplified products were scored for each individual as discrete characters. Amplified samples were scored for the presence (1) or absence (0) of the homologous bands to create banding matrix of the different RAPD genotypes. The pair wise genetic similarities between genotypes were estimated by DICE similarity coefficient. A cluster analysis based on similarity matrix was performed using the unweighted pair group method with arithmetic averages (UPGMA) using SAHN module.

Results and discussion

The yield stagnation under residual moisture conditions appears to be a consequence of narrow genetic base in the cultivated safflower species. The wild and weedy species of *Carthamus* have been used to a limited extent in safflower breeding. The *Carthamus* species offer a promise for incorporating genes for not only for biotic and abiotic stresses tolerance but also productivity related traits in to the cultivated safflower. 'The wild species of *Carthamus* seems to harbor significantly higher genetic diversity and useful source of favorable genes [2]. For effective utilization of this germplasm in the breeding programme, it is essential to determine the genetic diversity of different species with reference to cultivated species.

In the present study, four *Carthamus* species, one cultivated variety (*Carthamus tinctorius* L.) and three interspecific hybrids were analyzed for molecular diversity using random primers and all the primers produced the polymorphic bands (Fig. 1). The results indicate that RAPD markers are valuable in the study of *Carthamus* species, where extensive genetic characterization of nuclear genome is lacking. The 44 RAPD primers generated a total of 609 reliable fragments. The total number of amplified bands per primer varied from 6 (RKAZ 1) to 23 (RKAT 11) with an average of 13.84 per primer (Table 1). The total number of amplified products and production of unique and in variant markers are valuable parameters in determining genetic relationships among *Carthamus* species. Limited studies on molecular diversity in safflower showed little diversity [11, 12]. However, the present study showed very high polymorphism in *Carthamus* species suggesting the utility of these species in interspecific

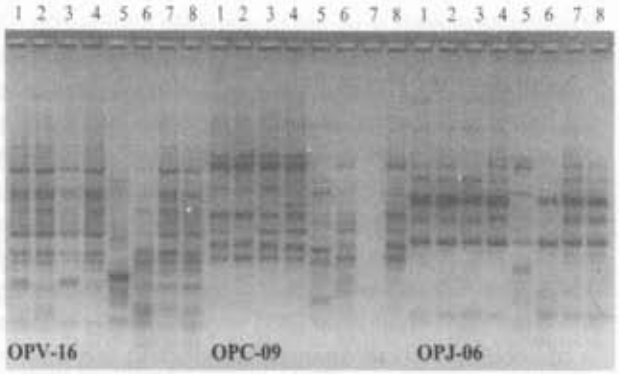

Fig. 1. RAPD banding pattern in different *Carthamus* species and interspecific hybrids. OPV-16, OPC-09 and OPJ-06 are random primers. (1. A-1 x *C. oxyacantha*; 2. A-1; 3. *C. oxyacantha*; 4. *C. palaestinus*; 5. *Carthamus* species (unidentified); 6. *C. glaucus*; 7. A-2 x *C. glaucus*; 8. A-2 x *C. palaestinus*)

Table 1. Number of polymorphic bands, number of monomorphic bands and percentage polymorphism for 44 RAPD primers in different species of *Carthamus* and interspecific hybrids

S.No.	Primers	No. of polymorphic bands	No. of monomorphic bands	% of polymorphism
1.	RKAT-2	14	1	93.30
2.	RKAT-4	16	1	94.10
3.	OPK-9(C)	12	5	70.58
4.	RKAT-6	12	3	80.00
5.	RKAT-5	14	4	77.77
6.	RKAT-9	9	3	75.00
7.	RKAT-11	21	2	91.30
8.	RKAT-15	12	3	80.00
9.	RKAT17	12	2	85.70
10.	RKAT-20	7	2	77.77
11.	RKAT-12	19	3	86.36
12.	RKAT-14	8	5	61.53
13.	RKAZ-1	4	2	66.66
14.	RKAZ-6	19	1	95.00
15.	RKAZ-9	10	5	66.66
16.	RKAZ-18	6	1	85.71
17.	RKAZ-17	13	2	86.66
18.	RKAZ-2	15	3	83.33
19.	RKAZ-3	10	1	90.90
20.	OPK-9(A)	9	2	81.80
21.	OPA-07(T)	15	0	100.00
22.	OPK-04	13	1	92.85
23.	OPK-06	13	1	92.85
24.	OPK-07	10	4	71.40
25.	OPK-10	11	0	100.00
26.	OPB-07	11	0	100.00
27.	OPB-10	9	2	81.80
28.	OPB-11	8	1	88.88
29.	OPAG-02	10	1	90.90
30.	OPAG-06	7	1	87.50
31.	OPAG-14	7	0	100.00
32.	OPB-17	5	4	55.50
33.	OPV-15	14	5	73.68
34.	OPV-16	18	2	90.00
35.	OPC-09	10	3	76.09
36.	OPJ-06	11	1	91.66
37.	OPC-13	16	0	100.00
38.	OPA-20	10	4	71.42
39.	OPF-09	11	3	78.57
40.	OPA-09	14	2	87.50
41.	OPA-12	16	5	76.19
42.	OPA-15	11	4	73.30
43.	OPA-19	6	2	75.00
44.	OPK-14	15	0	100.00
		11.65	2.20	83.75

hybridization to generate wide variability in cultivated safflower. Some of the markers used in the study were only amplified in one species. These bands could be potential species specific markers after checking that every individual from that species shows the markers in question. These markers could be used to detect interspecific hybrids and natural interspecific gene introgression. Selected species *C. oxyacantha, C. palaestinus* and *C. glaucus* are cross compatible with cultivated varieties [2].

The pattern of variation revealed in the dendrogram using 44 RAPD primers is largely in agreement with previous taxonomic studies [14,15]. The

estimated genetic similarity coefficients varied from as close as 0.98 to as diverse as 0.37 (Fig. 2) in *Carthamus* species. The *Carthamus* species (unidentified) recorded the least similarity not only with cultivated varieties but also with other *Carthamus* species. The *Carthamus* species studied have been grouped in to two independent clusters with one cluster having solitary accession (unidentified *Carthamus* species). This species is most diverse (similarity coefficient of 0.37, Fig. 2) from other species. This is distinct from other species in many morphological characters *viz.*, leaf size, shape, thorniness, branches, seed shape, size and flower colour. It is a weedy species. Among other species, *Carthamus oxyacantha* and *C. palaestinus* were most similar to cultivated types with similarity coefficient of more than 0.95. These species were morphologically closer to cultivated genotypes *of Carthamus* (data not shown). These accessions in any combination with *C. tinctorius* were fertile with high level of pollen fertility [16]. The cytogenetic studies suggest that the *Carthamus palaestinus* is a progenitor of *Carthamus oxyacantha* [15]. These in turn are considered as the parental species of the cultivated *Carthamus tinctorius* L. [15]. All these three species have the same genome BB [15] with the chromosome number 2n = 24. The evidence obtained through molecular markers support the conclusions obtained through cytogenetic and morphological studies. This reveals the potentiality of molecular markers for analyzing diversity in *Carthamus* species. However, there are more than 50 different accessions in *C. oxyacantha* and 9 accessions in *C. palaestinus* and are available in the germplasm bank of USDA. The molecular diversity analysis of these accessions would be useful to generate information on diversity levels within species before involving them in hybridization programmes. Attempts are being made to get these accessions. The other species, *Carthamus glaucus* formed a solitary cluster within the cluster-II. It is diverse from other species. C. *glaucus* is a weedy species with 10 pair of chromosomes. There are various sub-species in *glaucus* with genome designated as AA/A3A3. The difference in genome between *C. glaucus* and other species studied (BB) was potentially evident in molecular marker analysis supporting the earlier conclusions based on cytological and morphological studies.

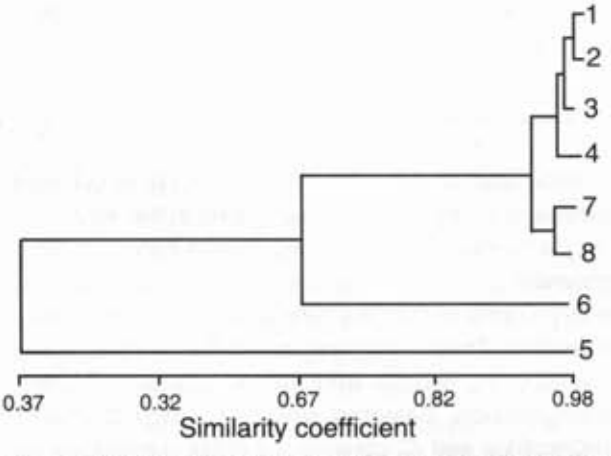

Fig. 2. Dendogram showing similarity coefficient among Carthamus species and interspecific hybrids (1. A-1 x *C. oxyacantha*; 2. A-1; 3. *C. oxyacantha*; 4. *C. palaestinus*; 5. *Carthamus* species. (unidentified); 6. *C. glaucus*; 7. A-2 x *C. glaucus*; 8. A-2 x *C. palaestinus*

The dendrogram shows that the interspecific hybrids A-1 x *C. oxyacantha*, A-2 x *C. glaucus* and A-2 x

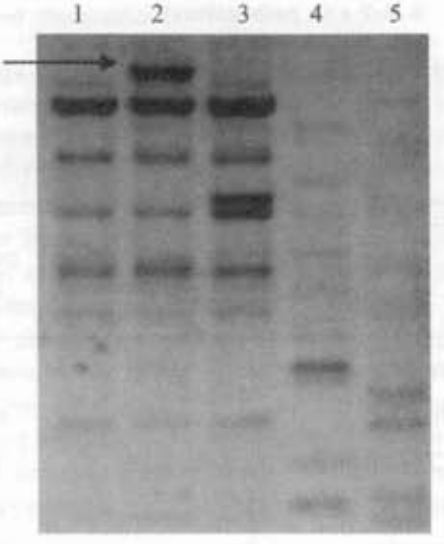

Fig. 3. Primer OPK-06 producing speices specific major band in *Carthamus oxyacantha* (1. A-1; 2. *C. oxyacantha*; 3. *C. palaestinus*; 4. *Carthamus* species (unidentified); 5. *C. glaucus*.

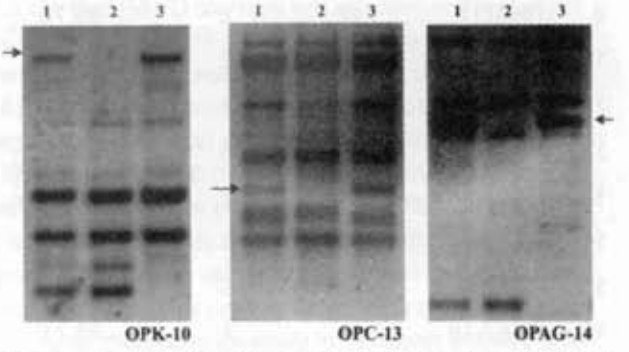

Fig. 4. Identification of true interspecific hybrid, A-1 x *C. oxyacantha* by markers. OPK-10, OPC-13 and OPAG-14 are random primers (1. Hybrid; 2. A-1; 3. *C. oxyacantha*)

C. palaestinus were more similar to cultivated species. The species *C. oxyacantha* and *C. palaestinus* were traditionally considered to be closely related to cultivated species; therefore, the interspecific hybrids were also similar to cultivated and parental species. On the other hand, the interspecific hybrid A-2 x *C. glaucus* was also relatively close to other interspecific hybrids and cultivated species. All the interspecific hybrids were fertile with normal seed set.

In the present study we identified the molecular marker with discriminating power and specific to different *Carthamus* species. The primers OPK-06 and RKAZ-1 produced an extra major bands specific in *C. oxyacantha,* which were not observed in other species (Fig. 3). Similarly primers OPB-07 and RKAZ-2 produced the extra species-specific bands in *C. palaestinus* and *C. glaucus*, respectively. The species-specific bands were used to identify true interspecific hybrids (Fig. 4). The true interspecific hybrid plants were identified and are being grown in the field to advance them to F_2 generation and subsequently for the development of interspecific recombinant inbred lines to map the genes and/or markers.

This study aimed at characterizing diversity at molecular level between different *Carthamus* species available in India. We have also identified molecular markers and primers, which have discriminatory power to differentiate the species and to identify true hybrids in interspecific hybridization. This is a first step towards utilization of these species for broadening the genetic base of cultivated safflower to improve the productivity and oil content, which is stagnant for several decades. The analysis of additional species and accessions in each species and the use of different molecular markers such as SSR, AFLP and ITS (Internally transcribed spacers) will improve the accuracy of resolution of genetic relationship between *Carthamus* species.

Acknowledgement

R. L. Ravikumar thankfully acknowledges, Dr. Vrijendra Singh, NARI, Phaltan, Maharastra, for supplying the seeds of *Carthamus* species.

References

1. **Anonymous.** 2006. Research achievement in safflower, AICRP on safflower. Directorate of Oilseeds Research Hyderabad, India, 111 p.
2. **Anjani K., Mukta N. and Lakshamamma P.** 2006. Crop improvement. In Research Achievements in safflower. Directorate of Oilseeds Research. Hyderabad. India, 111 p.
3. **Kumar H. and Agarwal R. K.** 1989. HUS 305 - a high yielding safflower variety. Indian Farming, **39**: 17-18.
4. **Ash G. J., Raman R. and Crump N. S.** 2003. An investigation of genetic variation in *Carthamus lanatus* in New South Wales, Australia, using interspecific sequence repeats (ISSR) analysis. Weed Res., **43**: 208-213.
5. **Anjani K.** 2005. Development of cytoplasmic genic male sterility in safflower. Plant Breed., **124**: 310-312.
6. **Knowles P. F.** 1969. Centers of plant diversity and conservation of crop germplasm; safflower. Eco. Bot., **23**: 324-329.
7. **Ashri and Rudich J.** 1964. Unequal reciprocal natural hybridization rates between two *Carthamus* L. species. Crop Sci., **5**: 190-191.
8. **Kumar H.** 1991. Cytogenetics of safflower. In chromosome engineering in plants: Genetics, Breeding, Evolution, Part B., Developments in plant Genetics and Breeding, 2B (eds. T. Tsuchiya and P.K Gupta) pp. 251-277.
9. **Guena F., Tochi M. and Bassi D.** 2003. The use of AFLP markers for cultivar identification in apricot. Plant Breed., **122**: 526-531.
10. **Lombard V., Baril C. P., Dubreuil P., Blouet F. and Zhang D.** 2000. Genetic relationship and fingerprinting of rapeseed cultivars using AFLP: Consequences for varietal registration. Crop Sci., **40**: 1417-1425.
11. **Deepmala Sehgal and Soomnath Raina.** 2005. Genotyping safflower (*Carthamus tinctorius*) cultivars by DNA fingerprints. Euphytica, **146**: 67-76.
12. **Ravikumar R. L., Shivapriya M., Patil B. S. and Satish D.** 2005. DNA profiling and fingerprinting of selected mutants for marker analysis in safflower (*Carthamus tinctorius* L.). VII International Safflower Conference, Istanbul, pp. 9-13.
13. **Edwards K., John stone C. and Thompson.** 1991. A simple and rapid method of preparation of plant genome DNA for PCR analysis. Nucleic Acids Res., **19**: 1849-1850.
14. **Imrie B. C. and Knowles P. F.** 1970. Inheritance studies in interspecific hybrids between *Carthamus flavescens* and *C. tinctorius*. Crop Sci., **10**: 346-352.
15. **Ashri A. and Knowles P. F.** 1960. Cytogenetics of safflower (*Carthamus* L.) species and their hybrids. Agron. J., **52**: 11-17.
16. **Knowles P. F.** 1959. Plant exploration reports for safflower and miscellaneous oilseeds. Near East and Mediterranean countries, March-October 1958. Dept. of Agronomy, University of California, Davis, C.A, in collaboration with UASD-ARS Crop Research Division, CR-43-5.