Indian J. Genet., 70(4): 386-389 (2010)

Short Communication

Molecular analysis of segregation in F_2 generation of inter-specific crosses in safflower

Lucy Kumari, R. L. Ravikumar[1], D. Satish, Deepak Kumar G. Shinde and C. D. Soregaon

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

(Received: September 2009; Revised: September 2010; Accepted: October 2010)

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

The development of linkage maps covering genome with molecular markers has provided new opportunities for the elucidation of complex polygenic traits enabling to identify and manipulate the genetic information necessary to obtain superior genotypes with combination of productive traits in many cereals like rice [1]. Unlike in cereals and other oilseed crops such as soybean, oilseed rape and sunflower, the development of molecular markers, mapping populations and molecular map in safflower is in infancy. Urgent need include the development of highly polymorphic mapping population, user friendly molecular markers and genome wide genetic map. Inter-specific crosses provide high level of polymorphism to develop basic maps in any crop plants and in most of the crop plants the basic molecular maps were based on inter-specific crosses. However, in inter-specific crosses of many crop plants large number markers showed deviation from normal segregation with high percentage of parental types [2, 3]. Therefore, it was observed in many crop plants that the inter-specific maps are not applicable in marker assisted breeding. However in safflower, the detectable variation in cultivated species is limited [4]. The genus *Carthamus* contains approximately 25 wild species of which *C. tinctorius* L., is cultivated. Inter-specific hybridization experiments have shown that *C. tinctorius* can be crossed with several wild relatives to produce fertile progeny [5, 6]. However, there are not many efforts to utilize inter-specific hybridization in crop improvement programmes of safflower. In the present investigation an attempt has been made to study the polymorphism between A1 and *C. palaestinus* by using ISSR and SSR markers and to analyze the segregation of the polymorphic markers in inter-specific F_2 population of A 1 x *C. palaestinus*. Further, F_2 and F_3 generations of many inter-specific crosses were also studied for frequency of sterile plants to understand segregation distortion in inter-specific crosses.

The experimental material consisted of F_2 generation of crosses A 1 x *C. palaestinus* and *C. palaestinus* x A 1 and F_3 generation of crosses A 2 x *C. palaestinus* and A 2 x *C. glaucus.* The F_2 seeds harvested from true F_1 plants (identified using molecular markers) of both the crosses were used to generate F_2 generation. The F_3 seeds of two crosses were obtained by selfing the individual F_2 plants grown during previous season. The selfed seeds were used to raise the F_3 and from each F_2 plant one F_3 plant was grown. The parents, F_2 and F_3 generations of both the crosses were grown in a single replication. All the agronomic practices like spacing, fertilizer application and plant protection measures were followed to raise a good crop. From each F_2 and F_3 population 150-165 plants were randomly selected. Two Capitula from each plant were covered with paper bags before anthesis to record observation on sterility. The plants which did not produce any seeds under bagging were considered as sterile.

For molecular analysis, the F_2 generation of the cross A 1 x *C. palaestinus* was chosen. The seeds of

[1]Corresponding author's present address: Department of Biotechnology, Agricultural College, HASSAN 573 225; e-mail: ravikumarrl@yahoo.co.in
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

parental lines and 72 F_2 plants of A 1 x *C. palaestinus* cross were sown in small earthen pots. The DNA from 10 days old seedlings was extracted by following CTAB method [7] with little modifications. Twelve ISSR and 16 EST-SSRs primers (Table 1) were used to study the parental polymorphism comprising the parents A 1 and *C. palaestinus*. The methodology followed for ISSR marker analysis is as given by Yang *et al.*, 2007 [8]. Initial denaturation was for 2 min at 95°C, followed by 39 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C, with a final extension of 8 min at 72C. The amplified products were separated on 2% Agarose gels and stained with ethidium bromide.

The polymerase chain reaction for EST-SSR markers was carried out with initial denaturation at 94°C for 3 min, followed by 35 cycles of 20 sec at 94°C, 30 sec at 60°C, 50 sec at 65°C, with a final extension of 5 min at 65°C. The amplified products were separated on 4% Agarose gels and the banding patterns were visualized on a UV transilluminator after staining the gels with ethidium bromide. From the polymorphic EST-SSR markers three SSR markers were chosen to study the segregation of markers in the F_2 generation. The segregation of markers in F_2 population of inter-specific cross A 1 x *C. palaestinus* was tested for monogenic segregation ratio of 1:2:1 using Chi-Square test.

Twelve ISSR primers were used to study parental polymorphism (A 1 and *C. palaestinus*). Totally 69 bands were produced of which 9 were polymorphic (Table 2). The primers ISSR 5 (60%), ISSR 9 (40%) and ISSR 12 (40%) produced very high number of polymorphic bands between the parents. The ISSR markers have been successfully applied to study the diversity in safflower genotypes [8]. ISSR-PCR technique is highly reproducible and hence can be used to study the diversity in safflower and genome mapping. There are very limited reports of using SSR markers in safflower. A total of 16 EST-SSRs were used for the study and seven SSR primers *viz.*, SES-33, SES-85, SES-86, SES-99, SES-100, SES-143 and SES-144 produced polymorphic bands in the parents. More than 42% of the SSR primers used showed polymorphism between parents suggesting EST-based SSRs are highly polymorphic in *Carthamus* species. The level of genetic diversity detected in the present study is sufficient enough for providing valuable inputs for the potentiality of SSR molecular markers to generate genetic map in safflower, thus paving way for the development of molecular maps and marker-assisted selection (MAS) in this crop.

Out of seven polymorphic SSRs, three (SES-33, SES-100 and SES-144) were selected to study the segregation of molecular markers in inter-specific F_2 population (Table 3). The segregation patterns of these markers would provide information on suitability of this inter-specific cross in developing permanent mapping population. The segregation distortion and linkage drag are the major problems in inter-specific crosses as the recombination is normally restricted between different genomes. Such recombination may cause severe drawbacks in genetic map, as the physical distance between markers could be much larger than expected from recombination. All the three markers showed

Table 1. EST-SSR Primers used for analysis and their sequences

Primer name		Sequence (5'-3')
SES- 33	(F)	CGTTCTAGGACGACTACTCC
	(R)	ACTGCTTTTTGTCTCTTTCC
SES-81	(F)	GCAATACCATCATCATCCTCAC
	(R)	AGGAGGTGAAAGGGAAGAG
SES-85	(F)	GGGTTCACTTCTTTCTCTCTC
	(R)	AGTACTCCTCCAGTGACATACAG
SES-86	(F)	ACCCTAGATTCATTCATTCC
	(R)	GATTACAGTCTGAGAAACATCG
SES-91	(F)	CATTCCGTCATCTATTTTGC
	(R)	GAAGTAATCGACTAACCAACG
SES-98	(F)	ACCTCACATGGCGAAGAG
	(R)	GATTTCCGGAATGAAACAG
SES-99	(F)	TTCTCTACTCTTCACGATTTGG
	(R)	CCATCTGTCTTAAGCTGTTCC
SES-100	(F)	CATCCAACAAGAACACACC
	(R)	CGCTATGATCCTAGTGTATCC
SES-104	(F)	TCCGTTCCTAACTGAATCC
	(R)	AGCTCAGATCAATCACTTTCC
SES-106	(F)	GGGGCTTTCTTTACTTCC
	(R)	TATTGCTGTTGTTGTCTAGGG
SES-122	(F)	GGGATGAGACTGAGATCG
	(R)	GACAGTTTGGAAGGTGTAGC
SES-129	(F)	CTCTTTATTTCGACTGGAACTG
	(R)	ATGCTTGTTGTTGCCTTATC
SES-139	(F)	TTTGCGTGTCGATAATCC
	(R)	TATCCTCATCGTAACATCATCC
SES-142	(F)	AAGATCTCATCTGGGTTTCC
	(R)	AGAATGAATCAATGGGTAGG
SES-143	(F)	ACCACCTCATGCTCTTACC
	(R)	AGCTATGAGTAGGAAGAATTGG
SES-144	(F)	CACCACCTCATGTTCTTACC
	(R)	GAGGAGAAGAGAGTTTACAACC

Table 2. ISSR banding patterns generated using 13 primers and two parents in safflower

Primer	Sequence	Total	Poly-morphic	Mono-morphic	%poly-morphism
ISSR 1	5'-$(AG)_8$T-3'	6	0	6	0
ISSR 2	5'-$(AG)_8$C-3'	5	1	4	20
ISSR 3	5'-$(GA)_8$T-3'	7	1	6	14.29
ISSR 4	5'-$(GA)_8$C-3'	7	0	7	0
ISSR 5	5'-$(CT)_8$G-3'	5	3	2	60
ISSR 6	5'-$(CA)_8$G-3'	6	0	6	0
ISSR 8	5'-$(TC)_8$C-3'	9	0	9	0
ISSR 9	5'-$(TC)_8$G-3'	5	2	3	40
ISSR 10	5'-$(AC)_8$C-3'	5	0	5	0
ISSR 11	5'-$(AC)_8$G-3'	7	0	7	0
ISSR 12	5'-$(TG)_8$G-3'	5	2	3	40
ISSR 13	5'-$(GA)_8$-3'	2	0	2	0
Total		69	9	60	14.52

segregation in the F_2 population of the inter-specific cross. The segregation of the markers was tested for a monogenic ratio of 1:2:1 using Chi-square analysis. Only one primer SES-33 recorded the expected 1:2:1 monogenic ratio (Fig. 1), remaining two primers SES-100 and SES-144 showed significant deviation from the expected ratio (Table 3). There was more number of parental types suggesting suppression of recombination between parental genome. Such deviation from normal segregation of markers in inter-specific crosses has been observed in different crop species [1, 2]. In addition, in all the populations high frequency (>20%) of sterile plants were observed. The production of such sterile plants in early segregating generations of inter-specific crosses has been reported earlier in safflower [9] and many other crops. It indicates the unequal pairing between the chromosomes and irregular meiosis. Sterile hybrids were obtained by crossing *C. tinctorius* and *C. lanatus* due to incomplete pairing of chromosomes during meiosis I [10]. The *Carthamus* species used for the present study were fairly polymorphic, which is

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

Table 3. Segregation of polymorphic SSR markers in interspecific F_2 generation of A 1 x *C. palaestinus*

Primer	Marker size (bp) A 1	Marker size (bp) C. palaestinus	Homozygous for A 1	Homozygous for C. palaestinus	Heterozyg	χ^2 value (1:2:1)
SES-33	201	173	18	18	36	NS
SES-100	172	157	23	22	27	4.51
SES-144	151	163	27	35	10	39.3

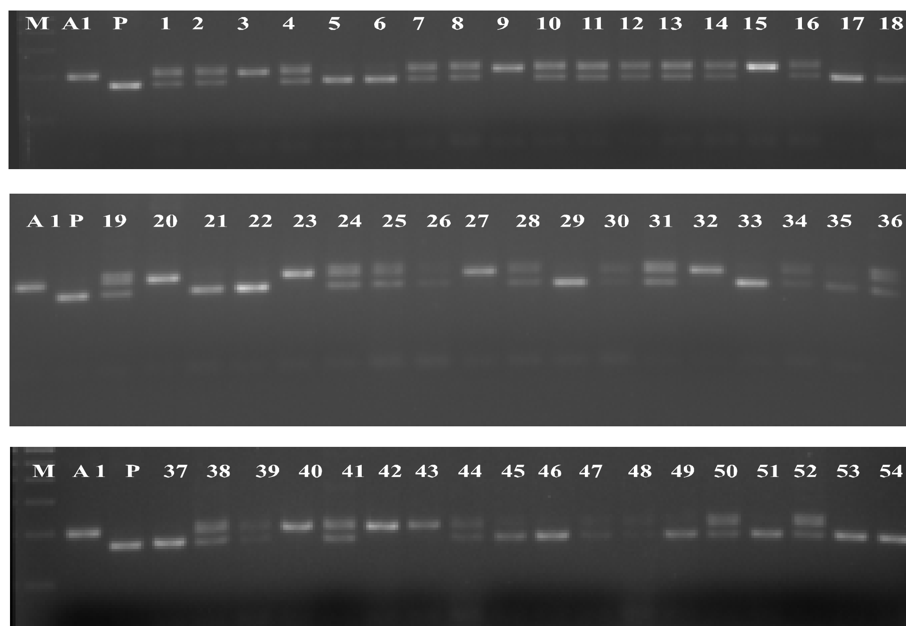

Fig. 1. Parental polymorphism and segregation of EST-SSR marker SES-33 in the interspecific population of cross A 1 x *C.palaestinus* (1 to 54 F_2 plants); (M= DNA ladder, *P = Carthamus palaestinus)*

important in the development of mapping population and genetic map. However, segregation distortion of markers and production of high frequency of sterile plants in inter-specific cross may be a big hindrance. Therefore, the inter-specific crosses may be useful to generate the basic map in the crop species because of high polymorphism in parents, but may not very useful in Marker Assisted Selection involving cultivated genotypes as physical distance may be more than the obtained from such crosses. The importance of using intra-specific mapping population has been realized in many crop plants as the genetic information generated using inter-specific cross is not useful in Marker Assisted Breeding programmes.

Acknowledgement

The authors thankfully acknowledge Dr. V. Dinesh Kumar, Principal Scientist, DOR, Hyderabad for providing the EST-SSR primer sequences.

References

1. **Rangel P. H. N., Brondani C., Brondani R. P. V., Rangel P. N. and Zimmerman J. F.** 2005. Development of rice lines with introgression from *Oryza glumaepahela* by the AB-QTL methodology. CBAB, **5**: 10-19.
2. **Sandler L. and Novitski E.** 1957. Meiotic drive as an evolutionary force. Am. Nat., **41**: 105-110.
3. **Lyttle T. W.** 1991. Segregation distorters. Ann. Rev. Genet., **25: 5**11-557.
4. **Ravikumar R. L., Roopa V. K., Soregaon C. D. and Satish D.** 2008. Molecular Diversity in *Carthamus* species and development of inter-specific mapping population toward development of the first molecular map in safflower. 7th International Safflower Conference, Wagga Wagga, NSW, Australia, Nov. 3-6.
5. **Schank S. C. and Knowles P. F.** 1964. Cytogenetics of hybrids of *Carthamus* species (Compositae) with ten pairs of chromosomes. American J. Bot., **51**: 1093-1102.
6. **Estilai A. and Knowles P. F.** 1978. Relationship of *Carthamus leucocaulos* to other *Carthamus* species (Compositae). Can. J. Genet. Cytol., **20**: 221-233.
7. **Saghaimaroof M. A., Soliman K. M., Jargensen R. A. and Allard R. W.** 1984. Riosomal spacer length polymorphism in barley. Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci., USA, **81**: 8014-8019.
8. **Yang Y., Wu W., Zheng Y. L., Cen L., Liu R. J. and Huang C.Y.** 2007. Genetic diversity and relationships among safflower (*Carthamus tinctorius*) analyzed by inter-simple sequence repeats (ISSRs). Genet Resour Crop Evol., **54**: 1043-1051.
9. **Anjani K., Mukta N. and Lakshmamma P.** 2006. Crop improvement: In research Achievements in Safflower. Directorate of oilseeds Research, Hyderabad, India, p. 11.
10. **Ashri A. and Knowles P. F.** 1960. Cytogenetics of safflower (*Carthamus* L.) species and their hybrids. Agron. J., **52**: 11-17.

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017