The Pharma Innovation Journal 2022; 11(5): 1911-1914

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(5): 1911-1914
© 2022 TPI
www.thepharmajournal.com
Received: 07-02-2022
Accepted: 18-04-2022

Yengkhom Linthoingambi Devi
Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Rajeev Shrivastava
Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Sunil Verma
Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Yengkhom Linthoingambi Devi
Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Hybridity test in safflower f_1 plants for purity testing using SSR markers

Yengkhom Linthoingambi Devi, Rajeev Shrivastava and Sunil Verma

Abstract
Safflower is an often-cross pollinated crop so both self and cross pollination occurs in safflower. Checking the purity of the hybrid is very much necessary in any crop if we want to continue for further generation. A total of six safflower hybrids were generated from six parents and the polymorphism of the parents were checked with 16 SSR markers, out of which eleven markers, SES-33, SES-81, SES-85, SES-86, SES-91, SES-99, SES-100, SES-139, SES-142, SES-143 and SES-144 were showed polymorphism of the parents. Same markers were used to test the trueness of the hybrids produced from these Parents. If the female parent gets selfed, it deteriorates the quality of hybrid seed production. Thus SSR markers were helpful in identifying the hybrids which were pure which will be helpful in hybrid seed production programme and in advancement of further generations.

Keywords: Safflower, often-cross pollinated, hybrids, SSR marker

Introduction
Safflower (*Carthamus tinctorious* L.) belongs to Asteraceae or Compositae family with chromosome numbers, 2n=24 and is the only cultivated species among the 25 species under the genus *Carthamus*. Safflower holds a genome size of 1.4 GB (Garnatje *et al*., 2006) [1]. In hindi, it is commonly known as *kusum* or *karrah*. Safflower is considered to be a multipurpose crop as it can be used as oilseed crop, medicinal crop and also in industrial applications. Safflower cultivation dates back in China around 2200 years ago. Safflower is cultivated around the world for its cooking purpose. The crop is being originated from Mediterrannean and the native countries like those of Ethiopia and Afganistan. The crop is self-pollinated but environmental factors and availability of insect can favor the cross pollination to occur till 5-10% so the crop is considered to be an often-cross pollinated crop species. Safflower crop is considered to be a hardy crop which can be grown well in drought and salinity stress condition as compare to other oilseed crops (Weiss, 2000) [7]. However, oil content and seed yield of safflower is less as compare to other oilseed crops like rapeseed, groundnut, soybean and sunflower. So, the best way to increase the yield is through development of hybrids as heterosis is reported both for seed oil and seed yield. Also it is important to know the genetic purity of the hybrid before their distribution to the farmers. One traditional way of checking the genetic purity is performing the Grow Out Test (GOT) which is the growing of plants to maturity and assessing by comparing their morphological performance. Since we know that conventional method always takes more time and there is presence of environmental factors. So, it necessitates the development and use of more cost effective, rapid and accurate method of hybrid purity accessment. DNA markers are known for their use even at seed stage. One most suitable molecular marker which can be used in hybridity test is PCR-based, simple sequence repeats (SSRs) markers as it can easily determine the heterozygosity of the hybrids by comparing with the parental alleles. Safflower has an enormous variability and several traits that could be genotyped through the available molecular marker systems. Through the molecular level, a better understanding of the variation in the genotypes could be illustrated. Hybridity test for the F1s along with the parents helps in the purity of the hybrids comparing with the parents (Patel and Shrivastava, 2016) [4].

Materials and Method
The experimental material consists of six parents and a total of six F1s were used to test for the purity of the hybrid. The list of the parents and hybrids is given in the table1. The parents and the F1s were grown during the *Rabi*, 2020-2021 in the research cum instructional farm of IGKV, Raipur using Randomized block design for its layout. Young leaves were used for the

extraction of genomic DNA following CTAB method (Cetyl tri methyl ammonium) described by Saghai-Maroof *et al.* (1984) [5]. The extracted DNA were then quantified by using Nano Drop Spectrophotometer and qualified using PAGE. A total of 16 EST-SSR markers were used to check the polymorphism of the parents and the list of the primer is given in table 2. Those markers which were found to show polymorphism among the parents were again used for the hybridity testing of the hybrids. The amplified products were then separated using 5% polyacrylamide gel electrophoresis and stained with ethidium bromide. The amplified products were then scored based on the bands of the SSR amplified markers. The list of parents and F1s used in the study are shown in table-1 and the list of EST-SSR markers used in the study are given in table-2.

Table 1: List of parents and the F1s used in the study

Sl. No.	Parents	Sl.No.	F1's
1	GMU- 6854	7	GMU 6854 X GMU 1217
2	EC 755664	8	EC 755673 X GMU 2830
3	EC 755673	9	EC 755664 X GMU 1217
4	GMU-2830	10	EC 755673 X GMU 6891
5	GMU-1217	11	EC 755664 X GMU 2830
6	GMU-6891	12	EC 755673 X GMU 1217

Table 2: List of primers used in the study

Sl no.	EST-SSR Primers	Primer Sequence	
		Forward	Reverse
1	SES-33	CGTTCTAGGACGACTACTCC	ACTGCTTTTTGTCTCTTTCC
2	SES-81	GCAATACCATCATCATCCTCAC	AGGAGGTGAAAGGGAAGAG
3	SES-85	GGGTTCACTTCTTTCTCTCTC	AGTACTCCTCCAGTGACATACAG
4	SES-86	ACCCTAGATTCATTCATTCC	GATTACAGTCTGAGAAACATCG
5	SES-91	CATTCCGTCATCTATTTTGC	GAAGTAATCGACTAACCAACG
6	SES-98	ACCTCACATGGCGAAGAG	GATTTCCGGAATGAAACAG
7	SES-99	TTCTCTACTCTTCACGATTTGG	CCATCTGTCTTAAGCTGTTCC
8	SES-100	CATCCAACAAGAACACACC	CGCTATGATCCTAGTGTATCC
9	SES-104	TCCGTTCCTAACTGAATCC	AGCTCAGATCAATCACTTTCC
10	SES-106	GGGGCTTTCTTTACTTCC	TATTGCTGTTGTTGTCTAGGG
11	SES-122	GGGATGAGACTGAGATCG	GACAGTTTGGAAGGTGTAGC
12	SES-129	CTCTTTATTTCGACTGGAACTG	ATGCTTGTTGTTGCCTTATC
13	SES-139	TTTGCGTGTCGATAATCC	TATCCTCATCGTAACATCATCC
14	SES-142	AAGATCTCATCTGGGTTTCC	AGAATGAATCAATGGGTAGG
15	SES-143	ACCACCTCATGCTCTTACC	AGCTATGAGTAGGAAGAATTGG
16	SES-144	CACCACCTCATGTTCTTACC	GAGGAGAAGAGAGTTTACAACC

Results and Discussion

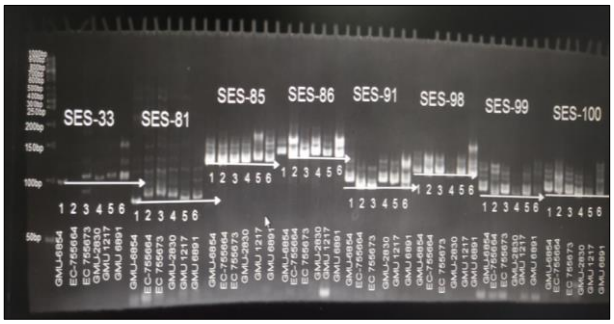

Fig 1: Checking polymorphism of Safflower parents with first 8 markers

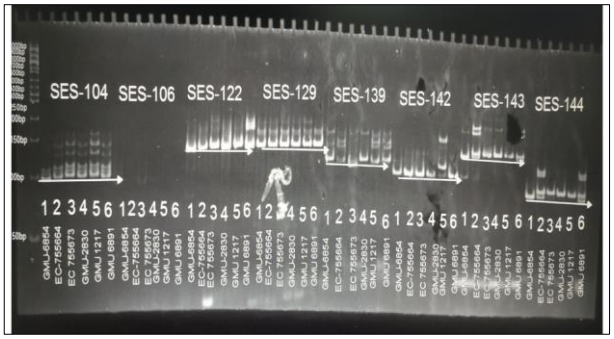

Fig 2: Checking polymorphism of Safflower parents with last 8 markers

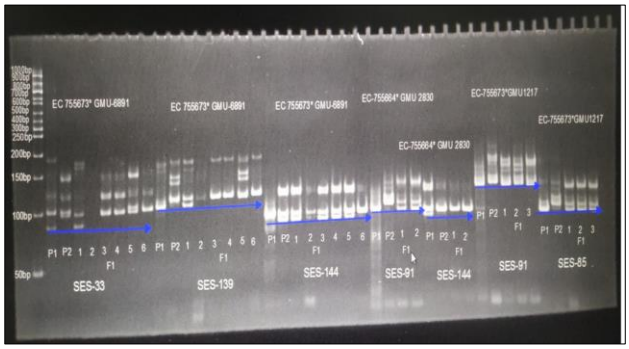

Fig 3: Hybrid purity test of first three crosses

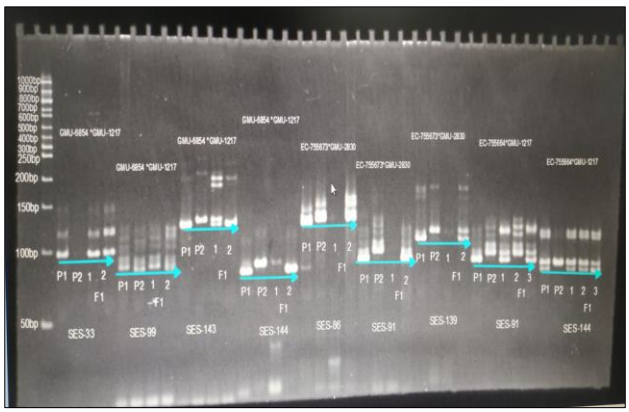

Fig 4: Hybrid purity test of last three crosses

The 6 parents were first tested for the polymorphism by using 16 SSR markers and out of the 16 SSR markers, 11 EST-SSR markers were found to show polymorphism. The markers which were shown polymorphism were SES-33, SES-81, SES-85, SES-86, SES-91, SES-98, SES-99, SES-100, SES-139, SES-142, SES-143 and SES-144. SES-33 showed polymorphism between parent 1(GMU- 6854) and 3(EC 755673), 1(GMU- 6854) and 5 (GMU-1217) and 3(EC 755673) and 6(GMU-6891). SES-81 marker showed polymorphism between parent 1 (GMU- 6854) and 2 (EC 755664), also for parent 1(GMU- 6854) and 3(EC 755673). SES-85 were found to show polymorphism of parent 5(GMU-1217) and 1 (GMU- 6854), 5(GMU-1217) and 2(EC 755664), 5(GMU-1217) and 3(EC 755673) and 5(GMU-1217) and 4(GMU-2830). SES-86 markers showed polymorphism between parent 1(GMU- 6854) and 2(EC 755664), 1(GMU-6854) and 3(EC 755673), 1(GMU- 6854) and 6(GMU-6891). SES-91 marker showed polymorphism for the parent 1(GMU-6854) and 2(EC 755664), 1(GMU- 6854) and 3(EC 755673), 2 (EC 755664) and 4 (GMU-2830), 3(EC 755673) and 4(GMU-2830), 2(EC 755664) and 5(GMU-1217) and 3 (EC 755673) and 5(GMU-1217). SES-99 showed polymorphism for the parent 1(GMU- 6854) and 2(EC 755664) and 1(GMU-6854) and 5 (GMU-1217). SES-100 showed polymorphism between parents 1(GMU- 6854) and 4(GMU-2830), 1 (GMU-6854) and 6 (GMU-6891) and 1 (GMU- 6854) and 5(GMU-1217). SES-139 showed polymorphism between parents 3 (EC 755673) and 4 (GMU-2830), 3 (EC 755673) and 6 (GMU-6891) and 1 (GMU- 6854) and 6 (GMU-6891). SES-142 showed polymorphism for the parents 1 (GMU- 6854) and 6 (GMU-6891). SES-143 showed polymorphism for the parent 1 (GMU- 6854) and 3 (EC 755673), 1 (GMU- 6854) and 4 (GMU-2830), 1 (GMU- 6854) and 5 (GMU-1217), 3 (EC 755673) and 6 (GMU-6891), 4 (GMU-2830) and 6 (GMU-6891) and 5 (GMU-1217) and 6 (GMU-6891). While SES-144 showed polymorphism for parent combinations 1(GMU- 6854) and 3 (EC 755673), 1 (GMU- 6854) and 4 (GMU-2830), 1 (GMU- 6854) and 5 (GMU-1217), 2 (EC 755664) and 3 (EC 755673), 2 (EC 755664) and 4 (GMU-2830), 2 (EC 755664) and 5 (GMU-1217), 3 (EC 755673) and 6 (GMU-6891), 4 (GMU-2830) and 6 (GMU-6891) and 5 (GMU-1217) and 6 (GMU-6891). Among the cross combination, the F1s which were used in the study were the combination of 1(GMU- 6854) and 5 (GMU-1217), 3 (EC 755673) and 4 (GMU-2830), 2 (EC 755664) and 5 (GMU-1217), 3 (EC 755673) and 6 (GMU-6891), 2 (EC 755664) and 4 (GMU-2830) and 3 (EC 755673) and 5 (GMU-1217). So, to check the purity of the hybrid, F1 from parent 1 (GMU- 6854) and 5 (GMU-1217), we used the markers SES-33, SES-99, SES-143, SES-144. To check their purity of F1s of cross 3 (EC 755673) and 4 (GMU-2830), we used markers SES-86, SES-91, SES-139. F1 plants from the cross 2 (EC 755664) and 5 (GMU-1217) were used for purity checking by using markers SES-91 and SES-144 to check the purity of the hybrid. The F1 of the cross 3 (EC 755673) and 6 (GMU-6891 was tested with markers SES-33, SES-139, SES-144. And for the F1 of cross 2 (EC 755664) and 4(GMU-2830), we used markers SES-91 and SES-144. Likewise, the F1s of the cross 3 (EC 755673) and 5(GMU-1217), were tested with markers SES-91 and SES-85. The gel pictures of the polymorphism of the parents with the marker were showed in figure 1 and 2.

Thus, the F1s were checked with the parents to check their purity. The 7,8,9,10,11,12 F1s have 2,2,3,6,2,3 individual plants, respectively. The purity of the F1s can be checked by observing the bands, if the band formed is the same as the male parent, then the purity of the hybrid is justified and if the band formed is same with the female parent then instead of crossing, the seeds are rather self-pollinated. Another way of checking the purity of the F1 is the separation of the bands of the F1s of both the parents as we were using SSR markers which have co-dominant nature. The marker SES-33 used in the cross EC-755673 × GMU-6891 showed polymorphism in the 5th F1 plant while the other F1 plants were not true hybrid while marker SES-139 justified the 1st F1 as true hybrid as there is formation of co-dominant nature of the band. For the cross EC-755673× GMU-1217, marker SES-91 did not show any pure hybrid as the bands formed were all monomorphic and dominant while marker SES-85 showed pure hybrid of all the F1 plants used in the cross. For the cross GMU-6854 × GMU-1217, markers SES-33, SES-99 and SES-144 could not give any pure hybrid F1 while marker SES-143 showed presence of pure hybrid of the first F1 plant. For the cross EC-755673 × GMU 2830, markers SES-86, SES-91 could not clearly justify for the purity of the hybrid while marker SES-139 could justify the purity of hybrid in second F1 plant. For the cross, EC-755664 × GMU-1217, both marker SES-91 and SES144 could justify the purity of the hybrid because of co-dominant nature of the band. SES-91 justified the purity of the first and third F1s while SES-144 justified the purity of hybrid of all the F1 plants used in the cross. Thus those pure hybrids of each cross were then collected and they could be used for further research work. Similar results were also found by Kumar *et al.*, 2016 for hybrid purity checking in Mango, in Eucalyptus by Subashini *et al*., 2014 and also in sunflower by Pallavi *et al*., 2011. The gel pictures for the hybrid purity test of the 6 crosses were showed in figure 3 and 4.

Conclusion

The study showed that EST-SSR markers could be used for checking the polymorphism of the parents as well as purity of the hybrid in safflower. The markers which were showed producing co-dominant nature of the band of F1 could be used further for checking the purity of safflower hybrid. And those pure hybrid plants could be helpful in continuing the research work in a more justified and shorter way as maintaining purity is the prime most important task in any breeding programme.

Acknowledgement

The author acknowledge Dr. Girish Chandel, HOD, and Dr. Sunil Verma, Assistant Professor, Dept. of Plant Molecular biology and Biotechnology, IGKV, Raipur, Chhattisgarh for guiding and assisting my work.

References

1. Garnatje T, Garcia S, Vilatersana R, Vallès J. Genome Size Variation in the Genus Carthamus (Asteraceae, Cardueae): Systematic implications and additive changes during allopolyploidization. Ann. Bot. 2006;97:461-467.
2. Kumar K, Srivastav M, Singh SK. Ascertaining hybridity of progenies in mango (*Mangifera indica* L.) using microsatellite (SSR) markers. J of Agri. and Eco. 2016;2:1-10
3. Pallavi HM, Gowda R, Shadakshari YG, Bhanuprakash K, Vishwanath K. Identification of SSR markers for hybridity and seed genetic purity testing in sunflower

(*Helianthus annuus* L.). Helia. 2011;34(54):59-66.
4. Patel NB, Shrivastava R. Hybrid purity assessment of safflower (*Carthamus tinctorius* L.) F1 s using inter-simple sequence repeats (ISSRs). J Oilseeds Res. 2016;33(3):193-195.
5. Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW. Extraordarily polymorphic microsatellite DNA in barly: species diversity, chromosomal locations, and population dynamics. Proceedings of the National Academy of Sciences, USA. 1984;91:5466-5470.
6. Subashini V, Shanmugapriya A, Yasodha R. Hybrid purity assessment in Eucalyptus F 1 hybrids using microsatellite markers. 3 Biotech. 2014;4(4):367-373.
7. Weiss EA. Safflower. In: Oilseed Crops. Blackwell Science Ltd., Victoria, Australia, 2000, 93-129pp.