I J T A
© Serials Publications
National Academy of Agricultural Science (NAAS) Rating : 3. 03

DNA Fingerprinting of a Newly Released Sunflower Hybrid RSFH-1887 Through SSR Marker

***Vikas Kulkarni[1], I. Shankergoud[1] and Rudragouda Mali Patil[2]**

ABSTRACT: *Sunflower (Helianthus annuus L.) being a cross-pollinated crop, genetic adulteration is a vested problem and phenotypic varietal description is cumbersome. An investigation was carried out to confirm hybridity and novelty of a newly released hybrid along with their parental lines both for morphometric traits and PCR based SSR markers. A total of 44 sunflower SSR primers were screened among them 17% were polymorphic. The hybrid RSFH-1887 could be clearly identified by using ORS-484, based on banding pattern on 3.5% agarosegel electrophoresis. Primer ORS-484 amplified allele size at 160bp was specific to male parent(R-127-1) and 120 bp was specific to female parent(CMS-38A). These two bands of allele size 160bp and 120bp were found only in hybrid RSFH-1887. The amplicon size in other sunflower varities/ hybrids ranged from 180bp to 195bp. Hence, the SSR marker ORS-484 can be used as referral marker for unambiguous identification and protection on the basis of novelty of sunflower hybrid RSFH-1887.*

Keywords: *Sunflower, SSR marker, amflification, novelty.*

INTRODUCTION

Sunflower (*Helianthus annuus*) is one of the important edible oilseed crops grown in the world after soybean and groundnut. It is an important source of edible and nutritious oil. It is used in the manufacturing paints, resins, plastics, soap, cosmetics and oil cake is rich in high quality protein (40–44%) and is used as cattle and poultry feed. The oil is also used for manufacturing hydrogenated oil. Sunflower seed oil can be exploited easily with the existing machinery in the country including simplest process of ghani. Sunflower oil is a rich source of lenoleic acid (64%) and 25-30 per cent of oleic acid which helps in washing out cholesterol deposition in the coronary arteries of the heart and thus is good for heart patients.

Introduction of sunflower to india considerably increased country's oilseed production. The area is increased continuosly due to its day neutrality, wide adaptability, short duration, high yielding ability and good quality oil.

In world, sunflower being cultivated over an area of 20 million hectares and production around 30 million tonnes. In India, sunflower is being grown over an area of 0.69 million hectares with a production of 0.54 million tonnes with the productivity of 791 kg per ha. The major sunflower producing state in India are Karnataka (0.30mt), Andhra Pradesh (0.09mt) and Maharashtra (0.04mt) constituting about 79.6 per cent of India's sunflower production [1].

Sunflower being a highly cross pollinated crop ideally suited for exploitation of heterosis. Commercial exploitation of heterosis has been possible using cytoplasmic male sterility-restorer system. The discovery of Cytoplasmic Male Sterility by Leclercq [7] and followed by fertility restoration system by Kinman [4] provided the required breakthrough in the development of hybrids. The first sunflower hybrid BSH-1was released in the year 1980 for Indian farmers and then onwards several hybrids have been released both by public and private sector for commercial cultivation.

[1] AICRP on Sunflower, MARS, Raichur
[2] Department of Genetics and Plant Breeding, AC, Raichur
* *E-Mail : vik_gene@rediffmail.com; vik_gene@rediffmail.com*

As per national and international registration of variety/ hybrid it needs to pass the criteria of DUS tests. Variety/ hybrid identity and purity are the critical in assessing the grain entering factories and seed production. To check the identity and varietal purity seed sample is investigated for seed admixture by distinguishing closely related species/varieties. In order to fulfil these requirements variety description must be discriminative, free from environmental effects, interpretable in genetic terms and reflective of pedigree and genetic constitution which can be achieved through finger printing technique. The classical taxonomic approach has been traditionally used method of varietal identification which can provide a unique identification of cultivated varieties/hybrids. However, morphological traits are influenced by environment and in most cases genetic control of morphological traits are unknown but increased number of genetically related variety or hybrid releases by plant breeders has made unique identification more difficult. Therefore, it is impossible to determine how completely the genome is sampled by morphological descriptors. The PCR based molecular markers *viz*., microsatellite markers are co-dominant nature, abundant enough to cover whole genome and not influenced by environment very helpful to verify purity and identity of any hybrid/variety.

MATERIAL AND METHODS

The experiment material consist of six public sector hybrids and one variety which are listed in Table 1.

Pure seeds of all sunflower genotypes along with female and male parent of RSFH-1887 were sown in plastic pots. The genomic DNA is extracted by following modified CTAB protocol. About 2g of young leaf tissue from each sample was homogenized in liquid nitrogen and incubated at 65 C for 60 min with 5 ml of CTAB buffer. Then 5 ml of chloroform: isoamyl alcohol mixture (24:1) was added and blended thoroughly. After centrifugation (10 minutes, 15,000 rpm), supernatant layer was pipette out into a new eppendorf tube, and equal volume of chilled ethanol was added. After storage at –20°C for over night precipitated DNA was centrifuged (10 minutes, 15,000 rpm), and pellet was washed with 70% ethanol, dried and finally stored in T10E1 buffer at –20°C.

PCR Amplification

Forty four SSR primer pairs were used in the study listed in table 2. Sterile microfuge tubes were added with 1.0 µl of template DNA, containing 19 µl of master mix (2 µl buffer, 2 µl of 0.2mM dNTP's, 1 µl of 0.25 µM primers and 1 unit Taq. Polymerase) was added to all the tubes and was given a short spin to mix the contents. Thermal Cycler was programmed for 35 cycles of denaturation (94°C for 1 min), annealing of, (23-65°C for 2 min) and extension (72°C for 2 min) followed by final extension at 72°C for 10 min. PCR products were used for electrophoresis on 3.5 per cent agarose gels stained with ethidium bromide and photographed using gel documentation unit under UV light.

Table 1
Sunflower hybrids/variety used

Sl. No.	*Hybrid/ variety*	*Year of release*	*CMS line*	*R- line*	*Released centre*
1.	RSFH-1	2005	CMS-103A	R-64NB	UAS, Raichur
2.	RSFH-130	2009	CMS-104A	R-630	UAS, Raichur
3.	RSFV-901 (Sunflower variety)	2012	–	–	UAS, Raichur
4.	RSFH-1887	2015	CMS-38A	R-127-1	UAS, Raichur
5.	KBSH-44	2002	CMS-17A	RHA95 C-1	GKVK, Bangalore
6.	KBSH-53	2008	CMS-335 A	RHA 95 C-1	DOR, Hyderbad
7.	DRSH-1	2006	ARM-243A	6D-1	DOR, Hyderbad

RESULTS AND DISCUSSION

Characterization and identification of cultivars are crucial for varietal release and in seed production programme. It is mandatory to maintain the genetic purity of hybrid seed for the successful crop production. Unequivocal of characteristic patterns of hybrids can be obtained using DNA fingerprinting. Use of markers to obtain genotype specific profile

Table 2
List of microsatellite primers used in the study

1.	ORS-287	16.	ORS-324	31.	ORS-677
2.	ORS -290	17.	ORS-332	32.	ORS-769
3.	ORS-296	18.	ORS-333	33.	ORS-780
4.	ORS-300	19.	ORS-339	34.	ORS- 807
5.	ORS-301	20.	ORS-337	35.	ORS- 811
6.	ORS-309	21.	ORS-358	36.	ORS- 852
7.	ORS-310	22.	ORS-378	37.	ORS- 930
8.	ORS-311	23.	ORS-388	38.	ORS- 938
9.	ORS-315	24.	ORS-407	39.	ORS- 959
10.	ORS-316	25.	ORS- 484	40.	ORS- 1068
11.	ORS-318	26.	ORS-546	41.	ORS- 1088
12.	ORS-319	27.	ORS-552	42.	ORS- 1159
13.	ORS-321	28.	ORS-578	43.	ORS- 1220
14.	ORS-322	29.	ORS- 628	44.	ORS- 1245
15.	ORS-323	30.	ORS- 671		

has advantage over morphological and biochemical methods, as morphological markers are influenced by environmental conditions and isozymes are less polymorphic in nature [8]. However, DNA markers overcome most of these disadvantages of morphological and biochemical marker that can be useful to distinguish hybrids, its potential lines and off-types. The usefulness of DNA fingerprinting technique for cultivar identification was demonstrated by Dallas [3] for first time in rice. In this paper we report the identification of sunflower hybrid RSFH-1887 and its parents based on unique banding pattern.

The molecular fingerprinting of the hybrids obtain parental lines assume utmost importance for protecting the plant breeder's rights on them and ensuring their genetic purity. It is proved that this technique can be successfully applied to distinguish and identify hybrids from their parents and other hybrids. Besides, SSR has more polymorphism than most other DNA markers and is co-dominant. Therefore, the high polymorphic information content of SSR has promoted the application of microsatellite as a molecular marker in fingerprinting of crop varieties [2].

Among these hybrids and parental lines studied hybrids RSFH-1887 could be distinguishable from parental lines using specific SSR-primers. Based on complementary banding patterns between hybrids and their parents, The SSR marker ORS-484 was identified as a specific marker to identify hybrid RSFH-1887 from their parental lines and distinguish from other hybrids.

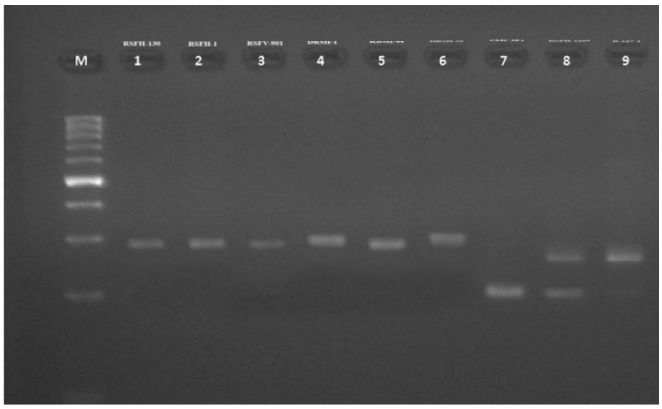

Plate 1 : SSR profile of Sunflower hybrids from ORS-484 marker

M: 100bp marker; Lane 1: RSFH-130; Lane 2: RSFH-1; Lane 3: RSFV-901; Lane 4: DRSH-1; Lane 5: KBSH-44; Lane 6: KBSH-53; Lane 7: CMS-38A; Lane 8: RSFH-1887 and Lane9: R-127-1

The ORS-484 marker shown a band of specific allele of a size 120bp in F1 hybrids, seed parent (CMS-38A) but not on its pollen parent (R-127-1) and the same marker ORS-484 amplified a allele of size 160bp in pollen parent (R-127-1) which restores fertility in male sterile parent and band of same size is also expressed in F1 hybrid but not on its female parent. Thus, these two bands indicates that 120bp band is very specific to female parent (CMS-38-A) and a band of 160bp is specific to male parent (R-127-1). Therefore the presence of both bands of male and female parental alleles in hybrid RSFH-1887 is the result of crossing between two parents and is highly specific hybrid RSFH-1887 which is not observed in other tested hybrids. The unique banding pattern indicates distinctness and novelty of a newly released hybrid RFSH-1887 and the F1 hybrid confirms heterozygosity by having two parental bands. The same ORS-484 marker shown different bands in the range of 180 to 195bp for other hybrids RSFH-1, RSFH-130, RSFV-901, KBSH-44, KBSH-53, and DRSH-1 tested (Plate 1). All these results suggests that SSR markers provide information for the identification of sunflower hybrid genotypes [6].

Morphological Characterization of Newly Released Hybrid RSFH-1887

The F1 hybrid has shown an average plant height of 175-190 cm almost similar to that of its female parent, where as its male parent dwarf in nature about only 120-140 cm (Table 3).

The RSFH-1887 has many distinguishable morphological characters of such as non branching type, very dark green head and pendulus bending at maturity which can easily used to distinguish from other varieties/hybrids. These morphological character will provide a unique identity to the hybrids and can be easily identified and distinguished from the other genotype during seed production and also helpful for rouging off any off-types and admixture plants.

Branching habit in sunflower determines plant type of particular line or parent branching directly or indirectly influences the yielding ability of the genotypes [5]. The male parent (R-127-1) of the hybrid is highly branched with central head branches terminating into small auxillary flowers but the

Table 3
Morphological descriptors of hybrid RSFH-1887 and its parent.

Sl. No	*Character*	*Female parent*	*Hybrid*	*Male / Restorer parent*
	CMS-38A	*RSFH-1887*	*R – 127-1*	
1.	Plant height (cm)	160-190	175-190	120-140
2.	Morphology			
	(a) Plant type	Non branching	Non branched, vigorous and tall	Fully branched with central head, branches terminating in to small auxiliary flowers
	(b) Leaf colour	Dark green	Dark green	Dark green
	(c) Leaf shape	Cordate and broad	Cordate and large	Cordate and medium
	(d) Leaf margin	Medium serration	Medium serration	Medium serration
	(e) Leaf apex	Slightly acute	Slightly acute	Slightly acute
	(f) Head shape	Flat to convex	Flat to convex	Flat
	(g) Head size (cm)	16-18 Medium	17-21	10-12 Small
	(h) Inflorescence	Mono head	Mono head	Multiple heads with auxiliary branches main head slightly bigger in size
	(i) Ray florets	Yellow and ovate type	Yellow and ovate	Yellow and ovate type
	(j) Disc Florets	Male sterile and stigma slightly pigmented	Male fertile	Male fertile produces abundant pollen and stigma slightly pigmented
	(k) Days to 50% flowering	58–63 days	58-62	60–65 days
	(l) Days to maturity	90–95 days	95-100	95–100 days
	(m) Seed size and colour	Bold and black	Black	Small, black with few stripes
3.	Synchronization of parents	To be planted 2 days after to male parent planting	–	To be planted 2 days earlier to female line planting
4.	Planting ratio	3	–	1
5.	Reaction to disease	Tolerant to viral necrosis and *Alternaria* leafspot disease	Tolerant to viral necrosis and *Alternaria* leaf spot	No major disease incidence
6.	Reaction to pest	No major damage	No severe damage	No major damage

hybrid RSFH-1887 produced using this as a male parent is of non branching type which is similar to its female parent CMS-38A. The hybrid has cordate leaf shape with large leaf size which is similar to both parents with respect to its shape however, hybrid has larger leaf compared to parents indicating expression of heterosis.

Head diameter is more heterotic than that of the both parents which is important for increased seed yield in hybrid. The female and male parent has an head size of 16-18 cm (medium) and 10-11cm(small), respectively but the hybrid has an average head size of 17-21cm which is larger than both parents. Both parents and hybrid belong to same maturity group of medium as per DUS guidelines.

The newly released hybrid requires 58-62 Days for 50% per cent flowering which is on par with that of its female parent which is having 58-63 days for 50 per cent flowering but male parent requires about a 60-65 days for 50% flowering. To ensure proper and complete seed setting male parent has to be planted two days earlier to that of female parent due to its delay in flowering.

CONCLUSION

Sunflower hybrid could be distinguishable clearly from their parental lines and other public sector hybrids using SSR marker. The microsatellite marker ORS-484 can be used as specific marker for identification of RSFH 1887 as well for its protection. The hybrids and parental lines showing similar morphological traits could be distinguished at molecular level using appropriate DNA marker. The study demonstrates the possibility of establishing a standard set of primers to distinguish and characterize different released hybrids, which can be used for their protection through DNA fingerprinting.

REFERENCES

Anonymous, (2015), *Director's Rep. on Sunflower*, Indian Institute of Oilseed Research, Hyderabad, pp. 14-15.

Ashikawa, I., Kurata, N., Saji, S., Umehara, Y., and Sasaki, T., (1999) Application of restriction fragment fingerprinting with a rice microsatellite sequence to assembling rice YAC clones. Genome **42**: 330–337.

Dallas, J.F., (1988), Detection of DNA "fingerprints" of cultivated rice by hybridization with a human minisatellite DNA probe. Proc. Nad. Acad. Sci. USA, **85**: 6831-6835.

Kinman, M. L., (1970), New development in the USDA and State Experiment Station, sunflower breeding programme. In: *Proc. of the Fourth Int. Sunflower Conference,* Memphis, Tennessa, pp. 181-183.

Kulkarni, V. V., Shankergoud, I., and Govindappa, M, R., (2015), Evaluation and characterization of sunflower germplasm accessions for quantitative characters. Electronic journal of Plant Breeding., **6**(1): 257-263.

Lawson, W. R., Henry. R. J., Kochman, J. K. and Kong, G.A., (1994), Genetic diversity in sunflower (*Helianthus annus* L.) as revealed by random amplified polymorphic DNA analysis. *Aust. J. Agric. Res.* **45**: 1319-1327.

Leclercqe, P., (1969), Line sterile cytoplasmique chezk tournesol. *Ann. Amelior Planta,* **12**: 99-106.

Lucchese, C., Dinelli, G., Miggiano, A. and Lovato, A., (1999), Identification of pepper (*Capsicum* spp.) cultivars by field and electrophoresis tests. Seed Science and Technology **27**: 37-47.

■■■