The Bioscan 10(2): 773-779, 2015 (Supplement on Plant Pathology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

MOLECULAR FINGERPRINTING AND ASSESSMENT OF GENETIC PURITY OF SUNFLOWER *(HELIANTHUS ANNUUS* L.) HYBRIDS BY USING RAPD AND SSR MARKERS

S. M. BHOSLE[1], R. L. CHAVHAN[1]*, V. R. HINGE[1] AND M. K. GHODKE[2]
[1]Department of Plant Biotechnology,
College of Agricultural Biotechnology, Marathwada Krishi Vidyapeeth, Parbhani - 431 402
[2]Oilseed Research Station, Nanded Road, Latur - 413 512
e-mail: rlchavhan@gmail.com

KEYWORDS
Fingerprinting
Genetic purity
Grow out test
Sunflower hybrids
RAPD
SSR

Received on :
16.11.2014

Accepted on :
10.06.2015

***Corresponding author**

ABSTRACT
The investigation pertaining to the hybrid identification in sunflower (*Helianthus annuus* L.) through RAPD and SSR analysis was conducted on six genotypes and their hybrids. Among fifty three primers comprising 25 RAPD and 18 SSR primers, 2 primers for LSFH-10128 (ORS5 and OPA11), 3 for LSFH-1706 (ORS662, OPC16, OPE16) and 2 for LSFH-7345 (ORS5 and OPA03) were showing both female and male parent specific markers and identified as co-dominant marker for hybrid identification. The genetic relationship study among hybrid and their parental lines revealed their close relationship with 92.0 % to 78.7 % similarity. The parental line CMS-17A was found out grouped at 42 % dissimilarity from members of other clusters in RAPD data. And male parent J/6 was found genetically more divergent and showed 21 % dissimilarity with cluster I and II in SSR data. Thus data analysis could indicate the close similarity of hybrid individual/offspring with their respective parent is the indicative of confirmation of hybrids. This study suggested that molecular marker analysis can be utilized for both reliable and less time consuming identification of hybrids.

INTRODUCTION

Sunflower (*Helianthus annuus* L.), a member of compositae family, is a significant staple oilseed crop of special economic importance in an oilseed industry worldwide. The oilseed revolution was mainly attributed to the development and adoption of high yielding varieties and hybrids. The success of this hybrid seed technology depends on the timely production and adequate supply of genetically pure hybrid seeds to the farmers. Conventionally, genetic purity of hybrids is ensured by grow-out test (GOT), The GOT is an expensive, time consuming procedure, influenced by environmental factors and results are skewed. Hence it is essential to develop a more rapid, accurate and cost-effective method for the identification of sunflower hybrids.

DNA marker technology offer an efficient alternative to this approach which provide powerful tools for cultivar identification and seed quality control in various crops with advantages of time-saving, less labour consumption and more efficiency (Liu *et al.,* 2007; Garg *et al.,* 2006). Genetic purity of hybrid is an essential requirement for its commercial success, conventionally genetic purity testing was done through GOT, which requires one full season and expenditure in storage and hence increased hybrid seed cost. Considering the disadvantages of conventional method of hybrid identification methods, molecular marker based method could be a better alternative. The RAPD and SSR marker system has been previously utilized as simple, safe and cost effective method for molecular analysis (Bellester and de Vicente, 1998). RAPD and SSR markers are widely used in genetic diversity which useful in selection diverse parental combination to acquire better heterosis pattern in hybrids (Bahurupe *et al.,* 2013, Chandra *et al.,* 2013, Joshi *et al.,* 2013, Zala *et al.,* 2014). Hybrid identification in crop species through DNA fingerprinting is an effective tool to increase the speed and quality of backcrossing conversion, thus reducing time taken to produce crop varieties with desirable characteristics (Ali *et al.,* 2008). RAPD and SSR, molecular markers based hybrid purity tests have been developed and are in routine use in many species such as Rice (Yashitola *et al.,* 2002; Sundaram *et al.,* 2008), Sorghum (Arya *et al.,* 2014), Sunflower (Pallavi *et al.,* 2011), Tomato (Liu *et al.,* 2007), Maize (Asif *et al.,* 2006; Hipi *et al.,* 2013), Cotton (Dongre and Pakhi 2005; Ali *et al.,* 2008; Dongre *et al.,* 2011), Safflower (Naresh *et al.,* 2009), Pigeonpea (Saxena *et al.,* 2010), Watermelon (Kwon *et al.,* 2013) and Cassava (Mohan *et al.,* 2013). Being locus specific, PCR based co-dominant markers, SSR are the most suitable marker for hybrid identification as the hetrozygosity of the hybrids can be easily determined by the presence of both the parental alleles. Hence these marker systems could be effectively used for seed genetic purity testing and cultivar identification. Nevertheless, EST-SSR data have been

successfully developed in sunflower (Pashley *et al.,* 2006) and being exploited in development of SSR markers towards their application in genetic diversity analysis, marker assisted selection and hybrid confirmation studies. SSR marker technology has utilized for hybrid identification and assessment of genetic diversity in sunflower (Pallavi *et al.,* 2011. Keeping in view of these facts, present study was conducted to assess hybrid purity of three sunflower hybrids and their genetic relationship between hybrid and their parental lines by using molecular markers.

MATERIALS AND METHODS

Plant materials and genomic DNA extraction

The present investigation was carried out at Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, India. The plant material for this study comprised, three sunflower hybrids and their parents *viz.*, LSFH-10128 (CMS-343A X J/6), LSFH-1706 (CMS-17A X NDLR-06) and LSFH-7345 (CMS-7-1A X AK-345) developed at Oilseed Research Station (ORS), Latur, Marathwada Krishi Vidyapeeth, Parbhani. Features of the both parents and their hybrids has given in Table no. 1, 2 and 3 respectively.

Genomic DNA was extracted from the tender leaves of *in vitro* germinated seedlings using CTAB method (Doyle and Doyle 1987). The isolated DNA was assessed qualitatively and quantitatively using agarose gel electrophoresis and spectrophotometeric analysis, respectively. Further this DNA samples were used for setup RAPD and SSR reactions for identification and confirmation of hybrids.

RAPD analysis

A total of 25 RAPD primers were obtained from Operon Technologies (Eurofins MWG Operon Inc., USA). RAPD PCR was performed in thermocycler (Eppendorf). The PCR procedure followed the method of Yang and Park (1998) with slight modification. The reaction mixture consisted template genomic DNA of 25 ng in 25μl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2μM primers and 1.0 U of *Taq* DNA polymerase in 25μl reaction. Thermal profile followed was 94^{0}C for 7 min (initial denaturation), followed by 45 cycles of denaturation at 94^{0}C for 45 s, annealing at 36^{0}C for 1 min, extension at 72^{0}C for 1 min, and final extension was set at 72^{0}C for 10 min. The RAPD amplified products were separated on 1.5% agarose gel, stained with ethidium bromide (0.5μg/ml) and images were captured using alpha imager gel documentation system (Sambrook *et al.,* 1989). The size of amplification products were determined by comparing with 1 kb DNA ladder (Fermentas).

SSR analysis

Eighteen oligonucleotide primers for SSR analysis were synthesized by Imperial Life Science (ILS) as per microsatellite sequences reported (Liu *et al.,* 2004; Antonova *et al.,* 2006). The polymerase chain reaction (PCR) was carried out in 25μl volume consisting 20 ug template DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2μM primers and 0.5 U *Taq* DNA polymerase as given by Yang and Park 1998). Amplification was performed by using Eppendorf mastercycler gradient PCR programmed for initial denaturation at 94^{0}C for 5 min followed by 35 cycles consisting of denaturation at 94^{0}C for 30 sec, annealing at 50-60^{0}C for 30 sec, extension at 72^{0}C for 1 min and final extension was set at 72^{0}C for 10 min. The amplified products were separated on 3 % agarose gel and visualized.

Grow out trials

Conventionally genetic purity of hybrids is ensured by grow out test (GOT), which involves growing plants to the maturity and assessing several morphological and floral characteristics that distinguish the hybrids.

Three different hybrids and their respective male and female parent were grown in the greenhouse. Regular irrigation, fertilization and crop protection measures were adopted and purity through visual evaluation was conducted on the main important morphological characters throughout the growth period.

Cluster analysis

The microsatellite and RAPD amplified fragments were scored as present or absent of a binary character when 1 = present and 0 = absent. The Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) Version 2.02 (Rohlf 1990) was used to determine Jaccard's similarity coefficient (Jaccard 1908), Principal Co-ordinate analysis, also used for dendrogram preparation based on RAPD, SSR and RAPD + SSR data.

RESULTS

Hybrid identification

RAPD analysis

The polymorphic data obtained in RAPD fingerprint pattern were utilized as marker for hybrid identification. The RAPD profile of parents and their hybrids were correlated to find out direct introduction of character/ genes in to the hybrids through male or female parent by possessing male and female parent specific bands among RAPD profiles of three hybrids. Nevertheless, it was exploited to document polymorphism and relationship between three hybrids and their parents. In hybrid LSFH-10128, four primers could identify this hybrid by amplifying a common fragment between hybrid and female parent while 3 primers produced male parent specific amplicons. The primer OPA11 produced a female parent specific (FPS) marker of $OPA11_{1000}$ (1000bp) and a male parent specific (MPS) marker of $OPA11_{600}$ (600bp) simultaneously (Fig. 1). While in hybrid LSFH-1706 three primers produced

Table 1: Details of CMS lines

Sr.No.	CMS Lines	Feature	Source
1	7-1A & B	Susceptible to downy mildew and necrosis, high seed yield, big head diameter	DOR, Hyderabad
2	17 A & B	High seed yield, susceptible to downy mildew and necrosis, low oil content	AICRP, Bangalore
3	234 A & B	Early, high oil content, susceptible to downy mildew, necrosis and *alternaria*.	AICRP, Bangalore

Table 2: Details of Restorer lines (R lines)

Sr.No.	Restorer	Feature	Source
1	AK- 345	Multihead, high seed yield potential	ORS, Latur
2	NDLR-06	Monohead.	ORS, Latur
3	J-6	Monohead, downy mildew, necrosis and *alternaria* resistant (multiple resistance), High seed yield and high oil content	ORS, Latur

Table 3: Hybrids with their parents

Sr.No	Name of hybrid	Parents	source	Features
H6	LSFH-10128	343A X J/6	ORS, Latur	high seed yield
H9	LSFH-1706	17A X NDLR-06	ORS, Latur	seed yield
H10	LSFH-7345	7-1A X AK-345	ORS, Latur	seed yield

Table 4: Six types of SSR markers observed in hybrids and their parents

Type of markers	Male(M)	Hybrid(H)	Female(F)	No of polymorphic bands	Polymorphism(%)	Remark
1	+	+	-	9	500	* Hybrid confirm
2	+	-	+	0	00	Polymorphic parent
3	-	+	+	5	2777	* Hybrid confirm
4	+	-	-	2	1111	Polymorphic parent
5	-	+	-	1	555	Polymorphic parent
6	-	-	+	1	555	Polymorphic parent
Total	18					

Table 5: Similarity matrix based on Jaccard's similarity coefficient value obtained from RAPD analysis

Hybrid/Parent	CMS-343A	H1	J/6	CMS-17A	H2	NDLR-06	CMS-7-1A	H3	AK345
CMS-343A	100								
H1	085	100							
J/6	085	079	100						
CMS-17A	058	064	060	100					
H2	071	073	073	058	100				
NDLR-06	069	071	071	052	069	100			
CMS-7-1A	071	073	069	058	075	093	100		
H3	067	069	065	054	075	085	087	100	
AK345	075	081	073	054	075	077	079	079	100

female parent specific (FPS) bands, 6 primers produced MPS bands while two primers produced both FPS and MPS bands. The primer OPC16 could generate one FPS marker of $OPC16_{1000}$ (1000 bp) and 3 MPS marker of $OPC16_{850}$ (850 bp), $OPC16_{1500}$ (1500 bp) and $OPC\ 16_{2000}$ (2000 bp) simultaneously (Fig.2 a). The primer OPE16 also could generate a FPS marker of $OPE16_{1000}$ (1000 bp) and 3 MPS marker of $OPE16_{250}$ (250 bp), $OPE16_{300}$ (300 bp) and $OPE16_{1100}$ (1100 bp) simultaneously (Fig.2 b). In hybrid LSFH-7345, four primers produced FPS bands, 5 primers produced MPS bands and one primer OPA03 could produce 1 FPS marker of $OPA03_{700}$ (700 bp) and 1 MPS marker of $OPA03_{500}$ (500 bp) (Fig.3).

SSR analysis

In present investigation among 18 SSR primers exploited for assessment of purity of three sunflower hybrids, seven primers were found polymorphic while six primers have generated monomorphic amplicons and remaining seven have shown non-specific amplification. Among microsatellite primers six different types of SSR polymorphic marker pattern had been identified which could help to identify purity of hybrids (Table 4)

Out of six polymorphic SSR primers three primers produced co-dominant markers pattern for three hybrids. LSFH-10128 produced polymorphic bands with two primers ORS5 is shown the presence of both female and male parent specific markers in hybrid individuals. The hybrid LSFH-10128 was identified by primer ORS5 through generating both FPS marker $ORS5_{300}$ (300 bp) and a MPS marker of $ORS5_{330}$ (330 bp) (Fig. 4).

However in hybrid LSFH-1706, a primer ORS662 could generate a FPS marker of $ORS662_{220}$ (220 bp) and a MPS marker of $ORS662_{320}$ (320 bp) simultaneously (Fig.5) and confirmed the identity of this hybrid individual. ORS5 was identified polymorphic marker in hybrid LSFH-7345 also, as it has shown different FPS and MPS marker compare to LSFH-10128. ORS5 amplified a FPS marker of $ORS5_{330}$ (330 bp) and a MPS marker of $ORS5_{310}$ (310 bp) (Fig.6). While microsatellite marker Ha1327 could produce only FPS marker of $Ha1327_{200}$ (200 bp) in hybrid LSFH-7345. On an average considering percent polymorphic pattern, the male and female parent specific banding pattern was found 50% and 27.77% sequentially between parent and offspring. Present study shows that the higher degree of similarity between male parent and offspring/hybrid compared to female parent and offspring along with male specific bands of Type I markers is an indication of successful cross and true hybrids (Table 4).

Table 6: Similarity matrix based on Jaccard's similarity coefficient value obtained from SSR analysis

Hybrid/Parent	CMS-343A	H1	J/6	CMS-17A	H2	NDLR-06	CMS-7-1A	H3	AK345
CMS-343A	100								
H1	085	100							
J/6	081	081	100						
CMS-17A	069	085	073	100					
H2	085	085	081	085	100				
NDLR-06	085	085	081	077	092	100			
CMS-7-1A	088	081	069	073	088	088	100		
H3	067	069	065	054	075	085	087	100	
AK345	088	088	085	081	081	073	077	088	100

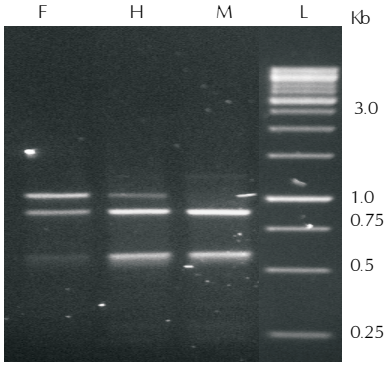

Figure 1: RAPD analysis of LSFH-10128 with identified primer OPA11 Lane F- Female parent, M- Male parent, H- Hybrid and L- 1 kb ladder. The arrow indicates male and female parent specific markers

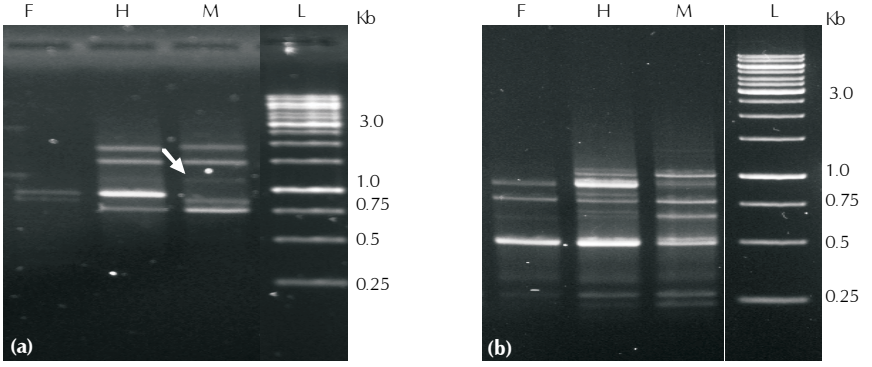

Figure 2: RAPD analysis of LSFH-1706 with identified primers OPC16 (a) and OPE 16 (b) Lane F- Female parent, M- Male parent, H- Hybrid and L- 1 kb ladder. The arrow indicates male and female parent specific markers

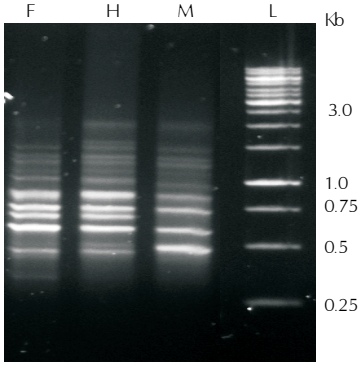

Figure 3: RAPD analysis of LSFH-7345 with identified primer OPA03 and Lane F- Female parent, M- Male parent, H- Hybrid and L- 1 kb ladder. The arrow indicates male and female parent specific markers

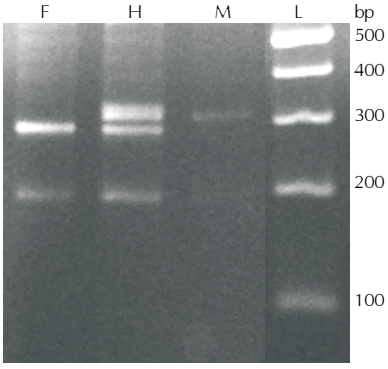

Figure 4: SSR analysis of LSFH-10128 with identified primer ORS5

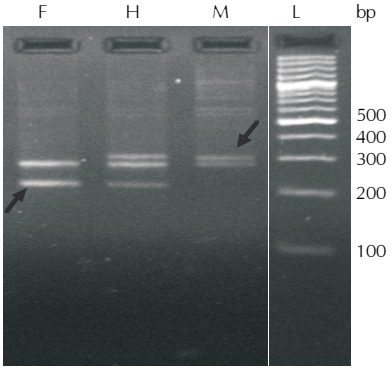

Figure 5: SSR analysis of LSFH-1706 with identified primer ORS662

Hybrid Authentication by genetic similarity coefficients and cluster analysis

The 25 random primers and 18 SSR primer generated DNA fingerprint pattern of three sunflower hybrid and their parents were assessed for genetic similarity analysis and hybrid identification. By using 25 random primers a total of 322 RAPD amplicons were generated. The RAPD fingerprint data were used to estimate genetic similarity on the basis of number of shared amplification products which were denoted by cluster dendrogram (Fig. 7) and coefficient of similarity matrix (Table 5). Based on cluster dendrogram generated through similarity matrix obtained with unweighted pair group method (UPGMA), three hybrids and their parents were categorized into three major clusters (Fig. 7). Cluster I comprised hybrid LSFH-10128 and their parents (CMS-343A and J/6) together showing more than 83% similarity with each other. The hybrid

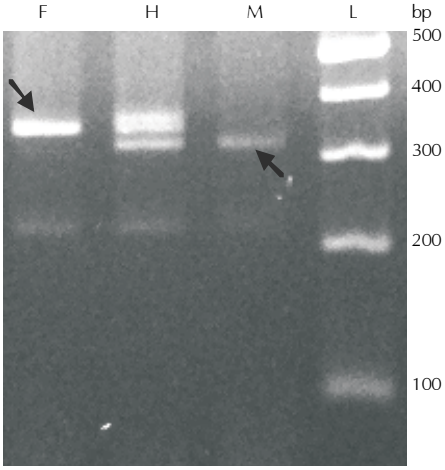

Figure 6: SSR analysis of LSFH-7345 with identified primer ORS5 Lane F- Female parent, M- Male parent, H- Hybrid and L- 50 bp ladder. The arrow indicates male and female parent specific markers

LSFH-10128 showed 85 % similar pattern with female parent CMS-343A. While group II comprised Hybrid LSFH-7345 and their parents (CMS-7-1A and AK-345) together with 78.7% similarity. However, the hybrid LSFH-1706 had shown 75 % similarity with hybrid LSFH-7345 and categorized into the same cluster. The parental line CMS-17A was found more divergent and has been isolated into separate cluster III showing 58 % similarity with members of other clusters.

Similarly 18 SSR primers could generate 181 amplicons and based on SSR fingerprint data analysis sunflower hybrids and their respective parents were categorized into three major clusters (Fig. 8). Cluster I comprised hybrid LSFH-1706 (H2 and parental line NDLR06, CMS-7-1A and CMS-343A in one group showing genetic similarity in the range of 88.6% to 92.0 %. The hybrid LSFH-1706 (H2 has showed 92% similarity with its male parent NDLR06). Cluster II pertained hybrid LSFH-10128, LSFH-7345 and parental line AK-345 and CMS-17A together showing 83.6% similarity. The genetic similarity between hybrids LSFH-10128, LSFH-7345 was 92.0 %. However male parent J/6 was found genetically more divergent and showed 79% similarity with members of cluster I and II. Thus data analysis could indicate the close similarity of hybrid individual/offspring with their respective parent is the indicative of confirmation of hybrids.

Grow out test of the sunflower hybrids

In the grow out trials, purity assessment was conducted on morphological traits including shoot length, plant height, leaf shape, number of whorl of achene per head, anthocyanin pigmentation etc. the characters of LSFH-10128 such as anthocyanin pigmentation, indeterminate growth, heart shaped leaves were much similar to those of male parental line and which was also supported by molecular marker assessment.

Hybrid LSFH-1706 and LSFH-7345 were assessed on field based observation. The plant of these hybrids individuals showed characters like pigmentation, erect shoot rounded, heart shaped leaves with serrate margin except large leaves in case of LSFH-7345. These characteristic features was much similar to their respective male and female parents which shown consonance with molecular testing of purity.

DISCUSSION

In the present study, DNA molecular marker systems *viz.*, RAPD and SSR were employed for sunflower hybrid identification and genetic relationship among hybrids and their respective parents. To the exploitation of full potential of heterosis and enhancement of hybrid development it is essential to characterize parental lines at genetic as well as morphological level. The genetic divergence study helps breeder to concentrate on possible promising parent combinations. To obtain high yield, exploitation of heterosis is a good way (Makani *et al.*, 2013). In RAPD and SSR fingerprinting, parental line CMS-17A was found more divergent showed 58 % similarity with members of other clusters in RAPD data and male parent J/6 was found genetically more divergent and

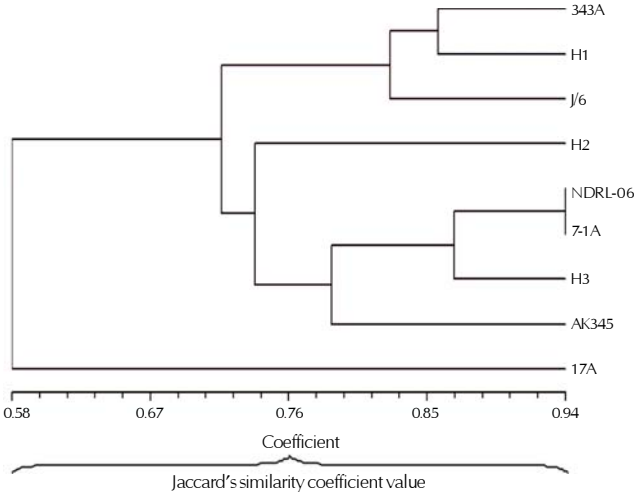

Figure 7: Dendrogram constructed using UPGMA based on 25 RAPD markers demonstrating relationship among three hybrids with their parents based on Jaccard's similarity coefficient value

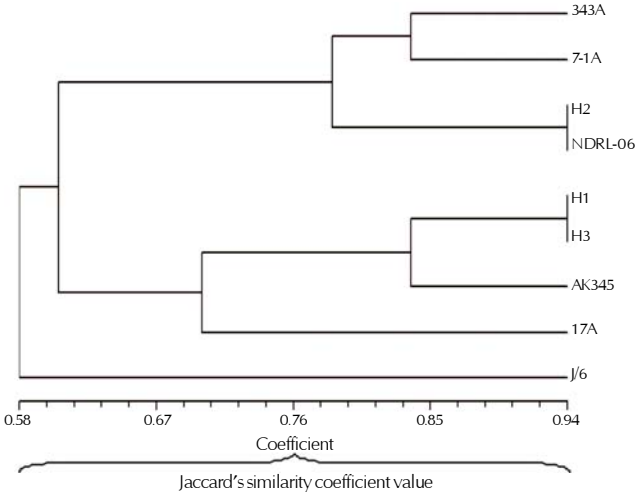

Figure 8: Dendrogram constructed using UPGMA based on 18 SSR primers demonstrating relationship among three hybrids with their parents based on Jaccard's similarity coefficient value

showed 79 % similarity with cluster I and II in SSR data. Therefore adequate genetic diversity information in parental lines should be useful for selecting crossing parental genotypes that may enhance the hybrid vigour.

Among 25 RAPD and 18 SSR primers used, 2 primers for LSFH-10128, 3 for LSFH-1706 and 2 for LSFH-7345 were identified as codominant marker for hybrid identification. The hybrid nature of progeny from cross between *H. rigidus* and *H. annuus was* confirmed by RAPD markers, which also revealed considerable variability between F_1 and BC_1F_1 plants (Pankovic, 2007)

Considering the residual hetrozygosity in parental line and overall purity it can be concluded that dominant RAPD marker was found an optimal method for the purity testing of three sunflower hybrids. Similarly RAPD marker was significantly utilized in purity assessment of hybrids in various crops (Bellester and de Vicente 1998, Liu *et al.,* 2007, Hashizume *et al.,* 1993, Ali *et al.,* 2008, Ilbi 2003 and Akhare *et al.,* 2008). As for purity testing in many crops whose genome were not well studied RAPD marker with universal primers could be of good choice.

SSR marker was another good tool for hybrid identification test. In present study two SSR markers (ORS5 and ORS662 could successfully identified three sunflower hybrids by producing both male and female parent specific markers. Some of SSR markers (Ha1327, Ha1442, ORS13, ORS5, ORS536, ORS243, and ORS662 could produce either of female or male parent specific bands. It is due to of residual hetrozygosity could accounts for the occasional occurrence that some true hybrids exhibited the absence of female or male parent specific markers (Liu *et al.,* 2007). Similarly, the hetrozygosity of hybrids can be easily determined by the presence of both parental alleles (Naresh *et al.,* 2009). A few SSR primers were developed in sunflower and readily used for purity testing of hybrids (Antonova *et al.,* 2006, Kumar *et al.,* 2009 and Pallavi *et al.,* 2011. The use of SSR markers for assessing seed purity of hybrids is almost routine for several crops example Rice (Yashitola *et al.,* 2002, and Nandakumar *et al.,* 2004), Safflower (Naresh *et al.,* 2009), Cotton (Ali *et al.,* 2008, Dongre *et al.,* 2011 and horticultural crops like Tomato (Smith and Register 1998 and Paran *et al.,* 1995), Cabbage (Liu *et al.,* 2004), and Melon (Kwon *et al.,* 2013, Jianli *et al.,* 2006 and Hashizume *et al.,* 1993). Although some studies reported the suitability of even single marker for hybrid purity assessment tests (Yashitola *et al.,* 2002 and Nandakumar *et al.,* 2004). The present study reports suitability of SSR markers for hybrid identification test of sunflower. Also the co dominant SSR marker system was found more informative and effective over RAPD marker system.

ACKNOWLEDGEMENT

The authors are gratefully acknowledged to the Associate Dean and Principal, College of Agricultural Biotechnology, Latur for their constant support during the study period.

REFERENCES

Akhare, A., Sakhare, S. B., Kulwal, P. L., Dhumale, D. B. and Abhilasha, K. 2008. RAPD profile studies in sorghum for identification of hybrids and their parents. *Internat J. Integ. Biol.* **31:** 18-24.

Ali, M. A., Muhammad, T. S., Awan, S., Shahid, N., Shiraz, A. and Abbas, A. 2008. Hybrid authentication in upland cotton through RAPD analysis. *Australian J. Crop Science*. **2:** 141-149.

Antonova, T. S., Gucheti, S. Z., Ramasanova, T. A. and Ramasanova, S. A. 2006. Development of markers system for identification and certification of sunflower lines and hybrids on the basis of SSR-analysis. *Helia*. **29:** 63-72.

Arya, L., Verma, M. and Lakhanpaul, S. 2014. Diagnostic set of microsatellite markers for hybrid purity testing and molecular identification of hybrids and parental lines in sorghum. *J. Plant Sci. Res.* **1:** 103.

Asif, M., Rahman, M. U. and Zafar, Y. 2006. Genotyping analysis of six maize (*Zea mays* L) hybrid using DNA fingerprinting technology. *Pakistan J. Bot.* **38:** 1425-1430.

Bahurupe, J. V., Sakhare, S. B., Kulwal, P. L., Akhare, A. A. and awar, B. D. 2013. Genetic diversity analysis in chilli (*Capsicum anuum* L.) using RAPD markers. *The Bioscan*. **8(3):** 915-918.

Bellester, J. and de Vicente, M. C. 1998. Determination of F_1 hybrid seed purity in pepper using PCR-based markers. *Euphytica*. **103:** 223-226.

Chandra, V., Usha Pant, Ram Bhajan and Singh, A. K. 2013. Studies on Genetic Diversity Among Alternaria Blight Tolerant Indian Mustard Genotypes Using SSR Markers. *The Bioscan*. **8(4):** 1431-1435.

Dongre, A. and Pakhi, V. 2005. Identification of cotton hybrid through the combination of PCR based RAPD, ISSR and microsatellite markers. *J. Plant Biochem Biotechno.* **14:** 53-55.

Dongre, A. B., Raut, M. P., Bhandarkar, M. R. and Meshram, K. J. 2011. Identification of genetic purity testing of cotton F_1 hybrid using molecular markers. *Ind. J. Biotechnol.* **10:** 301-306.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*. **19:** 11-15.

Garg, A., Singh, A. K., Parbhu, K. V., Mohapatra, T., Tyagi, N. K., Nandkumar, N., Singh, R. and Zaman, F. U. 2006. Utility of a fertility restorer gene linked marker for testing genetic purity of hybrid seeds in rice (*Oryza sativa* L.). *Seed Sci. Technol.* **34:** 9-18.

Hashizume, T., Sato, P. and Hirai, M. 1993. Determination of genetic purity of hybrid seed in watermelon (*Citrullus lanatus*) and tomato (*Lycopersicon esculentum* L) using random amplified polymorphic DNA (RAPD). *Japanese J. Breed.* **43:** 367-375.

Hipi, A., Surahman, M., Ilyas, S. and Giyanto 2013. Seed genetic purity assessment of maize hybrid using microsatellite markers (SSR). *Int. J. Appl. Sci. Technol.* **3:** 66-71.

Ilbi, H. 2003. RAPD marker assisted varietal identification and genetic purity test in pepper (*Capsicum annum* L). *Scientia Hortic.* **97:** 211-218.

Jaccard, P. 1908. Nourvelles recherche sur la distribution florale Bull Soc Vaud. *Sci Na*. **44:** 223-270.

Jianli, L., Xiaohua, S., Shouchunn, Z. and Jianshe, W. 2006. Purity test of melon hybrid with SSR molecular markers. *Mol. Plant Breed.* **4:** 23-26.

Joshi, M. Verma, S. K., Singh, J. P. and Barh A. 2013. Genetic Diversity Assessment in Lentil (Lens Culinaris Medikus) Genotypes Through ISSR Marker. *The Bioscan*. **8(4):** 1529-1532.

Kumar, D., Haritha, V. B., Anusha, S. and Ashfaq, M. A. 2009. SCAR and RAPD markers for genetic purity assessment of sunflower hybrid DRSH1. *J. Oilseeds. Res.* **26:** 192-194.

Kwon, Y. S. 2013. Use of EST-SSR markers for genetic characterization of commercial varieties and hybrid seed purity testing. *Seed Sci. Technol.* **41:** 254-256.

Liu, L. W., Hou, X. L., Gong, Y. Q., Zhang, Y. M., Wang, K. R. and Zheng, J. F. 2004. Application of molecular marker in variety identification and purity testing in vegetable crops. *Mol Plant Breed.* **2:** 563-568.

Liu, L. W., Wanga, Y., Gonga, Y. Q., Zhaob, T. M., Liua, G., Lia, X. Y. and YUC, F. M. 2007. Assessment of genetic purity of tomato (*Lycopersicon esculentum* L.) hybrid using molecular markers. *Sci. Hortic, (Amsterdam).* **115:** 7-12.

Makani, A. Y., Patel, A. L., Bhatt, M. M. and Patel, C. C. 2013. Heterosis for yield and its contributing attributes in brinjal (*Solanum melonhena* L.). *The Bioscan.* **8(4):** 1369-1371.

Mohan, C., Shanmugasundarami, P. and Senthil, N. 2013. Identification of true hybrid progenies in cassava using simple sequence repeats (SSR) markers. *Bangladesh J. Bot.* **42:** 155-159.

Nandakumar, N., Singh, A. K., Sharma, R. K., Mohapatra, T., Prabhu, K. V. and Zaman, F. U. 2004. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica.* **136:** 257-264.

Naresh, V., Yamini, K. N., Rajendrakumar, P. and Dinesh Kumar, V. 2009. EST-SSR marker-based assay for the genetic purity assessment of safflower hybrids. *Euphytica.* **170:** 347-353.

Pallavi, H. M., Rame, G., Shadakshari, Y. G., Bhanuprakash, K. and Vishwanath, K. 2011. Identification of SSR markers for hybridity and seed genetic purity testing in sunflower (*Helianthus annuus* L.). *Helia,* **34:** 59-66.

Pankovic, D. S. 2007. Application of molecular markers in sunflower breeding. *Genetika.* **391:** 1-11.

Paran, I., Horowitz, M., Zamir, D. and Wolf, S. 1995. Random Amplified Polymorphic DNA marker is useful for purity determination of tomato hybrids. *Hortscience.* **30(2):** 377.

Pashley, C. H., Ellis, J. R., McCauley, D. E. and Burke, J. M. 2006. EST databases as a source for molecular markers: lessons from *Helianthus*. *J. Hered.* **97:** 381-388.

procedure in *Capsicum annuum* L. *Korean J. Breed.* **30:** 204-211.

Rohlf, F. J. 1990. NTSYS-pc Numerical and multivariate analysis system version 2.02. *Applied Biostatistics New York.*

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold

Saxena, R. K. and Saxena, K. 2010. Application of SSR markers for molecular characterization of hybrid parents and purity assessment of ICPH 2438 hybrid of pigeonpea. *Mol Breed.* **26:** 371-380.

Smith, J. D. C. and Register, J. C. III. 1998. Genetic purity and testing technologies for seed quality: a company perspective *Seed. Sci Res.* **8:** 285-293.

Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sundaram, R. M., Naveenkumar, B., Biradar, S.K., Balchandran, S. M., Mishra, B., IlyasAhmad, M., Viraktamath, B. C., Ramesha, M. S. and Sarma, N. P. 2008. Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *Euphytica.* **163:** 215-224.

Yang, T. J. and Park, H. G. 1998. Optimization of the random amplified polymorphic DNA analyses

Yashitola, J., Thirumurugan, T., Sundaram, R. M., Naseerullah, M. K., Ramesha, M. S., Sarma, N. P. and Ramesh, V. S. 2002. Assessment of purity of rice hybrids using microsatellite and STS markers. *Crop Science.* **42:** 1369-1373.

Zala, H. Bosamia, T., Kulkarni, K., and Shukla Y. 2014. Assessment of Molecular Diversity in Wheat (Triticum aestivum L. and Triticum durum L.) Genotypes Cultivated in Semi-Arid Region Of Gujarat. *The Bioscan.* **9(2):** 731-737.