Original Research Article

Genetic purity testing of F_1 hybrids of cotton using DNA markers

ABSTRACT

Cotton is one of the most important fiber crops of the world. Traditionally it has been the practice to carry out Grow-Out-Tests (GOT) to assess the genetic purity of hybrid seeds using morphological characters and is not always apparent and cannot be recognized easily. The recent developments in molecular biology have resulted in simple and easily assayable PCR based DNA marker techniques such as RAPD, ISSR and microsatellite markers which provide alternative approach for assessment of seed purity. Out of twenty-six primers only seven primers, namely BNL 2709, NAU 1190, BNL 3594, JESPR 297, NAU 1230, BNL 3995 and JESPR 151 were polymorphic between the parents for only five hybrids and were then used for genetic purity assessment of the five hybrids, namely NH 324, SVHH 139, RAHH 455, Super 971 BG II and NHH 715 and none of the 26 tested primers was suitable for assessing the genetic purity of Phule Suman hybrid. It can be concluded from the study that SSR markers are reliable, efficient and useful in discriminating the parental lines of the hybrids as well as for evaluating the genetic purity of hybrid seed lot. This will empower seed producers to screen and keep up adequate levels of genetic purity at every stage of seed production.

INTRODUCTION

Assessment of genetic purity of F_1 hybrids is a quality control requirement of most importance in plant breeding and seed production. Genetic purity is used by seed producers as a quality assurance tool to identify out-crosses, selves (where applies), seed mixes, seed swaps and regulatory compliance testing. Maintaining genetic purity is of utmost importance and keeping uniformity to meet the demands of farmers, processors and consumers, precisely characterize and measure genetic diversity for PGR management, intellectual property protection and predicting agronomic performance enables farmers to exploit the full potential of hybrids. Genetic purity

test is conventionally conducted by Grow-Out-Test (GOT) to record morphological traits of a cultivar (Selvakumar *et al.,* 2010), but it is time consuming and also limited variation observed for morphological characters make it difficult for varietal identification and has great disadvantage in commercial seed production. Field based testing for genetic purity assessment is being replaced by DNA based molecular markers as an alternative method. The DNA markers have several advantages over morphological traits, as the latter are the resultant of genotype and environmental interactions, while, DNA markers are resultant of only genotype of the cultivar and are independent of the environmental interactions (Selvakumar *et al*., 2010). Molecular marker techniques offer valuable alternative for genetic purity analysis (Selvakumar *et al*., 2010; Dongre*et al*., 2011; Kumar *et al.,* 2015; Rao *et al*., 2015; Sudharani *et al*., 2015) which are very fast, reliable and require less quantity of tissue for experiment (Rana *et al.,* 2006). DNA markers such as RFLP (Dongre and Parkhi. 2005), RAPD (Satish Kumar *et al.*, 2016; Rashid Minhas *et al.,* 2014), AFLP (Rana and Bhat, 2004), SSR (Rana 2003; Dongre and Parkhi, 2005) and ISSR (Rana *et al.,* 2006) have been utilized in different crops including cotton for quick screening of genetic purity of hybrid seed lots.

MATERIALS AND METHODS

The experiments for the present study were performed in the Division of Genomic Resources, ICAR-NBPGR, Pusa Campus, New Delhi.

The plant material for the present study consisted of six cotton hybrids which are commercially grown in the country. The details of these hybrids along with their respective parents are provided below in the Table 1 below:

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Table 1: List of cotton hybrids used in the present study for testing seed purity

S. No.	Hybrid	Female Parent	Male Parent	Source
1	NH324	NH615	ARB908	Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (MH)
2	SVHH139	SVGCA-40	SVGCA02	Shakti Vardhak Hybrid Seeds Pvt. Ltd. Hisar, Haryana
3	RAHH455	SCSPL4	SCSPL3	AICRP (cotton), MARS, Raichur,

				Karnataka
4	Super 971 BG 11	Super 971 BG 11	Super 971 BG 11	Super Seeds Pvt. Ltd., Hisar, Haryana
5	Phule Suman (RHH1007)	RHC566/1-1	RHcr 060	Mahatma Phule Krishi Vidyapeeth, Rahuri (MH)
6	NHH 715	PH 1076	NDLH 1938	Regional Agricultural Research Station, Nandyal, Andhra Pradesh

Seeds of hybrids and their respective parents were sown in paper towels and the total genomic DNA was extracted from 10-12 days old seedlings using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Saghai-Maroof *et al*., 1984) with slight modifications.

A total of 26 simple sequence repeat (SSR) primers were employed for screening using five samples of each hybrid and one sample each of the respective parents. At first a blend of the regular constituents (PCR buffer, $MgCl_2$, dNTPs, *Taq* DNA polymerase and sterile distilled water) of PCR was prepared. PCR reaction was carried out in an Applied Biosystems PCR Thermal Cycler accessible in the laboratory. The temperature specification for the denaturation of DNA strands, annealing of primers and extension steps were followed as given in the **table 2.**

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Table 2: PCR thermal conditions for SSR amplification in cotton

Step	**Temperature (ºC)**	**Time (minute)**	
1.Initial denaturation	95	5	
2. Denaturation	94	1	x 40 cycles
3. Annealing	55	1	
4. Extension	72	1	
5. Final Extension	72	8	
6. Final storage	4	∞	

PCR amplified products were resolved for SSR analysis in cotton hybrids and parents in three percent (3%) metaphore agarose gel solution after that electrophoresis was carried out and the gel images were captured using a gel documentation system.

RESULTS

Primer identification

A total of 26 simple sequence repeats (SSR) primers were used using five samples of each hybrid (DNA of 10 individuals bulked for each sample) and one sample(bulk of 10 individual seeds) of the respective male and female parent. Finally, seven primers were selected for genetic purity analysis of five hybrids, namely NH 324, SVHH 139, RAHH 455, Super 971 BG II and NHH 715 based on the polymorphism and sharpness of the bands. In the hybrid Phule Suman (RHH1007) no primer out of the 26 primers showed polymorphism between the male and the female parent.

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Genetic purity analysis

Twenty-six SSR primers were used to screen six cotton hybrids along with their respective male and female parental lines using PCR. Initially primers were screened using bulk DNA of the hybrids and parental lines and the genetic purity of the hybrids was tested using 50 individuals of the hybrid. PCR products were electrophorised on three percent metaphor agarose gel. Details of the SSR primers and the markers identified for genetic purity testing of cotton hybrids included in the study are presented in the below table**.**

Table 3: Useful SSR markers for the genetic purity testing of cotton hybrids in the study

S. No.	Primer	Hybrid	Female parent-specific marker	Male parent-specific marker
1	BNL 2709	NH 324	130 bp	160 bp
2	NAU 1190	NH 324	220 bp	210 bp
3	BNL 3594	SVHH 139	200 bp	180 bp
4	JESPR 297	SVHH 139	190 bp	160 bp
5	BNL 2709	RAHH 455	140 bp	140 bp
6	NAU 1230	Super 971 BG II	230 bp	240 bp
7	BNL 3995	Super 971 BG II	200 bp	190 bp
8	JESPR 151	Super 971 BG II	170 bp	140 bp

9	BNL 2709	NHH 715	140 bp	120 bp
10	NAU 1190	NHH 715	220 bp	210 bp

In the hybrid NH 324, two primers namely, BNL 2709 and NAU 1190 were found polymorphic and used in genetic purity testing which revealed 76% and 74% genetic purity, respectively. Primers BNL3594 and JESPR 297 showed polymorphism in the hybrid SVHH 139 and observed 80% and 85% genetic purity, respectively. For the hybrid RAHH 45, no polymorphism was observed between its two parents using the primer BNL 2709. However, unexpectedly, the individual hybrid seeds showed polymorphism for two bands (120 bp and 140 bp) and 87% of genetic purity has been observed with respect to the primer BNL 2709 in the hybrid RAHH 45. For the hybrid Super 971 BG II, three primers namely NAU 1230, BNL 3995 and JESPR 151 revealed 77%, 86% and 53% genetic purity, respectively for this hybrid. The hybrid NHH 715 produced polymorphic bands using the primers BNL 2709 and NAU 1190, which helped in testing the genetic purity of the hybrid NHH 715 and was observed to be 64% and 68%, respectively.

Table 4: Molecular markers in testing genetic purity of cotton hybrids

Hybrids	**Primers**	**Number of seeds Tested**	**Number of Off-type Plants**	**Genetic Purity (%)**
NH 324	BNL 2709	50	12	76
	NAU 1190	50	13	74
SVHH 139	BNL 3594	50	10	80
	JESPR 297	50	7	85
RAHH 455	BNL 2709	50	6	87
Super 971 BG II	NAU1230	50	11	77
	BNL 3995	50	7	86
	JESPR 151	50	23	53
NHH 715	BNL2709	50	18	64
	NAU 1190	50	16	68

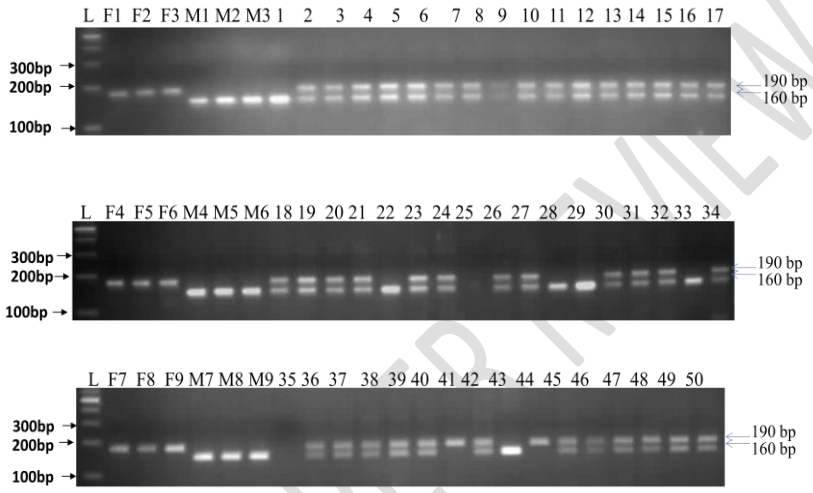

Figure 1: SSR amplification profiling of cotton hybrid SVHH-139 for assessing seed purity at JESPR-297 locus. Lanes marked F1 to F9 are the individuals of the female parent and lanes marked M1 to M9 are the individuals of the male parent. Lane marked L is the 100 bp DNA ladder (BR-Biochem). Lane numbers 1 to 50 are the individuals of the hybrid SVHH 139.Alleles of sizes 190 bp and 160 bp correspond to the female and the male parents, respectively.

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DISCUSSION

For the hybrid NH 324, the genetic purity was found to be 76% and 74% using primers BNL 2709 and NAU 1190, respectively. For the hybrid SVHH 139, using primer BNL3594 and JESPR 297 the genetic purity of 80% and 85% was observed respectively. In the hybrid NHH 715, two primers, namely, BNL 2709 and NAU 1190 showed differences in the genetic purity of

the hybrid and it ranged between 64 - 68%. The slight differences observed could be due to some segregation in the parental lines of this hybrid. Similar results were shown by Selvakumar *et al.*, (2010) where he got percent purity ranging between 65-70% in the hybrid TCHB 2310 using four different markers, and the range was 78-84% and 90-94 % for the hybrids TCHB 4510 and TCHB 213, respectively. Similar results were obtained by Arunkumar *et al.*, (2014) who got 74% and 82% genetic purity, in brinjal hybrid PH 5 using primers emd18C06 and emd11001, respectively. The primer BNL 2709 showed polymorphism between the parents of three hybrids, namely NH 324, RAHH 455 and NHH 715 and was used for genetic purity analysis of the same three hybrids. Rao *et al*., (2015) showed the same results wherein primer BNL 3255 amplified alleles in four cotton hybrids. In the hybrid Super 971 BG II three different primers namely, NAU 1230, BNL 3995 and JESPR 151 showed different magnitude of percent purity and it was found to be 77%, 86% and 53%, respectively. Dongre *et al*., (2011) showed that two primers each of RAPD, ISSR, and SSR marker techniques helped in assessing genetic purity of the hybrid Phule 388. We observed an instance where SSR primer was not polymorphic between the parents but still a random investigation revealed the co-dominant pattern in the hybrid individuals. For example, the primer BNL 2709 showed no polymorphism and amplified band of 140 bp in both the male and the female parents but on testing the purity of hybrid individual plants, amplified bands of sizes of 140 bp and 120 bp in the hybrid RAHH 455. This highlights the point that parental seed are not pure or the supplied seed is an admixture.

CONCLUSION

In the present pilot study an attempt has been made to find hybrid-specific markers for only six cotton hybrids and these have been further used for genetic purity assessment. Markers for testing genetic purity were identified for only five hybrids and none of the 26 tested primers was suitable for assessing the genetic purity of one hybrid, namely, Phule Suman which necessitated the screening of additional markers to find the ones suitable for this hybrid. There are over hundred hybrids that are presently grown in the country and newer hybrids are being continuously developed and released. So, in order to provide genetically pure seed of these hybrids to the farmers we need to find out SSR markers for each of the hybrid for successfully strengthening the conventional procedure of GOT for seed purity analysis.

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