Indian J. Genet., 74(1): 108-111 (2014)

Short Communication

Comparative assessment of genetic purity of cotton hybrids by GOT and RAPD markers

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(Received: February 2013; Revised: November 2013; Accepted: November 2013)

Abstract

In the present investigation, a comparative study was made between gro-out test (GOT) and molecular markers in assessing genetic purity in the seeds of hybrid cotton. In GOT, the genetic purity was detected based on color of the anthers, while in molecular studies 20 RAPD markers were used for the same purpose. The genetic purity in the seeds of hybrid Vishwanath ranged from 92.9 to 95.1%, while the same through RAPD markers (OPA-07) ranged from 94 to 96%. Results obtained in seeds lot of hybrid DCH-32 and Varalaxmi were similar. The results of GOT and RAPD markers were comparable. RAPD can be an efficient replacement for otherwise tedious and time consuming GOT tests.

Key words: Genetic purity, grow-out test, hybrid, cotton, RAPD

In order to determine genetic purity of seeds, field grow out test (GOT) is conducted which is laborious, tedious and time consuming. This has led to the exploration and adoption of alternative techniques which offers efficient, quick and reliable assessment of genetic purity. Molecular marker analysis offers an efficient alternative to this approach as genetic relationships are estimated on the basis of genotypes only. This investigation was carried out to assess the genetic purity of three cotton hybrids *viz*., Vishwanath, DCH-32, Varalaxmi and their parents by using field grow out test and RAPD markers.

The parentage of hybrids under testing is given below:

	Hybrid	Female	Male	Source of seed
1.	Vishwanath (3 lots) NCH2006-109001-lot 1 NCH2006-109002-lot 1 NCH2006-109009-lot 3	Unnamed	Unnamed	Nath Biogene
2.	DCH-32 (*G. hirsutum* x *G. barbadense*)	DS-28	SB (YF-425)	UAS Bangalore
3.	Varalaxmi (*G. hirsutum* x *G. barbadense*)	Laxmi	SB-289E	UAS Bangalore

The field grow out test was conducted during *kharif* 2007 in the experimental farm of the Division of Seed Science and Technology, IARI, New Delhi. Hybrid plants (400) were raised under standard field conditions along with 50 parent plants, maintaining row to row distance of 60 cm and plant to plant distance of 40 cm. Presence of selfed female and off-type plants was evaluated separately in 400 hybrid plants on the basis of anther colour of the flowers for genetic purity testing.

Total genomic DNA was isolated by CTAB method following a modified procedure of Edward *et al*. [1]. A set of 20 RAPD primers obtained from M/s Operon technology was used. The PCR amplification was done as per Williams *et al*. [2]. The PCR reaction

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Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
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carried out in 0.2 ml PCR tubes containing 2.5 µl of 10 x assay buffer (Bangalore Genei India), 0.5 µl of dNTP (MBI Fermentas), 0.2 µl of primer (Sigma Aldrich), 2.2 µl of 25 ng of tempelate DNA, 1.0 µl of *Taq* polymerase (Bangalore Genei India)and 0.5 µl of $MgCl_2$. Amplification conditioned were maintained at 95^{o}C for 5 min and the thermal cycler was programmed for 40 cycles of 1 min at 94^{o}C (denaturation), 32.5^{o}C for 1 min (annealing) and 72^{o}C for 1.30 min (extension) followed by final extension at 72^{o}C for 8 min. The amplification products were resolved in 1.5% agrose gel along with 1 Kb DNA ladder (MBI Fermentas) and visualized under UV light and the images were stored in Gel Doc system. The frequency of RAPD polymorphism was calculated based on presence (+) or absence (0) of common bands.

For testing of hybrid seed purity, grow out test (GOT) was performed. In the GOT, only one morphological character i.e., colour of the anthers could differentiate the female parent from their respective hybrids. The flowers of the hybrid plants had yellow colored anthers while flowers of the female parent had the white color. Out of the three tested commercial lots of Vishwanath, lot no. 3 (109009) was rejected based on GOT results as frequency of selfed female seeds (12.6%) were more than the permissible limit [3]. For similar reason, lot no. 1 of DCH-32 hybrid was rejected as it had 2.2% off-type seeds exceeding permissible limit (Table 1) [3]. However, both the lot of Varalaxmi hybrid had selfed and off-type impurities within the permissible range and hence accepted in GOT (Table 1).

For RAPD studies, 20 random primers were selected of which 13 primers amplified and only four primers, OPA-07, OPA-08, OPA-10, and OPA-12 produced polymorphic bands (20%) in the range of 500 to 4000 bp. Amongst all, the primer OPA-07 showed highly reproducible polymorphic bands. In earlier study also, this primer was successfully used to establish hybridity in cotton [4]. In Vishwanath, this primer generated amplified products ranging from 700 to 4000 bp. A male specific band of size 2200 bp (approx.) was found in the hybrid which could effectively differentiate the female parent (Fig. 1). Absence of this male-specific band (2200 bp) in the profiles of any seed lot indicates impure seeds or mixtures of female seeds. This has been successfully demonstrated in the single seed analysis of Vishwanath hybrid (Fig. 2). In a similar manner, a male specific band of 700 bp from primer OPA-11 was used effectively in testing the purity of cotton hybrids H-6 [5]. In hybrid DCH-32, a band of 1400 bp (approx.) which was male-specific could differentiate the female parent from its hybrid and could be used successfully in detecting the selfed seeds in the hybrid seed lots (Fig. 3).

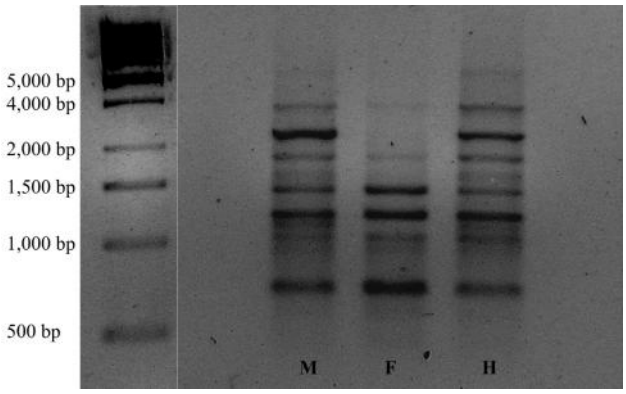

Fig. 1. Hybrid specific bands of vishwanath with OPA-07 RAPD primer. M : Male, F : Female, H : Hybrid

Table 1. Percentage of impurities observed in commercial seed lots by grow-out test

Hybrid	Lot No.	Total No. of plants observed	Observed impurities		Percentage of purity	Remarks
			Selfed plants	Off-type plants		
Vishwanath	1 (NCH-109001)	408	29	0	92.9	Accepted
	2 (NCH-109002)	408	20	0	95.1	Accepted
	3 (NCH-109009)	410	52	0	87.4	Rejected
DCH-32	DCH Lot No 1	405	2	9	97.3	Rejected
	DCH Lot No. 3	403	4	6	97.5	Accepted
Varalaxmi	Lot No. 3	404	8	4	97.0	Accepted
	Lot No. 5	409	7	4	97.3	Accepted

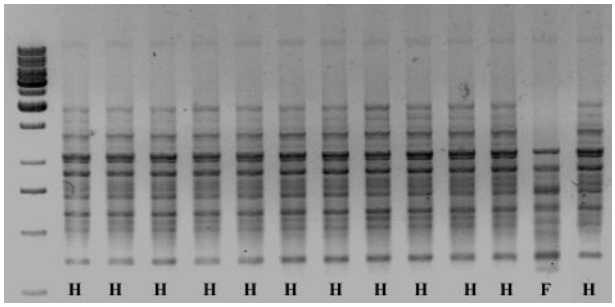

Fig. 2. Single seed analysis of Vishwanath hybrid with OPA-7 primer for genetic purity. H : Hybrid, F : Female

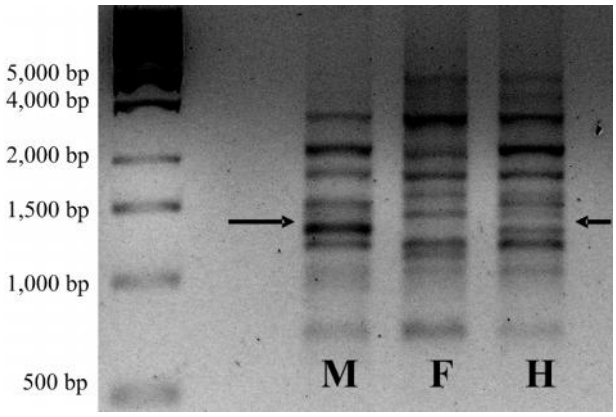

Fig. 3. Hybrid specific bands of DCH-32 with OPA-07 RAPD Marker

Primer OPA-07 showed reproducible polymorphism between hybrid Varalaxmi and parents. It produced 11 bands in the range of 700 to 3500 bp. Two bands of sizes 1300 bp and 2000 bp were found to be male parent specific. On the other hand, the female parent produced three distinct bands of sizes 1700, 1200 and 1000 bp. The true hybrids produced

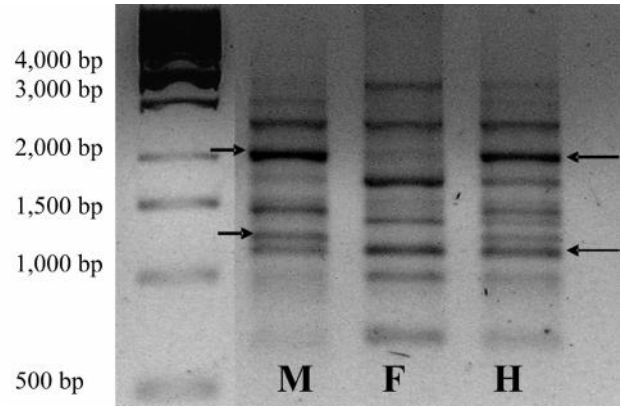

Fig. 4. Hybrid specific band of varalaxmi with OPA-07 RAPD marker. M : Male, F : Female, H : Hybrid

four bands of which two were male-specific (2000 and 1300 bp) and rests were female-specific (1700 and 1200 bp). Hence, these bands could be effectively used in differentiating the hybrids from the mixtures (Fig. 4). Absence of male-specific bands (2000 bp) indicated presence of selfed or female seeds in the seed lot of Varalaxmi. In a similar manner, OPA-20 and OPA-10 were used to test the genetic purity of cotton hybrid Varalaxmi and DHB-105 [4]. Male-specific bands *viz*., 1000bp and 700bp produced by OPA-07 were used in testing genetic purity of hybrid Nathbaba and PKVDH-1,6 respectively [5]. Similarly, OPA-08 primer resulted in amplification of 300 bp and 500 bp male specific amplicons as well as 1300 bp female specific amplicon, and were also seen in hybrid cotton phule-388. Thus it showed that RAPD markers can be conclusively used for hybridity testing [6]. OPA-07 RAPD primers also used for testing genetic purity of cotton F_1 hybrids DHH-11 [7].

Table 2. Percentage of impurities observed in cotton hybrids by RAPD marker (OPA-07)

Hybrid	Lot No.	Total No. of hybrid seed tested	Observed impurities		Percentage of purity	Remarks
			Female	Off-type		
Vishwanath	NCH-109001	50	3	0	94	Accepted
	NCH-109002	50	2	0	96	Accepted
	NCH-109009	50	5	0	90	Rejected
DCH-32	DCH Lot No 1	50	3	2	90	Rejected
	DCH Lot No. 3	50	2	0	96	Accepted
Varalaxmi	Lot No. 3	50	2	0	96	Accepted
	Lot No. 5	50	1	0	98	Accepted

Mixtures of female seeds in the hybrid seed lots detected through OPA-07 were 6%, 4% and 10% for hybrid Vishwanath lot no. 1 (109001), lot no. 2 (109002) and lot no. 3 (109009), respectively (Table 2). Hence, lot no.3 was rejected as it contained female seeds beyond permissible limit. The results obtained through GOT and RAPD analyses are similar and dependable. As found in this study, use of RAPD as a substitute of GOT in seed certification program has already been reported [7, 8].

The genetic purity detected in the present investigation through GOT in the two lots of Vishwanath were 92.9% and 95.1%, respectively while the same through RAPD analysis was 94% and 96%, respectively. For DCH-32, genetic purity detected through GOT and RAPD was 97.5% and 96% respectively. Similarly the genetic purity of the two lots of Varalaxmi as revealed by GOT was 97% and 97.3% each and for RAPD it was 96% and 98%, respectively.

The results of the present investigation indicated that the testing by molecular markers is comparable with the GOT for assessing the genetic purity of commercial cotton hybrids. The study thus re-confirmed that RAPD is a powerful tool, which can be exploited for assessing the genetic purity in the cotton hybrids.

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