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Characterization of DCH-32 cotton hybrid and parental lines by SDS-PAGE and RAPD marker*

Cotton, the most important fiber crop of the world, has 49 species, includes four cultivated, 43 wild diploid and two are tetraploids. Out of these *Gossypium hirsutum* and *Gossypium arboreum* are the two widely grown species in the Indian subcontinent and their improvement has been primarily based on intraspecific crosses (Kumar *et al.,* 2003). Reliable, quick and accurate methods of characterization and cultivar identification are essential for their effective utilization in breeding programme, granting Plant Variety Protection (PVP), certification and also in genetic resource management. Morphological markers are the most commonly used but they are not much reliable. Though morphological description of varieties does not require sophisticated laboratory techniques, the generation of data are time consuming, laborious and the characters are liable to be influenced by a complex genotype and environment interaction. Hence, it is essential to develop alternative methods that are rapid, reliable and less influenced by environment. Recently protein and DNA markers have been reported to be an important tool for cultivar identification in cotton (Vinod Kumar *et al.,* 2007, Iqbal *et al.,* 1997 and Multani and Lyon 1995) due to technical simplicity and speed of methodology (Gepts, 1993). Thus the present investigation was undertaken to characterize cotton hybrid and its parental lines based on total seed protein and RAPD marker.

Mature and well filled seeds of DCH-32 and its parental lines were decoated, crushed and defatted in 3-4 changes of defatting solvent mixture (2:1:1 chloroform : methanol : acetone). Dried seed meal was dissolved in 0.3 ml of 0.6 M working protein extraction buffer [4.25 ml of stock, (2 g SDS, 10 mg Pyronin G, 10.4 ml of 0.6 M Tris-HCl (pH 6.6), 7.9 ml distilled water, 10 ml glycerol) + 0.75 ml of mercaptoethanol and making final volume to 10 ml with distilled water]. The samples were left for 2h at room temperature and then kept in refrigerator overnight. The samples were then kept in a water bath at 40°C for 10 minutes, cooled and centrifuged at 15000 rpm for 10 min. The clear supernatant was taken as protein source for electrophoresis of total seed soluble proteins in 15 per cent separating and 6 per cent stacking gel of 1 mm thickness. 25 µl of each sample was loaded in the wells and electrophoresis was carried out initially at 18 mA till the samples migrated in to the running gel and subsequently at 36 mA until the tracking dye reached the bottom of the gel. After the completion of electrophoresis, the gel was immersed in Commassic Brilliant blue R-250 for over night under room temperature and then destained in a solution of methanol and water (1:1) and 10 per cent acetic acid until the bands were clearly visible. The relative mobility (Rm) values of protein bands were determined from the relation

$$Rm = \frac{\text{Distance traveled by protein band}}{\text{Distance traveled by the tracking dye}}$$

For RAPD analysis DNA was extracted from 3-4 week old seedlings of DCH-32 and its parental lines by CTAB method (Saghai-Maroof *et al.,* 1984). Extracted DNA was purified, quantified and further diluted using TE buffer. PCR reaction mixture were performed in final volume of 20 µl containing 10X buffer, 25 mM MgCl2 (Bangalore Genei), 0.5 units of Taq DNA polymerase (Bangalore Genei), 2.5 mM each of dNtP's (Bangalore Genei), 5pmols/reaction of random primers and 50ng of template DNA. The PCR was performed in MWG thermocycler by initial denaturation at 94°C for 5 minutes followed by 40 cycles of denaturation at 94°C for one minute, annealing at 36°C for one minute, extension at 72°C for two minutes and final elongation at 72°C for 10 minutes. The PCR products were electrophoresed on 1.2 per cent agarose gel prepared in 1X TAE containing 0.5µg/ml of ethidium bromide at 80 V. The gel was photographed under UV transilluminatore. RAPD gels were scored manually for presence (coded as 1) and absence (coded as o) of a band in each line. Very sharp, easily scorable and reproducible bands were taken into consideration.

SDS-PAGE analysis of total seed proteins of DCH-32 hybrid and its parents revealed a total of 19 bands with Rm values ranging from 0.056 to 0.773 (Fig. 1 and 2). A wide variation was

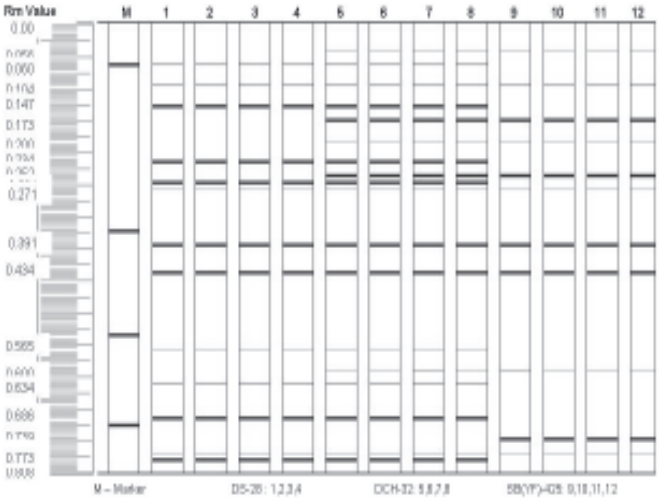
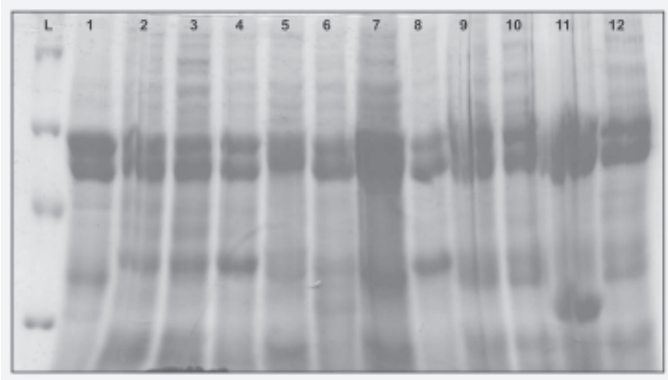


Fig 1. Zymogram representation of banding pattern of DCH-32 cotton hybrid and its parents



L - Marker
1,2,3,4 - DS-28
5,6,7,8 - DCH-32
9,10,11,12 - SB (YF)-425

Fig. 2. Single seed protein profile of DCH-32 cotton hybrid and its parents

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Table 1. Intensity and relative mobility of total soluble seed proteins of DCH-32 cotton hybrid and its parents

Band number	Rm value	DS-28				DCH-32				SB(YF) - 425			
1	0.056	-	-	-	-	+	+	+	+	+	+	+	+
2	0.060	++	++	++	++	++	++	++	++	-	-	-	-
3	0.104	++	++	++	++	++	++	++	++	++	++	++	++
4	0.147	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-
5	0.173	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++
6	0.200	-	-	-	-	+	+	+	+	+	+	+	+
7	0.234	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-
8	0.252	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++
9	0.261	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-
10	0.271	+	+	+	+	+	+	+	+	+	+	+	+
11	0.391	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
12	0.434	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
13	0.565	+	+	+	+	+	+	+	+	-	-	-	-
14	0.600	-	-	-	-	+	+	+	+	+	+	+	+
15	0.634	++	++	++	++	++	++	++	++	-	-	-	-
16	0.686	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-
17	0.739	-	-	-	-	-	-	-	-	+++	+++	+++	+++
18	0.756	+	+	+	+	+	+	+	+	+	+	+	+
19	0.773	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-

\+ Light intensity ++ Medium intensity +++ Dark intensity

observed in protein profiles with respect to Rm values and intensity. The DCH-32 hybrid banding pattern was distinct and could be able to differentiate from its parental lines. The protein profile of male parent (SB (YF)-425) (Table 1) differed from its female parent (DS-28) by a presence of band number 1st, 5 th, 6 th, 8 th and 14th. These bands were also present in hybrid DCH-32. The band number 3 rd (Rm: 0.104), 10th (Rm: 0.271), 11th (Rm: 0.391), 12th (Rm: 0.434) and 18th (Rm: 0.756) were common to hybrid and parental lines, this might be due to prominence of proteins. Similarly the band number 2nd, 4th, 7th, 9th, 13th, 15th, 16th and 19th were absent in SB (YF)-425 parent and which were present in DCH-32 and DS-28 parent, which indicated that contribution of that protein from female parent to hybrid. The importance of varietal characterization through electrophoretic banding pattern was earlier reported in cotton genotypes by Kapse and Nerkar (1985), Agarwal *et al.* (1988) and Dadlani *et al.* (1994).

The protein profile of hybrid DCH-32 revealed that male line (SB(YF)-425) exhibited band at Rm 0.739 supposed to be appeared in hybrid but they were absent. This might be due to dominant nature of protein and was not expressed in heterozygous condition of hybrid. By SDS-PAGE, the total soluble seed proteins could be fractionated into 19 bands. The highest (18) and lowest (11) number of bands were observed in DCH-32 and SB (YF)-425 respectively which showed heterogeneity among hybrid and parental lines. In cotton, SDS-PAGE of total soluble seed proteins has been used by a number of workers for varietal identification and characterization. The invariability in the electrophoretic profiles of total Tris- soluble proteins were studied by Kapse and Nerkar (1985) in four intra *G. hirsutum* hybrids, two *G. hirsutum* x *G. barbadense* hybrids, the parents of respective hybrids and two varieties each of *G. hirsutum* and *G.arboreum* species of cotton. They reported that cultivar could be identified from electrophoregram of soluble proteins of single seed. Wang *et al.* (1990) also evaluated 73 accessions of *G.herbaceum* by electrophoretic profiles of SDS-PAGE analysis of seed proteins. Abdel *et al.* (1993) identified cotton cultivars on the basis of electrophoretic profiles of SDS-PAGE analysis of seed proteins.

In the present study DCH-32 hybrid and its parents were characterized on the basis of RAPD profile. Based on the complimentary banding pattern between the hybrid and their parents (Table 2), the seven RAPD primers *viz.,* OPA-15, OPA-16, OPA-19, OPI-10, OPI-20, OPC-06 and OLI-631 were identified as the effective primers to distinguish F1 hybrid from its parental lines (Fig. 3). The primer OPA-15 amplified a specific allele of size 500 bp and 700 bp in F1 hybrid and its male parent SB (YF)-425, while the allele size of 500 bp and 700 bp was absent in female parent. The same primer had amplified allele of size 300 bp in female parent and F1 hybrid but not in male parent (SB (YF)-425). The presence of both female and male parent allele was observed as a resultant of crossing between two parents (F1 hybrid), thus it confirm the crossing and hybridity between two parents.

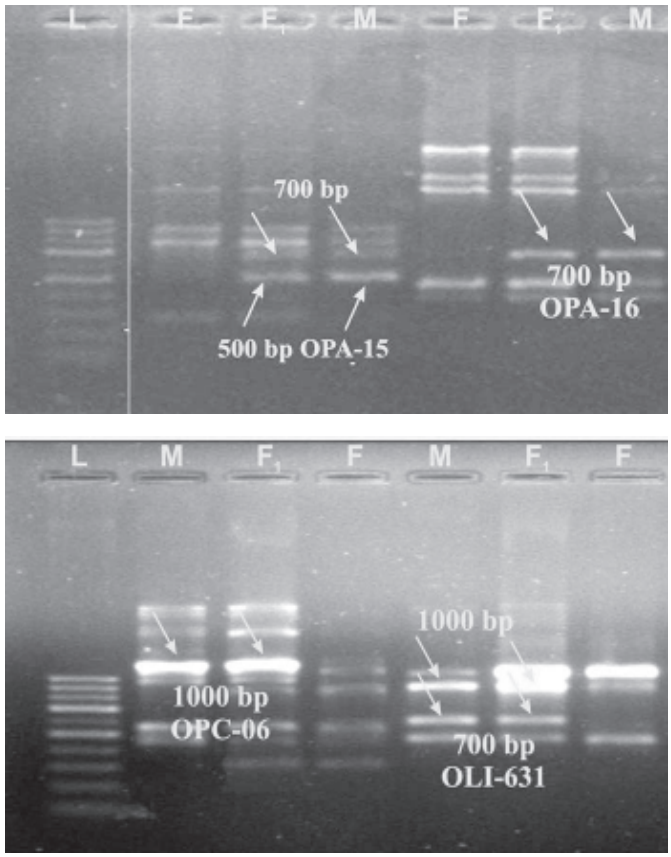
Similarly, the OPA-16 primer had also resulted in amplifying allele of size 3000 bp and 4000 bp in female parent (DS-28) and hybrid (DCH-32). While, the male parent had an amplicon at 700 bp, which was absent in female parent. However, the F1 hybrid exhibited both the alleles of the parents confirming the heterozygosity condition of the hybrid by having bands at 3000 bp, 4000 bp and 700 bp.

The primer OPA-19 had amplicon of size 4000 bp, 900 bp and 300 bp from its female parent. The same primer had amplicon of size 600 bp in male parent (SB (YF)-425). The banding pattern of this hybrid showed both the amplicon at 4000 bp, 900 bp, 300 bp and 600 bp. Thus it is confirm the genuine crossing and heterozygotic condition of the hybrid.

Table 2. Random amplified polymorphic DNA profiles of DCH-32 cotton hybrid and its parents

Primer	OPA-15			OPA-16			OPA-19			OPI-10			OPI-20			OPC-06			OLI-631		
fragments (bp)	DS-28	DCH-32	SB (YF)-425	DS-28	DCH-32	SB (YF)-425	DS-28	DCH-32	SB (YF)-425	DS-28	DCH-32	SB (YF)-425	DS-28	DCH-32	SB (YF)-425	DS-28	DCH-32	SB (YF)-425	DS-28	DCH-32	SB (YF)-425
5000																					
4000			1	1	0	1	1	0													
3000				1	1	0	1	1	1												
2000				1	1	1				0	1	1				1	1	1			
1000							1	1	1				1	1	1	0	1	1	1	1	1
900	1	1	1				1	1	0				1	1	1	1	1	0	1	1	1
800	1	1	1							1	1	1	1	1	1						
700	0	1	1	0	1	1				0	1	1									
600							0	1	1	1	1	1	0	1	1	1	1	1	0	1	1
500	0	1	1	1	1	1				1	1		1	1	0	1	1	1	1	1	1
400				1	1	1															
300	1	1	0				1	1	0	0	1	1				1	1	0			
200																					
100																					

0 – Absent of band; 1 – Presence of band



L - Marker; F1 - DCH-32; F - DS-28; M - SB(YF)-425
Fig. 3: DNA profile of DCH-32 cotton hybrid and its parents

OPI-10 primer amplified an allele of size 700 bp and 300 bp in hybrid (DCH-32) and its male parent SB (YF)-425, which was absent in female parent.

Similarly OPI-20, OPC-06 and OLI-631 primers had also resulted in amplifying allele of size 500 bp with OPI-20 and 900 bp and 300 bp with OPC-06 primer in female parent and hybrid (DCH-32), which was absent in male parent [SB(YF)-425].While the male parent had an amplicon at 600 bp, 1000 bp and 600 bp with OPI-20, OPC-06 and OLI-631 respectively. However, the F1 hybrid exhibited both the alleles of the parents confirming the heterozygosity condition. The identified RAPD marker in F1 hybrids showed complimentary banding pattern of both the parents. It was valuable to distinguish the F1 from their male and female parents.

Multani and Lyon (1995) have reported fairly reproducible results of RAPD analysis in cotton and found cultivar specific markers, which were consistently stable and could be used for varietal identification and classification. They also observed 98 per cent of 50 primers to be polymorphic for cotton varieties. Tatineni *et al.* (1996) reported that 27 primers (33.8%) did not show any polymorphism among 19 cotton genotypes, while Iqbal *et al.* (1997) obtained 98 per cent polymorphism and high level of genetic similarity. They also observed that RAPD marker can clearly establish the distinctness of the *arboreum* cotton variety from the *hirsutum* varieties. RAPD had a greater efficiency in varietal identification and diversity assessment as RAPD exploit random sites of the genome to express

polymorphism (Vinodkumar *et al.*, 2007). The three primers (OPC-06, OPA-16 and OLI-631) were identified as effective primers to distinguish F1 hybrid from its parental lines. Therefore RAPD marker is found to be useful in hybrid and parental line identification and characterization.

Overall comparison of RAPD and protein profiling was indicative of greater efficiency of RAPD for varietal identification because protein profiles are based on expressed genes, which are more conserved whereas RAPD could exploit random sites of the genome to express polymorphism.

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