Indian J. Plant Physiol., Vol. 12, No. 2, (N.S.) pp. 115-119 (April-June, 2007)

RAPD AND PROTEIN PROFILES OF COTTON VARIETIES

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Received on 16 Nov., 2006, Revised on 7 June, 2007

SUMMARY

RAPD and SDS-PAGE techniques were employed for identification and genetic diversity estimation of six tetraploid (*Gossypium hirsutum*) and two diploid (*G. arboreum*) cotton cultivars. 17 RAPD primers produced 86 amplicons, out of which 55 were polymorphic having 63.95 % polymorphism and 8 primers produced variety specific bands. The maximum discriminating power was obtained from Primers OPA-17 and OPA-19. SDS-PAGE of seed storage proteins resolved into 30 bands. Out of 30 bands, only one variety specific band was found, while 12 bands were species specific and appeared only in one of the species. The discrimination power of seed protein profile was 0.53, which was less than the discriminatory power of most of the RAPD profiles generated for individual primers. Cluster analysis of both RAPD and SDS-PAGE data produced two clusters, separating diploid with tetraploid cultivars. RAPD was found to be more efficient for cultivar identification and genetic diversity estimation.

Key words: *Gossypium* spp., RAPD, SDS-PAGE, variety discrimination

INTRODUCTION

Cotton the most important fiber crop of the world, has 49 species, includes four cultivated, 43 wild diploid and two wild tetraploids. Out of these *Gossiypium 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 germplasm characterization and cultivar identification are essential for their effective utilization in breeding programme, granting plant variety protection (PVP) and utility patent, certification and also in genetic resource management. Morphological markers are most commonly used but they are not much reliable due to gene environment interaction. Recently isozymes, protein markers and DNA markers have been used, which are more reliable and stable. Seed protein profiling has been widely used as an important tool for determining relationship and identification of cultivar in many crops (Goyal and Sharma 2003). Besides large number of molecular markers, RAPDs have also been used in cotton (Multani and Lyon 1995, Tatineni *et al.* 1996, Willium *et al*. 1990, Iqbal *et al.* 1997) for cultivar analysis and identification in most plants due to technical simplicity and speed of RAPD methodology (Gepts 1993). The present study was undertaken in order to identify and evaluate the genetic diversity present among cotton varieties, using both seed protein profile and RAPD patterns, since, comparative study on Indian varieties using both the techniques is meager.

MATERIALS AND METHODS

Six varieties of *G. hirsutum* (tetraploid cotton) and two of *G. arboreum* (diploid cotton) were used for the extraction of DNA from 3-4 weeks old seedlings by using the method of Doyle & Doyle (1990) with slight

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modification. The quantitation of DNA in RNA free sample was done using a UV visible spectrophotometer (UNICAM). PCR reactions were performed in final volume of 25 µl containing 10X Assay Buffer (Bangalore Genei), 0.5 units of Taq DNA polymerase (Bangalore Genei), 200 µM each of dNTPs (Bangalore Genei), 10 pmols / reaction of random primers and 50 ng of template DNA. RAPD was done by using 17 primers of OPA and OPB series obtained from 'OPERON TECHNOLOGIES' (Inc. Alameda, Calif.). The PCR was performed in 'Biometra Thermocycler' by Initial denaturation at 94°C for 5 minutes following by 43 cycles of denaturation at 94°C for one minute, annealing at 37° for one minute and extension at 72°C for two minutes and final elongation at 72°C for 7 minutes. The PCR products were electrophoresed on 1.2%. Agarose gel (Himedia), prepared in 1X TBE buffer containing 0.5 µg/ml of the Ethidium Bromide at 50 V for 5 hrs with cooling. The gel was photographed under UV transillluminator. The PCR reaction was repeated twice for each primer.

For SDS-PAGE analysis, seed powder was treated with hexane and acetone to remove fats and oils (Stegmann and Pietsch 1983). Protein was extracted by using 0.1 M Tris-borate (pH 8.5) from the seed powder. Protein concentration was estimated using Biuret method and finally equal amount of protein was loaded on 12% and 20% resolving gel for better resolution of larger and smaller polypeptides, respectively. The gel was electrophoresed at 200v and then stained in Commassie Brilliant blue R-250 for 2-3 hours and then destained in a solution of methanol and water (1:1) and 10% acetic acid.

For RAPD, the presence of each band was scored as 1 and its absence as 0. Faint bands were not scored but a major band corresponding to faint bands was considered for scoring. The band appearing in any of the two replicates was considered as present. For SDS-PAGE the presence of each band was also scored as 1 and its absence as 0. The bands were designated on the basis of their molecular weight according to Protein Molecular Weight Marker (PMW, Bangalore Genei). Jaccard's similarity coefficient values for each pair-wise comparison between cultivars and a similarity coefficient matrix was constructed. The matrix was subjected to unweighted pair group method of arithmetic average analysis (UPGMA) to generate dendogram. The statistical calculations were done using NTSYS version 2.3 software.

Variety specific bands and banding patterns generated by different primers were used for identification of varieties. The discriminating power (D) of each primer was estimated to compare the efficiency of the markers. If C is the confusion probability, i.e. the probability that two randomly chosen individuals from the sample of varieties have identical banding patterns, then $D = 1 - C$ represents the probability that two randomly chosen individuals have different patterns, and thus are distinguishable from one another. In a set of N individuals, it is possible to draw $N(N - 1)/2$ different pairs. For the ith pattern of the given jth primer, present at frequency p_i in this set of varieties, the confusion probability C_i is:

$$Ci = p_i \quad \frac{(Npi - 1)}{N - 1}$$

For the ith primer, the confusion probability Cj is equal to the sum of the different c_i for all I patterns generated by the primer:

$$C_j = \Sigma\ c_i = \Sigma\ p_i \quad \frac{(Npi - 1)}{N - 1}$$

Thus, the discriminating power of the jth primer is equal to:

$$D_j = 1 - Cj = 1 - \Sigma\ p_i \quad \frac{(Npi - 1)}{N - 1}$$

(Tessier *et al.* 1999).

Similarly the discriminating power (D) of SDS-PAGE profile was also calculated.

RESULTS AND DISCUSSION

Seventeen selected RAPD primers produced a total of 86 amplicons of which 55 were found to be ploymorphic, and the level of polymorphism was 63.95%. The maximum discriminating power (0.93) was assessed for OPA-17 and OPA-19 which produced maximum different types of banding patterns (6). The discriminating

power of OPB-4 (0.858) closely followed the maximum discriminating power with 5 banding patterns. While the discriminating power of OPA-7 was 0 due to monomorphic banding pattern (Table 1). Primers OPA-1, OPA-2, OPA-9, OPA-17, OPA-20, OPB-2 and OPB-3 produced variety specific bands for four varieties in which two were tetraploid, RST-9 and RS-2013 and the remaining two were diploid, RG-8 and RG-18. No single primer produced unique banding patterns for all the varieties and only combination of primers could identify all the varieties. When classified and categorized, data for banding pattern were scrutinized for suitable primer combination for identification, only a few combinations were detected which could identify all the varieties. Primers OPA-17 and OPA-19, which individually produced maximum type of banding patterns, were found to identify all these varieties.

Table 1. Primer discriminative power (D) of eight 8 cotton varieties.

Primer	Number of markers	Numbered banding pattern	D at 37°
OPB-1	4	3	0.72
OPB-2	5	3	0.46
OPB-3	4	3	0.68
OPB-4	9	5	0.86
OPB-5	3	3	0.72
OPA-1	6	4	0.64
OPA-2	5	3	0.47
OPA-5	8	3	0.46
OPA-7	3	1	0.00
OPA-9	9	3	0.46
OPA-11	2	2	0.43
OPA-14	2	2	0.43
OPA-15	7	2	0.43
OPA-16	3	3	0.61
OPA-17	7	6	0.93
OPA-19	6	6	0.93
OPA-20	3	4	0.64
Protein Profile	30	6	0.53

The calculated Jaccard's similarity coefficient of RAPD analysis showed the genetic similarity between 0.43 (RS-2013 and RG-8) to 0.97 (RST-9 and BN), whereas, the average genetic similarity coefficient was 71.82% (Table 2). The dendogram generated by UPGMA could differentiate between diploid and tetraploid groups at 49.41 % similarity, demonstrating (a) the genetic similarity between diploids and tetraploid was low and (b) the current set of decamer primers was sufficient to identify diploid and tetraploid groups. Mean genetic similarity among tetraploids was higher (0.88) than that for diploids (0.77). This shows that tetraploids harbor less genetic diversity than diploids. Among the tetraploid GA got further different from other cluster consisting of 5 cultivars, i.e. RS-810, RS-875, RST-9, BN, and RS-2013. Within tetraploid RS-875 and RST-9 (0.97) / RST-9 and BN (0.97) showed higher value of similarity coefficients, indicating the close resemblance between them (Fig. 1A).

Table 2. Jaccard's similarity coefficient for eight varieties of cotton based on RAPD profiling.

Varieties	RS-810	RS-875	RS-2013	RST-9	BN	GA	RG-9	RG-18
RS-810	1.00							
RS-875	0.91	1.00						
RS-2013	0.85	0.88	1.00					
RST-9	0.94	0.97	0.88	1.00				
BN	0.94	0.94	0.88	0.97	1.00			
GA	0.86	0.85	0.80	0.86	0.88	1.00		
RG-8	0.47	0.45	0.43	0.45	0.45	0.48	1.00	
RG-18	0.57	0.53	0.50	0.53	0.53	0.54	0.77	1.00

Jaceard's similarity coefficients based on SDS-PAGE analysis are presented in Table 3. The range of genetic similarity was found between 0.47 to 1.00 with average genetic similarity of 73.21%. The similarity matrix showed the maximum similarity among RS-810 and BN (1.00) and RST-9 and RS-2013 (1.00). The dendogram splited the cultivars into diploid and tetraploid groups at 48.25 per cent similarity (Fig. 1B). Thus diploid

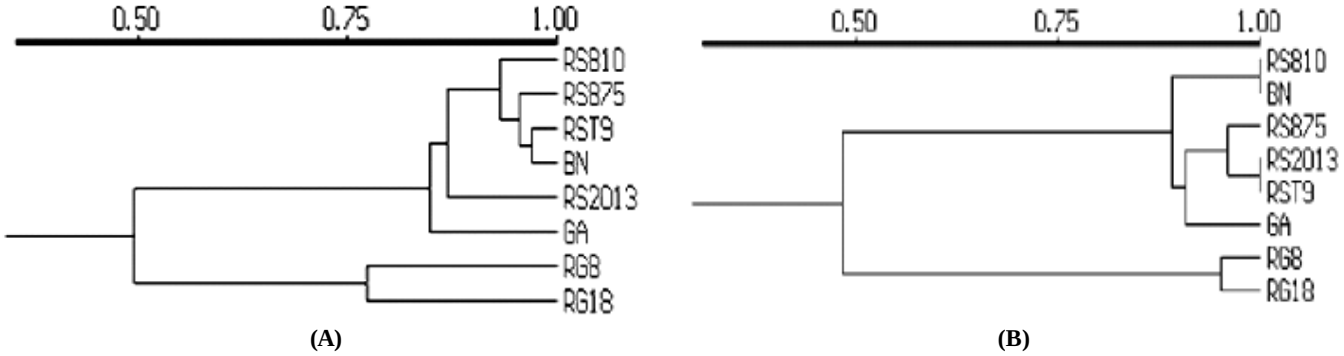


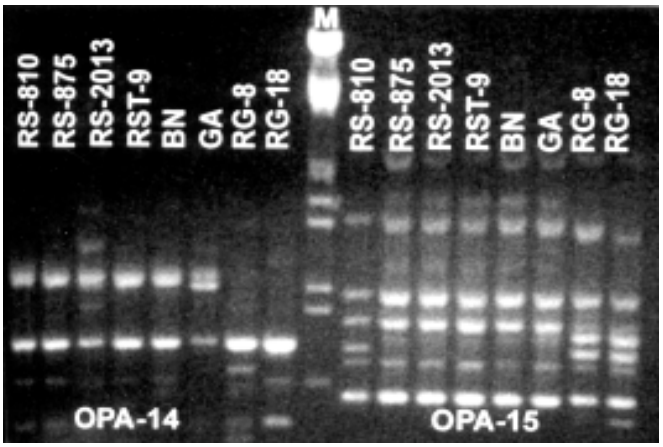
Fig. 1. Dendogram generated for eight cotton cultivars using UPGMA cluster analysis based on Jaccard's similarity estimates for RAPD (A) and SDS-PAGE (B).

and tetraploid species could easily be discriminated on the basis of SDS-PAGE banding patterns. However, discrimination power of this method was very low within tetraploid and within diploid varieties, showing very high average genetic similarity within tetraploid, i.e. 91.73 and this value also was very high within diploid RG-8 and RG-18 (95%). (RS-810 and BN) and (RST-9 and RS-2013) were 100%similar according to their Jaccard's similarity coefficient (Table 3).

Table 3. Jaccard's similarity coefficient for eight varieties of cotton based on polypeptide profiles developed for seed storage proteins.

Varieties	RS-810	RS-875	RS-2013	RST-9	RG-8	RG-18	BN	GA
RS-810	1.00							
RS-875	0.92	1.00						
RS-2013	0.88	0.96	1.00					
RST-9	0.88	0.96	1.00	1.00				
RG-8	0.48	0.48	0.47	0.47	1.00			
RG-18	0.50	0.50	0.48	0.48	0.95	1.00		
BN	1.00	0.92	0.88	0.88	0.48	0.50	1.00	
GA	0.88	0.88	0.92	0.92	0.47	0.48	0.88	1.00

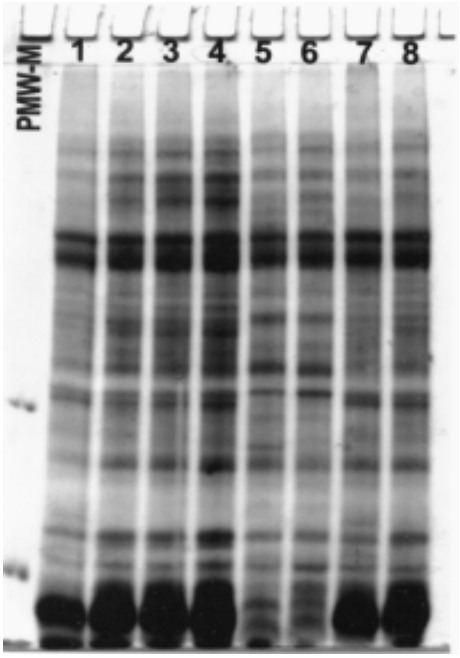
Overall comparison of RAPD and protein profiling was indicative of greater efficiency of RAPD for identification and diversity assessment. RAPD profiling could identify all the varieties under study with



M-Marker Lambda DNA Hind III /EcoRI Double Digest

Fig. 2. RAPD profile generated by primer OPA-14 and OPA-15 in different cotton varieties

combinations of 2-3 primers (Fig. 2). While seed protein profiles, though contained 30 bands could not identify all the varieties (Fig. 3). This is expected as protein profiles are based on expressed genes, which are more conserved whereas RAPD could exploit random sites of the genome to express polymorphism. This phenomenon is conspicuously evident by more average diversity recorded through RAPD compared to protein profile. Though protein profile was less efficient to distinguish accessions within species but could separate diploids with tetraploid at a comparable level to RAPD. There is indirect evidence that RAPD discriminate purely on genetic basis (DNA), whereas protein profile may put agronomically similar varieties together.



PMW-M protein molecular weight marker-M (14,200-97,400 D)
1. RS-810; 2. RS-875; 3.RS-2013; 4.RST-9; 5.RG-8; 6.RG-18; 7.BN; 8. GA

Fig. 3. Seed protein profile of different cotton cultivars generated by SDS-PAGE

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