Genetic diversity analysis in elite cotton cultivars using RAPD markers

S.N. SALUNKHE*, Y.A. DESHMUKH[1] AND V.R. HINGE[2]
Mahatma Gandhi Mission College of Agriculture Biotechnology, AURANGABAD (M.S.) INDIA

ABSTRACT

Genetic variability and relationship between varieties are of great importance for cotton breeding. RAPD marker system was used for identification and genetic diversity analysis of elite *G. hirsutum*, *G. arboreum* and introgressed lines. 12 cotton genotypes were subjected to RAPD analysis using 20 RAPD primers. PCR products were subjected to agarose gel electrophoresis and the banding patterns were compared among 12 elite cotton varieties of diploid, tetraploid and introgressed cotton. Out of 20 random primers tested, 15 primers produced reproducible results yielding 123 markers with 92 (74%) being polymorphic. The random primer OPA 17 generated the maximum number of polymorphic markers with a specific band of 300bp for variety PA-255. A dendogram constructed from RAPD data classified 12 cotton genotypes into two major clusters, one containing six genotypes belonging to *G. hirsutum* cultivars and the other contained 4 genotypes belonging *G. arboreum* cultivars. Two introgressed cultivars PAIG-8/1 and PAIG-27 showed highest level of genetic similarity with *G. arboretum* varieties. RAPD technique was thus found to be efficient method for detecting DNA polymorphism useful for molecular evaluation in cotton.

Key words : Genetic diversity, Molecular markers, RAPD, Cotton

INTRODUCTION

Cotton 'The white gold' is the world's leading natural fiber crop and it is the corner stone of textile industries world wide. The cultivated cottons include *Gossypium arboreum* (L) *and Gossypium herbacium* (L) (Old World species), both diploid species with an AA genome native to southern Asia, Africa and two allotetraploid species *Gossypium barbadense* (L) and *Gossypium hirsutum (L)* (New World species) with AD genome from Central, North and South America. Although small gains in yield and fiber quality continue to be made by conventional breeding programs, genetic improvement of agronomic traits is beginning to plateau as a result of an increasing narrow germplasm base for selection. Genetic diversity is desirable for long term crop improvement and reduction of vulnerability to important crop pests. Genetic diversity resulting from interspecific introgression can be evaluated with morphological characteristics, seed proteins, isozymes and DNA markers. To have reliable estimates of genetic relationship, a large number of polymorphic markers are required. This limits the use of morphological characteristics and isozymes, which are few, or lack adequate levels of polymorphism in *Gossypium* spp. Therefore there is a need to study polymorphism at the DNA level which can be indicative of genetic diversity in cotton.

DNA markers have proven to be valuable in crop breeding especially in studies of genetic diversity and in cultivar identification. Polymerase chain reaction (PCR) based molecular markers, e.g. ISSR, RAPD, SSR, STS; AFLP etc. are useful for various applications in the plant breeding. RAPD markers involve the amplification of random DNA segments using arbitrary sequences of 10-15 base pairs without any prior knowledge of DNA sequence (Welsh *et al.*, 1990). The present molecular diversity analysis was carried out to analyze genetic relationship and genetic diversity of the cultivars using RAPD markers.

MATERIALS AND METHODS

Plant material and DNA extraction:

The list of elite cotton cultivars used in the present study is as below.

Elite *G. hirsutum* cultivars: 1. pH-93 2. pH-325 3. pH-348 4. NH-452 5. NH-545

Elite *G. arboreum* cultivars. 1. PA-402 2. PA-255 3. PA-405

Elite introgressed cultivars: 1. PAIG- 8/1 2. PAIG-27

Parents of introgressed cultivars: 1. PA-140 (*G. arboreum*) 2. Poornima (*G. hirsutum*).

The seeds of the above 12 cotton cultivars were obtained from the Cotton Research Station, Nanded; Cotton Research Station, Mahboob Baugh Farm; and the Cotton Research Scheme, Marathwada Agricultural University, Parbhani. Total genomic DNA was extracted from 4g of bulked leaf sample by a modified procedure of Edwards *et al.,* (1991).

* **Author for correspondence.** Present address: Dept. of Agril. Botany, Marathwada Agricultural University, PARBHANI (M.S.) INDIA
[1]MAHYCO, Life Sicences Research Centre, Crop Gene Function and Manipulation Lab, JALNA (M.S.) INDIA
[2]Department of Agricultural Botany, B.A. College of Agricultural, Anand Agricultural University, ANAND (GUJARAT) INDIA

Molecular analysis:

A total of 20 primers from the OPA series from Operon Technologies, USA were used for this study. The RAPD reaction profile was preceded by a single 94°C soak for 6 minutes, 36 cycles consisting each of a denaturing step of 45 seconds at 94°C, a primer annealing step of 1 minute at 36°C and a primer extension step of 1 minute at 72°C. At the end of 36 cycles, a single 72°C extension was applied for 10 minutes for polishing the ends of PCR products.

Data analysis:

DNA fragment size was estimated by comparing the DNA bands against a Lambda/ Hind III, pUC 18 / Sau 3A-pUC 18/ Taq I base pair ladder (Bangalore Genie, India). The amplified DNA bands were scored on gel under a UV transilluminator as 1 for the presence and 0 for the absence of bands and assembled in the data matrix table. The pair wise comparisons were calculated using Nei and Li's coefficient (Nei *et al.,* 1979). The similarity values found were utilized to group individuals via the unweighted pair group method with arithmetic average (UPGMA). NTSYS-PC was sed to perform all the analysis (Rolf *et al.,* 1993).

RESULTS AND DISCUSSION

Polymorphism as detected by RAPD analysis:

Among 20 random OPA series primers initially screened against the DNA of 12 cotton genotypes only 15 produced reproducible results. These fifteen reproducible primers generated a total 123 markers of which 92 were polymorphic *i.e.* 74 per cent amplified loci were polymorphic. The average number of polymorphic markers generated per primer was 6.13. The size of RAPD amplicons was between 150bp in OPA 06 and OPA 12 and 3000bp in OPA 12, OPA 15, OPA 16 and OPA 17. In this respect investigated the genetic diversity of 23 elite cotton varieties using 50 random decamer primers (Iqbal *et al.,* 1997). A total of 349 bands were amplified, 89.1% of which were polymorphic. 31 Gossypium species recorded a level of polymorphism of 99.8% (Khan *et al.,* 2000).

Highly polymorphic profiles were obtained with 10 of the total primers used such as OPA 06, OPA 17 and OPA 19. These 10 primers detected 79 polymorphic markers. However, none of the primers individually was so informative as to differentiate all the genotypes. The random primer OPA 17 generated the maximum number of markers *i.e.*16 and OPA 03 generated the least number of markers (Table 1). The primer OPA 17 showed a specific band of 3000bp for the cultivar PA-255 (Fig 1a). The primer OPA 16 generated 7 polymorphic markers and 3 monomorphic markers. OPA 16 also generated a specific band of 700bp for the cultivar PA-255 (Fig 1b). Primer OPA12 produced specific band of 500bp for variety pH 348. Primer OPA08 produced 4 polymorphic markers and 2 monomorphic markers.

Cluster analysis:

Nei and Li's similarity coefficient between 12 cotton cultivars using RAPD markers ranged from 0.5 to 0.92. *G. arboreum* cultivar PA-255 and the introgressed cultivar PAIG-8/1 were highly similar (Nei *et al.,* 1997). Similarly, a high degree of similarity was evident between cultivar PA-405 (*G. arboreum*) and PA-402 (*G. arboreum).* Maximum RAPD diversity was evident between the introgressed cultivar PAIG-27 and *G.arboreum* varieties such as PA-405 and PA-402. A dendrogram resulting from cluster analysis based on similarity values of 12 cotton cultivars generated from RAPD data (Fig. 2) revealed
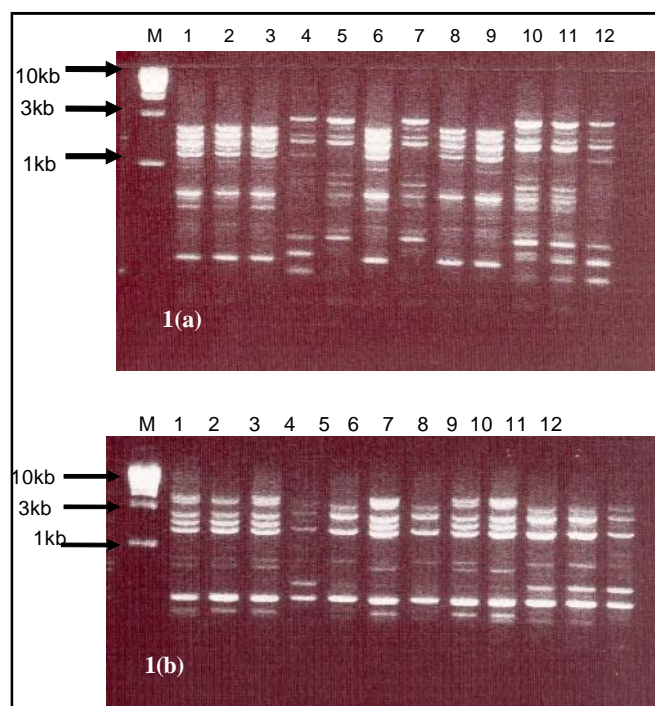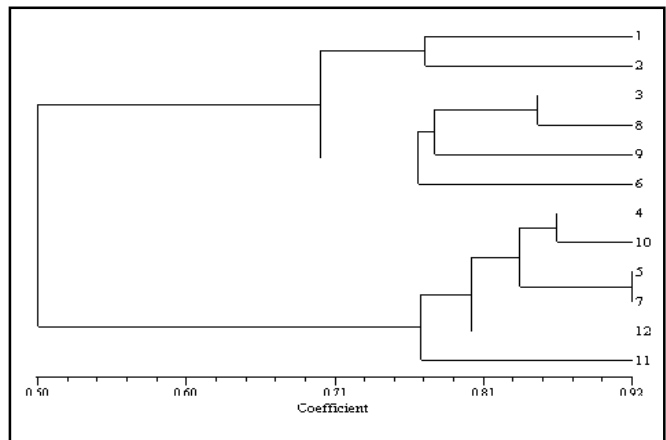

Fig.1 : **(1a & 1b) RAPD Polymorphism among 12 Cotton Varieties using primer a)OPA17 b)OPA16. Details of lanes: 1. NH-545 (*G.h.*) 2. PH-93 (*G.h.*) 3.PH-325 (*G.h.*) 4. PA-255 (*G.a.*) 5. PA-405 (*G.a.*) 6. NH-452 (*G.h.*) 7. PA402 (*G.a*) 8. pH-348 (*G.h.*) 9.Poornima (*G.h.*) 10. PAIG-8/1 (I) 11. PAIG-27 (I) 12.PA-140 (*G.a*) and M for marker 1 Kb ladder and Lambda/hindIII, pUC18/Sau 3AI-pUC18/Taq I Digest. (Note - *G.h* - *G.*hirsutum and *G.a* - *G. arboreum*. and I - Introgressed cotton varieties)**

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Table 1 : List of RAPD primers used their sequence and level of polymorphism detected among 12 cotton genotypes			
Primer	Primer Sequences 5'-3'	No. of markers generated	No. of polymorphic markers
PA 01	5'-CAGGCCCTTC-3'	4	3
OPA02	5'-TGCCGACGTG-3'	3	2
OPA03	5'-AGTCAGCCAC-3'	1	-
OPA04	5'-AATCGGGCTG-3'	N R	-
OPA05	5'-AGGGGTCTTG-3'	N R	-
OPA06	5'-GGTCCCTGAC-3'	9	9
OPA07	5'-GAAACGGGTG-3'	9	8
OPA08	5'-GTGACGTAGG-3'	6	4
OPA09	5'-GGGTAACGCC-3'	N S	-
OPA10	5'-GTGATCGCGC-3'	13	9
OPA11	5'-CAATCGCCGT-3'	11	10
OPA12	5'-TCGGCGATAG-3'	8	5
OPA13	5'-CAGCACCCAC-3'	5	5
OPA14	5'-TCTGTGCTGG-3'	N S	-
OPA15	5'-TTCCGAACCC-3'	10	5
OPA16	5'-AGCCAGCGAA-3'	10	7
OPA17	5'-GACCGCTTGT-3'	16	12
OPA18	5'-AGGTGACCGT-3'	N R	-
OPA19	5'-CAAACGTCGG-3'	12	9
OPA20	5'-GTTGCGATCC-3'	6	4
Total		123	92(74%)

NR – Non reproducible, NS – Non scorable

Fig.2 : Dendrogram generated by RAPD analysis of Diploid, Tetraploid and Introgressed cotton varieties. Details of lanes: 1. NH-545 (*Gh.*) 2. PH-93 (*Gh.*) 3. PH-325 (*Gh.*) 4. PA-255 (*Ga.*) 5. PA-405 (*Ga.*) 6. NH-452 (*Gh.*) 7. PA-402 (*Ga.*) 8. PH-348 (G.h.) 9. Poornima (*Gh.*) 10. PAIG - 8/1 (I) 11. PAIG-27 (I) 12. PA-140 (*Ga.*)
(Note-*Gh* - *G.hirsutum* and *G.a* - *G. arboreum.* and I - Introgressed cotton varieties)

that the 12 cotton cultivars could be separated into 2 major groups (I and II). The first major group consisted of 6 *G. hirsutum* cultivars and the second major group consisted of the 4 *G. arboreum* cultivars and the 2 introgressed lines. Each of these major groups could be further subdivided. In group I, Two *G. hirsutum* cultivars, NH-545 and PH-93, clustered separately from rest of the *G. hirsutum* cultivars *viz.*, pH-325, pH-348, Poornima and NH- 452. In group II, the 4 *G. arboreum* cultivars and

the introgressed cultivar PAIG-8/1 clustered separately from the cultivar PAIG-27 also found distinct cluster formation of accessions belonging to *G. hirusutum* and *G. arboretum* groups (Iqbal *et al.,* 1997). *G. hirusutum* was separated from *G. arboreum* genotypes based on RAPD analysis and morphological characteristics (Kumar *et al.,* 2003)

Introgression study:

Two introgressed cultivars PAIG-8/1 and PAIG – 27 and their parents Poornima (*G. hirsutum*) and PA-140 (*G. arboreum*) were analyzed for detecting introgression using 20 RAPD primers (Table 2). The number of parent specific bands present in both introgressed cultivars

Table 2: Parent specific bands generated by introgressed lines by RAPD analysis						
Primer	Introgressed variety PAIG-8/1	Parent-1 (Poornima) specific band	Parent-2 (PA140) specific band	Introgressed variety PAIG-27	Parent-1 (Poornima) specific band	Parent –2 (PA-140) specific band
OPA17	8	2	3	8	2	3
OPA10	5	0	2	4	0	1
OPA06	3	1	1	3	1	1
OPA08	1	0	1	1	0	1
OPA12	7	2	3	7	2	2
OPA15	7	0	1	7	0	1
OPA16	5	0	1	5	0	1
OPA11	6	0	1	4	0	2
Total	42	57	13	39	5	12

showed that they have the greatest homology with parent PA 140. Primer OPA 12 showed a specific band of 750bp which is present in both introgressed cultivars and the parent Poornima. The primer OPA 6 revealed a 1500bp band in both introgressed cultivars which is specific to the parent Poornima. The primer OPA 17 revealed 800bp band in both cultivars which is specific to parent Poornima but absent in parent PA -140. Similarly, analyzed introgression of *A. cardenasii* chromosome segments using 70 RAPD primers in 46 introgression lines from a cross between *Arachis hypogaea* and *A. cardenasii* (Garcia *et al.,*1995) Two introgressed cultivars PAIG-8/1 and PAIG-27 also clustered in the *G.arboreum* group. These two introgressed cultivars were developed from interspecific crosses between *G. arboreum* (PA-140) and *G.hirsutum* (Poornima) and are the selection lines from back crossed F_1 populations[1]. Since the maximum number of genes of *G. arboreum* have been recovered in the introgressed cultivars, these showed the highest level of genetic similarity with *G. arboreum*. Although a few genes like those for fibre quality, boll size and ginning outturn have been introgressed from *G. hirsutum*. Therefore the results of the present investigation confirmed the efficiency of RAPD markers in estimation of genetic relatedness and genetic diversity among cotton genotypes of the cultivars.

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