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RAPD-based assessment of genetic diversity in cotton (*Gossypium hirsutum* L.) race stock accessions

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Abstract

Twenty-five cotton (*Gossypium hirsutum*) accessions from Africa, Australia, USA and India were subjected to RAPD analysis using 86 random oligonuceotide primers. The major objectives of the study were to study the extent of genetic variation and find out the duplicates if any. Sixty three primers detected polymorphism. A total ot 296 DNA fragments were generated by the 63 primers, of which 113 were polymorphic. The accessions revealed genetic divergence ranging from 0.13 to 0.33 among themselves. RAPD analysis using SIMQUAL-Dice Coefficient of NTSYS-pc showed that the 25 accessions could be split into 2 groups of 24 and 1 accessions at 67% similarity. The first group of 24 accessions could be divided into cluster A consisting of 20 accessions and cluster B consisting of 4 accessions. Accessions AC 53 and AR 43 were found to be 100 percent similar based on molecular analysis, but could not be considered as duplicate, because of very low reproducibility of the RAPD markers.

Key words: Cotton, RAPD, genetic diversity

Introduction

The genus *Gossypium* is very diverse and consists of 36 diploid and six tetraploid species. *G. arboreum* and *G. herbaceum*, the old world cottons and *G. hirsutum* and *G. barbadense*, the new world cottons are commercially grown for their seed and fiber characteristics [1]. Many successful cotton cultivars have been developed from closely related parents, but limited yield gains in recent years have led some to advocate more extensive use of exotic germplasm [2]. Assessment of genetic diversity in this regard is of paramount importance for long term crop improvement and reduction of vulnerability to important crop pests. Prior to the availability of DNA based markers most genetic diversity analysis studies in cotton were carried out using morphological markers. Polymerase Chain Reaction (PCR)-based molecular markers such as RAPD, SSR, STS, AFLP, ISSR have developed into powerful tools to analyze genetic relationships and genetic diversity. Among them Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) utilize single arbitrary primers. RAPD [3, 4] is a powerful technique and its resolving power is several folds higher than morphological and isozyme markers. It is much simpler and technically less demanding than other techniques. RAPD markers have been used for diversity analysis and other applications in a vast array of field crops including cotton [5, 6, 7 & 8]. In present study RAPD technique was employed in a set of 25 accessions of *Gossypium hirsutum* to study the extent of genetic variation and find out the duplicates if any among those accessions.

Materials and methods

The materials included in the present study consisted of twenty five accessions of *Gossypium hirsutum* of different origins. The plants were grown at C.I.C.R., Nagpur, (Table 1).

Table 1. *Gossypium hirsutum* accessions and their source

S. No.	Accession	Source	S. No.	Accession	Source
1.	A56-347	Africa	14.	AS 132-59	Gadag
2.	A218 (932)	Africa	15.	AS 136	Nandyal
3.	A 7215	Africa	16.	Ashmoton	Not known
4.	AC 53	Hisar	17.	ATH×444-2	Ivory Coast
5.	Acala 1518-D64-65	USA	18.	Atlas 59-63	USA
6.	AR 43	Darsi (AP)	19.	Austin 64-65	USA
7.	AR 46	Darsi (AP)	20.	B7-955	Indore
8.	AR 47	Nandyal	21.	B55-50	Indore
9.	Arkansas 61-28	USA	22.	B55-53	Indore
10.	Arkot	Australia	23.	B 56-33-6	Indore
11.	AS 32-59	Karnataka	24.	B 57-740	Indore
12.	AS 100	Srivilliputtur	25.	B 57-819	Indore
13.	AS 122	Siruguppa			

DNA was isolated following a modified procedure of Edward *et al.*, [9] in which 2% polyvinyl pyrreledone (PVP) in extraction buffer was added to avoid co-isolation of phenolics and polysaccharides in the DNA. The DNA was resuspended in Tris-EDTA buffer, pH 8.0 and

quantified through agarose gel electrophoresis. The reaction mixture for DNA amplification consisted of 20ng sample DNA; 10 × Taq buffer (with 15 mM $MgCl_2$) - 2.5µl; 100 mM dNTPs - 2.5 µl; primer - 50ng, 1µl; Taq polymerase-5 units and made up to 25 µl with sterile distilled water for 1 reaction. Amplification was carried out on a thermal cycler (Biometra Programmable Thermal Cycler) with initial denaturation at 94°C for 6 minutes, followed by 36 cycles of denaturation at 94°C for 45 seconds, annealing at 36°C for 1 minute, and primer extension at 72°C for 1 minute. A final extension at 72°C for 10 minutes was given at the end of the cycles and the samples were held at 4°C till retrieval [3]. Fifteen microlitre of amplified product was loaded on 1.6% agarose gel containing ethidium bromide and electrophoresed at 100 mA. In all, eighty six 10-mer random oligonucleotide primers from Operon technologies, kit A (20), B (17), C (14), D (13), E (11) and F (11) respectively were used for the study.

Scoring

RAPD bands were designated based on their molecular weight calculated using the kilobase (Kb) ladder used as marker. Amplified bands were scored as present (1) or absent (0). The dice similarity coefficient was calculated from the data using the programme Similarity for Quantitative data (SIMQUAL) [10]. Cluster analysis was carried out by the UPGMA method and dendrogram was generated using SAHN subroutine of NTSYS-PC [10].

Results and discussion

Eighty six RAPD primers were used to amplify DNA from 25 *Gossypium hirsutum* accessions, of which sixty three primers detected polymorphism. A total of 296 amplified DNA fragments that ranged in size from 200-2000 base pairs were generated by the 63 primers. 113 (38.17%) of which appeared polymorphic. Average polymorphism was 1.8 bands per primer. RAPD gel patterns generated from two primers (OPA-14, and OPD-18) are presented in Fig. 1. Most of the primers from E and F kits did not produce scorable results. The RAPD data was used to generate a similarity matrix. Similarity ranged from 67 to 87%. The genetic divergence of 0.13 to 0.33 reflected that cotton accessions have considerable variation at the DNA level. The 25 accessions could be split into 2 groups of 24 and 1 accessions at 67% similarity (Fig. 2). First group of 24 accessions could be divided into, cluster A consisting of 20 accessions and cluster B consisting of 4 accessions within them. These two clusters reflected the genetic divergence of 0.30 and were found to be somewhat related sharing about 70% similarity. Although genetic divergence ranging from 0.13 to 0.33 was obtained using DNA markers, but still there was a group within which accession AC 53 and accession AR 43 shared 100% similarity.

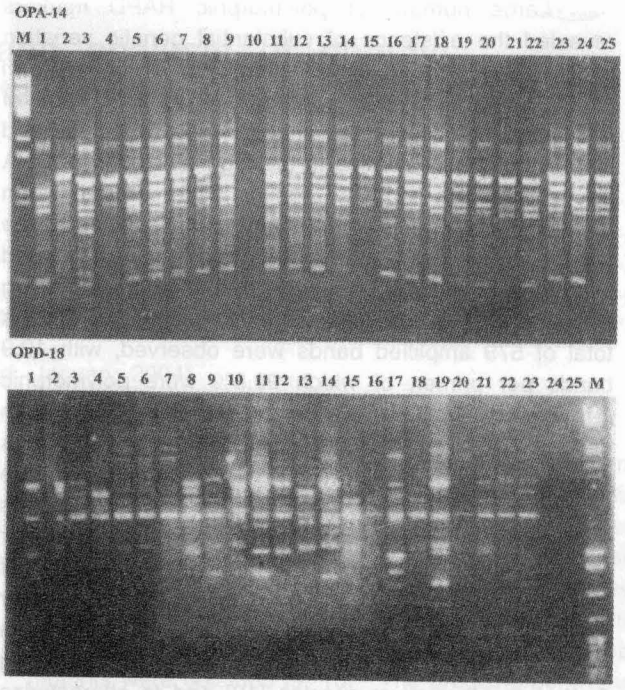

Fig. 1. Ethidium bromide stained 1.6 percent agarose gel showing RAPD profiles of twenty five Gossypium hirsutum accessions based on 10-mer random oligonucleotide primers

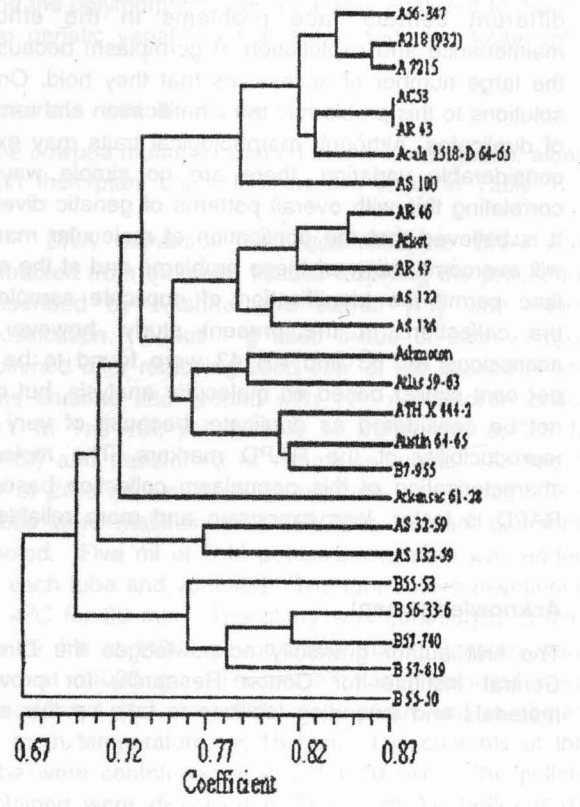

Fig. 2. Dendrogram based on dissimilarity indices (SIMQUAL-Dice Coefficient) among *Gossypium hirsutum* accessions for RAPD

Large number of polymorphic RAPD markers revealed the existence of substantial genetic variation at DNA level among the 25 genotypes of *G. hirsutum* studied. The SCIs ranged from 0.67 to 0.87. One of the important observations made during this study and also in the earlier studies is the high resolution of DNA based markers. Similar results were found when molecular phylogeny of 31 *Gossypium* species, three subspecies and one interspecific hybrid was estimated to evaluate genetic diversity by DNA fingerprinting using RAPD markers. From 45 random decamer primers, a total of 579 amplified bands were observed, with 12.9 bands per primer, of which 99.8% were polymorphic [1]. Similarly, RAPD markers generated by 30 random decamer primers were used to fingerprint 12 cultivars and a breeding line of *Gossypium hirsutum* and the *Gossypium barbadence*. Ten of the *G. hirsutum* cultivars were characterized individually based upon cultivar specific RAPD markers indicating the possibility of differentiating closely related cultivars by molecular markers [8]. Successful use of RAPDs has been documented to analyse genomic affinity among Australian *Gossypium* species [12] and to characterize three Egyptian cotton varieties [13]. The repositories of biodiversity available for cotton are a valuable source of useful genes for plant breeders. However, it is a difficult task to maintain such a large collection and different centers face problems in the efficient maintenance and exploitation of germplasm because of the large number of accessions that they hold. One of solutions to this problem is the identification and removal of duplicates. Although morphological traits may exhibit considerable variation, there are no simple ways of correlating this with overall patterns of genetic diversity. It is believed that the application of molecular markers will overcome many of these problems and at the same time permit the identification of duplicate samples in the collection. In the present study, however the accessions AC 53 and AR 43 were found to be 100 per cent similar based on molecular analysis, but could not be considered as duplicate, because of very less reproducibility of the RAPD markers. The molecular characterization of this germplasm collection based on RAPD is faster, less expensive and more reliable.

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