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Analysis of Genetic Diversity in 20 Cotton Germplasm Lines Using Random Amplified Polymorphic DNA Marker

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ABSTRACT
Cotton plant is one of the most important cash crops cultivated globally in several countries. It provides raw material for cotton textile industry and also is the source for edible oil. In this study, it carried out to analysis of genetic diversity of 20 cotton (*Gossypium* spp.) gremplasm line using Random Amplified Polymorphic DNA (RAPD) molecular markers. A total of 22 RAPD primers were used for the screening of 20 germplasm line out of which 2322 fragments were amplified with 20 random primers and 73.7% were polymorphic. Genetic similarity matrix based on Jaccard similarity coefficients of cotton genotypes were ranging from 0.14-0.79. These coefficients were used to construct a dendrogram using the Unweighted Pair Group of Arithmetic Means (UPGMA). All 20 cotton genotypes were grouped into seven clusters. The largest cluster consists of 7 genotypes. The highest similarity among the cotton varieties were observed between NBRI-77 and NBRI-75 (0.768). Present study indicated a great deal of germplasm diversity among these 20 genotypes. Our study also reveal that RAPD technology is potentially simple, rapid, reliable and effective method of detecting polymorphism for assessing genetic diversity between genotype and help in the selection of parent for hybridization.

Key words: *Gossypium hirsutum*, RAPD marker, UPGM, genetic diversity

INTRODUCTION

Cotton plant is one of the most important cash crops cultivated worldwide. India, China, Pakistan USA and Uzbekistan are the leading cotton growing countries of the world (Khadi *et al*., 2010; Mehboob-ur-Rahman *et al*., 2012). It provides raw material for cotton textile industry and also is the source for edible oil. India cotton industry is growing significantly for production of 5.335 Mt in 2008 to 29 Mt in 2013/2014. India contributes 61% of cotton in international market. Currently, *Gossypium* includes 50 species, four of which are cultivated, 44 are wild diploids and two are wild tetraploids (Percival and Kohel, 1990). Out of the four cultivated species, *Gossypium hirsutum* L. and *Gossypium barbadense* L. Commonly called as new world cottons are tetraploids (2n = 4x = 52), whereas, *Gossypium herbaceum* L. and *Gossypium arboreum* L. is diploids (2n = 2x =26) and are commonly called as old world cottons (Simth, 1995).

The study of genetic diversity is important in a crop breeding programmed for selection of suitable diverse parent to obtain heterotic hybrids as well as for the conservation and characterization of germplasm. To analysis genetic possessions for their efficiency, quality parameters and stress tolerance field test are usually time consuming. Therefore, molecular markers and DNA technology are used to evaluate diversity in the gene pool to recognize genes of interest and to expand a set of markers for screening progenies (Karp *et al*., 1993). The genetic diversity of cotton cultivar has been carried out by the use of morphological, biochemical and molecular markers and study provides helpful information on the selection of parents in the development of cotton cultivar as well hybrid (Wu *et al*., 2006; Ullah *et al*., 2012). Karp *et al*.

(1993) described the basic categories of molecular markers as (i) Hybridization based (non-PCR) markers like RFLP and VNTR (ii) Arbitrary (or semi arbitrary) primed markers like RAPD, AP-PCR, ISSR, DAF and (iii) Site targeted PCR techniques like sequencing and STMS. Zhang *et al.* (2008) reported that DNA based marker have been subjugated broadly for molecular characterization and DNA finger printing of cotton. Different types of PCR and non PCR-based DNA markers such as RAPD, SSR, AFLP and RFLP have been used in cotton genome research. Amongst these molecular tools, Randomly Amplified Polymorphic DNA (RAPD) being comparatively expensive simple and reliable markers. It is provide a simple and fast approach to detect DNA Polymorphism for cultivars identification and diversity analysis (Preetha and Raveendran, 2008; Sheidai *et al.*, 2007; Rana and Bhat, 2005; Welsh and McClelland, 1990; Williams *et al.*, 1990). In this method, short oligonucleotides, an arbitrary sequence is used singly which binds at two different sites tying within an amplifiable distance with each other i.e., between 100 bp to more than 3 KB (Tingey and del Tufo, 1993; Witkiin *et al.*, 1994). Differences in amplified fragment a pattern is visualized by gel electrophoresis are attributed to mutations at primer binding sites, preventing or causing the annealing of a primer (Reiter *et al.*, 1992).

MATERIALS AND METHODS

Germplasm line: Twenty germplasm lines of Cotton viz., NBRI-31, NBRI-72, NBRI-63, NBRI-40, NBRI-60, NBRI-37, NBRI-68, NBRI-82, NBRI-84, NBRI-75, NBRI-5, NBRI-7, NBRI-77, NBRI-78, NBRI-85, NBRI-81, NBRI-86, NBRI-67, NBRI-39 and NBRI-41 were obtained from MGM college farm, Aurangabad.

Preparation of sample: Seeds were surface sterilized with 1% (w/v) mercuric chloride and 70% ethanol and then rinsed with deionised water. Seedlings are grown in pot of 2 weeks old were used. Two gram fresh leaves of 2 weeks old germinated seedling grown pot were used for DNA extraction.

DNA extraction: For each cultivar, genomic DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) by the method of Doyle and Doyle (1990) with some modifications. The DNA was quantified by using spectrophotometer at 260 nm. For quality assessment, DNA was electrophoresed on 0.8% agarose gel. The dilution of extracted DNA were verified again by spectrophotometery.

Rapd analysis: PCR was performed in a thermal cycler with 20 ng DNA as template, 25 mL Amplification reaction contained 1 mL of Taq DNA polymerase (Chromosome Biotech), 6.5 mL of 10X PCR buffer (Chromosome Biotech), 4 mL of dNTPs (Chromosome Biotech), 1 mL of $MgCl_2$ (Qualigens), 1.0 mL of Primer (Genei), 10.5 mL of Nuclease free water (Chromosome Biotech), 1.0 mL of Template DNA. The DNA amplification protocol was 94°C for 5 min, followed by 40 cycles of 94°C for 1.30 min 35°C for 1 min, 38°C for 1 min, 72°C for min 2.30 min and finally 72°C for 10 min. The amplification products were stored at 4°C till electrophoresis was performed. At the time of electrophoresis and 3 μL of 6 X loading dye (GeNei, Bangalore) was added into PCR products. All amplification products were electrophoresed on 2% (w/v) agarose gels at 60 V for 3 h, stained with Ethidium bromide, The sizes of amplified fragments were determined using standard 100 to >1000 bp DNA ladder mix (GeNei, Bangalore). Gel was photographed using a Gel documentation system (UVP MultiDoc-It).

Data scoring and analysis: Amplification products in the gel images were scored for presence (1) or absence (0) missing and doubtful case were scored. Homology of bands based on the distance of migration of amplified DNA fragments according to their molecular weights in the gel was determined. Molecular weights of the bands were estimated using 100 bp DNA ladder (Genei, Banglore) as standard. Jackard IJ similarity coefficients (F) were calculated using the programme SIMQUAL.

The similarity matrix was subjected to UPGMA (Unweighted pair group method for arithmetic mean) for cluster analysis and a dendrogram was generated (Sokal and Michener, 1958). These computations were performed using the programme NTSYS-pc version 2.0, Exeter software, New York (Rohlf, 1993).

The polymorphic percentage of obtaining band was calculated of by using the following equation:

$$\text{Polymorphic (\%)} = \frac{\text{Polymorphic bands}}{\text{Total bands}} \times 100$$

RESULTS AND DISCUSSION

Universal random primers viz., RPI-1, RPI-2, RPI-3, RPI-4, RPI-6 RPI-7 RPI-8, RPI-9, RPI-10, RPI-11, RPI-12, RPI-14, RPI-15, RPI-16, RPI-17, RPI-19, RPI-20, RPI-21, RPI-22, RPI-23 were used for the study and they generated 2322 RAPD amplification products among all 20 cotton germplasm lines. Among RAPD markers, RPI-23 produced the maximum number of bands (161 in all genotypes) followed by RPI-2 (158) (Fig. 1) and RPI-3 (153). While RAPD marker RPI-19 generated a minimum number of bands (54) in the genomic pool. Polymorphic bands in screened markers ranged from 14-138 and the maximum was observed in RPI-2 (138) (Fig. 1) followed by RPI-3 (135) Level of monomorphic bands in screened markers as it was observed in RPI-20 (80) (Fig. 2), RPI-21 (60), RPI-10 (20) (Fig. 3), RPI-23 (40), RPI-15 (40), RPI-19 (40) and RPI-1 (20). The percent amplified bands in banding pattern was calculated and it was highest in RPI-3 (88.23%) followed

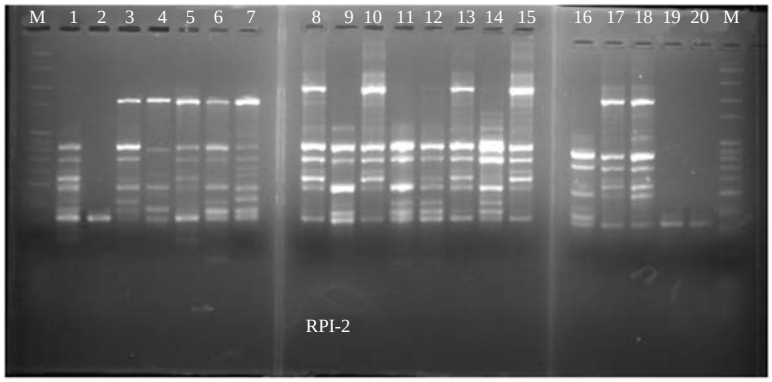

Fig. 1: RAPD banding pattern of twenty cotton genotypes using RPI-2 primers

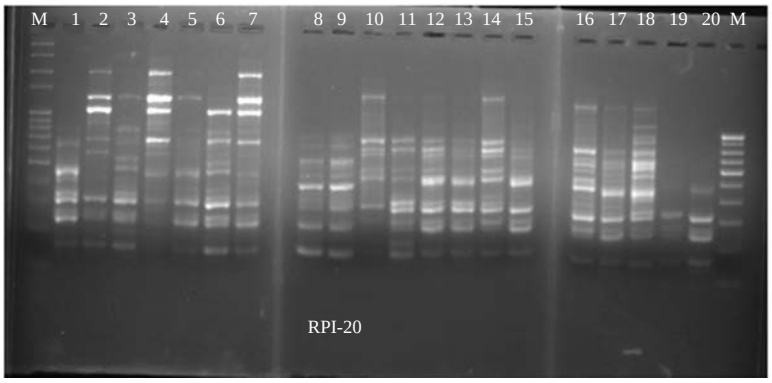

Fig. 2: RAPD banding pattern of twenty cotton genotypes using RPI-20 primers

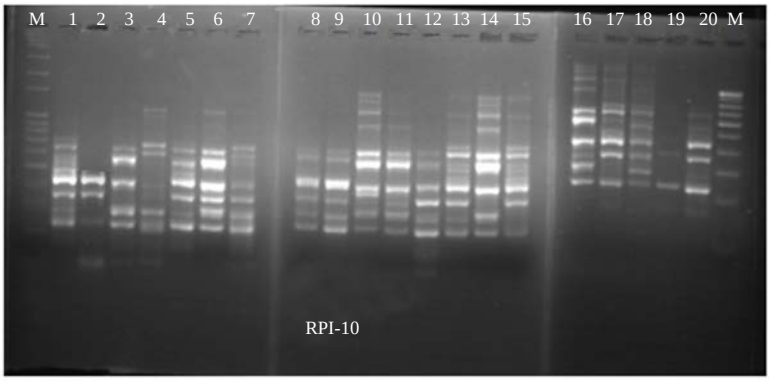

Fig. 3: RAPD banding pattern of twenty cotton genotypes using RPI-10 primers

by RPI-2 (87.34%) while the minimum was recorded from RPI-19 (25.92%) (Table 1). Calculations for Polymorphic Information Content (PIC) be completed using the formula of the expected heterozygosity (Smith *et al*., 2000) as:

$$PIC = 1-\sum f^2i$$

where, f is the percentage of genotypes in which the fragment is present. The PIC value is a sign of a high probability of obtaining polymorphism using that primer combination. Among Random Amplified Polymorphic DNA (RAPD) markers have more Polymorphism Information Content (PIC) value among RAPD markers the highest PIC value was observed from RPI-2 (0.91) followed by RPI-3 (0.90). The minimum PIC value was observed from RPI-1 (0.45) (Table 1). The genetic relationship between cotton genotype was determined on the basis of Jackard IJ pairwise similarity coefficient values. The value of similarity coefficients ranged

from 0.14-0.79. The genotype NBRI-85 and NBRI-84 represent highest average similarity coefficient value (0.79) the genotype NBRI-84 and NBRI-75 represent lowest average similarity coefficient value (0.14). Nei's similarity used to carry out the cluster analysis and to generate a dendrogram showing the relationships among the selected genotypes. All 20 genotypes were grouped into seven clusters (Fig. 4). Second cluster is largest consist of 7 genotypes i.e., NBRI 37 NBRI 41, NBRI-77, NBRI-75, NBRI-81, NBRI-60 and NBRI-86 in which NBRI-77 and NBRI-75 (0.768) are closest and NBRI-37 and NBRI-41 (0.725) are moderately close. The second largest cluster is the 1st cluster which consists of 5 genotypes i.e., NBRI-85, NBRI-84, NBRI-39 NBRI-69 and NBRI-31 in which NBRI-85 and NBRI-84 are closest. Then come 3rd cluster in which NBRI-7 and NBRI-72 (0.473) are close then come 5th cluster in which NBRI-63 and NBRI-78 (0.432) are closely related. 4th (NBRI-5), 6th (NBRI-40) and 7th (NBRI-82) are solitary clusters. Present results depicted efficient use of RAPD technique to determine genetic distance among genotypes. It is therefore, concluded that RAPD marker is potentially simple, rapid, reliable and effective method of detecting polymorphism for assessing genetic diversity between genotype and these help in the selection of parent for hybridization.

Table 1: Characteristics of the amplification products obtained with 20 primers for RAPD

Primer	Total bands	Monomorphic bands	Polymorphic bands	Percent polymorphism (%)	PIC value
RPI-1	93	20	73	78.49	0.45
RPI-2	158	20	138	87.34	0.91
RPI-3	153	20	135	88.23	0.91
RPI-4	92	20	72	78.20	0.90
RPI-6	119	20	99	83.10	0.87
RPI-7	150	40	110	73.30	0.84
RPI-8	130	20	110	84.61	0.89
RPI-9	107	20	87	81.30	0.88
RPI-10	134	20	114	85.07	0.88
RPI-11	88	20	68	77.20	0.84
RPI-12	111	20	91	81.98	0.87
RPI-14	106	20	86	81.10	0.88
RPI-15	104	40	64	61.53	0.82
RPI-16	99	20	79	79.80	0.87
RPI-17	115	20	95	82.60	0.90
RPI-19	54	40	14	25.92	0.70
RPI-20	149	80	69	46.30	0.88
RPI-21	118	60	58	49.10	0.85
RPI-22	76	20	56	73.68	0.83
RPI-23	161	40	121	75.10	0.90
	2322	600	1722		

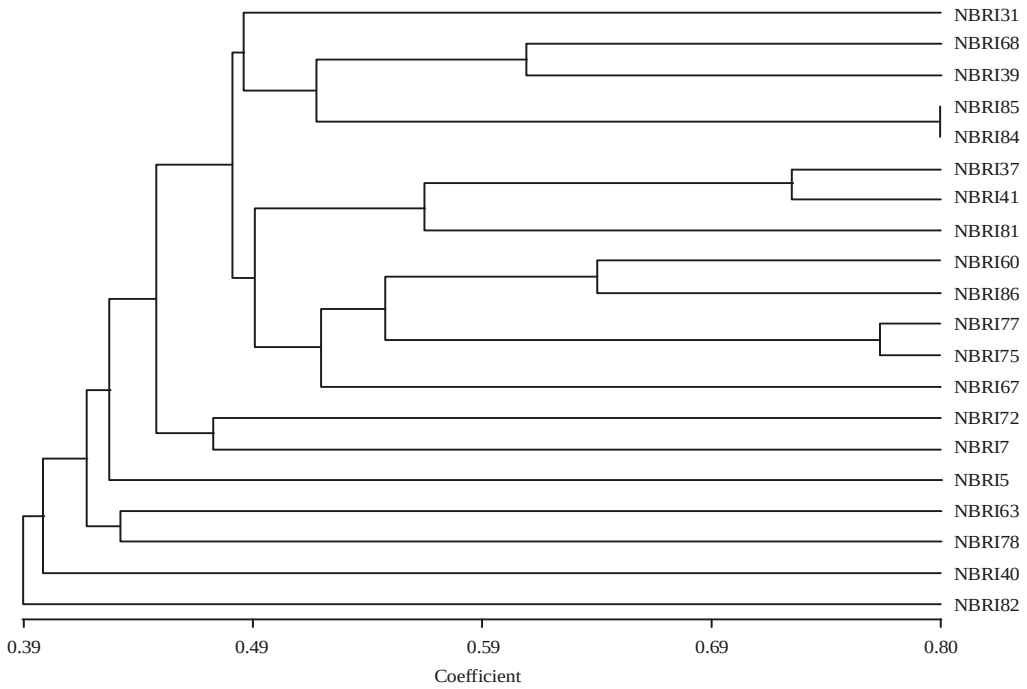

Fig. 4: Dendrogram showing genetic diversity for RAPD markers in *Gossypium* spp.

The consideration of estimated genetic distance is important for comparative analysis of diversity levels (Roldan-Ruiz *et al*., 2000). The RAPD technique is useful in areas of genetic diversity and DNA fingerprinting analysis. Ali *et al.* (2009) developed the linkage map of leaf red color in cotton using RAPD and SSR markers. They have found the basic information about the inheritance of red color in cotton using RAPD and SSR for linkage analysis and construction of genetic linkage map.

Khan *et al.* (2010) investigated RAPD marker associated with heat tolerance in 11 cotton genotypes among 53 RAPD primers, 32 were polymorphic and 21 were monomorphic. Thirty two polymorphic primers produced 273 fragments. The GLC 20 primer produces 31 polymorphic bands while two primer GLB-5 and GLC-12 produced one polymorphic band. Highest genetic similarity was observed between FH-945 and BH-160 (88.89%) whereas the lowest value was found between NIAB-801/2 and FH-945 (42.48) Patil *et al.* (2007) used 19 random decamer primers to asses genetics diversity in four cotton genotypes of them. Ninety seven were polymorphism out of 123 RAPDs loci. Primer GH-10, H-1, L-13, L-14 showed 100% polymorphism and found maximum and the lowest genetic distance between verities. Jayadhar and LRA-5166 showed a maximum distance (533.29%) and abadhita and LRA-5166 showed a lowest genetic distance (9.68%). From the present study, it is evident that RAPD analysis is potentially simple, rapid, reliable and effective method of detecting polymorphism for evaluate genetic diversity among the genotype and these assist in the collection of parent for hybridization.

CONCLUSION

The overall findings from this study indicated that RAPD analysis sufficiently detected genetic diversity to differentiate cotton cultivars. RAPD technique is useful in areas of genetic diversity and DNA fingerprinting analysis. As the need to protect proprietary germplasm as it is likely to increase in the future, RAPD will have an important role in securing a plant variety right by virtue of its unique efficiency in distinguishing closely related germplasm. Future thrust will be directed towards the holistic use of RAPD primers for DNA fingerprinting, genetic analysis and linkage mapping in cotton.

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