International Scholars Journals

African Journal of Plant Breeding ISSN 2375-074X Vol. 5 (10), pp. 001-005, October, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

Full Length Research Paper

Estimation of genetic divergence among some cotton varieties by RAPD analysis

L. Chaudhary[1]*, A. Sindhu, M. Kumar, R. Kumar[2] and M. Saini[3]

[1]Department of Biotechnology Engineering, Ambala College of Engineering and Applied Research, Mithapur, Ambala Cantt., Haryana, India- 133 101.
[2]Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar-125 004, India.
[3]Department of Plant, Soil and Agricultural System, Southern Illinois University, Carbondale-62901, USA.

Accepted 26 November, 2017

Total genomic DNA from 15 cotton varieties were analysed to evaluate genetic diversity among them through random amplified polymorphic DNA (RAPD) analysis, with 30 random decamer primers using the polymerase chain reaction (PCR). A total of 370 bands were observed, with 12.3 bands per primer, of which 91.6% were polymorphic. OPM-16 produced the maximum number of fragments while the minimum number of fragments was produced with the primer OPM-18. Cluster analysis by the unweighted paired group method of arithmetic means (UPGMA) showed that 15 varieties can be placed in five groups with a similarity ranging from 0.48 - 0.86. Maximum similarity was observed between H-777, H-974 and H-1098 (0.86). Interestingly, these varieties have been developed at one breeding center. The analysis revealed that the intervarietal genetic relationship of several varieties is related to their center of origin. Most of the varieties have a narrow genetic base. These results were well in accordance with previous reported results. The RAPD analysis indicates that it may be a more efficient marker than morphological marker, isozyme and restriction fragment length polymorphism (RFLP) technology. The results obtained can be used in selecting divergent parents for breeding and mapping purposes.

Key words: Cluster, cotton varieties, diversity, RAPD, genetic similarities.

INTRODUCTION

Cotton is the most important textile fiber crop and is the second most important oil seed crop in the world. Cotton belongs to genus *Gossypium* and comprises of 50 different species, distributed in eight genomes. Of the 50 species, only four species are cultivated in India. *Gossypium arboreum and Gossypium harbaceum* belong to the old world diploid group, where as the new world tetraploid cultivated species are *Gossypium hirsutum and Gossypium barbedance*. India is among the top three cotton producing countries with an annual production of nearly 23.2 million bales. A large number of cotton varieties grown in India originated from intraspecific crosses of *G. hirsutum* and this practice resulted in a narrow genetic base for the new varieties. Previously morphological markers, with their complex and undeciphered genetic control, were used for individual identification. Morphological features are indicative of genotypes but are represented by only a few loci because there is not a large enough number of a character available. Moreover, they can also be affected by environmental factors and growth practices. Protein markers can be used to provide varietal profiles because the variations for these markers are ubiquitous and this variation can be understood in genetic terms. Although proteins are products of the primary transcripts of DNA, environmental factors can affect qualitative and quantitative levels of protein. Wendel et al. (1992) studied the genetic distances of a large number of accessions of upland cotton from different locations by isozyme analysis. However, isozyme analysis has certain limitations due to the availability of limited number of marker loci. Within the last few years Restriction Fragment Length Polymorphism (RFLP) technology have been applied to several cotton species to study the

*Corresponding author. E-mail: lakshmi_gpb@rediffmail.com.

Table 1. List of cotton varieties used in genetic analysis studies.

Sr. No.	Variety	Year	Pedigree	Center of origin
1.	HS-6	1991	(BN X K3199) BN	CCSHAU, HISAR
2.	H-777	1978	Reselection BN	CCSHAU, HISAR
3.	H-974	1993	(H14X 45 red AK) H777	CCSHAU, HISAR
4.	H-1098	1997	(LH354 X SBI 71)H777	CCSHAU, HISAR
5.	H-1117	2002	Selection	CCSHAU, HISAR
6.	F-414	1978	Bikaneri narma	PAU, RS, FARIDKOT
7	LH-900	1987	(LH223 -480) LH223-343	PAU, LUDHIANA
8.	F-505	1987	F414X A231	PAU, RS, FARIDKOT
9.	F-846	1994	F452X LH223-481	PAU, RS, FARIDKOT
10.	LH-1556	1996	(LH886 X LH900) LH952	PAU, LUDHIANA
11.	RST-9	1992	BN X PS 10-27-1	RAU, RS, SRIGANGANAGAR
12.	RS-810	2000	RS644 X Khandwa 3	RAU, RS, SRIGANGANAGAR
13.	RS-2013	2002	Selection	RAU, RS, SRIGANGANAGAR
14.	Bikaneri Narma	1978	Local selection	RAU, BIKANER
15.	Ganganagar Ageti	1982	RS89	RAU, RS, SRIGANGANAGAR

evolution, population genetics, phylogenetic relationships and genome mapping (Yu et al., 1997), but it creates low variation in cotton compared to other plant taxa (Brubaker and Wendel, 1994). The random amplified polymorphic (RAPD) technique of Williams et al. (1990) provides an unlimited number of markers which can be used for various purposes like cultivar analysis and species identification in most plants. DNA fingerprinting studies to assess genetic purity with RAPD have already been conducted in cotton (Soregaon, 2004) . Multani and Lyon (1995) reported that RAPD markers could be used to distinguish closely related varieties. Keeping in view of these findings the present work was planned to study the genetic divergence of popular cultivated varieties of *G. hirsutum.*

MATERIALS AND METHODS

Plant material

The plant material used in the study consisted of 15 cotton varieties (Table 1). The plants were grown in pots in a greenhouse.

DNA isolation

Total genomic DNA was isolated with the modified CTAB method (Saghai-Maroof et al., 1984) . Approximately 5 g leaf material was ground to a fine powder using liquid nitrogen and quickly transferred into 25 ml of prewarmed (60°C) isolation buffer in a capped polypropylene tube, incubated for 1 hour at 65°C in a water bath and mixed by gentle swirling after every 10 min. To these tubes, equal volume of Chloroform: Isoamyl alcohol (24:1) was added and the contents were shaken for 10 min by hand. The tubes were centrifuged for 10 min at 8000 rpm; the upper aqueous layer was extracted twice with fresh Chloroform: Isoamyl alcohol and the final aqueous layer were transferred to a centrifuge tube. To these tubes, 0.6 volume of pre-chilled isopropanol was added and shaken gently for precipitation. By using a glass hook, DNA was spooled out in the form of whitish fibers and transferred to washing solution and dried. DNA was dissolved in an appropriate volume of 1X TE buffer.

For purification, RNase A was added to the tube (50 g/ ml) and the mixture incubated for 1 h at 37°C. DNA was extracted with CI by centrifuging the tubes at 10,000 rpm for 5 min at room temperature. DNA was precipitated with 2 volume of pre-chilled absolute ethanol and was recovered by centrifuging the tubes at 5000 rpm for 10 min; the pellet was washed with 70% ethanol and dissolved in appropriate volume of IX TE buffer.

PCR and gel electrophoresis

The random decamer oligonucleotide primers for the PCR were obtained commercially from Operon Technologies, Alameda, Calif; 30 random primers (OPM- 01 to 20 primers; OPA - 01 - 10) were used in this study. PCRs were carried out in 0.05 cm^3 reaction volumes each containing 50 ng of genomic template DNA, 0.2 M of the particular primer, 100 M of each dNTP, 2 l of Taq poly-merase 10X buffer, 1 unit Taq polymerase (Perkin Elmer) and 2.5 mM $MgCl_2$. PCR amplification was performed on a PTC 100 Thermal Cycler (MJ Research, Inc. Watertown, MA, USA) under the following conditions: Initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 10 min. The amplification products were resolved on 1.0% agarose gel and visualized under UV light following staining with ethidium bromide.

Data analysis

The frequency of RAPD polymorphism was calculated based on presence (taken as 1) or absence (taken as 0) of common bands (Ghosh et al., 1997). The binary data were used to compute Pair-wise similarity coefficient (Jaccard, 1908) on NTSYS-PC. A dendrogram based on similarity coefficient was generated by using the unweighted pair group of arithmetic means (UPGMA).

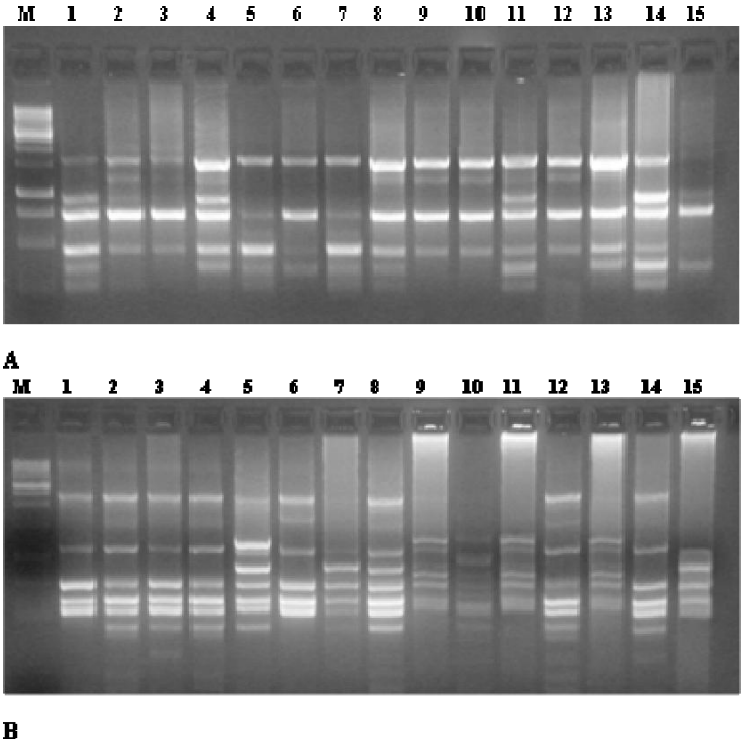

Figure 1. Amplified profile of 15 cotton varieties with primers (A) OPM-08 and (B) OPM-13. Lane M = 1kb size marker; Lane 1 = HS-6; Lane 2 = H-777; Lane 3 = H-974; Lane 4 = H-1098; Lane 5 = H-1117; Lane 6 = F-414; Lane 7 = LH-900; Lane 8 = F-505; Lane 9 = F-846; Lane 10 = LH-1556; Lane11 = RST-9; Lane 12 = RS-810; Lane 13 = RS-2013; Lane14 = Bikaneri Narma; Lane 15 = Ganganagar Ageti.

RESULTS AND DISCUSSION

DNA of 15 cotton varieties was amplified with 30 different random primers purchased from Operon Technologies. All 15 genotypes with the 30 primers revealed a unique banding pattern and so can be used for variety identification. This might be indicative of a wide genetic base of the cotton varieties studied. Different primers produced a different level of polymorphism among the different varieties (Figure 1A and B).

A total of 370 DNA fragments were amplified, with an average of 12.3 RAPD markers per primer. Out of 370 amplified fragments 31 (8.4%) were found to be monomorphic. The remaining 339 (91.6%) amplicons were polymorphic in one or other of the 15 varieties studied. The amplitude of polymorphisms was high even there was not a single primer (out of 30 studied) which could differentiate clearly all the varieties. The size of the amplified fragments also varied with different primers. The approximate size of the largest fragment produced was 3.0 kb and the smallest fragment produced was approximately 0.25 kb. Out of the 15 varieties studied, LH-1556 produced the maximum number of DNA amplified fragment (248), while LH-900 produced 153 bands, which is the minimum number. Other varieties produced common bands in the range of 189 - 236. The variety H-974 and F-846 produced maximum number of common bands that is 223. A maximum of 14 fragments were amplified with primer OPM-16 and a minimum of 8 bands with primer OPM-18.

To estimate the genetic similarities of the cotton varieties a similarity matrix was obtained using Jaccard's similarity coefficient (1908) and is shown in Table 2. These similarity coefficients were used to generate a dendrogram (Figure 2) by UPGMA analysis in order to determine the grouping of different varieties. Maximum similarity was observed between H-777, H -974 and H-1098 (0.86). Interestingly, these varieties have been developed at one breeding center. On the basis of the RAPD data their genetic bases look very narrow. From the similarity matrix, the least similar variety is LH-900. Its similarity ranges from 0.48 - 0.57. The coefficient of similarity for most of the other varieties was found to be in the range 0.48 - 0.82. Similarly, Multani and Lyon (1995) studied a number of Australian cultivars and found 92.1 - 98.9% genetic similarity among nine cultivars of *G. hirsutum* L, while *G. barbedance* L. variety Pima S-7 showed about 57% similarity with *G. hirusutum* L. varieties. Tatineni et al. (1996) assessed genetic diversity among 19 cotton genotypes and compared the RAPD data with the taxonomic data. In their studies, 33.3% of the primers did not produce any polymorphism, while

Table 2. Jaccard similarity coefficient among 15 cotton varieties as revealed from RAPD marker analysis.

	HS-6	H-777	H-974	H-1098	H-1117	F-414	LH-900	F-505	F-846	LH-1556	RST-9	RS-810	RS-2013	Bikaneri Narma	Ganganagar Ageti
HS-6	1.00														
H-777	0.67	1.00													
H-974	0.65	0.86	1.00												
H-1098	0.65	0.82	0.83	1.00											
H-1117	0.69	0.60	0.55	0.60	1.00										
F-414	0.58	0.72	0.74	0.74	0.56	1.00									
LH-900	0.63	0.57	0.53	0.55	0.69	0.50	1.00								
F-505	0.60	0.75	0.75	0.76	0.59	0.74	0.57	1.00							
F-846	0.60	0.76	0.75	0.77	0.53	0.74	0.54	0.77	1.00						
LH-1556	0.61	0.75	0.78	0.81	0.58	0.72	0.51	0.76	0.76	1.00					
RST-9	0.62	0.76	0.75	0.75	0.55	0.74	0.48	0.74	0.77	0.82	1.00				
RS-810	0.58	0.72	0.71	0.74	0.61	0.72	0.54	0.73	0.75	0.82	0.81	1.00			
RS-2013	0.60	0.72	0.72	0.76	0.59	0.71	0.50	0.78	0.76	0.78	0.76	0.76	1.00		
Bikaneri Narma	0.60	0.70	0.70	0.72	0.58	0.70	0.52	0.70	0.72	0.70	0.72	0.68	0.76	1.00	
Ganganagar Ageti	0.63	0.76	0.73	0.74	0.57	0.70	0.55	0.75	0.74	0.74	0.73	0.74	0.77	0.75	1.00

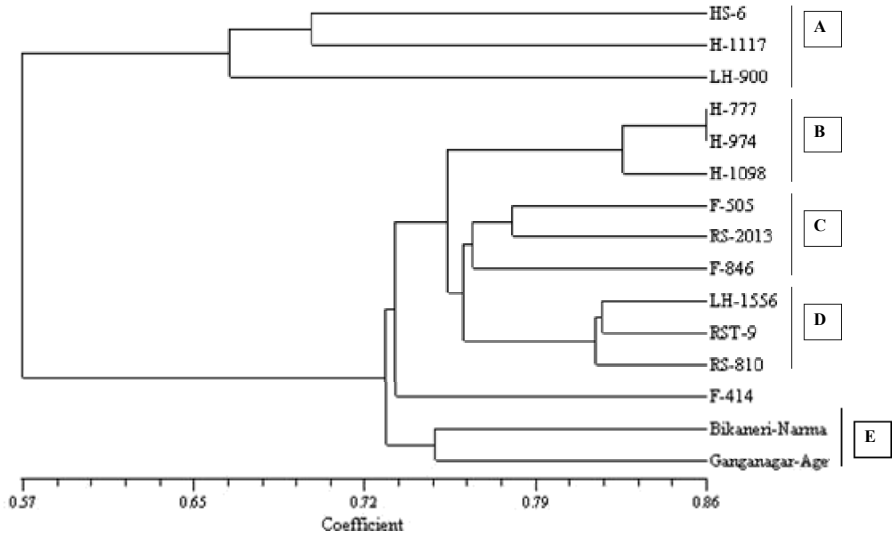

Figure 2. Dendrogram showing the genetic similarity among 15 cotton varieties as derived from RAPD data using the unweighted pair group method of arithmetic means (UPGMA).

Iqbal et al. (1997) reported the existence of 70 - 90% similarity coefficient in 22 *G. hirusutum* varieties but with very low level of genetic divergence. Brubaker and Wendel (1994) also demonstrated that the level of RFLP diversity was low in *G. hirusutum* L. cultivars as compared to other reported taxa. Cluster analysis using RAPD resulted in five main cluster groups. The dendrogram (Figure 2) assigned the varieties into groups which correspond well with their centers or sub centers of release and / or pedigree relationship. In cluster 'B' three varieties H-777, H-974 and H-1098 are more closely related as compared to any other variety. They have high estimates of genetic identity (0.86). In cluster 'A' out of three *G. hirsutum* varieties HS- 6 and H-1117 clustered together indicating that they are more closely related as compared to LH-900. The cluster 'C' and 'D' comprises three varieties each namely F-505, RS-2013 and F-846 and LH- 1556, RST-9 and RS-810, respectively. Their similarity ranges between 0.70 - 0.77 and 0.70 - 0.82, respectively. The variety F -414 showed a similarity index of 0.74 with the rest of the varieties of cluster B, C and D. The cluster 'E' comprises of two varieties Bikaneri-Narma and Ganganagar -Ageti. Earlier, subclustering had been reported in some elite cotton genotypes developed at one breeding station (Iqbal et al., 1997). The clustering of the varieties might be due to selection of the elite lines from a single population. Esbroeck et al. (1998) shown that genetic uniformity of U.S. cotton cultivars is greater today than it was 25 years ago. This was also reported in Basmati rice by Bligh et al. (1999). Moreover, breeders mostly shares the elite lines of other breeding stations in cotton improvement programs, making the breeding material identical which ultimately result in close kinship of the varieties. The genetic similarity obtained from the analysis will be useful in selecting divergent parents for breeding and mapping purposes.

REFERENCES

Bligh HFG, Blackhall NM, Edwards KJ, McClung AM (1999). Using amplified fragment length polymorphism and simple sequence length polymorphism to identify cultivars of brown and white milled ride. Crop Sci. 39: 1715-1721.

Brubaker CL, Wendel JF (1994). Re-evaluating the origin of domesticated cotton (*Gossypium hirsutum*; Malvaceae) using nuclear restriction fragment length polymorphisms (RFLPs). Am. J. Bot. 81: 1309-1326.

Esbroeck Van GA, Bowman DT, Calhoun DS, May OL (1998). Changes in the genetic diversity in cotton in the USA from 1970 to 1995. Crop Sci. 38: 33-37.

Ghosh S, Karanjawala ZE, Hauser ER (1997). Methods for precise sizing, automated inning of alleles and reduction or error rates in large scale genotyping using fluorescently labeled dinucleotide markers. Genome Res. 7: 165-178.

Iqbal MJ, Aziz N, Saeed NA, Zafar Y, Malik KA (1997). Genetic diversity evaluation of some cotton varieties by RAPD analysis. Theor. Appl. Genet. 94: 139-144.

Jaccard P (1908). Nauvelles recherches sur la distribution florale. Bull. Soc. Vaudoise Sci. Nat. 44: 223-270.

Multani DS, Lyon BR (1995). Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome 38: 1005-1008.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984). Ribosomal DNA spacer length polymorphism in barley: Mandelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. (USA). 81: 8014-8018.

Soregaon CD (2004). Studies on genetic introgression in interspecific crosses of cotton (*Gossypium* spp) M.Sc. (Agri.) Thesis, UAS, Dharwad, India. p. 130.

Tatineni V, Cantrell RG, Davis DD (1996). Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPD. Crop Sci. 36: 186-192.

Wendel JF, Brubaker CL, Percival AE (1992). Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. Am. J. Bot. 79: 1291-1310.

Williams JGK, Kubelik AR, Levak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphism amplification by arbitrary primers are useful as genetic markers. Nucleic Acid Res. 18: 6531-6535.

Yu ZH, Park YH, Lazo GR, Kohel RJ (1997). Molecular mapping of the cotton genome. Agron. Abstracts, ASA, Madison, Wiconsin. p. 147.