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Molecular Diversity Analysis in Cotton (*Gossypium hirsutum L.*) Using RAPD Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAW conducted the research and wrote the article. Authors SAS and AI helped author in lab works and analyzing the data. Author MEH supervised the study and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Cotton (*Gossypium hirsutum* L*)* is considered as major textile fiber around the world. This research aimed to analyze the molecular diversity among 9 cotton genotypes collected from Cotton Development Board, Bangladesh using the 7 Random Amplified Polymorphic DNA (RAPD) markers. Some total of 18 DNA bands were obtained and among them, 13 were polymorphic bands. The range of DNA amplification varied from 180 to 800 bp. The percentage of polymorphism was about 50.71. Genetic diversity ranged from 0.22 to 0.44 with an average value of 0.34. Nei's genetic distance ranging from 0.1667 to 0.6667 and most importantly PIC value ranged from 0.18 to 0.35 with an average value of 0.27. The PIC value indicated that most of the studied cotton genotypes were moderately diversified and homogenous as well as no heterozygosity found. A dendrogram indicating the relative genetic similarity of the Bangladeshi cotton genotypes was constructed which followed in two major clusters (A and B) among the studied material. The Cotton Development Board (CDB), Bangladesh committed to working on cotton improvement and this investigation will be helpful for the selection of diversified genotypes in varietal improvement in cotton. The output of this research will be a baseline for future molecular research work on cotton genotypes in Bangladesh.

Keywords: RAPD Markers; genetic diversity; cotton; polymorphism; dendrogram.

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1. INTRODUCTION

Cotton fiber is one of the highly acclaimed natural fibers under the genus of Gossypium. *Gossypium* includes 50 species and out of the four are cultivated [1]. Out of the four cultivated species, *Gossypium hirsutum* L. and Gos*sypium barbadense* L. commonly called as new world cottons are tetraploids (2n = 4x = 52), whereas, *Gossypium herbaceum* L. and *Gossypium arboretum* L. are diploids (2n = 2x = 26) and are commonly called as old-world cottons. It is grown primarily as a fiber crop, but after the lint, the long twisted unicellular hairs are removed by ginning, the seed can be crushed to extract vegetable oil, is used in a multitude of products including soap, cosmetics, and margarine and protein-rich animal food. Cotton can be easily processed into several products that we use on a daily basis, like coffee filters, bookbinding, paper, and bandages. Even stalks and leaves from the cotton plant can be made useful; stalks are ploughed underground to enrich the soil, and fiber extracted from them is used to make pressed paper and cardboard. Additionally, cotton serves a function in just about every industry, from pharmaceuticals to rubber and plastics Cotton linters (the short fibers that remain on the plant after it is ginned) are used in x-rays, swabs, and cotton buds in the medical field, as well as used in mattresses, furniture, automobile cushions, and even flat-screen televisions.

Cotton is the second important cash crop in Bangladesh after Jute. It is the main raw material of the textile industry. The annual requirement of raw cotton for the textile industryof Bangladesh is estimated at 8.3 million bales. Around 2-3% of the national requirement isfulfilled through the local production (nearly 0.17 million bales) from 44 thousand hectares of land, the remaining 97% requirement is fulfilled by importing raw cotton from India, USA, Pakistan, Turkmenistan, Uzbekistan, and from African countries [2]. In 2019, approximately7.3 million 480-pound bales of cotton had been imported in Bangladesh [3]. Cotton production potential can be raised to 5.0 lakh bale by expanding cotton cultivation in the saline and drought-prone areas, hilly areas, char land areas through implementing research, extension services, and training. The cultivation was not increased to its expected peak due to the lack of facilities and modern technologies. In order to increase the production and productivity of cotton, organized cotton breeding programs for open-pollinated, hybrids, mutant variety and Bt cotton must be developed. Such a program will require the development of techniques to determine the genetic diversity of the cotton varieties and races available in Bangladesh.

The existence of genetic diversity is an essential requirement for a successful hybridization program. In order to take the program of development of hybrid cotton successfully, the choice of the suitable parent through careful and critical evaluation is of paramount importance. The application of biotechnological approaches in breeding programs provides alternative avenues for cotton improvement through direct manipulation of genes and can produce improvements that are not possible through the classical breeding programs. Cotton grown in different regions shows a very less genetic difference [4]. A narrow genetic background itself poses a threat to the survival of a plant as it makes the plant vulnerable to environmental changes and natural catastrophes. Therefore to make the plants well adapted and more resistant to diseases and other environmental stresses there is always a need to improve the quality of a plant''s genome i.e.to increase its genetic div ersity. Molecular markers have been widely used in genetic analyses, breeding studies, and investigations of genetic diversity and the relationship between cultivated species and their wild parents [5]. RAPD (Randomly amplified polymorphic DNA) which is a PCR based markers are based on the amplification of unknown DNA sequences using single, short, and random oligonucleotide sequences of arbitrary nature as primers, requiring a minute amount of genomic DNA, does not need blotting and radioactive detection, etc [6]. RAPD does not need any prior knowledge of DNA sequence, however, still reveals a high level of polymorphism. The aim of this research to produce genetic variation and relatedness of cotton cultivars by PCR-based RAPD technique and phylogenetic relationship study among the cotton genotypes.

2. MATERIALS AND METHODS

2.1 Experimental Location and Source Materials

The experiment was done at the Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207. The sources of cotton seeds of the collected cotton cultivars and genotypes are presented in Table 1.

Table 1. List of cotton genotypes and their sources

Sl. No.	Genotypesname	Source of collection area
1.	CB-12	Cotton Research Center, Mahiganj, Rangpur
2.	CB-13	Cotton Research Center, Mahiganj, Rangpur
3.	CB-14	Cotton Research Center, Mahiganj, Rangpur
4.	CB-15	Cotton Research Center, Mahiganj, Rangpur
5.	Ra-16	Cotton Research Center, Mahiganj, Rangpur
6.	SR-16	Cotton Research, Training & Seed multiplication farm Sreepur, Gazipur
7.	SR-17	Cotton Research, Training & Seed multiplication farm Sreepur, Gazipur
8.	DM-3	CDB, Khamarbari, Dhaka
9.	Rupali-1	CDB, Khamarbari, Dhaka

2.2 DNA Extraction

Genomic DNA extraction was done in 3 to 4 leaf stage of seedling about 30 days of germination by using a standard protocol with the phenol-chloroform method. 1% agarose gel was used to visualize the extracted DNA. On the basic of DNA band thickness necessary dilution was made for PCR reaction. DNA quantification was done only by electrophoresis.

2.3 RAPD Analysis

Seven decamer RAPD primers viz. OPBA 03, OPBB 06, OPG 03, OPG 05, OPX 01, OPB 17 and OPD 20 (Operon Technologies, Inc., Alameda, California, USA) were used for PCR reaction on 9 cotton genotypes for their ability to produce the polymorphic band. DNA amplification was performed in a thermal cycler (Q- cycler Germany). 10 µl reaction mixture containing 5.0 µl 2X Taq Master Mix, 1.50 µl primers, 2.0 µl sample DNA, and 1.5 µl de-ionized water (Biobasic, Canada) were used to perform the PCR reaction.

Pre-denaturation with 95°C for 4 mins; denaturation with 95°C for 45sec, annealing at 35°C (based on Tm value of primer) for 40 sec, extension at 72°C for 1 min, final extension at 72°C for 5 mins continuing with 31 cycles and finally stored at 4°C.

2.4 Electrophoretic Separation of the Amplified Products

Running 2.0% agarose gel containing 0.75 µl ethidium bromide in 1x TBE buffer at 90 V for 75 minutes was used to confirm the PCR product. One relative molecular mass markers 100 bp (Bioneer, Korea and Bio Basic, Canada) was loaded on the left side of the gel. Under Ultra Violet (UV) light on a trans-illuminator, RAPD bands were observed and documented by taking photographs employing a Gel Cam camera.

2.5 RAPD Data Analysis

Since RAPD markers are dominant, hence all the DNA bands represented the phenotype at one allelic locus [7]. The band-size for each marker was scored using the Alpha Ease FC 4.0 software. The scores attained using all primers within the RAPD analysis were then pooled to form one data matrix. The concise statistics including the number of alleles per locus, major allele frequency, gene diversity, and Polymorphism Information Content (PIC) values were definite using POWER MARKER version 3.25 [8], a factor data analysis software. The allele frequency data from POWER MARKER was accustomed to export the info in binary format (presence of allele as “1” and absence of allele as “0”) for analysis with NTSYS-PC (Numerical Taxonomy and Multiware Analysis System) Version 2.2 software [9]. The unweighted pair grouping method, using arithmetic average (UPGMA), was used to determine the similarity matrix following the Dice coefficient with the SAHN subprogram. The polymorphism information content (PIC) value was described by Botstein [10].

$$PIC = 1-\sum pi^2$$

Where *pi* is the frequency of the i^{th} allele.

3. RESULTS AND DISCUSION

This chapter comprises the presentation and discussion of the results of the experiment. The results were obtained from 9 genotypes of cotton using finally seven RAPD primers.

3.1 Banding Pattern and Polymorphism Survey of Cotton (*G. hirsutum*) Genotypes

Five RAPD primers were screened and chosen for 9 cotton genotypes to evaluate their suitability for amplification of the cotton DNA fragments. The primers, which gave minimum smearing, high resolution and maximum reproducible and distinct polymorphic amplified bands were selected and RAPD primers viz. OPG 03, OPG 05, OPB 17, OPBB 06 and OPBA 03 showed reproducible amplified bands.

Strong and weak bands were produced in the RAPD reactions. Weak bands result from low homology between the primer and pairing site on the DNA strand [11]. The present study agrees with those of Hardy et al. [12] and Williams et al. [7].

The RAPD primer OPBA 03 produced different DNA fragments in different cotton varieties. It produced total 4 DNA fragments which were ranged from 300 bp to 500 bp and 3 of them were polymorphic (Fig. 1). The primer OPBB 06 was able to produced 5 DNA fragments in total and which ranged from 250 to 700 bp. Four bands out of 5 bands were polymorphic (Fig. 2). Two DNA fragments were amplified by the primer OPG 05 in different cotton genotypes which ranged from 280 to 330 bp and produced 1 polymorphic band. The genotype DM-3 was not amplified in this reaction (Fig. 3). Four DNA fragments amplification were noticed by the primer OPG 03 in 9 cotton genotypes which were ranged from 180 to 800 bp in which 2 bands were polymorphic in nature (Fig. 4). The RAPD primer OPB 17 was able to amplify 3 DNA fragments among all the individuals. The DNA fragments ranged from 150 to 700 bp. and all bands were polymorphic (Fig. 5).

The 5 primers regenerated total 18 DNA fragments with an average 3.6 per primer among the 9 cotton germplasm. Out of 18 DNA bands, 13 DNA fragments were polymorphic and the average percent of polymorphism was 50.71. The highest number of polymorphic band (4) was produced by the primer OPBB 06 and the lowest number of polymorphic band (1) was observed OPG 05. Maximum 100% of polymorphism was recorded in the primer OPB 17.

80% polymorphism was recorded in the primer OPBB 06. Seven of RAPD markers scored for each individual of 9 cotton genotypes for each primer is presented in Table 2.

Polymorphism in cotton in different studies could be attributed to the nature of the genetic material under investigation. The low degree of polymorphism in our study compared to other reports, could be due to the less diverse material which belonged to some cultivated varieties of cotton.

Harisaranraj et al. [13] found 45.5% polymorphism using 20s RAPD primers. Mia et al. [14] investigated 62.50% polymorphism with 12 RAPD primers.

Relatively higher level of polymorphism is also found in various experiments. Iqbal et al. [15] also observed that 98% of the primers in their study produced polymorphic profiles. Lu and Myers [16] observed a low level of DNA variation among ten varieties of *Gossypium hirsutum L*. as they observed only 13.5% polymorphism.

Bilwal et al. [17] observed total 171 amplicon among them 156 were polymorphic with 91.22% polymorphism.

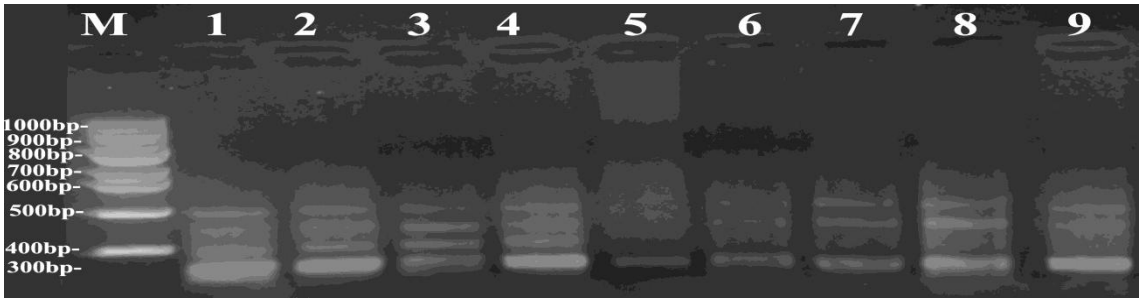

Fig. 1. PCR amplification with RAPD primer OPBA 03

(M=100 bp DNA ladder (Bioneer, Korea), Lane 1= CB-12, Lane 2= CB-13, Lane 3= CB-14, Lane 4= CB-15, Lane 5= Ra-16, Lane 6= SR-16, Lane 7= SR-17, Lane 8= DM-3, Lane 9= Rupali-1)

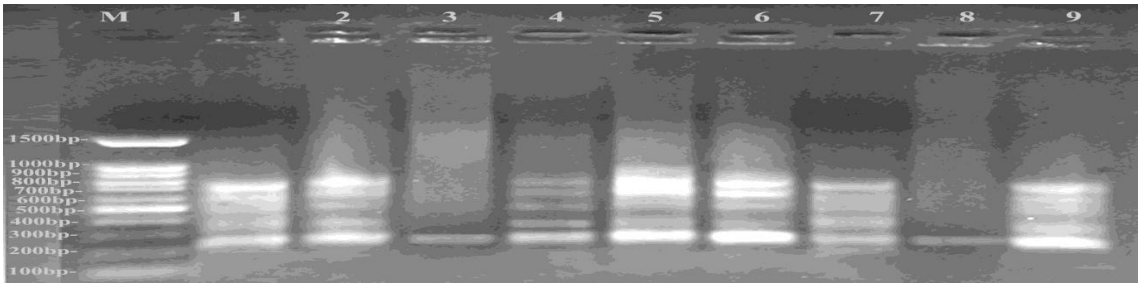

Fig. 2. PCR amplification with RAPD primer OPBB 06

(M=100 bp DNA ladder (Bio-Basic, Canada), Lane 1= CB-12, Lane 2= CB-13, Lane 3=CB-14, Lane 4= CB-15, Lane 5= Ra-16, Lane 6= SR-16, Lane 7= SR-17, Lane 8= DM-3, Lane9= Rupali-1)

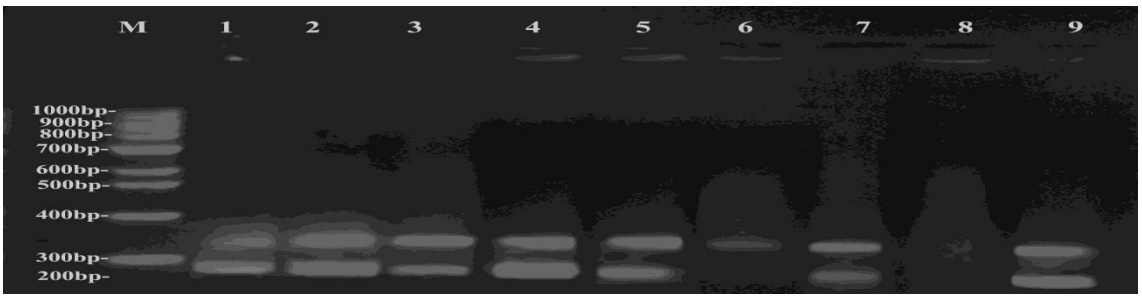

Fig. 3. PCR amplification with RAPD primer OPG 05

(M=100 bp DNA ladder (Bioneer, Korea), Lane 1= CB-12, Lane 2= CB-13, Lane 3=CB-14, Lane 4= CB-15, Lane 5= Ra-16, Lane 6= SR-16, Lane 7= SR-17, Lane 8= DM-3, Lane 9= Rupali-1)

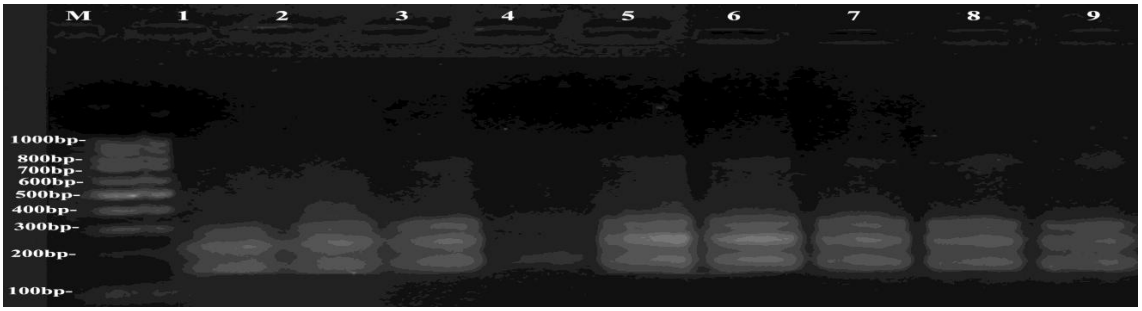

Fig. 4. PCR amplification with RAPD primer OPG 03

(M=100 bp DNA ladder (Bio-Basic, Canada), Lane 1= CB-12, Lane 2= CB-13, Lane 3= CB-14, Lane 4= CB-15, Lane 5= Ra-16, Lane 6= SR-16, Lane 7= SR-17, Lane 8= DM-3, Lane 9= Rupali-1)

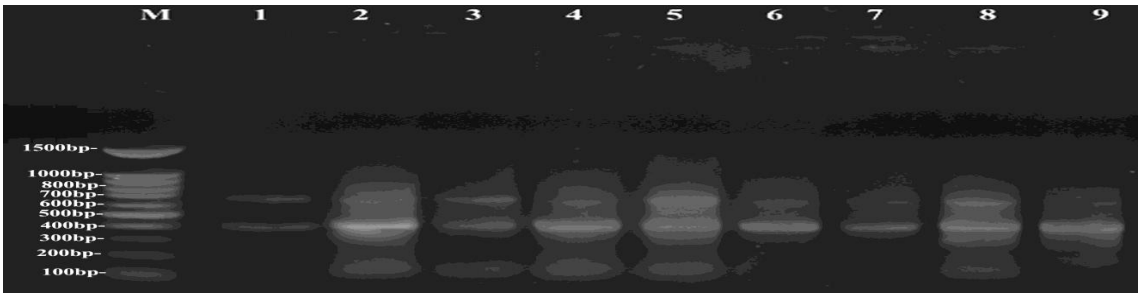

Fig. 5. PCR amplification with RAPD primer OPB 17

(M=100 bp DNA ladder (Bio-Basic, Canada), Lane 1= CB-12, Lane 2= CB-13, Lane 3= CB-14, Lane 4= CB-15, Lane 5= Ra-16, Lane 6= SR-16, Lane 7= SR-17, Lane 8= DM-3, Lane 9= Rupali-1)

3.2 Gene Diversity, Gene Frequency and PIC Values

Representing different alleles of the five markers which were scored as binary data whether present (1) or absent (0) was used for estimation of genetic distance and similarity co- efficientsthe binary matrix. Absence of bands may be caused by failure of primers to anneal a site in some individuals due to nucleotide sequence differences or by insertions or deletions between primer sites [18]. The summary statistics including major allele frequency, gene diversity and polymorphism information content (PIC) values are given in Table 3.

The gene frequency of the major allele ranged between 0.6667 (OPG 05) to 0.8891 (OPG 03) with an average value of 0.78. Polymorphic Information Content (PIC) value for the 5 markers ranged from 0.18 to 0.35 and the average PIC value was 0.29. The highest PIC value (0.35) was obtained for OPG 05 and the lowest PIC value (0.18) was obtained for OPG 03. PIC value revealed that OPG 05 was considered as the best marker for 9 cotton genotypes followed by OPBA 03, OPBB 06 and OPB 17 respectively. Gene diversity ranged between 0.44 (OPG 05) to 0.22 (OPG 03) with an average of 0.34. The results indicated that the 9 cotton genotypes present a high degree of homozygosity and also considerable intra-varietal group diversity and a certain degree of genetic differentiation and polymorphism.

The PIC was a good index for genetic diversity evaluation. Botstein et al. [10] reported that PIC index can be used to evaluate the level of gene variation, when PIC>0.5, the locus was in high diversity; when PIC<0.25, the locus was in low diversity and the locus was of intermediate diversity, when PIC between 0.25 and 0.5.

Data in that table show all the studied primers produced polymorphic amplification products. In cotton, Rana and Bhat [19] reported low gene diversity value 0.02 to 0.34 in 20 OPA- RAPD primers, which indicate that some RAPD markers are usefulfor differentiating between closely related genotypes.Sagar et al. [20] observed maximum PIC value (0.68) in ISSR 32 and UBC 842. Bilwal et al. [17] reported genetic similarity ranged from 0.74 to 0.93 percent. Dhatri et al. [21] observed PIC value ranged 0.66 to 0.92 with average of 0.83.

Table 2. RAPD primers with corresponding banding pattern and polymorphism observed in 9 cotton genotypes

Sl. No	Primer	Primer Sequence	(G+C) %	Band Scored	Number of Polymorphic Bands	%of Polymorphism	Ranges of fragment Size
1.	OPBA 03	GTGCGAGAAC	60	4	3	75	300- 500
2.	OPBB 06	CTGAAGCTGG	60	5	4	80	250-700
3.	OPG 05	CTGAGACGGA	60	2	1	50	280-330
4.	OPG 03	GAGCCCTCCA	70	4	2	50	180-800
5.	OPB 17	AGGGAACGAG	60	3	3	100	150-700
Total		-	-	18	13	355	-
Mean		-	-	3.6	1.86	50.71	-

Table 3. Gene diversity, gene frequency and PIC value of cotton genotypes by RAPD primers

Primers	Observation No.	Allele No	Major Allele Frequency	Genetic Diversity	PIC Values
OPBA 03	9	3	0.7778	0.37	0.34
OPBB 06	9	2	0.7778	0.35	0.29
OPG 05	9	2	0.6667	0.44	0.35
OPG 03	9	2	0.8891	0.20	0.18
OPB 17	9	2	0.7778	0.35	0.29
MAX		3	0.8891	0.44	0.35
MIN		2	0.6667	0.20	0.18
Mean	-	2.2	0.78	0.34	0.29

3.3 Principal Component Analysis (PCA)

Being a PCA statistical analysis method, it is capable of reducing the dimensionality of multivariate data. Data reduction is done to clarify the relationship between two or more characters and to divide the total variance of the original characters into a limited number of uncorrelated new variables called principal components.

Principal component analysis (PCA) is a technique used to emphasize variation and bring out strong patterns in a dataset. It's used to visualize genetic distance and relatedness between populations to confirm similarity or dissimilarity among the studied genotypes. Principal components analysis (PCA) analysis was conducted also using the PAST software. All 9 cotton genotypes were classified into four groups and showed in two dimensional scatter plot. All the groups were separated from each other (Fig. 6). Highest distance was showed between Group ii and Group iii.Group ii represents CB-12 and Group iii represents CB-14.Ra-16, SR-16, SR-17 and Rupali-1, CB-15 constructed Group-1.Group-4 represents CB-13 and DM-3. It indicates that, material selection for cotton improvement program should be taken from this group.

3.4 Nei's Genetic Distance and Genetic Identity

Genetic distance refers to the genetic deviation between species or between populations within a species. It is measuxred by a variety of parameters like Nei's standard genetic distance. This distance assumes that genetic differences arise due to mutations and genetic drift, but this distance measure is known to give more reliable population trees than other distances particularly for DNA data. Similarity indices measure the amount of closeness between two.

Individuals, the larger the value the more similarity between two individuals. There is a variety of alternative measures for expressing similarity, like Jaccard's coefficient of similarity which can be used for binary data and often is applied in RAPD-based studies. Other genetic diversity estimates in cotton have been reported using RAPD markers to range from 1 to 8% among Australian cultivars [22]. This coefficient is based on number of positive matches between two individuals whereas joint absences are excluded Smaller genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic relationship. Genetic distance can be used to

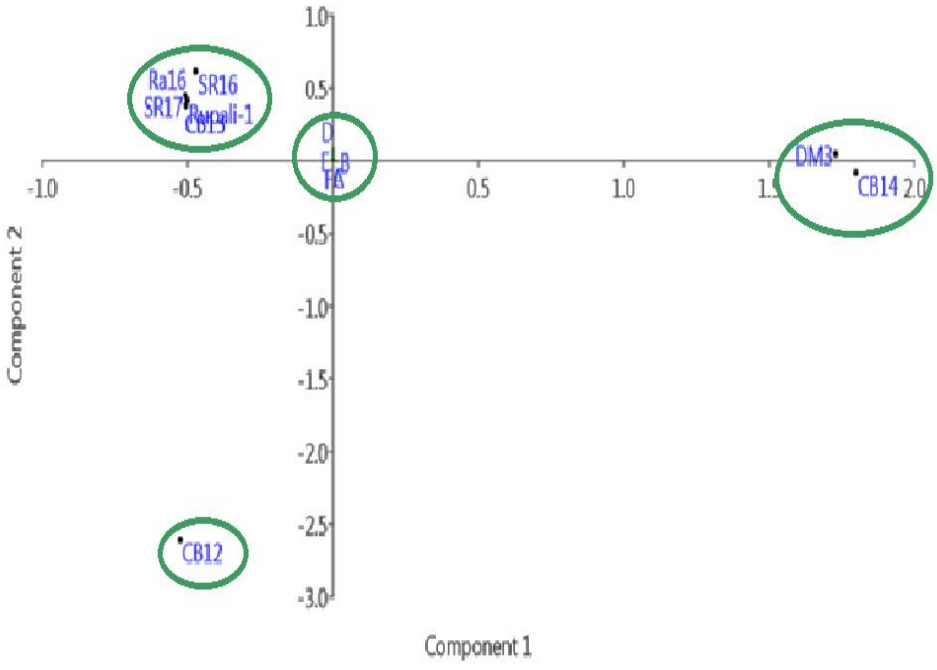

Fig. 6. PCA analysis represent PCA-1 and PCA-2 respectively by RAPD primer on 9 cotton genotypes

compare the genetic similarity between different species. Genetic diversity studies help in formulating proper conservation, preservation and selection of material for breeding program. The value of pair-wise comparisons Nei's [23] genetic distance between 9 cotton genotypes were computed from combined data through 6 primers, ranging from 0.1667 to 0.6667. The highest genetic distance 0.6667 was observed in DM-3 vs. CB-12 or Ra-16, CB-14 vs CV-12 or Rupali-1 or Ra-16 varietal pair whereas lowest value was observed in CB-12 vs CB-13 or CB-15 or SR-17, Ra-16 vs CB-13 or CB-15 or SR-17 and Rupali-1 vs CB-13 or CB-15 or SR-17 varietal pair (Table 4).

The similarity indices(Sij) for different accession pairs with six different primers and their average re shown in Table 5. In this experiment, inter varietal means of the pair wise similarity indices (Sij) ranged from 20 to 337.22. The highest similarity indices of 337.22 was observed in CB-12 and CB-14 varietal pair.So, genetic distance was lower between that pair than rest of the varietal pairs. On the other hand, Rupali-1 vsCB-13 or CB-15 or SR- 17 pair showed least Inter variety similarity indices of 20 and genetic distance was higher between that pair than rest of the varietal pairs. Rest of all the varietal pairs were not homogenous at different number of loci and with different primers. Therefore, this study clearly indicates that there was moderately genetic variation among the 9 cotton genotypes. Dhatri et al. [21] observed similarity co-efficient ranged 0.26 to 0.87.

3.5 Cluster Analysis

Dendogram based on Nei's [23] genetic distance using Unweighed Pair Group Method of Arithmetic Means (UPGMA) indicated the segregation of 9 genotypes of cotton into two main clusters: A and B. The first major cluster 'A' had divided only two minor group , genotypes DM-3 and CB-14 and the second major cluster 'B' had rest of genotypes. The second major cluster was subdivided into two minor clusters C & D in which cluster D was minor cluster included SR-16. The other sub cluster C had rest of the six. This minor cluster was also subdivided into two clusters E & F. where cluster E is included only one genotype Rupali-1 and sub cluster F divided into two sub clusters G and H. Ra-16 were grouped in cluster Gand cluster H grouped into two subdivision I and J. Group I included genotype CB-12and the subdivision of cluster J formed CB-13, CB-15 and SR-17 (Fig. 7).

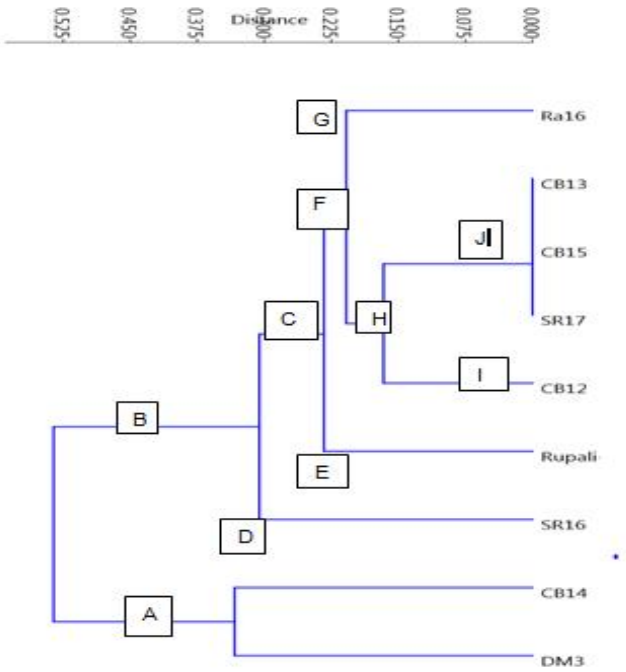

Fig. 7. UPGMA method based similarity matrix dendogram of 9 cotton genotypes by RAPD markers with PAST software

Table 4. Summary of Nei's genetic identity above diagonal) and genetic distance (below diagonal) values among 9 cotton genotypes

	CB-12	CB-13	CB-14	CB-15	Ra-16	SR-16	SR-17	DM-3	Rupali-1
sCB-12	***	0.1667	0.6667	0.1667	0.3333	0.5000	0.1667	0.6667	0.3333
CB-13	0.1667	***	0.5000	0.0000	0.1667	0.3333	0.0000	0.5000	0.1667
CB-14	0.6667	0.5000	***	0.5000	0.6667	0.5000	0.5000	0.3333	0.6667
CB-15	0.1667	0.0000	0.5000	***	0.1667	0.3333	0.0000	0.5000	0.1667
Ra-16	0.3333	0.1667	0.6667	0.1667	***	0.3333	0.1667	0.6667	0.3333
SR-16	0.5000	0.3333	0.5000	0.3333	0.3333	***	0.3333	0.5000	0.5000
SR-17	0.1667	0.0000	0.5000	0.0000	0.1667	0.3333	***	0.5000	0.1667
DM-3	0.6667	0.5000	0.3333	0.5000	0.6667	0.5000	0.5000	***	0.3333
Rupali-1	0.3333	0.1667	0.6667	0.1667	0.3333	0.5000	0.1667	0.3333	***

Table 5. Similarity and distance indices of cotton genotypes by RAPD primers

Acc NO.	CB-12	CB-13	CB-14	CB-15	Ra-16	SR-16	SR-17	DM-3	Rupali- 1
CB-12	0	79.000	337.217	79.000	82.006	90.615	79.000	328.199	81.492
CB-13	79.000	0	327.832	0.000	22.000	44.385	0.000	318.550	20.000
CB-14	337.217	327.832	0	327.832	328.569	325.186	327.832	82.462	328.441
CB-15	79.000	22.000	327.832	0	22.000	44.385	0.000	318.550	20.000
Ra-16	82.006	0.000	328.569	22.000	0	44.35	22.000	319.308	29.732
SR-16	90.615	44.385	325.186	44.385	44.385	0	44.385	315.825	48.683
SR-17	79.000	0.000	327.832	0.000	22.000	44.385	0	318.550	20.000
DM-3	328.199	318.550	82.462	318.550	319.308	315.82	318.550	0	317.921
Rupali1	81.492	20.000	328.442	20.000	29.732	48.683	20.000	317.921	0

CB-13,CB-15 and SR-17 are present in same group that means they are in same group but the percentage of similarity among these is unknown by this analysis.

Similarly 4 clusters were obtained by Sagar et al. [20], 7 clusters by Dhatri et al. [21], 2 clusters by Bilwal et al. [17], 7 clusters by Ashraf et al. [24] and 3 clusters by Dongre et al. [25] on the basis of different markers. The main objectives for this study were to study genetic diversity among Bangladeshi cotton genotypes using RAPD analyses. Consideration of estimated genetic distance is important for comparative analysis of diversity levels [26].

RAPD analysis appeared to provide more accurate estimates and utility of genetic diversity measurements. These methods have advantages and disadvantages for practical applications under different circumstances. Consequently, should continue rendering valuable services to farmers, breeders and genetic resource curators. The overall findings from this study indicated that RAPD analysis to a certain extent detect genetic diversity to differentiate Bangladeshi cotton genotypes. Genetically distinct varieties were identified that could be potentially important sources of germplasm for cotton improvement. Although all methods did not provide exactly the same description of relationships between genotypes. RAPDs analysis are more efficient and provide exciting insights [27,28,16].

4. CONCLUSION

Cotton (*Gossypium hirsutum L*) is one of the most important cash crop grown today in Bangladesh as well as in the world. Cotton is a major source of foreign exchange for many countries around the world. This research activities performed mainly the molecular characterization of nine cotton genotypes of Bangladesh.

In general, the choice of a molecular marker technique is based on reliability, statistical power, and level of polymorphisms. In this respect, RAPD marker is suitable to screen the genotype for molecular characterization.

Some total of nine cotton germplasms were used RAPD-PCR analysis and was done by using seven primers. Some total of 18 DNA bands were obtained and among them, 13 were polymorphic bands. The range of DNA amplification varied from 180 to 800 bp. The percentage of polymorphism was about 50.71. Maximum 100% of polymorphism was recorded in the primer OPB 17. 80% polymorphism was recorded in the primer OPBB 06. The overall genetic diversity was ranged from 0.22 to 0.44 in this study.In principle component analysis (PCA) on 9 cotton genotypes by five RAPD primers showed into two dimensional scatter plot with four groups. Nei's genetic distance ranging from 0.1667 to 0.6667The highest similarity indices of 337.22 was observed in CB-12 and CB-14 varietal pair and rest of varietal pairs showed low indices. So, this study indicates moderately genetic variation among the different cotton genotypes.

The dendogram (UPMGA) constructed from the fingerprint data obtained from nine cotton genotypes showed into two major cluster (cluster A and Cluster B).These findings demonstrate the utility and importance of molecular markers for genetic diversity analysis. Such studies can be extremely helpful for plant breeders to improve the cotton varieties for breeding program.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

There are no ethical issues involved in this research work.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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