Research Article

Studies on Genetic Diversity of Cotton Using RAPD Markers

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ABSTRACT

The study was conducted to assess the genetic diversity in 12 cotton varieties (*Gossypium hirsutum*). Diverse gene pool is significantly important in variety development programs and assessment of genetic variability is the first step in any crop improvement programs. The results showed considerable variability in the genotypes under study. Four RAPD primer series (A, C, J & K) were used in this study. Most of the primers used showed significant polymorphism. The genotypes were clustered into four groups (A, B, C and D). Four groups contained cluster and sub clusters which showed the similarity and dissimilarity of the genotypes. In group A, there were four genotypes, FH-169, FH-170, FH-172 and FH-113 while in group B, FH-941, CIM-241, CRIS-134 and PB-900 were present. In group C and D, there were three varieties i.e. CIM-XCP 29, CIM-506, CIM-448 and one genotype (CIM-240) respectively. This information would be useful for cotton breeding programs and in the hybridization experiments.

Key words: Cotton, Genetic Diversity, PCR, RAPD Markers

Introduction

Cotton fiber is the biggest import cash harvest of Pakistan known as “white gold” [1]. Pakistan is the fourth biggest producer of cotton, third largest exporter of raw cotton fiber, fourth largest consumer of cotton and the largest exporter of cotton yarn in the world. About 1.3 million farmers (out of a total of 5 million) cultivate cotton over 3 million hectares, covering 15 per cent of the cultivable area in the country [2]. Economic system of Pakistan is heavily dependent on cotton and textile sectors which accounts for 8.2 percent of the value-added in agriculture and about two percent of GDP [3]. In addition to cotton fiber, edible oil is also extracted from cotton seeds. It is also used in livestock and poultry feed [4].

Genetic diversity is the basic part of biological diversity and is the base of biological polymorphism. Genetic diversity and parenthood of germplasm play a significant role in cotton breeding. The specific evaluation of the genetic diversity of excellent germplasm provides a guide for selecting parents and predicting the degree of inheritance, variation, and level of heterosis, which are essential for realizing the breeding goal [5]. Study of the genetic diversity of Pakistan's present cotton cultivars and hybrids is very important for the development of new cotton varieties. Previous studies revealed that cultivated cotton exhibits a very low level of genetic diversity. While need for cultivar specific DNA markers in a cotton breeding program for cultivar registration, plant patents, and breeder's right protection and early

revealing of agronomic and monetary qualities as an aid to marker assisted selection is still needed [6].

RAPD analysis is one of the polymerase Chain Reaction (PCR) based system and involves genomic DNA amplification having the single primer arbitrary nucleotide sequence [7]. DNA markers are being used for determination of genetic diversity in different crops including cotton. In addition, they are also used to describe the quantitative and qualitative traits in different crops. These DNA markers have priority over morphological qualities, and are polymorphic with no epistatic or pleiotropic effects. They are not affected by environmental factors. They assist in determination of traits at earlier stages [7]. Keeping in view the importance of DNA markers, the project was designed to study the genetic variability of selected local cotton cultivars using RAPD markers.

Materials and Methods

Twelve cotton varieties (FH-169, FH-170, FH-172, FH-113, FH-941, CIM-241, CIM-XCP-29, CIM-506, CIM-448, CIM-240, CRIS-134 and PB-900) used in this study were highly adoptable and high yielding and belongs to different research institutes. The seed of the varieties was collected from the local market.

Growing of Seedlings

The twelve lines were grown in the green house of the Center of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan during the month of March. The leaves were plucked when seedlings were of 8 to 10 days old for DNA extraction.

DNA Extraction

The DNA was extracted by using CTAB-based method from cotton (*Gossypium hirsutum*) leaves previously collected. The procedure developed by Doyle and Doyle [8] was followed. The leaves were collected and immediately stored at -80°C for further analysis.

RAPD Primers

The series (A, C, J, K) of RAPD primer were used in the study for RAPD analysis and 24 polymorphic primers were selected to amplify the genomic DNA (Table-1). The fingerprints were examined under ultra violet Transilluminator and photographed using SyneGene Documentation System. All amplification products were scored as present (1) or absent (0) for each of the 12 varieties with all primers. Ambiguous bands that could not be clearly distinguished were not scored. The bands were counted by starting from top of the lanes to their bottom.

Development of Dendrogram

A dendrogram was developed by using Nei's similarity coefficient. PopGen software was used to obtain a dendrogram.

Results

Different primers produced different bands. A total of one thirty six bands were amplified in 12 varieties (an average 9 amplification per variety). CIM-506 produced the maximum of 35 bands. The minimum bands were obtained in FH-170 which amplified only at 20 loci. The monomorphic bands are invariable bands and cannot be used to study diversity whereas polymorphic bands revealed differences and can be used to examine and establish systematic relationships among the genotypes. K-20 produce only 14 bands, ten were monomorphic while four were polymorphic. CRIS-134 produced only polymorphic bands.

DNA fingerprinting was done to estimate the genetic diversity among 12 genotypes. The decamer primers produced a variety of RAPD blueprints. Total seventeen oligo-nucleotide primers were used for this purpose, out of which 15 oligo-nucleotides were amplified and give amplification. Gel pictures of amplified DNA using K-20 and K-18 primers are shown in Figure-1 & 2.

Dendrogram showed four distinct sub groups as shown in Figure-3.

Group- A

This group contained four varieties FH-169, FH-170, FH-172, FH-113. These varieties were present in paired cluster. FH-169 and FH-170 were clustered together while FH-113. FH-169 clustered together. FH-169 and FH-170 were 94% similar. FH-172 and FH-113 showed 94 % similarity and FH-169 showed 88% similarity with FH-172, FH-113 varieties. FH-

170 showed 82% similarity with FH-172 and FH-113.

Group-B

This group contained four varieties CIM-241, FH-941, CRIS-134, PB-900. The CRIS-134, PB-900 varieties were paired in a cluster and showed 91% similarity. CIM-241 showed 82% similarity with CRIS-134 and 85% with FH-941 and PB-900. CIM-241 did not cluster with any varieties and is distinct from all the other varieties.

Group-C

Group-C contained three varieties CIM-XCP 29, CIM-506 and CIM-448. CIM-506 and CIM-448 clustered together. They were 88% similar with each other. CIM-506 showed 82% similarity with CIM-XCP 29 while CIM-448 showed 88% similarity with CIM-XCP 29.

Group-D

This group contained one variety. This variety does not match with any other varieties. It is distinct from all other varieties.

Discussion

The fragments obtained from different primers showed a variable number of percentages among varieties. The Overall performance on the basis of diversity showed clear differences among different lines. FH-169 and FH-170 were 6% dissimilar whereas FH-172 and FH-113 showed 94 % similarity. But FH-169 showed 88% similarity with FH-172, FH-170 showed 82% similarity with FH-172. This showed 18 % dissimilarity which clearly indicated that these lines could be used in the breeding program. Plaschke et al. [9] reported the similar results who worked on another crop. CIM-241 showed 82% similarity with CRIS-134 and 85% with FH-941. CIM-506 showed 82% similarity with CIM-XCP 29. CIM-448 showed 88% similarity with CIM-XCP 29. Same results were found by Murtaza [6] who used RFLP markers in different lines of cotton. The same results were found in other crops by using different restriction and random markers [10,11]. Other researchers also found the same results that were in a range of 79-93% similarity [12]. CIM-240 is the most distinct variety among all the varieties under study. So, it is recommended to use this variety for the further breeding programs.

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Table-1: RAPD primers used in this study and their Sequences

S. No.	Primer Name	Primer Sequence (5' – 3')
1	GL Decamer A-02	TGCCGAGCTG
2	GL Decamer A-07	GAAACGGGTG
3	GL Decamer A-10	GTGATCGCAG
4	GL Decamer A-11	CAATCGCCGT
5	GL Decamer A-20	GTTGCGATCC
6	GL Decamer C-01	TTCGAGCCAG
7	GL Decamer C-02	GTGAGGCGTC
8	GL Decamer C-03	GGGGGTCTTT
9	GL Decamer C-06	GAACGGACTC
10	GL Decamer C-08	TGGACCGGTG
11	GL Decamer C-09	CTCACCGTCC
12	GL Decamer C-10	AGTCTGGGTG
13	GL Decamer C-11	AAAGCTGCGG
14	GL Decamer C-12	TGTCATCCCC
15	GL Decamer C-14	TGCGTGCTTG
16	GL Decamer C-15	GACGGATCAG
17	GL Decamer C-18	TGAGTGGGTG
18	GL Decamer C-19	GTTGCCAGCC
19	GL Decamer C-20	ACTTCGCCAC
20	GL Decamer J-03	TCTCCGCTTG
21	GL Decamer J-07	CCTCTCGACA
22	GL Decamer J-20	AAGCGGCCTC
23	GL Decamer K-18	CCTAGTCGAG
24	GL Decamer K-20	GTGTCGCGAG

Figure-1: Random amplication of 12 genotypes of cotton by Primer-K20. Lane M: 1 kb DNA Ladder, Lane 1-12: DNA from cotton varieties.

Figure-2: Random amplication of 12 genotypes of cotton by Primer-K18. Lane M: 1 kb DNA Ladder, Lane 1-12: DNA from cotton varieties.

Figure-3: Dendrogram developed using similarity coefficient data of RAPD analysis.

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