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Analysis of Genetic Diversity of Cotton Genotypes using RAPD PCR Technique*

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Abstract: Random Amplified Polymorphic DNA (RAPD) profiles for four cotton genotypes were generated with 19 random decamer primers. The primers generated 123 RAPD loci, of which 97 were polymorphic. The primers like GH-10, H-1, H12, L-13 and L-14 exhibited 100 per cent polymorphism. Maximum genetic distance was found between Jayadhar and LRA-5166 (53.29%), while the lowest genetic distance was between Abadhita and LRA-5166 (9.68%). The dendrogram of cotton genotypes showed two major clusters. The genotypes Jayadhar and Rahs-14 in one cluster and Abadhita and LRA-5166 in other cluster.

Key words : Genetic diversity, cotton, RAPD PCR technique, molecular marker

Introduction

Genetic variation is a prerequisite for any crop improvement programme. Assessment of the extent and distribution of genetic variation in a crop species and its relatives is essential in understanding pattern of diversity and evolutionary relationships between accessions that help to sample genetic resources in a more systematic fashion for conservation and plant improvement. Traditionally, genetic diversity is assessed based on morphological features such as plant height, reproductive features, day length sensitivity, local adoptation etc. through such, characters exhibit enormous variation for the particular use of the crop, only a part of the total genetic germplasm accessions for drought tolerance based on few discrete morphological characteristics may not provide an accurate indication of hte genetic divergence among cultivals/ speicies. In recent years, limitations of morphological and biochemical markers has been overcome by molecular markers. Among the different molecular markers, some are relatively cheaper and simple to use in variety of applications in plant research. One of such markers is Random Amplified Polymorphic DNA (RAPD) and is one of the Polymerase Chain Reaction (PCR) based DNA marker, defined as "an assay based on the amplification of genomic DNA with single primer of arbitrary nucleotide sequence (Weising *et al*., 1995). RAPD can be used in studying genetic diversity, varietal identification etc. It is simple to operate and does not involve radioactive labelling. The information on polymorphism using RAPD in a set of genotypes is useful in tagging genes of interest and genetic mapping in long run to facilitate marker assisted selection. With this in view, four coton genotypes were subjected to RAPD analysis for molecular characterisation.

Material and Methods

A total of four cotton genotypes were studied during the year 2004. DNA was extracted by CTAB method (Saghai-Maroof *et al.*, 1984). Seed were raised in plastic cups. Two grams of fresh young leaf from the shoot apex was taken for DNA isolation. The sample was ground to fme powder using liquid nitrogen with pre-chilled pestile and mortar. The powder was transferred into 2 ml eppendorf tube containing 1 ml extraction buffer. The solution was vortexed for 30-40 seconds, centrifuged at 4000 rpm for 10 minutes. Supernatant was discarded and 600 µl lysis buf fer was added. The solution was inverted and incubated at 650C for 60 min in a water bath. Chloroform and isomamyl alcohol (CIA) solution prepared in the ratio of 24: 1 was added to the tubes @ 500 µl per tube. The solution was inverted and centrifuged @ 8000 rpm for 10 min. The aqu,eous supernatant solution was pipetted out and. transferred to a fresh eppendorf tube. Ice chilled isopropanol of 10 ml was added to each tubes. Mixed by inverting and kept at -200C for over night. The solution was centrifuged at 8000 rpm for 10 min. Supernatant was carefully decanted, retaining the pellet. The pellet was washed with 70 per cent alcohol for 30 min and centrifuged @ 8000 rpm for 20 min, recovering the pellet. The pellet was allowed to dry at room temperature until the alcohol is completely evaporated. The pellet was resuspended in 200µ of Tris-EDTA buffer and incubated at 4°C. RAPD analysis was done individually with 19 random decamer primer. The thermal cycling conditions were;

Denaturation	:	94^0C for 1 min
Primer annealing	:	36^0C for 1 min
Primer extension	:	72^0C for 2 min

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The amplification products along with 2 µ of loading dye (bromophenol blue) were separated on 1.2 per cent agarose gel at 80 volts using Ix TAE buffer of pH 8.0 containing Ethidium bromide (0.5 µl 1/10 ml of gel). The gel were viewed under UV-transilluminator and photographed for documentation. Out of 30 primers used 19 primer produced recognizable bands. Scorable bands for a primer in each genotype were compared and allotted 0 (absence) or 1 (presence) values.' Band patterns (0, 1 matrix) were tabulated for individual primers separately and the data pooled to obtained a combined matrix for four genotypes off 19 primers. Diversity coefficient for each primer (number of polymorphic bands/total number of bands) and pair-wise genetic distance were calculated.

Results and Discussion

A total of 123 scorable bands were produced in four genotypes with 19 primers (Table 1). This data was utilized for further computations. The average number of bands per primer was 6.47. Out of 123 bands, 97 bands were polymorphic (77.73%). noted in the present investigation was much higher than the diversity (56.7%) previously detected in cotton by Rana and Bhat (2004). Further, the diversity was lower than the diversity (89.1%) previously detected in cotton by Iqbal *et al.* (1997). The similarity coefficient among the genotypes Jaydhar and Rahs-14 was 0.74 and the genotypes Abadhita and LRA-5166 was 0.89. High level of polymorphism based on RAPD technique has been reported among the genotypes of cotton (Khan *et al.*, 2000). The primer H-8 and H11 amplified 200 bp and 680 bp Jayadhar specific bands and the primer BH-12 and AC-4, which amplified 820 bp and 1020 bp Rahs-14 specific band.

The genetic distance was computed considering all the genotypes from the pooled data and the dendrogram was constructed. The distance matrix is presented in table 2. The maximum genetic distance of 53.29 per cent was found between Jayadhar and LRA-5166, followed by 5`.48 per cent between Jayadhar and Abadhita. The lower genetic distance of 9.68 per cent was recorded between LRA-5166 and Abadhita. Part of hte

Table 1. Scorale DNA bands generated by different random decamer primer through PCR

Primer	Total no. of bands produced	No. of polymorphic band	Per cent produced polymorphism
AC-4	8	7	87.50
AC-lO	6	4	66.70
AT-5	6	5	83.33
AT-20	4	2	50.00
BH-2	4	3	75.00
G-9	7	3	42.90
G-lO	9	9	100.00
G-ll	6	5	83.33
H-l	3	3	100.00
H-8	9	6	66.70
H-ll	7	4	57.14
H-12	8	8	100.00
H-14	11	10	90.90
1-18	6	5	83.33
L-ll	6	4	66.70
L-12	6	5	83.33
L-13	8	8	100.00
L-14	4	4	100.00
M-3	5	2	40.00
Pooled	123	97	1476.86
Average	6.47	5.11	77.73

The average number of polymorphic RAPD bands was 9 per primer. The primers such as G-1 0, H -1, H -12, L-13 and L-14 produced 100 per cent polymorphism with all the identifiable RAPD bands being polymorphic. Primers AC-4, AT-5, G-11, H-14, 1-18, L-12 produced 87.50, 83.33, 83.33, 90.90,.83.33, 83.33 per cent polymorphism, respectively (Table 1). The diversity diversity was attributed to wide spead (geograpical diversity) and domestication of cotton from wild species. Although, the genotypes choosen for the present study were not random accessions but were choosen based on drought tolerance is a highly complex trait. It is hte combination of several morphological and physiological components contributing for

drought tolerance. Each genotype could tolerate drought through different combinations of component traits. Therefore, although, the selected genotypes are uniformly drought tolerrant , there could be lot of variation among the genotyeps for component traits and their combination, could be the reason for high level of polymorphism observed for the selected genotypes. The genetic distance was used for cluster analysis and dendrogram was constructed for the pooled data (Fig. 1). The dendrogram separated the genotypes into two major clusters. The two genotypes, LRA-5166 and Abadhita were found in one cluster and the. genotype Jayadhar and Rahs-14 were found in other cluster. The genotypes, which were found in both clusters, were further divided into two sub-clusters. The genotypes LRA-5166 and Abadhita was found in one sub-cluster each and the other sub-clusters had the genotypes Jayadhar and Rahs-14. Considering the dendrogram constructed from the pooled data, it is evident that set of primers choosen for hte study were able to group the genotypes into phenotypically intended category such as drought tolerant and drought susceptible. Further, hte close asociation of yeild with physiobiochemcial parameters suppports the clustering of hte genotypes with 19 primers (Table 3). Thus, the categorization of genotypes as drought tolerant (Jaydhar and Rahs -14) and drought susceptible (LRA - 5166 and Abadhita) based on yield and physio biochemcial observations. Like LAD, SLW, RWC, CSI, proline content, chlorophyll fluorescence and leaf water potential is well reflected in molecular groupings.

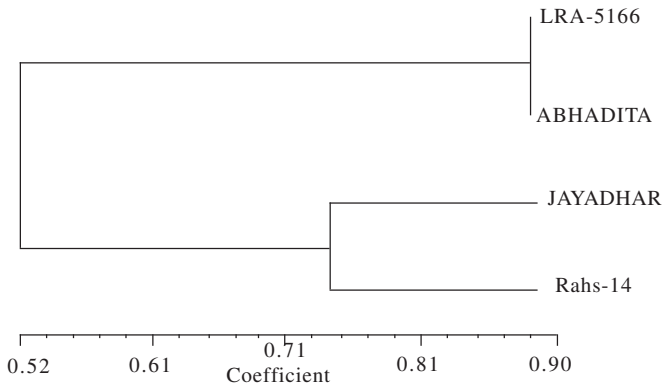

Fig. 1. Dendrogram depicting four cotton genotypes based on the genetic distance generated by 19 random primers

Table 2. Genetic distance (%) based on RAPD pooled over the 19 primers in four cotton genotypes

	LRA-SI66 *(G. hirsutum)*	Abadhita *(G. hirsutum)*	Jayadhar *(G.. herbaceum)*	Rahs-14 *(G.herbaceum)*
LRA-5166	0.00			
Abadhita	9.68	0.00		
Jayadhar	53.29	51.48	0.00	
Rahs-14	43.21	45.35	25.88	0.00

Table 3. Correlation coefficient (r) of seed cotton yield with physio biochemical characters of cotton

Sl. No.	Particulars	'r' value
1.	Leaf area duration (LAD)	0.90*
2.	Specific leaf weight (SLW)	0.98*
3.	Relative water content (RWC)	0.93*
4.	Chlorophyll stability index (CSI)	0.96*
5.	Proline	0.77*
6.	Leaf water potential (LWP)	0.99*
7.	Chlorophyll fluorescence	0.98*

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