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Analysis of genetic diversity in cotton genotypes using DNA marker

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Abstract
Cotton plant which is also known as white gold is cultivated all around the world. It is the main source of raw material for the textile industry and also contributes to edible oil. In this study to analyze the genetic diversity of 30 cotton genotypes, Randomised Amplified Polymorphic DNA (RAPD) was used. A total of 21 RAPD primers were used for screening 30 germplasm lines out of which 152 fragments were amplified with 21 random primers, out of which 76.44% were polymorphic. Genetic similarity matrix based on Jaccard similarity coefficient of cotton genotypes ranged from 0.62-1.00. These coefficients were used to construct a dendrogram using the Unweighted Pair Group of Arithmetic Means (UPGMA). All 30 cotton genotypes were grouped into two clusters, which were further subdivided into sub-clusters. The highest similarity has been observed between GISV208 and GISV 246. The present study indicated a great diversity among these 30 genotypes. The RAPD technology proved to be a potentially simple, rapid, reliable, and effective method for detecting polymorphism to assess genetic diversity among genotypes and very helpful in selecting diverse parents for hybridization.

Keywords: randomised amplified Polymorphic DNA, jaccard similarity coefficient, primers, dendrogram, cluster, subcluster

Introduction
Upland Cotton (*Gossypium hirsutum* L.) is one of the most important fiber and textile crops of world significance that is cultivated in subtropical and tropical regions of more than 60 nations (Jeffery *et al*. 2007 and Feng *et al*. 2017) [4]. Cotton is also famous for its name as “king of the fiber plants". It has many uses in the form of lint fibers and as other cotton byproducts. Upland cotton is likewise referred to as “pure white gold” (Kumar and Katageri, 2017) [10]. Cotton belongs to the genus *Gossypium* of the Malvaceae family which contains 50 species. Out of these, four species are under commercial cultivation *viz*., *Gossypium herbaceum* (2n=26), *Gossypium arboreum* (2n=26), *Gossypium hirsutum* (2n=52), and *Gossypium barbadense* (2n=52). Cotton is a fiber, oil, and protein yielding crop of global significance. Cotton is a multipurpose crop that gives rise to many primary merchandise like seed, lint, oil, hull, and linters.
Molecular markers play a vital position in crop improvement programs as they have been used substantially to estimate genetic diversity among parental lines. Molecular markers have several advantages as compared with morphological markers which include excessive polymorphism and independence from outcomes related to environmental situations and the physiological level of the plant. The random amplified polymorphic DNA (RAPD) technique of Williams *et al*. (1990) [15] is considerably utilized in many crops to have a look at diversity, gene identity, and identifying markers linked with desired traits. Irrespective of the plant age, RAPD patterns are consistent and very beneficial for germplasm characterization, estimation of genetic relatedness, and conservation of plant genetic resources (Welsh and McClelland, 1990) [14].

Material and Methods
Plant material
The plant material used in the study consisted of 30 cotton genotypes. The plants were grown in trays in a greenhouse.

DNA isolation
Total genomic DNA was extracted from the fresh leaves of 21 days old plants by using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987) [3] with some

modifications. DNase-free RNase A (Fermentas, USA) 5 µl was added. The samples were stored at -20 °C deep freeze. To test the purity of DNA, extracted DNA samples were run on 0.8% agarose medium gel. After running gel electrophoresis for 2 hours we obtained a single compact band pattern indicating the intact and quality DNA. Quantification of DNA was done with the help of spectrophotometry using Nanodrop (Thermo, USA) at an absorbance ratio of 260/280 nm wavelength.

PCR and gel electrophoresis

The random decamer oligonucleotide primers for the PCR were obtained commercially from Eurofins genomics; 21 random primers (K, OPA, and OPD series) were used in this study. PCR was carried out in 25 µl reaction volumes each containing 1.0 µl of genomic template DNA, 1.0 µl of the particular primer, 2.0 µl of dNTP (2.5 mM each, Genei), 2.5 µl of PCR buffer (10 X, Genei), 0.3 µl Taq polymerase (5 µ/µl, Genei) and 18.2 µl of Millipore sterile distilled water. 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 30 sec, annealing at 36 °C for 30 sec, extension at 72 °C for 2 min and final extension at 72 °C for 7 min. The amplification products were resolved on the agarose gel. Agarose (1.2 g) was dissolved in 100 ml 1X TBE buffer. The 1.2% agarose solution (Lonza) was heated in the microwave oven to dissolve completely and later cooled to 40 °C, followed by the addition of 5.0 µl ethidium bromide solution (0.5 µg/ml, Sigma) swirled gently, the gel was poured into the cast and comb was fixed in caster tray. When the gel was set, the comb was removed carefully, and the gel was kept in the gel electrophoresis unit. The gel electrophoresis tank was filled with 1 X TBE buffer. After placing the gel, amplified PCR products with a particular primers were loaded in each well. One µl of amplified PCR product was added into the 1 µl of loading dye (BR BIOCHEM, Life science), mixed well, and loaded inside the well slowly by placing the tip of micropipette tip in a slanting position. DNA ladder (100 bp, SMOBiO) was loaded in one of the wells to check the quality. 3.65 volts current was applied for 1:30 hours. The gel was visualized under the UV using the BIO-RAD Gel Doc Imaging system.

Data analysis

The frequency of RAPD polymorphism was calculated based on the presence (taken as 1) or absence (taken as 0) of common bands (Ghosh *et al*. 1997) [5]. The binary data were used to compute Pairwise 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).

Results and Discussion

A total of21 RAPD primers *viz*., K 17, OPA 2, OPA 3, OPA 4, OPA 7, OPA 8, OPA 9, OPA 10, OPA 11, OPA 12, OPA 13, OPA 14, OPA 15, OPD 2, OPD 3, OPD 5, OPD 7, OPD 10, OPD 12, OPD 13 and OPD 15 were screened and all the primers amplified unambiguous, readable and reproducible bands. A total of 152 amplification products were produced from the selected 21 primers out of which 121 bands were polymorphic. The number of bands varied from 4 to 14 and the size ranged from 150 to 2000 bp. 100bp Molecular ladder was used for analysis. Among the RAPD markers, OPA 13 produced the maximum number of bands (14), while the minimum number of bands (4) was observed with primer OPA 9, OPA 14, and OPD 15 with an average of 7.23 bands per primer. The highest numbers of polymorphic bands were observed for primer OPA 13. OPA 14 and OPD 15 primers were found to be monomorphic. Khan *et al.* (2010) [9] investigated 32 polymorphic primers which produced 273 fragments. The GLC 20 primer produces 31 polymorphic bands while two primer GLB-5 and GLC-12 produced one polymorphic band. The percentage of polymorphism ranged from 28.50% to 100% with an average of 76.44% polymorphism per primer. The percent polymorphism recorded in the present study was much higher than the diversity (56.7%) previously detected in cotton by Rana and Bhat (2005) [12]. Further, the diversity was lower than the diversity (89.1%) previously detected in cotton by Iqbal *et al*. (1997). Primer K 17, OPA 2, OPA 3, OPA 4, OPA 7, OPA 8, OPA 10, OPA 12, OPA 13, OPD 3, OPD 10, OPD 12, and OPD 13 revealed the highest polymorphism (100%), whereas the primer OPA 15 exhibited the lowest polymorphism (28.50%). The polymorphic information content (PIC) value ranged from 00 to 0.45. The highest PIC value was obtained for primers OPA 10 and the lowest PIC value was obtained for OPD 12 and OPA 14. All these details are presented in Table 1. The gel pictures of primers OPD 12 and OPA 9 are presented in Fig.1 and Fig.2.

The Jaccard's similarity coefficient ranged from 0.62 to 1.0. Dhatri *et al*. (2018) [1] observed a similarity co-efficient ranged 0.26 to 0.87. In dendrogram 30 cotton genotypes were divided into two main clusters (cluster 1 and cluster 2). Similarly, 4 clusters were obtained by Sagar *et al*. (2020), 7 clusters by Dhatri *et al.* (2018) [1], and 3 clusters by Dongre *et al.* (2004) [2]. Both clusters are divided into two sub-clusters, resulting in 4 sub-clusters (cluster 1A, cluster 1B, cluster 2A, and cluster 2B). Alike Patil *et al*.(2007) [11] divided genotypes which were found in both clusters into two sub-clusters. The genotypes LRA5166 and Abadhita were found in one sub-cluster each and the other sub-clusters had the genotypes Jayadhar and Rahs-14. Out of which cluster 1A consisted of seven genotypes i.e. GISV 28, GISV 33, GISV 103, GISV 109, GISV 114, GISV 130, and GISV 136, out of which three genotypes i.e. GISV 28, GISV 33, and GISV 103 fall under a single clade which shows a high degree of similarity between them compared to other genotypes of the group. Cluster 1B includes eight genotypes GISV 147, GISV 162, GISV 175, GISV 181, GISV 185, GISV 200, GISV 186, and GISV 201 in which GISV 162 and GISV 175 are closest. These two sub-clusters fall under cluster 1. Cluster 2A consisted of eight genotypes GISV 208, GISV 246, GISV 255, GISV 264, GISV 277, GISV 278, GISV 23, and GISV 25/1699 in which GISV 208 and GISV 246 found closest. The cluster 2B consisted of seven genotypes GISV 300, GISV 315, GISV 317, GISV 284, GISV 285, GISV 314 and GISV 320. Out of which two genotypes i.e. GISV 285 and GISV 314 belonged to a single clade showing a high degree of similarity between them compared to other genotypes of the group.

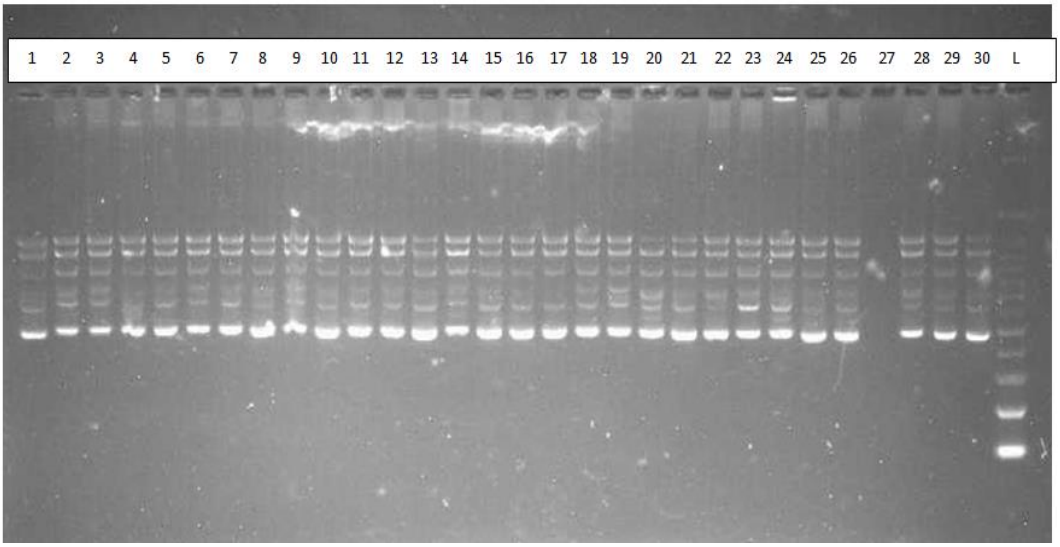

Fig 1: RAPD banding pattern of 30 cotton genotypes using OPD 12

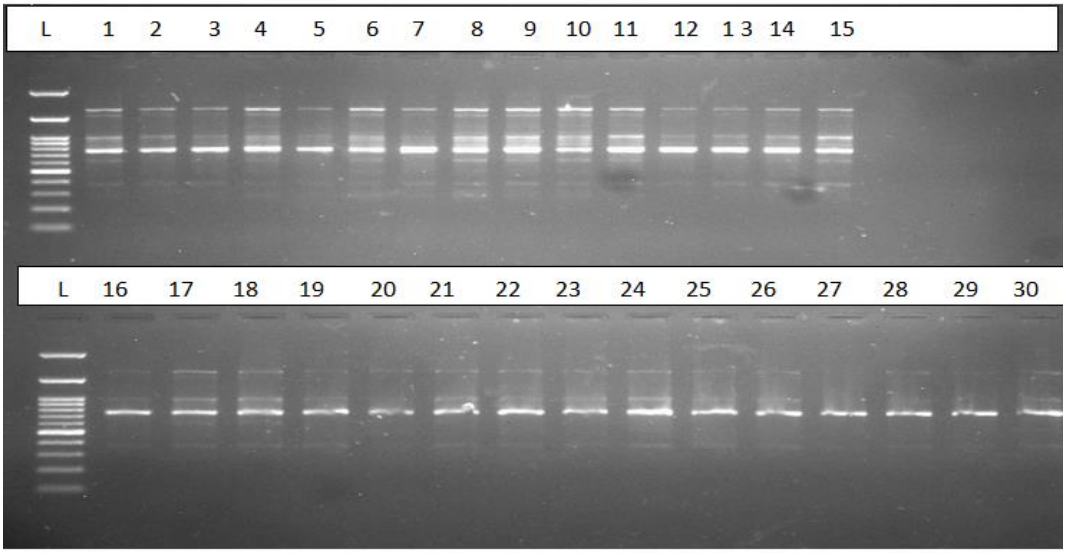

Fig 2: RAPD banding pattern of 30 cotton genotypes using OPA 9

Table 1: The total number of bands, polymorphism (%), and PIC value generated by different random decamer.

Primer	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism (%)	PIC
K 17	9	0	9	100	0.36
OPA 2	7	0	7	100	0.27
OPA 3	5	0	5	100	0.29
OPA 4	10	0	10	100	0.10
OPA 7	7	0	7	100	0.26
OPA 8	9	0	9	100	0.36
OPA 9	4	2	2	50	0.12
OPA 10	5	0	5	100	0.45
OPA 11	7	2	5	71.40	0.24
OPA 12	7	0	7	100	0.39
OPA 13	14	0	14	100	0.37
OPA 14	4	4	0	0	0
OPA 15	7	5	2	28.50	0.05
OPD 2	11	6	5	45.40	0.12
OPD 3	7	0	7	100	0.20
OPD 5	10	7	3	30	0.25
OPD 7	5	1	4	80	0.06
OPD 10	9	0	9	100	0.42
OPD 12	6	0	6	100	0
OPD 13	5	0	5	100	0.09
OPD 15	4	4	0	0	0.14
	152	31	121		

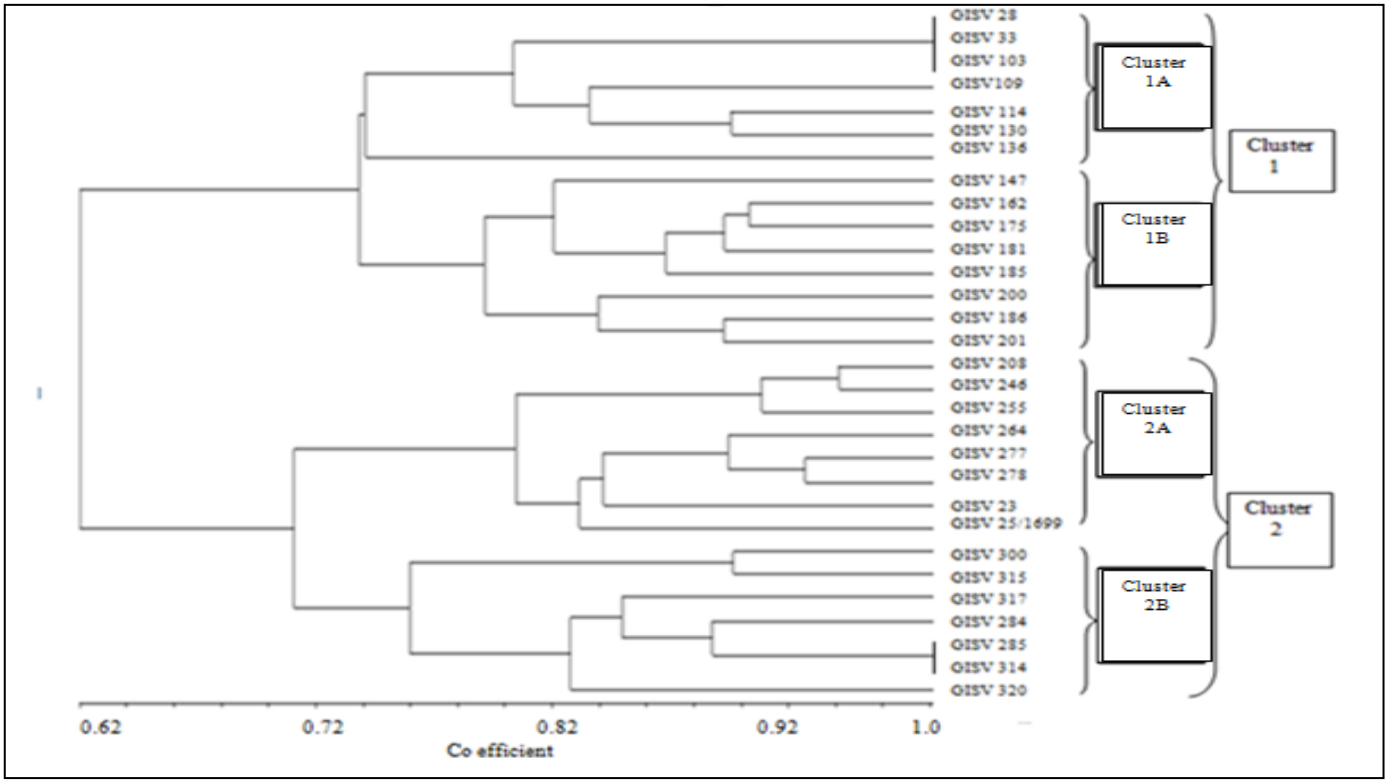

Fig 3: Dendrogram showing the genetic similarity among 30 cotton varieties.

Conclusion

Molecular analysis using RAPD markers gave a clear picture of the existence of diversity in genotypes at the genetic level as DNA markers are not influenced by environmental effects or plant growth stages whereas morphological markers do. RAPD markers proved to be worth assessing genetic diversity in cotton germplasm. Genotypes used in our present study possessed a wide range of variability, so these can be utilized in further crop improvement programs. Genotypes from different clusters should be selected as parents for further hybridizing programs in order to exploit maximum heterosis.

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