GENETIC DIVERSITY ANALYSIS OF COTTON (*Gossypium hirsutum* L.) GENOTYPES USING RAPD MARKERS

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ABSTRACT

Cotton (Gossypium hirsutum L. 2n=52, family: Malvaceae) is one of the most important commercial fiber and oil yielding crop in India. Genetic variability and relationships among fifteen cotton genotypes were investigated using six RAPD primers. These primers amplified a total of 61 bands/alleles out of which 55 bands were polymorphic (90.16 %) with an average of 9.16 bands per primer. The primer OPA-07, OPA-18 and OPD-05 demonstrated 100 % polymorphism. The polymorphic information content (PIC) was recorded from 0.858 to 0.903. The phylogenetic tree constructed by UPGMA method generated two main clusters, (cluster I and II) with a genotype G. Cot-18 grouped as solitary in cluster I. The cluster II consisted of rest of the genotypes grouped together in their respective sub-clusters. Large numbers of single type of markers could be screened for genetic diversity in cotton genotypes and utilized diverse genotypes in crop improvement programme to enhance crop productivity of cotton.

KEY WORDS: RAPD, genetic diversity, cotton, polymorphism, PIC.

INTRODUCTION

Cotton is one of the most important commercial fibre and oil yielding crops playing a key role in economic, political and social affairs of the world. Because of its worldwide economic importance, new cultivars are constantly being released in the world. India is the world's second largest cotton producer after China, produced 365 lakh bales of cotton from an area of 117.78 lakh hectares with productivity of 518 kg per hectare. Gujarat produced 93 lakh bales from an area of 24.97 lakh hectares with productivity of 633 kg per hectare (Anonymous, 2013).

Molecular investigations of germplasm are essential for their collection, conservation and its utilization in breeding programmes. The knowledge of genetic diversity in a crop species is fundamental to its improvement. DNA marker technology would provide a tool to the plant breeders to select desirable plants directly on the basis of genotype instead of phenotype. The use of molecular marker system (RAPD) offers significant advantages for species identification in that they are rapid, relatively cheap, independent of environmental conditions, eliminate the need to grow plants upto maturity. The use of molecular markers for the evaluation of genetic diversity is receiving much attention than morphological characterization. The

random amplified polymorphic DNA (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 crop plants. DNA fingerprinting studies to assess genetic purity with RAPD have already been conducted in cotton (Soregaon, 2004). Keeping in view the above, the present investigation was planned to study molecular characterization of upload cotton (*Gossypium hirsutum* L.) genotypes through RAPD markers.

MATERIAL AND METHODS

Plant materials

The experimental materials consisted of fifteen genotypes of cotton (*Gossypium hirsutum*), which were collected from Cotton Research Station, Junagadh Agricultural University, Junagadh.

DNA extraction

Total genomic DNA was isolated from young leaves of different cotton plants grown in pots. DNA extraction was carried out by CTAB method as described by Doyle and Doyle (1987) with minor modifications. Leaf tissues were cut into small pieces, homogenized and digested with extraction buffer (pH= 8.0): 1 M Tris HCl, 0.5 M EDTA (Ethylene diaminetetraacetic acid), 5 M NaCl, 2 X CTAB, 4 % PVP and β-mercaptoethanol. After incubation at 65 ^{0}C in water bath for one hour with gentle swirling, the mixture was emulsified with an equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1). Equal volume of ice-cold iso-propanol was added to precipitate DNA and pelleted by centrifugation. The pellets were washed with 70 % alcohol, air dried and resuspended in 100 µl of TE buffer (1 M Tris HCl, 0.5 M EDTA, pH 8.0) and finally treated with 1 µl of RNase. DNA was loaded into the sample spot of Nanodrop Spectrophotometer (Thermo Scientific, U.S.A.) and the concentration of DNA and absorbance at 260 nm and 280 nm were measured. The A_{260}/A_{280} ratio was automatically calculated by the software.

RAPD analysis

The method given by Rana *et al.* (2006) with minor modifications was followed for molecular characterization through RAPD. The RAPD assays were performed using random 10-mer oligonucleotide primers from Operon Technology Inc., USA (Table 1). The amplified products of RAPD were analyzed using 1.5 % agarose gel in TBE buffer.

Statistical analysis

In order to score and preserve banding pattern, photograph of the gel was taken in a Gel Documentation System, under UV trans-illuminator. The presence of each band was scored as '1' and its absence as '0'. The data matrix was read by NTSYS-pc version 2.02 developed by Rohlf (2000) and analyzed by the SIMQUAL (similarity for qualitative data) program with Jaccard's similarity coefficient. The resultant similarity matrix was entered into SAHN (sequential, agglomerative, hierarchical, and nested clustering method) clustering program, a tree matrix was produced and a dendrogram was constructed using UPGMA. Clustering methods create clusters of the data, no matter whether there are true clusters in the data or not, so a check was made for the existence of true clusters. This was done by using the tree matrix produced by SAHN to calculate the cophenetic values of similarity or dissimilarity by the program COPH (cophenetic values). The cophenetic value matrix was compared with the original tree matrix for goodness of fit of the cluster analysis to the data. This type

of cophenetic correlation was done by the MXCOMP (matrix comparison) program (Rohlf, 2000). The program MXCOMP plots the cophenetic value matrix against the original tree matrix, and computes the Cophenetic correlation coefficient (r) and the Mantel test statistic (Z). The PIC value for each locus was calculated on the basis of allele frequency (Anderson *et al*., 1993).

RESULTS AND DISCISSION

Polymorphism as detected by RAPD analysis

Out of eighteen RAPD primers used, six primers *viz*,. OPA-07, OPA-18, OPB-18, OPC-05, OPD-05 and OPG-12 showed the amplification. They amplified a total of 61 bands/alleles in which 55 bands/alleles were polymorphic with average 9.16 bands per primer and six were monomorphic. Two unique polymorphic bands were observed in two genotypes i. e. GTHV-95/145 by OPC-05 (1128 bp) and OPG-12 (719 bp) and BS-279 by OPA-18 (1220 bp) and OPB-18 (640 bp). From the data, it was observed that 9.84 % monomorphic bands and 90.16 % of polymorphic bands were observed (Fig. 1 and Table 1).

El-Zanaty *et al.* (2012) examined 19 RAPD and eight agronomic traits to estimate the genetic diversity in Egyptian cotton. RAPD primers produced a total of 101 amplicons, which generated 86.25 % polymorphism. Number of amplification products ranged from 2 to 7. Similar findings were also recorded by Iqbal *et al.* (1997) and Vafaie-Tabar *et al.* (2003). Iqbal *et al.* (1997) detected forty-nine primers polymorphism in all twenty-three cotton varieties, while one produced monomorphic amplification profile. They reported that a total of 349.0 bands were amplified, of which 89.1 % were polymorphic. Vafaie-Tabar *et al.* (2003) reported 87 % polymorphism and a genetic similarity of 30 % in testing 50 random decamer primers on 22 cotton genotypes belonging to tetraploid and diploid cotton species.

The primer OPC-05 produced highest thirteen bands followed by OPA-07 and OPA-18 with twelve bands and eleven bands, respectively. OPG-12 produced nine bands and OPB-18 and OPD-05 both produced eight bands. The average percentage of polymorphism recorded was 90.16 % per primer for all the six RAPD primers. The primer OPA-07, OPA-18 and OPD-05 gave maximum polymorphism (100 %), while primer OPB-18, OPC-05 and OPG-12 exhibited 87.5 %, 76.92 % and 77.77 % polymorphism, respectively. In present study, the amplified fragments were in the range of 147 bp (OPA-18) to 1940 bp (OPC-05). Similar findings were also reported by Surgun *et al.* (2003) and Dongre *et al.* (2004). Surgun *et al.* (2003) reported that 34 primers showed amplification of 319 fragments ranging from 200 bp to 2800 bp in nine different genotypes of cotton. Dongre *et al.* (2004) worked with 21 RAPD primers which generated 150 markers in which 15 RAPD primers were polymorphic and produced 76 markers in cotton with size ranged between 100 bp and 2000 bp.

The polymorphic information content (Table 1) was recorded from 0.858 to 0.903. The highest PIC value of 0.903 was recorded by OPA-07, while lowest PIC value of 0.858 was recorded by OPB-18. RAPD primer index (RPI) ranged from 6.864 (by OPC-05) to 11.64 (by OPB-18) with an average of 9.008 per primer.

Genetic relationship among cotton genotypes

Jaccard's coefficient of similarity between 15 cotton genotypes ranged from 31.9 % to 90.3 %. Patil *et al*. (2007) generated RAPD profiles for four cotton genotypes using nineteen random decamer primers with genetic similarity ranged from 46 % to 91 %. Likewise, Sharaf *et al*. (2009) also recorded genetic similarity among seven cotton genotypes which ranged from 64.8 % to 93.2 % which supported the present findings.

Fifteen cotton genotypes were grouped into two main clusters I and II with an average similarity of 49 % (Fig. 2). The cluster I consisted of solitary genotype of G. Cot-18. The cluster II consisted of fourteen genotypes and these were divided into two subclusters, IIA and IIB. The subcluster IIA consisted of thirteen genotypes and these were again further divided into two sub subcluster IIAi and IIAii. The sub subcluster IIAi was grouped into twelve genotypes viz., G. Cot-12, MR-786, GISV-254, BS-27, GJHV-460, BC-68-2, 76-IH-20, GBHV-148, GJHV-503, H-1316, BS-279 and GTHV-95/145. G. Cot-12 and MR-786 shared a similarity of 90.3 %. Likewise, GISV-254 and BS-27 shared similarity of 88 %; GISV-254 and GBHV-148 (86 %); BC-68-2 and 76-IH-20 (85.1 %); GBHV-148 and GJHV-503 (84.9 %); and H-1316 and GJHV-460 (79.2 %); BC-68-2 and GJHV-503 (78.8 %). The sub subcluster IIAii consisted of only one genotype GBHV-170. The subcluster IIB also consisted of only one genotype (LRA-5166). The cluster analysis showed the highest (90.3 %) similarity was observed between the genotypes G. Cot-12 and MR-786, while lowest (31.9 %) similarity between G. Cot-18 and GBHV-148.

To test the goodness of fit of the clustering of RAPD data, matrixes of cophenetic values were also computed using the program COPH (Fig. 3). In the present study also the Mantel test statistic-Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation r = 0.9411) was found to fall under the category of **"very good fit"**, as categorized by Rohlf (2000). Similar findings were also reported by Abd-Elsalam *et al*. (2003), Noormohammadi *et al*. (2011) and Noormohammadi *et al*. (2013) which supported present study.

CONCLUSION

The conclusions drawn from the present investigation are as under:

1. The formation of several subclusters within cluster I suggested the presence of moderate genetic diversity among the fifteen cotton genotypes studied. The geographical diversity was not associated with genetic diversity.
2. Genetic diversity analysis through RAPD marker gave highest (100 %) polymorphism percentage with three primers *viz.*, OPA-07, OPA-18 and OPD-05. Therefore, these primers were most useful for genetic diversity analysis to generate DNA fingerprinting in cotton genotypes.

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Table 1: Size, number of amplified bands, per cent polymorphism, PIC and RPI obtained by RAPD primers

Sr. No.	RAPD Primers	Allele/Band Size (bp)	Total No. of Allele (A)	Polymorphic Bands (B)			Monomorphic Bands	% Polymorphism (B/A)	PIC Value	RPI (PIC×A)
				S	U	Total Bands (T)				
1	OPA-07	147-1034	12	12	0	12	0	100	0.903	10.83
2	OPA-18	147-1220	11	10	1	11	0	100	0.898	9.878
3	OPB-18	441-1345	8	6	1	7	1	87.5	0.858	6.864
4	OPC-05	154-1940	13	9	1	10	3	76.92	0.896	11.64
5	OPD-05	387-1472	8	8	0	8	0	100	0.870	6.96
6	OPG-12	251-1720	9	6	1	7	2	77.77	0.876	7.88
TOTAL			61	51	4	55	6	-	-	-
AVERAGE			-	-	-	9.16	-	**90.16**	**0.883**	**9.008**

S = Shared; ***U = Unique;*** ***T = Total Polymorphic Bands;***
PIC = Polymorphism information content; ***RPI = (RAPD Primer Index)***

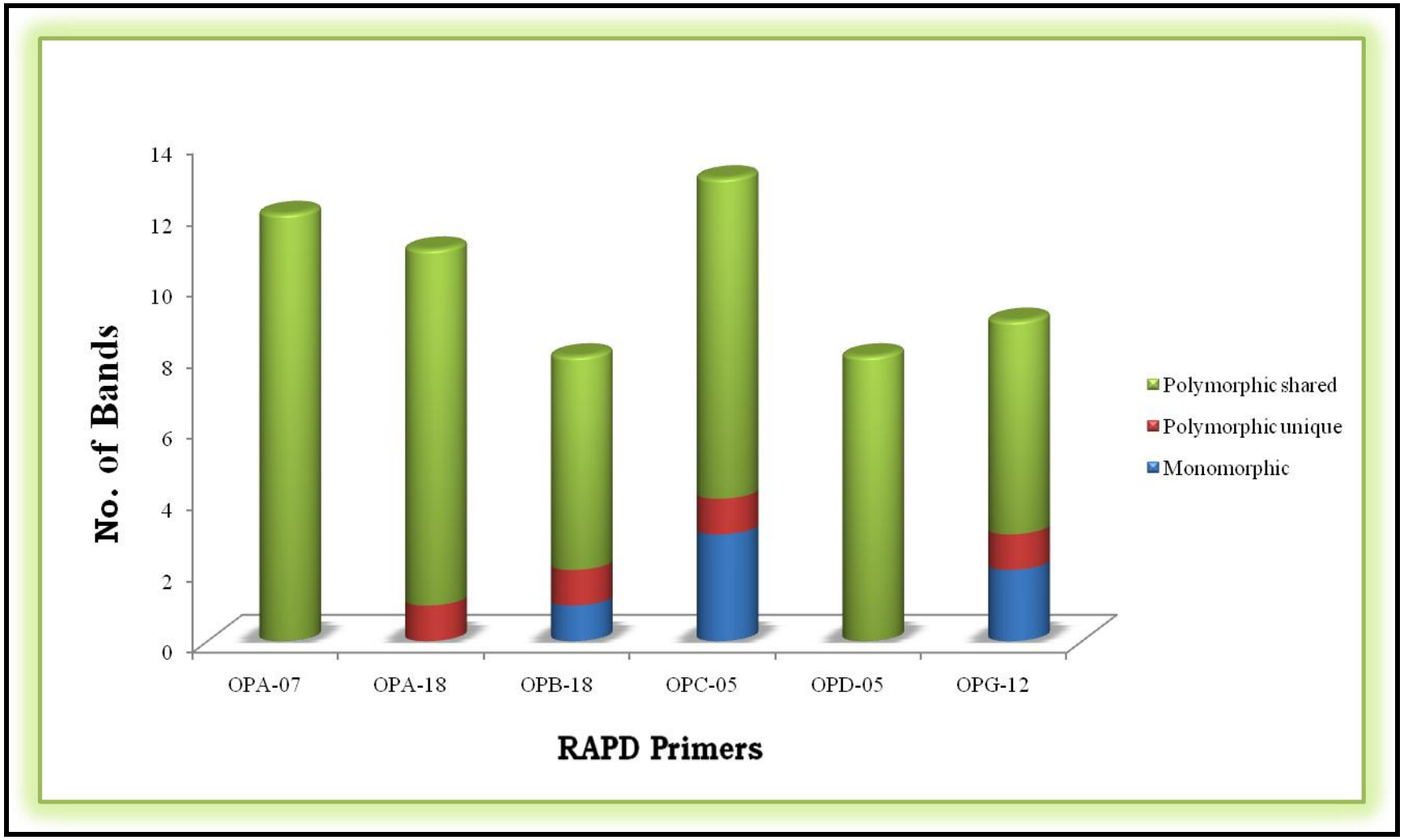

Figure 1: Properties of polymorphic and monomorphic bands amplified by the RAPD primers

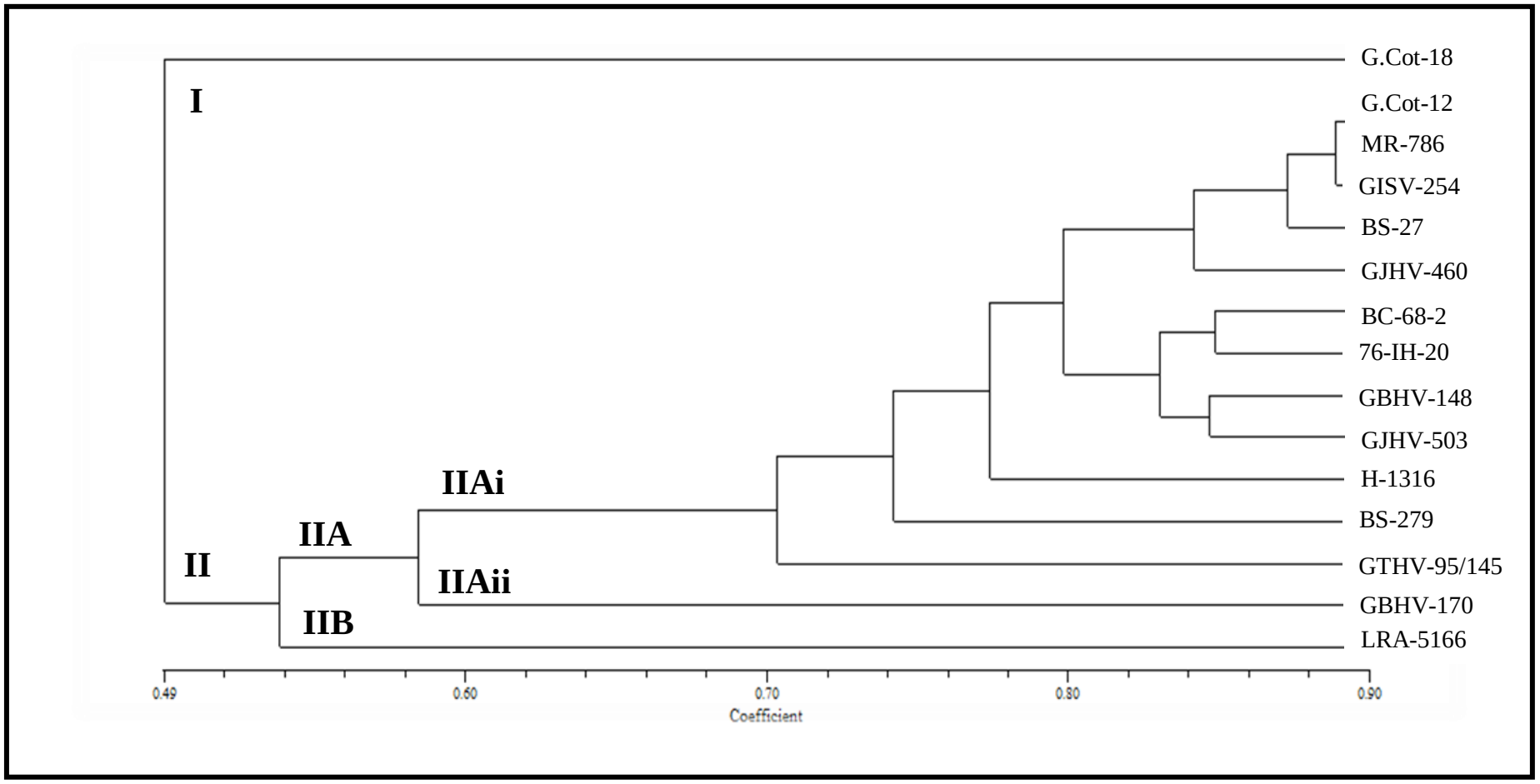

Figure 2: Dendrogram depicting the genetic relationship among 15 cotton genotypes based on RAPD markers

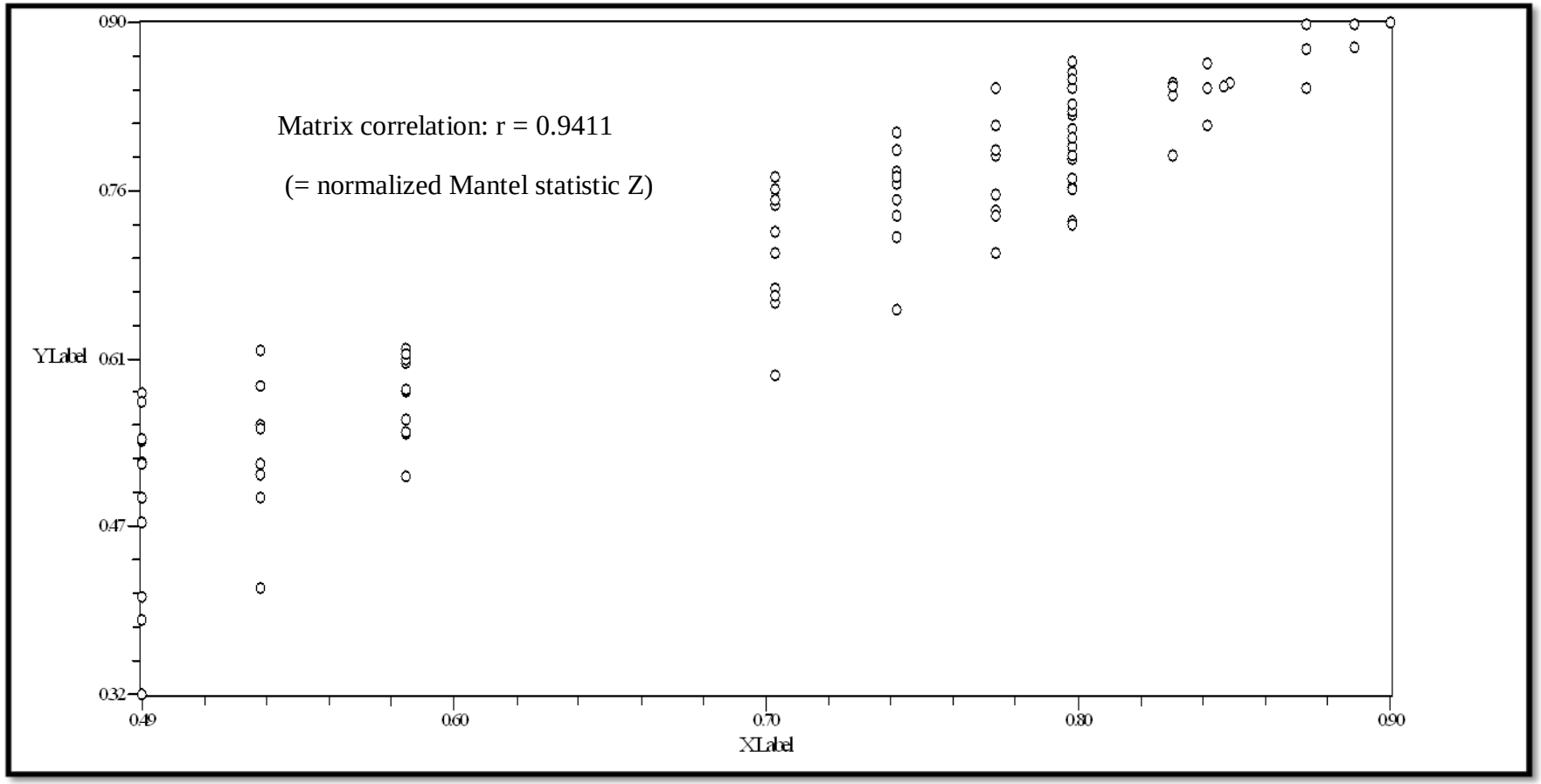

Figure 3: Cophenetic values against Jaccard's similarity coefficients from RAPD data of cotton genotypes

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