Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6718

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (5):** 367-376 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Comparison of Molecular and Morphological Diversity Analysis of Tetraploid Cotton (*Gossypium hirsutum* L.)

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Received: 12.07.2018 | Revised: 25.08.2018 | Accepted: 4.09.2018

ABSTRACT

The present investigation carried out to find out molecular diversity using 30 RAPD primers and to estimate morphological diversity in 36 genotypes of cotton through Mahalanobis's D^2 analysis for 8 morphological characters, the field experiment was laid out in randomized block design with three replications. The number of RAPD fragments generated per primer ranged from 3 (OPC 14) to 14 (OPD 01). Twenty one primers resulted in 100 % polymorphism. The PIC value ranged between 0.66 to 0.92 with an average of 0.83.The similarity coefficients ranged from 0.26 to 0.87, which indicate high variability among 36 cotton genotypes. Maximum similarity 87% was observed between CSH-2931 and F-2454 and lowest similarity 26% was observed between GSHV-164 and BPHI-537. Based on D^2 statistics, 36 cotton genotypes were grouped into 7 clusters. Among the 7 clusters, cluster I was the largest with 18 genotypes followed by cluster II, III and IV with 9, 3 and 3 genotypes, respectively. The cluster V, VI and VII were solitary clusters. Clustering from morphological data as well as RAPD data by NTSYS-pc version 2.02 programmes, the same clustering pattern observed. In addition to that, collectively fifty percent genotypes occupied same clusters. This indicated that the clustering of genotypes through NTSYS-pc version 2.02 programme is more reliable as compared to D^2 analysis in the present investigation.

Key words: *Tetraploid cotton, D^2 analysis, Morphological and Molecular diversity*

INTRODUCTION

Gossypium is a large, rich and economically important genus. Cotton belongs to *Gossypium* genus and classified under the tribe *Hibisceae* in the family *Malvaceae*. The *Gossypium* genus constitutes about 50 species. These species are grouped into nine genomic types with designations: AD, A, B, C, D, E, F, G, and K[22], From which 45 are diploid with 26 chromosomes and five are allotetraploid species with 52 chromosomes[6]. Out of these 50 species four species are under cultivation of which two are new world cotton species {*G. hirsutum* L. [n=2x=26, (A_1D_1)], *G. barbadense* L. [n=2x=26, (A_2D_2)]} and two are old world cotton species {*G. herbaceum* L. (n=x=13, A_1) *and. G. arboreum* L. (n=x=13, A_2)} [1, 10].

Cite this article: Patel, D., Patel, J.A. and Gami, R.A., Comparison of Molecular and Morphological Diversity Analysis of Tetraploid Cotton (*Gossypium hirsutum* L.), *Int. J. Pure App. Biosci.* **6(5):** 367-376 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6718

In crop improvement programs, the study of genetic relationships (diversity) is important for the selection of suitable diverse parents to obtain heterotic hybrids, predict progeny performance, conserve, and characterize used germplasm. The main objective of cotton breeding programs is enhancement of seed cotton yield and quality traits. Thus, evaluation of genetic diversity related to these characters is important for sustainable production of cotton. A narrow genetic base of cultivated germplasm was one of the major factors causing the recent cotton yield and quality declines[8, 20]. The level of genetic diversity of crop species is an essential element of sustainable crop production in agriculture, including cotton. The amplitude of genetic diversity of *Gossypium* species is exclusively wide, encompassing wide geographic and ecological niches[32]. Cotton productivity and the future of cotton breeding efforts tightly depend on the level of the genetic diversity of cotton gene pools and its effective utilization in cotton breeding programs. Genetic diversity has been conventionally estimated on the basis of morphological traits. *Gossypium* species exhibit amazing morphological variation. Morphological features are traditionally used for assessed genetic diversity and serve as one of the major criteria to users for assessing genetic diversity in cotton genotypes for identification of genetic distances. Within species, *Gossypium hirsutum* L. shows great phenotypic diversity[34].

Morphological markers can be monitored visually without specialized biochemical or molecular techniques. Although agronomical characterization provides useful information to users, these characteristics are normally subjected to environmental influences and must be assessed during a fixed vegetative phase of the crop[31]. Molecular markers reveal differences of natural sites at the DNA level. These variations are not seen in phenotype and each might be a single nucleotide differences in a gene or a piece of repetitive DNA. The DNA marker technique provides simple approach to study the diversity at molecular level. The other noted benefits of this approach are; markers are potentially unlimited in number, are not affected by the environment, and can be organized into linkage maps. The best process to assess the genetic relationship is to combine both morphological traits with molecular markers in order to build better genetic figure of the nature of genetic relationship than using one of them[2]. Therefore, the present investigation was designed with aim to characterize cotton (*G. hirsutum* L.) genotypes with respect to their morphological traits and molecular markers.

MATERIAL AND METHODS

The present investigation partitioned into two categories *i.e.*, Molecular diversity analysis and Morphological diversity analysis.

Molecular diversity analysis:

The molecular work carried out at Department of Plant Molecular Biology and Biotechnology, C. P. College of Agriculture, S. D. Agricultural University by using 30 RAPD primers.

Band scoring

Data were scored for computer analysis on the basis of the presence or absence of the bands obtained. If a band was present in a genotype, it was designated as '1' and if absent; it was designated as '0'. The data were maintained in the spread sheet format for further analysis. The data were entered in to binary matrix and subsequently analyzed by using NTSYS-pc version 2.02[25].

Data Analysis

The data were subjected to statistical analysis for the calculation of 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 cluster analysis by UPGMA (unweighted pair-group method with arithmetic averages) using NTSYS-pc version 2.02 based software[13, 26, 28].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[25]. The polymorphism percentage was calculated as per the method suggested by[4].

$$\text{Polymorphism}(\%) = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Polymorphic Information Content

Polymorphic information content (PIC) values were calculated for each RAPD primers[5].

$$PIC = 1 - \sum (P_{ij})^2$$

Where,

P_{ij} = frequency of the j^{th} allele of the i^{th} loci.

Morphological diversity analysis:

The field experiment was carried out at Cotton Research Station, S. D. Agricultural University, Talod. The field experiment was laid out in randomized block design with three replications to estimate morphological diversity in 36 genotypes of cotton (Table 1.) through Mahalanobis's D^2 analysis for 8 morphological characters *viz.*, days to 50% flowering, plant height, number of sympodia per plant, number of monopodia per plant, number of ball per plant, ball weight, ginning percentage (ginning outturn) and seed cotton yield. The analysis of variance was calculated with the method suggested by Panse and Sukhatme[19]. Mahalanobis's D^2 statistics was followed for genetic divergence study. Grouping of genotypes in different clusters was done by using Tocher's method[24]. The intra-cluster and inter-cluster distances were calculated by the formula given by[27].

RESULT AND DISCUSSION:

Molecular marker diversity:

The primers used in present investigation produced high degree of polymorphism with an average 93.65 per cent and ranged from 71.42-100.00 per cent. Similarly, 89.1 per cent polymorphism reported by[12], 63.2 per cent polymorphism detected by Hussain *et al.*[11], 84.95 per cent polymorphism observed by[9]. Among the 30 primers 21 *viz.*, OPA-09, OPA-11, OPB-01, OPB-08, OPB-11, OPB-17, OPB-18, OPC-04, OPC-06, OPC-11, OPC-14, OPC-15, OPC-18, OPC-19, OPD-01, OPE-04, OPP-01, OPP-02, OPP-03, OPP-04 and OPP-16 revealed 100 percent polymorphism. Similarly, 11 out of 23 RAPD primers resulted in 100% polymorphism[9], 5 primers out of 18 were resulted in 100% polymorphism[21].

The genetic similarity was computed considering all the genotypes from the pooled data. The overall range of the genetic similarity among 36 genotypes was found to be very wide ranging from 0.26 to 0.87 which indicated that there was high variability among the cotton genotypes under study. Likewise 0.38-0.98 similarity was observed in their investigation by[23] and 9.68-53.29 similarity detected by[21]. Based on similarity matrix and clustering pattern, the genotype F-2454 and CSH-2931 were found to have maximum similarity coefficient 0.87. This indicating narrow genetic base among such genotypes and upon using them in future breeding programme may give inadequate results. The lowest similarity coefficient 0.26 was observed in between SCS-1214 and BPHI-537 followed by BS-51-1 and BPHI-537 (0.29) similarity, which shows high degree of variation among them. By utilizing genotypes having wide variation may give superior characteristics upon breeding them. These results are in accordance with the findings of Esmail *et al.*[9] and Rohlf[27].

The dendrogram based on Jaccard's similarity coefficient was constructed using UPGMA after analysis of banding patterns

generated by all 36 cotton genotypes with 26 primers. The dendrogram and similarity coefficient values give an idea about the nature of the individual sample in the whole sample set. The cluster analysis was carried out based on the RAPD profile. The results based on the RAPD profile broadly grouped the 36 cotton genotypes into 2 main clusters i.e., Cluster A and B (Fig 1.)

The cluster A further divided in to three sub clusters i.e. A1, A2 and A3. Group A1 comprises SCS-1211, CPD-1301, SCS-1210 and ARBH-1301. Group A2 comprises NDLH-1976, HS 292, CCH 13-1, GSHV-164, SCS-1214, P-5629, CSH-2931, BS-51-1, HS-293, CPD-1302, GSHV-171, ARBH-1301, GSHV-173, CSH-3175, H-1476, GSHV-169, CCH-13-2, BS-1, GJHV-516, CNH-1116, F-2451, TCH-1742, RS-2733, CSH-2931, F-2454, LH-2255, BPHI-537, NDLH-1975 and Bihani-301. Group A3 comprises only HS-293, whereas group B have two genotypes *viz.*, G.Cot-20 and CHN-19 (Fig 1 & Table 2). Esmail *et al*.[9], Rana[23] and Rohlf [27] also recorded similar results.

Morphological markers based diversity

Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods. The data often involved numerical measurements and in many cases, combinations of different types of variables[17]. Phenotypic characters are genetically informative; they impart the understanding of the genetic distance between the populations of same species and permit to classify them in groups showing qualitative differences between them[16].

The analysis of variance for different characters in cotton (*G. hirsutum* L.) revealed highly significant differences among the genotypes for all characters under investigation, indicating the presence of considerable amount of variation in the material. Similar results were confirmed the study of Kumar *et al*[15].

The D^2 technique based on multivariate analysis developed by Mahalanobis is method for quantifying the degree of genetic diversity among genotypes, which helps in selecting the parents for hybridization. It is generally accepted that genetically diverse parents when crossed will show maximum heterosis and offer the maximum chance of isolating transgressive segregants. Earlier, geographic diversity among parents was generally taken as an index of genetic diversity. However, as per the review, much diversity analysis did not agree with these views and it was pointed out that geographical diversity may not be an indication of genetic diversity. Single character is not much importance as the combined merit of number of desirable traits like yield. Hence, for improving the grain yield, selection of parents based on number of characters having quantitative divergence is required can be assessed by Mahalanobis's D^2 statistics which enables to discriminate genotypes according to the diversity present. It gives clear idea about the diverse nature of the populations. The clusters formed according to Tocher's method proposed by Rao[24] were used to know the distances between and within the cluster.

On the basis of the magnitude of the D^2 values, the genotypes were grouped in to seven clusters (Table 3). Cluster I was the largest with 18 genotypes followed by cluster II, III and IV which includes 9, 3 and 3 genotypes, respectively. The cluster V, VI and VII were monogenotypic or solitary clusters. The solitary clusters may be due to total isolation preventing the formation of gene flow or natural or human selection for diverse adaptive complexes. This indicates wide diversity from the rest of the genotypes and from each other. Jayaprakash *et al*.[14] were also confirmed the same results in cowpea [*Vigna unguiculata* (L.) Walp.]. The inter cluster distance from present investigation by D^2 analysis ranged from 169.42 to 6490.72. The highest D value (6490.72) was observed between cluster I and VI, while the lowest D value (169.42) was observed between cluster II and VII. The maximum intra-cluster distance (D = 214.07) was observed in cluster IV and minimum intra-cluster distance (D = 0.00) recorded in cluster V, VI and VII. This

revealed the presence of divergent genotypes within the clusters (Table 4 & Fig.3). Similar results were also obtained in the study carried out by Sundar *et al*[30].

In general, intra cluster distances were lower than the inter cluster distances. Thus, the genotypes included within a cluster tended to diverse less from each other. This was indication of diversity present in the material evaluated. The genetic diversity among the different clusters can be attributed to the combined effects of geographical differences, genetic drift, spontaneous variation, history of selection, heterogeneity and selection under diverse environments[18]. Cluster I and VI with 18 (SCS-1211, CPD-1301, CSH-3175, H-1476, G. Cot-20, GSHV-169, CCH-13-2, ARBH-1302, BS-1, NDLH-1976, HS-292, CCH-13-1, SCS-1214, CSH-2931, BS-51-1, HS-293, Bihani-301 and GSHV-173) and 1 (ARBH-1301) genotype, respectively were the most divergent groups with maximum inter-cluster distance. The findings of present study in are in accordance with the findings of Sonawane *et al*.[29], Viswanathan *et al*.[33] in cowpea [*Vigna unguiculata* (L.) Walp.].

Comparison of molecular marker and morphological marker based diversity

Based on combined results for morphological and RAPD genetic diversity estimates, genotype 'BPHI-537' was found to be distinct from other genotypes and can be exploited to harness their unique features in breeding programs. Moreover, based on genetic distance, different cluster and *per se* performance 11 cross combinations of diverse lines are suggested. By keeping in view these three criteria *i.e.*, diverse parents having less than 0.5 distance, belonging to different morphological and molecular clusters and having more than average *per se* performance for seed cotton yield were selected and summarized in Table 5.

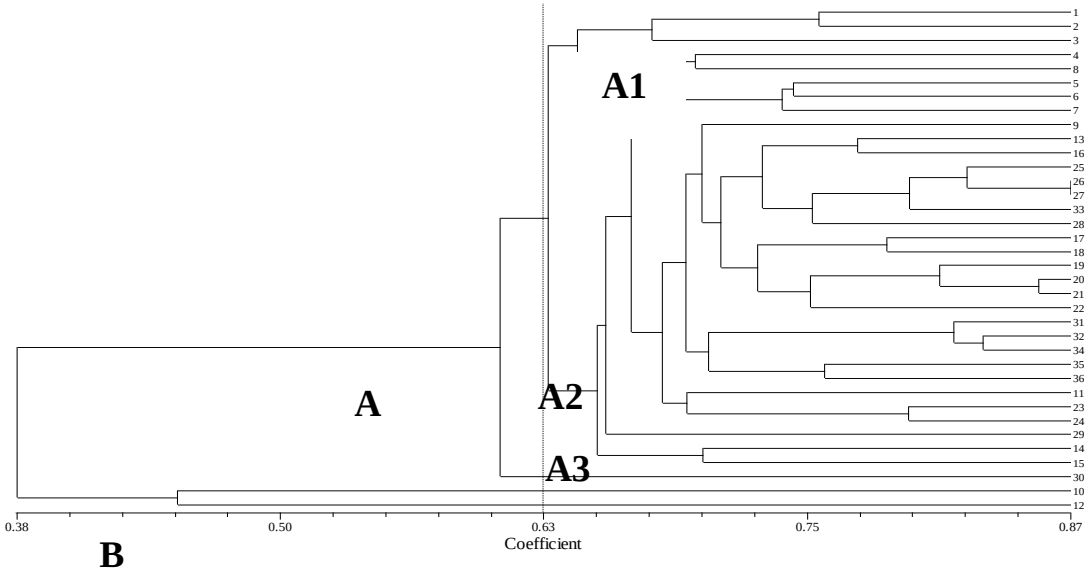
Based on molecular data of the present investigation it was observed that genotype BPHI-537 is most diverse genotype, as it found placed in a separate group (group B) than rest of the genotypes. This genotype was evaluated in all India coordinate cotton improvement project in 2013-14 across the India and ranked fifth in both, south cotton zone with 6 locations and central cotton zone with 5 locations[3].

In present investigation, 7 distinct clusters were formed through D^2 analysis whereas, main two clusters (A & B) with three sub clusters (A_1, A_2 and A_3) in major cluster A were formed through NTSYS-pc version 2.02 programme from morphological data (Fig 2). More over very few genotypes were found common in different clusters made through morphological data analysis through D^2 analysis and RAPD data in present investigation. When NTSYS-pc version 2.02 programme is used for clustering from morphological data as well as RAPD data, same clustering pattern was observed. In addition to that collectively fifty percent genotypes occupied same clusters in present investigation. So it seemed that clustering of genotypes through NTSYS-pc version 2.02 programme is more reliable as compared to D^2 analysis.

Table 1: List of genotypes of cotton (*G. hirsutum* L.) used in the present investigation

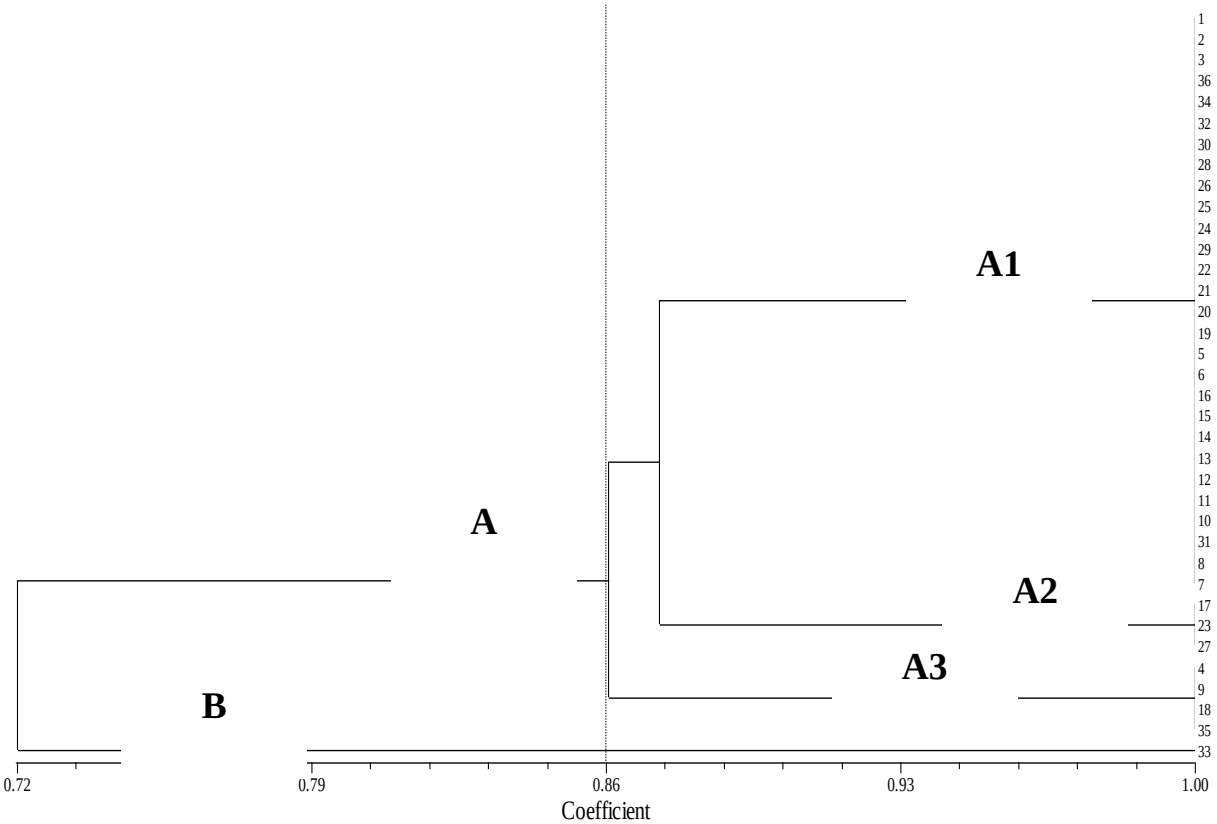
Sr. No.	Genotypes	Sr. No.	Genotypes
1.	ARBH-1301	19.	BS-1
2.	SCS-1211	20.	NDLH-1976
3.	CPD-1301	21.	HS-292
4.	SCS-1210	22.	CCH-13-1
5.	CSH-3175	23.	RS-2733
6.	H-1476	24.	SCS-1214
7.	LH-2255	25.	P-5629
8.	GJHV-516	26.	CSH-2931
9.	RS-2728	27.	F-2454
10.	G.Cot-20	28.	BS-51-1
11.	GSHV-169	29.	GSHV-164
12.	BPHI-537	30.	HS-293
13.	NDLH-1975	31.	Bihani-301
14.	CNH-1116	32.	CPD-1302
15.	F-2451	33.	CNH-19
16.	CCH-13-2	34.	GSHV-171
17.	TCH-1742	35.	GSHV-172
18.	ARBH-1302	36.	GSHV-173

Figure 1: UPGMA cluster analysis-based dendrogram from RAPD data (using NTSYS-pc 2.02) depicting genetic relationships among the 36 genotypes of cotton (*G. hirsutum* L.)



1: ARBH-1301	2: SCS-1211	3: CPD-1301	4 : SCS-1210	5: CSH-3175
6: H-1476	7: LH-2255	8: GJHV-516	9: RS-2728	10: G.Cot-20
11: GSHV-169	12: BPHI-537	13: NDLH-1975	14: CNH-1116	15: F-2451
16: CCH 13-2	17: TCH-1742	18: ARBH-1302	19: BS-1	20: NDLH-1976
21: HS-292	22: CCH-13-1	23: RS-2733	24: SCS-1214	25: P-5629
26: CSH-2931	27: F-2454	28:BS-51-1	29: GSHV-164	30: H- 293
31: Bihani-301	32: CPD-1302	33: CNH-19	34:GSHV-171	35: GSHV-172
36: GSHV-173				

Figure 2: UPGMA cluster analysis based dendrogram from morphological data (using NTSYS-pc 2.02) depicting genetic relationship among the 36 genotypes of cotton (*G. hirsutum* L.)



1: ARBH-1301	2: SCS-1211	3: CPD-1301	4 : SCS-1210	5: CSH-3175
6: H-1476	7: LH-2255	8: GJHV-516	9: RS-2728	10: G.Cot-20
11: GSHV-169	12: BPHI-537	13: NDLH-1975	14: CNH-1116	15: F-2451
16: CCH 13-2	17: TCH-1742	18: ARBH-1302	19: BS-1	20: NDLH-1976
21: HS-292	22: CCH-13-1	23: RS-2733	24: SCS-1214	25: P-5629
26: CSH-2931	27: F-2454	28:BS-51-1	29: GSHV-164	30: H- 293
31: Bihani-301	32: CPD-1302	33: CNH-19	34:GSHV-171	35: GSHV-172
36: GSHV-173				

Figure 3: Clustering of 36 cotton (*G. hirsutum* L.) genotypes by Tocher's method

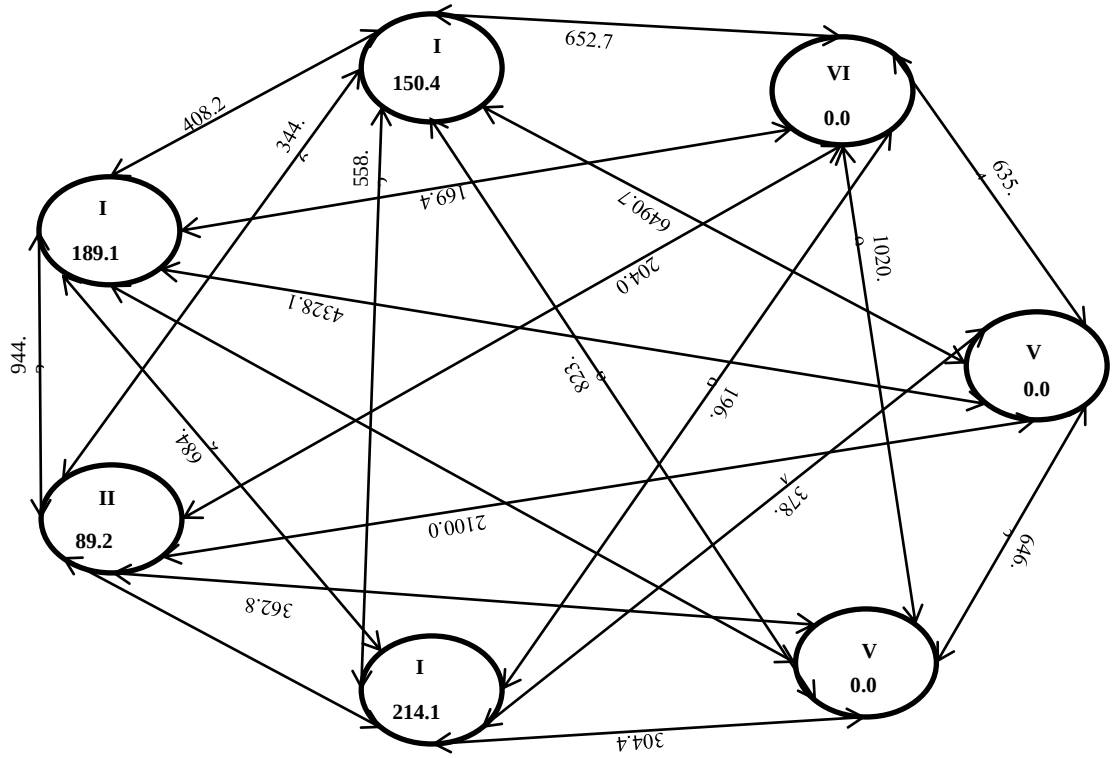


Table 2: Grouping of genotypes on the basis of morphological and RAPD data by using NTSYS-pc 2.02 programme

Morphological Group	Genotypes	Molecular group	Genotypes	Common genotypes
A	SCS-1211, CPD-1301, NDLH 1976, HS-292, CCH-13-1, GSHV-164, SCS-1214, P-5629, CSH-2931, BS 51-1, HS-293, CPD-1302, GSHV 171, ARBH-1301 and GSHV-173	**A**	SCS-1211, CPD-1301, SCS-1210 and ARBH-1301	SCS-1211, CPD-1301 and ARBH-1301
A2	CSH-3175,H 1476,G.Cot-20,GSHV-169,CCH 13-2,S-1,GJHV-516,SCS-1210,CNH-1116,F-2451,TCH-1742,RS-2733,CSH-2931,F-2454, LH-2255, BPHI-537, NDLH-1975 and Bihani-301	**A2**	NDLH 1976, HS 292, CCH 13-1, GSHV-164, SCS 1214,P-5629,CSH-2931,BS 51-1,HS 293,CPD-1302,GSHV 171,ARBH-1301,GSHV 173,CSH-3175,H 1476,GSHV-169,CCH 13-2,BS-1,GJHV-516,CNH-1116,F-2451,TCH-1742,RS-2733,CSH-2931,F-2454,LH-2255,BPHI-537,NDLH-1975 and Bihani-301	CSH-3175, H-1476, GSHV-169,CCH-13-2, BS-1, GJHV-516, CNH-1116,F-2451,TCH-1742,RS-2733,CSH-2931,F-2454, LH-2255,NDLH 1975 and Bihani-301
A3	ARBH-1302 and RS-2728	**A3**	HS-293	
B	GSHV-172 and CHN-19	**B**	G.Cot-20 and BPHI-537	

Table 3: Composition of cluster based on D^2 values of 36 genotypes of cotton (*G. hirsutum* L.)

Clusters	Number of genotypes	Name of genotypes
I	**18**	SCS-1211, CPD-1301, CSH-3175, H-1476, G.Cot-20, GSHV-169, CCH-13-2, ARBH-1302, BS-1, NDLH-1976, HS-292, CCH-13-1, SCS-1214, CSH-2931, BS-51-1, HS-293, Bihani-301, GSHV-173
II	**9**	SCS-1210, CNH-1116, F-2451, TCH-1742, RS-2733, CSH-2931, F-2454, GSHV-164, CPD-1302
III	**3**	LH-2255, BPHI-537, NDLH-1975
IV	**3**	GJHV-516, GSHV-171, GSHV-172
V	**1**	RS-2728
VI	**1**	ARBH-1301
VII	**1**	CHN-19

Table: 4 Average inter and intra cluster distance ($D=\sqrt{D^2}$) values of 36 genotypes of cotton (*G. hirsutum* L.)

	I	II	III	IV	V	VI	VII
I	**150.45**	408.25	344.65	558.21	823.92	6490.72	652.75
II		**189.07**	944.26	684.63	433.08	4328.11	169.42
III			**89.29**	459.41	362.81	2100.01	204.01
IV				**214.07**	304.40	378.45	196.96
V					**0.00**	646.21	1020.99
VI						**0.00**	635.42
VII							**0.00**

Table 5: Best possible crosses suggested from diverse parent based on genetic distance, clustering and *per se* performance for seed cotton yield

Sr. no	Cross	Morpho-logical clusters	Mole-cular clusters	Morpho-logical distance	Mole-cular distance	*Per se* performance							
						DF	PH	NOS	NOM	BP	BW	GP	SCY
1	BPHI-537	III	B	450.87	0.26	53	116.67	20*	2.3*	25.37	4.5*	33.1	110.7*
	GSHV-164	II	A2			48*	110	11.3	1.3	25.36	3.5	34.77	87.12
2	BPHI-537	III	B	233.15	0.29	53	116.67*	20*	2.3*	25.37*	4.5	33.1	110.7*
	SCS-1214	I	A2			56	101.56	15.3	1.2	15.47	5.3*	34.17	82.72
3	BPHI-537	III	B	110.36	0.30	53	116.67	20	2.3*	25.37*	4.5	33.1	110.7*
	H-1476	I	A2			49*	108.56	20.7	1.7	20.95	4.2	35.47	89.48
4	BPHI-537	III	B	461.6	0.30	53	116.67*	20	2.3*	25.37	4.5	33.1	110.7
	F-2451	II	A2			52	95.27	21.4	1.7	23.21	4.9	37.37*	114.55
5	BPHI-537	III	B	272.57	0.31	53	116.67*	20	2.3*	25.37*	4.5	33.1	110.7*
	P-5629	I	A2			46*	101.58	18.3	1.3	16.68	4.3	33.63	72.23
6	BPHI-537	III	B	130.06	0.32	53	116.67*	20*	2.3*	25.37*	4.5	33.1	110.7*
	HS-292	I	A2			47*	89.88	14	1.3	21.37	4.4	37.7*	94.92
7	BPHI-537	III	B	112.11	0.32	53	116.67*	20*	2.3*	25.37*	4.5	33.1	110.7*
	BS-1	I	A2			50	88.31	13.7	1.7	18.51	4.7	34.13	87.33
8	BPHI-537	III	B	265.42	0.34	53	116.67	20*	2.3*	25.37	4.5*	33.1	110.7
	GSHV-171	IV	A2			52	116.91	13.3	2	39.33*	2.6	36.27*	103.77
9	BPHI-537	III	B	400.31	0.35	53	116.67	20*	2.3*	25.37*	4.5	33.1	110.7*
	CCH 13-2	I	A2			51	115	16.7	1	16.46	4.8	35.43	79.82
10	BPHI-537	III	B	303.56	0.37	53	116.67	20*	2.3*	25.37	4.5	33.1	110.7
	GJHV-516	IV	A2			47*	116.67	14	1.3	26.28	4.4	35.97	117.37
11	CPD-1301	I	A2	238.67	0.52	52	98.27	18.7	1.7	15.52	4.9	33.63	80.25
	F-2451	II	A2			52	95.27	21.4*	1.7	23.21*	4.9	37.37*	114.55*
S.Em. ±						1.33	5.26	0.71	0.08	1.39	0.3	0.88	4.48
C.D. at 5%						3.74	14.83	2.01	0.24	3.92	0.83	2.48	12.63
C.V %						4.59	8.98	8.27	9.98	13.22	11.64	4.4	9.85
No. of crosses showing significant differences between parents						5	5	8	10	8	3	4	8

DF: Days to 50% flowering **PH:** Plant Height **NOS:** Number of sympodia **NOM:** Number of monopodia

BP: Ball per Plant **BW:** Ball weight **GP:** Ginning Percentage **SCY:** Seed cotton yield

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