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Insights of Genetic Divergence through Mahalanobis' D^2 Statistics among American Cotton (*Gossypium hirsutum* L.) Genotypes

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ABSTRACT: In any crop improvement programme, genetic diversity provides opportunity for researchers to develop new improved varieties with desirable traits, which accommodates both farmers and breeders preferred traits. The aim of this study was to characterize the genetic diversity of twenty-five genotypes of cotton and the genotypes were evaluated in randomized complete block design with four replications during *kharif*, 2019 at Agronomy Instructional Farm, SDAU, Sardarkrushinagar. The results of multivariate analysis revealed that the genotypes grown had significant genetic divergence and the genotypes grouped into six clusters using *Tocher*'s method in D^2 analysis. The cluster with the maximum genotypes was Cluster I, followed by Cluster VI. Cluster VI had the greatest intra-cluster distance and maximum inter cluster distance was calculated between cluster V and cluster VI. Inter cluster distances were greater than intra cluster distances, indicating that the genotypes differed significantly. Crosses between genotypes of these clusters can be made based on these studies to create suitable transgressive segregants. According to Mahalanobis' D^2 statistic, fibre strength, followed by lint yield per plant, number of bolls per plant and oil content, contributed the most to the overall genetic divergence. It can be concluded that choosing divergent parents based on these attributes would be advantageous in terms of maximising diversity.

Keywords: Cluster, Cotton, Diversity, Genotypes, Selection.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.), a crop of prosperity, is an industrial commodity of worldwide importance. It is one of the most ancient and important commercial crop next only to food grains. Cotton is grown for its lint, which is a major textile raw material, and for its seed cotton, which produces oil and protein. It is also known as "white gold" because of its importance in agriculture as well as in industrial economy. Cotton belongs to *Malvaceae* family and the *Gossypium* genus. According to Percival and Kohel (1990) the genus Gossypium includes 49 species, four are cultivated, 43 are wild diploids and two, wild tetraploids, of the four cultivated species, *G.hirsutum* and *G. barbadense* are allotetraploids (2n=4x=52), commonly called as new world cotton. *G. hirsutum* also known as upland cotton, long staple cotton or Mexican cotton, produces 90 per cent of the world's cotton; *G. barbadense*, also known as Sea Island cotton, extra-long staple cotton, American Pima or Egyptian cotton, contributes 8 per cent of the world's cotton; Whereas *G. arboreum* and *G. herbaceum* are diploids (2n=2x=26) and commonly called as old world or Asiatic cotton. *G. herbaceum*, known as Levant cotton and *G. arboreum*, known as Tree cotton, together provides 2 per cent of the world's cotton (Zhang *et al*., 2008). India has a pride place in the global cotton scenario due to several reasons, including the largest cotton growing area, cultivation of all four cultivated species, a large area under tetraploid cotton, one of the largest producers of long and extra-long staple cotton, possibly the only country to grow hybrid cotton, the native home of old cultivated cotton, and a wide range of agro-climatic conditions under which cotton is grown. India is the world's largest cotton producer, with 13.47 million hectares and 36.07 million bales of cotton lint produced at an average productivity of 455 kg per ha (Anonymous, 2020). The cultivation of Bt cotton, favourable seasons, and good agronomic practises are the main reasons for high yield and productivity. *G. hirsutum*'s higher yield potential and adaptability have been improved through breeding and genetic manipulation. Similarly, the superior fibre

characteristics of the *G. barbadense* species have been emphasised in the development of commercial cultivars in that species, despite the fact that *G. barbadense* has a unique nature of high quality fibre, its production is limited due to its lower yield potential. Despite the fact that *G. hirsutum* leads fibre production, modern spinning techniques and end users have created demands to improve its quality (Felker *et al.,* 2001). Advancement in the cotton improvement programme is largely dependent on genetic diversity in the base population's metric traits.

It is necessary to estimate the amount of variability present in a population in order to exploit it for trait improvement, which is required for genetic improvement in any crop species. Genetic distance estimations are used in hybrid crop breeding to select parental combinations with appropriate genetic diversity and to classify germplasm into distinct heterotic groups (Chakraborty *et al*., 2021). Cotton breeders' ultimate goal is to develop varieties and hybrids with desirable fibre traits and high seed cotton yield. It is necessary to measure the genetic diversity among the genotypes in order to select elite parents for the hybridization programme, as genetic diversity plays an important role in generating heterosis in hybrids between genotypes. Mahalanobis' D^2 statistics is an effective too for determining the degree of genetic divergence at the genotypic level, as well as a measure of the relationship between geographic distribution and genetic diversity based on generalised distance (Mahalanobis, 1928). Multivariate analysis, utilizing Mahalanobis' D^2 statistics has been found to be a potent biometrical tool in quantifying the degree of divergence in germplasm collection of various crop plants (Rao, 1952). Without making crosses before starting the hybridization programme, the D^2 statistic can be used to select parent combinations (Bhatt, 1970).

An experiment by Kulkarni *et al*. (2011) revealed that genetic diversity is not entirely dependent on geographical diversity. Inter-cluster distances were found to be greater than intra-cluster distances, indicating that the genotypes studied have a high level of genetic diversity. High magnitude of heterosis or desirable segregants can be produced by taking inter-cluster distances into account during the hybridization process. Based on Mahalanobis D^2 analysis, Asha *et al*. (2013) studied genetic diversity in forty cotton genotypes for fifteen quantitative characters, which were grouped into seven clusters, with clusters I and III being the largest, each with eight genotypes, and cluster IV having seven genotypes. The lack of parallelism between geographical and genetic diversity was revealed by the random distribution of genotypes. Cotton genetic diversity was studied by Bhimate *et al*. (2019), *Tocher*'s method was used to divide the 36 genotypes into nine clusters and eleven traits. Cluster I had the most genotypes (21) and Cluster VIII had the fewest (7), while Cluster VII and Cluster VIII had the greatest cluster distance D=59.36, followed by Cluster VI and IX (50.13). Days to 50% flowering were the most important factor in genetic divergence (28.89%), followed by seed index (21.59%) and seed cotton yield per plant (both 21.59%). (13.02 %). Satish (2021) investigated genetic diversity in upland cotton using 55 genotypes by D^2 statistics for yield and contributing traits. The 55 genotypes were divided into six groups, which explained 91.8 percent of the variability. The maximum divergence among genotypes L1785, L1687, L1748, L1780, L1783, and L 604 was detected in the multivariate analysis, indicating that crosses between these genotypes will produce highly heterotic hybrids.

In light of the foregoing, the current study was conducted to look into the genetic diversity of yield, yield contributing traits and fibre traits.

MATERIALS AND METHODS

The current study was conducted at the S. D. Agricultural University's Agronomy Instructional Farm in Sardarkrushinagar during *Kharif*, 2019. The experimental material consisted of twenty-five cotton genotypes obtained from the Cotton Research Station, Talod, and evaluated using Randomized Block Design (RBD) with four replications in *Kharif*, 2019-20. Each genotype was sown in 6 m long rows with a 90 cm row spacing and 60 cm between plants. To raise a healthy crop, recommended agronomical and plant protection practises were followed throughout the crop season. In each replication, five competitive plants per genotype were chosen at random and labelled for recording observations. Data were statistically analyzed using the mean of the five plants chosen. At CIRCOT, Mumbai, observations on fibre quality traits in each replication were recorded using a High Volume Instrument (HVI) in ICC mode. The standard procedure suggested by Fisher was used to analyse the variance of the observations recorded on different characters (1925). According to Rao (1952), Mahalanobis' D^2 statistics were used to estimate genetic diversity.

RESULTS AND DISCUSSION

The degree of values suggested that there was significant variability in the material studied which led to genetic diversity. Using *Tocher*'s approach (Rao, 1952), the twenty-five genotypes were divided into six clusters with the condition that intra-cluster average D^2 values should be less than inter-cluster D^2 values. The distributions of genotypes into six clusters presented in Table 1 and diagrammatically in Fig. 1.

The cluster I had highest number of genotypes (19) followed by cluster VI (2). The cluster II, III, IV and V were mono-genotypic. Geographic obstacles restricting gene movement or intensive natural and human selection for varied and adaptable gene complexes may be responsible for the establishment of different isolated clusters. With rare exceptions, the observed genotype grouping pattern was irrespective of their geographical origin.

Table 1: Clustering pattern of twenty-five cotton genotypes by Tocher's method.

Cluster No.	No. of genotypes	Name of the genotypes
Cluster I	19	GTHV 15/22, GJHV 534, GTHV 15/220, RB 611, G. Cot-16 (LC), GSHV 185, GJHV 568, GBHV 185, TCH 1837, GSHV 209, GDHV 221, GSHV 229, GJHV 548, GJHV 553, GISV 322, RHC 1307, GISV 319, GN. Cot-22 (CC), GTHC 13/35
Cluster II	1	GJHV 546
Cluster III	1	G. Cot-20 (CC)
Cluster IV	1	GBHV 186
Cluster V	1	GISV 332
Cluster VI	2	RAH 1075, SURAJ

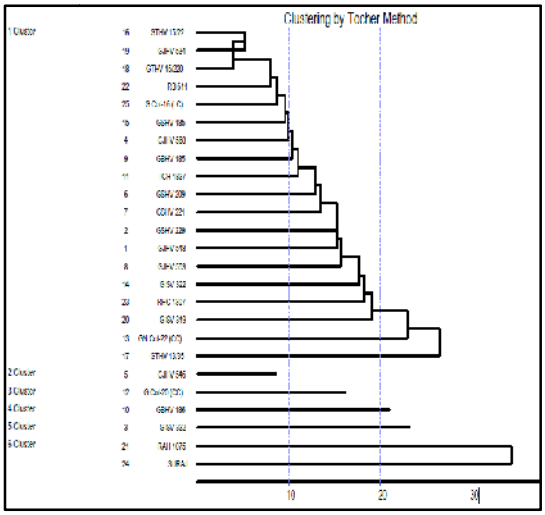

Fig. 1. Dendrogram showing relationship among twenty-five cotton (*Gossypium hirsutum* L.) genotypes in 6 clusters based on Mahalnobis D^2 value.

Cluster I had the most genotypes, with 19 genotypes from various regions, which could be due to the free flow of breeding material from one location to another, as well as the unidirectional selection practised by breeders in different locations. Furthermore, genotypes from various eco-geographical regions were grouped together in the same cluster, implying that there was no link between geographic and genetic diversity. Genotypes from the same cluster are more closely related than genotypes from different clusters. Similar findings of Kulkarni *et al.* (2011); Asha *et al.* (2013); Kavithamani *et al.* (2013) corroborated that the distribution of genotypes from different eco-geographical regions into clusters was at random, indicating geographical distribution does not necessarily exhibit genetic divergence. Asha *et al.* (2013); Bhimate *et al.* (2019), showed cluster I contained the maximum number of genotypes.

The intra and inter-cluster D^2 and D values among six clusters are given in Table 2 and the mutual relationship between clusters is represented diagrammatically (Fig. 2) by taking average intra and inter cluster Euclidean distances. The clustering pattern revealed that varieties from different sources were grouped together in one group, while varieties from the same source formed separate clusters, indicating that there was no correlation between geographic and genetic divergence. Murthy and Arunachalam (1966) stated that genetic drift and selection in different environment could cause greater diversity than geographical distance. Intra cluster average D^2 values ranged from 17.97 to 34.29. Among the clusters, Cluster VI had the maximum intra-cluster distance (34.29) while the minimum intra cluster distance was observed for cluster I (17.97). The zero intra clusters distance was observed for cluster II, III, IV and V. These four clusters (II, III, IV and V) were a solitary cluster. Cluster VI has a high intra-cluster distance, indicating that the genotypes within this cluster have a lot of genetic diversity.

Table 2: Average intra (bold) and inter-cluster values (D^2)among six clusters in twenty-five genotypes of cotton.

Cluster No.	I	II	III	IV	V	VI
I	**17.97**	29.74	31.08	41.79	53.09	64.12
II		**0**	38.61	90.27	35.04	67.13
III			**0**	54.44	90.91	87.20
IV				**0**	105.65	91.10
V					**0**	106.28
VI						**34.29**

Cluster V and Cluster VI had the greatest inter cluster distance (106.28), followed by Cluster IV and V. (105.65). This indicated that these clusters have a good amount of genetic diversity. Crosses between genotypes of these clusters can be made based on these studies to obtain desirable transgressive segregants. Cluster I and II were found to have the shortest inter-cluster distance (5.41).

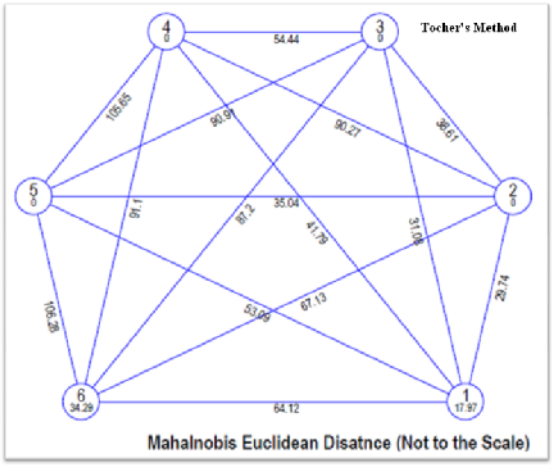

Fig. 2. Intra and inter cluster distance of twenty-five cotton (*Gossypium hirsutum* L.) genotypes in 6 clusters based on Euclidean distance.

Cluster I had the largest distance from cluster VI (64.12) followed by cluster V (53.09) and cluster IV (41.79). The inter cluster distance between cluster I and II (29.74) and cluster I and III (31.08) was comparatively of two magnitude. The distance between cluster II and IV (90.27) was highest followed by cluster VI (67.13), cluster III (38.61) and cluster V (35.04). The closest cluster from the cluster II was cluster I (29.74). The cluster V (90.91) was far away from cluster III followed by cluster VI (87.20), cluster IV (54.44) and cluster II (38.61). The cluster I (31.08) was nearest to cluster III. Cluster IV depicted maximum distance from cluster V (105.65), which was followed by cluster VI (91.10), cluster II (90.27) and cluster III (54.44). Whereas, it had minimum D^2 value with cluster I (41.79). The distance between cluster V and cluster VI (106.28) was highest followed by cluster IV (105.65), cluster III (90.91) and cluster I (53.09). Whereas, the cluster II (35.04) was nearest to cluster V. Cluster VI had the largest distance from cluster V (106.28), which was followed by cluster IV (91.10), Cluster III (87.20) and cluster II (67.13). The cluster I (64.12) was nearest to cluster VI. Choice of the particular cluster and selection of particular genotype from selected cluster are the two important points to be considered before initiating the crossing programme. The hybrids between varieties of different clusters will express high heterosis and throw more useful segregants. Further, one or two varieties from different clusters may be chosen for further genetic studies either by diallel or line × tester analysis.

Table 3 shows the mean cluster performance for 16 characters. Days to flowering, days to boll bursting, plant height (cm), number of monopodia per plant, number of sympodia per plant, number of bolls per plant, boll weight (gm), ginning percent, lint yield per plant, seed index, seed cotton yield per plant, oil content (%), 2.5 percent span length, micronaire (106 g/inch), and fibre strength (g/tex) all showed significant inter cluster variation. It is imperative from the results that character wise contribution differs from cluster to cluster, so far improvement of a particular character can be carried out by utilizing genotypes from the respective cluster having the highest mean values for it. Thus, clustering pattern and deciding the cross combinations which may generate the highest variability for various traits. The superior genotypes for the breeding program can also be selected based on cluster means and inter cluster distance. Higher mean values for boll weight were seen in clusters V (3.77 g) and VI (3.65 g) and higher mean for number of bolls per plant was observed in clusters VI (31.15) and II (19.87), which are the major contributors in improving seed cotton yield per plant in cotton. Based on mean values, series of diallel analysis may prove highly successful breeding programme.

In Table 4 and Fig. 3, the contribution of each character to total genetic diversity is shown. A breeder's understanding of the characters that influence divergence is crucial. According to character rank, no single character contributed more to total genetic divergence than lonely.

Fibre strength (22.00 %), lint yield per plant (21.67 %), number of bolls per plant (14.00 %), and oil content (14.00 %) contributed the most to total divergence of the 16 characters studied. Other traits such as days to flowering (0.33 %), micronaire (0.33 %), lint index (0.33 %), 2.5% span length (1.00 %), seed cotton yield per plant (2.00 %), and sympodia per plant (2.33 %) played a minor role in the divergence. Similar findings were also reported by Vijayalaxmi *et al.* (2008); Gopinath *et al.* (2009). The success of the plant breeding program depends largely on the selection of appropriate parents. The use of divergent parents in hybridization is expected to result in promising recombinants. The amount of genetic variability in the population has a big impact on genetic improvement.

Table 3: Mean values of six clusters estimated by Tocher's method from twenty-five genotypes of cotton.

Clusters	Days to flowering	Days to boll bursting	Plant height (cm)	No. of monopodia per plant	No. of sympodia per plant	No. of bolls per plant	Boll weight (g)	Ginning per cent (%)
Cluster I	68.93	114.66	106.88	1.51	14.70	15.57	2.93	37.02
Cluster II	65.75	110.25	90.20	1.65	15.28	19.87	3.23	37.59
Cluster III	72.50	122.75	117.55	2.05	15.37	15.92	2.68	37.66
Cluster IV	70.75	118.75	94.58	1.50	9.62	12.01	2.80	36.87
Cluster V	72.25	122.75	96.98	0.95	16.08	17.39	3.77	38.28
Cluster VI	66.13	116.00	125.70	1.18	16.95	31.15	3.65	37.05

Clusters	Lint yield per plant (g)	Seed index (g)	Lint index (g)	Seed cotton yield per plant (g)	Oil content (%)	2.5% Span length (mm)	Micronaire (10^{-6} g/inch)	Fibre strength (g/tex)
Cluster I	14.23	7.87	4.65	38.34	17.55	26.65	4.32	21.35
Cluster II	18.82	8.14	4.91	50.00	16.64	25.10	4.57	19.75
Cluster III	13.20	8.14	4.92	35.05	15.77	25.34	4.41	21.75
Cluster IV	8.82	8.39	4.89	23.90	18.43	27.08	4.19	25.18
Cluster V	27.05	7.88	4.89	70.65	17.67	26.76	4.30	21.30
Cluster VI	12.32	9.21	5.48	34.33	17.87	26.93	4.21	21.45

Table 4: Contribution of different characters towards genetic divergence in twenty-five cotton genotype.

Sr. No.	Source	Contribution (%)	Times ranked first
1.	Days to flowering	5.00	15
2.	Days to boll bursting	0.33	1
3.	Plant height (cm)	4.67	14
4.	No. of monopodia per plant	7.67	23
5.	No. of sympodia per plant	2.33	7
6.	No. of bolls per plant	14.00	42
7.	Boll weight (g)	0.67	2
8.	Ginning per cent (%)	0.00	0
9.	Lint yield per plant (g)	21.67	65
10.	Seed index (g)	4.00	12
11.	Lint index (g)	0.33	1
12.	Seed cotton yield per plant (g)	2.00	6
13.	Oil content (%)	14.00	42
14.	2.5% Span length	1.00	3
15.	Micronaire (10^{-6} g/inch)	0.33	1
16.	Fibre strength (g/tex)	22.00	66

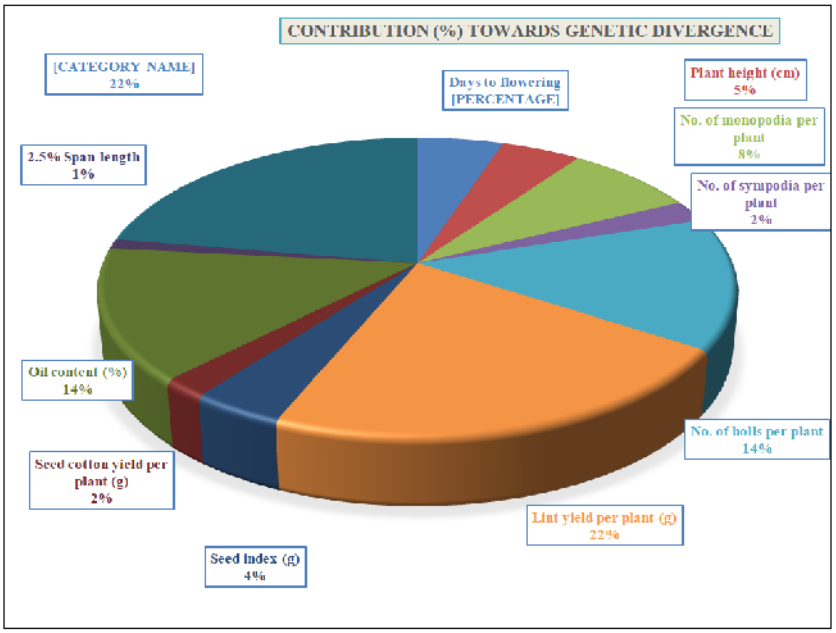

Fig. 3. Contribution of different characters towards genetic divergence in twenty-five cotton (*Gossypium hirsutum* L.) genotypes.

CONCLUSION

Because genotypes from the same area were scattered into different clusters and genotypes from different areas were grouped in the same cluster, genetic diversity was independent of geographic regions. As a result, rather than relying solely on geographical diversity, the breeder must evaluate his material for genetic diversity. The current findings suggest that in the future, when developing a breeding programme to improve seed cotton yield, more emphasis should be placed on the number of bolls per plant, boll weight, ginning percentage and lint yield per plant.

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Conflicts of Interest. None.

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