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Genetic divergence studies in American cotton (*Gossypium hirsutum* L.)

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Abstract
This study aimed to assess the genetic diversity and relationships among the *G. hirsutum* genotypes using multivariate Mahalanobis D^2 statistics. The analysis of variance for dispersion revealed significant differences among the genotypes, which were grouped into five clusters. The largest group, Cluster I, contained 21 genotypes sourced from various locations. Cluster II was the second largest, with 15 genotypes. Clusters III and IV each represented a single genotype, while Cluster V included 2 genotypes and exhibited no intra-cluster D^2 value. The trait that contributed most significantly to genetic divergence was seed cotton yield per plant, accounting for 48.32% of the variation. This was followed by halo length (13.21%), number of bolls per plant (8.72%), ginning percentage (5.38%), seed index (4.49%), and number of monopodia per plant (4.23%). The maximum inter-cluster distance between Cluster V and Cluster II was observed, with a D value of 9.33. Therefore, the genotypes in Clusters V and II may be valuable for breeding programs aimed at enhancing heterosis for yield and related traits, such as the number of bolls per plant, boll weight, seed index, seed cotton yield per plant, days to 50% flowering, and days to 50% boll bursting.

Keywords: D^2 statistics, cluster, diversity, cotton, genotypes

Introduction
Cotton (*Gossypium* spp.) is the most important renewable natural fiber crop globally and remains the predominant and sustainable fiber source in the Indian textile industry. It provides raw materials such as cotton lint, which is a key export item, as well as crude oil and cottonseed cake for the oil and livestock industries, respectively. Cotton is widely cultivated as a profitable fiber crop, contributing significantly to the national economy and attracting considerable research attention. It plays a crucial role in trade, industrial activities, employment, and foreign exchange earnings. Due to its multiple uses, including lint and byproducts, cotton is often referred to as "white gold." In its wild state, cotton is a perennial plant; however, most cultivated varieties are annuals. The plant has a taproot with secondary roots that branch laterally from the primary root. The main stem is erect and highly branched. Each cotton leaf petiole has two buds at its base: the true axillary bud, which develops into a vegetative branch that produces only leaves and no flowers, and the accessory bud, which generally develops into a sympodial or fruiting branch. Only the fruiting branches bear flowers, with a tendency for lower branches to be vegetative and upper branches to be fruiting. The flowers of cotton are characteristic of the Malvaceae family. The flower buds, which appear as small, pyramidal-shaped green structures, are referred to as 'squares.' Each square consists of three triangular leafy structures surrounding the flower bud. The flowers typically open 18 to 24 days after the squares appear. When the cotton boll is ripe, the capsule splits along its sutures. The cotton fiber itself is an elongation or outgrowth of an epidermal cell from the seed coat. The long outgrowths form the 'staple' or 'lint,' while shorter outgrowths form the 'fuzz.' There is considerable variability within cotton germplasm (Grewal *et al*., 1994) [6], which can be utilized for developing new genotypes with higher yields and better quality. Genetic diversity is crucial for the adaptability of a species; populations with a greater degree of genetic variability exhibit more variation. This genetic diversity significantly influences the success of hybridization programs. Breeders have long recognized the importance of genetic diversity in crop improvement. Conversely, relying on single genetic cultivars can lead to genetic erosion and the extinction of primitive and

adaptive genes. Therefore, research focuses on selecting more diverse parents within acceptable fitness limits, which increases the chances of producing heterotic F1 hybrids and a broader spectrum of variability in the segregating generation (Arunachalam, 1981; Falconer, 1989) [2, 5]. Thus, the first step to initiating a hybridization program is to assess genetic diversity and identify genetically diverse parents.

Material and Methods

The experiment was conducted at the Cotton Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, during the kharif season of 2020. The study was designed using a randomized block design with three replications and a spacing of 60 x 30 cm². Forty genotypes of upland cotton were sourced from the Cotton Research Unit. Five plants from each genotype were randomly selected and tagged in all three replications. Observations were recorded for 11 characters: days to 50% flowering, days to 50% boll bursting, plant height (cm), number of monopodial branches per plant, number of sympodial branches per plant, number of bolls per plant, boll weight (g), ginning percentage (%), seed index (g), halo length (mm), and seed cotton yield per plant (g). The Mahalanobis D^2 statistic is a powerful tool for quantifying genetic divergence in germplasm collections based on the traits considered together. Genetic divergence among the 40 genotypes was analyzed using the Mahalanobis D^2 statistics method (1928), and the genotypes were grouped into clusters following the Tocher's method described by Rao (1952) [11].

Results and Discussion

Analysis of variances exhibited significant differences among the forty genotypes for all studied Eleven characters.

Analysis of dispersion (Wilk's criterion)

The analysis of dispersion and the simultaneous test of significance based on Wilk's criterion for the pooled effect of eleven characters showed highly significant differences among forty genotypes studied in the present investigation ($X^2 = 90.5$ at d.f. = 429).

Estimation of D^2 values

We used the plot means of forty genotypes across eleven characters (X1-X11) and transformed them into standardized, uncorrelated means (Y1-Y11). We then calculated the D2 values for 121 possible comparisons, taking two genotypes at a time. According to the tabulated values, the critical value of X2 at a 1% level of significance with 10 degrees of freedom is 23.20. All calculated D2 values exceeded this threshold, indicating that the D2 values are statistically significant. Table 1 presents the percentage contribution of each of the eleven characters to genetic divergence. Understanding which characters influence divergence is crucial for breeders. The contributions rank shows that no single character accounted for a greater portion of the total genetic divergence. The character with the maximum contribution was seed cotton yield per plant, accounting for 48.32%. This was followed by halo length at 13.21%, the number of bolls per plant at 8.72%, ginning percentage at 5.38%, seed index at 4.49%, number of monopodia per plant at 4.23%, plant height at 4.10%, number of sympodia per plant at 4.00%, days to 50% flowering at 3.33%, boll weight at 1.41%, and days to 50% boll bursting at 1.28%.

Grouping of genotypes into various clusters

The 40 genotypes were categorized into 5 clusters using Tocher's method. The distribution of these genotypes across the clusters is shown in Table 3. Cluster I contains 21 genotypes, Cluster II has 15 genotypes, and Cluster V includes 2 genotypes. Clusters III and IV are solitary, each containing a single genotype. This grouping pattern suggests that genetic diversity does not necessarily correlate with geographical diversity. Instead, it may result from various factors, including natural selection, the exchange of breeding materials, genetic drift, and environmental variation. Consequently, the selection of genotypes for hybridization should prioritize genetic diversity rather than geographical diversity. Previous studies by Satish *et al*. (2016) [9], Haritha and Ahamed (2013) [7], Asha *et al*. (2013) [3], Tulasi *et al*. (2014) [13], Kumar *et al*. (2017) [1], Sharma *et al*. (2016) [12], Naik *et al*. (2016) [9], and Anil *et al*. (2017) [1] have also reported a lack of parallelism between genetic divergence and geographical divergence among genotypes.

Average intra-and inter-cluster D^2 values

The average intra-and inter-cluster distances for eleven characters, estimated using Tocher's method, are presented in Table 2 and illustrated in Fig. 2. The maximum intra-cluster distance was found in cluster V (3.13), followed by cluster II (2.8) and cluster I (2.74); both clusters III and IV had an intra-cluster distance of zero. The high intra-cluster distance in cluster V indicates a significant level of genetic diversity among the genotypes within this cluster. In terms of inter-cluster distances, the maximum average inter-cluster distance was observed between clusters V and II (D = 9.33), followed by the distance between clusters V and IV (D = 9.26), and between clusters V and I (D = 6.97). The inter-cluster distance between clusters IV and III was 6.15, and between clusters III and II, it was 6.13. These findings suggest a considerable genetic diversity between these clusters. Based on this analysis, crossing genotypes from these clusters could lead to the production of desirable transgressive segregants. The comparison of intra-and inter-cluster distances indicates that inter-cluster distances are greater than intra-cluster distances. Consequently, hybrids resulting from the combinations of genotypes from different clusters are likely to exhibit high heterosis and produce more useful segregants.

Table 1: Contribution of different characters towards genetic divergence in 40 cotton (*Gossypium hirsutum* L.) genotypes.

Sr. No.	Characters	Times ranked Ist	Contribution in percentage
1	Days to 50 percent flowering	26	3.33
2	Days to 50% boll bursting	10	1.28
3	Plant height (cm)	32	4.10
4	Number of monopodia per plant	33	4.23
5	Number of sympodia per plant	31	4.00
6	Number of bolls per plant	68	8.72
7	Boll weight (g)	11	1.41
8	Ginning percentage (%)	42	5.38
9	Seed index (g)	35	4.49
10	Halo length (mm)	103	13.21
11	Seed cotton yield per plant (g)	377	48.32

Table 2: Average intra and inter-cluster D^2 values among 5 clusters in 40 genotypes of cotton (*Gossypium hirsutum* L.)

Clusters	I	II	III	IV	V
I	2.74	3.82	3.83	4.37	6.97
II		2.8	6.13	3.69	9.33
III			0	6.15	4.57
IV				0	9.26
V					3.13

Table 3: Clustering pattern of 40 cotton (*Gossypium hirsutum* L.) genotypes by Tocher's method.

Clusters	Total No. of genotypes	List of genotypes
		AKH-JKP4/4-5, AKH-JKP4/4-28, AKH-PB-75, AKH-PB-135, AKH-
		JKP4/4-64, AKH-JKP4/4-2, AKH-JKP4/4-9, AKH-PB-8, AKH-PB-26,
I	21	AKH-JKP4/4-29, AKH-PB-19, AKH-JKP4/4-62, AKH-PB-124, AKH-
		JKP4/4-33, AKH-JKP4/4-1, AKH-PB-48, AKH-JKP4/4-21, AKH-
		JKP4/4-66, AKH-JKP4/4-13, AKH-PB-60 and AKH-PB-115
		AKH-PB-14, AKH-PB-36, AKH-JKP4/4-4, AKH-PB-67, AKH-PB-138,
II	15	AKH-JKP4/4-6, AKH-JKP4/4-32, AKH-PB-21, AKH-PB-107, AKH-PB-33, AKH-PB-143, AKH-JKP4/4-7, AKH-JKP4/4-34, AKH-PB-16
		and AKH-PB-110
III	1	AKH-PB-106
IV	1	AKH-PB-78
V	2	AKH-PB-112 and AKH-PB-82

Cluster mean values

The cluster mean values for 11 characters are presented in Table 4. The data indicated a wide range of mean values between the characters. Higher mean values for boll weight were seen in clusters V and I and higher means for the number of bolls per plant were observed in clusters V and III which are major contributors in improving seed cotton yield per plant in cotton. Based on mean values, a series of crosses in a diallel fashion may prove highly successful. The success and usefulness of Mahalanobis D^2 analysis in quantifying genetic divergence have been studied by Rajamani and Rao (2009) [10], Satish *et al.*, (2016) [9], Asha *et al.*, (2013) [3], Sharma *et al.*, (2016) [12] and Dahiphale and Deshmukh (2018) [4].

Thus the present study identified divergent genotypes from clusters II and V as they have high inter cluster distance AKH-PB-14, AKH-PB-36, AKH-JKP4/4-4, AKH-PB-67, AKH-PB-138, AKH-JKP4/4-6, AKH-JKP4/4-32, AKH-PB-21, AKH-PB-107, AKH-PB-33, AKH-PB-143, AKH-JKP4/4-7, AKH-JKP4/4-34, AKH-PB-16, AKH-PB-110, AKH-PB-112 and AKH-PB-82 and they should be used for further improvement in heterosis in yield targeted traits with creation of wider variability.

Table 4: Mean values of 5 clusters estimated by Tocher's method from 40 genotypes of cotton (*Gossypium hirsutum* L.)

Clusters	Days to 50% Flowering	Days to 50% boll bursting	Plant height (cm)	Number of monopodia per plant	Number of sympodia per plant	Number of bolls per plant	Boll weight (g)	Ginning percentage (%)	Seed index (g)	Halo length (mm)	Seed cotton yield per plant (g)
I	63.32	128.43	92.39	1.47	12.12	9.76	3.52	36.71	7.16	26.58	32.1
II	64.64	130.62	78.56	1.5	10.47	7.82	3.08	36.72	7.33	27.32	20.38
III	63.67	127.67	117.53	1.3	14.67	15.27	3.4	36.13	6.17	25.27	46
IV	66	137.33	41.2	1.57	5.33	7.2	3.13	35.17	2.5	26.4	20.37
V	61.5	126.17	103.56	1.92	14.33	18.9	3.8	37.05	7.67	27.38	63.92

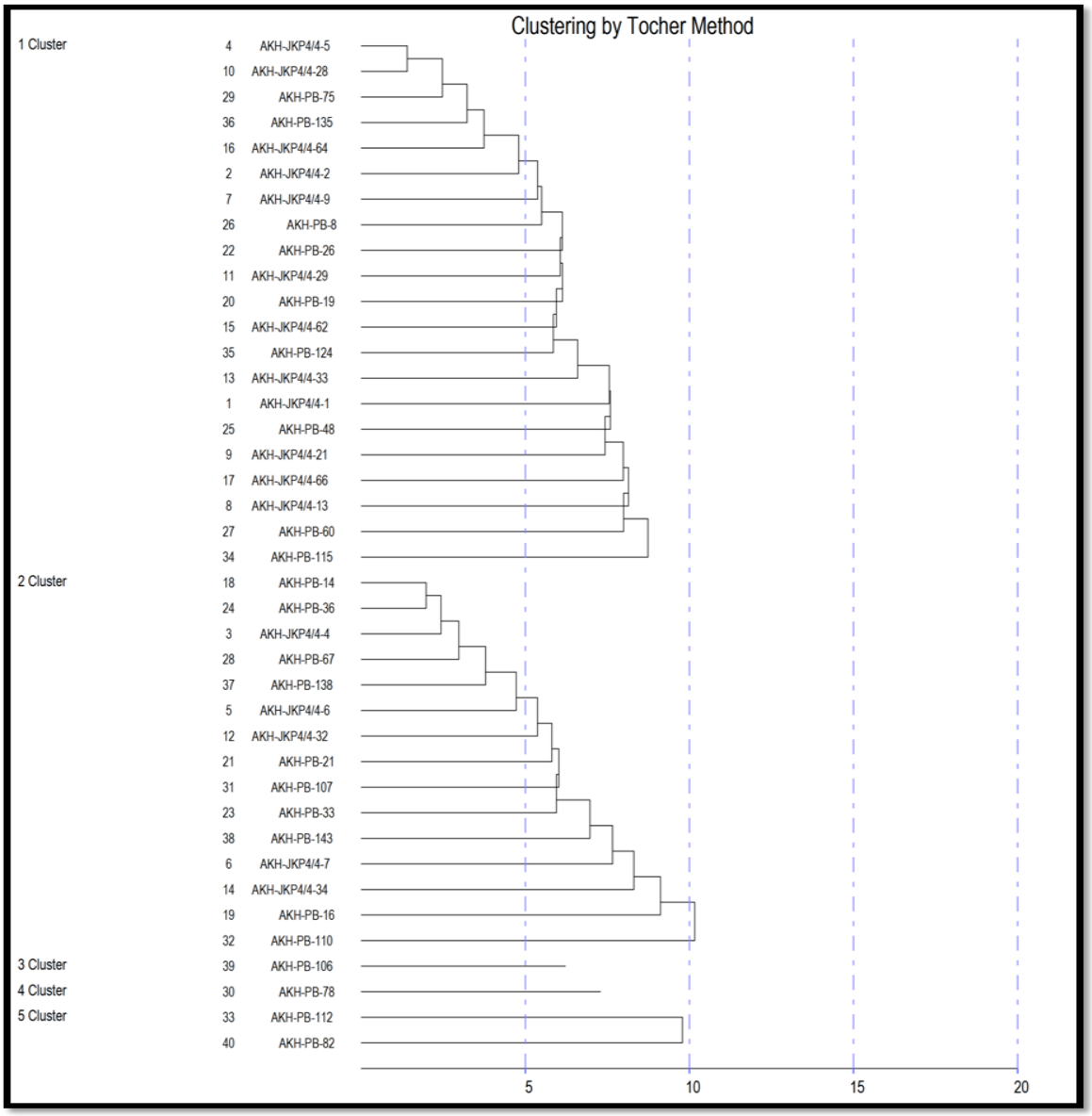

Fig 1: Dendrogram

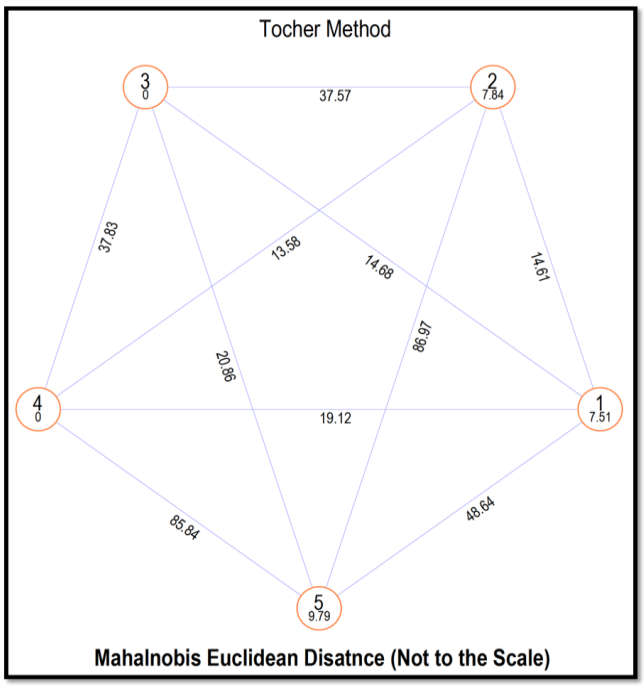

Fig 2: Cluster diagram

Conclusion

The genotypes used in the present study were sourced from various geographical regions. The findings reveal that genotypes from the same area were distributed across different clusters, while genotypes from different regions were found within the same cluster. This indicates that genetic diversity is not solely determined by geographical distribution. Therefore, it is essential to evaluate genetic diversity before utilizing any genetic material or genotypes in crop breeding.

Future Scope

Looking ahead, when planning breeding programs aimed at improving seed cotton yield, it is important to focus on specific traits such as boll weight, the number of bolls per plant, and lint yield.

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