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Research Note

Genetic diversity analysis of upland cotton (*Gossypium hirsutum* L.) genotypes using cluster analysis

Ch. Mohan Mani Kanta[1*], N. Chamundeswari[2], K. V. Siva Reddy[2] and B. Sreekanth[3]

[1]Department of Genetics and Plant Breeding, Acharya N. G Ranga Agricultural University, Agricultural College, Bapatla, Andhra Pradesh
[2]Department of Genetics and Plant Breeding, Acharya N. G Ranga Agricultural University, Lam, Guntur, Andhra Pradesh
[3]Department of Crop Physiology, Acharya N. G Ranga Agricultural University, Lam, Guntur, Andhra Pradesh
***E-Mail**: chmohanmanikanta@gmail.com

Abstract
The D^2 statistic enables the evaluation of genetic diversity among numerous genotypes and aids in selecting genetically distinct parents for hybridization programmes. The present research work on genetic diversity involved 56 genotypes of upland cotton, evaluated in alpha lattice design with two replications and data was collected on 19 characters. Multivariate analysis revealed substantial genetic diversity among the 56 genotypes evaluated. Using Tocher's method in D^2 analysis, the genotypes were classified into six distinct clusters. Among the traits studied, total seed cotton yield plant^{-1} contributed more towards genetic divergence followed by seed cotton yield plant $^{-1}$ at 140 days after sowing, proline content, ginning out turn and seed index. The cluster II had highest (19) number of genotypes followed by cluster I (16) genotypes, cluster III (10) genotypes and cluster IV (8) genotypes. For most of the traits inter cluster distances were higher than intra cluster distance which indicates considerable diversity among the genotypes. The greatest inter-cluster distance was observed between clusters IV and VI followed by clusters V and VI and clusters II and IV indicating that crossing genotypes from these clusters could generate a broad range of variation in the segregating population and potentially produce hybrids with high heterosis.

Keywords: Upland cotton, genetic diversity, cluster, Tocher's method, D^2 statistics

Cotton is a major natural fibre and is globally preferred for textiles and garments. The word cotton derived from the Arabic word "alqatan" and popularly known as "White Gold" (Malathi and Rajesh, 2019). Cotton is primarily valued for its fibre, which serves as a key raw material for the textile industry in the production of various clothing products. Besides fibre, cotton seeds also contain approximately 18% oil (Abdullah *et al*., 2016). Cotton contributes greatly to employment generation, directly supporting approximately 51 million people and indirectly benefiting around 68 million individuals. It also contributes significantly to the economy, accounting for 14% of industrial output and 11% of export revenues (Kumar *et al*., 2017). Cotton, a member of the genus *Gossypium* within the family *Malvaceae*, consists of 50 species, among these 44 are diploids, 2 are wild tetraploid and only four species are cultivated (Percival and Kohel, 1990). Among the domesticated cotton species, *Gossypium arboreum* L. and *Gossypium herbaceum* L. are diploid (2n=2x=26). They are also known as "Old World" or Desi cotton. In contrast, *Gossypium hirsutum* L. and *Gossypium barbadense* L. are allo-tetraploid (2n=4x=52) and are recognized as "New World" cotton. Among them, *Gossypium barbadense* is distinguished for producing superior-quality fibres. *Gossypium hirsutum* commonly referred to as upland cotton or American cotton, has become the predominant species in the global cotton industry (Ganapathy and Nadarajan, 2008) and it has gained considerable scientific attention from plant breeders owing to its economic significance in terms of yield and adaptability to diverse environments (Sun *et al*., 2019).

Even though India has reached self-reliance in seed cotton production, the cultivated area is steadily decreasing as a consequence of rising input costs. Although India has attained self-sufficiency in seed cotton production, the area under cotton cultivation is gradually declining due to rising input costs. Additionally, the productivity of cotton in India remains significantly lower than the global average. Therefore, there is an urgent need to develop new, high-yielding cotton cultivars with superior fibre quality to enhance the overall production of this valuable crop (Ujjainkar, 2024).

In a breeding programme aimed at developing high-yielding cotton cultivars, genetic information on various yield related traits, including fibre quality parameters, plays a crucial role. Such knowledge assists breeders in improving the genetic makeup of cotton to achieve specific breeding objectives, particularly enhancing overall productivity (Abbas *et al.,* 2008 and Ujjainkar, 2022).

Genetic diversity is crucial for a species adaptability and is fundamental to the effectiveness of hybrid breeding programmes. Greater diversity among parents, within the limits of fitness, increases the likelihood of producing heterotic F_1 hybrids and ensures greater genetic variability in the segregating generations (Arunachalam, 1981; Falconer, 1989). Evaluating diversity in morphological traits is crucial for creating improved cultivars suitable for use in various breeding programmes (Rathinavel *et al*., 2017). A lack of genetic diversity restricts progress in breeding cotton varieties with higher yield, better stress resistance and superior quality (Esmail *et al.,* 2008). Thus, evaluation of genetic diversity and identification of genetically diverse parents constitute the initial step in developing a hybridization program. The 'Mahalanobis D^2 statistic', introduced by Mahalanobis (1936), is a commonly used approach for evaluating genetic diversity among available genotypes. This technique provides reliable estimates of genetic divergence and supports the evaluation of many genotypes at a time for genetic diversity.

The study was conducted during *kharif,* 2024-25 at Regional Agricultural Research Station, Lam Farm, Guntur, Andhra Pradesh. The experimental site is situated at 16°2' North and 80°3' East longitude at a height of 31.5 m above mean sea level. The soils are black cotton type with clay texture. The experimental material used in the present study comprised of 56 genotypes (*G. hirsutum* L.) developed in ANGRAU with different genetic backgrounds. Among the 56 genotypes, NDLH-1938 and NDLH-2035-5 were obtained from RARS, Nandyal while remaining genotypes from RARS, Lam. The experiment was laid in Alpha lattice design with two replications adopting a spacing of 105x60 cm and a plot size of 12.3 m^2. To ensure healthy crop stand, recommended agronomic practices and integrated plant protection measures were diligently implemented all through the evaluation time.

Data was recorded for nineteen characters *viz.,* days to 50% flowering, plant height (cm), number of monopodia $plant^{-1}$, number of sympodia $plant^{-1}$, number of bolls $plant^{-1}$, boll weight (g), seed index (g), lint index (g), seed cotton yield $plant^{-1}$ (g) at 140 days after sowing, seed cotton yield $plant^{-1}$ (g) at 165 days after sowing, total seed cotton yield $plant^{-1}$ (g), ginning outturn (%), upper half mean length (mm), micronaire (µg/inch), tenacity (g/tex), uniformity index (%), relative water content (%), proline content (%) and seed oil content (%). To evaluate the fibre quality attributes, the seed cotton from the studied plants was mixed, ginned and the resulting lint was analysed. Fibre quality analysis was conducted at the Central Institute for Research on Cotton Technology (CIRCOT), Regional Unit, RARS, Lam, Guntur, Andhra Pradesh utilizing the HVT Expert 1201 high volume fibre tester instrument. Additionally, the percentage of oil content in cotton seeds was analysed for each sample using Soxhlet apparatus at IIOR, Hyderabad.

The D^2 statistic of Mahalanobis (1928) was used to assess genetic divergence, following the procedure outlined by Rao (1952). Based on Rao's (1952) method, all the D^2 values were grouped into clusters by Tocher's method. The intra and inter cluster distances were calculated according to formulae given by Singh and Chaudhari (1977).

Grouping of genotypes into various clusters: Using Tocher's method the 56 cotton genotypes were classified into six clusters based on D^2 value such that the genotypes grouped within a common cluster (intra-cluster) exhibited lower D^2 values compared to those distributed among different clusters (inter-cluster). The distribution of 52 cotton genotypes and four checks into six clusters is presented in **Table 1**.

The analysis showed that Cluster II had highest number of genotypes (19) whereas, cluster VI had lowest with only one genotype. Cluster I comprised of 16 genotypes *viz.,* L- 1643, L- 1786, L- 1858, L- 1816, L- 1801, LHDP - 62, LHDP - 65, L- 1702, LHDP - 68, LHDP - 57, LHDP - 60, LHDP - 83, L- 1827, L- 1854, LHDP - 82, L- 1711. The genotypes grouped into cluster II were L- 1675, L- 1829, LHDP - 70, LHDP - 86, L- 1823, L- 1578, L- 1804, L - 1787-1, LHDP - 88, LHDP - 5, L- 1765, LHDP - 1, L- 1742, L- 1815, L- 1828, L- 1851,

Table 1. Tocher's clustering pattern for yield and fibre quality traits in 56 cotton genotypes

Cluster group	Number of genotypes	List of genotypes
Cluster I	16	L- 1643, L- 1786, L- 1858, L- 1816, L- 1801, LHDP - 62, LHDP - 65, L- 1702, LHDP - 68, LHDP - 57, LHDP - 60, LHDP - 83, L- 1827, L- 1854, LHDP - 82, L- 1711
Cluster II	19	L- 1675, L- 1829, LHDP - 70, LHDP - 86, L- 1823, L- 1578, L- 1804, L - 1787-1, LHDP - 88, LHDP' - 5, L- 1765, LHDP - 1, L- 1742, L- 1815, L- 1828, L- 1851, LHDP - 74, NDLH-2035-5, L- 1835
Cluster III	10	NDLH - 1938, LHDP - 69, L- 1756, LHDP - 55, L- 1821, L- 1839, LHDP - 59, LHDP - 64, LHDP - 35, LHDP - 81
Cluster IV	8	L- 1822, L- 1809, L- 1743, L- 1767, L- 1833, LHDP - 71, LHDP - 75, L- 1849
Cluster V	2	L- 1830, LHDP - 80
Cluster VI	1	LHDP - 58

LHDP - 74, NDLH-2035-5, L- 1835. Cluster III comprised of 10 genotypes including NDLH - 1938, LHDP - 69, L- 1756, LHDP - 55, L- 1821, L- 1839, LHDP - 59, LHDP - 64, LHDP - 35, LHDP – 81. Cluster IV included 8 genotypes *viz.,* L- 1822, L- 1809, L- 1743, L- 1767, L- 1833, LHDP - 71, LHDP - 75, L- 1849. Cluster V comprised of L- 1830 and LHDP – 80, while Cluster VI had only one genotype LHDP-58.

In a similar way, Adsare *et al.* (2017) studied twenty genotypes of upland cotton for eight quantitative characters *viz.* days to 50% flowering, plant height (cm), number of bolls plant^{-1}, boll weight (g), seed cotton yield plant^{-1} (g), ginning outturn (%), seed index (g), lint index (g) and grouped into two clusters; Handi *et al.* (2017) grouped three hundred twenty germplasm lines of upland cotton into eighteen clusters based on morphological and yield contributing traits indicating high amount of divergence; Malathi and Rajesh (2019) studied thirty-five genotypes of parental and interspecific hybrids of cotton for 11 yield and yield attributing traits and grouped into four clusters; Pujer *et al.* (2020) evaluated sixty-eight genotypes of upland cotton for days to first flower, plant height (cm), number of monopodia plant^{-1}, number of bolls plant^{-1}, boll weight (g), seed cotton yield plant^{-1} (g), ginning outturn (%), seed index (g), lint index (g) and fibre quality traits and grouped into eight clusters; Fatima *et al.* (2025) evaluated 50 cotton genotypes for 10 morpho-physiological characters and grouped into four clusters; Kaleri *et al*. (2025) studied 8 cotton genotypes for plant height (cm), number of monopodia plant^{-1}, number of sympodia plant^{-1}, boll weight (g), number of bolls plant^{-1}, seed cotton yield plant^{-1} (g), ginning outturn (%), staple length and grouped into four clusters.

Average intra and inter-cluster d^2 values: According to the procedure proposed by Singh and Chaudhary (1977), the estimated mean intra and inter-cluster D² values are summarized in **Table 2**. The extent of genetic closeness and divergence among the six clusters is summarized in **Table 3**. Data revealed that the inter cluster distance-D^2 value ranged from 2162.20 to 16197.63. The highest inter-cluster distance (D = 16197.63) was recorded between cluster IV and VI, succeeded by between V and VI (10497.63); II and IV (8979.06); III and VI (8246.54); III and V (8084.23). The minimum inter-cluster distance was observed between cluster II and VI (2162.20), indicating a high degree of similarity between the genotypes within these clusters, Therefore, hybridization between genotypes from these clusters will not produce desirable segregants. The intra-cluster distance ranged from 298.30 (cluster-V) to 1336.85 (cluster-III). The cluster VI contained single genotype and therefore, their intra-cluster distance was zero. The average intra and inter cluster distance were measured for each cluster and also each pair of clusters in all possible combinations respectively, in terms of D^2 values (**Table 2 and Fig.1**).

In the present study, greater inter cluster distances compared to intra cluster distances revealed the presence of considerable genetic variability among the genotypes, implying that crosses involving genetically distant genotypes may produce rare and superior segregants.

Cluster mean for different characters: The cluster mean values reflect the average performance of all genotypes within a specific cluster. These estimates are useful for identifying potential donor genotypes that could be used to enhance particular traits. **Table 4** summarizes the cluster mean values for yield and fibre quality traits evaluated in 52 cotton genotypes along with four checks.

In the current study, the cluster means showed considerable variation across different traits. For days to 50% flowering, values ranged from (65.50) days in Cluster VI to (70.00) days in Cluster V reporting that earliness in cluster VI and late duration in cluster V. Plant height varied from (106.76 cm) in Cluster IV to (135.20 cm) in Cluster VI. The number of monopodia plant^{-1} ranged from (0.61) in Cluster VI to (3.44) in Cluster V, while number of sympodia plant^{-1} ranged from (13.93) in Cluster IV to (15) in Cluster VI. Cluster V had the lowest number of bolls plant^{-1} (29.05), whereas Cluster VI had the highest (35.10). Boll weight ranged from (3.54 g) in Cluster III to

(4.07 g) in Cluster V. Seed index ranged from (7.60 g) in Cluster III to (7.95 g) in Cluster I. Lint index ranged from (3.39 g) in Cluster VI to (5.30 g) in Cluster V. Ginning outturn was lowest in Cluster VI (30.39 %) and highest in Cluster V (39.4 %).

Table 2. Intra and inter-cluster D^2 values among the six clusters

	Cluster distances					
	Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Cluster VI**
Cluster I	**1060.46**	3657.00	3242.90	2379.44	2186.62	8028.66
Cluster II		**1246.67**	4265.73	8979.06	6105.91	2162.20
Cluster III			**1336.85**	5024.46	8084.23	8246.54
Cluster IV				**602.97**	3368.29	16197.63
Cluster V					**298.30**	10497.63
Cluster VI						**0.00**

Table 3. The nearest and the farthest cluster from each cluster based on D^2 values

Cluster Number	Nearest cluster with D^2 values	Farthest cluster with D^2 values
I	V (2186.62)	VI (8028.66)
II	VI (2162.20)	IV (8979.06)
III	I (3242.90)	VI (8246.54)
IV	I (2379.44)	VI (16197.63)
V	I (2186.62)	VI (10497.63)
VI	II (2162.20)	IV (16197.63)

Note: Values in parentheses indicate D^2 values

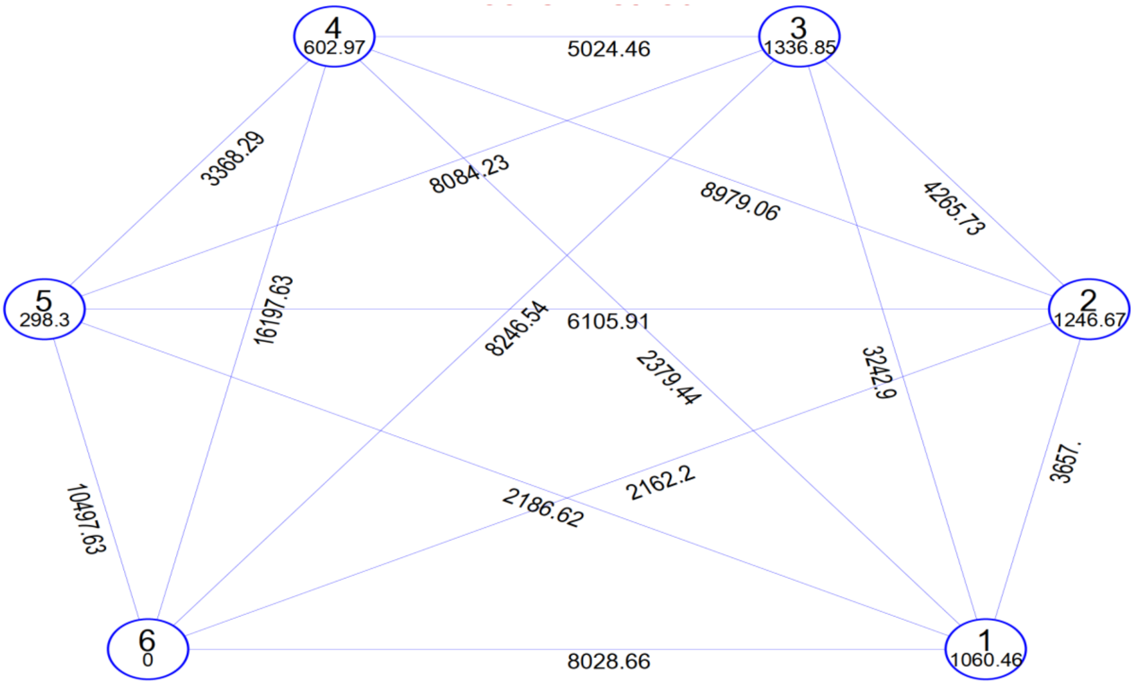

Fig.1. Intra and inter-cluster distances among six clusters formed from 56 cotton genotypes

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Seed cotton yield plant^{-1} at 140 days after sowing ranged from (51.52 g) in Cluster I to (57.86 g) in Cluster IV, while Seed cotton yield plant^{-1} at 165 days after sowing ranged from (31.19 g) in Cluster V to (41.01 g) in Cluster II. Total seed cotton yield plant^{-1} ranged from (83.15 g) in Cluster V to (96.22 g) in Cluster III. For fibre quality traits, upper half mean length ranged from (26.40 mm) in Cluster VI to (27.40 mm) in Cluster V; micronaire ranged from (3.40 µg/inch) in Cluster V to (4.14 µg/inch) in Cluster II; tenacity ranged from (27.01 g/tex) in Cluster III to (27.89 g/tex) in Cluster IV; uniformity index ranged from (82.50 %) in Cluster V to (81.70 %) in Cluster III. Relative water content ranged from (64.56 %) in Cluster VI to (72.39 %) in Cluster V. Proline content ranged from (1.59 µ mol/g) in Cluster VI to (8.52 µ mol/g) in Cluster III. Seed oil content ranged from (14.89 %) in Cluster I to (16.29 %) in Cluster V.

Among the six clusters, Cluster I exhibited the highest mean value for seed index. Cluster II recorded the highest mean for seed cotton yield plant^{-1} at 165 days after sowing. Cluster III showed the highest mean values for total seed cotton yield plant^{-1} and proline content. Cluster IV was characterized by the highest mean values for seed cotton yield plant^{-1} at 140 days after sowing and tenacity. Cluster V recorded superior mean values for days to 50% flowering, number of monopodia plant^{-1}, boll weight, lint index, ginning out turn, upper half mean length, uniformity index, relative water content and seed oil content. Meanwhile, Cluster VI exhibited the highest means for plant height, number of sympodia plant^{-1}, number of bolls plant^{-1} and micronaire value.

In cluster III, genotypes LHDP-35 had highest no. of sympodia plant^{-1}, boll weight, seed cotton yield plant^{-1} whereas, LHDP-59, LHDP-81, NDLH-1938 had highest upper half mean length, proline content and no. of bolls plant^{-1}. Cluster I had genotypes L-1858, LHDP-83, LHDP-68, L-1786, L-1801 with high mean values for lint index, seed index and for fibre quality traits. The selection of genotypes from these clusters is suggested for use in future crop improvement programmes aimed at developing high yielding cotton genotypes with superior fibre quality traits.

Among all, cluster V and VI recorded highest desired mean values for GOT and fibre quality traits whereas cluster III had genotypes with earliness, more number of bolls plant^{-1}, total seed cotton yield plant^{-1} and proline content suggesting that hybridization between the genotypes of cluster V and III could yield good segregants for seed cotton yield and fibre quality traits.

*Relative contribution of individual characters towards divergence:*A perusal of the results on per cent contribution of 56 cotton genotypes for 19 characters towards genetic divergence are presented in **Table 5.** The results revealed maximum contribution towards genetic

Table 4. Cluster mean values for seed cotton yield, yield attributing traits and fibre quality traits in 56 cotton genotypes

S. No.	Characters	Clusters					
		I	II	III	IV	V	VI
1	Days to 50% flowering	68.25	69.92	66.55	68.88	70.00	65.50
2	Plant height	108.86	108.33	117.03	106.76	110.70	135.20
3	Number of monopodia plant^{-1}	2.45	1.25	1.28	3.21	3.44	0.61
4	Number of sympodia plant^{-1}	14.01	14.76	14.78	13.93	14.30	15.00
5	Number of bolls plant^{-1}	30.69	31.01	33.08	30.73	29.05	35.10
6	Boll weight	3.57	3.74	3.54	3.72	4.07	3.77
7	Seed index	7.95	7.69	7.60	7.64	7.74	7.69
8	Lint index	4.83	4.66	4.10	4.41	5.30	3.39
9	Ginning out turn	37.72	37.07	34.56	36.34	39.54	30.39
10	Seed cotton yield plant^{-1} at 140 days after sowing	51.52	56.62	57.78	57.86	51.96	57.06
11	Seed cotton yield plant^{-1} at 165 days after sowing	35.41	41.01	36.73	33.17	31.19	34.26
12	Total seed cotton yield plant^{-1}	86.93	96.11	96.22	91.04	83.15	91.32
13	Upper half mean length	27.28	27.36	26.64	27.25	27.40	26.40
14	Micronaire	4.13	4.14	4.02	4.05	3.40	4.40
15	Tenacity	27.81	27.71	27.01	27.89	27.55	27.50
16	Uniformity index	82.25	82.42	81.70	82.25	82.50	82.00
17	Relative water content	65.19	66.44	65.58	66.55	72.39	64.56
18	Proline content	4.97	3.36	8.52	7.13	1.94	1.59
19	Seed oil content	14.89	15.35	15.33	15.24	16.29	15.89

Table 5. Percent contribution of different characters towards genetic divergence in 56 cotton genotypes

S. No.	Traits	Contribution %
1	Total seed cotton yield plant^{-1}	15.00
2	Seed cotton yield plant^{-1} at 140 days after sowing	9.12
3	Proline content	8.75
4	Ginning out turn	8.12
5	Seed index	7.21
6	Micronaire	6.32
7	Seed oil content	6.13
8	Boll weight	5.50
9	Number of bolls plant^{-1}	5.16
10	Uniformity index	4.98
11	Relative water content	4.50
12	Number of sympodia plant^{-1}	4.21
13	Number of monopodia plant^{-1}	3.21
14	Seed cotton yield plant^{-1} at 165 days after sowing	3.20
15	Days to 50% flowering	2.34
16	Lint index	2.19
17	Tenacity	2.12
18	Plant height	1.62
19	Upper Half Mean Length	0.32

divergence by total seed cotton yield plant^{-1} (15.00 %), followed by seed cotton yield plant^{-1} at 140 days after sowing (9.12 %), proline content (8.75 %), ginning out turn (8.12 %), seed index (7.2 %), micronaire (6.32 %), seed oil content (6.13 %), boll weight (5.50 %), number of bolls plant^{-1} (5.16 %), uniformity index (4.98 %), relative water content (4.50 %), number of sympodia plant^{-1} (4.21 %), number of monopodia plant^{-1} (3.21 %), seed cotton yield plant^{-1} at 165 days after sowing (3.20 %), days to 50% flowering (2.34 %), lint index (2.19 %), tenacity (2.12 %) and plant height (1.62 %). The lowest contribution was observed by upper half mean length (0.32 %) indicating more homogeneity for the trait in the material evaluated.

In a similar study, Sirisha *et al.* (2022) reported that maximum contribution towards genetic divergence was made by uniformity ratio, lint index, boll weight, no. of monopodia plant^{-1}, micronaire, bundle strength, 2.5 % span length, bolls plant^{-1}, days to 50 % flowering, fibre elongation, seed index and plant height.

Multivariate analysis revealed substantial genetic diversity among the 56 genotypes evaluated. Using Tocher's method in D^2 analysis, these genotypes were classified into six distinct clusters. The cluster II had highest number of genotypes (19). Cluster III showed the highest intra-cluster distance, indicates variable genotypes were grouped in this cluster. The genotype, LHDP – 35, noted its superiority for total seed cotton yield plant^{-1} (170.20g), number of sympodia plant^{-1} (18.70) and boll weight (5.03g). The genotype, L-1804 was superior for uniformity index (85.00%) and earlier in days to 50% flowering (61.50) whereas, genotype, L-1830, showed the highest seed oil content (18.35%). These genotypes can be utilized to enhance seed cotton yield and for the development of heterotic hybrids in future breeding programmes. Based on the *per se* performance and genetic distance, the genotypes *viz.,* LHDP-35 and NDLH-1938 (from Cluster III), L-1833, L-1743, and L-1849 (from Cluster IV), L-1830 (from Cluster V) and LHDP-58 (from Cluster VI) were identified as promising. Hybridization among these genotypes from genetically diverse clusters could generate a broad spectrum of variation in the segregating population and potentially produce hybrids with high heterosis.

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