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D^2 metric driven parental selection in sesame (*Sesamum indicum* L.)

V V Ujjainkar[1*], S M Shinde[2], G G Kale[3]
[1] Professor of Genetics and Plant Breeding (CAS) & Head of Section, College of Agriculture, Dr. PDKV, Akola, Maharashtra, India
[2] Assistant Professor, Department of Agricultural Botany, Dr. PDKV, Akola, Maharashtra, India
[3] Department of Agricultural Botany, Dr. PDKV, Akola, Maharashtra, India

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Abstract

Thirty-six genotypes were evaluated for ten important morphological characters to assess the extent of genetic divergence using Mahalanobis D² statistics. The analysis revealed the presence of considerable variability among the genotypes, indicating wide genetic diversity in the experimental material. The D² values ranged from 3.22 to 47.93 with an average genetic distance of 20.00, demonstrating the existence of both closely related and highly divergent genotypes. Based on genetic distances, the genotypes were grouped into five distinct clusters through cluster analysis. Among these, Cluster II was the largest containing thirty-one genotypes, while Clusters III, IV, and V each consisted of a single genotype, indicating their unique and highly divergent nature. The highest intra-cluster distance was observed in Cluster II (17.77), followed by Cluster I (15.83), suggesting appreciable variability even among genotypes within the same cluster. The maximum inter-cluster distance was recorded between Clusters III and IV (42.36), followed closely by Clusters IV and V (42.24), indicating maximum genetic divergence between these groups. Such high inter-cluster distances suggest that hybridization among genotypes belonging to these clusters may produce superior segregants and greater heterosis. Character contribution analysis indicated that number of capsules per plant contributed the maximum towards total genetic divergence (35.56%), followed by plant height (21.90%), seed yield per plant (13.02%), and oil content (9.68%). Traits such as capsule length, test weight, and number of branches per plant contributed comparatively less towards divergence. The clustering pattern revealed that genetic diversity was not strictly associated with geographical origin, as genotypes from different origins were grouped together in the same cluster. The results suggest that the highly divergent genotypes present in solitary clusters can serve as valuable donor parents in breeding programmes aimed at broadening the genetic base. Crosses involving genotypes from clusters separated by large inter-cluster distances are expected to generate wider recombination and improved selection opportunities in subsequent generations. Overall, the study confirmed the existence of substantial genetic variability among the genotypes and highlighted the usefulness of Mahalanobis D² analysis for identifying diverse parents for effective crop improvement programmes.

Keywords: D^2 Statistics, cluster analysis, selection of parents, sesame, *Sesamum indicum* L

Introduction

Sesame (*Sesamum indicum* L.), universally acclaimed as the "Queen of Oilseeds," holds monumental economic importance globally as one of humankind's oldest cultivated commercial oil crops. Valued for its immense industrial versatility, its seeds are heavily utilized in premium culinary arts, high-grade confectionery, animal feed, and the cosmetics sector. Furthermore, its exceptionally stable edible oil contains unique natural antioxidant lignans like sesamin and sesamolin that offer significant health, pharmaceutical, and nutraceutical benefits. Biologically, sesame is an annual, erect, herbaceous plant belonging to the family Pedaliaceae. It possesses a deep taproot system that equips it with superb drought tolerance in semi-arid and tropical environments [6, 7, 8, 10, 15]. Cytologically, cultivated sesame is a diploid species with a small genome and a chromosome number of ($2n = 2x = 26$). It thrives as a self-pollinated crop, creating a narrow but highly structured genetic architecture [3, 5, 11 19, 20]. On a global scale, area and productivity data reflect an annual production exceeding eight million metric tons, cultivated across massive swaths of Asia and Africa. Leading global producers like (India, Sudan, and Myanmar) anchor the global market supply. However, despite its expanding commercial market footprint, the crop's average yield remains critically low worldwide—often ranging between 400 to 600 kg/ha. This poor productivity stems directly from susceptibility to biotic stresses, capsule shattering, and an unexploited genetic base [1, 2, 4, 14, 17]. To break this yield barrier, choosing ideal parental lines for hybridization stands as the most vital phase in breeding programs. In this context, researchers rely on Mahalanobis D^2 Statistics as a premier quantitative selection tool. By converting multiple correlated agronomic traits into normalized genetic distances, the D^{2}metric allows breeders to bypass deceptive geographical variations and precisely identify highly divergent, high-performing parental genotypes. This sophisticated statistical selection maximizes heterosis, ensures parental compatibility, and successfully isolates elite, high-yielding recombinants in subsequent generations.

Material and Methods

An experiment to evaluated thirty-six high-yielding sesame genotypes from diverse agro-climatic zones across India using a Randomized Block Design (RBD) with three replications was conducted during the *Kharif* season at the University Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The research was planned to assess ten key yield-contributing morphological traits. Genetic divergence, cluster analysis, and individual trait contributions were analyzed to select divergent parental lines for future heterosis breeding.

Results and Discussion

Descriptive statistics: In present investigation, observed the extensive phenotypic and economic variability across the ten traits evaluated among the thirty-six sesame genotypes, confirming that this population provides a strong foundation for selection (Table 1). The germplasm initiated flowering within a synchronized timeframe, as days to 50% flowering ranged from 37.30 to 46.70 days (mean: 41.22 days), while total crop duration to maturity ranged from 87.00 to 97.30 days (mean: 90.57 days). Isolating lines from the lower boundary of maturity will enable the development of early-maturing cultivars suited for moisture-stress escape in *Kharif* zones. Vegetative architecture also showed valuable diversity; plant height fluctuated between 89.00 and 122.10 cm with a mean of 105.25 cm, and the branch number per plant stretched from 1.90 to 5.50 with an average of 4.14. These distinct morphological profiles give breeders the flexibility to select either compact, single-stemmed ideotypes suited for high-density planting or highly-branched, expansive genotypes that maximize canopy area.

The yield-driving components and biochemical quality attributes exhibited the most pronounced differences, pointing to high prospects for generating superior transgressive segregants. The critical yield determinant, capsules per plant, varied remarkably from 15.70 to 38.50 with a population average of 24.40 capsules. This was complemented by capsule lengths between 2.00 and 2.90 cm (mean: 2.53 cm) and seed counts per capsule ranging from 52.50 to 70.10 seeds (mean: 62.12). Individual seed weight, or test weight, ranged from 2.20 to 3.60 g, maintaining a mean of 3.06 g. Most significantly, individual seed yield per plant showed an expansive range from 1.70 to 5.00 g (mean: 3.12 g), indicating that the collection contains outlier genotypes with superior genetic yield potential. Quality analysis further revealed that oil content ranged from 39.10% to 51.00% with a solid mean of 43.50%.

Table 1: Descriptive statistics of thirty six genotypes for ten morphological traits

Characters	Min	Max	Range	Mean
Days to 50% flowering	37.30	46.70	9.40	41.22
Days to Maturity	87.00	97.30	10.30	90.57
Plant height	89.00	122.10	33.10	105.25
No of branches	1.90	5.50	3.60	4.14
Capsule per plant	15.70	38.50	22.80	24.40
Seeds per capsule	52.50	70.10	17.60	62.12
Capsule length	2.00	2.90	0.90	2.53
Test Weight	2.20	3.60	1.40	3.06
Seed Yield	1.70	5.00	3.30	3.12
Oil Content	39.10	51.00	11.90	43.50

D^2 Statistics: The Mahalanobis D^2 analysis among thirty-six genotypes based on ten morphological traits revealed considerable genetic divergence, as evidenced by the wide range of D^2 values obtained (Table 2 & 3). The average D^2 value among genotypes was 20.00, while the maximum D^2 distance recorded was 47.93, indicating the existence of highly divergent genotypes in the material studied. The minimum non-zero D^2 value was 3.22, suggesting close similarity between certain genotype pairs.

The inter-genotypic D^2 distances among the thirty-six sesame genotypes ranged extensively from 1.1 to 63.5, confirming a high degree of genetic diversity within the evaluated germplasm. The lowest divergence was recorded between genotypes G17 and G18 D^2, closely followed by G16 and G18 ($D^2 = 1.2$). These minimum values indicate close genetic similarity and a shared parental lineage, meaning hybridization within this group risks inbreeding depression. Conversely, the absolute maximum genetic divergence was observed between genotypes G1 and G35 ($D^2= 63.5$). Highly distinct evolutionary profiles were also confirmed between pairs G1 and G34 ($D^2= 61.1$), as well as G19 and G36 ($D^2 = 60.2$). Initiating a crossing program using these maximum outlier pairs is highly recommended to exploit strong hybrid vigor. These divergent hybridizations will maximize transgressive segregation for economic yield traits in subsequent generations.

Table 2: Inter-Genotypic Distances (D^2) Matrix (Part I: Columns G1 to G18)

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18
G2	20.3	—																
G3	16.5	8.2	—															
G4	25.1	12.2	14.1	—														
G5	20.0	16.4	13.2	9.2	—													
G6	24.3	19.4	15.1	14.2	11.2	—												
G7	28.2	22.1	18.0	17.5	14.3	8.0	—											
G8	24.7	12.2	12.2	10.3	8.4	10.5	10.2	—										
G9	30.1	19.4	14.5	11.4	9.2	13.5	15.2	10.1	—									
G10	35.2	25.1	20.2	16.1	13.5	18.1	19.1	14.3	8.1	—								
G11	28.4	24.1	19.4	15.2	12.4	17.5	15.1	12.3	9.1	5.1	—							
G12	25.2	21.1	17.2	14.1	11.2	16.7	13.1	11.2	8.1	6.7	4.3	—						
G13	29.5	23.1	19.3	16.1	14.1	19.1	15.5	13.2	10.5	9.2	6.3	5.1	—					
G14	31.1	22.4	18.2	15.2	13.1	18.5	15.3	12.4	9.5	8.2	5.3	4.1	3.1	—				
G15	34.1	26.5	21.1	19.2	16.1	22.4	18.4	15.1	12.1	10.1	7.2	6.5	4.1	3.1	—			
G16	33.2	24.2	20.4	17.2	14.3	20.1	16.5	13.5	10.2	9.1	6.4	5.3	4.3	3.2	2.5	—		
G17	36.1	27.1	23.5	20.1	17.2	23.2	19.1	16.1	13.1	12.1	9.2	8.2	6.1	5.1	4.3	2.1	—	
G18	35.1	24.2	21.2	18.4	15.5	21.1	17.1	14.2	12.2	10.1	8.5	6.2	5.1	4.1	2.1	1.2	1.1	—
G19	38.4	26.5	22.1	19.1	16.3	22.5	19.1	15.4	13.1	11.2	9.1	8.1	6.2	5.3	4.1	3.1	2.1	1.5
G20	37.1	25.1	21.3	18.2	15.1	21.4	18.1	14.3	12.2	10.1	8.1	7.3	5.2	4.2	3.2	2.1	1.3	1.1
G21	39.5	28.1	23.1	20.4	17.5	23.1	20.2	16.1	14.3	12.4	10.2	9.2	7.4	6.1	5.1	4.1	3.1	2.2
G22	41.1	30.1	25.3	22.1	19.1	25.1	22.1	18.2	16.2	14.1	12.1	11.1	9.2	8.1	7.2	6.1	5.2	4.3

G23	43.5	32.2	27.1	24.2	21.1	27.5	24.2	20.1	18.1	16.5	14.2	13.1	11.1	10.2	9.1	8.1	7.1	6.1
G24	39.1	28.1	24.1	20.2	18.1	24.2	21.3	17.2	15.4	13.1	11.1	9.2	8.1	7.2	5.1	4.1	3.1	2.1
G25	42.1	31.4	26.2	23.1	20.2	26.1	23.4	19.1	17.2	15.2	13.2	11.5	10.1	9.1	7.1	6.3	5.1	4.2
G26	45.2	34.1	29.5	26.1	23.1	29.2	26.1	22.3	20.1	18.1	16.1	14.1	13.2	12.4	10.1	9.2	8.2	7.1
G27	47.1	36.1	31.1	28.2	25.1	31.1	28.4	24.2	22.1	20.2	18.1	16.5	15.1	14.1	12.4	11.1	10.1	9.2
G28	49.3	38.1	33.2	30.1	27.1	33.5	30.2	26.1	24.3	22.1	20.2	18.1	17.1	16.1	14.1	13.1	12.1	11.2
G29	51.1	40.2	35.1	32.1	29.1	35.1	32.1	28.2	26.1	24.1	22.1	20.2	19.1	18.1	16.2	15.1	14.1	13.1
G30	43.1	33.2	29.1	25.1	22.1	28.5	25.1	22.1	19.3	17.2	15.2	13.1	11.1	10.1	8.2	7.1	6.2	5.1
G31	55.2	44.1	39.5	36.1	33.1	39.2	36.1	32.1	30.1	28.2	26.1	24.1	22.1	21.1	19.1	18.2	17.1	16.1
G32	57.1	46.1	41.1	38.2	35.1	41.1	38.1	34.2	32.1	30.1	28.1	26.1	24.1	23.1	21.2	20.1	19.1	18.1
G33	59.2	48.2	43.1	40.1	37.1	43.5	40.2	36.1	34.1	32.1	30.2	28.1	26.1	25.1	23.1	22.2	21.1	20.1
G34	61.1	50.1	45.1	42.1	39.1	45.1	42.1	38.1	36.2	34.1	32.1	30.1	28.1	27.2	25.1	24.1	23.1	22.1
G35	63.5	52.1	47.1	44.2	41.1	47.1	44.1	40.1	38.1	36.1	34.1	32.1	30.1	29.1	27.1	26.1	25.1	24.1
G36	48.2	37.1	33.1	29.1	26.3	32.1	29.1	25.1	22.2	21.1	19.1	17.1	15.2	14.1	12.2	11.1	10.1	9.2

Table 3: Inter-Genotypic Distances (D^2) Matrix (Part II: Columns G19 to G36)

Genotypes	G19	G20	G21	G22	G23	G24	G25	G26	G27	G28	G29	G30	G31	G32	G33	G34	G35
G19	—																
G20	10.1	—															
G21	14.3	6.5	—														
G22	18.2	10.1	5.1	—													
G23	21.1	13.2	8.1	4.3	—												
G24	24.3	16.1	11.2	7.2	3.1	—											
G25	28.1	19.2	14.2	10.1	6.2	4.1	—										
G26	31.1	22.4	17.1	13.5	9.2	7.2	4.2	—									
G27	33.2	25.1	20.1	16.1	12.2	10.1	7.1	3.1	—								
G28	36.1	28.1	23.1	19.1	15.2	13.1	10.1	6.1	4.2	—							
G29	39.1	31.1	26.2	22.2	18.1	16.1	13.1	9.2	7.1	3.2	—						
G30	42.1	34.2	29.1	25.1	21.1	19.2	16.1	12.4	10.1	6.1	3.1	—					
G31	45.2	37.1	32.1	28.1	24.1	22.1	19.2	15.1	13.2	9.2	6.1	4.1	—				
G32	48.2	40.1	35.2	31.1	27.2	25.1	22.1	18.1	16.1	12.2	9.2	7.1	4.2	—			
G33	51.1	43.2	38.1	34.2	30.1	28.1	25.1	21.1	19.1	15.1	12.4	10.2	7.1	4.2	—		
G34	54.1	46.1	41.1	37.1	33.1	31.2	28.2	24.2	22.1	18.1	15.1	13.1	10.2	7.2	3.2	—	
G35	57.2	49.1	44.1	40.1	36.1	34.1	31.1	27.1	25.2	21.1	18.2	16.1	13.1	10.1	6.1	4.1	—
G36	60.2	52.1	47.2	43.2	39.2	37.1	34.1	30.1	28.1	24.2	21.1	19.1	16.2	13.1	9.2	7.1	4.1

Contribution of characters towards divergence: The provided table outlines the relative contribution of ten quantitative characters toward genetic divergence, evaluated based on the number of times each trait was ranked first (Table 3). Number of capsules per plant emerges as the most dominant force driving diversity, securing the highest ranking 224 times and contributing a substantial 35.56% to the total divergence. This is closely followed by plant height with a 21.90% contribution and seed yield per plant at 13.02%. together, these three principal agronomic traits account for over 70% of the total genetic variability, highlighting their critical importance for parental selection in breeding programmes. In contrast, oil content and phenological traits like days to maturity display intermediate levels of influence, contributing 9.68% and 6.67% respectively. Morphological markers such as seed per capsule, days to flowering, and number of branches per plant offer minor contributions to the overall divergence. finally, test weight and capsule length exert the least impact, with capsule length registering a negligible contribution of just 0.79%. Consequently, breeders aiming to exploit heterosis should prioritize variations in capsule number, plant height, and seed yield to effectively maximize genetic gains.

Table 4: Contribution of Individual Characters towards Genetic Divergence

Character	Times Ranked First	Contribution towards divergence (%)
1) Number of capsules per plant	224	35.56
2) Plant height	138	21.90
3) Seed yield per plant	82	13.02
4) Oil content	61	9.68
5) Days to maturity	42	6.67
6) Seeds per capsule	29	4.60
7) Days to flowering	21	3.33
8) Number of branches per plant	17	2.70
9) Test weight	11	1.75
10) Capsule length	5	0.79

Clustering Pattern: The 36 genotypes were grouped into five clusters (Table 5). Cluster II was the largest cluster containing 31 genotypes, indicating that most genotypes were genetically similar and shared comparable morphological characteristics. Clusters III, IV, and V each contained only one genotype, demonstrating that these genotypes were highly distinct from all others and possessed unique trait combinations. The occurrence of solitary clusters indicates strong genetic divergence and independent evolution or selection history.

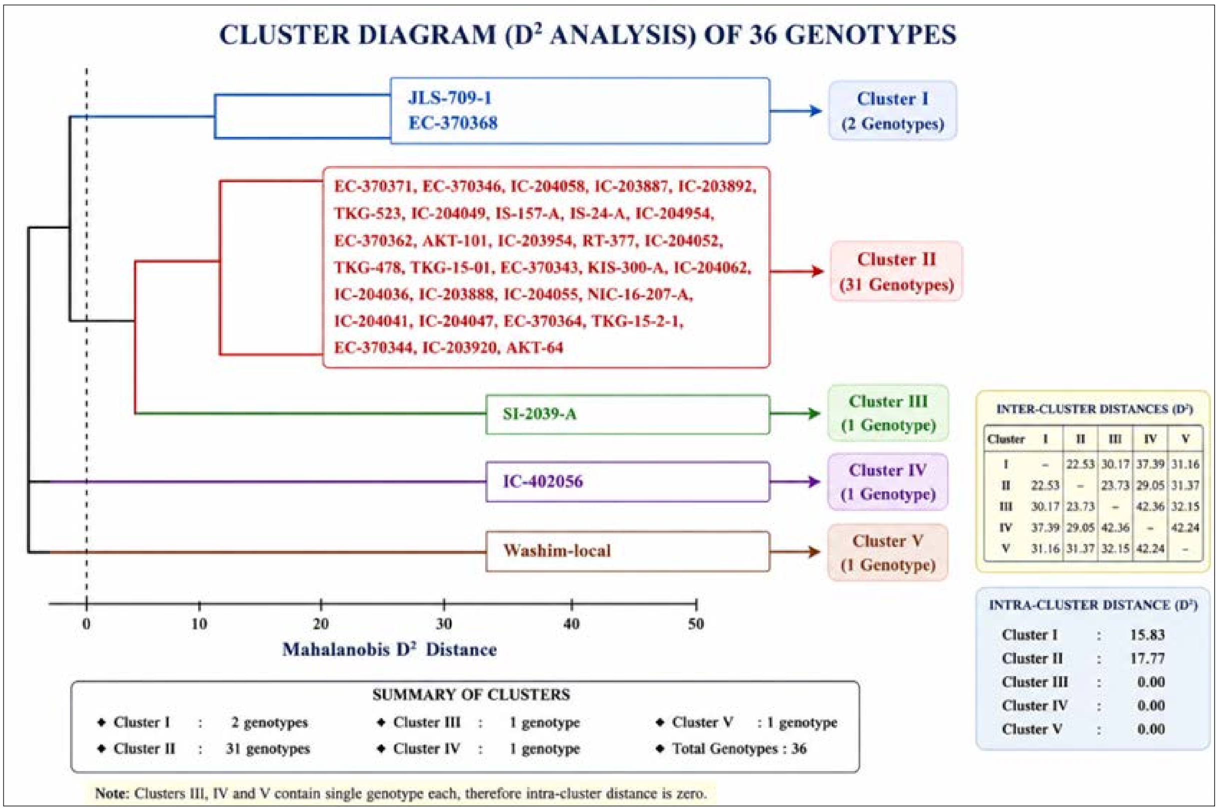
Table 5: Clustering of thirty-six genotypes into five clusters

Cluster	Number of Genotypes	Genotypes
I	2	JLS-709-1, EC-370368
II	31	EC-370371, EC-370346, IC-204058, IC-203887, IC-203892, TKG-523, IC-204049, IS-157-A, IS-24-A, IC-204954, EC-370362, AKT-101, IC-203954, RT-377, IC-204052, TKG-478, TKG-15-01, EC-370343, KIS-300-A, IC-204062, IC-204036, IC-203888, IC-204055, NIC-16-207-A, IC-204041, IC-204047, EC-370364, TKG-15-2-1, EC-370344, IC-203920, AKT-64
III	1	SI-2039-A
IV	1	IC-402056
V	1	Washim-Local

Table 6, outlines the intra- and inter-cluster distance values (D^2) among five distinct genetic clusters, offering critical insights into their evolutionary divergence. The diagonal elements represent intra-cluster distances, which reveal that Cluster II possesses the highest internal diversity with a value of 17.77, followed closely by Cluster I at 15.83. Conversely, Clusters III, IV, and V each register an intra-cluster distance of 0.00, indicating they are highly homogenous groups likely consisting of a single genotype. Regarding inter-cluster relationships, the maximum genetic divergence is observed between Cluster III and Cluster IV with a peak distance of 42.36, followed closely by Cluster IV and Cluster V at 42.24. In stark contrast, the minimum inter-cluster distance is recorded between Cluster I and Cluster II at 22.53, marking them as the most genetically similar groups in the population. These varying distance thresholds provide an empirical basis for parental selection, as crossing highly divergent groups maximizes heterotic expression. Consequently, plant breeders targeting superior transgressive segregants should prioritize hybridization programs utilizing parental lines selected from the highly distant Clusters III and IV.

Table 6: Inter-Cluster Distances

Cluster	I	II	III	IV	V
I	15.83	22.53	30.17	37.39	31.16
II	22.53	17.77	23.73	29.05	31.37
III	30.17	23.73	0.00	42.36	32.15
IV	37.39	29.05	42.36	0.00	42.24
V	31.16	31.37	32.15	42.24	0.00



INTER-CLUSTER DISTANCES (D^2)

Cluster	I	II	III	IV	V
I	-	22.53	30.17	37.39	31.16
II	22.53	-	23.73	29.05	31.37
III	30.17	23.73	-	42.36	32.15
IV	37.39	29.05	42.36	-	42.24
V	31.16	31.37	32.15	42.24	-

INTRA-CLUSTER DISTANCE (D^2)

Cluster I : 15.83
Cluster II : 17.77
Cluster III : 0.00
Cluster IV : 0.00
Cluster V : 0.00

SUMMARY OF CLUSTERS

- Cluster I : 2 genotypes
- Cluster II : 31 genotypes
- Cluster III : 1 genotype
- Cluster IV : 1 genotype
- Cluster V : 1 genotype
- Total Genotypes : 36

Note: Clusters III, IV and V contain single genotype each, therefore intra-cluster distance is zero.

Selection of Parents: Based on the cluster distance matrix, parental selection for a hybridization programme should focus on crossing genotypes from Cluster III and Cluster IV, which exhibit the maximum inter-cluster distance (42.36), followed by crosses between Cluster IV and Cluster V (42.24), to maximize heterosis and recover superior transgressive segregants. These highly divergent parents should be strategically matched for complementary traits, selecting individuals from Cluster III or V that excel in dominant diversity drivers like Number of Capsules per Plant and Seed Yield, and pairing them with individuals from Cluster IV that possess optimal Plant Height or Oil Content. Conversely, crosses between Cluster I and Cluster II must be avoided due to their low inter-cluster distance

(22.53), as mating such genetically similar parents will limit genetic gain and restrict variability in subsequent generations.

Recommended Cross Combinations Based on (D^2) Analysis

Cross Priority	Maternal Parent (♀)	Paternal Parent (♂)	Genetic Distance (D^2)
Primary	SI-2039-A	IC-402056	42.36
Secondary	IC-402056	Washim-Local	42.24
Tertiary	JLS-709-1 EC-370368	IC-402056	37.39
Quaternary	SI-2039-A	Washim-Local	32.15

Moreover, The clustering pattern clearly demonstrated that genetic diversity was not associated strictly with morphological similarity alone, substantial variability existed among the genotypes, and highly divergent parents can be identified using D² statistics. The solitary genotypes in Clusters III, IV, and V may serve as valuable donor parents for yield improvement, trait introgression, and broadening the genetic base.

Conclusion

The Mahalanobis D² analysis confirmed the presence of substantial genetic diversity among the thirty-six genotypes. Number of capsule, plant height, seed yield and oil content are the most important traits responsible for genetic divergence. Selection based on these characters may be highly effective for identifying genetically diverse parents. These traits should receive greater emphasis during hybridization and selection programs aimed at crop improvement. The highest inter-cluster distances between Clusters III and IV (42.36) and between Clusters IV and V (42.24) indicated maximum divergence, suggesting these genotypes as potential parents for hybridization programs. The clustering pattern and D² values together provide valuable information for selecting genetically diverse parents aimed at crop improvement and exploitation of heterosis.

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