International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(8): 1643-1647
www.biochemjournal.com
Received: 18-05-2025
Accepted: 21-06-2025

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Study on genetic diversity in sunflower (*Helianthus annuus* L) 32 genotypes

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i8Sw.5426

Abstract
This study assessed thirty-two sunflower (*Helianthus annuus* L.) genotypes, including two control varieties, to analyse genetic diversity. The research was conducted during the *Kharif* season (20242025) at the College of Agriculture's Research Farm in Pune. Observations were made on ten quantitative traits, and the Tocher clustering method categorized the genotypes into ten distinct groups. Among these, Cluster I and III were the largest, containing 10 and 8 genotypes, respectively, followed by Cluster II (5 genotypes) and Cluster IV (3 genotypes). The remaining clusters (V to X) each consisted of a single genotype, resulting in zero intra-cluster distance. By evaluating cluster mean values, the most genetically diverse genotypes can be identified for future hybridization programs.

Keywords: Sunflower, genotypes, diversity, cluster analysis

Introduction
Sunflower (*Helianthus annuus* L.) has gained prominence in India's oilseed sector due to its high-quality, low-cholesterol oil. Recognized as a "versatile all-season crop", it offers numerous benefits, including adaptability to varying light and temperature conditions, short growth cycle, high productivity, and nutritionally superior oil. Classified under the Asteraceae (Compositeae) family, sunflower is a diploid (2n = 34), protandrous species, where pollen and stigma maturation occur at different times, making it predominantly crosspollinated.

Given its significant role in reducing the edible oil deficit, selecting genetically diverse parental lines is crucial for successful hybridization and the development of high-yielding genotypes. According to the latest USDA (2025) data, global sunflower cultivation spanned 28.23 million hectares in 2024-2025, producing 52.45 million metric tons with an average yield of 1.85 metric tons per hectare. In contrast, India's cultivation area was 0.18 million hectares, yielding 0.11 million metric tons at 0.63 metric tons per hectare.

To assess genetic divergence, Mahalanobis's D^2 statistic serves as a reliable method for identifying superior parental combinations for breeding programs. Thus, evaluating genetic variability among sunflower genotypes remains essential for crop improvement.

The current investigation aimed to characterize the breeding potential of sunflower genotypes as parental material for hybridization initiatives. Previous research has established the efficacy of D^2 analysis for genotype classification and relationship mapping in *Helianthus annuus* L., with notable contributions from (Marinkovic *et al.* 1992) [6] and (Teklewold *et al.* 2000) [12]. This study employed Mahalanobis' D^2 methodology to examine genetic variation across 32 sunflower genotypes (including two checks), focusing on ten critical agronomic traits associated with yield performance, yield-determining factors, and early growth vigor parameters. The analytical outcomes are systematically organized in subsequent sections.

The research specifically targeted the quantification of genetic diversity within the experimental germplasm pool. Contemporary breeding research (Reddy *et al.* 2024) [9] emphasizes that deliberate selection of phenotypically divergent parental stocks fundamentally enhances hybrid breeding programs through two mechanisms: (1) optimization of heterotic effects and (2) expansion of the available gene pool for cultivar development. The D^2 statistical approach serves as a robust tool for isolating parental candidates demonstrating both substantial genetic polymorphism and stable trait heritability.

Materials and Methods

Thirty-two germplasm accessions were collected from the ICAR-Indian Institute Oilseeds Research (IIOR)
Rajendranagar, Hyderabad, Oil seed Research station, Latur and Zonal Agricultural Research Station, Solapur. Which included 30 sunflower germplasm accessions and 2 checks. DRSF-108 (National checks) and Phule Bhaskar (Local check grown in Maharashtra).

The study was conducted at the botany research farm, College of Agriculture, Pune (18.5366°N, 73.8441°E; 560 m elevation) during the 2024-25 *kharif* season. The experiment followed a Randomized Block Design with three replications, using a 60 × 30 cm spacing configuration with 4.5 m row lengths. Seeds were sown using the dibbling method, placing two seeds per hill to ensure proper germination, while maintaining standard agronomic practices throughout the trial. Ten quantitative traits were evaluated: days to 50% flowering, maturity duration, plant height, head diameter, seed volume (100 ml/g), seed filling percentage, 100-seed weight, per plant yield, hull content, and oil content. Data collection involved random sampling of five plants from each genotype and control plot per replication, with individual plant measurements recorded separately before calculating genotype-specific means for each parameter. All statistical analyses were performed using appropriate software packages.

Results and Discussion

Effective parent selection represents a critical yet challenging aspect of crop improvement initiatives. Genetic variability plays a pivotal role in breeding programs, as it facilitates heterotic expression in F1 hybrids and generates substantial variation in subsequent generations. Breeding strategies particularly emphasize parental divergence when pursuing heterosis, since greater genetic dissimilarity (within optimal limits) enhances the probability of improving target traits in progeny. Among various analytical methods, Mahalanobis' D^2 analysis serves as a particularly valuable statistical approach for categorizing genetically distinct parental lines using quantitative characteristics, thereby supporting strategic hybridization efforts.

Genetic diversity represents the collective variation of inherited traits within a species' gene pool. This biological variation enables populations to evolve in respose to environmental changes. Populations exhibiting greater genetic variability have increased probability of containing individuals with advantageous allele combinations that enhance survival. Those individuals possessing favourable genetic variations demonstrate greater survival and reproductive success, enabling the transmission of beneficial alleles to future generations. This natural selection process ensures population continuity across successive generations. In our analysis, genotypes were systematically categorized into distinct groups based on their genetic divergence. The results comprehensively display: (1) the distribution patterns of genotypes across clusters, (2) mean values for each cluster group, (3) quantitative measurements of both withincluster and between-cluster divergence (D^2 values), and (4) the proportional contribution of each evaluated trait to the observed genetic variation. These findings provide valuable insights for selective breeding programs and population genetics studies.

Clustering pattern

In the present investigation, all the thirty-two genotypes taken for genetic divergence analysis differed significantly with regard to the characters studied and displayed marked divergence. They were grouped in to ten clusters on the basis of Tocher's method of clustering utilizing D^2 values (Table 1). Cluster I comprised ten genotypes namely GMU249, DRSF-108 ©, GMU-494, GMU-78, GMU-569, GMU360, EC-3999512-1, IB-228, EC-75717, RHA 1-1. Cluster
III comprised eight genotypes namely P. Bhaskar ©, GMU474, TSG-53-90, EC-178168-2, GMU-577, GMU481, EC178155, EC-198078. Cluster II comprised five genotypes namely GMU-1000, GMU-770, GMU-841, GMU-616, GMU-312.Cluster IV comprised three genotypes namely GMU-934, EC-93901-5, LTRR-341. Rest six other monogenic clusters i.e., cluster V, VI, VII, VIII, IX, X namely IB-6, EC-494389, EC-6011901, GMU-317, EC601951, GMU-1042 respectively showed zero intra-cluster confirming to the result of (Patel *et al.* 2021) [8], also observed similar clustering pattern of genotypes among cluster as some clusters were unique having only single genotypes (Kumar *et al.* 2020) [3]. These results are in conformity with the observations made by (Anand Kumar *et al.* 2018) [1], (Sujatha *et al.* 2012) [11] and (Dudhe *et al.* 2010) [2].

Table 1: Distribution of thirty-two genotypes of sunflower in different cluster

Cluster No.	Number of Genotypes Included	Genotypes
I	10	GMU-249, DRSF-108 ©, GMU-494, GMU-78, GMU-569, GMU-360, EC-3999512-1, IB-228, EC-75717, RHA 1-1
II	5	GMU-1000, GMU-770, GMU-841, GMU-616, GMU-312
III	8	P. Bhaskar ©, GMU-474, TSG-53-90, EC-178168-2, GMU-577, GMU-481, EC-178155, EC-198078
IV	3	GMU-934, EC-93901-5, LTRR-341
V	1	IB-6
VI	1	EC-494389
VII	1	EC-6011901
VIII	1	GMU-317
IX	1	EC-601951
X	1	GMU-1042

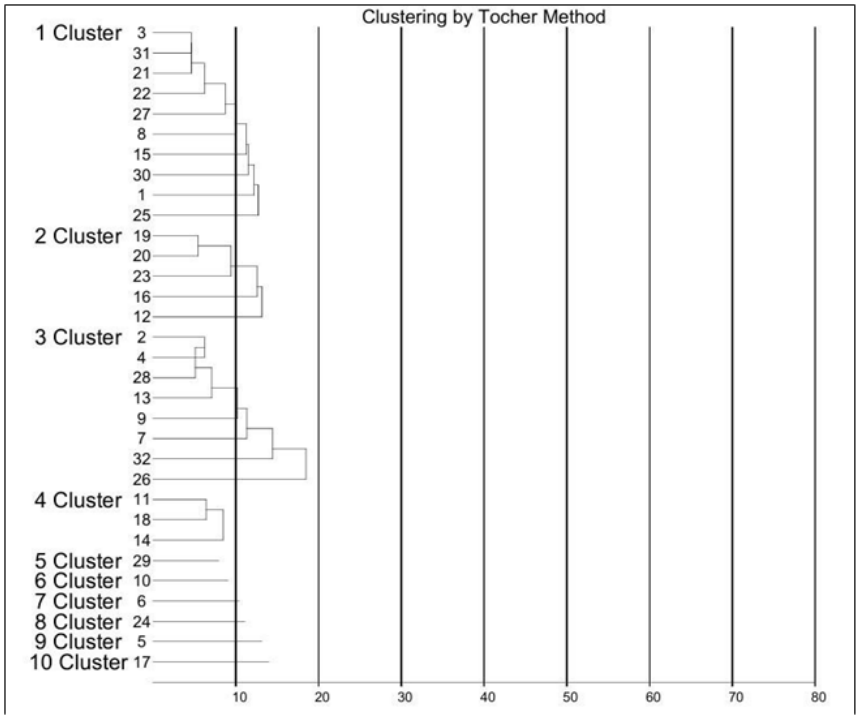

Fig 1: Distribution of 32 genotypes of sunflower into different cluster.

Cluster mean for characters in sunflower

The mean performances of cluster values of ten characters are presented in Table 2. A considerable inter cluster variation in respect of cluster was observed among the various clusters for ten characters studied. Cluster III recorded highest Head diameter (19.68 cm), 100 seed weight (6.17 g), seed yield per plant (41.40 g) and less hull content (23.21%). Seed filling percentage was highest for

Table 2: Cluster mean for characters in sunflower.

Clusters	Days to 50% flowering (No.)	Days to maturity (No.)	Plant height (cm)	Head diameter (cm)	100 seed weight (g)	Hull content (%)	Seed filling percentage (%)	Oil content (%)	Volume weight (g/100 ml)	Seed yield per plant (g)
I	58.67	88.50	144.94	16.27	5.04	26.00	80.05	35.71	44.11	26.98
II	50.67	80.67	108.03	16.01	4.70	25.49	77.18	35.51	43.39	23.80
III	57.50	87.79	148.38	19.68	6.17	23.21	85.85	36.58	45.50	41.40
IV	52.11	82.89	120.39	17.82	4.73	23.34	86.26	34.69	40.10	39.36
V	58.33	87.00	116.50	13.70	4.08	29.70	75.37	33.80	42.20	16.37
VI	55.00	85.00	148.83	15.33	5.66	29.07	76.83	31.90	41.33	19.53
VII	50.67	82.33	161.90	11.03	5.51	28.90	76.50	34.27	45.12	16.97
VIII	50.33	81.33	156.73	15.67	4.86	25.47	76.10	39.80	48.32	18.13
IX	53.33	84.00	180.87	14.40	4.51	28.73	72.83	32.13	40.35	15.53
X	63.67	93.67	151.87	13.13	4.79	30.57	70.70	34.00	43.80	17.33
Mean	54.87	85.24	142.20	15.13	4.97	26.93	77.62	34.77	43.36	22.09
SD	4.43	3.99	22.50	2.44	0.61	2.67	5.04	2.28	2.54	9.57

Intra and inter cluster distance

The intra-cluster distance is highest for cluster II (3.87) followed by cluster III (3.86), while it is minimum for the mono-genotypic clusters V, VI, VII, VIII, IX, and X. The inter cluster distance is maximum between the cluster X and IV followed by the clusters IX and IV, while it is minimum between the cluster X and IX. (Lagiso *et al.* 2021) [4]. suggested that hybridizing clusters with large inter-cluster distances would be a logical approach for recombining desirable traits in sunflower hybrid development programs. Mono-genotypic clusters (V-X) with zero intra-cluster distance, as observed here, were similarly reported by (Lakshman *et al.* 2021) [5] reported the presence of single genotype in five clusters and highlighted the role of solitary clusters in harnessing heterosis in hybrid development in sunflower.

Table 3: Cluster distances

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	12.39 (3.52)	38.68 (6.22)	27.35 (5.23)	32.26 (5.68)	25.30 (5.03)	15.84 (3.98)	32.71 (5.72)	19.53 (4.42)	26.52 (5.15)	21.52 (4.64)
II		14.97 (3.87)	63.52 (7.97)	26.93 (5.19)	20.43 (4.52)	34.45 (5.87)	48.02 (6.93)	32.14 (5.67)	64.48 (8.03)	60.52 (7.94)
III			14.89 (3.86)	31.02 (5.57)	68.22 (8.26)	39.81 (6.31)	64.16 (8.01)	45.02 (6.71)	60.68 (7.79)	60.52 (7.78)
IV				12.39 (3.25)	43.16 (6.57)	40.70 (6.38)	61.30 (7.83)	43.03 (6.56)	70.72 (8.41)	73.27 (8.56)
V					0.00 (0.00)	18.57 (4.31)	33.17 (5.76)	27.87 (5.28)	37.45 (6.12)	23.61 (4.86)
VI						0.00 (0.00)	10.43 (3.23)	17.55 (4.19)	13.10 (3.62)	16.08 (4.01)
VII							0.00 (0.00)	19.09 (4.37)	15.76 (3.97)	25.50 (5.05)
VIII								0.00 (0.00)	19.18 (4.38)	29.92 (5.47)
IX									0.00 (0.00)	15.21 (3.90)
X										0.00 (0.00)

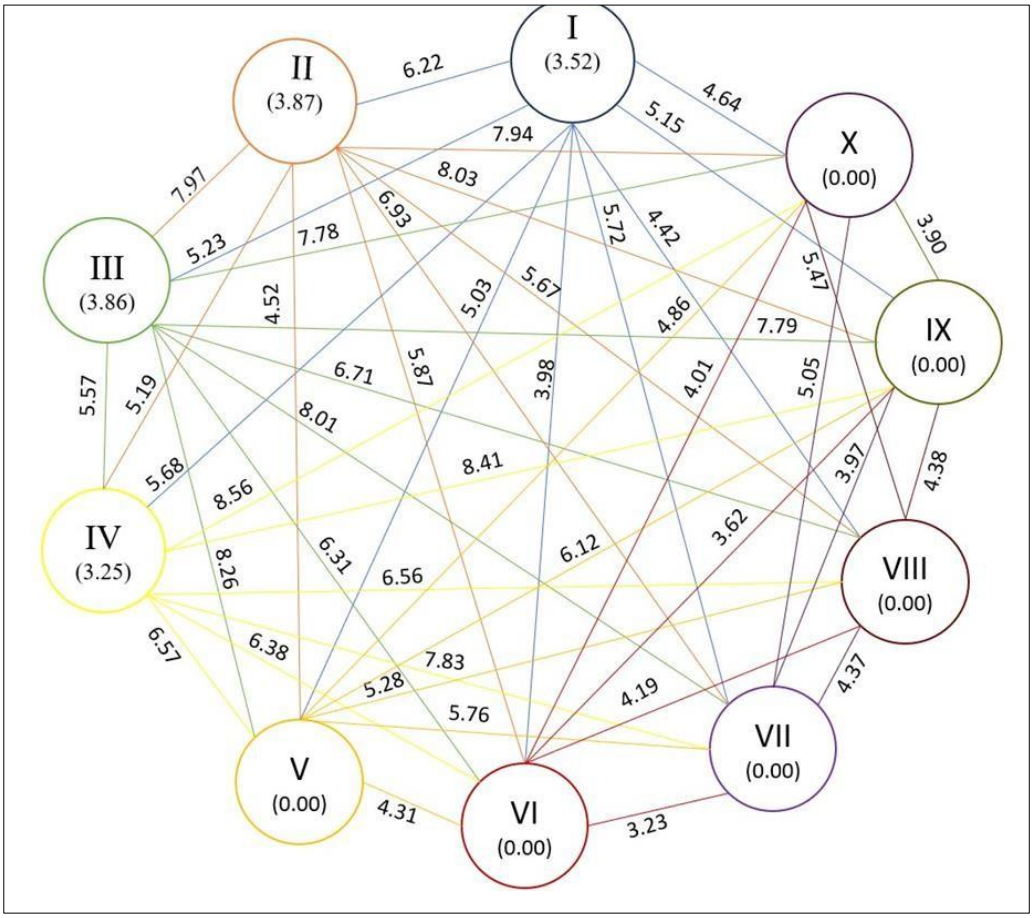

Fig 2: A cluster diagram showing inter relationship between eleven clusters in sunflower

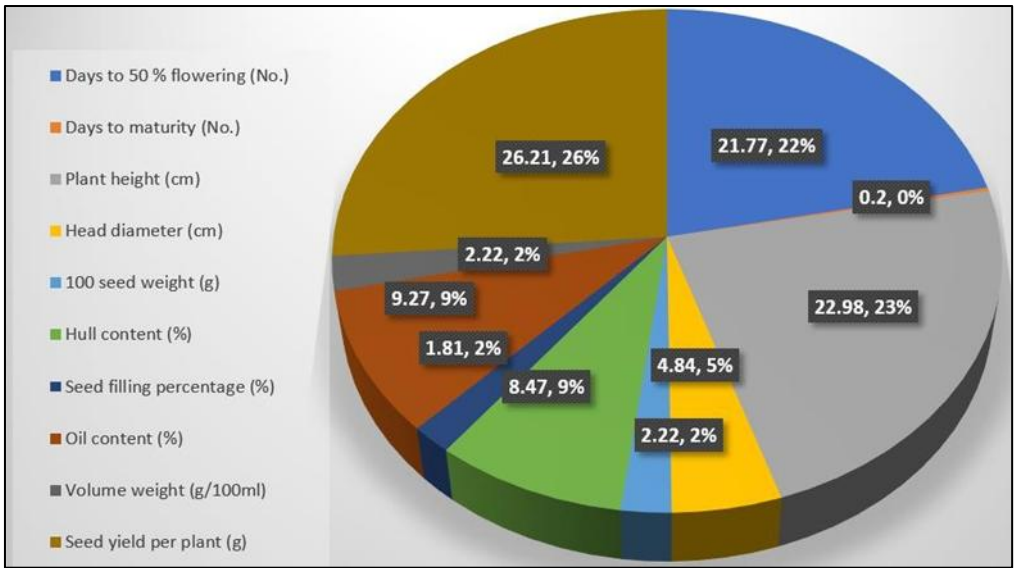

Fig 3: Per cent contribution of 10 characters for divergence in Sunflower

Percent contribution of 10 characters for divergence in sunflower

Out of 10 characters studied, the maximum contribution to divergence was reported in characters seed yield per plant (26.21%), Plant height (22.98%) and days to 50% flowering (21.77%). It was followed by oil content (9.27%) remaining characters followed the divergence (Neelima *et al.* 2016) [7]. observed that hundred seed weight played a most significant role in total genetic diversity after that plant height and days to maturity and highlighted the role of classifying the genotypes based on diversified traits in sunflower hybrid development programmes.

Conclusion

Genetic diversity is of major interest to plant breeders, more diverse the parents, greater are the chances of obtaining heterotic expression in F1 with possibility of broad spectrum of variability in segregating generations. While selecting appropriate sunflower germplasm, the breeder looks for genetically diverse and superior genotypes, which could be utilized in population and heterosis breeding. The present study exhibited very high differences among the genotypes for seed yield and almost all the yield component characters which may favour the selection and its further utilization in recombination breeding programmes. The genetically diverse sunflower germplasm identified from the present study could be utilized in development of diverse inbreds which may be utilized in future heterosis breeding. Promising trait specific superior sunflower germplasm accessions identified will serve as donors for the development of trait specific heterotic gene pools, which can be further exploited in sunflower. Based on comprehensive divergence analysis and superior per se performance, ten genotypes emerge as prime candidates for sunflower improvement programs: EC-399512-1, EC178168-2, EC-198078, EC-178155, EC-93901-5, GMU481, GMU-1000, GMU-841, GMU-474, and GMU-577.

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