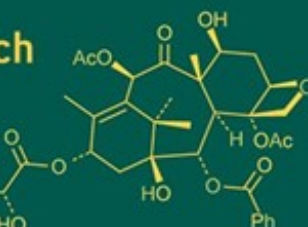
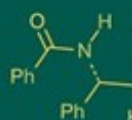
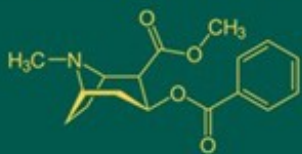


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Genetic diversity in sunflower (*Helianthus annuus* L.) genotypes assessed through Mahalanobis D² analysis

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ABSTRACT

Genetic divergence is an important component of sunflower breeding because the choice of genetically distinct parents influences the breadth of variability available in subsequent generations. Methods: Thirty sunflower genotypes, comprising 28 experimental entries and two standard checks, Phule Bhanu and Phule Bhaskar, were evaluated during Kharif 2025 at Baramati, Maharashtra, in a randomized block design with three replications. Ten quantitative characters were used to estimate genetic divergence through Mahalanobis D² statistics and Tocher's clustering method. Results: The genotypes exhibited wide divergence, with D² values ranging from 25.91 between SS-25-01 and SS-25-10 to 1731.14 between SS-25-27 and SS-25-07. The genotypes were grouped into nine clusters. Cluster I was the largest with 15 genotypes, whereas Clusters VII, VIII and IX were solitary. The maximum inter-cluster distance was recorded between Clusters IV and VI (1647.75), followed by Clusters III and IV (1433.97) as reported in the source analysis. Days to maturity contributed most to total divergence (18.51%), followed by seed yield per plant (17.58%) and plant height (12.33%). Cluster I showed the highest mean seed yield per plant (32.97 g), while Clusters IV and V recorded the highest mean oil content (38.34%). Conclusion: The evaluated material possessed substantial genetic diversity. Divergent clusters with complementary desirable means, particularly combinations involving Clusters IV and VI, provide useful parental resources for sunflower improvement. Parent selection should consider both genetic distance and trait performance.

Keywords: Cluster analysis, Genetic divergence, *Helianthus annuus*, Mahalanobis D², Sunflower, Tocher's method

1. Introduction

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in which improvement of seed yield, oil content, maturity duration and adaptation depends on the availability and effective use of diverse breeding material. Genetic diversity provides the raw material for selection and hybridization and allows breeders to assemble complementary traits in subsequent generations. Quantitative assessment of divergence is therefore useful for identifying parents that are sufficiently distinct while also possessing desirable agronomic attributes.

Mahalanobis D² analysis provides a multivariate measure of divergence among genotypes and has been widely applied to classify germplasm into genetically distinct groups. Tocher's method further facilitates the interpretation of these distances by grouping genotypes according to their relative similarity. Such information can assist parental choice and help broaden the genetic base of breeding populations. Previous sunflower studies have reported substantial divergence among inbred lines and germplasm accessions and have identified yield, phenology, plant architecture and quality traits as important contributors to diversity (Sujatha *et al.*, 2002; Reddy *et al.*, 2012; Lakshman *et al.*, 2021) [2, 3, 1].

The present investigation was undertaken to characterize genetic diversity among thirty sunflower genotypes using Mahalanobis D² statistics, identify distinct clusters, determine the magnitude of intra- and inter-cluster divergence, and identify clusters and genotypes with complementary attributes for use in future hybridization programmes.

2. Materials and methods**2.1 Experimental site and genetic material**

The experiment was conducted during Kharif 2025 at the Research Farm, Department of Genetics and Plant Breeding, Dr. Sharadchandra Pawar College of Agriculture, Baramati, Maharashtra, India. The experimental material consisted of thirty sunflower genotypes, including twenty-eight experimental genotypes and two

standard checks, Phule Bhanu and Phule Bhaskar. The crop was sown on 8 July 2025.

2.2 Experimental design and observations

The genotypes were evaluated in a randomized block design with three replications. Five randomly selected plants from each plot were used for recording observations. Ten quantitative characters were considered for diversity analysis: seed yield per plant, head diameter, hull content, days to 50% flowering, 100-seed weight, days to maturity, oil content, plant height, seed filling percentage and bud necrosis.

2.3 Statistical analysis

Genetic divergence among the genotypes was estimated using Mahalanobis D^2 statistics (Mahalanobis, 1936) [4]. The resulting distances were used to classify the genotypes into clusters following Tocher's method as described by Rao (1952) [5]. Average intra-cluster and inter-cluster distances were calculated to assess the relative divergence within and among clusters. Cluster means were computed for the ten characters to identify groups with desirable trait combinations. The relative contribution of individual characters to total divergence was also estimated.

3. Results

3.1 Variation among genotypes

The evaluated genotypes displayed considerable variation for all ten characters. Seed yield per plant ranged from 18.93 to 34.53 g, with a mean of 26.17 g. Head diameter ranged from 10.90 to 19.60 cm, while 100-seed weight varied from 4.53 to 6.30 g. Days to 50% flowering ranged from 48.67 to 70.00 days and maturity duration from 81.00 to 105.00 days. Oil content varied from 26.25 to 41.27%, plant height from 96.73 to 167.30 cm, seed filling percentage from 75.48 to 87.58%, hull content from 21.53 to 30.43%, and bud necrosis from 0 to 7.26%.

3.2 Magnitude of genetic divergence

Mahalanobis D^2 values showed a wide range of divergence among the thirty genotypes. The smallest distance was 25.91 between SS-25-01 and SS-25-10, indicating close multivariate similarity, whereas the largest distance was 1731.14 between SS-25-27 and SS-25-07, indicating pronounced divergence. The broad distance range confirmed substantial diversity within the evaluated material.

3.3 Cluster composition

Tocher's method grouped the thirty genotypes into nine clusters. Cluster I contained 15 genotypes: Phule Bhanu, SS-25-08, SS-25-31, SS-25-03, SS-25-34, SS-25-21, SS-25-12, SS-25-07, SS-25-26, SS-25-30, SS-25-22, SS-25-32, SS-25-11, SS-25-23 and SS-25-13. Cluster II comprised SS-25-10, SS-25-33 and SS-25-29, while Cluster III contained SS-25-01, SS-25-04 and SS-25-14. Clusters IV, V and VI contained two genotypes each. Clusters VII, VIII and IX were solitary and comprised SS-25-02, SS-25-09 and SS-25-28, respectively.

3.4 Intra- and inter-cluster divergence

Intra-cluster D^2 values ranged from 120.82 in Cluster IV to 227.24 in Cluster VI; the three solitary clusters had zero intra-cluster distance. The largest inter-cluster distance was 1647.75 between Clusters IV and VI, followed by 1433.97 between Clusters III and IV and 1108.45 between Clusters IV and VII. Other comparatively large distances were observed between Clusters I and V (932.63) and Clusters III and VII (861.21). The smallest inter-cluster distance was 271.88 between Clusters I and IV.

3.5 Cluster mean performance

Cluster means differed substantially for the traits studied. Cluster I recorded the highest mean seed yield per plant (32.97 g), together with a high mean head diameter (19.10 cm) and 100-seed weight (6.18 g). Cluster IV showed the earliest mean maturity (81.83 days), whereas Cluster IX had the latest mean flowering (69.00 days) and

the lowest mean head diameter (11.73 cm). The highest mean oil content was observed in Clusters IV and V (38.34%). Cluster VIII recorded the highest mean seed filling percentage (86.74%). Cluster IX had the lowest mean 100-seed weight (4.53 g) and plant height (102.40 cm).

3.6 Contribution of characters to divergence

Days to maturity made the largest contribution to total genetic divergence (18.51%), followed by seed yield per plant (17.58%) and plant height (12.33%). Seed filling percentage (9.42%), bud necrosis (9.23%), 100-seed weight (8.50%) and head diameter (8.25%) also contributed appreciably. Oil content, days to 50% flowering and hull content contributed 6.30, 6.19 and 3.69%, respectively.

Figure 4.7. Relative contribution of different quantitative characters towards genetic divergence in sunflower

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Figure 4.7. Percent contribution of different quantitative characters towards genetic divergence in sunflower

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Table 1. Cluster composition of thirty sunflower genotypes based on Mahalanobis D^2 analysis

Cluster	No. of genotypes	Genotypes
I	15	Phule Bhanu, SS-25-08, SS-25-31, SS-25-03, SS-25-34, SS-25-21, SS-25-12, SS-25-07, SS-25-26, SS-25-30, SS-25-22, SS-25-32, SS-25-11, SS-25-23, SS-25-13
II	3	SS-25-10, SS-25-33, SS-25-29
III	3	SS-25-01, SS-25-04, SS-25-14
IV	2	Phule Bhaskar, SS-25-25
V	2	SS-25-05, SS-25-06
VI	2	SS-25-24, SS-25-27
VII	1	SS-25-02
VIII	1	SS-25-09
IX	1	SS-25-28

Table 2. Average intra- and inter-cluster D^2 values among sunflower genotypes

Cluster pair	D^2	Cluster pair	D^2
I-I	178.32	IV-VI	1647.75
II-II	141.60	III-IV	1433.97
III-III	138.42	IV-VII	1108.45
IV-IV	120.82	I-V	932.63
V-V	222.91	III-VII	861.21
VI-VI	227.24	I-IV	271.88
VII-VII	0.00	I-II	296.60
VIII-VIII	0.00	II-IX	315.03
IX-IX	0.00	VI-VII	319.08

4. Discussion

The wide range of Mahalanobis D^2 values demonstrated substantial multivariate diversity among the evaluated sunflower genotypes. The occurrence of nine clusters from only thirty entries indicates that the

breeding material contained distinct combinations of phenological, yield, quality and plant architectural traits. Comparable diversity has been reported in sunflower germplasm by *Sujatha et al. (2002)* [2], *Reddy et al. (2012)* [3] and *Lakshman et al. (2021)* [1], supporting the usefulness of multivariate divergence analysis for parental identification.

Table 3. Cluster mean performance for selected characters

Cluster	Seed yield/plant (g)	Head diameter (cm)	100-seed weight (g)	Maturity (days)	Oil content (%)
I	32.97	19.10	6.18	94.67	38.02
II	25.36	17.41	5.92	85.89	38.34
III	25.03	18.52	5.92	81.83	36.77
IV	25.10	14.73	5.92	104.83	35.61
V	21.92	16.15	4.72	99.83	38.34
VI	26.40	14.93	5.97	103.00	35.67
VII	25.27	16.03	5.90	94.00	26.25
VIII	26.40	11.73	4.53	92.00	36.72
IX	25.80	16.70	5.60	89.80	38.06

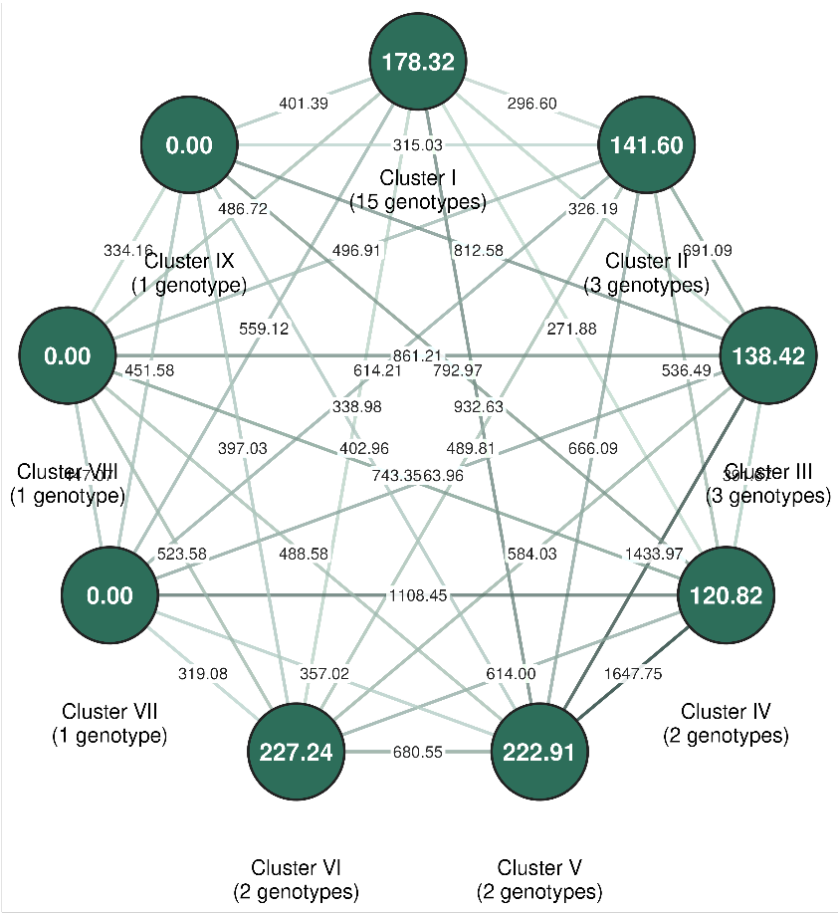


Figure 4.6. Distance among nine clusters by Tocher's method

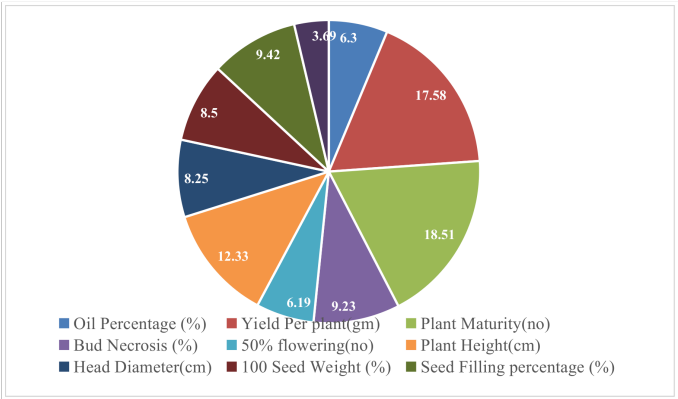


Figure 4.6. Distance among nine clusters by Tocher's method

The large inter-cluster distance between Clusters IV and VI indicated strong divergence between these groups. However, genetic distance alone is insufficient for parent selection. Cluster means showed that different groups possessed complementary attributes: Cluster I combined high seed yield, larger heads and greater seed weight, whereas Clusters IV and V were characterized by comparatively high oil content. Thus, crossing divergent clusters that differ favourably for complementary traits may broaden segregating variability and increase the likelihood of recovering useful recombinants. The contribution analysis identified days to maturity as the major discriminator among the genotypes, followed by seed yield per plant and plant height. This indicates that phenological duration, productivity and plant architecture were important sources of diversity in the present material. The contribution pattern differed from studies in which stem girth, seed yield or flowering traits were the leading discriminators, emphasizing that the relative contribution of characters

can vary with the genetic constitution of the population under study (Sujatha *et al.*, 2002; Reddy *et al.*, 2012) [2, 3].

Table 4. Relative contribution of characters to total genetic divergence

Character	Contribution (%)
Days to maturity	18.51
Seed yield per plant	17.58
Plant height	12.33
Seed filling percentage	9.42
Bud necrosis	9.23
100-seed weight	8.50
Head diameter	8.25
Oil content	6.30
Days to 50% flowering	6.19
Hull content	3.69

The solitary clusters deserve particular attention. SS-25-02 was characterized by relatively late flowering and maturity with high seed filling, SS-25-09 had markedly low oil content, and SS-25-28 expressed late flowering with low 100-seed weight, small head diameter and short plant stature. Their distinct multivariate profiles explain their separation from the major groups and may make them useful when specific contrasting traits are required in crossing programmes.

The cluster pattern also indicates that geographical or nominal origin should not be used as the sole basis for choosing parents. Genetic divergence estimated from the actual trait data provides a more direct basis for identifying complementary parents. Accordingly, combinations involving highly divergent clusters should be considered together with the agronomic merit of individual genotypes and the breeding objective. The present results are consistent with the broader principle that divergence coupled with desirable performance is more informative for parent selection than distance alone (Arunachalam *et al.*, 1984) [6].

5. Conclusion

Mahalanobis D² analysis established substantial genetic diversity among the thirty sunflower genotypes and separated them into nine clusters. The greatest divergence occurred between Clusters IV and VI, while Cluster I showed the highest mean seed yield per plant. Days to maturity, seed yield per plant and plant height were the major contributors to total divergence. The contrasting performance of the clusters suggests opportunities for combining high yield, favourable

maturity, oil content, seed filling and other desirable traits through targeted hybridization. In particular, highly divergent clusters should be crossed only after considering their complementary cluster means and the intended breeding objective. The identified diverse genotypes therefore constitute useful parental resources for broadening the genetic base and developing improved sunflower populations.

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