



Genetic Diversity Analysis in Bread Wheat (*Triticum aestivum* L.) Using Mahalanobis D² Statistics

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was undertaken to assess the genetic diversity among 48 genotypes of bread wheat (*Triticum aestivum* L.) using Mahalanobis D² statistics, with the aim of identifying genetically diverse and high-performing lines suitable for future breeding programs. The genotypes were evaluated under a randomized block design with two replications for ten quantitative traits at the farm of Wheat and Maize research Unit, Parbhani during *rabi* season 2023-24. Based on D²

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values, the genotypes were grouped into 11 distinct clusters, indicating the presence of substantial genetic variability. Cluster II included the maximum number of genotypes (17), followed by Cluster I with 15 genotypes and Cluster IV with 8 genotypes. The remaining clusters, namely Cluster III, Cluster V, Cluster VI, Cluster VII, Cluster VIII, Cluster IX, Cluster X, and Cluster XI, each comprised a single genotype. The maximum inter-cluster distance was observed between Cluster V and Cluster VII, indicating the genotypes in these clusters are genetically most diverse and could serve as ideal parents for hybridization programs aimed at exploiting heterosis or generating transgressive segregants. Cluster mean analysis revealed that Cluster VII recorded superior mean values for key traits such as grain yield per plant, 1000 grain weight and number of grains per spike, while Cluster III and Cluster VI had moderate performance for most characters. The traits contributing most to genetic divergence were number of grains per spike (20.17%), harvest index (15.68%), spike length (12.90%), and plant height (12.67%). The results suggest that genotypes from diverse clusters, particularly those showing high inter-cluster distances and superior trait performance, can be effectively used in crossing programs to improve yield and genetic variability in wheat.

Keywords: *Wheat germplasm; genetic divergence; multivariate analysis; genotype clustering; plant breeding.*

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a globally significant cereal crop that plays a key role in food and nutritional security. In India, it is the second most widely cultivated cereal after rice, covering 31.82 million hectares with an annual production of 112.74 million tonnes (Government of India, 2023). In Maharashtra, wheat is cultivated on approximately 1.05 million hectares, producing 1.99 million tonnes with a productivity of 1,899 kg/ha during the 2023–24 rabi season. Despite its large cultivation area, wheat productivity has plateaued in many regions due to challenges such as climate variability, biotic and abiotic stress, and a narrow genetic base in modern cultivars. This lack of diversity poses a major constraint in breeding programs aimed at improving yield potential and stress resilience. Genetic diversity analysis is a key tool in plant breeding, as it enables the identification of genetically divergent genotypes that can serve as potential parents in hybridization programs. Mahalanobis D^2 statistics is a powerful multivariate technique that allows the assessment of genetic divergence based on multiple quantitative traits simultaneously. Grouping genotypes into distinct clusters based on D^2 values enables breeders to select parents that are likely to produce transgressive segregants and heterotic combinations (Singh et al., 2018; Kumar et al., 2021).

Therefore, the present study was undertaken to assess the genetic diversity among 48 bread wheat genotypes using Mahalanobis D^2 statistics, with the aim of identifying genetically

divergent and agronomically superior genotypes for use in future wheat improvement programs.

2. MATERIALS AND METHODS

2.1 Experimental Site and Experimental Design

The experiment was conducted during rabi season 2023-24 at the Wheat and Maize research Unit, Parbhani. The experimental material for the present study comprised of 48 Genotypes of wheat obtained from The International Maize and Wheat Improvement Center (CIMMYT), Mexico were planted in a Randomized Block Design with two replications having plot size 4.0 X 0.20 m² and plant spacing of 20cm and 10 cm respectively. All recommended agronomic practices were followed throughout the crop growth period, including the application of a basal dose of NPK fertilizers at the time of sowing and irrigation at critical growth stages such as crown root initiation, flowering and grain filling.

2.2 Characters Studied

The data were recorded from five randomly selected plants from each genotype on ten quantitative traits viz., days to 50% heading, days to maturity, plant height (cm), number of tillers per plants, spike length (cm), number of grains per spike, 1000-grains weight (g), biological yield per plant (g), harvest index (%) and grain yield per plant (g). Days to 50% heading and days to maturity observations were recorded on plot basis only once. Days to 50%

heading was measured as the number of days from sowing to when approximately 50% of plants in a plot had emerged spikes. Days to maturity was noted as the number of days from sowing to when most plants reached physiological maturity. Plant height (cm) was measured from the base of the plant to the tip of the spike, excluding awns. The number of tillers per plant was manually counted from each sampled plant. Spike length (cm) was recorded from the base to the tip of the spike. The number of grains per spike was counted after threshing individual spikes. The 1000-grain weight (g) was obtained by weighing 1000 well-filled grains from each plot. Biological yield per plant (g) included the total dry matter of individual plants at harvest. Harvest index (%) was calculated as the ratio of grain yield to biological yield, expressed in percentage. Grain yield per plant (g) was recorded after threshing and weighing the grain from each sampled plant. The methods for trait measurement followed the procedures described by Singh and Choudhary (1977) and Panse and Sukhatme (1985).

2.3 Statistical Analysis

Genetic divergence among genotypes was assessed using Mahalanobis D^2 statistics following standard multivariate procedures. Clustering of genotypes was performed using the Tocher's method as outlined by Rao (1960). Intra and inter-cluster distances were computed using the formula provided by Singh et al. (1977), while the overall divergence was quantified as per the methodology described by Singh and Pawar (2005).

3. RESULTS AND DISCUSSION

3.1 Cluster Formation

Mahalanobis D^2 analysis grouped the 48 wheat genotypes into 11 distinct clusters, highlighting substantial genetic diversity in the studied material. This clustering reflects variability in the traits analyzed, suggesting that the material holds potential for breeding programs aimed at genetic improvement. The distribution pattern of genotypes across clusters is presented below:

Cluster II included the maximum number of genotypes (17): PBN 234, PBN 238, PBN 224, PBN 240, PBN 217, PBN 205, PBN 203, PBN 211, PBN 227, PBN 119, PBN 220, PBN 194, PBN 223, PBN 199, PBN 228, PBN 225, PBN 197 followed by Cluster I with 15 genotypes (PBN 216, PBN 239, PBN 221, PBN 201, PBN 235, PBN 218, PBN 207, PBN 200, PBN 215, PBN 206, PBN 214, PBN 241, PBN 208, PBN 230, PBN 237) and Cluster IV with 8 genotypes (PBN 204, PBN 210, PBN 196, PBN 198, PBN 195, PBN 209, PBN 213, PBN 202). The remaining clusters, namely Cluster III (PBN 236), Cluster V (PBN 232), Cluster VI (PBN 229), Cluster VII (PBN 231), Cluster VIII (PBN 212), Cluster IX (PBN 226), Cluster X (PBN 233), and Cluster XI (PBN 222), each comprised a single genotype. The presence of these singleton clusters suggests a high level of uniqueness and genetic divergence in these individual genotypes, making them potential candidates for use in hybridization to introduce novel traits.

Table 1. Grouping of 48 wheat genotypes into 11 clusters on the basis of D^2 analysis

Cluster	Total Genotypes	Genetic material
Cluster 1	15	PBN 216, PBN 239, PBN 221, PBN 201, PBN 235, PBN 218, PBN 207, PBN 200, PBN 215, PBN 206, PBN 214, PBN 241, PBN 208, PBN 230, PBN 237
Cluster 2	17	PBN 234, PBN 238, PBN 224, PBN 240, PBN 217, PBN 205, PBN 203, PBN 211, PBN 227, PBN 119, PBN 220, PBN 194, PBN 223, PBN 199, PBN 228, PBN 225, PBN 197
Cluster 3	1	PBN 236
Cluster 4	8	PBN 204, PBN 210, PBN 196, PBN 198, PBN 195, PBN 209, PBN 213, PBN 202
Cluster 5	1	PBN 232
Cluster 6	1	PBN 229
Cluster 7	1	PBN 231
Cluster 8	1	PBN 212
Cluster 9	1	PBN 226
Cluster 10	1	PBN 233
Cluster 11	1	PBN 222

Table 2. Intra and Inter cluster distances (D^2) among 11 clusters for 48 wheat genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster XX	Cluster XI
Cluster I	31.92	72.93	51.70	52.27	86.30	63.84	77.97	107.74	53.73	97.42	75.34
Cluster II		36.24	100.60	74.48	57.46	56.40	197.68	60.37	71.23	74.48	112.57
Cluster III			0.00	47.75	148.35	122.54	72.08	84.64	129.28	166.67	103.23
Cluster IV				48.44	102.41	105.47	101.61	92.93	94.28	104.65	119.68
Cluster V					0.00	30.14	236.85	153.76	92.74	82.26	185.23
Cluster VI						0.00	195.44	125.44	53.58	102.82	100.80
Cluster VII							0.00	222.01	113.21	175.56	134.10
Cluster VIII								0.00	129.96	130.19	110.67
Cluster IX									0.00	53.73	59.44
Cluster X										0.00	178.76
Cluster XI											0.00

Table 3. Cluster mean among 11 clusters for ten characters for 48 genotypes of wheat.

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster XX	Cluster XI
Days to 50 % heading	50.97	52.47	60	54	49.5	51	54	57	47.5	50	49.5
Days to maturity	93.43	94.44	96	94.81	92.5	91.5	95.5	95	92.5	94	95
Plant height (cm)	77.36	76.52	76	76.86	83.3	79.4	77.8	70	76.4	78.7	70.1
Number of tillers per plant	7.85	7.18	8.05	7.01	7.85	7.55	7.30	7.70	6.35	7.65	7.70
Spike length (cm)	8.83	8.42	9.30	9.21	7.95	7.70	10.15	8.40	8.70	8.95	8.25
Number of grains per spike	61.39	49.15	67.17	60.80	53.61	51.97	66.65	51.03	46.30	42.74	54.32
1000 grains weight (g)	46.05	42.17	41.12	41.48	42.15	42.54	52	41.58	51.22	55.53	46.10
Biological yield per plant (g)	7.68	7.80	8.42	7.35	7.48	6.62	8.56	9.64	6.80	8.47	5.57
Harvest index (%)	75.53	65.14	72.71	65.36	66.62	76.69	82.92	57.40	81.88	63.44	91.12
Grain yield per plant (g)	5.83	5.08	6.13	5.62	4.97	5.84	7.07	5.56	5.57	5.32	5.62

Cluster-wise distribution of genotypes is summarized in Table 1 and fig 1. The experimental results were supported by the similar findings of Dutamo et al. (2015) (clustered 60 genotypes of bread wheat into six clusters), Niyazi et al. (2023) (clustered 20 genotypes into five clusters) and Kumar et al. (2015) (grouped 50 genotypes into 10 diverse clusters). The formation of many singleton clusters (III to XI) indicates the presence of highly divergent individual genotypes, which are of particular interest for breeding purposes.

3.2 Intra and Inter Cluster Distances

The average intra and inter cluster D^2 values are presented in Table 2. These values provide insights into the genetic relationships and variability both within and between clusters. The intra cluster distance (D^2) range from 0.00 to 48.44 whereas inter cluster distance (D^2) ranges

from 30.14 to 236.85. Among multi-genotype clusters, Cluster IV showed the highest intra-cluster distance ($D^2 = 48.44$) followed by Cluster II ($D^2 = 36.24$) indicating more variability within these clusters. The maximum inter-cluster distance was recorded between Cluster V and Cluster VII ($D^2 = 236.85$), followed by Cluster VII and Cluster VIII (222.01) and Cluster II and Cluster VII (197.68). These wide distances suggest that the genotypes in these clusters are genetically highly diverse and may serve as ideal parents for hybridization to exploit heterosis and transgressive segregation. Conversely, the lowest inter-cluster distance was observed between Cluster V and Cluster VI ($D^2 = 30.14$), indicating relatively closer genetic relationships. Similar finding was also reported by Sharma and Panwar (2007), Mohanty et al., (2017), Naik et al., (2016), Gupta et al., (2002), Singh et al. and Verma et al. (2006), Mahalanobis, (1936).

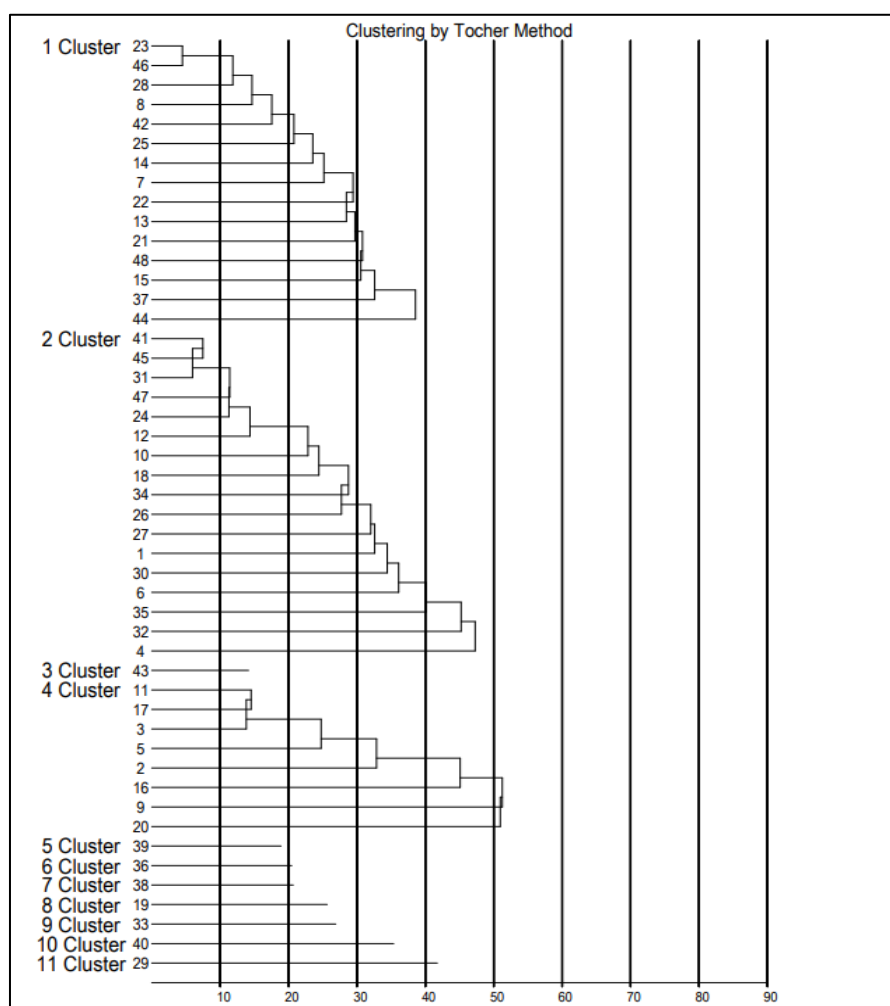


Fig.1. Dendrogram showing clustering pattern of total 48 genotypes of wheat by Tocher method

3.3 Cluster Means for Quantitative Traits

The cluster means for each of the 10 characters are presented in (Table 3). The genotypes belong to cluster III showed highest mean value (60) for days to 50 % heading, while cluster IX showed the least mean value (47.5). cluster III showed highest mean value of (96 days) for days to maturity, while cluster VI showed the least mean value for days to maturity (91.5 days). For plant height, genotypes belong to cluster V showed highest mean value of (83.3 cm), while cluster XI showed the least mean value of plant height (70.1 cm). cluster III showed the highest mean value (8.05) for number of tillers per plant, while cluster IX showed the least mean value for number of tillers per plant (6.35). cluster VII showed the highest mean value (10.15 cm) for Spike length, while cluster VI showed the least mean value (7.70). For number of grains per spike, genotypes belong to cluster III showed highest mean value (67.17), while cluster X showed the least mean value (42.74). The genotypes belong to cluster X showed the highest mean value for 1000 grain weight (55.53 g) while cluster III showed the least mean value for 1000 grain weight (41.12 g). cluster VIII showed the highest mean value for biological yield per plant (9.64 g), while cluster XI showed the least mean value for biological yield per plant (5.57 g). cluster XI showed the highest mean value for Harvest index (91.12 %) while cluster VIII showed the

least mean value for Harvest index (57.40%). cluster VII showed the highest mean value for grain yield per plant (7.07 g), while cluster V showed the least mean value for grain yield per plant (4.97 g). These trait-specific strengths highlight clusters with desirable combinations for yield and its components. Similar findings were observed by Jaiswal et al., (2010) & Ahmad et al. (2014).

3.4 Percent Contribution towards Total Divergence

The percentage contribution towards genetic divergence by all the characters is presented in Table 4. and Fig. 2. Trait-wise contributions to overall genetic divergence showed that: Number of grains per spike contributed the most (20.17%) Followed by Harvest index (15.68%), Spike length (12.90%), Plant height (12.67%) and 1000 grain weight (10.42%). These five traits cumulatively accounted for the majority of the observed genetic variability, indicating their significant role in differentiating genotypes. The high contribution of these traits implies that they are the most variable among the genotypes studied and should therefore be prioritized during the selection of parents for hybridization. Emphasis on these traits in breeding programs could lead to the development of improved genotypes with enhanced yield potential, better resource use efficiency, and adaptability to diverse environments.

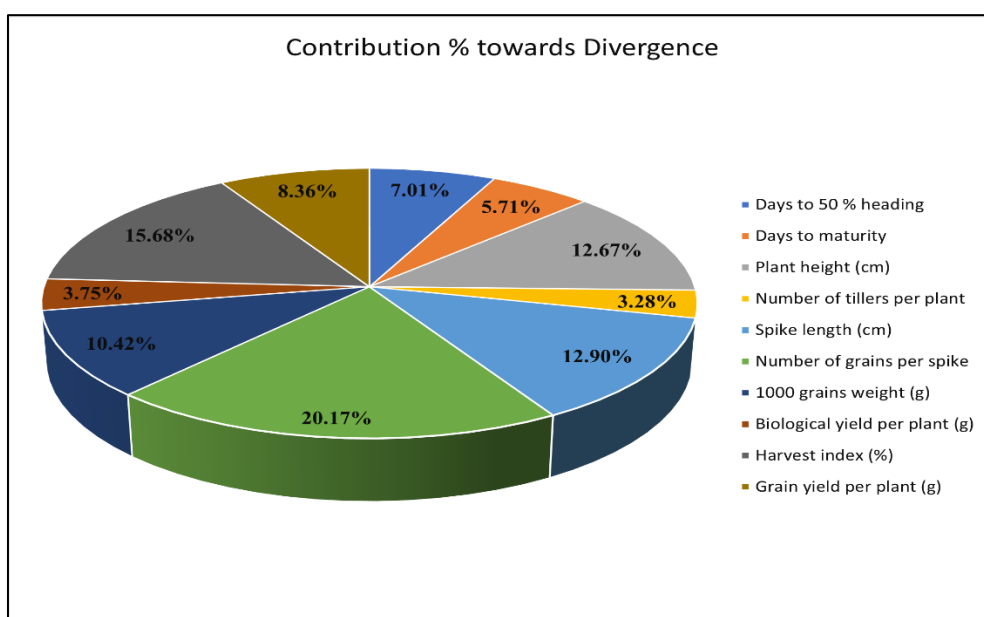


Fig. 2. Pie chart representation of different traits and their contribution towards genetic divergence

Table 4. Contribution to each character to genetic Divergence in wheat

Sr. No.	Traits	Contribution % towards Divergence
1	Days to 50 % heading	7.01%
2	Days to maturity	5.71%
3	Plant height (cm)	12.67%
4	Number of tillers per plant	3.28%
5	Spike length (cm)	12.90%
6	Number of grains per spike	20.17%
7	1000 grains weight (g)	10.42%
8	Biological yield per plant (g)	3.75%
9	Harvest index (%)	15.68%
10	Grain yield per plant (g)	8.36%

4. CONCLUSION

The study confirmed the presence of substantial genetic diversity among the evaluated wheat genotypes. The clustering pattern and inter-cluster distances provide clear evidence for selecting divergent parents. Genotypes in clusters with maximum inter-cluster distances and desirable trait performance (e.g., Cluster V, VII, and VIII) are recommended as divergent and high-yielding donors. Crosses involving genotypes from Cluster V and Cluster VII may generate heterotic hybrids with superior yield potential. Traits like number of grains per spike, harvest index, and spike length were key contributors to diversity. Genotypes from clusters showing high inter-cluster distances and superior mean performance can be recommended for use in future breeding programs aimed at enhancing genetic gain and yield potential in bread wheat.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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