

Genetic Divergence and Seed Quality Assessment of Indian Mustard (*Brassica Juncea* L.) Genotypes using Mahalanobis D² Statistics

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Abstract

Indian mustard (*Brassica juncea* L.) is one of the most important edible oilseed crops cultivated in India and serves as a major source of vegetable oil. The development of high-yielding cultivars with superior seed quality requires comprehensive information on the extent of genetic diversity available among breeding materials. The present investigation was conducted to assess genetic divergence and seed quality parameters among twenty-five Indian mustard genotypes using Mahalanobis D² statistics. The experiment was conducted during the Rabi season of 2022–23 in a Randomized Complete Block Design with three replications. Twelve agronomic and yield-related traits were recorded, and genetic divergence was estimated using Tocher's clustering method. The twenty-five genotypes were grouped into six distinct clusters, indicating the existence of considerable genetic diversity. Cluster I accommodated sixteen genotypes, Cluster II contained five genotypes, whereas Clusters III, IV, V and VI each consisted of a single genotype. The maximum inter-cluster distance was observed between Clusters V and VI, indicating the highest level of genetic divergence, while Cluster II exhibited the highest intra-cluster distance, suggesting greater variability within that cluster. Among the studied traits, seed yield per plant contributed the highest proportion towards total genetic divergence, followed by test weight, days to maturity and number of siliquae per plant. Seed quality evaluation revealed substantial variation among genotypes for germination percentage, seedling length and seed vigour index. Genotype DRMRDR-2141 exhibited the highest seed vigour index and seedling length, whereas DRMR 2020-15 and DRMRDR 2203 recorded superior germination percentages. The observed diversity among the evaluated genotypes provides valuable opportunities for selecting genetically divergent parents for hybridization programmes aimed at improving seed yield and seed quality in Indian mustard.

Keywords: *Brassica juncea*, Mahalanobis D² statistics, genetic divergence, Tocher's clustering, seed vigour index, seed germination, genetic diversity.

1. Introduction

Indian mustard (*Brassica juncea* L. Czern & Coss.) is one of the most important oilseed crops cultivated worldwide and contributes substantially to edible oil production, particularly in South Asian countries. India occupies a leading position in both acreage and production of rapeseed-mustard, where the crop serves as an important source of edible oil, livestock feed and industrial raw material. Despite continuous genetic improvement, the productivity of Indian mustard remains lower than its potential because of limited exploitation of available genetic diversity and the narrow genetic base of many breeding populations.

Genetic diversity is a fundamental prerequisite for crop improvement programme. The availability of genetically diverse parental material enhances the probability of obtaining superior recombinants possessing desirable combinations of agronomic, yield and quality traits. Therefore, characterization of germplasm based on genetic divergence provides valuable information for the selection of suitable parents in hybridization programmes. Among several multivariate techniques, Mahalanobis D^2 statistics has been widely employed for assessing genetic divergence because it simultaneously considers multiple quantitative traits and accurately estimates the genetic distance among genotypes. The grouping of genotypes into distinct clusters facilitates the identification of genetically divergent parents that can produce transgressive segregants and broaden the genetic base of breeding populations.

Besides genetic diversity, seed quality is another critical determinant of crop establishment and productivity. High seed vigour and germination ensure rapid emergence, uniform crop establishment and improved field performance. Seed quality traits such as germination percentage, seedling length and seed vigour index are therefore considered important supplementary criteria during genotype evaluation and varietal improvement programmes. The simultaneous assessment of genetic divergence together with seed quality characteristics provides a comprehensive understanding of breeding materials and assists in identifying promising parental lines with superior agronomic performance and enhanced physiological seed quality.

Although several studies have reported genetic variability in Indian mustard, comparatively fewer investigations have integrated genetic divergence analysis with seed quality parameters using the same set of genotypes. Therefore, the present study was undertaken to estimate the genetic divergence among twenty-five Indian mustard genotypes using Mahalanobis D^2 statistics and to evaluate their seed germination, seedling length and seed vigour index for identifying promising genotypes suitable for future breeding programmes.

Objectives

1. To estimate the genetic divergence among Indian mustard genotypes using Mahalanobis D^2 statistics.

2. To classify the genotypes into distinct clusters based on multivariate analysis.
3. To determine the relative contribution of different agronomic traits towards total genetic divergence.
4. To evaluate seed germination, seedling length and seed vigour index among the studied genotypes.
5. To identify genetically diverse and physiologically superior genotypes for use in future hybridization and crop improvement programmes.

2. Materials and Methods

2.1 Experimental site

The present investigation entitled “**Genetic Divergence and Seed Quality Assessment of Indian Mustard (*Brassica juncea* L.) Genotypes Using Mahalanobis D² Statistics**” was conducted during the **Rabi season of 2022–23** at the experimental farm of the **All India Coordinated Research Project on Rapeseed and Mustard (AICRP-R&M), Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-Jammu), Chatha, Jammu, India**. The experimental site is situated in the subtropical agro-climatic region of Jammu, which is suitable for rapeseed-mustard cultivation.

2.2 Experimental material

The experimental material comprised **twenty-five genetically diverse Indian mustard (*Brassica juncea* L.) genotypes** collected from different breeding programmes. These genotypes represented a broad spectrum of genetic variability for agronomic, yield and seed quality traits and were evaluated to identify promising parental lines for future breeding programmes.

2.3 Experimental design and crop management

The experiment was laid out in a **Randomized Complete Block Design (RCBD)** with **three replications**. Each genotype was grown in a plot measuring **7.2 m²**, maintaining recommended agronomic practices throughout the crop growth period. Standard cultural operations, including fertilizer application, irrigation, weed management and plant protection measures, were followed uniformly to minimize environmental variation.

Observations were recorded on **five randomly selected competitive plants** from each genotype in every replication, except for traits recorded on a plot basis.

2.4 Traits recorded

Twelve quantitative characters were recorded following the standard descriptors for *Brassica* species:

- Days to 50% flowering

- Days to maturity
- Plant height (cm)
- Length of main shoot (cm)
- Number of primary branches per plant
- Number of secondary branches per plant
- Silique length (cm)
- Number of seeds per silique
- Seed diameter (mm)
- Test weight (g)
- Number of siliquae per plant
- Seed yield per plant (g)

In addition, the seed quality parameters recorded included:

- Seed germination (%)
- Seedling length (cm)
- Seed vigour index

2.5 Statistical analysis

Analysis of variance

Analysis of variance (ANOVA) was performed to determine the significance of variation among the evaluated genotypes following the procedure described by Gomez and Gomez (1984). The significance of treatment effects was tested using the F-test at the 5% and 1% probability levels.

Genetic divergence analysis

Genetic divergence among the twenty-five genotypes was estimated using **Mahalanobis' D² statistics** (Mahalanobis, 1936). Pairwise generalized distances were computed using twelve quantitative traits. Based on the D² values, genotypes were grouped into different clusters following **Tocher's clustering method** as described by Rao (1952).

The clustering pattern was used to estimate:

- Number of clusters
- Distribution of genotypes
- Intra-cluster distance
- Inter-cluster distance
- Cluster mean values
- Relative contribution of individual traits towards total genetic divergence

Greater inter-cluster distance was considered indicative of higher genetic divergence. Therefore, greater potential for selecting parents in hybridization programmes.

Seed quality evaluation

Seed germination was determined using the standard laboratory germination test following the procedures recommended by the **International Seed Testing Association (ISTA)**. Germination percentage was calculated based on normal seedlings counted after the prescribed incubation period.

Seedling length was measured by recording the combined root and shoot length of normal seedlings and expressed in centimeters.

The **Seed Vigour Index (SVI)** was calculated according to the method proposed by Abdul-Baki and Anderson (1973):

Seed Vigour Index = Germination (%) × Seedling Length (cm)

This index was used to compare the physiological quality and establishment potential of the evaluated genotypes.

2.6 Data interpretation

Cluster means were used to identify superior genotypes for individual traits. The relative contribution of each character toward total genetic divergence was estimated based on the frequency of first-ranked contributions in pairwise comparisons. Genotypes belonging to clusters separated by large inter-cluster distances and possessing desirable cluster mean values were considered promising parents for future hybridization programmes aimed at improving yield and seed quality.

3. Results and Discussion

3.1 Genetic divergence analysis

Assessment of genetic diversity among breeding materials is an essential prerequisite for selecting suitable parents in hybridization programmes. In the present investigation, genetic divergence among twenty-five Indian mustard (*Brassica juncea* L.) genotypes was estimated using Mahalanobis' D² statistics, and the genotypes were grouped according to Tocher's clustering method.

The analysis revealed considerable genetic diversity among the evaluated genotypes, resulting in the formation of **six distinct clusters**. The presence of multiple clusters indicates substantial variability among the genotypes and provides opportunities for selecting genetically diverse parents for breeding programmes.

3.2 Distribution of genotypes into clusters

The twenty-five genotypes were grouped into six clusters based on multivariate analysis (Table 1). Cluster I was the largest, comprising sixteen genotypes, followed by Cluster II with five genotypes. Clusters III, IV, V and VI each contained a single genotype.

The occurrence of a large number of genotypes within Cluster I suggests a relatively close genetic relationship among these accessions despite their different origins. Conversely, the formation of four solitary clusters indicates the presence of highly divergent genotypes possessing unique genetic constitutions. Such isolated genotypes may serve as valuable parental sources for developing broad-spectrum variability in breeding programmes.

Table 1. Distribution of Indian mustard genotypes into different clusters

Cluster	Number of genotypes
I	16
II	5
III	1
IV	1
V	1
VI	1

The clustering pattern further demonstrates that geographical origin alone does not necessarily correspond to genetic divergence. Genotypes originating from different breeding programmes may cluster together because of similar selection histories, while genetically distinct lines may occur as solitary clusters.

These findings agree with previous reports by Verma (2000), Lodhi *et al.* (2013), Mohan *et al.* (2017) and Tudu *et al.* (2018), who also observed that clustering in Indian mustard is governed primarily by genetic constitution rather than geographical distribution.

3.3 Inter- and intra-cluster distances

The average intra- and inter-cluster distances are presented in **Table 2**.

Among all clusters, **Cluster II exhibited the highest intra-cluster distance (68.40)**, followed by **Cluster I (46.55)**. Greater intra-cluster distance indicates comparatively higher variability among genotypes within the same cluster, suggesting that further selection among these genotypes may still be rewarding.

Clusters III, IV, V and VI each consisted of a single genotype and therefore recorded **zero intra-cluster distance**, reflecting the absence of within-cluster variation.

The inter-cluster distances were considerably higher than the intra-cluster distances, indicating substantial genetic divergence among the clusters. The **maximum inter-cluster distance was observed between Clusters V and VI (214.60)**, followed by **Clusters II and IV (197.66)**. Such widely separated clusters are expected to possess maximum genetic diversity, and crosses involving genotypes from these clusters are likely to generate greater heterosis and wider segregation in subsequent generations.

On the other hand, the **lowest inter-cluster distance was recorded between Clusters IV and V (55.59)**, followed by **Clusters I and III (66.04)**, indicating relatively close genetic relationships among these clusters.

Table 2. Major inter- and intra-cluster distances

Parameter	Cluster combination	Distance
Highest intra-cluster	Cluster II	68.40
Second highest intra-cluster	Cluster I	46.55
Highest inter-cluster	Cluster V × Cluster VI	214.60
Second highest inter-cluster	Cluster II × Cluster IV	197.66
Lowest inter-cluster	Cluster IV × Cluster V	55.59

He present findings suggest that hybridization between genotypes belonging to **Clusters V and VI** or **Clusters II and IV** may produce superior recombinants because of the greater genetic divergence between these groups. Selection of parents from genetically distant clusters has been widely recommended to maximize heterosis and broaden the genetic base of breeding populations.

Similar observations have been reported by Gupta *et al.* (2015), Devi *et al.* (2017), Kumar *et al.* (2017) and Vanukuri *et al.* (2022), who also emphasized the importance of inter-cluster distances in selecting parents for hybridization.

Interpretation for breeders

The clustering pattern indicates that the germplasm possesses adequate genetic diversity for crop improvement. Solitary clusters (III–VI) represent unique genetic resources and should be conserved for future breeding programmes. Crosses involving genotypes from clusters separated by large inter-cluster distances, particularly **PHR-2 (Cluster V)** and **DRMRCI (Q)-57 (Cluster VI)** or representative genotypes from **Cluster II** and **Cluster IV**, are expected to generate broad genetic variability and may facilitate the development of high-yielding mustard cultivars with improved seed quality.

3.4 Cluster Mean Performance

Cluster mean analysis provides valuable information for identifying clusters possessing superior mean values for economically important traits. Selection of parents from clusters with desirable mean performance and large inter-cluster distances enhances the probability of obtaining superior recombinants in segregating generations. The mean performance of the six clusters for twelve quantitative traits is presented in **Table 3**.

Cluster I exhibited the earliest flowering (48.33 days), whereas Cluster III recorded the tallest plants (224.33 cm). Cluster II was characterized by superior values for several

important yield-contributing traits, including length of main shoot (65.00 cm), number of secondary branches (20.47), number of siliquae per plant (268.60) and seed yield per plant (13.30 g). These observations indicate that genotypes belonging to Cluster II possess desirable yield attributes and may serve as valuable donor parents in future breeding programmes.

Cluster V recorded the highest siliqua length (5.10 cm) and seed diameter (1.87 mm), whereas Cluster IV exhibited the highest test weight (5.00 g). Cluster VI showed the highest number of primary branches (12.67), while Cluster III recorded the maximum number of seeds per siliqua (12.00).

The wide variation observed among cluster means indicates the presence of substantial genetic variability for different agronomic traits. Therefore, parent selection should not be based solely on genetic distance but should also consider cluster mean performance for target traits.

Table 3. Superior clusters for important traits

Character	Best Cluster	Mean
Earliness (Days to 50% flowering)	Cluster I	48.33
Plant height	Cluster III	224.33 cm
Length of main shoot	Cluster II	65.00 cm
Primary branches	Cluster VI	12.67
Secondary branches	Cluster III	23.00
Siliqua length	Cluster V	5.10 cm
Seeds per siliqua	Cluster III	12.00
Seed diameter	Cluster V	1.87 mm
Test weight	Cluster IV	5.00 g
Number of siliquae per plant	Cluster VI	310.00
Seed yield per plant	Cluster II	13.30 g

3.5 Contribution of Individual Traits towards Genetic Divergence

The relative contribution of different characters towards total genetic divergence is presented in **Table 4**.

Among the twelve characters studied, **seed yield per plant contributed the highest proportion (15.22%)** towards total divergence, followed by **test weight (12.54%)**, **days to maturity (11.00%)**, and **number of siliquae per plant (10.80%)**. These findings indicate that yield-related characters played a major role in differentiating the evaluated genotypes.

Conversely, **plant height contributed only 0.33%**, indicating that this trait had minimal influence on overall genetic divergence. Likewise, siliqua length and seed diameter showed relatively low contributions.

The predominance of yield-associated traits in determining divergence suggests that these characters should receive greater emphasis while selecting parents for hybridization programmes. Improvement in these traits is expected to contribute substantially towards genetic gain in future breeding efforts.

Table 4. Relative contribution of different characters towards total genetic divergence

Trait	Contribution (%)
Seed yield per plant	15.22
Test weight	12.54
Days to maturity	11.00
Number of siliquae per plant	10.80
Days to 50% flowering	9.00
Number of seeds per siliqua	8.90
Number of primary branches	7.67
Length of main shoot	7.00
Number of secondary branches	6.78
Seed diameter	5.76
Siliqua length	5.00
Plant height	0.33

The present findings are consistent with earlier reports indicating that seed yield and its component traits contribute substantially towards genetic divergence in Indian mustard and therefore represent important selection criteria for breeding programmes.

3.6 Seed Germination, Seedling Length and Seed Vigour Index

Considerable variation was observed among the twenty-five mustard genotypes for seed germination percentage, seedling length and seed vigour index, demonstrating the existence of substantial variability for physiological seed quality traits.

Seed germination ranged from **74.00% to 96.66%**. The highest germination percentage was recorded in **DRMR 2020-15 (96.66%)**, closely followed by **DRMRDR 2203 (96.33%)**, whereas **DRMRDR 2156 (74.00%)** and **ROHINI (SC) (74.33%)** exhibited comparatively lower germination.

Marked differences were also observed for seedling length, which ranged from **4.00 cm to 9.46 cm**. The genotype **DRMRDR-2141** recorded the longest seedlings (9.46 cm), indicating greater seedling vigour and enhanced early establishment potential.

Similarly, seed vigour index varied widely among genotypes, ranging from **294.93 to 792.66**. The highest vigour index was observed in **DRMRDR-2141 (792.66)**, followed by **NRCHB 101 (738.60)** and **DRMR 2020-15 (737.46)**. In contrast, **ROHINI (SC)** recorded the lowest vigour index (294.93).

These results clearly indicate that substantial variability exists among the evaluated genotypes for physiological seed quality. Genotypes exhibiting high germination and vigour index are expected to establish more rapidly under field conditions and therefore constitute valuable parental material for developing high-quality seed lots.

The observed variability in seed quality traits agrees with previous findings reported in Indian mustard and further confirms the usefulness of incorporating seed physiological traits into breeding programmes.

Breeding implications

Based on both genetic divergence and seed quality performance, the following genotypes emerge as particularly promising:

- **DRMRDR-2141** – Exceptional seedling length and highest seed vigour index.
- **DRMR 2020-15** – Excellent germination combined with high seed quality.
- **DRMRDR 2203** – Superior germination percentage.
- **PHR-2** and **DRMRCI (Q)-57** – Represent highly divergent genetic resources due to their placement in separate clusters.

Hybridization involving genetically distant parents that also possess superior seed quality may increase the likelihood of obtaining high-yielding, vigorous and genetically diverse progenies.

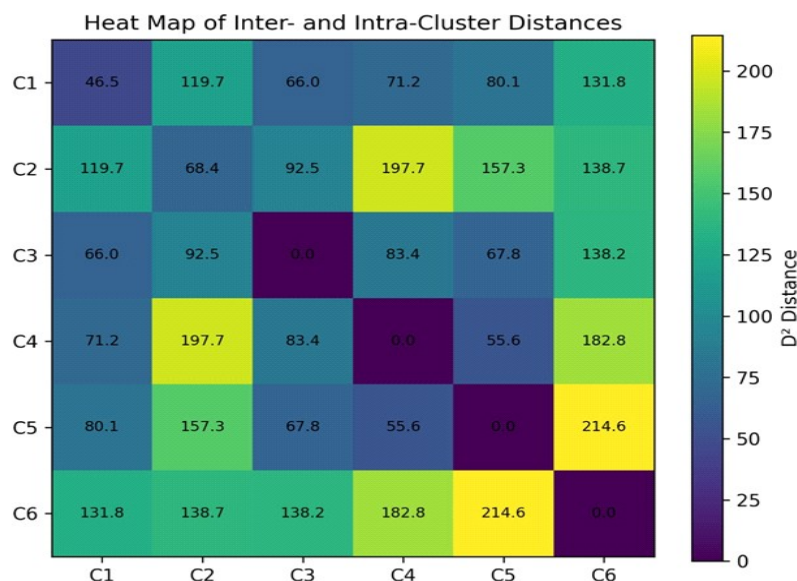


Fig 1. Contribution of different traits towards genetic divergence (Heat map).

Fig. 2. Contribution of different traits towards genetic divergence (Pie chart).

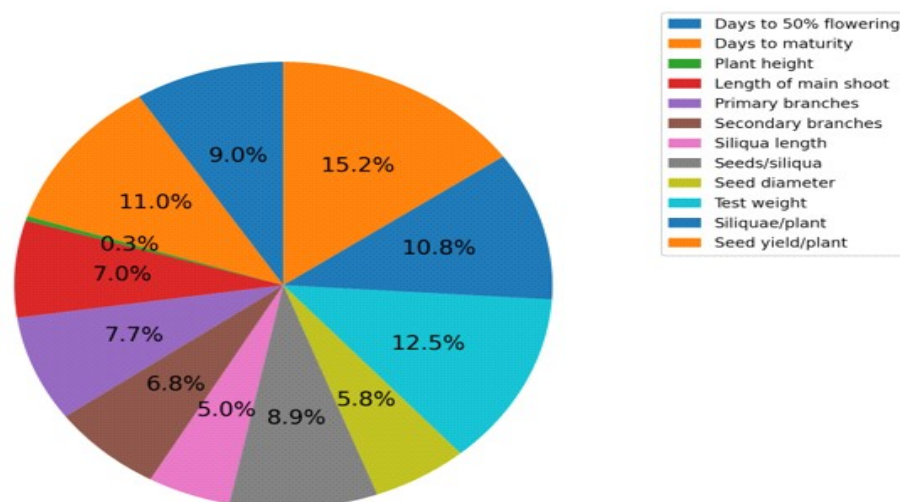
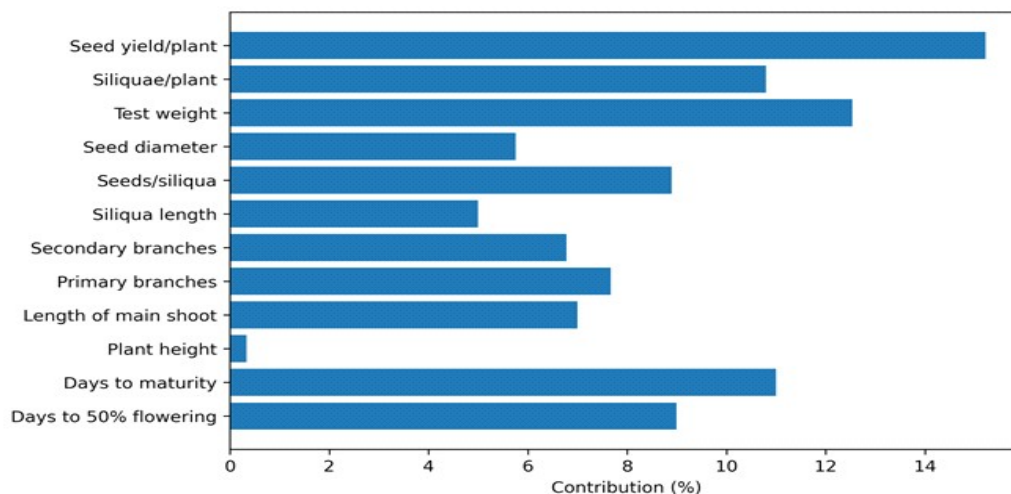


Fig 3. Contribution of different traits towards genetic divergence (Bar chart).



4. Conclusion

The present investigation demonstrated the existence of substantial genetic diversity among twenty-five Indian mustard (*Brassica juncea* L.) genotypes evaluated using Mahalanobis D² statistics. The genotypes were grouped into six distinct clusters, reflecting

a broad range of genetic variability that can be effectively utilized in future breeding programmes.

The occurrence of maximum inter-cluster distance between **Clusters V and VI** indicated the presence of highly divergent genotypes, suggesting that crosses between these clusters are likely to generate greater heterosis and wider genetic recombination. In contrast, the comparatively higher intra-cluster variability observed in Cluster II provides opportunities for selection even within the same cluster.

Among the evaluated traits, **seed yield per plant, test weight, days to maturity, and number of siliquae per plant** contributed the greatest proportion toward total genetic divergence, highlighting their importance in parent selection and breeding strategies.

Seed quality evaluation further revealed considerable variability among genotypes for germination percentage, seedling length, and seed vigour index. **DRMRDR-2141** exhibited superior seedling length and the highest seed vigour index, whereas **DRMR 2020-15** and **DRMRDR 2203** recorded excellent germination performance.

Overall, the combined evaluation of genetic divergence and seed quality parameters identified several promising genotypes that may serve as valuable parents for developing high-yielding, genetically diverse, and physiologically superior Indian mustard cultivars.

Breeding Recommendations

Based on the present investigation, the following recommendations are proposed:

- Crosses between **PHR-2 (Cluster V)** and **DRMRCI (Q)-57 (Cluster VI)** are expected to generate maximum genetic variability because of the highest inter-cluster distance.
- **Cluster II** contains genotypes with superior yield attributes and should be considered an important donor group for breeding.
- **DRMRDR-2141** may be utilized as a parent for improving seed vigour and early seedling establishment.
- **DRMR 2020-15** and **DRMRDR 2203** may be exploited for improving seed germination and overall seed quality.
- Simultaneous selection for **seed yield, test weight, and number of siliquae per plant** is likely to improve breeding efficiency because these traits contributed most to genetic divergence.

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