Assessment of Genetic Diversity in Indian Mustard (*Brassica juncea* L. Czern & Coss) Genotypes Using D² Analysis

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Authors' contributions

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

Original Research Article

ABSTRACT

A total 44 varied genotypes of Indian mustard were assessed for Genetic divergence during the 2021-22 Rabi season, using a randomized complete block design (RCBD) comprising 3 replications. Fourteen distinct characteristics, including days to 50% flowering, days of maturity, plant height, No. of primary and secondary branches/ plant, pod length, No. of pod on main shoot, length of main shoot, No. of seeds per pod, test weight, B.Y, H.I, oil content and grain yield were

observed. D^2 values employed to classify the genotypes into seven clusters, with largest cluster being cluster II, comprising 10 genotypes. Cluster III exhibited maximum intra-cluster distance, while most significant inter-cluster distance was observed between cluster II and IV. This suggested that genotypes in cluster II possess a broad range of genetic diversity. Notably, days to maturity played a crucial role in contributing to Genetic divergence among the traits studied.

Keywords: Indian mustard; D^2 analysis; genetic divergence.

1. INTRODUCTION

In India, the oilseeds Brassica are commonly referred to as Rapeseed-mustard crops. This category includes both traditionally cultivated indigenous species, such as Toria (*Brassica compestris* var. toria), Brown sarson (*Brassica compestris* var. brown sarson), Yellow sarson (*Brassica compestris* var. yellow sarson), Indian mustard (*Brassica juncea*), Black mustard (*Brassica nigra*) & Taramira (*Eruca sativa*). These indigenous species meant to be cultivated approximately 3,500 B.C.E. In addition to these traditional varieties, there are unconventional sp. as Gobhi sarson (*Brassica napus*, 2n (AACC) = 38), Ethiopian mustard or Karan rai (*Brassica carinata*, 2n (BBCC) =34). The plants are characterized by their tall stature (ranging from 90 to 200 cm), erect growth habit, and extensive branching. The base of the leaves is dilated, and they are wide, and pinnately compound leaves. The slender, long fruit (siliquae) ascend strongly or stand erect accompanied by small, round beaks. Mustard seed colour is brown or dark brown with a rough/ uneven seed coat. *Brassica juncea* primarily a self-pollinating crop, as indicated by Labana et al. [1]. Nevertheless, in certain environments, the rate of outcrossing ranges from 7.6% to 22%. In India, the area under cultivation, production & productivity of Rapeseed and Mustard are 6.69 million ha., 10.11 million tonnes, and 1511 Kg per ha, respectively. Specifically in Uttar Pradesh, the production & productivity of Rapeseed and Mustard are 0.7 million ha., 0.99 million tones, and 1412 Kg/ha, respectively (Source: Ministry of Agriculture and Farmers Welfare, Department of Agriculture, Cooperation and Farmers Welfare, Directorate of Economics & Statistics, Agriculture Statistics Division, 2020-2021).Mustard seeds serve diverse purposes, including medicinal applications, spice production, and incorporation into salads, juices, curries, and pickles. Evaluating the genetic diversity within and between germplasm groups is crucial, aiding in the selection of suitable parents to achieve heightened heterosis and obtain valuable recombinants. Various methods have been proposed to assess D^2 in crops [2], with Mahalanobis generalized distance, calculated through the D^2 statistic by Rao in 1952, standing out as a distinctive tool. This approach discriminates between populations by considering a set of parameters collectively, as opposed to relying on indices derived from morphological matching, eco-geographical variations, evolutionary relationships. The current study aimed to determine the genetic divergence in 44 Indian mustard genotypes using the D^2 statistical approach.

2. MATERIALS AND METHODS

The study was performed at the CRC of SVPUA&T in Meerut, Uttar Pradesh in the Rabi season of 2021-2022. Meerut is situated at 29°01' latitudes in the North, 77°45' longitudes, and an altitude at 277 meters above MSL, representing North Western plain zone. The experimental field had fertile sandy loam soil. A total of 44 germplasms were used as experimental materials and were cultivated in 3 replications following the Randomized Complete block design. Every genotype was planted in three rows with a length of 5.0 meters, maintain row to row spacing 45 cm and plant to plant spacing 15 cm. Proper thinning was done to ensure the required spacing between plants. Standard agricultural practices were adhered to for optimal crop growth. Three competitive plants out of every plot were randomly selected for take down observations on various quantitative characters, including days to 50% flowering, days of maturity, plant height (cm), No. of 1' branches / plant, No. of 2' branches/ plant, No. of pods on the main shoot, length of the main shoot (cm), pod length (cm), No. of seeds/pod, 1000 grain weight (g), B.Y/ plant (g), H.I (%), oil content (%), and G.Y/ plant (g).

Days to 50% flowering and days to maturity was observed on a plot base. Oil content was determined by use of Fourier Transform Near-Infrared Reflectance Spectroscopy (FT-NIRS) at the Central Soil Salinity Research Institute in Karnal. Genetic divergence among the 44

genotypes of Indian mustard was assessed using Mahalanobis D2 [3] as described by Rao [4].

3. RESULTS AND DISCUSSION

The 44 genotypes categorized in 7 clusters on the basis of their D^2 values. Clustering pattern Shown in detailed in Table 1. The second cluster stood out as the largest, encompassing ten genotypes: RH-1934, PM-30, RH-1807, CS-2005-143, RH-1975, RH- 1706, Kranti, LES-60, JC-36, CS-54. Cluster seven followed closely with nine genotypes, including JM-16-5, HUJM-10-6, DRMRCI-147, RH-749, ORM-18-29-5, PBR-939, RMM-1918, CS-60, Pusa Vijay. Cluster one consisted of seven genotypes: HUJM-20-9, KMR-21-4, DRMRIJ-18-62, ACN-237, Giriraj, Kranti, DRMR-2019-19. Cluster four comprised five genotypes: RB-109, NPJ-252, DAUM-21-1, SKM-1744, SKM-1801. Cluster five included five genotypes: DM -1521, NPJ-253, RH-2049, PRB-2016-1, RH-1974. Cluster three contained four genotypes: RGN-507, PDZ-14, PDZ-1, PDZ-15. Similarly, cluster six also had four genotypes: KMR-21-3, RGN-524, PR-2019-3, PM-29. The clustering shows that genotypes in the identical cluster were genetically similar, while those spread across different clusters contributed to the apparent high level of diversity. There was a lack of correlation B/W genetic diversity & the geographic origin of the genotypes. Similar findings were noted in research carried out by Bind [5], Saleem [6], Nagda [7], Singh [8], and Nandi [9]. The current investigation has identified significant genetic variations among clusters based on the mean comparison of fourteen characteristics shown in Table 3, Cluster III (51.58 days) exhibited early flowering, whereas Cluster VI (59.83 days) showed a delayed flowering pattern.

Genotypes in Cluster I (142.81 days) displayed early maturity, while those in Cluster VII (147.93 days) demonstrated late maturity. The genotypes with higher plant height were grouped in Cluster VI (190.84), while those in Cluster I (160.48) were characterized as dwarf. The No. of 1' branches/plant express the maximum mean (6.11) in Cluster III and the lowest mean (4.73) in Cluster I. The No. of 2' branches/plant reached maximum mean (13.30) in Cluster III and the minimum mean (6.93) in Cluster V. Clusters V and III exhibited the highest (63.69) and lowest (44.47) mean for the length of the main shoot, respectively.

The No. of pods on the main shoot had highest mean (41.79) in Cluster III and the lowest mean (33.03) in Cluster I. Pod length displayed the highest mean (6.56) in Cluster V and the lowest mean (5.26) in Cluster VI. The mean for the No. of seeds / pod was the highest (13.75) and lowest (10.03) in Clusters I and IV, respectively. The test weight mean was the highest (6.69) in Cluster V and min. (4.59) in Cluster IV. Cluster IV exhibited the maximum value (39.41) for oil content, while the minimum (38.69) was noted in Cluster VI. The mean for harvest index was the highest (35.83%) in Cluster V and the lowest (21.86%) in Cluster VI. Biological yield per plant had the highest mean (60.42) in Cluster II and the lowest (38.22) in Cluster IV. The maximal average grain yield per plant, recorded at 20.08, was shown in cluster II, whereas the minimal mean yield, at 9.44, was found in cluster VI. These results suggest that genotypes with both high and low mean values for specific traits tend to cluster together, with clusters exhibiting elevated mean values for their respective characteristics.

In terms of Intra-cluster distances displays in Table 2, cluster III displayed greatest distance at 2.933, followed by cluster II at 2.848, cluster V at 2.616, cluster VI at 2.606, cluster VII at 2.405, and cluster IV at 2.250. Conversely, cluster I exhibited the smallest intra-cluster distance, measuring at 2.132. This unveiled the presence of diverse genotypes among various clusters. The likelihood of generating desirable segregates through the interbreeding of genotypes from the same cluster, characterized by a low intra-cluster distance, is quite slim. Consequently, it would be logical to explore crosses between genotypes of cluster III (RGN- 507, PDZ-14, PDZ-1, PDZ-15), which exhibit larger inter-cluster distances compared to genotypes in other clusters. The choosing of cluster genotypes for crossbreeding can be in accordance with their superior mean values, seed yield, and yield components. Clusters second and fourth demonstrated the highest inter-cluster distance values at 5.467, followed by clusters second and sixth (5.439), third and fifth (5.414), first and sixth (5.330), fifth and sixth (5.302), third and sixth (5.132), first and third (4.863), third and fourth (4.756), first and second (4.449), second and fifth (4.057), fourth and fifth (4.021), second and third (4.006), sixth and seventh (3.785), third and seventh (3.737), fourth and seventh (3.688), fourth and sixth (3.663), first and fourth (3.495), first and seventh (3.250), first and fifth (3.085), and fifth and seventh (3.012), while the minimal inter-cluster distance were shown between clusters second & seventh (2.906). The maximum inter cluster

Table 1. Clustering pattern of forty-four genotypes of Indian mustard (*Brassica juncea* L.) on basis of D² cluster analysis

Clusters	No of genotypes	Genotypes
I	7	HUJM-20-9, KMR-21-4, DRMRIJ-18-62, ACN-237, Giriraj, Kranti and DRMR-2019-19
II	10	RH-1934, PM-30, RH-1807, CS-2005-143, RH-1975, RH-1706, Kranti, LES-60, JC-36 and CS-54
III	4	RGN-507, PDZ-14, PDZ-1 and PDZ-15
IV	5	RB-109, NPJ-252, DAUM-21-1, SKM-1744 and SKM-1801
V	5	DM-1521, NPJ-253, RH-2049, PRB-2016-1 and RH-1974
VI	4	KMR-21-3, RGN-524, PR-2019-3 and PM-29.
VII	9	JM-16-5, HUJM-10-6, DRMRCI-147, RH-749, ORM -18-29-5, PBR-939, RMM-1918, CS-60 and Pusa Vijay

Table 2. Inter and Intra cluster distances among 7 clusters of 44 genotypes of Indian Mustard

Clusters	I	II	III	IV	V	VI	VII
I	2.132						
II	4.449	2.848					
III	4.863	4.006	2.933				
IV	3.495	5.467	4.756	2.250			
V	3.085	4.057	5.414	4.021	2.616		
VI	5.330	5.439	5.132	3.663	5.302	2.606	
VII	3.250	2.906	3.737	3.688	3.012	3.785	2.405

Table 3. Cluster mean for fourteen characters of forty-four genotypes in Indian mustard (*Brassica juncea* L.)

Clusters	Days to 50% flowering	Days to maturity	Plant height	No. of primary branches per plant	No. of secondary branches per plant	No. of pod on main shoot	Length of main shoot (cm)	Pod length (cm)	No. of seeds per pod	Test weight (g)	Oil content (%)	Harvest index (%)	Biological yield (g/plant)	Grain yield (g/plant)
I	51.81	142.81	160.48	4.73	8.10	33.03	56.17	6.08	13.75	5.36	39.37	30.76	40.45	12.41
II	53.57	144.73	172.18	5.49	12.99	41.79	62.47	6.34	13.31	6.18	38.86	33.54	60.42	20.08
III	51.58	147.75	183.21	6.11	13.30	35.39	44.47	5.34	13.08	5.03	38.89	31.85	53.15	17.01
IV	56.80	146.80	167.87	5.40	8.18	33.53	55.91	5.66	10.03	4.59	39.41	24.83	38.22	9.48
V	52.80	145.13	172.28	4.76	6.93	34.31	63.69	6.56	10.71	6.69	39.33	35.83	42.48	15.32
VI	59.83	148.17	190.84	5.14	7.06	41.15	60.58	5.26	11.61	4.79	38.69	21.86	44.25	9.44
VII	55.70	147.93	174.56	4.74	9.93	36.89	57.33	6.33	13.07	5.83	38.94	30.52	50.33	15.17

Table 4. Contribution (%) of fourteen characters towards genetic divergence in Indian mustard (*Brassica juncea* L.)

Characters	Days to 50% flowering	Days to maturity	Plant height	No. of primary branches per plant	No. of secondary branches per plant	No. of pod on main shoot	Length of main shoot (cm)	Pod length (cm)	No. of seeds per pod	Test weight (g)	Oil content (%)	Harvest index (%)	Biological yield (g/plant)	Grain yield (g/plant)
Contribution	6.48	9.94	8.48	6.98	7.54	6.74	8.30	4.50	8.43	6.17	9.90	8.24	4.62	3.69

distance between clusters indicates that the second and fourth genotypes are not closely linked.

However, the minimal/lowest inter cluster distance between clusters suggest a strong correlation among the genetic types within these groupings. Notably, the second and seventh genotypes exhibit the lowest inter-cluster distance, indicating a close association. It is recommended to strengthen hybridization programs between genotypes of Cluster II (RH-1934, PM-30, RH-1807, CS-2005-143, RH-1975, RH-1706, Varuna, LES-60, JC-36, CS-54) and also between genotypes of Cluster IV (RB-109, NPJ-252, Baum-21-1, SKM-1744, SKM-1801) to potentially obtain transgressive segregants, thereby providing opportunities to select genetically diverse genotypes. Additionally, exploiting heterotic cross combinations could be advantageous for developing hybrids in Mustard. Similar findings have been reported by Kumar [10], Bind [5], Nagda [7], Rout [11], Singh [8], Nandi [9], and Margam [12].

The most significant influence on the manifestation of genetic diversity was observed in the days to maturity (9.94), which had the highest contribution to divergence. This was followed by oil content (9.90), the No. of 1' branches/plant (8.48), length of the main shoot (8.43), pod length (8.30), No. of pods on the main shoot (8.24), days to 50% flowering (7.54), No. of seeds per pod (6.98), G.Y/plant (6.74), plant height (6.48), and 1000 grain weight (6.17). On the other hand, characters such as harvest index (4.62), number of 2' branches per plant (4.50), and B.Y/ plant (3.69) made comparatively lower contributions to genetic divergence that shown in Table 4. Among the 14 studied characters, days to maturity, No. of 1' branches/plant, oil content, length of the main shoot, pod length, and No. of pods on the main shoot were identified as significant contributors to genetic diversity. These parameters could be considered in opting of diverse parents for hybridization programs aimed at further improving yield. Similar findings were reported by Lodhi [13], Singh [8], Tahira [14], Nagda [7], Rout [11], Gadi [15], Singh [8] and Nandi [9,16].

4. CONCLUSION

Cluster III exhibited highest intra-cluster distance, while Cluster I had minimum. Therefore, it would be reasonable to explore crosses between genotypes within Cluster III (RGN-507, PDZ-14,

PDZ-1, PDZ-15) that are separated by larger intra-cluster distances compared to genotypes in other clusters. The limited diversity and the selection of parents within a cluster with a higher mean for a specific trait could also prove beneficial in the further creation of high-yield cultivars of Indian mustard. The maximal inter-cluster distance was observed between Cluster II and IV, suggesting genotypes within Cluster II.

(RH-1934, PM-30, RH-1807, CS-2005-143, RH-1975, RH-1706, Varuna, LES-60, JC-36, CS-54) and Cluster IV (RB-109, NPJ-252, Baum-21-1, SKM-1744, SKM-1801) possess a wide spectrum of genetic diversity. A hybridization program between genotypes of these clusters may yield transgressive segregants, providing an opportunity to select genetically variable genotypes. Heterotic cross combinations could also be explored for the development of hybrids in mustard. Genotypes from these clusters could be chosen based on their high individual performance for inclusion in hybridization programs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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