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Genetic divergence in Indian mustard [*Brassica juncea* (L.) Czern and Coss] under sub-Himalayan region

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ABSTRACT

A study was carried out with 71 genotypes of Indian mustard [Brassica juncea (L.) Czern and Coss] under sub-Himalayan condition to examine the genetic diversity within them. All the 71 genotypes were tested in randomized complete block design with three replications during rabi 2017-18. The genetic divergence was studied among the genotypes of Indian mustard [Brassica juncea (L.) Czern & Coss] using Mahalanobis D^2 statistics followed by Rao (1952). Genotypes were found to be grouped into seven clusters. Cluster I had the largest number of genotypes (31) followed by cluster IV (21), V (12), II (two), III (two), VI (two) and VII (one). Maximum intra cluster divergence was found in cluster VI followed by cluster V, IV and I. Maximum inter cluster distance was found between cluster VII and V followed by cluster VII and VI, cluster VII and IV and cluster VII and I, which indicates that efficient breeding programme can be formulated to improve yield potential by hybridization between genotypes from these clusters. Based on the maximum intra cluster distance value the crosses could be made among the genotypes having the highest divergence like PHR-2, RNWR-09-3, Giriraj, Kranti, SKJM-05, DRMR-15-16, RW-85-59 and NPJ-194 from various clusters like IV, V, VI and VII to get desirable transgressive segregants. Cluster III having the highest seed yield (11.80 g plant^{-1}) had not shown highest genetic divergence from the other clusters. However, other clusters like cluster IV, V, VI, and VII had shown higher genetic divergence among themselves. Plant height (18.71) contributed maximum towards genetic divergence followed by 1000 seed weight (18.35) as well as penetration force (18.35), aphid count (12.76) and seed yield per plant (10.62). For the characters like plant height,1000 seed weight, penetration force, seed yield and aphid count contributing substantially high to the total genetic divergence, it was found that genetically divergent clusters namely IV, V, VI and VII performed optimally and amongst these clusters only. Cluster VII was the poorest seed yielder. This clearly reflected that the genetically divergent genotypes were distributed in the different clusters like cluster IV, V, VI and VII.

Keywords **:** D^2 statistics and genetic divergence

Brassica juncea (L.) Czern & Coss., also known by the name of Indian mustard, belongs to the plant family *Brassiceae* (*Cruciferae*) or the mustard family. India is world's fourth largest edible oil economy after the U.S., China and Brazil. The average contribution of rapeseed-mustard to the total oilseed production in India was 25.2per cent, with its average productivity 1304 (kg ha^1) during 2016-17 (www.srmr.org.in/nbc/). Though, rapeseed-mustard is placed 2nd in terms of production, after soybean, it ranks 1st in terms of oil yield among all oilseed crops. The estimated area, production and yield of rapeseed-mustard in the world was 36.68 million ha, 72.42 million tons and 1974 kg ha^{-1}, respectively during 2017-18. Globally, India accounts for 19.8 and 9.8 per cent of the total acreage and production (USDA 2016-17). During the last seven years, there has been a considerable increase in productivity from 1840 kg ha^{-1} in 2010-11 to 1974 kg ha^{-1} in 2017-18 and production has also increased from 61.64 m t in 2010-11 to 72.42 m t in 2017-18. (www.srmr.org.in/nbc/). India is the second largest importer of edible oilseeds after China. However, attempts to enhance its productivity significantly are not fully successful due to their cultivation under diverse and mostly constrained ecologies. Climate change can further limit the productive potential of the crop. Therefore, breeders always look for genetic divergence among traits to select desirable types. Genetic diversity plays an important role in plant breeding because hybrids between lines of diverse origin generally display a greater heterosis than those between closely related strains (Singh, 1983) which permits to select the genetically divergent parents to obtain the desirable recombination in the segregating generations. Hence, the present study was planned to estimate the genetic diversity through Mahalanobis D^2 technique, among the 71 Indian mustard genotypes, in respect of eleven characters influencing seed yield under the sub-Himalayan zone.

MATERIALS AND METHODS

The field trail was carried out at Instructional Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, during the *rabi* season of 2017-18. The experimental material consisted of 71 diverse genotypes of mustard collected from three diverse sources namely Pulses and Oilseed Research Station

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(PORS), Berhamapur, West Bengal, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh and Directorate of Rapeseed and Mustard Research (ICAR-DRMR), Bharatpur, Rajasthan. The experiment was conducted in Randomized Complete Block Design in three replications. Experiment was sown in a 3 row plot of 3 meter length. The row to row spacing was kept 60 cm and plant to plant distance was maintained at 15 cm by proper thinning. All cultural practices essential for the good crop of mustard were applied for obtaining healthy and competitive crop stand. Five randomly selected competitive plants from each genotype in each replication were used for the purpose of recording the observations on eleven characters. The data was recorded on 11 characters *viz*., Plant height(cm), height upto first fruiting branch (cm), days to 50% flowering, primary branches plant^{-1}, secondary branches per plant, siliquae plant^{-1}, seeds per siliquae, 1000 seed weight (g), penetration force (kpascal), aphid count (% incidence) and seed yield per plant (g).

Probe Penetration experiment (Mondal et al., 2017)

The positive and negative pressures were measured with the help of instrument called Texture analyzer through following protocol;

1. Stable micro system; 2 mm needle stainless (Plant code-P/2N)
2. Sequence manure; (Texture analyzer)

Caption	Value	units
Test mode	Compression	
Pre-test mode	1.00	/mm/sec
Test speed	1.00	/mm/sec
Post-test Speed	10.00	/mm/sec
Target mode	Distance	
Distance	1.00	/mm
Trigger type	Auto (force)	
Trigger force	5.0	g
Advance option	off	

The positive pressure was calculated and expressed in forms of various graphs in the system attached to the Texture analyzer. Positive pressure is the pressure that needs to puncture the tissue of the tough plant twig. This pressure is exerted by the aphid while it punctures to the twig.

The genetic divergence was estimated using Mahalanobis D^2 statistics (1936) followed by Rao (1952). The theory of the D^2 statistics is as follows:

Let us assume X_1, X_2, X_2, ... X_p are the multiple measurements(characters) on each individuals and d_1, d_2, d_3..... d_p are the differences between the mean of the two population for multiple characters viz., $\bar{X}_1^1$, $\bar{X}_1^2$, $\bar{X}_2^1$, $\bar{X}_2^2$.....$\bar{X}_p^1$, $\bar{X}_p^2$ respectively.

Then, D^2 statistic of Mahalonobis is as follows :

$D^2 = w^{ij}\left(\bar{x}_i^1 - \bar{x}_i^2\right)\left(\bar{x}_j^1 - \bar{x}_j^2\right)$, where, w^{ij} is the inverse of variance and co- variance matrix.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) was carried out to test the significance of variance among 71 diversified genotypes of mustard for all the eleven traits. The mean sum of square for all the traits is given in the table 1. Analysis of variance revealed significant differences among the material, used in the present investigation, for all the eleven characters studied *viz*; Plant height (cm), height upto first fruiting branch (cm), days to 50% flowering, primary branches plant^{-1}, secondary branches per plant, siliquae plant^{-1}, seeds per siliquae, 1000 seed weight (g), penetration force (kpascal), aphid count (% incidence) and seed yield per plant (g) which indicated wide spectrum of variation among the genotypes. Similar type of results were also reported by Burton *et al.* (2004), Hopkins *et al.* (2006), Alie *et al.* (2009) and El-Esawi *et al.* (2016).

The chi- square test indicated that the population of the mustard was divergent and therefore the D^2 analysis was carried out. The seventy one genotypes of mustard when subjected to D^2analysis, using eleven yield attributing characters revealed that seven clusters were formed. From the pattern of clustering it could be inferred that sufficient divergence was present to enable the formation of individual clusters. The clustering pattern could be utilized in selection of parents for crossing and deciding the best cross combinations which may generate the highest possible variability for various traits. Mahalanobis D^2 analysis of quantitative traits is a powerful tool for measuring genetic divergence among the material selected even from the same geographic region, reported by Mahalanobis (1936) followed by Rao (1952). Based on D^2 values seventy one genotypes of mustard were grouped into seven clusters (Table 2**).** A total of thirty one genotypes were grouped into cluster I namely B-85 (Seeta), RW-351 (Bhagarathi), RW-85-59 (Sarna), RW-4C-6-3 (Sanjukta Asech), NPJ-194, TM-276, Rohini (SC), KMR-15-4, PR-2012-9, Divya-88, RL-JEB-52, Kranti- NC, DRMRIJ-15-85, RH1202, NPJ-196, RMM-09-10, JMM-927-RC, RRN-871, KM-126, SKM-1313, RB-77, DRMR-15-5, KMR-53-3, RL-JEB-84, Ganga, RGN-73-JC, RH-1209, PR-2012-12, RGN- 385, NPJ-195 and Maya-C; twenty one genotypes in cluster IV namely SKJM-05, SVJ-64, Sitara-Sreenagar, RH-0923, DRMR-15-16, NPJ-198, JMM-927-RC, DRMR-15-47, RAURD-214, DRMR-15-14, DRMR-4001, RGN-384, NPJ-197, RB-81, NPJ-200, DRMR-15-9, KMR-L-15-6, PRD-2013-9, DRMRIJ-15-66, RH-1368, RH-406 and CS 54; twelve genotypes in

Table 1: Analysis of variance (ANOVA) for eleven characters in Indian mustard

Sources of variation	d.f.	Plant height (cm)	Height upto first fruiting branch (cm)	Days to 50 % flowering	Primary branches per plant	Secondary branches per plant	Siliquae per plant	Seeds per siliqua	1000 seed weight (g)	Aphid count (% incidence)	Penetration force (kpascal)	Seed yield per plant(g)
Treatment	70	727.92**	999.71**	67.10**	2.37**	12.44**	4181.39**	1.66**	2.90**	42.40**	2069.92**	16.71**
Error140	2.82	13.64	0.85	0.20	0.75	213.49	0.70	0.015	16.65	428.39	5.72	

*Note : ** Significant at 1% probability level*

cluster V namely RNWR-09-3, PRD-2013-2, GIRIRAJ, NRCHB-101, RGIN-73, DRMR-IJ-31, NRCHB-101, DRMR-150-35, RH-749, Pusa mustard 25 (NPJ112), Pusa mustard 26 (NPJ113) and Pusa mustard 27 (EJ17); two genotypes in cluster II namely JMM-927-RC, RH-1325), as well as in cluster III namely RGN-389 and RGN-386; two genotypes in cluster VI namely RL1358 and Kranti and one genotype in cluster VII namely PHR2. Similarly 19 diverse genotypes of Indian mustard were grouped into five clusters by Sinha and Singh (2004). Thirty-three diverse genotypes of Indian mustard were grouped into eight different clusters by Thul *et al.* (2004). Monalisa *et al.* (2005) carried out similar type of genetic divergence study in nine genotypes of Indian mustard and grouped them into six clusters using Tocher's method. Malik *et al.* (2006) studied 30 lines and cultivars of Indian mustard for 12 quantitative characters and grouped them into six clusters using Mahalanobis D^2 statistics. The clustering pattern of genotypes showed that the genotypes of different origin collected from Pulses and Oilseed Research Station, Berhampur, West Bengal, Banaras Hindu University and Directorate of Rapeseed- Mustard Research, Bharatpur were clubbed in one cluster, whereas the genotypes belonging to same origin were grouped in different clusters indicating that the geographic distribution didn't considered to be the sole criterion of genetic diversity. Similar type of work was also reported by Alie *et al.* (2011). Pattern of distribution of genotypes among various clusters reflected the considerable genetic diversity present in the genotypes under study.

In the present investigation the inter cluster and intra cluster distance was estimated among eleven characters (Table 3). The maximum intra cluster distance was recorded in cluster VI (943.87) followed by cluster V (524.72), cluster IV (472.12), cluster I (350.00), cluster III (22.21), cluster II (19.69) and cluster VII (0.00). The maximum intra cluster distance in cluster VI was because of wide genetic diversity among its genotypes. Similarly Bind *et al.* (2015) reported maximum intra cluster divergence was found in cluster III followed by cluster IV and cluster VI. Cluster III exhibited maximum intra cluster distance which indicated that genotype may be used to produce superior hybrid and transgressive segregants. Minimum intra cluster distance was observed for the cluster I reported by Gupta *et al.* (2015). Earlier studied performed by Chandra *et al.* (2018) reported cluster VI exhibited maximum intra cluster distance.

The maximum inter cluster distance was between cluster VII and V (3147.81) followed by cluster VII and VI (2564.55), cluster VII and IV (2560.63) and cluster VII and I (2507.42). On minute observation of distance

Table 2: Distribution of seventy one genotypes of Indian mustard in seven clusters

Cluster	No. of genotypes	Genotypes
I	31	B-85(Seeta), RW-351(Bhagarathi),RW-85-59 (Sarna),RW-4C-6-3 (Sanjukta Asech), NPJ-194, TM-276, Rohini(SC), KMR-15-4, PR-2012-9, Divya-88, RL-JEB-52, Kranti- NC, DRMRIJ-15-85, RH1202, NPJ-196, RMM-09-10, JMM-927-RC, RRN-871, KM-126, SKM-1313, RB-77, DRMR-15-5, KMR-53-3, RL-JEB-84, Ganga, RGN-73-JC, RH-1209, PR-2012-12, RGN- 385, NPJ-195, Maya-C
II	2	JMM-927-RC, RH-1325
III	2	RGN-389, RGN-386
IV	21	SKJM-05,SVJ-64, Sitara-Sreenagar, RH-0923, DRMR-15-16, NPJ-198, JMM-927-RC, DRMR-15-47, RAURD-214, DRMR-15-14, DRMR-4001, RGN-384, NPJ-197, RB-81, NPJ-200, DRMR-15-9, KMR-L-15-6, PRD-2013-9, DRMRIJ-15-66, RH-1368, RH-406, CS 54
V	12	RNWR-09-3, PRD-2013-2, GIRIRAJ, NRCHB-101, RGIN-73, DRMR-IJ-31, NRCHB-101, DRMR-150-35, RH-749, Pusa mustard 25 (NPJ112), Pusa mustard 26 (NPJ113), Pusa mustard 27 (EJ17)
VI	2	RL1358, Kranti
VII	1	PHR2

Table 3: Average intra (diagonal) and inter-cluster (off-diagonal) D^2 values in Indian mustard

Cluster	I	II	III	IV	V	VI	VII
I	**350.00**	325.19	542.99	414.56	491.54	523.97	2507.42
II		**19.692**	83.99	303.98	410.53	372.20	1954.86
III			**22.21**	496.72	682.97	587.32	1591.24
IV				**472.12**	515.42	538.13	2560.63
V					**524.72**	557.07	3147.81
VI						**943.87**	2564.55
VII							**0.00**

Table 4: Description of the genetically divergent clusters and distance (D^2 value) between the genotypes selected

Cluster combination	Inter cluster distance (D^2 value)	Genotype selected from the cluster	Distance between the genotypes selected (D^2 value)
Cluster VII and cluster V	3147.808	PHR -2 in cluster VII and RNWR-09-3 in cluster V	4662.847
Cluster VII and cluster V	3147.808	PHR -2 in cluster VII and Giriraj in cluster V	4342.083
Cluster VII and cluster VI	2564.546	PHR -2 in cluster VII and Kranti in cluster VI	3811.183
Cluster VII and cluster IV	2560.633	PHR -2 in cluster VII and SKJM-05 in cluster IV	6745.971
Cluster VII and cluster IV	2560.633	PHR -2 in cluster VII and DRMR-15-16 in cluster IV	3636.379
Cluster VII and cluster I	2507.415	PHR -2 in cluster VII and RW-85-59(Sarna) in cluster I	4950.465
Cluster VII and cluster I	2507.415	PHR -2 in cluster VII and NPJ-194 in cluster I	5853.214

Table 5: Cluster mean for the eleven characters in Indian mustard

Clusters	Plant height (cm)	Height upto first fruiting branch (cm)	Days to 50 % flowering	Primary branches per plant	Secondary branches per plant	Siliquae per plant	Seeds per siliqua	1000-seed weight (g)	Aphid count (% incidence)	Penetration force (k pascal)	Seed yield per plant (g)
I	167.43	57.85	44.94	3.72	10.09	182.24	12.94	4.55	10.17	97.47	9.46
II	182.48	57.87	49.00	4.30	8.53	145.30	13.00	5.02	9.05	58.37	9.48
III	192.77	58.93	52.00	4.00	10.73	178.13	13.23	4.97	8.94	56.44	11.80
IV	170.77	56.27	46.00	4.27	9.60	161.08	13.03	4.87	8.45	79.96	9.83
V	167.66	52.36	43.33	4.13	7.64	148.44	13.02	5.43	13.87	109.11	8.67
VI	168.17	76.43	48.00	4.53	6.43	125.37	13.70	5.45	9.40	106.64	5.81
VII	212.93	153.00	57.00	6.20	8.13	132.47	11.87	2.20	11.13	60.85	3.19

between the genotypes into different divergent clusters, it was revealed that PHR-2 in cluster VII and RNWR-09-3 in cluster V had a very high genotypic distance (D^2= 4662.847). Similar findings with high genetic distance between the genotypes in other clusters like PHR-2 in cluster VII and Giriraj in cluster V had high genotypic distance (D^2=4342.083). Information from other clusters like PHR-2 in cluster VII and Kranti in cluster VI had high genotypic distance (D^2=3811.183). Similarly PHR-2 in cluster VII and SKJM-05 in cluster IV had very high genotypic distance (D^2=6745.971) followed by PHR-2 in cluster VII and DRMR-15-16 in cluster IV (D^2= 3636.379). PHR-2 in cluster VII and RW-85-59 (Sarna) in cluster I very high genotypic distance (D^2= 4950.465) followed by PHR-2 in cluster VII and NPJ-194 in cluster I (D^2=5853.214). Hence, on the basis of the higher inter cluster distance value, the crosses could be made among the genotypes of cluster VII and cluster V (PHR 2 and RNWR-09-3; PHR 2 and Griraj), cluster VII and cluster VI (PHR 2 and Kranti), cluster VII and cluster IV (PHR 2 and SKJM-05 ; PHR 2 and DRMR-15-16), cluster VII and cluster I (PHR 2 and RW-85-59 (Sarna); PHR 2 and NPJ-194) as per their D^2 values for expecting better segregants. This clearly indicates that the genotypes included in these clusters are having broad spectrum of genetic diversity and could very well be used in hybridization programme for improving seed yield. Therefore, it would be logical to attempt crosses between the genotypes from the above mentioned clusters. Similarly Doddabhimappa *et al.* (2012) Cluster I and II showed maximum inter cluster distance followed by between cluster II and VII and cluster I and V. Khan *et al.* (2013) highest inter cluster distance between cluster I and V. Maximum inter cluster distance was found in cluster V and cluster VI by Bind *et al.* (2015).

The estimates of average intra and inter cluster distance value of seven clusters revealed that the genotypes belonging to the same cluster (intra cluster) have less genetic divergence as compared to genetic diversity between the genotypes of different clusters (inter cluster). The genotypes grouped into the same cluster displayed the lowest degree of divergence from one another and in case crosses are made between genotypes belonging to the same cluster, no transgressive segregant is expected from such combinations. Therefore, hybridization program should always be formulated in such a way that the parents belonging to different clusters with maximum genetic distance divergence could be utilized to get desirable transgressive segregants.

Highest cluster mean value (Table 5) for plant height was recorded in case of cluster VII (212.93), for height

Table 6: Contribution towards genetic divergence by the eleven characters in Indian mustard

Sl. No.	Characters	Contribution (%)
1.	Plant height (cm)	18.71
2.	Height upto first fruiting branch (cm)	3.86
3.	Days to 50 % flowering	8.09
4.	Primary branches per plant	0.60
5.	Secondary branches per plant	2.86
6.	Siliquae per plant	5.47
7.	Seeds per siliqua	0.32
8.	1000-seed weight (g)	18.35
9.	Aphid count (% incidence)	12.76
10.	Penetration force (k pascal)	18.35
11.	Seed yield per plant (g)	10.62

upto first fruiting branch highest cluster mean value was recorded in case of cluster VII (153.00), days to 50 % flowering in case of cluster VII (57.00), primary branches plant^{-1} highest cluster mean value was recorded in case of cluster VII (6.20), secondary branches plant^{1} in case of cluster III (10.73), siliquae plant^{-1} in case of cluster I (182.24), seeds per siliqua in case of cluster VI (13.70), 1000-seed weight in case of culture VI (5.45), aphid count in case of cluster V (13.86), penetration force in case of cluster V (109.11), seed yield plant^{-1} in case of cluster III (11.80). The results obtained in the present study are in accordance to the findings of Khan *et al.* (2013), Shekhawat *et al.* (2014) and Singh *et al*. (2018).

An interesting finding from the cluster mean for the seed yield is that the cluster III having the highest seed yield (11.80 g plant^{-1}) had not shown highest genetic divergence from the other clusters. However, other clusters like cluster IV (9.83 g plant^{-1}), cluster V (8.87 g plant^{-1}), cluster VI (5.81 g per plant) and cluster VII (3.19 g plant^{-1}) showed higher genetic divergence amongst themselves. For the characters like plant height, 1000 seed weight, penetration force, seed yield and aphid count contributing substantially high to the total genetic divergence, it was found that genetically divergent clusters namely IV, V, VI, and VII performed optimally and amongst these clusters only. Cluster VII was the poorest seed yielder. This clearly reflected that the genetically divergent genotypes were distributed in the different clusters like cluster IV, V, VI, VII. This is in confirmation with our own findings in this experiment with respect to the selection of genetically divergent genotypes from the clusters having the highest genetic divergent.

The percentage contribution (Table 6) of plant height (18.71%) has been maximum to divergence followed by 1000 seed weight (18.35%) as well as penetration force (18.35%), aphid count (12.76%), seed yield per plant (10.62%) and days to 50 % flowering (8.09%), contributed most towards genetic divergence, where as remaining characters contributed very little towards genetic divergence *i.e.,* siliquae plant^{-1} (5.47), height upto first fruiting branch (3.86), secondary branches plant^{-1} (2.86), primary branches plant^{-1} (0.60) and seeds per siliqua (0.32). In contrast, Shalini (1998) and Somu (2001) indicated that the no. of siliquae plant^{-1} followed by plant height and days to 50% flowering were the major contributors towards genetic divergence. Jahan *et al.* (2013) observed primary branches plant^{-1}, no. of secondary branches plant^{-1} and days to 50% flowering contributed maximum towards divergence. Similarly Devi *et al.* (2017) observed highest contribution percentage for no. of siliqua per plant followed by 1000 seed weight.

A proper follow up of this experiment would be justifiable if a suitable crossing programme is carried out using the eight most genetically divergent mustard genotypes identified on the basis of the inter cluster distance, intra cluster distance and the D^2 distance between the individual genotypes, namely PHR 2, RNWR-09-3, Giriraj, Kranti, SKJM-05, DRMR-15-16, RW-85-59 (Sarna) and NPJ-194.

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