Electronic Journal of Plant Breeding

Research Note

Diversity assessment among exotic and indigenous lines of Indian mustard for exploitation in breeding programme

Huynh Thi Phuong Lien, Ram Avtar, Nisha Kumari, Neeraj Kumar*, Vivek K. Singh, Manjeet Singh and Mahavir Bishnoi

Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125004
*E-Mail: neerajkummar8@gmail.com

Abstract
Genetic divergence using D^2 statistics was assessed for 13 different traits in a collection of 75 exotic and indigenous Indian mustard lines at CCS HAU Hisar. Based on genetic diversity, all were grouped into 15 clusters, of which cluster I had the maximum of 46 genotypes, followed by cluster V (8 genotypes), cluster III (7 genotypes) and cluster XII (3 genotypes). The remaining 11 clusters, *i.e.,* cluster II, cluster IV, cluster VI, cluster VII, cluster VIII, cluster IX, cluster X, cluster XI, cluster XIII, cluster XIV and cluster XV, had only one genotype each. Cluster XII showed the maximum intra-cluster distance (15.51), while the maximum inter-cluster distance between cluster XII and cluster XV was observed (38.15). The maximum contribution to genetic divergence was recorded for the trait 1000-seed weight (33.26%) followed by days to 50 per cent flowering (26.09%), oil content (23.93%), days to maturity (26.09%) and seed yield per plant (%). The genotypes IC-570302, IC-520747, IC-122427, EC-766480, RH 0749, EC-766040 and IC-470935, were observed promising for most of the important yield traits based upon high cluster means. The use of these genotypes was suggested in the crossing programme for further improvement in Indian mustard.

Key words: Indian mustard, genetic divergence, D^2 analysis, cluster analysis

Indian mustard [*Brassica juncea* (L.) Czern & Coss.], from the Cruciferae family, is a predominant crop among all the crops belongs to this family and accounting for nearly 80-85 per cent of the total area under these crops. It is cultivated in 69.4 lakh ha at the national level, with 72.0 lakh tonnes of production and 1.03 tonnes/ha of productivity (Kumar *et al.,* 2020). Despite achieving a remarkable increase in this crop's productivity, there is an urgent need to further increase productivity because oil demand has risen with an increase in both population and living standards (Singh *et al*., 2020).

The decision of the right parents is of enormous consequence to plant breeders. The first step in a breeding programme is to assemble diverse genotypes and analyse divergence in existing germplasm (Manjunath *et al.*, 2017). This is mainly to promote the crossing of diverse parents to exploit new gene combinations. Diverse parents provide more heterotic F_1s and a broad range of segregants for seed yield and component characters, which appear in the F_2 and subsequent generations. Because of this context, the present research was conducted to analyse the genetic divergence in exotic and indigenous Indian mustard lines by using Mahalanobis D^2 statistics.

This study has been carried out at the Research Farm of Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during *Rabi*, 2016-17. Seventy-five diverse indigenous and exotic lines with different geographical origins were planted in paired rows of 4 m length in a randomised complete block design with three replicates each (**Table 1**). At sowing time, the distance of 30 cm was maintained between row to row. Thinning was performed

Table 1.List of genotypes evaluated in the present study

S. No.	Genotype	S. No.	Genotype	S. No	Genotype
1.	IC-12209	26.	EC-766032	51.	EC-766480
2.	IC-73225	27.	EC-766176	52.	EC-766489
3.	IC-121661	28.	EC-766189	53.	EC-766539
4.	IC-122281	29.	IC-766198	54.	EC-766592
5.	IC-122348	30.	IC-766214	55.	EC-766597
6.	IC-122415	31.	IC-766236	56.	EC-766599
7.	IC-122427	32.	IC-766237	57.	IC-491462
8.	IC-253066	33.	EC-766257	58.	IC-491299
9.	IC-267705	34.	EC-766260	59.	IC-491344
10.	IC-296507	35.	EC-766283	60.	IC-491463
11.	IC-296690	36.	EC-766300	61.	IC-491626
12.	IC-361670	37.	EC-766321	62.	IC-491008
13.	IC-417480	38.	EC-766380	63.	IC-491120
14.	IC-470935	39.	EC-766391	64.	IC-491127
15.	IC-560698	40.	EC-766401	65.	IC-491390
16.	IC-571236	41.	EC-766402	66.	IC-520747
17.	IC-571685	42.	EC-766404	67.	IC-570302
18.	EC-287711	43.	EC-766421	68.	EC-766422
19.	EC-333591	44.	EC-766417	69.	IC-589660
20.	EC-347853	45.	EC-766426	70.	IC-342780
21.	EC-385604	46.	EC-766444	71.	IC-426337
22.	EC-400082	47.	EC-766448	72.	IC-426389
23.	EC-414319	48.	EC-766460	73.	RH30
24.	EC-491579	49.	EC-766477	74.	RH8812
25.	EC-699035	50.	EC-766478	75.	RH0749

21 days after sowing to keep the plant's distance within the row of 10 to 15 cm. All recommended package of practices was followed to raise a healthy crop. The data were recorded on thirteen characters *viz*., days to 50% flowering, days to maturity, plant height (cm), the number of primary branches/plant, the number of secondary branches/plant, main shoot length (cm), the number of siliquae on the main shoot, siliqua density, siliqua length (cm), the number of seeds/siliqua, seed yield/plant (g), 1000-seed weight (g) and oil content (%). D^2 analysis was performed according to the method suggested by Mahalanobis (1936) and extended by Rao (1952) and Murty and Arunachalam (1966). Intra-and inter-cluster distances have been calculated using the formula given by Singh and Choudhary (1985), while the clustering of D^2 values was executed using the Tocher method as specified by Rao (1952).

The ANOVA analysis results showed that mean squares were found significant for all the characters except the number of primary and secondary branches per plant, indicating the presence of sufficient genetic variability among genotypes (**Table 2).** Clustering using the Tocher approach (Rao, 1952) grouped all 75 genotypes into 15 clusters. The number of genotypes in a cluster varied from 1 to 46. The largest number of genotypes emerged in Cluster I (46), followed by Cluster V (8 genotypes), Cluster III (7 genotypes), and Cluster XIII (3 genotypes).

The remaining 11 clusters had just one genotype each, *i.e.,* cluster II, cluster IV, cluster VI, cluster VII, cluster VIII, cluster IX, cluster X, cluster XI, cluster XIII, cluster XIV and cluster XV (**Table 3**). The clustering trend indicated no correlation between genetic diversity and geographical origin, but the distribution of genotypes was unpredictable and distinct. Perhaps this may be due to natural selection, genetic drift and other environmental factors that create variations rather than actual genetic distances. Roy *et al.* (2016) had divided 40 diverse genotypes of Indian mustard into five groups. Diversity analysis was also carried out by Kumar *et al*. (2013), and grouped 24 distinct genotypes of Indian mustard into six clusters; 45 Indian mustard genotypes were grouped into seven clusters by Devi *et al*. (2017) and 41 Indian mustard lines were also grouped into seven clusters by Kumar *et al*. (2017).

In **Table 4**, the cluster mean values were given for 13 different traits. Cluster II recorded the highest mean cluster values for seed yield/plant (28.65 g), length of siliqua (4.78 cm) and 1000-seed weight (6.57 g). Cluster XIV had a maximum number of secondary branches/plants (16.4), higher yield/plant (27.5 g), early flowering (32 days) and medium height (135.6 cm). The cluster X had maximum oil content (40.55%), but it exhibited a low seed yield/plant (14.00 g). Cluster IV included the highest cluster mean values for characters *viz*., the number of primary branches/plant (5.4), main shoot length (79.4

Table 2. Analysis of variance (mean squares) for different characters in Indian mustard

Source of variation	Replication	Genotypes	Error	C.V. (%)
Days to 50% flowering	1.13	44.71**	0.65	1.89
Days to maturity	0.03	5.66**	0.18	0.29
Plant height	1423.27	678.49**	127.00	6.11
Number of primary branches per plant	9.83	0.84	0.78	18.43
Number of secondary branches per plant	7.28	6.46	4.66	19.78
Main shoot length	13.11	110.50**	50.55	10.28
Number of siliquae on main shoot	11.65	69.55**	26.34	12.01
Siliqua length	0.27	0.37**	0.06	6.14
Number of seeds per siliqua	2.83	3.21**	0.84	7.23
Siliqua density on main shoot	0.01	0.02**	0.01	13.61
1000- seed weight	0.37	1.91**	0.03	3.23
Seed yield per plant	27.74	44.48**	7.00	12.18
Oil content	0.67	1.00**	0.03	0.41

**: Significant at 0.01 level of probability

Table 3.Grouping of Indian mustard genotypes into different clusters

S. No.	Clusters	Number of genotypes	Name of genotypes
1	I	46	EC-766448, EC-766460, EC-766421, EC-766417, EC-766380, IC-491626, EC-766402, IC-589660, IC-491299, EC-400082, IC-122415, EC-766426, EC-766321, EC-766391, EC-414319, IC-296690, IC-560698, EC-766478, IC-417480, EC-766444, IC-122281, EC-766477, IC-361670, EC-766401, EC-766283, IC766198, EC-766597, EC-766032, EC-766539, EC-766599, IC-766214, EC-766257, EC-766300, IC-267705, IC-491390, EC-766592, IC-73225, IC-122427, RH8812, IC-491008, RH30, IC-491120, EC-766480, IC-491344, IC-491463, IC-342780
2	II	1	RH0749
3	III	7	IC-122348, EC-287711, IC-253066, IC-121661, EC-491579, EC-766480, EC-766176
4	IV	1	IC-470935
5	V	8	IC-571685, EC-385604, EC-766189, IC-426389, IC-571236 , IC-12209, EC-699035, IC-766237
6	VI	1	EC-333591
7	VII	1	EC-347853
8	VIII	1	IC-426337
9	IX	1	IC-296507
10	X	1	EC-766404
11	XI	1	EC-766422
12	XII	3	IC-491462, IC-491127, IC-766236
13	XIII	1	EC-766260
14	XIV	1	IC-520747
15	XV	1	IC-570302

cm) and 1000-seed weight (6.99 g). This cluster is also high in oil content (39.9%). Cluster XV had the genotypes which were earliest in flowering (31 days) and maturity (140 days) and had lesser height (109.9 cm). The highest cluster means value for the main shoot's siliqua density was recorded in cluster VIII (0.71) followed by cluster V (0.70). Cluster mean value for the number of siliqua on the main shoot was observed maximum (47.8 siliqua) in cluster XI. The maximum number of seeds/siliqua (16.16 seeds) was recorded in cluster IX. The remaining clusters (I, VI, VII, XII and XIII) had lower cluster mean values for different characters. These results were supported by

Lodhi *et al*. (2013), Kumar *et al*. (2017) and Devi *et al*. (2017).

Intra-cluster D^2 values ranged within the cluster from 0.00 to 15.51 and among clusters *i.e.,* inter-cluster value varied from 6.28 to 38.15 (**Table 5**.). This showed that the genotypes present within clusters were homogeneous and among clusters were heterogeneous. Cluster VII consisted of more diverse genotypes because it had maximum intra-cluster distance (15.51). Between clusters XII and XV maximum inter-cluster distance (38.15) was observed, followed by clusters II and XV (37.27); clusters

Table 4.Clusters mean for different traits in Indian mustard

Cluster	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of primary branches per plant	Number of secondary branches per plant	Main shoot length (cm)	Number of siliquae on the main shoot	Siliqua length (cm)	Number of seeds per siliqua	Siliqua density on the main shoot	1000-seed weight (g)	Seed yield per plant (g)	Oil content (%)
I	42.11	144.78	183.58	4.70	10.73	70.05	42.34	3.90	12.48	0.60	5.28	21.76	39.37
II	45.50	144.50	173.00	4.80	8.50	72.30	37.20	4.78	14.08	0.52	6.57	28.65	39.85
III	49.50	144.71	200.43	5.16	11.17	69.71	45.12	3.89	12.19	0.67	5.00	26.12	39.54
IV	40.00	143.00	175.90	5.40	9.70	79.40	41.40	4.28	12.48	0.53	6.99	19.65	39.90
V	48.38	146.00	199.61	5.29	11.63	67.84	47.09	3.55	12.94	0.70	3.07	21.16	39.06
VI	35.50	146.00	161.30	4.00	8.80	61.40	33.90	3.54	11.72	0.56	5.22	12.00	38.45
VII	35.50	146.00	191.70	4.10	10.40	67.50	42.40	3.73	13.61	0.63	4.52	20.15	38.45
VIII	36.00	144.00	178.20	5.00	10.80	57.90	40.40	3.26	13.26	0.71	4.48	14.35	38.70
IX	36.00	144.00	167.90	5.10	10.40	71.90	45.40	3.64	16.16	0.63	4.02	21.50	39.20
X	40.00	145.00	187.50	4.20	10.60	65.30	34.60	4.10	13.10	0.53	4.31	14.00	40.55
XI	40.00	141.00	170.50	4.00	10.50	75.50	47.80	3.74	12.52	0.65	4.89	20.35	38.50
XII	44.67	146.00	192.70	4.70	10.10	68.23	43.30	4.20	12.01	0.64	6.27	19.77	38.58
XIII	31.00	147.00	160.00	5.30	13.40	64.70	36.70	4.32	13.66	0.57	4.55	21.07	38.55
XIV	31.50	142.00	135.60	4.80	16.40	59.30	40.00	3.19	13.02	0.68	3.88	27.50	38.30
XV	31.00	140.00	109.90	5.00	13.30	54.70	33.80	3.56	15.31	0.65	3.09	13.80	39.10
Mean	39.11	144.27	172.52	4.77	11.10	67.05	40.76	3.85	13.24	0.62	4.81	20.12	39.07

Table 5.Intra and inter-cluster distances among different clusters in Indian mustard

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
I	**11.75**	14.41	15.52	14.87	20.39	14.98	15.14	14.91	15.91	14.56	14.90	16.50	20.01	22.50	29.14
II		**0.00**	14.53	10.61	25.86	21.85	22.95	23.41	23.89	20.30	20.19	14.59	27.32	30.69	37.27
III			**12.76**	19.69	18.81	23.44	22.78	22.40	22.44	18.59	20.16	18.71	28.36	29.66	35.25
IV				**0.00**	29.36	18.52	21.33	20.04	22.06	19.49	16.99	16.72	25.11	27.22	33.19
V					**14.63**	24.21	21.39	21.17	19.86	19.18	22.65	25.97	26.27	26.70	30.92
VI						**0.00**	6.28	8.65	13.09	17.91	14.57	18.38	9.73	16.51	25.33
VII							**0.00**	7.47	9.83	16.96	14.54	19.96	8.14	14.60	23.84
VIII								**0.00**	6.82	14.57	10.55	21.96	12.40	11.13	18.50
IX									**0.00**	13.29	12.91	24.45	13.38	10.98	16.85
X										**0.00**	18.44	24.00	19.95	21.13	23.94
XI											**0.00**	20.43	20.26	15.44	21.54
XII												**15.51**	24.73	30.12	38.15
XIII													**0.00**	14.98	23.84
XIV														**0.00**	12.14
XV															**0.00**

Values in bold faces are intra-cluster distances.

III and XV (35.25); clusters IV and XV (33.19); clusters V and XV (30.92); clusters II and XIV (30.69); clusters XII and XIV (30.12) and clusters III and XIV (29.66) indicating genotypes exhibiting a broad range of phenotypes were present in these clusters. More inter-cluster distance means that the genotypes grouped in these clusters may be exhibiting contrasting phenotypes. This indicated the presence of enough genetic diversity in the material used in this study. Genotypes from cluster II with XV, cluster XII with XV, cluster III with XV and cluster IV with XV may produce considerable heterosis and can

give good transgressive segregants in the segregating generations. Gene introgression may have occurred among the genotypes of different geographical origins. This is indicated by the presence of genotypes with a different background in the same cluster. Similar findings were also stated earlier by Singh *et al*. (2010) and Lodhi *et al*. (2013) in Indian mustard. The contribution of various characters towards genetic divergence (**Table 6**) indicated more contribution of six characters to divergence. 1000-seed weight contributed maximum per cent to the genetic divergence (33.26%) followed by days to 50% flowering

Table 6. Per cent contribution of different characters in genetic divergence

S.No.	Contributing character	*Per cent* contribution
1	Days to 50% flowering	26.09
2	Days to maturity	11.96
3	Plant height	0.00
4	Number of primary branches per plant	0.04
5	Number of secondary branches per plant	0.00
6	Main shoot length	0.11
7	Number of siliqua on the main shoot	0.04
8	Siliqua length	1.51
9	Number of seeds per siliqua	0.50
10	Siliqua density on the main shoot	0.04
11	1000- seed weight	33.26
12	Seed yield per plant	2.52
13	Oil content	23.93

Table 7. Promising genotypes identified for different traits In Indian mustard

S. No.	Characters	Genotypes	Clusters
1	Days to 50% flowering	IC-570302, EC-766260, IC-520747	XV, XIII, XIV
2	Days to maturity	IC-570302	XV
3	Plant height	IC-570302	XV
4	Number of primary branches per plant	IC-470935	IV
5	Number of secondary branches per plant	IC-520747	XIV
6	Main shoot length	IC-470935	IV
7	Number of siliquae on main shoot	EC-766422	XI
8	Siliqua length	RH 0749	II
9	Number of seeds per siliqua	IC-296507	IX
10	Siliqua density on main shoot	IC-426337	VIII
11	1000-seeds weight	IC-470935	IV
12	Oil content	EC-766040	X
13	Seed yield per plant	RH0749, IC-520747, IC-122427, EC-766480,	II, XIV,I

(26.09%), oil content (23.93%), days to maturity (26.09%), seed yield/plant (2.52%) and siliqua length (1.51%). The other traits, *viz*., the main shoot length, plant height, the number of siliqua on the main shoot, the number of primary branches per plant, the number of seeds per siliqua, the number of secondary branches per plant, and siliqua density on main shoot showed very low or nearly no contribution towards genetic divergence. At the first sight, these results may be seen as unobvious. This material constituted of diverse lines having exogenous and indigenous origins. Roy *et al*. (2018) reported a significant positive correlation of 1000-seed weight with oil content and although a non-significant positive correlation of 1000-seed weight with phenological traits. These characters also have high heritability in comparison to other characters, reported by Kumari *et al*. (2019). It is possible that, characters with high heritability may be contributed more to genetic divergence. The combined effect of heritability and correlation may be producing such type of trend in which few characters contributed largely in divergence and remaining traits contribution was observed negligible. A similar type of characters contribution to genetic divergence for one or more characters was reported by Singh *et al*. (2010), Pandey *et al*. (2013), Devi *et al*. (2017), Nagda *et al*. (2018), Tripathi *et al*. (2019) and Meena *et al*. (2020) in Indian mustard. But there are some variations in the findings also and this is due to the different materials used under study.

Based on this study, the promising genotypes from different clusters were identified and given in **Table 7**. The genotype IC-570302 was observed as early in flowering and maturity along with short stature. Similarly, IC-520747 was also early, along with good seed yield and having a large number of secondary branches. These strains can be used in the breeding program to develop early, short and high-yielding varieties. For seed yield per plant, the genotypes IC-122427, EC-766480 and RH 0749 were observed promising. RH 0749 also had a higher siliqua length. In Indian mustard, oil content and 1000-seed weight are also essential traits. The genotypes EC-766040 and IC-470935 showed superiority, respectively for these traits. The genotype IC-470935 was also somewhat good for the main shoot length and number of primary branches. Similarly, the number of siliqua on main shoot EC-766422 and the number of seeds per siliqua

IC-296507 showed their superiority. These genotypes may be used as donor parents to develop Indian mustard varieties with high seed yields and high oil content.

REFERENCES

Devi, T. R., Devi, N. D., Vivekananda, Y. and Sharma P. R. 2017. Genetic diversity analysis in Indian mustard [*Brassica juncea* (L.) Czern and Coss.] genotypes using agro-morphological parameters. *Electron. J. Plant Breeding,* **8**(3): 749-753. [Cross Ref]

Kumar, B., Pandey, A. and Singh, S. K. 2013. Genetic diversity for agro-morphological and oil quality traits in Indian mustard (*Brassica juncea* L. Czern & Coss*). The Bioscan,* **8**(3): 771-775.

Kumar, N., Avtar, R., Ahlawat, N. and Punia, R. 2020. Molecular diversity analysis using Simple sequence repeats (SSRs) in 'A' and 'R' lines of Ogura CMS system in Indian mustard. *Int. J. Chem. Stud.*, **8**(4): 1276-1282. [Cross Ref]

Kumar, R., Singh, H., Kaur, S., Singh, I. and Kaur, R. 2017. Quantitative analysis for yield and its components in IC lines of Indian mustard [*Brassica juncea* (L.) Czern and Coss.]. *J. Pharmacogn. Phytochem.,* **6**(5): 2257-2260.

Kumari, S., Srivastava, K., Srivastava, A. and Akanksha. 2019. Genetic parameters and character association of yield and its attributes in Indian mustard (*Brassica juncea* L.). *J. Oilseed Brassica,* **10**(2): 140-148.

Lodhi, B., Thakral, N. K., Singh, D., Avtar, R. and Bahadur, R. 2013. Genetic diversity analysis in Indian mustard (*Brassica juncea*). *J. Oilseed Brassica,* **4**(2): 57-60.

Mahalanobis, P. C. 1936. Study on the generalized distance in statistics. Proceedings of the National Institute of Sciences of India, Calcutta., **2**: 49-55.

Manjunath, H., Phogat, D. S., Kumari, P. and Singh, D. 2017. Genetic analysis of seed yield and yield attributes in Indian mustard [*Brassica juncea* (L.) Czern and Coss.]. *Electron. J. Plant Breeding,* **8**(1)**:** 182-186. [Cross Ref]

Meena, H. M., Meena, P. K., Singh, K., Meena, H. P. and Meena, D. 2020. Genetic Divergence Analysis in Indian Mustard [*Brassica juncea* (L.) Czern & Coss.]. *Int. J. Curr. Microbiol. App. Sci.*, **10**(02): 2185-2192. [Cross Ref]

Murty, B. R. and Arunachalam, V. 1966. The nature of divergence in relation to breeding system in some crop plants. *Indian J. Genet. Plant Breeding,* **26A**: 188-198.

Nagda, R., Dubey, N., Avinashe, H. and Tamatam, D. 2018. Assessment of genetic diversity in mustard genotypes. *Plant Arch.,* **18** (2) : 2091-2096.

Pandey, R., Kumar, B. and Kumar, M. 2013. Genetic divergence for quantitative traits in Indian mustard (*Brassica juncea* L. Czern & Coss). *American-Eurasian J. Agric. Environ. Sci.,* **13**(3): 348-351.

Rao, C. R.1952. Advanced Statistical Methods in Biometrical Research.; Ist Edn. Johan Wiley and Sons, New York.

Roy, R. K., Kumar, A., Kumar, S. and Kumar, A. 2016. Genetic variability, correlation, path analysis and genetic diversity studies in late sown mustard (*Brassica juncea* L. Czern & Coss.). *The Bioscan,* **11**(4)**:** 3117-3124.

Roy, R. K., Kumar, A., Kumar, S., Kumar, A. and Kumar, R. R. 2018. Correlation and path analysis in Indian mustard (*Brassica juncea* L. Czern & Coss) under late sown condition. *Environ. Ecol.,* **36** (1): 247-254.

Singh, D., Arya, R. K., Chandra, N., Niwas, R. and Salisbury P. 2010. Genetic diversity studies in relation to seed yield and its component traits in Indian mustard (*Brassica juncea* L. Czern & Coss.). *J. Oilseed Brassica,* **1**(1): 19-22.

Singh, M., Avtar, R., Pal, A., Punia, R., Singh, V. K., Bishnoi, M., Singh, A., Choudhary, R. R. and Mandhania, S. 2020. Genotype-Specific Antioxidant Responses and Assessment of Resistance Against *Sclerotinia sclerotiorum* Causing Sclerotinia Rot in Indian Mustard. *Pathogens*, **9**(11): 1-17. [Cross Ref]

Singh, R. K. and Chaudhary, B. D. 1985. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi, India.

Tripathi, A., Singh, M. and Tomar, A. 2019. Genetic diversity analysis for qualitative and quantitative traits in Indian mustard (*Brassica juncea* L. Czern & Coss). *The Pharma Innov.,* **8**(1): 637-639.