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 and 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

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*Brassica juncea* (L.) Czern & Coss., also known by the name of Indian mustard, belongs to the plant family *Brassicaceae* (*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.
of the two population for multiple characters viz.,
measurements (characters) on each individual and
siliquae plant−1, seeds per siliquae, 1000 seed weight (g),
Mahalanobis D2 statistics (1936) followed by Rao
twig. Positive pressure is exerted by the aphid while it punctures to the
needs to puncture the tissue of the tough plant twig. This
Texture analyzer. Positive pressure is the pressure that
in forms of various graphs in the system attached to the
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)

The genetic divergence was estimated using
Mahalanobis D2 statistics (1936) followed by Rao (1952). The theory of the D2 statistics is as follows:
Let us assume $X_1$, $X_2$, $X_p$ ....... $X_p$ are the multiple measurements (characters) on each individual and
$d_1$, $d_2$, $d_p$ ......... $d_p$ are the differences between the mean of the two population for multiple characters viz.,
$\bar{X}_1$, $\bar{X}_2$, $\bar{X}_p$ ......... $\bar{X}_{p}$ $\bar{P}_1$, $\bar{P}_2$ respectively.

Then, $D^2$ statistic of Mahalanobis is as follows :
$$D^2 = w^0 \left( \bar{X}_i - \bar{X}_j \right) \left( \bar{P}_i - \bar{P}_j \right),$$
where, $w^0$ 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 up to 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 (kPascale), 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)

<table>
<thead>
<tr>
<th>Caption</th>
<th>Value</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test mode</td>
<td>Compression</td>
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</tr>
<tr>
<td>Pre-test mode</td>
<td>1.00</td>
<td>/mm/sec</td>
</tr>
<tr>
<td>Test speed</td>
<td>1.00</td>
<td>/mm/sec</td>
</tr>
<tr>
<td>Post-test speed</td>
<td>10.00</td>
<td>/mm/sec</td>
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<tr>
<td>Target mode</td>
<td>Distance</td>
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<tr>
<td>Distance</td>
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<td>/mm</td>
</tr>
<tr>
<td>Trigger type</td>
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<tr>
<td>Trigger force</td>
<td>5.0</td>
<td>g</td>
</tr>
<tr>
<td>Advance option</td>
<td>off</td>
<td></td>
</tr>
</tbody>
</table>

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 D2 statistics (1936) followed by Rao (1952). The theory of the D2 statistics is as follows:

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$$D^2 = w^0 \left( \bar{X}_i - \bar{X}_j \right) \left( \bar{P}_i - \bar{P}_j \right),$$
where, $w^0$ is the
inverse of variance and co-variance matrix.
Genetic divergence in Indian mustard [Brassica juncea (L.) Czern]

Cluster V namely RNWR-09-3, PRD-2013-2, GHIRIRAJ, NRCHB-101, RGIN-73, DRMR-II-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). 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

<table>
<thead>
<tr>
<th>Cluster</th>
<th>No. of genotypes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>2</td>
<td>JMM-927-RC, RH-1325</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>JMM-927-RC, RH-1325</td>
</tr>
<tr>
<td>VI</td>
<td>2</td>
<td>RL1358, Kranti</td>
</tr>
<tr>
<td>VII</td>
<td>1</td>
<td>PHR2</td>
</tr>
</tbody>
</table>

Table 3: Average intra (diagonal) and inter-cluster (off-diagonal) D² values in Indian mustard

<table>
<thead>
<tr>
<th>Cluster</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>350.00</td>
<td>325.19</td>
<td>542.99</td>
<td>414.56</td>
<td>491.54</td>
<td>523.97</td>
<td>2507.42</td>
</tr>
<tr>
<td>II</td>
<td>19.69</td>
<td>83.99</td>
<td>303.98</td>
<td>410.53</td>
<td>372.20</td>
<td>1954.86</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>22.21</td>
<td>496.72</td>
<td>682.97</td>
<td>587.32</td>
<td>1591.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>472.12</td>
<td>515.42</td>
<td>538.13</td>
<td>2560.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>524.72</td>
<td>557.07</td>
<td>3147.81</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>943.87</td>
<td>2564.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Description of the genetically divergent clusters and distance (D² value) between the genotypes selected

<table>
<thead>
<tr>
<th>Cluster combination</th>
<th>Inter cluster distance (D² value)</th>
<th>Genotype selected from the cluster</th>
<th>Distance between the genotypes selected (D² value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster VII and cluster V</td>
<td>3147.808</td>
<td>PHR -2 in cluster VII and RNWR-09-3 in cluster V</td>
<td>4662.847</td>
</tr>
<tr>
<td>Cluster VII and cluster V</td>
<td>3147.808</td>
<td>PHR -2 in cluster VII and Giriraj in cluster V</td>
<td>4342.083</td>
</tr>
<tr>
<td>Cluster VII and cluster VI</td>
<td>2564.546</td>
<td>PHR -2 in cluster VII and Kranti in cluster VI</td>
<td>3811.183</td>
</tr>
<tr>
<td>Cluster VII and cluster IV</td>
<td>2560.633</td>
<td>PHR -2 in cluster VII and SKJM-05 in cluster IV</td>
<td>6745.971</td>
</tr>
<tr>
<td>Cluster VII and cluster IV</td>
<td>2560.633</td>
<td>PHR -2 in cluster VII and DRMRIJ-15-16 in cluster IV</td>
<td>3636.379</td>
</tr>
<tr>
<td>Cluster VII and cluster I</td>
<td>2507.415</td>
<td>PHR -2 in cluster VII and RW-85-59(Sarna) in cluster I</td>
<td>4950.465</td>
</tr>
<tr>
<td>Cluster VII and cluster I</td>
<td>2507.415</td>
<td>PHR -2 in cluster VII and NPJ-194 in cluster I</td>
<td>5853.214</td>
</tr>
</tbody>
</table>
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 Giriraj), 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>
<thead>
<tr>
<th>Clusters</th>
<th>Plant Height (cm)</th>
<th>Height up to 50% flowering (cm)</th>
<th>Days to first fruiting (branch)</th>
<th>Secondary branches per plant</th>
<th>Seed yield (g)</th>
<th>Penetration Force (kPa)</th>
<th>Seed weight (g)</th>
<th>Siliquae per plant</th>
</tr>
</thead>
</table>
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.

**ACKNOWLEDGEMENT**

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