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Assessment of agro-morphological and molecular diversity among Indian mustard (*Brasssica juncea*) genotypes

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ABSTRACT

An experiment was conducted in randomized block design with three replications during winter (*rabi*) season 2015–16 at Instructional farm, RCA, MPUAT, Udaipur. Genetic diversity of the 10 Indian mustard [*Brassica juncea* (L.) Czern. & Coss] varieties was evaluated by Random Amplified Polymorphic DNA markers and morphological characters. Fifteen RAPD primers were used out of which three were amplified. These three amplified primers produced total 148 amplicons, of which 128 amplicons (86.48%) were polymorphic. The polymorphic information content value ranged from 87.50% (OPA-2) to 100% (OPA-11) with an average of 93.33%. A dendrogram based on UPGMA analysis separated 10 Indian mustard varieties in four clusters at 0.58 similarity coefficient. Genetic divergence using Mahalanobis D^2 statistics was worked out and based on D^2 values, the varieties were grouped into four different clusters. Cluster 1 included maximum (seven) genotypes and cluster 2, 3 and 4 had only one genotype. Maximum divergence was observed between clusters 1 and 4 followed by cluster 1 and 2. Number of siliquae per plant was the main trait contributing to this divergence followed by test weight, seed yield per plant, number of seeds per siliqua, days to 50% flowering and number of secondary branches per plant. The genotypes PM-27 and Pusa Vijay were the most diverse on the basis of UPGMA clustering analysis and genotypes PM-27 and Bio-902 were most divergent on the basis of D^2 value clustering. The results obtained can be utilized in hybridization and selection for breeding programmes.

Keywords: Genetic diversity, Indian mustard, Principal components, RAPD

Indian mustard [*Brassica juncea* (L.) Czern. & Coss] is the second most important edible oilseed crop of India after groundnut (Kumar *et al.* 2018). It is globally used as oilseed, vegetable and condiment. It contributes about 27% of edible oil pool in India and 13% of edible oil pool globally (Meena *et al.* 2017). It is highly promising *rabi* crop cultivated in the states of Rajasthan, Uttar Pradesh, Haryana, Madhya Pradesh, Gujarat, West Bengal, Assam, Bihar and Punjab. It accounts for about 75–80% of the total area under *Brassica* crop group in India (Rathore *et al.* 2018). The important objective of Indian mustard breeding programs is to develop high yielding varieties to fulfil the ever-increasing oil demand.

Genetic diversity analysis is frequently used method by breeders to select more distinct parental material for a particular trait. Among different types of molecular markers, Random Amplified Polymorphic DNA (RAPD) markers are commonly used because they are quick and simple to obtain. The objective of this work was to estimate the genetic variation and relatedness among 10 Indian mustard varieties released from different parts of the country.

MATERIALS AND METHODS

The experimental material comprising a set of 10 Indian mustard varieties (PM30, RGN-73, Bio-902, DR-M-RIJ-31, PM27, Laxmi, RGN-498, Pusa Vijay, RGN-229, RB-50) released from different parts of the country was evaluated in randomized block design with three replications during winter (*rabi*) season 2015–16 at Instructional farm, RCA, MPUAT, Udaipur. Various parameters related to morphological characterization, viz. days to 50% flowering, days to maturity, plant height, total number of primary branches, total number of secondary branches, length of main branch, number of siliquae per plant, length of siliqua, number of seeds per siliqua, 1000 seed weight, seed yield per plant, oil content and protein content were recorded during *rabi* 2016–17. Genetic divergence was estimated by Mahalanobis D^2 statistics and clustering of genotypes was done on the basis of D^2 values according to Tocher's method as described by Rao (1952). Principal factor was carried out using SPSS 10.0 (Morrison 1978). Principal factor analysis was carried out using Principal Component method for factor extraction. The principal components having eigen values

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more than one were considered for further interpretation. Total cellular DNA was isolated from the leaflet tissues of each mustard genotype using a modified acetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1990). Polymerase chain reaction was performed. The gel was visualized under UV using gel documentation system. The data were analyzed using NTSYS pc 2.02 (Numerical Taxonomy System) software and Jaccard's similarity coefficient was computed. Cluster analysis was performed using NTSYS pc 2.02 statistical software.

RESULTS AND DISCUSSION

The analysis revealed significant difference amongst the genotypes for all the characters studied. High variability was noticed with respect to number of secondary branches per plant, number of siliquae per plant and seed yield per plant. Ten genotypes were grouped into four clusters, using the Tocher's method (Fig 1). The pattern of clustering revealed that cluster 1 was the largest comprising seven genotypes. Distribution of genotypes among various clusters reflected presence of considerable amount of genetic variability. Cluster 2, cluster 3 and cluster 4 were solitary, each had a single genotype. The maximum genotypes in a single cluster (cluster 1) depict their same geographical origin or continuous selection for a particular trait in a desirable direction. The solitary clusters may be due to intensive natural or artificial selection by human for diverse adaptive gene complexes. The average D^2 values of intra and inter cluster distances (Table 1) showed that intra-cluster D^2 values ranged from zero (cluster 2, 3 and 4) to 10.75 (cluster 1). This reveals that genotypes occupying the same cluster have low level of diversity and selection of parents within the cluster may not be considered promising. Maximum inter cluster distance was observed between cluster 1 and cluster 4 (28.40) followed by cluster 2 and 4 (23.51), lowest inter cluster divergence was noticed between cluster 3 and 4 (12.50). High inter-cluster distance between the clusters indicated high diversity among the genotypes of these clusters. These results are in agreement with earlier findings by Roy *et al.* (2016), Kumar *et al.* (2017), Gupta *et al.* (2018) and Nagda *et al.* (2018).

Table 1 Average inter-cluster and intra-cluster distance based on D^2 values

Cluster	1	2	3	4
1	10.75	13.20	17.56	28.40
2	-	0.00	12.64	23.51
3	-	-	0.00	12.50
4	-	-	-	0.00

The cluster means for each of twelve characters are presented in Table 2. The magnitude of intra cluster distances measure the extent of genetic diversity between the genotypes belonging to same cluster, while inter cluster distance is a measure of genetic distance between genotypes belonging from two different clusters. The data indicated that the cluster mean for days to 50% flowering and days to maturity were highest in cluster 2 and lowest in cluster 4. Plant height was highest in cluster 4 and lowest in cluster 1. Number of primary branches per plant was highest in cluster 4 and lowest in cluster 3. Cluster 4 recorded the highest secondary branches per plant and the lowest was recorded in the cluster 2. Length of main branch was highest in cluster 4 and lowest in cluster 2. Number of siliquae per plant was recorded highest in cluster 4 and lowest in cluster 1. Cluster 4 recorded highest number of seeds per siliqua and the lowest was recorded in the cluster 2. Test weight was highest in cluster 4 and lowest in cluster 3. Seed yield per plant was highest in cluster 4 and lowest in cluster 1. Cluster 4 exhibited highest value for oil and protein content and lowest was expressed in cluster 1. Differences in cluster means and *per se* performance of the characters recorded indicated the responsiveness of the studied characters for the presence of genetic divergence. The transgressive segregates for yield and yield contributing components can be obtained from mating of genotypes from more diverse clusters for further improvement. These results have also been reported by Devi *et al.* (2017), Mohan *et al.* (2017) and Monika *et al.* (2019).

The relative per cent contribution of each character towards total genetic divergence among the genotypes was

Table 2 Cluster means and per cent contribution of different characters in Indian mustard

	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches per plant	No. of secon-dary branches per plant	Length of main branch	No. of siliqua per plant	No. of seeds per siliqua	Test weight	Seed yield per plant	Oil content	Protein content
Cluster 1	52.33	116.24	181.43	6.75	13.25	85.34	389.53	5.29	14.69	4.74	29.42	37.75
Cluster 2	54.00	119.67	185.00	6.47	10.60	72.67	447.43	4.88	14.60	5.36	34.91	38.26
Cluster 3	47.67	114.00	186.00	6.27	16.23	79.67	434.91	5.42	15.33	5.94	33.21	40.09
Cluster 4	44.00	110.67	199.33	7.87	18.53	88.00	525.22	5.88	16.40	6.22	41.17	41.15
Time ranked First	3	0	0	0	1	0	22	4	10	4	1	0
Per cent contribution	6.67%	0.00%	0.00%	0.00%	2.22%	0.00%	48.89%	8.89%	22.22%	8.89%	2.22%	0.00%

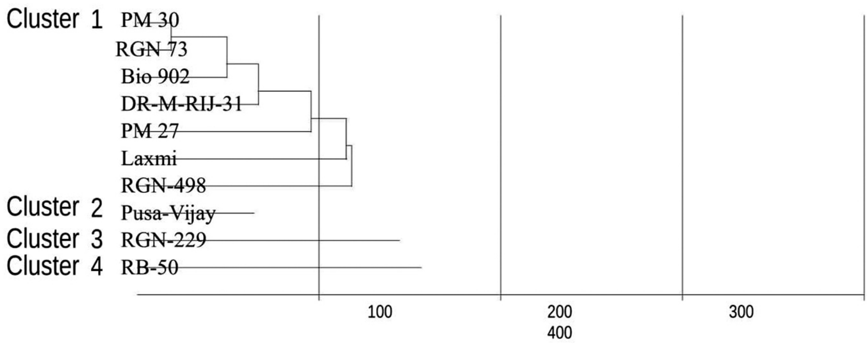

Fig 1 Dendrogram derived from cluster analysis using morphological descriptors.

studied. The data revealed that number of siliquae per plant was the main trait contributing to this divergence which accounted for 48.89%, followed by test weight, seed yield per plant and number of seeds per siliqua, days to 50% flowering, number of secondary branches per plant and oil content. The traits contributing maximum towards the divergence need to be given great emphasis for deciding the cluster to be chosen for hybridization and subsequent selection of the parents from the clusters based on their *per se* performance. In mustard crop, maximum contribution from traits towards divergence has been reported to be different from different sets of materials studied by Lodhi *et al.* (2013) and Gupta *et al.* (2019).

The results of principal component analysis explained the genetic variation among the genotypes for all agro-morphological and quality characters studied. The three principal components, viz. PC1, PC2 and PC3 explained above 90% of the total variation among the 12 characters (Fig 2). PC1 exhibited maximum variability with an eigenvalue of 893.96 and loaded with characters, viz. length of main branch, days to 50% flowering and days to maturity and contributed in positive direction whereas number of secondary branches per plant, number of siliquae per plant, protein content and test weight contributed in negative direction. PC2 accounted for 14.96% of the total variation with an eigenvalue of 184.40 and loaded with the characters, viz. test weight, oil content, days to maturity, and seed yield per plant. PC3 had contribution from the characters like days to 50% flowering, oil content and protein content which accounted for 5.082% of the total variation with an eigenvalue 62.6. It clearly showed that the variation in this component is contributed by the combination of yield as well as quality characters. The results of principal component analysis corroborated with results obtained by Neeru *et al.* (2015), Rao *et al.* (2017) and Gupta *et al.* (2019).

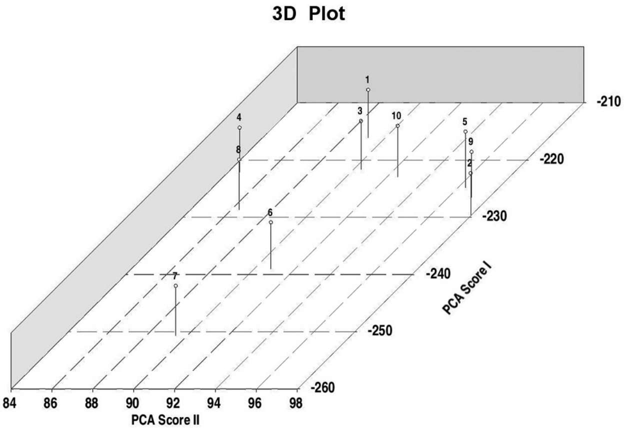

Fig 2 Distribution of Indian mustard genotypes across first two components based on PCA.

The genetic diversity among 10 Indian mustard genotypes was also evaluated by RAPD markers using 15 decamer primers (OPERON) showing high G+C content. Out of 15 primers used, amplification was obtained with only three primers. Amplified primers showed variable degree of polymorphism ranging from 87.50% (OPA-2) to 100% (OPA-11). Overall polymorphism was found to be 93.33%. Primer OPA-2 produced 8 scorable bands of which 7 were polymorphic amounting to 87.50% polymorphism. Primer OPA-3 produced 12 scorable bands of which 11 bands were polymorphic amounting to 91.66% polymorphism. Primer OPA-11 produced 10 scorable bands, all the bands were polymorphic which amount to 100% polymorphism. The maximum number of amplicons were produced by the primers OPA-3 followed by OPA-1. The minimum numbers of amplicon were produced by the primer OPA-11. Among all the primers tested, OPA-3 proved to be the best primer. The diversity studies on the basis of dendrogram revealed that the 10 genotypes could be placed into 4 groups at 0.58 similarity coefficient. Total 60% genotypes were covered in cluster 2 and hence can be considered closely similar. Nevertheless, the genotypes Pusa Vijay and Bio-902 were most diverse as compared to any other paired combinations. RAPD markers have been successfully used to evaluate genetic diversity in *Brassica* species (Gupta *et al.* 2014, Iqbal *et al.* 2015, Harsha *et al.* 2016, Gupta *et al.* 2018 and Monika *et al.* 2019).

It may be concluded that there is ample amount of variability in morphological traits and RAPD polymorphism among 10 Indian mustard genotypes. The 92.61% of the total variability was explained by the first two principal components. This study on genetic variability from the morphological descriptor and molecular analysis found a difference in the pattern of clustering. The results of clustering pattern suggest that breeding programme involving genotypes from cluster 1 with 2, 3, 4 may give heterosis and transgressive segregation due to more genetic diversity. The genotype Pusa Vijay and Bio-902 were farthest as per the Jaccard's similarity coefficients among 10 Indian mustard genotypes. These diverse parents could be effectively utilized in breeding programme and in maintaining diversity in genetic resources, required for employing the genetic potential of genotypes for improvement of desirable traits.

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