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Original scientific article

GENETIC DIVERSITY ANALYSIS THROUGH CLUSTER CONSTELLATION IN BRINJAL (*Solanum melongena* L.)

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In present investigation, 110 locally developed genotypes from different breeding programmes in brinjal were classified into eleven clusters on the basis of their D^2 values computed from Mahalanobis D^2 statistics of twelve morphological traits, wherein inter- and intra-cluster distances highlighted the genetic divergence of the genotypes grouped among and within different clusters. Among all, fourth cluster was the largest with 33 genotypes; however, each of second, fifth, ninth, tenth and eleventh clusters contained only single genotype. The genotypes of eighth and tenth clusters were highly diverse (1584.40) followed by third and eighth (1431.31), eighth and ninth (1302.69), sixth and eighth (1126.33) and first and eighth (1042.91) clusters. Intra-cluster (within cluster) variation was the highest in seventh cluster (74.43) followed by eighth (61.20) and sixth (54.36) that described the diverse nature of eighteen, five and nineteen genotypes in these groups, respectively. However, PBL-268, PBGL-401, PBL-243, PSR 308 and PBOB-518 were grouped individually in II^{nd}, V^{th}, IX^{th}, X^{th} and XI^{th} clusters, respectively. Overall, fifth cluster had most vigorous and high yielding ((2.82 kg/plant) genotype (PBGL-405); eighth cluster included genotypes with big round fruits and maximum fruit weight (317.43g); and tenth cluster had the earliest genotype (PSR-308) with the maximum number of fruits per plant (43.17). Out of twelve morphological traits, 94.19% diversity was brought by average fruit weight (67.86%), number of fruits per plant (17.26%), fruit yield per plant (5.37%) and fruit breadth (3.70%), however, other traits had negligible share towards the variation. This study created the foundation for future hybridization

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programmes in brinjal, where the parents can be selected on the basis of highly diverse groups as well as traits.

Keyword: Brinjal, morphological diversity, inter-cluster distance, Mahalanobis D^2 statistics

INTRODUCTION

Brinjal (*Solanum melongena* L.) is commonly known for its highest diversity in India and is also being cultivated in many other countries of south-east Asia and Africa, Europe and United States. India has been considered as centre of its origin and diversity (TAHER *et al.,* 2017), but most recent studies on DNA sequencing suggested its earlier existence in Africa (LI *et al.,* 2010; WEESE and BOHS, 2010). Therefore, it might have indirectly resulted from the wild specie *S. incanum* that was among the several species evolved earlier in Africa and spread to the South-East Asia as the wild progenitor of *S. melongena*. It exists as wild or semi wild form in India, where its domestication may have resulted into the greatest diversity.

Various natural as well as breeding activities such as domestication, natural intercrossing, mutation, selection and hybridization created broad genetic diversity of this crop in India and other parts of the world (FRARY *et al.,* 2007; CAKIR *et al*., 2018; SIFAU *et al* ., 2018). Cultivars have become diverse in vegetative growth as well as various fruit characteristics such colour, shape, size and quality etc. The genetic diversity for fruit color (light to dark purple, almost black, green, or whit), thickness, length, weight, shapes, bearing habit (solitary or in clusters) and many other physiological as well as biochemical properties has been described by many researchers (KUMAR *et al*., 2008; ULLAH *et al.,* 2014). However, the success of any crop improvement programme largely depends upon the nature and magnitude of the genetic variability existing in breeding material as well as the parental selections in set hybridization programmes (MEENA and BAHADUR, 2013; TAHER *et al.,* 2017). Brinjal is an often cross pollinated crop, where hybrid vigour has been commercially exploited, because of high number of seeds obtained from a single cross. Hybridization between genetically diverse parents results in heterotic and/or transgressive recombinants. Higher the diversity of the parents, better are the chances of improving economic traits under consideration in the offspring. Collection of germplasm and its genetic analysis can help to get suitable genotypes for higher yield or any other desirable character. Years of efforts are required to create genetic diversity of a crop at particular centre. However, the use of this genetic divergence for crop improvement is not possible without morphological characterization of these genotypes as the plant breeder selects the diverse inbred lines as parents on the basis of their morphological performance. In brinjal, there are specific local preferences for colour, shape and taste. Therefore, it is essential to explore the local diversity and improve the yield potential of available genotypes through suitable breeding programme.

Mahalanobis D2 statistics has been suggested as a useful technique for the grouping of resembling genotypes in crops on the basis of their morphological traits (BHANDARI *et al*., 2017). It involves the multivariate analysis for quantifying the degree of genetic divergence among the genotypes (RAVALI *et al*., 2017) and it is reliably and extensively used for the assessment of genetic variation in different crops (BEGUM *et al*., 2013; SHINDE *et al.,* 2012; VIDHYA and KUMAR, 2014). Therefore, the present study was undertaken to assess the genetic diversity

among the genotypes developed in different breeding programmes and to identify suitable donors for a successful crop improvement programme among these 110 accessions in brinjal.

MATERIAL AND METHODS

The present investigation comprised of one hundred and ten accessions of brinjal germplasm. Most of the accessions were developed and maintained at Punjab Agricultural University, Ludhiana. The germplasm included 22, 58, 17 and 13 accessions in round, long, oblong and small round groups of brinjal, respectively. All the germplasm lines were raised during rainy season in randomized block design (RBD) with three replications. Ten plants were selected for taking observation on each of twelve morphological traits viz; plant height (cm), number of primary branches, petiole length (cm), leaf blade length (cm), leaf blade width (cm), days to 50% flowering, pedicel length (cm), fruit length (cm), fruit breadth (cm), number of fruits per plant, fruit yield per plant (kg) and average fruit weight (g) using standard descriptors given by NBPGR (2001). The morphological observations of all the mentioned traits were compiled and replicated data was subjected to Mahalanobis D^2 statistics given by MAHALANOBIS (1936) and as explained by BHANDARI *et al.*, (2017). Mahalanobis's generalized distance (D^2) between two genotypes was estimated on the basis of the 'p' characters as explained by the equation:

$$D^2 = \sum_{i=1}^{p}\sum_{j=1}^{p} wij(Xi1 - Xi2)(Xj1 - Xj2)$$

Where, wij = variance-covariance matrix, w^{ij}= reciprocal of (wij), (i j = 1, 2……, p), X_{i1} = sample mean for i^{th} character for first sample and X_{i2} = sample mean for i^{th} character for second sample. In present study, twelve quantitative characters (p = 1 to 12) were used to perform the above analysis. Mahalanobis's generalized distances (D^2) were calculated for each pair of genotypes and all the genotypes were grouped into different clusters according to Tocher 's method. Average Intra and Inter cluster distances were calculated as per the method provided by SINGH and CHAUDHURY (1985). A computer software programme named INDOSTAT (Indostat Statistical Software Package developed by Indostat Pvt. Ltd., Hyderabad, India) was used for conducting D^2 statistics for the assessment of genetic divergence among the germplasm lines of brinjal.

RESULTS AND DISCUSSION

Cluster constellation

One hundred and ten genotypes were classified into eleven clusters on the basis of D^2 values calculated from the analysis of twelve morphological traits (Fig 1). Among all the clusters, fourth cluster being the largest consisted of 33 genotypes that was followed by first cluster (25 genotypes), seventh cluster (18 genotypes), third cluster (15 genotypes), sixth cluster (9 genotypes) and eighth cluster (5 genotypes). Each of other five clusters has only one genotype (Table 1). The variable number of accessions in different clusters pointed toward the presence of wide range of genetic diversity within as well as between the clusters. The inclusion of

genotypes into diverse clusters did not adopt any set pattern of particular group on the basis of fruit shape and size. Each cluster was found variable with respect to the fruit shape, size and colour. The genotypes with thin long fruits and cluster bearing habit came together in sixth cluster. Mixed the genotypes with long, oblong and medium round fruits and single or cluster bearing habit have been grouped in seventh cluster. Eighth group contained big round genotypes with single bearing habit. Second, fifth, ninth, tenth and eleventh clusters included PBL-268 (long group with single bearing habit), PBGL-405 (thick long fruits with single bearing habit), PBL-243 (thin long fruits and single bearing habit), PSR-308 (small fruits and cluster bearing habit), PBOB-512 (oblong fruits and single bearing habit), respectively.

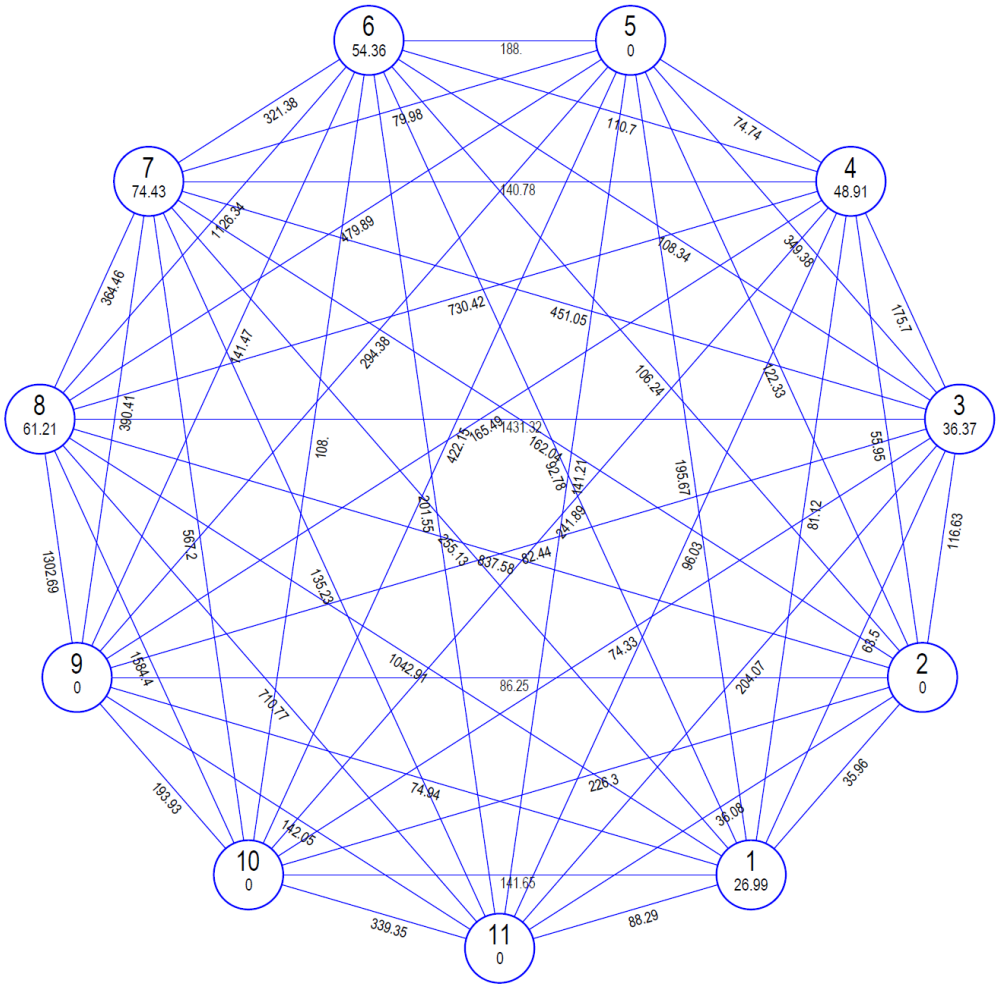

Fig. 1. Inter and intra cluster distances for one hundred and ten genotypes in brinjal

Table 1. Group constellation of one hundred and ten genotypes through D^2 analysis in brinjal

S. No.	Cluster	Number of genotype (s)	Genotype (s)
1	I	25	PBL-244, PBL-257, PSL426, PSL-424, PBR-137, PBOB-510, PBGL 332, PSR 333, PBRG-224, PSR 334, PSR-303, PSR 320, PSL-419, PSL-427, PSR-322, PBRW-136, PBL-253 ,PBL-246, PBL-258, PBL-256, PBR-101, PSR 305, PWL-406, PWL-502, PWL-302
2	II	1	PBL-268
3	III	15	PBL-418, PSL-428, PBGL 415, PBOB-504, PSR 307, PSL-318, PSL-310, PSR 314, PSR 327, PSL-420, PBR-144, PSR 324, PBOB-511, PSR 313, PBR-138
4	IV	33	PSL-425, PBOB-516, PBOB-503,PBL-247, PBOB-505, PBOB-512, PBGL-412, PBL-241, PBRW-124-1, PBOB-507, PBL 216, PBOB-506, PSR-335, PBL 207, PBOB-509, PBL-255, PBOB-515, PBR-131, PBL-250, PBL-261, PBOB-514, PBR-134, PBL-249, PSL-422, PBL-267, PBL-259, PBL-245, PBL-264, PSL-423, PBRG-111, PBL-251, PBL-248, PBL-254
5	V	1	PBGL-405
6	VI	9	PBL 208, PWL-231, PBL-203, PWL-303, PBGL 417, PBL 214, PBL-232, PBL-266, PBL-252
7	VII	18	PSL-421,PBL-260, PBGL 217, PBL-263, PBOB-519, PBOB-513, PBR-143, PBR-113, PBMR-494-1, PBOB-508, PBRW-135, PBL-262, PBR-139, PBOB-517, PBL-265, PBR-142, PBR-133, PBGL-401
8	VIII	5	PMRG-322, PBR-140, PBRG-123, PBR-141, PMR-322
9	IX	1	PBL-243
10	X	1	PSR-308
11	XI	1	PBOB-518

The group constellation of different 110 genotypes was also plotted in the form of a dendrogram in Fig. 2, where euclidian distances in dendrogram highlighted the genetic divergence between and within the clusters. First, third, fourth, sixth, seventh and eighth clusters have represented a range of variability among the member genotypes, however PMR-322 in eighth, PBGL-401, PBR-133, PBR-142 and PBL-265 in seventh, and PBL-252 and PBL-266 in sixth cluster also diverged considerably from the other members of their respective groups. The crosses between the members of these clusters shall be expected to exhibit high heterosis and diverse genetic recombinants in F_2 generation with the desirable traits. The grouping of dissimilar genotypes in same cluster might be resulted from unidirectional selection carried out by the breeders during the period of development of potential genotypes. The results of our study were substantiated with the similar reports on diversity study provided by QUAMRUZZAMAN *et al.* (2009); BEGUM *et al*, (2013); DEVI *et al.* (2016); RAVALI *et al.* (2017); DAS and DAS (2017) and QUAMRUZZAMAN *et al.* (2009) who assembled brinjal germplasm into 5 clusters (19 genotypes), 10 clusters (92 genotypes), 4 clusters (24 genotypes), 10 clusters (35 accessions), 11 groups (26 accessions), and 5 clusters (21 genotypes) respectively.

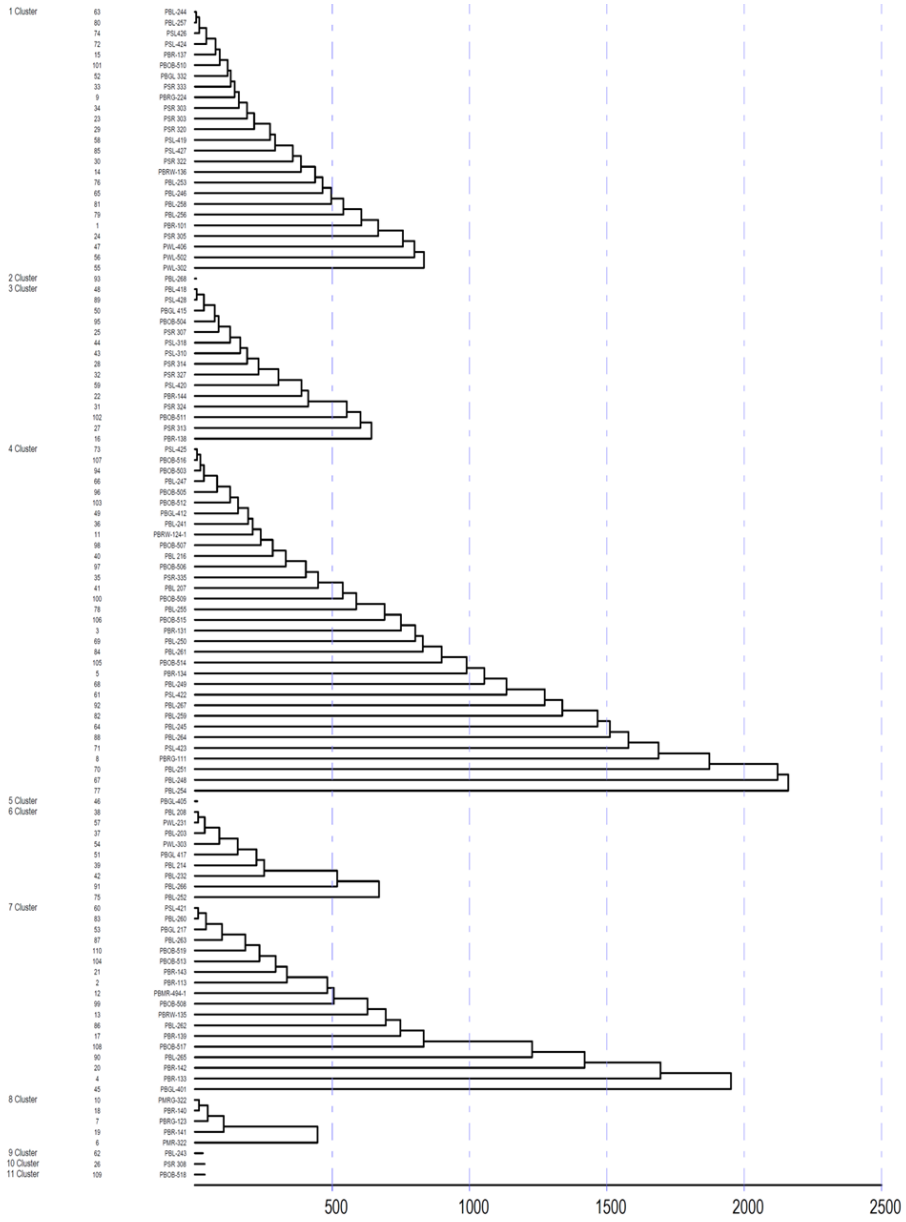

Fig. 2. Dendrogram showing Euclidean distances based on 12 quantitative traits among 110 brinjal genotypes

Average inter as well as intra-cluster distances computed through Mahalanobis (D^2) analysis of all the accessions under investigation are presented in Fig 1. The inter-cluster distances ranged from 35.96 (first and second) to 1584.40 (eighth and tenth). The lowest and the highest values of D^2 revealed less and more genetic divergence among the genotypes of the mentioned clusters. Therefore, the genotypes included in eighth and tenth cluster (1584.40) displayed maximum diversity among each other that was followed by third and eighth cluster (1431.31), eighth and ninth cluster (1302.69), sixth and eighth cluster (1126.33) and first and eighth cluster (1042.91). The prevalence of high genetic variation between the members of these clusters could be exploited for hybridization program to get better recombinants in the segregating generations**.** On the other side, the lesser inter-cluster distances between first and second cluster (35.96), followed by second and fourth cluster (55.94), first and third cluster (63.50), third and tenth cluster (74.331) and fifth and seventh cluster (79.33) enlightened the lower degree of divergence and close genetic makeup of these genotypes. The intra-cluster distances for the second, fifth, ninth, tenth and eleventh clusters were the lowest (0.00), because of the inclusion of single genotype in each. However, the maximum intra-cluster distance was displayed in seventh cluster (74.43) with eighteen genotypes, followed by eighth (61.20), sixth (54.36), fourth (48.91), third (36.66) and first (35.96) clusters with five, nine, thirty-three, fifteen and twenty-five genotypes, respectively. The much lower Intra-cluster distances indicated homogenous nature of genotypes within the clusters, while higher values displayed heterogeneous nature of clusters (QUAMRUZZAMAN *et al.,* 2009; KUMAR *et al.,* 2013). Lower level of intra- cluster distances explained narrow genetic variation within the cluster. Therefore, the members of same cluster were not expected to yield desirable recombinants. In present investigation, seventh cluster with the highest intra-cluster distance had the maximum heterogeneity among the assembled genotypes that is also clear from the assembling pattern of the member genotypes in the dendrogram (Fig 1). It means that the genotypes in this cluster were highly diverse for the traits under study. These results for genetic variation within and between the clusters were substantiated with the reports of KUMAR *et al.* (2013); LOKESH *et al.* (2013); BEGUM *et al.* (2013); DEVI *et al.* (2016); DAS and DAS (2017); RAVALI *et al.* (2017); QUAMRUZZAMAN *et al.* (2019).

Cluster-wise performance

The considerable morphological diversity of different clusters could also be explained from the average performance of their member genotypes for 12 morphological traits as presented in Table 2. Among all the clusters, vigorous and tallest plants were observed in fifth and eleventh cluster (133 and 132cm, respectively). However, the highest number of primary branches was observed in sixth cluster followed by fourth (4.28) first (4.18) and fifth (4.00) clusters. The maximum (7.50 cm) and minimum (3.16 cm) petiole length were noticed in ninth and second clusters, respectively. The maximum vegetative growth with respects to longest and broadest leaf dimensions was seen in ninth followed by fifth cluster. The earliest flowering (59.00 days) was observed in tenth cluster. On an average, the longest fruits were harvested from ninth and fifth clusters due to the inclusions of the maximum genotypes with long fruit types. However, the broadest fruits were picked from sixth and seventh clusters because of round genotypes in these groups. The highest number of fruits (43.17) was harvested in tenth cluster followed by sixth cluster (37.43), whereas the highest yield (2.83 kg) and fruit weight (317.43 g)

were observed in fifth and eighth clusters, respectively. The single genotype of tenth group (PSR-308) was earliest in flowering and bore maximum number of fruits with less vegetative growth. Similar type of observations for morphological variability in brinjal have been earlier reported by UDDIN *et al.* (2015); SADARUNNISA *et al.*, (2015) and RAVALI *et al.* (2017).

Table 2. Mean values of different clusters for 12 morphological traits of 110 genotypes in brinjal

Trait	Clusters										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
PH	92.77	103.00	94.14	94.45	133.0	106.5	114.8	107.9	87.3	86.0	132.0
NPB	4.18	3.66	3.91	4.28	4.00	4.88	3.96	3.80	3.66	2.33	1.67
PL	3.99	3.16	4.10	4.40	4.93	3.95	3.99	4.18	7.50	4.00	4.50
LBL	13.88	13.20	13.78	16.11	18.06	13.97	15.76	15.62	20.50	13.33	12.66
LBW	8.66	8.33	8.77	9.97	11.50	7.66	9.75	9.30	15.50	7.50	7.90
DFF	66.76	65.00	65.02	66.39	64.00	65.59	67.18	67.33	70.00	59.00	73.33
PDL	4.33	5.16	4.35	4.59	5.83	4.80	4.67	4.48	7.60	4.66	7.16
FL	10.34	17.16	9.90	13.24	20.33	17.29	13.78	10.74	20.66	6.23	10.83
FB	4.89	4.73	4.52	4.97	5.00	3.37	6.28	7.65	3.70	4.60	5.83
NFP	15.55	9.85	21.63	22.61	26.07	37.43	13.11	9.58	11.95	43.17	1.46
AFW	93.19	116.67	54.85	132.97	172.22	93.82	193.93	317.43	73.33	50.00	138.89
FYP	1186.3	948.7	1225.2	2055.3	2828.3	1982.8	1612.3	1959.3	890.7	1733.6	472.8

Plant height (cm), Number of primary branches, Petiole length (cm), Leaf blade length (cm), Leaf blade width (cm), Days to 50% flowering, Pedicel length (cm), Fruit length (cm), Fruit breadth (cm), Number of fruits per plant, Average fruit weight (g), Fruit yield per plant (kg)

The high yield potential in brinjal has been reported to be associated with the number of primary branches, number of fruits per plant and fruit weight (SUJIN *et al.*, 2017; CHAITANYA and REDDY, 2017). In present study, the genotypes involved in Ist, IVth, Vth, VIth and XIth clusters carrying good number of primary branches and taller plants that can be used to improve plant vigour. However, the genotypes in IIIrd, IVth, Vth, VIth and Xth clusters yielded higher number of fruits per plant and Vth, VIIth, VIIIth and XIth clusters displayed more fruit weight. For the improvement in yield, highly diverse genotypes from these clusters may be used in hybridization program to get highly desirable recombinants in F_2 generation. The parents with better plant height, smaller petiole and peduncle length along with lesser foliage growth can be used to develop genotypes suitable for net house cultivation as longer plants with less vegetative growth are required for better light penetration during commercial cultivation. Additionally, the precise selections for specific characteristics may help in the development of trait-specific high-yielding inbreds. PSR-308 from tenth cluster can be included as parent to earliness in F_2 recombinants.

Among various groups, the genotypes were more distinct between VIIIth and Xth Clusters, where former had the heaviest fruits in terms of fruit weight and the later was characterized with the maximum number of fruits per plant along with the smallest fruits in terms of average fruit weight. Therefore, the hybridization of PMRG 322, PBR-140, PBRG-123, PBR-141 and PMR-322 from eighth cluster with PSR-308 from tenth cluster may result in earliness and can provide a range of fruit weight, fruit breadth, fruit yield per plant, number of

fruits per plant in first segregating generations. There are chances of getting better transgressive segregants after 3-4 generations of inbreeding. Similarly, the high yielding genotypes of fifth cluster can also be utilized. Superior recombinants with high heterotic values for yield and related traits may be developed through hybridization between parents across the groups. For hybridizations, the parents should be chosen from clusters expressing moderate to high D^2 values and crossed to obtain broad range of variability or segregation for the target traits. Initially, the clusters with target traits should be identified and the best genotypes from the same may be preferred for intercrossing to accomplish the better combinations in advanced generations.

Trait-wise contribution to diversity

Some morphological traits may represent higher contribution towards genetic variation as compared to the others. In this study, the contribution of twelve morphological traits towards the genetic divergence is presented in table 4. Among various morphological traits in this study, average fruit weight (67.86%) had the maximum involvement for the appearance of the diversity. Rest of the variation among different groups or genotypes was emerged because of the number of fruits per plant (17.26%), fruit yield per plant (5.37%), fruit breadth (3.70%), fruit length (2.30%) and petiole length (1.00%). However, the traits such as plant height (0.67%), number of primary branches (0.58%), leaf blade length (0.50%), leaf blade width (0.43%), days to 50% flowering (0.17%) and fruit pedicel length (0.15%) brought negligible share towards the variation present among the germplasm lines. Therefore, fruit weight, number of fruits per plant and fruit yield per plant with the maximum share towards total divergence should be preferred during selection of at least one parent for hybridization program. The importance of morphological traits in genetic diversity was also substantiated with the findings of SADARUNNISA *et al.* (2015); RAVALI *et al.* (2017); ISLAM *et al*. (2018) and NAND *et al.* (2018) in the different parts of the world.

Table 3. Per cent contribution of the traits towards genetic divergence in brinjal genotypes

S. No.	Source	Times ranked 1st	Contribution %
1	Plant height (cm)	40	0.67
2	Number of primary branches per plant	35	0.58
3	Petiole length (cm)	60	1.00
4	Leaf blade length (cm)	30	0.50
5	Leaf blade width (cm)	26	0.43
6	Days to 50% flowering	10	0.17
7	Fruit pedicel length (cm)	9	0.15
8	Fruit length (cm)	138	2.30
9	Fruit breadth (cm)	222	3.70
10	Number of fruits per plant	1035	17.26
11	Fruit yield per plant (kg)	322	5.37
12	Average fruit weight(g)	4068	67.86

CONCLUSIONS

It can be concluded that the germplasm lines developed in different breeding programmes and used in the present investigation had high morphological divergence and could be divided into eleven distinct clusters through Mahalanobis D^2 statistics of twelve morphological traits in brinjal. Overall, 94.19% divergence was created by average fruit weight, number of fruits per plant, fruit yield per plant and fruit breadth. In future breeding programmes for improvement in brinjal, the selection of parents can be affected from highly diverse groups and this selection can be focused on the above mentioned highly diverse and contrasting traits.

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KLASTER ANALIZA GENETIČKOG DIVERZITETA KOD PATLIDŽANA (*Solanum melongena* L.)

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Izvod

U ovom istraživanju, 110 lokalnih genotipova iz različitih oplemenjivačkih programa patlidžana klasifikovano je u jedanaest klastera na osnovu njihovih vrednosti D^2 izračunatih pomoću Mahalanobisa D^2 statistike za dvanaest morfoloških osobina, pri čemu su udaljenost unutar i između klastera pokazali genetičku divergentnost genotipova grupisanih između i unutar različitih klastera. Među svima, četvrti klaster je bio najveći sa 33 genotipa; međutim, drugi, peti, deveti, deseti i jedanaesti klaster sadržali su samo po jedan genotip. Genotipovi osmog i desetog klastera bili su veoma raznovrsni (1584.40), zatim trećeg i osmog (1431.31), osmog i devetog (1302.69), šestog i osmog (1126.33) i prvog i osmog klastera. Varijacija unutar klastera bila je najveća u sedmom klasteru (74,43), zatim osmom (61,20) i šestom (54,36) klasteru koji su opisivali raznoliku prirodu osamnaest, pet i devetnaest genotipova u ovim grupama. Međutim, PBL-268, PBGL-401, PBL-243, PSR 308 i PBOB-518 grupisani su pojedinačno u IInd, Vth, IXth, Xth i XIth klasteru, respektivno. Sveukupno, peti klaster je imao najvigorozniji i visoko prinosni (2,82 kg / biljci) genotip (PBGL-405); osmi klaster obuhvatao je genotipove sa velikim okruglim plodovima i maksimalnom težinom ploda (317,43 g); I deseti klaster sa najranijim genotipovima (PSR-308) sa maksimalnim brojem plodova po biljci (43,17). Od dvanaest morfoloških svojstava, 94,19% diverziteta bilo je zbog prosečne mase ploda (67,86%), broja plodova po biljci (17,26%), prinosa ploda po biljci (5,37%) i širine ploda (3,70%), međutim, ostale osobine imale su zanemarljiv udeo u varijaciji. Ova studija stvorila je osnovu za buduće programe hibridizacije kod patlidžana, gde roditelji mogu biti izabrani na osnovu visoko divergentnih grupa kao i osobina.

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