Exploring genetic diversity in brinjal (*Solanum melongena* L.) - Insights from D² analysis and principal component analysis

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Abstract

In the present study, genetic divergence was quantified in thirty brinjal accessions based on 22 morphological descriptors using multivariate techniques. The 30 brinjal genotypes were divided into nine clusters using D² statistic (Tocher's method). The distance between clusters IV and IX was the highest (214.67), followed by clusters IV and VIII (186.04). The largest distance between genotypes within a cluster was found in Cluster I (65.30), followed by Clusters IV, III, and II. For fruit breadth and single fruit weight, Cluster IV with genotypes SM-12 and SM-19 had the highest cluster means. Cluster VI with the genotype SM-14 had the highest cluster mean values for fruit yield per plant and yield per harvest. In Principal Component Analysis (PCA), first four PCs (Principal Components) contributed 59.661% of the total variation and the first seven components were with eigenvalues>1 for the 30 brinjal genotypes. The accessions SM-9, SM-12, SM-19, SM-14 and SM-27 were placed distinctly in the scatter plot of PCA as well as in different clusters with high inter-cluster distance in cluster analysis of Tocher's method. Based on this, it can be inferred that crosses between these genotypes can be used to produce heterotic hybrids in future crop improvement programs.

Key Words: Brinjal, D² analysis, Principal Component Analysis.

Introduction

Brinjal (*Solanum melongena* L.) (2n=2x=24) commonly called as eggplant, is a warm-weather crop belonging to the Leptostemonum clade of genus *Solanum* (Solanaceae). With an annual production of 13.02 million tonnes, brinjal is cultivated in India on an area of 0.75 million hectares (GoI, 2022). An increasing rate of consumer preference for high-quality and phytonutrient-rich fruits makes it necessary to explore the genetic diversity of brinjal at various levels to meet the needs of consumers. Compared to cultivated species, wild relatives have significantly more genetic diversity (Mutegi et al., 2015). They tend to grow even in extreme conditions with a wide range of day-night temperatures, swampy and water-logged areas (Knapp et al., 2013; Davidar et al., 2015). These characteristics make them an important component of brinjal breeding for climate change adaptation. However, there is very little use of wild accessions in brinjal breeding programs, unlike other solanaceous crops like tomato (Rotino et al., 2014). In addition, a progressive dominance of commercial varieties and especially hybrids over local cultivars in recent years resulted in narrowing the useful gene sources available in local germplasm in the longer term. Despite this threat, there are not enough studies on the genetic diversity of brinjal cultivars currently grown in the country. Evaluation of genetic diversity also acts as a major tool in hybridization

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programs to select potential parents for crossing. This necessitates classifying the readily available germplasm into various groups and estimating the degree of genetic diversity among them (Pandey, 2007). Multivariate techniques like Mahalanobis D^2 statistics and Principal Component Analysis can be used to assess the level of divergence within and between populations. With this background, the current investigation was made with the aim of estimating the genetic diversity among cultivated and wild accessions for fruit yield and its component characteristics and to identify better genotypes.

Materials and Methods

The investigation was conducted in the summer season of 2022 (February–June) at the Instructional Farm–I, College of Agriculture, Padannakkad, Kasaragod (12.2°N Latitude, 75.1°E Longitude). The design used for the experiment was RBD (Randomized Block Design) in three replications with ten plants in each replication. The plot size was 2.5m x 2.0m with spacing of 90cm x 60cm. The temperature ranged between 30°C-34°C for most of the duration. Soil of the experimental field was sandy with pH ranging from 4.5-5.0. The experimental material consisted of 30 brinjal genotypes (Table 1). One-month-old seedlings of 8-10 cm height were transplanted into the main field at a spacing of 90 cm x 60 cm. The management practices for the crop were followed as per Package of Practices (KAU, 2016). The genotypes were assessed for 22 quantitative characters. For each accession per replication, five plants were selected randomly, and mean values were derived for statistical analysis. The data for 22 characters were analysed using Principal Component Analysis and Mahalanobis D^2 statistics to examine genetic diversity. TNAUSTAT was used for D^2 analysis and KAU GRAPES packages (Gopinath et al., 2020) for PCA (Principal Component Analysis).

Results and discussion

Using Tocher's approach (Rao, 1952), the 30 brinjal genotypes were divided into nine clusters (Table 2). Cluster I, which had 14 genotypes, was the biggest of the nine clusters. Five accessions were in Cluster II, four were in Cluster III, two were in Cluster IV, and the remaining accessions were distributed across Clusters V, VI, VII, VIII, and IX. The wild progenitor *Solanum insanum* fell separately in Cluster IX. *Solanum insanum* and *Solanum incanum* are the closest wild relatives of brinjal, with *S.*

Table 1. List of 30 brinjal accessions used for evaluation

Source of accessions	Genotypes
Local accessions from North Kerala	SM-1, SM-2, SM-3, SM-4, SM-5, SM-7, SM-8, SM-9, SM-10, SM-31, SM-32 and SM-33
NBPGR, Thrissur	SM-12 ((IC111010), SM-13 (IC203604), SM-14 (IC253463), SM-15 (IC253967), SM-16 (IC256208), SM-17 (IC336793), SM-18 (IC383102), SM-19 (IC397299), SM-20 (IC439279), SM-21 (IC545901), SM-22 (IC618035), SM-23 (IC427008), SM-24 (IC241675), SM-25 (Local) and wild accessions SM-26 (IC620612) (*S. incanum)* and SM-27 (IC256161) (*S. insanum*)
KAU varieties	SM-28 (Ponny), SM-29 (Haritha) and Surya (SM-30)

Table 2. Grouping of 30 accessions on the basis of D^2 analysis

S.No.	Cluster number	Number of genotypes	Genotypes
1	I	14	SM-32, SM-33, SM-18, SM-8, SM-3, SM-2, SM-23, SM-1, SM-30 (Surya), SM-10, SM-5, SM-25, SM-16 and SM-17
2	II	5	SM-20, SM-26 (*Solanum incanum*), SM-24, SM-15 and SM-13
3	III	4	SM-28 (Ponny), SM-29 (Haritha), SM-9 and SM-4
4	IV	2	SM-19 and SM-12
5	V	1	SM-31
6	VI	1	SM-14
7	VII	1	SM-21
8	VIII	1	SM-22
9	IX	1	SM-27 (*Solanum insanum*)

insanum being the Asian-originated wild progenitor of the vegetable. Widely found in arid areas, *Solanum incanum* is said to have African origins. Molecular methods have demonstrated the close relationship between *S. insanum, S. melongena*, and *S. incanum*, while maintaining their distinctness (Knapp et al., 2013). The maximum contribution towards genetic divergence was identified for single fruit weight (51.5%) followed by fruit length (25.98%) and fruit width (8.28%) (Fig. 1). A similar
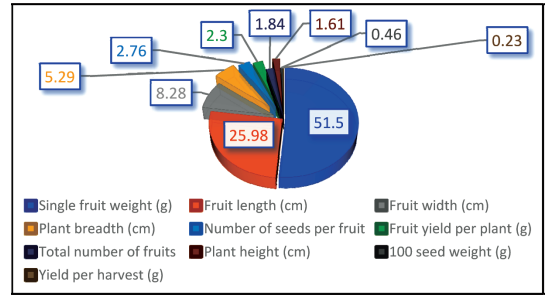

Figure1. Contribution percentages of characters towards divergence

result for fruit traits was reported by Barik et al. (2021).

The ANOVA of nine clusters (Table 3) revealed significant differences for all traits except for maturity traits (days to first flowering, days to fifty percent flowering, days to first fruiting, days to first harvest) and ratio of (long+medium) to (short+pseudo-short) style flowers. The cluster mean values for 17 traits are displayed in Table 4. For fruit width and single fruit weight, Cluster IV with genotypes SM-12 and SM-19 had the highest cluster means. Cluster VI with SM-14 displayed the highest cluster mean for fruit yield per plant and yield per harvest. This could be linked to the existence of specific functional genes in the accessions of clusters IV and VI, which have the

Table 3. ANOVA of cluster means for seventeen characters in nine clusters of brinjal accessions

Character	Mean Sum of Squares		
	Treatment	Replication	Error
Degrees of freedom	8	2	16
Plant height (cm)	274.84**	0.009	0.421
Plant breadth (cm)	283.91**	0.387	0.170
No. of primary branches	3.574**	0.318	0.062
No. of secondary branches	12.21**	0.473	0.175
Stem girth (cm)	0.09**	0.002	0.01
No. of inflorescences per plant	21.33**	6.132	2.537
No. of flowers per inflorescence	0.87**	0.002	0.006
No. of fruits per inflorescence	0.52**	0.016	0.016
Fruits per plant	559.52**	0.541	0.368
Fruit length(cm)	74.87**	0.006	0.014
Fruit width (cm)	27.44**	0.073	0.005
Single fruit weight (g)	1206.52**	3.691	0.166
No. of seeds per fruit	32007.51**	68.409	6.56
100- seed weight (g)	$3.1 \times 10^{-4**}$	2.7×10^{-4}	4×10^{-5}
Crop duration	45.69**	2.089	5.22
Yield per harvest(g)	144600.31**	1907.34	410.06
Fruit yield per plant(g)	1336936.97**	7564.017	1329.82

**significance at 1% level * significance at 5% level

Table 4. Cluster mean of seventeen characters in nine brinjal clusters

Character	C1	C2	C3	C4	C5	C6	C7	C8	C9
Plant height (cm)	70.67	57.85	66.75	63.16	50.71	55.94	60.11	71.73	42.15
Plant breadth (cm)	73.07	71.74	70.00	75.74	50.14	69.77	76.03	81.73	57.97
Number of primary branches	7.97	8.65	7.47	8.00	7.89	7.89	9.56	11.45	9.56
Number of secondary branches	17.49	22.73	18.86	23.00	18.22	22.67	20.89	22.00	21.67
Stem girth (cm)	0.87	1.00	1.10	1.30	0.95	0.93	1.18	0.70	0.96
Number of inflorescences per plant	11.73	13.73	10.86	16.39	10.00	17.00	15.89	13.78	14.89
Number of flowers per inflorescence	2.83	2.82	2.56	2.78	2.89	3.55	3.11	2.11	3.22
Number of fruits per inflorescence	2.37	2.20	2.06	2.00	2.22	3.67	2.22	1.45	3.22
Fruits per plant	16.09	23.87	23.56	28.11	18.11	53.00	21.89	12.67	45.89
Fruit length (cm)	12.10	8.69	20.34	10.81	10.49	5.67	9.74	20.22	10.35
Fruit width (cm)	11.92	10.99	14.15	19.38	10.68	11.28	9.65	9.68	11.63
Single fruit weight (g)	66.23	38.07	72.52	81.10	77.53	46.73	39.97	41.07	26.30
Number of seeds per fruit	584.26	538.11	625.69	642.95	572.45	689.00	856.11	524.67	696.66
100 seed weight (g)	0.47	0.46	0.47	0.48	0.45	0.46	0.46	0.46	0.45
Crop duration (Days)	148.97	145.18	147.31	145.06	156.78	145.00	152.78	148.67	148.22
Yield per harvest (g)	359.29	303.03	596.32	773.71	468.76	828.17	325.27	179.69	408.59
Fruit yield per plant (g)	1077.86	909.09	1788.97	2321.13	1406.27	2484.50	877.53	539.07	1225.77

Table 5. Inter and Intra (underlined) cluster distances (D^2) among 30 brinjal accessions

Cluster no.	1	2	3	4	5	6	7	8	9
1	65.30	104.29	95.44	107.51	91.81	111.12	115.17	114.75	154.65
2		58.97	147.66	170.32	133.69	76.69	78.79	97.37	96.64
3			61.83	115.29	96.22	160.22	161.44	122.50	176.57
4				62.80	121.42	162.81	177.98	186.04	214.67
5					0.00	128.27	152.82	154.28	153.57
6						0.00	83.33	141.39	90.03
7							0.00	108.20	115.81
8								0.00	137.42
9									0.00

potential to play a more significant role in contributing to the variation in yield and its component characters. Recognizing the origin of genes contributing to a specific trait is crucial for assessing genetic diversity within a germplasm, as highlighted by Tomooka (1991).

According to Table 5, there was substantial heterogeneity within a cluster as seen by the highest distance between genotypes inside Cluster I, which was followed by Clusters IV, III, and II. This suggests that genotypic divergence existed even after clustering. Similar findings in brinjal were reported by Sindhuja et al. (2019).The selection of parents with high genetic variation for the desired traits largely determines the efficacy of hybridization followed by selection, according to Murty and Arunachalam (1966). Therefore, the intra cluster heterogeneity among the component genotypes found in this study may serve as guidance when choosing parents for the recombination breeding strategy. Similar findings were made about eggplant by Kaur et al. (2019), who argued that hybridization between genotypes in such clusters can result in transgressive segregates.The remaining clusters, which are all solitary, exhibited no intra-cluster distance.Cluster IV (SM-19 and SM-12) and IX (*S. insanum*) were found to be the two clusters with the greatest distance between them followed by clusters IV and VIII (SM-22) indicating high diversity between these clusters. The accessions from clusters IV and IX could be the parents of interspecific hybridization for the development of introgression lines (ILs), a crucial step in expanding the genetic base of brinjal. According to Plazas et

al. (2016), the production of compatible hybrids with a high number of seeds per fruit and good germination when the *S. melongena* accession is used as the female parent in interspecific hybridization is what leads to the classification of the wild species *S. insanum* into the primary gene pool (GP1). In contrast to interspecific hybrids with other wild species, where fruits from hybrids are typically significantly more similar to the wild relative parent, interspecific hybrids between *S. melongena* and *S. insanum* are intermediate to both parents in fruit size and form.

Ranil et al. (2017) claim that this suggests that in backcross breeding operations employing *S. insanum* as donor parent, there will be a swift recovery of fruit size and typical traits of the fruit of *S. melongena*.The accessions from clusters IV and VIII and IV and VII can be chosen for intra-specific hybridization, allowing for a wide range of diversity in growth and yield traits. PCA is an effective method for reducing the number of dimensions in a data collection and highlighting the importance of the main source of variance. Among the 22 principal components derived, the initial four principal components stood out as significant,

Table 6. Eigen values, proportion of variation and total variation across axis

PC	Eigen value	% variance	Cumulative variability (%)
1	4.206	20.030	20.030
2	3.703	17.632	37.662
3	2.535	12.069	49.731
4	2.085	9.930	59.661
5	1.790	8.524	68.185
6	1.441	6.863	75.048
7	1.181	5.623	80.671

Table 7. Factor loadings of traits in brinjal genotypes for four principal components

Variables	PC1	PC2	PC3	PC4
Plant height (cm)	-0.230	0.268	-0.186	0.135
Plant breadth (cm)	0.015	0.108	-0.209	0.408*
Number of primary branches	0.225	-0.058	-0.080	0.434*
Number of secondary branches	0.046	-0.242	-0.082	0.445*
Stem girth (cm)	0.059	-0.152	-0.051	0.238
Number of inflorescences per plant	0.121	-0.274	-0.127	0.328*
Number of flowers per inflorescence	0.208	-0.15	-0.362	-0.266
Number of fruits per inflorescence	0.164	-0.116	-0.406	-0.217
Days to first flowering	0.284	-0.053	0.375*	0.040
Days to 50% flowering	0.060	-0.195	0.319*	0.053
Days to first fruiting	0.362*	0.015	0.315*	-0.145
Days to first harvest	0.235	-0.069	0.319*	-0.021
Number of seeds per fruit	-0.029	-0.297	0.086	-0.122
100 seed weight (g)	-0.293	0.115	0.182	0.126
Ratio of (long+medium) to (short+pseudo-short) style flowers	0.040	0.278	0.233	0.127
Fruit length (cm)	-0.253	0.122	0.146	0.037
Fruit width (cm)	-0.233	-0.211	0.160	0.223
Single fruit weight (g)	0.376*	0.054	0.037	-0.077
Fruits per plant	-0.031	-0.427	-0.006	-0.081
Marketable fruit yield (g)	-0.302	0.357*	0.063	-0.073
Fruit yield per plant (g)	-0.308	0.352*	0.063	-0.074

Table 8. Possible cross combinations of brinjal accessions based on superior yield performances and average inter-cluster distance

Cluster combination	Average cluster distance	Cross combination	Intra/Interspecific cross
IV x IX	46084.47	SM-19 x SM-27 (*S. insanum*)	Interspecific cross
		SM-12 x SM-27 (*S. insanum*)	
IV x VIII	34612.52	SM-19 xSM-22	Intraspecific cross
		SM-12 x SM-22	
II x IV	29007.85	SM-19 xSM-15	Intraspecific cross
		SM-12 x SM-15	
IV x VI	26507.76	SM-19 x SM-14	Intraspecific cross
		SM-12 x SM-14	
III x VI	25673.30	SM-28 (Ponny) x SM-14	Intraspecific cross
		SM-14 x SM-9	
II x III	21804.56	SM-28 (Ponny) xSM-15	Intraspecific cross
		SM-9 x SM-26 (*S.incanum*)	Interspecific cross
V x VI	16453.03	SM-14 xSM-31	Intraspecific cross

Figure 2. Biplot of 30 brinjal accessions using PC 1 and PC 2

collectively accounting for 59.661% of the total variance. Further more, the first seven components, each possessing eigen values > 1, collectively played a role in describing the characteristics of the 30 brinjal genotypes, as depicted in Table 6. The Table 7 illustrates the factor loadings of various traits associated with the four principal components obtained in the study. The first PC had high positive loadings for days to first fruiting and single fruit weight. The main traits that brought variation in PC 2 were marketable fruit yield and fruit yield per

plant. Days to first flowering, days to 50% flowering, days to first fruiting, and days to first harvest were the key loading factors for the third PC. The fourth PC was contributed by plant breadth, number of primary branches, number of secondary branches and number of inflorescences per plant. Similar results for PC 1 and PC 2 were reported by Ahmed et al. (2014). Marketable fruit yield, single fruit weight, and total fruits per plant showed larger phenotypic contribution to fruit yield in the biplot produced using PC 1 and PC 2 (Fig. 2). The genotypes SM-19, SM-12 and SM-10 are found to be superior due to their close proximity to the yield and its contributing attributes, while SM-9 and SM-14 were found to be more diverse among all the genotypes. In future selection processes, opting for traits that exhibit a positive correlation with yield would be a strategic and effective approach. Utilizing the yield potential and maximum genetic divergence as criteria, Table 8 provides a compilation of appropriate cross combinations. These combinations can be employed in forthcoming crop improvement initiatives to develop heterotic hybrids.

Conclusion

The current study identified phenotypic and genotypic variance among 30 genotypes of brinjal. The outcomes of the D² analysis and Principal Component Analysis were found to be consistent and in agreement with each other. Genotypes SM-9, SM-12, SM-19, SM-14, and SM-27 demonstrated diversity compared to all other genotypes in both methods. This genetic diversity provides promising possibilities for brinjal improvement through straightforward selection based on superior traits and the crossing of compatible parent plants.

Acknowledgement

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