

Morphogical and molecular markers based assessment of genetic diversity in eggplant

Amandeep Kaur* , Nirmaljit Kaur, Geeta Bassi, Navraj Kaur and A. S. Dhatt

Department of Vegetable Science, Punjab Agricultural University, Ludhiana 141004, Punjab

ABSTRACT

In the present study, twenty-six eggplant genotypes were characterized using morphological traits and seventy simple sequence repeat (SSR) markers. Information of the genetic diversity of eggplant is imperative to support management and conservation programme that will subsequently aid in sustainable production of this crop. Multivariate analysis (Principal component analysis, cluster analysis and Mahalanobis D2) and UPGMA was done in order to group the genotypes into clusters. On the basis of morphological traits, the genotypes grouped in cluster IV and VI can be selected in crop improvement program because of their highest inter-cluster distance (56.43). Genotypes of cluster II were heterogeneous and showed highest intra-cluster distance (12.71). PCA suggested that 76.73% of the total variation was explained by the first 8 PCs. Out of 70 SSR markers used, 40 were polymorphic and 30 monomorphic. The average PIC value and number of alleles were 0.50 and 2.55 per markers, respectively. On the basis of PIC values and number of alleles, emi03K06 was most informative polymorphic marker. The morphological and molecular evaluation of set of 26 eggplant genotypes will serve as source for generation of elite varieties with desirable characters.

Key words: *Solanum melongena*, Cluster analysis, PCA, SSR marker.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a member of family *Solanaceae* which is also known as Nightshade family. In India, it is cultivated in approximately 0.76 million ha area with an annual production of 12.99 million tonnes and productivity of 17.09 tonnes per ha (NHB, 11). It contributes about 7.65 percent of the total vegetable production of India and is placed at sixth position among the vegetables grown in the world (FAOSTAT, 8). Eggplant being native to India, is available in diverse forms that can be utilized in future investigations and breeding programmes. For any effective breeding programme, information concerning the extent and nature of genetic diversity within a crop is essential. It is useful for characterizing individual accessions and cultivars and as a general guide for the selection of parents for hybridization. Eggplant is a highly nutritious vegetable and has got multifarious uses as a dish item being a valuable source of minerals, particularly iron and vitamins such as A, C, B6, B1, folate and niacin **(**Singh, 16**).** Additionally, the eggplant peel is rich in anthocyanin having therapeutic potential against hyperlipidemia and cardiovascular diseases as it inhibits lipid peroxidation (Singh, 16).

Morphological traits have been important and serve as an informative index to evaluate the characteristics of a plant to identify different

germplasm resources. Although this path may be influenced by the environmental variables, yet the morphological traits have been the direct and acquired index. For enhancing accuracy level on the results based on morphological traits offer more information on genetic diversity and variation, studies supported by molecular markers combined with morphological traits are imperative (Mao *et al*., 9). Molecular characterization constitutes the foundation for an effective conservation procedure and adequate exploitation of the available gene pool. Molecular markers are an important tool for the plant scientists to ascertain the genetic diversity within and between plant populations. Markers like Random amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP) and Simple sequence repeats (SSR), Inter-simple sequence repeats (ISSR) are being used to assess genetic diversity, characterization of genotypes and linkage mapping in eggplant (Vilanova *et al*., 20; Nunome *et al*., 12; Stàgel *et al*., 18). SSR markers are a preferred tool for assessing individual and population dynamics, developing genetic linkage map and marker-assisted selection (MAS) owing to their simplicity, reliability, wide genomic distribution, co-dominant inheritance, bi-allelic nature and cost effectiveness (Chinnappareddy *et al*., 4).

In view of the extensive importance of genetic diversity in future breeding programmes, morphological characterization and genetic analysis

^{*}Corresponding author's E-mail: amandeepkaurvirk526@gmail.com, amandeepbot@pau.edu

of twenty-six eggplant genotypes was conducted at Punjab Agricultural University, using qualitative and Simple Sequence Repeat (SSR) markers.

MATERIALS AND METHODS

The twenty-six eggplant genotypes (Table 1) including six hybrids (PBH-3, PBH-4, BH-2, PHBR-41, PBHR-42 and PBHL-52) along with their parents procured from Department of Vegetable Science, Punjab Agricultural University (PAU), Ludhiana were selected for the present investigation. The studies were conducted in the Laboratories and Research Farm of Seed Technology Section, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana, Punjab, India, during 2017. The sowing of seeds was done in March 2017 and the crop was raised as per the package of practices for cultivation of vegetables.

Twenty-one qualitative characters were observed during different growth stages of the crop *viz*, seedling, vegetative, flowering and fruiting as per guidelines of PPV&FRA (The Protection of Plant Varieties and Farmers Right Authority) (Anonymous, 2). For the studies, ten healthy plants were selected from the central rows of each plot. The multivariate analysis (Principle Component Analysis, Cluster analysis and Mahalanobis's D^2) was carried out using INDOSTAT software and the dendrogram was obtained by using cluster analysis (Ward's method). The inter and intra- cluster distances were calculated by Mahalanobis's D 2 .

Genomic DNA was extracted from five plants of each sample following cetyl tri-methyl ammonium

Table 1. List of eggplant genotypes used in this study

Sr. No.	Genotype	Germ plasm type	Sr. No.	Genotype	Germ plasm type
1	PBH-3	Hybrid	14	BL-216	Line
2	PBH-4	Hybrid	15	PSB-7-2	Variety
3	BH-2	Hybrid	16	93SN-22-1-1-2	Line
4	PBHR-41	Hybrid	17	BL-2011-417-1-2	Line
5	PBHR-42	Hybrid	18	BLW-231	Line
6	PBHL-52	Hybrid	19	BMR-494-1	Line
7	P-67	I ine	20	KBSR-343-1	Line
8	BL-214	Variety	21	93PSB-1-1-2-1	Line
9	BL-201	Line	22	BR-113	Line
10	BL-219	Line	23	BR-116	Line
11	BR-104	Variety	24	BR-101	Line
12	MR-319	Line	25	Kanya-6-1	Line
13	BR-109	Line	26	SC-15-2	Line

bromide (CTAB) method (Doyle and Doyle, 7). The quality and concentration of DNA concentration was estimated by 0.8% agarose gel electrophoresis and spectrophotometer analysis (Nanodrop, Thermo Fisher, USA). Seventy SSR primers were selected from the previous studies of Chinnappareddy *et al*., 4; Nunome *et al*., 12 and Numome *et al*., 13. A working DNA concentration of 50 nguL⁻¹ was prepared and stored at 4°C until further use. Initial denaturation of DNA was done for 5 minutes at 94 °C followed by 35 cycles of denaturation at the same temperature for 1 minute, annealing was done at 49- 57°C depending upon the SSR primer temperature for 1 minute, extension at 72 °C for 1 minute, and final extension at 72 °C for 10 minutes. The amplified PCR product was analyzed by using 2.5% agarose gel electrophoresis, visualized and photographed in the Gel Documentation and Analysis Systems. Among the genotypes, the genetic diversity was computed using Computer Software Programme DARwin6.0 (Perrier and Jacqumoud-Collet, 14). The amplified fragments were recorded as 1 (presence) and 0 (absence) in each genotype. The Polymorphic information content (PIC) values were estimated by using equation of Anderson *et al.* (1). PIC=1- $\sum_{i=1}^{n}$ (Pij)2.

Where, P_i is the frequency of jth allele in ith primer and summation extends over "n" pattern. Similarity matrix for SSR primers was constructed using Dice coefficient of similarity to find genetic relationship. The data were subjected to Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis to generate dendrogram using DARwin 6.0 software. Data from 40 markers were used to estimate the dissimilarity based on the number of shared amplified bands. Tree was constructed by using Darwin6 on the basis of UPGMA.

RESULTS AND DISCUSSION

Eggplant is cultivated all over the world and in India and is known as the King of vegetables (Doganlar *et al*., 6). Only a handful of recent studies have analyzed eggplant genetic diversity at both the morphological and molecular levels (Munoz *et al*., 10). Such studies are, however, useful for the information that they supply for germplasm management and breeding efforts using collective genetic material. In this study, 26 eggplant genotypes including varieties, lines and hybrids were evaluated for morphological and molecular diversity.

Analysis of variance for different quantitative traits in Table 2, revealed highly significant mean squares of genotypes for all the traits under investigation. A few traits viz., leaf blistering, leaf spinniness and calyx spinniness were missing in all the genotypes evaluated. Among different traits evaluated with

Character	Mean Squares					
	Replications Genotypes		Error			
	(2)	(25)	(50)			
Leaf length (cm)	0.08	10.03	1.00			
Leaf breadth (cm)	0.03	8.24	0.42			
Length of petiole (cm)	0.07	1.09	0.32			
No. of flowers	0.17	16.90	0.33			
Fruit length (cm)	0.67	24.29	0.60			
Fruit diameter (cm)	0.24	4.57	0.18			
Fruit length diameter ratio	0.27	3.64	0.10			
Plant height at early stage (cm)	0.91	113.91	9.16			
Plant height at later stage (cm)	1.05	1136.71	49.07			
Plant spread at early stage (cm)	4.60	380.30	12.51			
Plant height at later stage (cm)	1.09	580.01	43.29			
Length of fruit peduncle (cm)	0.43	1.57	0.24			
Diameter of pistil scar (cm)	0.00	0.39	0.01			
Time of flowering (no. of days)	0.55	111.47	4.54			
Time of physiological fruit ripeness (no. of days)	0.01	554.98	3.41			

Table 2. Analysis of variance for different quantitative characters in eggplant.

respect to growth habit, stem and fruit characters, maximum variation was observed in pubescence, fruit shape and fruit color among the 26 genotypes.

Principal component analysis (PCA) was performed in order to determine which of the eggplant morphological descriptors accounted for the most of the variation obtained. PCA analysis revealed that first 8 PCs gave high eigen values (>0.1) and cumulatively accounted for 76.73% of the total variation indicating a high degree of variation for these characters (Table 3 and Fig. 1). More than 50% of the morphological variation was based on the first 4 principal components i.e., PC1 to PC4. The projections of the 26 accessions have been plotted in a two dimensional (2D) graph in Fig. 1. Based on the 2D graph analysis, three major groups were formed, but some accessions formed demarcation within one of the groups. The first quadrant contained most of the eggplant accessions i.e. twenty-four. The genotypes BLW-231 and P-67 formed completely different clusters in PCA as they were morphologically distinct. PCA based score distribution pattern was reported earlier among 35 genotypes by Solaiman *et al*. (17).

Clustering of genotypes and heat map analysis of all the traits demonstrated that the different eggplant genotypes grouped into six major clusters. The chi square grouped analysis of morphological data using 26 brinjal genotypes was laid down into clusters with variable number of entries that pointed toward the wide range of genetic diversity within as well as between the clusters (Table 4 and Fig. 2).

Fig. 1. Two-dimensional principle coordinates analysis of 26 genotypes based on morphology-based similarity coefficients (qualitative) traits.

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		PC ₁	PC ₂	PC ₃	PC4	PC ₅	PC6	PC7	PC8
Eigene Value (Root)		6.585	4.32	2.72	1.764	1.646	1.371	1.177	1.135
% Var. Exp.		24.389	15.998	10.076	6.534	6.096	5.076	4.359	4.204
Cum. Var. Exp.		24.389	40.387	50.462	56.996	63.092	68.17	72.527	76.731
$\mathbf{1}$	Seedling color	0.242	0.228	0.073	0.122	0.031	0.03	0.207	0.456
1a	Intensity of purple color	0.242	0.218	0.081	0.184	-0.013	-0.02	0.214	0.426
$\overline{2}$	Stem pubescence	-0.188	0.009	0.002	0.428	0.295	0.006	-0.051	-0.284
3	Stem Anthocyanin	0.256	-0.208	-0.026	-0.178	-0.118	0.037	-0.101	0.089
За	Intensity of anthocyanin (upper portion of stem)	0.281	0.072	$\pmb{0}$	-0.042	-0.074	0.463	-0.19	-0.115
3b	Intensity of anthocyanin (middle portion of stem)	0.234	0.139	0.034	-0.027	0.1	0.423	-0.016	-0.278
4	Leaf blistering	$\pmb{0}$	$\pmb{0}$	$\pmb{0}$	$\mathbf 0$	$\pmb{0}$	$\pmb{0}$	$\pmb{0}$	0
5	Leaf spininess	$\mathbf 0$	$\mathbf 0$	0	0	0	0	0	$\mathsf 0$
6	Leaf blade color	0.149	0.274	0.038	-0.382	0.302	-0.21	-0.017	-0.15
7	Leaf margin (sinuation)	-0.087	0.124	0.171	-0.014	0.526	-0.07	0.029	0.295
8	Vein color	0.27	-0.29	-0.174	-0.054	0.147	-0.12	-0.049	0.022
8а	Vein color: intensity of purple color	0.307	0.088	-0.062	0.153	-0.026	0.158	0.095	-0.035
9	Flower color	0.301	-0.019	0.06	-0.023	0.072	0.313	-0.023	0.007
10	Fruit shape	-0.186	0.017	-0.31	-0.312	-0.104	0.216	0.294	-0.064
11	Fruit Glossiness	0.008	-0.23	0.127	0.152	0.206	0.077	0.355	0.01
12	Fruit Stripes	-0.08	0.014	-0.563	0.071	0.168	0.064	0.065	0.122
12a	Intensity of stripes	-0.08	0.014	-0.563	0.071	0.168	0.064	0.065	0.122
13	Fruit Patches	0.149	0.274	0.038	-0.382	0.302	-0.21	-0.017	-0.15
14	Shape of apex of fruit	-0.032	-0.152	0.099	-0.299	-0.078	0.011	0.708	-0.156
15	Spininess of calyx	0	$\pmb{0}$	0	$\pmb{0}$	$\pmb{0}$	$\pmb{0}$	$\pmb{0}$	$\pmb{0}$
16	Calyx color	0.194	0.189	-0.207	0.103	-0.34	-0.33	0.049	-0.098
16a	Calyx:Intensity of purple color	0.235	0.23	-0.098	0.118	-0.275	-0.33	0.092	-0.119
17	Fruit color	0.27	-0.29	-0.174	-0.054	0.147	-0.12	-0.049	0.022
18	Fruit : Intensity of purple color	0.148	-0.319	0.176	0.219	0.017	-0.04	0.23	-0.077
19	Fruit: flesh color	0.27	-0.29	-0.174	-0.054	0.147	-0.12	-0.049	0.022
20	Plant growth habit	-0.142	-0.189	0.078	-0.342	-0.208	0.086	-0.2	0.452
21	Fruit color at physiological maturity	0.094	-0.322	0.102	0.001	0.04	-0.25	-0.134	-0.07

Table 3. Principle component analysis of morphological traits in eggplant genotypes.

The inter- and intra-cluster distances was computed through Ward analysis (Table 5). The inter-cluster distances varied between 15.78 to 56.43 indicating high genetic diversity among the genotypes falling in different clusters. In the present investigation, the maximum inter cluster distance was between cluster IV and VI (56.43) which indicated that the genotypes in cluster IV (93SN-22-1-1-2) and VI (P-67) were highly diverse from each other. The different genotypes which diverged into six different clusters were shown with the help of a dendrogram in Fig. 2. The dendrogram also

Table 5. The Inter and Intra (underlined) cluster distances $(D²)$ among 26 eggplant genotypes based on qualitative traits

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Table 4. Grouping constellation of 26 genotypes on the basis of chi square analysis in eggplant based on qualitative traits.

Red and geeen colours represents high and low levels, respectively. The units in the colour scale are standard deviations

highlighted the genetic variations within and between the clusters. However, the minimum inter-cluster distance was in between cluster III and VI (15.78) that explained the lower degree of divergence and close genetic makeup of these genotypes.

The intra-cluster distance for the clusters IV, V and VI was worked out to be 0.00, because of single genotype in each of these clusters. The highest intracluster distance was observed in cluster II (12.72) that unveiled the most heterogeneous nature of the six genotypes within this cluster. The magnitudes of the intra cluster distances were not always proportional to the number of genotypes in the clusters. Intra-cluster distances were much lower than the inter-cluster ones, indicating heterogeneous or homogenous nature of genotypes within the clusters (Ravali *et al.*, 15). These results for genetic variation in intra and inter clusters were substantiated with the report of Ravali *et al*. (15).

Seventy SSR primer combinations were used to characterize 26 eggplant genotypes. It is well known that co-dominant SSRs have the ability to detect genetic relationship in varieties that have common background or limited genetic diversity. Out of these amplified markers, 40 markers were found polymorphic (57.14%) and the remaining (30) were monomorphic (42.86 %). Number of SSR amplicons generated by primer combination and their respective polymorphic information content (PIC) values are presented in Table 6. Highest number of allele (5) were amplified by the marker emi03K06. The highest PIC value was recorded by emi03K06 (0.78) and the minimum by emf01L14 (0.07). In contrast, Stagel *et al*. (18) reported 28% of microsatellite markers informative within 38 *S. melongena* accessions. The overall variability detected in the current study was low since SSR markers produced 2.55 alleles per locus on an average and PIC value of 0.50 as against 3.1 allele per locus with an average PIC value of 0.38 from 11 EST-SSR markers reported by Stagel *et al*. (18). Vilonova *et al*. (20) detected higher average alleles per locus (4.33) and PIC value (0.50) by using SSR markers in 30 eggplant genotypes.

Using molecular data, four major groups emerged in the dendrogram generated by employing UPGMA method. The similarity coefficient was used (Table 7 and Fig. 3) through Darwin 6. Based on the similarity coefficient analysis of the 26 eggplant genotypes, high level of genetic distance was observed. The cluster I was further branched into sub-clusters IA, IB and IC with thirteen, one and eight genotypes in each cluster respectively. The cluster II consisted of only one genotype SC-15-2, while the cluster III had two genotypes BL-216 and BR-109 and the only genotype in the cluster VI was BH-2.

Fig. 3. UPGMA based molecular relationship among 26 genotypes in eggplant

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The genotype SC-15-2 varied from all other genotypes on the basis of molecular observations but showed similarity with other genotypes on the basis of qualitative traits within cluster I. Whereas, 93SN-22-1-1-2 genotype was placed in a cluster IV on the basis of qualitative traits but on the basis of molecular studies it was grouped in cluster IA with some other genotypes. Similar trend was seen when clustering analysis of the Turkish eggplants based on molecular data showed no relationship with morphological traits of eggplant (Tumbilen *et al*., 19). There are many studies where a similar situation has been reported (Cericola *et al*., 3), while others showed a reasonable level of phenotype / genotype correlation (Munoz *et al*., 10). It has been strongly suggested that combination of morphological and molecular studies is highly recommended than using single analysis for studying genetic diversity (Cortese *et al*., 5).

The present investigation focused on 26 genotypes provides an useful information on the diversity of *S. melongena*, their interrelationship and importance in defining groupings characterized by different levels of similarity. Thus the study indicates the genotype BLW-231 and P-67 are promising as they are highly diverse material. Morphological characterization is inexpensive and is generally the first recommended step initiating any DNA based studies. But morphological characters are highly influenced by environment. Hence, morphological studies were supported by the molecular characterization of the eggplant genotypes. The morphological and molecular evaluation of set of 26 eggplant genotypes will be useful in developing elite varieties with desired characters.

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