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Morphological and molecular characterization of *Solanum melongena* and related species

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Abstract

India is considered as the domestication center for eggplant and its diverse germplasm is notable. In present study, 84 germplasm lines including 79 breeding lines of cultivated eggplant along with five wild species were differentiated on the basis of 21 morphological trait descriptors and 40 simple sequence repeat (SSR) markers. According to principle component analysis (PCA) of morphological traits, 70.33% of the total variation was explained with first 8 PCs. Out of 40 SSR markers, only 19 (50-100%) revealed polymorphism among various cultivated and wild genotypes with a range of 2 and 10 alleles and a mean of 4.9 alleles per marker. On the basis of PIC values and number of amplified alleles per marker, EEMS28 was the highest polymorphic marker, which was followed by CSM31 and CSM27. Jaccard's similarity coefficient among genotypes varied from 0.38 -0.98 being the highest between cultivated genotypes and *S. torvum*. The morphological and SSR data were analyzed individually as well as combined using UPGMA cluster analysis. Morphogenetic divergence within the cultivated eggplant highlighted the possibility of their use in future breeding programmes. Wild species diverged into separate group on the basis of SSRs data, but merged into small round group of cultivated eggplant morphologically as well as morpho-genetically. *S. laciniatum* and *S. aethiopicum* were more close to the cultivated eggplant and can be used in introgression breeding.

Keywords: Genetic diversity, PCA, SSR, *Solanum* species

Introduction

Eggplant (*Solanum melongena* L.) is one of the most important *Solanum* crop, grown as cash crop by small farmers in tropical and subtropical regions across the world. It is placed at the sixth position among the vegetables grown in the world (FAOSTAT 2016). It is a good source of minerals (Calcium, Magnesium, Phosphorous and Iron), poly-unsaturated fatty acids (linoleic and lenolenic acid), vitamins (Group B) and act as antioxidant (San Jose et al. 2014). Additionally, its total nutritional quality is also comparable to tomato (Singh and Kumar 2007). Due to the high nutritional value of fruits, it has also been exploited in medicines to cure liver troubles, high blood cholesterol problem and stomach cancer (Daunay and Hazra 2012). India is considered as centre of origin and diversity of eggplant (Bhat and Vasnathi 2008, Meyer et al. 2012). Genetic diversity can be easily estimated by morphological characterization (Jugran et al. 2013). However, only phenotypic information is not reliable to study genetic diversity, because of change in performance by environmental factors and the developmental stages of the plant (Last et al. 2014). But, it is inexpensive, easily implemented and first recommended step before initiating DNA-based studies (Hoogendijk and Williams 2001). With distinction, molecular markers are more variable, less dependent on the environment and more informative at any developmental stage of a plant (Backes et al. 2003). Now-a-days, genetic diversity is appraised morphologically as well as with molecular markers (Wang et al. 2013). Many types of molecular markers like RAPD, AFLP and SSR have been used to study genetic diversity of the eggplant germplasm (Ali et al. 2011, Asad et al. 2015, Thangadurai et al. 2015). High reproducibility, multi-allelic nature, co-dominant inheritance, abundance and wide genome coverage made SSR's the most commonly used markers for unveiling genetic divergence of a crop. Many researchers used SSRs to study the genetic diversity among the eggplant genotypes (Stagel et al. 2008, Nunome et al. 2009, Ansari and Singh 2014).

Genetic diversity makes the foundation for crop improvement by providing the genetic material for high yield, insect-pest and disease resistance, better environmental adaptations and improved quality. Hybridization necessitates the use of genetically diverse

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parents for crop improvement (Fasoula and Fasoula 2002). Therefore, the development of new eggplant inbreeds using old as well as new breeding methods are highly dependent on the knowledge of genetic diversity in the germplasm. Eggplant also carries rich diversity of wild relatives such as *Solanum laciniatum, Solanum torvum, Solanum sisymbriifolium, Solanum macrocarpon, Solanum aethiopicum* which possess insect-pest and disease resistance (Daunay and Hazra 2012, Rotino et al. 2014) as well as high nutritive value (Mennella et al. 2010, Meyer et al. 2015) and diverse environmental adaptations (Knapp et al. 2013). Mostly the wild types are cross-incompatible with the cultivated type in this crop and if crossing occurred, post-fertilization barriers did not allow the development of normal seed. The introgression of these traits into the cultivated eggplant demands to overcome the cross incompatible barriers. The cultivated eggplant has a lot of variability and the genotypic response for cross-compatibility with wild relatives may be diverse. Therefore, the genetic relatedness with wild species should be scrutinized. Cultivated genotypes with high similarity coefficient with wild relatives may lead to the development of successful inter-specific hybrids in future. In view of the importance of genetic diversity in future breeding programmes, morphological characterization and genetic analysis of eggplant germplasm (84 eggplant genotypes) developed at Punjab Agricultural University along with wild relatives viz, such as *Solanum laciniatum, Solanum torvum, Solanum sisymbrifolium, Solanum macrocarpon, Solanum aethiopicum* using Simple Sequence Repeat (SSR) markers was carried out in present investigation.

Materials and Methods

Plant material: Total 84 accessions included 79 breeding lines of cultivated eggplant along with five wild species were used in this study (Table 1.). All the breeding lines and wild species were grown in replicated trial in 2015-16. The data of various qualitative and quantitative characteristics, especially, vegetative growth and fruit traits was collected during the crop season.

Morphological analysis: Morphologically twenty one traits were determined for plant growth habit (4 traits), leaf (6 traits), flower (4 traits) and fruit (7 traits). Quantitative measurements were carried out on 10 selected plants for each replication. Leaf and fruit dimensions (length and width) were measured using digital caliper with 0.1 cm precision. The mean value of three replications was used for the morphological analysis. One-way analysis of variance (ANOVA) was performed using the Statistical Package for Social Sciences program (SPSS 16.0, SPSS Inc., USA). Coefficients of variation (CV %) were determined as indicators of morphological variability. The simple correlation coefficient was calculated to determine the inter-relationships between the morphological variables using the Pearson correlation coefficient. Further, morphological variables were evaluated by Principal component analysis (PCA) in order to determine the most suitable traits for group wise classiûcation, using SPSS software. The morphological similarity coefficients according to the Euclidean method were calculated using the SIMINT program of the numerical taxonomy multivariate analysis system (NTSYS-pc v2.10) (Rohlf 2000), and the dendrogram was constructed with the SAHN clustering program using the unweighted pair-group method with arithmetic means (UPGMA).

Molecular analysis: Genomic DNA was extracted using a modiûed cetyl tri-methyl ammonium bromide method (Doyle and Doyle 1987). The DNA concentration was estimated using a Nanodrop (Thermo Fisher, USA). DNA quality was checked by gel electrophoresis on 0.8% agarose gel. A working DNA concentration of 50 ng μL^{-1} was prepared and stored at 4°C until use. PCR reaction was carried out in a volume of 10 µl containing 10 ng genomic DNA, 1.5mM $MgCl_2$, 0.2 µM dNTP mix, 0.2 µM of each primer, 1 unit of Taq DNA polymerase and 1x PCR buffer. Reactions were performed in a G- Storm Thermocycler (GMI, Inc. UK) using the following PCR profile: initial denaturation for 5 minutes at 94 °C followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at recommended SSR primer temperature for 1 minute and extension at 72 °C for 1 minute, and final extension at 72 °C for 5 minutes. PCR conditions for all the SSR primers used differed only in their annealing temperature. PCR products were analyzed by gel electrophoresis in a 6% non-denaturing polyacrylamide gel, stained with ethidium bromide. All clearly detectable SSR amplicons were scored as either present (1) or absent (0) and the matrix of SSRs data was assembled. Only well-deûned amplicons were scored. The polymorphic information content (PIC) values for all primers were estimated using the formula:

$$PIC = 1" “ x_i^2$$

Where, x_i is the relative frequency of the i^{th} allele of the SSR loci. Markers were classified as informative when PIC was e” 0.5. PIC values greater than 0.5 indicated loci of high polymorphism, between 0.25 - 0.5 showed loci of intermediate polymorphism and PIC value less than 0.25 indicate loci of low polymorphism (Ge *et al.* 2013). From the binary matrix, similarity matrices were computed using Sequential Hierarchial and Nested

Table 1: List of germplasm lines used for morphogenetic diversity analysis

Acce-ssion names	Source	Code No.	Accession names	Source	Code No.	Accession names	Source	Code No.	Accession names	Source	Code No.
SR-306	PAU, Ludhiana	1	BL-210	PAU, Ludhiana	22	93PSB-2-4-1-2	PAU, Ludhiana	43	RCSC-2-1	PAU, Ludhiana	64
BL-222	PAU, Ludhiana	2	BL-205	PAU, Ludhiana	23	R-307-109-9-2	PAU, Ludhiana	44	BLG-232	PAU, Ludhiana	65
BL-202	PAU, Ludhiana	3	BR-116	PAU, Ludhiana	24	MOB-307-109-9-2	PAU, Ludhiana	45	GL-408	PAU, Ludhiana	66
BR-332-2	PAU, Ludhiana	4	BL-201	PAU, Ludhiana	25	MR-319	PAU, Ludhiana	46	GL-417	PAU, Ludhiana	67
BR-122	PAU, Ludhiana	5	Sel-93C	PAU, Ludhiana	26	93SN-33-1-2-1	PAU, Ludhiana	47	GNR-414	PAU, Ludhiana	68
SR-312	PAU, Ludhiana	6	BR-332-1	PAU, Ludhiana	27	93PFC-22-4-1-3	PAU, Ludhiana	48	RSC-15-2	PAU, Ludhiana	69
SR-317	PAU, Ludhiana	7	Swami Mani	West Bengal	28	93SL-81-3-3-2	PAU, Ludhiana	49	GL-405	PAU, Ludhiana	70
BB-93C	Bhuvneshwar	8	SR-6	PAU, Ludhiana	29	CB-9991-121-1-1	PAU, Ludhiana	50	93SN-33-28-1	PAU, Ludhiana	71
BR-104	PAU, Ludhiana	9	BR-332-3	PAU, Ludhiana	30	RC-SC-11-1	PAU, Ludhiana	51	93SN-62-1-1-1	PAU, Ludhiana	72
SL-309	PAU, Ludhiana	10	BL-240	PAU, Ludhiana	31	W-230-42-45-1-2	PAU, Ludhiana	52	93PSB-3-3-1-1	PAU, Ludhiana	73
SR-301	PAU, Ludhiana	11	MOB-316	PAU, Ludhiana	32	GL-Abi-Collection-103-23	PAU, Ludhiana	53	CB-9991-215-1-1	PAU, Ludhiana	74
BL-211	PAU, Ludhiana	12	BL-219	PAU, Ludhiana	33	SLV-352-3-4	PAU, Ludhiana	54	UGSR-524-1	PAU, Ludhiana	75
BL-204	PAU, Ludhiana	13	93SL-21-3-1-2	PAU, Ludhiana	34	S-324-466-2-2	PAU, Ludhiana	55	MR-325	PAU, Ludhiana	76
BR-112	PAU, Ludhiana	14	UGSR-563-1	PAU, Ludhiana	35	93SL-21-6-1-3	PAU, Ludhiana	56	CH-375-2-1-4-1	IIVR	77
P-71	PAU, Ludhiana	15	93PSB-11-1-2-1	PAU, Ludhiana	36	WO-406	PAU, Ludhiana	57	KBSR-343-1	PAU, Ludhiana	78
BL-213	PAU, Ludhiana	16	GOB-411	PAU, Ludhiana	37	SR-323	PAU, Ludhiana	58	93DBL-23-4-8-21	PAU, Ludhiana	79
BL-235	PAU, Ludhiana	17	GL-403	PAU, Ludhiana	38	BRG-114	PAU, Ludhiana	59	*Solanum laciniatum*	Orissa	80
SR-302	PAU, Ludhiana	18	FM-SPN-11-24-1	PAU, Ludhiana	39	CB-9991-211-2	PAU, Ludhiana	60	*Solanum torvum*	PAU, Ludhiana	81
BL-220	PAU, Ludhiana	19	V-230-12-66-2-1-3	PAU, Ludhiana	40	SR-308	PAU, Ludhiana	61	*Solanum sisymbriifolium*	Orissa	82
BL-215	PAU, Ludhiana	20	7848-15-1	PAU, Ludhiana	41	42324-121-2-1	PAU, Ludhiana	62	*Solanum marcrocarpum*	Orissa	83
BR-123	PAU, Ludhiana	21	SOV-328	PAU, Ludhiana	42	S-324-187-1-2	PAU, Ludhiana	63	*Solanum aethiopicum*	NBPGR	84

(SHAN) clustering option of the NTSYS-pc 2.10 software package (Rohlf 2000). The Jaccard's similarity coefficient matrix obtained was utilized to construct a UPGMA- based dendrogram.

Morpho-genetical analysis: Euclidean similarity coefficient were calculated for combined data (SSR and morphological data) using the SIMINT program of the numerical taxonomy multivariate analysis system (NTSYS-pc v2.10) (Rohlf 2000), and the dendrogram was constructed with the SAHN clustering program using the unweighted pair-group method with arithmetic means (UPGMA).

Results and Discussion

Morphological diversity: The morphological variability in quantitative traits of eggplant germplasm (84 accessions) is presented in Table 2. All the quantitative traits were significantly different among the germplasm lines and the highest level of coefficient of variation was observed in number of primary branches (17.01%) followed by petiole length (10.21%) and peduncle length (9.88), while minimum was noticed in fruit length (7.13%). The number of primary branches, petiole length and peduncle length ranged from 1.3-7.3, 1.8-10.33 cm, and 1.03-7.2 cm, respectively. All the genotypes were phenotypically diverse also. Among qualitative traits (Table 3.), the maximum variability in germplasm was seen in fruit shape with very long (1%), long (24%), medium long (1%), small long (9%), big round (20%), medium round (4%), small round (20%), very small round (4%), medium oblong (8%), small oblong (4%), medium oval (1%) and small oval (4%) fruits. It was followed by fruit colour i.e. purple (32%) and purple black (32%) fruits dominated over green (18%), light purple (11%), whitish purple (3%), milky white (2%) and black (2%). The fruit colour was distributed uniformly in maximum lines (77%), while the rest were found regular stripped (5%) irregular stripped (10%) and mottled (8%). Most of the genotypes were upright (35%) and had narrow plant spread (50%). The vegetative growth characteristics such as leaf (67%), petiole (57%) and fruit calyx (56%) colour were green in maximum genotypes, but purple pigmentation was also there. Leaf blade and pedicel prickles were present in 4% and 13% population, respectively. The

Table 2: Variability in quantitative characters of eggplant germplasm

S. No.	Characters	Minimum	Maximum	Range	Mean±SD	CD (5%)	CV (%)
1.	Plant height (cm)	37.67	173.67	37.67-173.67	86.64±18.58	11.62	8.26
2.	No. of primary branches	1.3	7.3	1.3-7.3	4.59±1.11	1.27	17.01
3.	Petiole length (cm)	1.8	10.33	1.8-10.33	4.90±1.15	0.81	10.21
4.	Leaf blade length (cm)	2.5	21.33	2.5-21.33	16.24±3.03	2.08	7.91
5.	Leaf blade breadth (cm)	3.0	12.37	3.0-12.37	10.25±1.99	1.36	8.17
6.	Fruit peduncle length (cm)	1.03	7.2	1.03-7.2	4.50±1.03	0.72	9.88
7.	Fruit length (cm)	1.4	34.33	1.4-34.33	12.36±5.32	1.43	7.13
8.	Fruit breadth (cm)	0.8	9.8	0.8-9.8	5.17±2.13	0.77	9.24

morphological diversity in brinjal was substantiated with the finding of Ali et al (2011).

Further, principle component analysis (PCA) was performed in order to determine the contribution of morphological traits towards genotypic variation. Eigen vector values/coefficients determined the relative contribution of different principle components towards the morphological variation. In the present investigation, first 8 PCs with high Eigen values (>0.1) explained 70.33% of the total variation (Table 4). Out of 8 PCs the first component (PC1) contributed 16.98% and involved fruit breadth, fruit length, fruit peduncle length, leaf blade breadth, leaf length, number of primary branches and petiole length. The second component (PC2) accounted for 11.80% of the total variation due to calyx colour, plant growth habit, plant height, leaf blade breadth and leaf blade length, while the third component (PC3) accounted 8.56% of the total variation, featuring petiole colour, leaf blade colour and leaf blade breadth. Fourth component (PC4) showed 8.36% of the total variation, accounting calyx colour, corolla colour and petiole colour. However, fifth component (PC5) recorded 7.3%, sixth component (PC6) featured 6.51%, seventh component (PC7) involved 5.83% and eighth component (PC8) involved 4.97% of the total variation. The results indicated that 53% of total variation for morphological traits mainly aroused from five principle components. Leaf blade length, fruit peduncle length, petiole length, leaf blade breadth, fruit length, fruit breadth, number of primary branches and plant height remained key traits for maximum contribution to the genetic diversity. The results of principle component analysis were also corroborated with the findings of Kumar et al. (2016), where the fruit traits contributed maximum to the morphological dissimilarities.

Morphological cluster analysis on the basis of 14 morphological traits revealed that 84 genotypes were dissimilar from each other by Euclidean distance from 1.00 to 9.98. Similarity distance matrix based on UPGMA among all studied genotypes got divided into two distinct clusters (I& II) at Euclidean distance 9.98 (Fig. 1). The first main cluster (I) mainly included small round and very small round genotypes and was separated into two sub-clusters (A and B) at Euclidean distance 5.65. Sub-cluster A included all the germplasm lines of cultivated *S. melongena*, was further divided into two sub-clusters

Table 3: Variability in qualitative characters of eggplant germplasm

S. No.	Qualitative Characters	Percent of germplasm with particular character
1	Plant growth habit	Prostrate (26%), Intermediate (34%), Upright (35%)
2	Plant spread	Broad (43%), Very broad (7%), Narrow (50%)
3	Petiole colour	Violet (32%), Green (57%), Greenish violet (11%)
4	Leaf blade colour	Violet (2%), Green (67%), Dark green (20%), Greenish violet (9%)
5	Leaf blade prickles	None (96%), Few (4%)
6	Pedicel prickles	Many (4%), None (87%), Few (9%)
7	Corolla colour	Violet (52%), Pale violet (30%), Light violet (12%), White (6%)
8	Calyx colour	Dark purple (32%), Light purple (12%), Green (56%)
9	Calyx spininess	High thorny (6%), Medium thorny (14%), Smooth(80%)
10	Fruit shape	Big long (1%), Long (24%), Medium long (1%), Small long (9%), Big round (20%), Medium round (4%), Small round (20%), Very small round (4%), Medium oblong (8%), Small oblong (4%), Medium oval (1%), Small oval (4%)
11	Fruit density	Very compact (6%), Compact (68%), Loose (24%), Very loose (2%)
12	Fruit colour	Black (2%), Purple (32%), Purple black (32%), Light purple (11%), Whitish purple (3%), Green (18%), Milky white (2%)
13	Fruit color distribution	Uniform (77%), Regular stripped (5%), Irregular stripped (10%), mottled (8%)

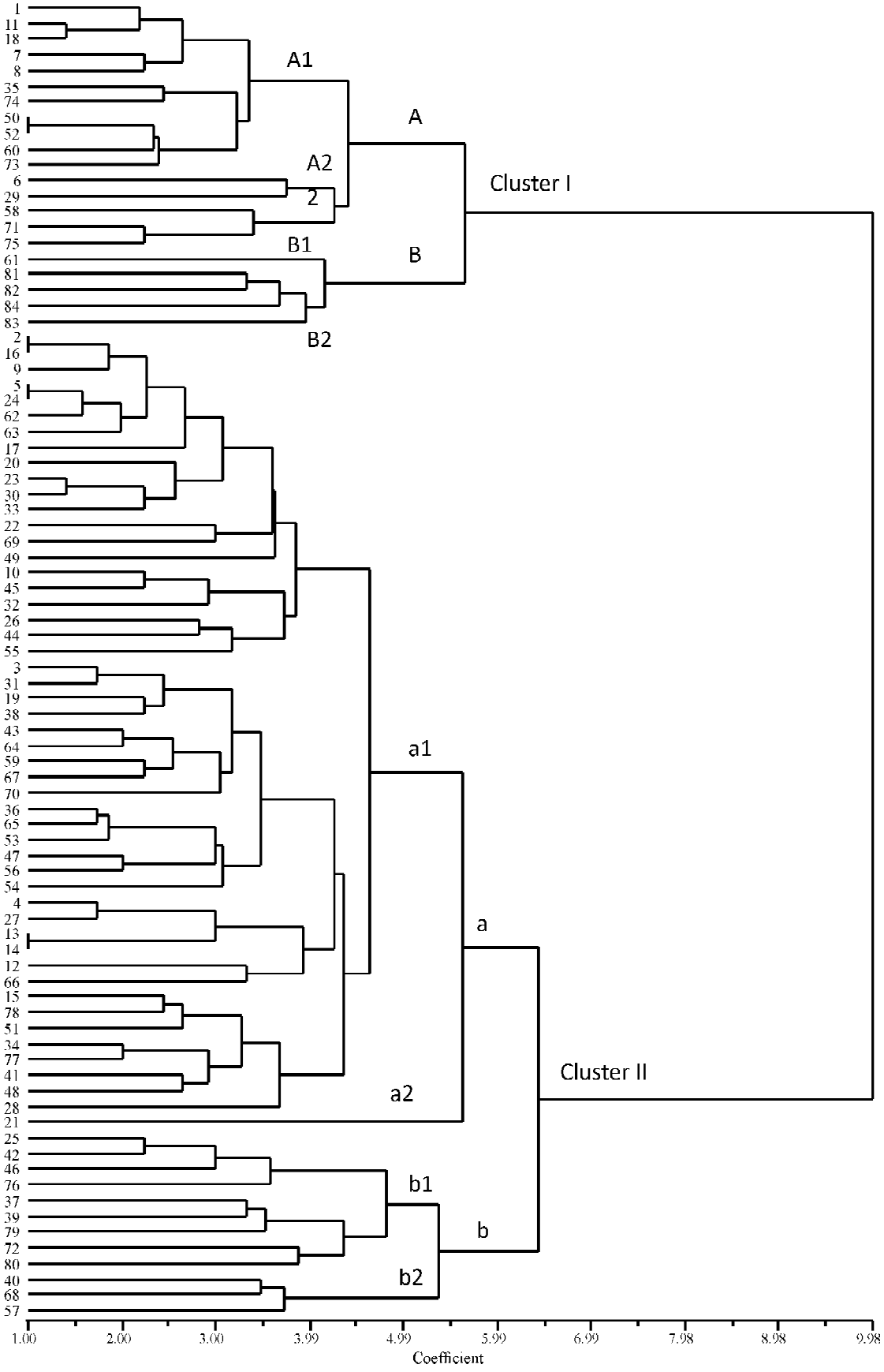

Fig. 1: UPGMA based morphological relationship (Euclidean coefficient) among 84 germplasm lines in eggplant

Table 4: Principal component analysis of morphological traits in eggplant germplasm

S. No.	Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
1	Plant growth habit	.044	.621	-.393	-.217	.067	.098	.174	.162
2	Plant spread	.089	.383	-.508	.085	-.179	-.324	.424	-.035
3	Plant height	.206	.611	.047	-.448	.289	-.082	-.272	.271
4	Number of primary branches	.434	-.389	-.144	.160	.396	.031	-.215	.100
5	Petiole length	.506	.161	.350	.182	.009	-.095	-.035	-.343
6	Petiole colour	-.173	.273	.342	.619	.218	.016	.184	.218
7	Leaf blade colour	.219	-.339	.323	-.482	.138	.072	.065	.204
8	Leaf blade prickles	-.523	.264	-.072	-.252	.381	-.141	-.281	.213
9	Leaf blade length	.648	.553	.234	-.114	.006	.079	.088	-.126
10	Leaf blade breadth	.483	.569	.302	-.236	-.040	.107	-.134	-.252
11	Calayx colour	-.214	.471	.272	.528	.158	-.125	.108	.256
12	Calyx spininess	.251	-.309	.328	-.037	-.086	.166	.194	.498
13	Corolla colour	-.376	.325	-.037	.398	-.168	-.066	-.403	.079
14	Pedicel prickles	.106	-.144	.346	.047	.696	-.190	.103	-.143
15	Fruit peduncle length	.606	.070	-.392	.109	.158	.418	.105	.017
16	Fruit length	.457	-.081	-.552	.222	.405	.206	.041	.163
17	Fruit breadth	.432	.059	.224	-.073	-.470	-.057	.077	.448
18	Fruit colour	-.641	.148	.067	-.195	-.051	.465	.239	.032
19	Fruit color distribution	-.028	.113	.198	.251	-.019	.727	.028	-.123
20	Fruit density	-.691	.117	-.002	-.180	.129	.354	-.152	-.062
21	Fruit shape	-.390	.025	.113	-.232	.269	-.136	.654	-.124
Eigen values		3.565	2.478	1.798	1.757	1.537	1.367	1.224	1.044
% of variance		16.978	11.799	8.563	8.365	7.32	6.508	5.83	4.973
Cumulative %		16.978	28.776	37.34	45.705	53.024	59.532	65.362	70.335

(A_1 and A_2) at Euclidean distance 4.39. Sub-cluster A_1 carried all the germplasm lines with small round and purple black fruits and purple fruits with green foliage. Cluster B grouped wild species along with one genotype from cultivated eggplant. It was divided into two small sub-clusters (B_1 and B_2). B_1 had only one genotype named SR-308. B_2 carried wild types with very small green fruits. Among wild species *S. torvum* and *S. sisymbriifolium* grouped together showing maximum morphological similarity, while *S. macrocarpon* and *S. aethiopicum* slightly diverged in this cluster. The second main cluster (II) had all the genotype of cultivated eggplant (*S. melongena* L.) was divided into two sub-clusters (a and b) at Eucledian distance 6.39. Sub-cluster (a) further divided into two sub-clusters (a_1 and a_2) at Euclidean distance 5.49. Sub-cluster a_1 contained 50 genotypes. Out of which, 21 genotypes with big round, oblong and long having purple black or black fruits, 6 genotypes with medium oblong, black purple or light purple fruits, 21 genotypes with mostly long, purple or light purple or green or white fruits and 8 genotypes of with variable shapes, purple fruits and pigmented leaves came together. However, sub-cluster a_2 had only one genotype BR123. Sub-cluster b was further divided into two further clusters (b_1 and b_2) at Euclidean distance 5.39. Sub-cluster b_1 had 9 genotypes including one wild species *S. laciniatum*. Sub-cluster b_2 contained 3 genotypes V-23012-66-2-1-3, GNR-414 and WO-406. Dendrogram based on morphological traits grouped *S. torvum* and *S. sisymbriifolium, S. macrocarpon* and *S. aethiopicum* along with germplasm lines having small round fruits and distantly related to other cultivated genotypes. Morphologically, *S. laciniatum, S. macrocarpon* and *S. aethiopicum,* had strong similarity to cultivated genotypes. Least similarity to cultivated genotypes was shown by *S. sisymbriifolium* and *S. torvum*. *S. laciniatum, S. macrocarpon* and *S. aethiopicum,* had potential for utilization in introgression breeding. *S. aethiopicum* has been used to induce male sterility in cultivated brinjal (unpublished) and the results also confirmed with the findings of Gowda et al. (1990) who evaluated F1 hybrids between *S. macrocarpon* and cultivated brinjal**.**

Molecular diversity: Out of randomly selected 40 SSR markers, 21 displayed monomorphic banding pattern in all the genotypes. Polymorphism was recorded in the amplification pattern of only 19 SSR markers. Among these polymorphic markers, 17 amplified more than two amplicons (3-10), while 2 markers (EEMS46 and CSM73) produced only two amplicons. PIC value for 19 primers ranged from 0.124 to 0.867 with an average of 0.643 (Table 5.). The highest PIC value was noticed in EEMS28 (0.867), followed by CSM31 (0.843) and CSM27 (0.826). High PIC value for the amplification of 16 SSR markers indicated that the germplasm lines used in present investigation were highly diverse and had potential prospectus in the improvement of cultivated

eggplant for various yield and other related traits. In an earlier report, average PIC value utilizing SSRs markers indicated more similarity among eggplant genotypes (Ge et al. 2013).

Jaccard's similarity coefficients between any two genotypes estimated based on SSR polymorphism varied from 0.32-0.98. UPGMA based cluster analysis of 84 genotypes using SSR markers (Fig. 2) opened into three major clusters (I, II and III) at similarity coefficient of 0.52. Cluster I had all the genotypes of cultivated *S. melongena*, while Cluster II and III had wild genotypes. Cluster II had two wild species, *S. laciniatum* and *S. aethiopicum,* with 74% similarity. Cluster III grouped three wild species, where *S. torvum* separated from *S. sisymbriifolium* and *S. macrocarpon* at similarity coefficient of 0.62. Later two wild species had 71% similarity. Our molecular results indicated that *S. sisymbriifolium* and *S. macrocarpon* were more distantly related to the genotypes of cultivated *S. melongena* with a least similarity of 0.32. Cluster I could be further divided into three groups (A, B and C) at similarity coefficient of 0.62. Group A was divided into two sub-clusters (A_1 and A_2). Sub-cluster A_1 had 38 genotypes while, the sub-cluster A_2 had only one genotype 8 (BB-93C). Group B had only one genotype 14 (BR-112). Group C was further divided into two sub-clusters (C_1 and C_2) at similarity coefficient of 0.72. Sub-cluster C_1 had 37 genotypes, however, C_2 had only two genotypes *i.e.* 78 (KBSR-343-1) and 73 (93PSB-3-3-1-1).

The divergence of cluster I into three groups revealed that these genotypes of group A were 38% diverse from group B & C and vice versa. On the other hand, wild species, *S. torvum, S. macrocarpon* and *S. sisymbriifolium* diversed in separate cluster and showed least similarity to the rest of genotypes (Fig. 2). In previous studies, *S. torvum* also showed high divergence to cultivated lines that leads to sterile inter-specific hybrids with cultivated types and excludes the possibility of its utilization in introgression breeding programs (Stagel et al. 2008). However, *S. laciniatum* and *S. aethiopicum* had 52% similarity with our cultivated germplasm and had the potential possibility of cross compatibility for the introgression of resistant traits as reported by Rizza et al (2002). Among the cultivated genotypes, BR-112 and BB93C were found more close to wild species and there are potential prospectuses for fertile hybrids for introgression of traits through inter-specific hybridization.

Morpho-genetical diversity: Combined data analysis based on UPGMA cluster pattern (Fig. 3) grouped 84 genotypes at Euclidean distance in a range from 3.16-10.58. Morphogenetic dendrogram diverged into two major clusters (I, and II) at Euclidean distance of 10.58. All the wild types grouped together as in SSR data, but diverged to cluster I of morphological analysis that contained all the genotypes with small round and medium

Table 5: Polymorphism of SSR markers in eggplant germplasm

S. No.	Primers Code	Number of amplicons			Range of amplicon size (bp)	PIC value	Polymorphism (%)
		Total amplicons	Monomorphic amplicons	Polymorphic amplicons			
1	EEMS20	5	1	4	190-400	0.737	80.00
2	EEMS28	10	0	10	200-500	0.867	100.00
3	EEMS34	3	1	2	250-375	0.282	66.67
4	EEMS37	6	0	6	100-150	0.562	100.00
5	EEMS46	2	1	1	250-400	0.124	50.00
6	EEMS48	5	0	5	175-300	0.782	100.00
7	EEMS50	6	0	6	200-300	0.701	100.00
8	CSM27	7	1	6	200-300	0.826	85.71
9	CSM31	8	0	8	225-400	0.843	100.00
10	CSM36	4	0	4	300-500	0.749	100.00
11	CSM40	5	0	5	275-450	0.784	100.00
12	CSM43	4	0	4	250-400	0.540	100.00
13	CSM45	4	0	4	160-200	0.726	100.00
14	CSM54	7	2	5	225-400	0.773	71.43
15	CSM57	4	0	4	190-300	0.571	100.00
16	CSM62	4	0	4	230-300	0.747	100.00
17	CSM73	2	0	2	200-250	0.331	100.00
18	CSM74	3	0	3	175-200	0.588	100.00
19	CSM78	4	0	4	300-550	0.676	100.00

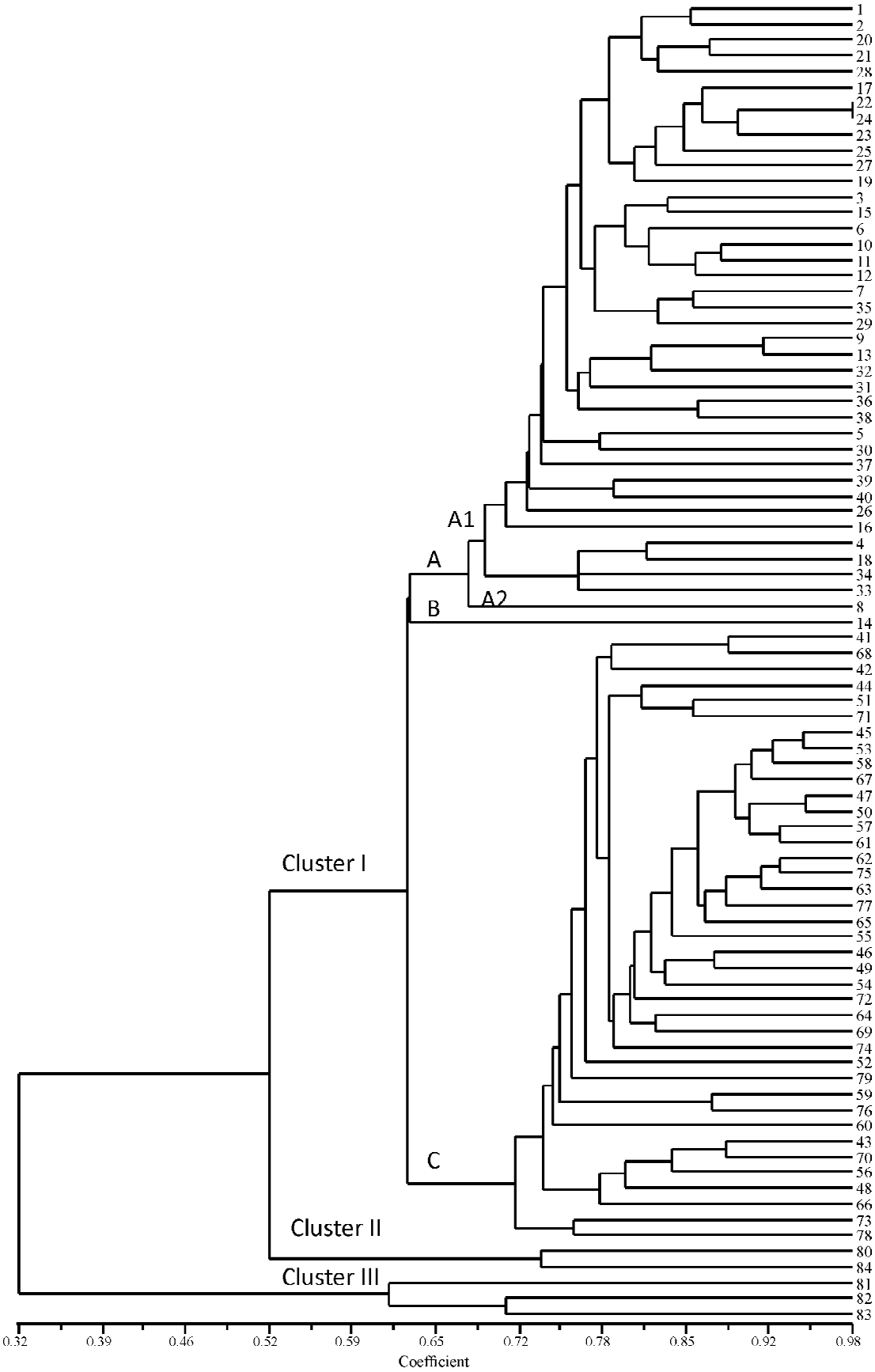

Fig. 2: UPGMA based molecular relationship (Jaccard's similarity coefficient) among 84 germplasm lines in eggplant

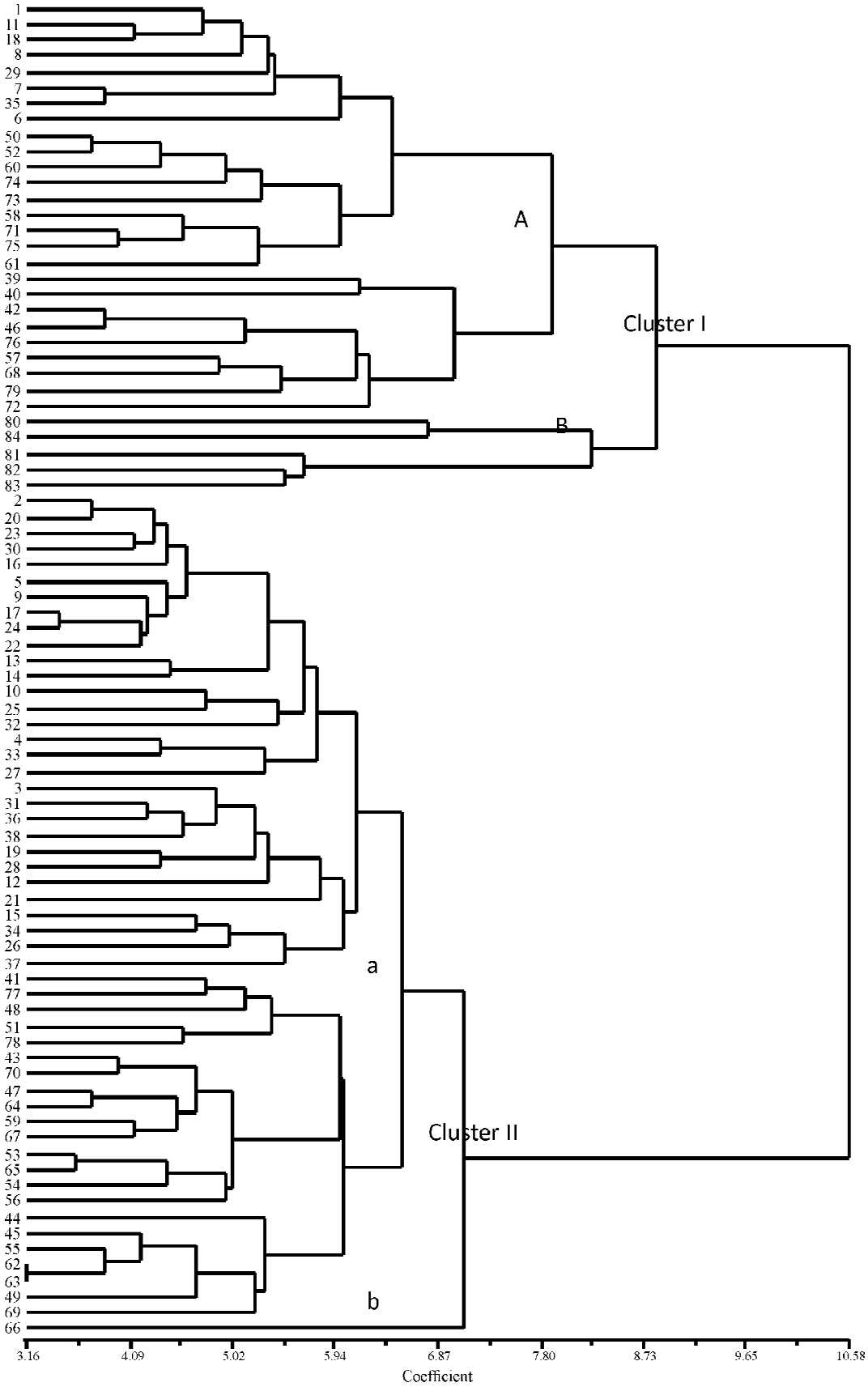

Fig. 3: UPGMA based combined (14 morphological traits and 19 SSR) analysis (Euclidean coefficient) among 84 germplasm lines in eggplant

round fruits. This cluster I was further diverted into two groups A and B at Euclidean distance of 8.99. Group A separated all the small round genotypes of cultivated *S. melongena* morpho-genetically from wild species in group B. Cluster II had only germplasm lines of cultivated eggplant and further departed into two clusters (a and b) at Euclidean distance 7.10. Cluster contained genotypes with big round, long and oblong fruits, while cluster b had only one genotype 66 (GL-408). Morpho-genetically, small round germplasm lines with cluster bearing came more close to the wild types and highlighted their importance for introgression of important wild traits into the cultivated eggplant. On the other hand, great divergence among small round, long, oblong or big round germplasm lines was apparent that can further be utilized in future breeding programmes. Our findings are concurrent with results of Stagel et al (2008), who showed least similarity of *S. torvum* followed by *S. sisymbriifolium* and *S. aethiopicum*. The research also reported the possible hybridization between cultivated brinjal and *S. aethiopicum* for the introgression of important traits (Collonnier et al. 2001 Rizza et al. 2002 and Gisbert et al. 2011). It is concluded from the present investigation that the cultivated genotypes had potential divergence. Small round types diverged from big and long fruited genotypes highlighting the possibility of their use in future breeding programmes. *S. sisymbriifolium* and *S. torvum* had the least similarity to the rest of cultivated germplasm. However, *S. macrocarpon, S. laciniatum* and *S. aethiopicum* were comparatively more close to cultivated genotypes especially BB-93C and BR112. Thus, these genotypes with greater genetic closeness to the wild types may be used in introgression breeding for insect pest and disease resistance into cultivated types.

सारांश

बैंगन मूलतः भारत का उद्‌गम एवं ग्राम्यन केन्द्र है और इसके विविध जननद्रव्य महत्वपूर्ण हैं। वर्तमान अध्ययन में कुल खेती योग्य 84 जननद्रव्यों, जिनमें 5 जंगली प्रजातियों सहित 79 प्रजनन लाइनों को 21 अकारकीय वर्णानात्मक गुणों तथा 40 सिम्पल सिक्वेनस रीपिट (एसएसआर) मार्कर्स के आधार पर अलग किया गया। अकारकीय गुणों के प्रिंसिपल कम्पोनेन्ट एनालिसिस (पीसीए) के अनुसार प्रथम 8 पी.सी. के साथ कुल विविधता 70.33 प्रतिशत स्पष्ट हुआ। कुल 40 एस.एस.आर.मार्कर्स में केवल 19 (50–100 प्रतिशत) खेती योग्य तथा जंगली प्रभेदों में बहुरूपता ज्ञात हुआ जिनमें विस्तार 2 व 10 एलील्स प्रति मार्कर व ई.ई.एम.एस 28 सबसे उच्च बहुरूपी पाया गया और इसके उपरान्त सी.एस.एम 31 तथा सी.एम.एम.–27 का स्थान रहा। प्रभेदों के मध्य जैकार्ड सिमिलैरिटी गुणांक विविधता 0.38–0.98 पाया गया जो विशेषतः खेती योग्य प्रभेदों व *सोलेनम टोरवम* के मध्य था। अकारकीय एवं एस.एस.आर. आंकड़ों को एकल एवं संयुक्त रूप से यू.जी.जी.एम.ए. क्लस्टर एनालिसिस का उपयोग कर किया गया। खेती योग्य किस्मों की अकारकीय अनुवांशिक भिन्नता से प्राथमिकता स्पष्ट है कि भविष्य में प्रजनन कार्यक्रमों में इनका प्रयोग किया जायेगा। जंगली प्रजातियाँ एस एस आर आकड़ें के आधार पर अलग समूह में विभक्त हो गये लेकिन बैंगन का छोटे गोल समूह में अकारकीय व अनुवांशिक रूप से समाहित हुए। *सोलेनम लासीनियेटम तथा सोलेनम इथियोपिकम* खेती योग्य बैंगन के नजदीक पाये गये व इनका उपयोग संयोजन प्रजनन में किया जा सकता है।

References

Ali Z, Xul ZL, Zhang DY, He XL, Bahadur S and Yi JX (2011) Molecular diversity analysis of eggplant (*Solanum melongena*) genetic resources. Genet Mol Res 10(2): 1141-1155.

Ansari AM and Singh YV (2014) Molecular diversity of brinjal (*Solanum melongena* L. and *S. aethiopicum* L.) genotypes revealed by SSR markers. Elect J Plant Breed 5(4): 722-728.

Asad HA, Meah MB, Begum SN, Khalil MI, Rafii MY and Latif MA (2015) Study of genetic variation of eggplant cultivars by using RAPD-PCR molecular markers and the relationship with Phomopsis blight disease reaction. Genet Mol Res 14(4): 17007-17018.

Backes G, Hatz B, Jahoor A and Fischbeck G (2003) RFLP diversity within and between major groups of barley in Europe. Plant Breed 122: 291-299.

Bhatt RV and Vasanthi S (2008) Antiquity of the cultivation and use of brinjal in India. Asian Agri-History 12: 169 – 178.

Collonnier C, Mulya K, Fock I, Mariska I, Servaes A, Vedel F, (2001) Source of resistance against Ralstoniasolanacearum in fertile somatic hybrids of eggplant (*Solanum melongena* L.) with *Solanum aethiopicum* L. Plant Sci 160(2): 301–313.

Daunay MC and Hazra P (2012) Eggplant. In: Peter K V and Hazra P (eds) Handbook of vegetables. Stadium Press, Houston, TX, USA. pp 257-322.

Devrajan T, Munjunath K, Jayabalan S and Mundaragi A (2015) The molecular assessment of genetic diversity of eggplant cultivars from Northern Karnataka in India using randomly amplified polymorphic DNA markers. Scientific Bullet Series F Biotechnologies 19:76-84.

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bullet 19: 11-15.

FAOSTAT (2016) FAOSTAT Production Databases. Available online at http://faostat3.fao.org/home/E

Fasoula VA and Fasoula DA (2002) Principles underlying genetic improvement for high and stable crop yield potential. Field Crops Res 75: 191-209.

Ge H, Liu Y, Jigang M, Zhang J, Han H and Cheng H (2013) Analysis of genetic diversity and structure of eggplant populations (*Solanum melongena* L) in China using simple sequence repeat markers. Scientia Hortic 162: 71-75.

Gisbert C, Prohens J, Raigón MD, Stommel JR, Nuez F (2011) Eggplant relatives as sources of variation for developing

new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and composition. Scientia Hortic 128(1):14–22

Gowda, PHR, Shivashankar K T and Joshi SH (1990) Interspecific hybridization between *Solanum melongena* and *Solanum macrocarpon*: study of the F_1 hybrid plants. *Euphytica* 48(1): 59-61.

Hoogendijk M and Williams DE (2001) Characterizing the genetic diversity of home garden crops: some examples from the Americas. In: Watson JW, Eyzaguirre PB (eds) Proceedings of the second international home gardens workshop: contribution of home gardens and *in situ* conservation of plant genetic resources in farming systems. IPGRI, Germany, pp 34-40

Jugran AK, Bhatt ID, Rawal RS, Nandi SK and Pande V (2013) Patterns of morphological and genetic diversity of *Valeriana jatamansi* Jones in different habitats and altitudinal range of West Himalaya, India. Flora 208: 13-21.

Knapp S,Vorontsova MS and Prohens J (2013) Wild relatives of the eggplant (*Solanum melongena* L.: Solanaceae): new understanding of species names in a complex group. PLoS ONE 8(2): e57039. https://doi.org/10.1371/journal.pone.0057039

Kumar SR, Arumugam T And Ulaganathan V (2016) Genetic diversity in eggplant germplasm by principal component analysis. SABRAO J Breed Genet 48 (2): 162-171.

Kumchai J, Wei YC, Lee CY, Chen FC and Chin SW (2013) Production of interspecific hybrids between commercial cultivars of the eggplant (*Solanum melongena* L.) and its wild relative *S. torvum*. Genet Mol Res 12(1): 755-764.

Last L, Luscher G, Widmer F, Boller B and Kolliker R (2014) Indicators for genetic and phenotypic diversity of Dactylisglomerata in Swiss permanent grassland. Ecolo Indicators 38: 181-191.

Mennella G, Lo Scalzo R, Fibiani M, D'Alessandro A, Francese G and Toppino L (2010) Chemical and bioactive quality traits during fruit ripening in eggplant (*S. melongena* L.) and allied species. J Agric Food Chem 47: 11821–11831.

Meyer RS, Karol KG, Little DP, Nee MH and Litt A (2012) P hylogeographic relationships among Asian eggplants and new perspectives on eggplant domestication. Mol Phylogen Evol 63: 685 – 701.

Meyer RS, Whitaker BD, Little DP, Wu SB, Kennelly EJ and Long CL (2015) Parallel reductions in phenolic constituents resulting from the domestication of eggplant. Phytochem 115: 194–206

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H, Ohyama A and Fukuka H (2009) Development of SSR- enriched genomic library of eggplant (*Solanum melongena* L). Theol Appl Genet 119: 1143-1153.

Rizza F, Mennella G, Collonnier C, Sihachakr D, Kashyap V and Rajam M, (2002) Androgenic dihaploids from somatic hybrids between *Solanum melongena* and *S. aethiopicum* group gilo as a source of resistance to *Fusarium oxysporum* f. sp. *melongenae*. Plant Cell Rep 20(11): 1022–1032.

Rohlf F J (2000) NTSYS-pc: numerical taxonomy and multivariate system, version 2.1. Exter Software. Setauket, New York, USA.

Rotino GL, Sala T and Toppino L (2014) Eggplant in alien gene transfer. In: Pratap A and Kumar J (Eds) Crop plants. Springer, New York, 2: 381–409.

San Jose R, Sanchez-Mata M C, Camara M and Prohens J (2014) Eggplant fruit composition as aûected by the cultivation environment and genetic constitution. J Sci Food Agri 94: 2774-2784.

Singh M and Kumar R (2007) Eggplant (*Solanum melongena* L.). In: Singh R J (ed) Genetic resources, chromosome engineering and crop improvement. CRC Press, Boca Raton, FL, USA, pp 473–495.

Stagel A, Portis E, Toppino L, Rotino GP and Lanteri S (2008) Gene-based microsatellite development for mapping and phylogeny studies in eggplant. BMC Genomics 9: 357

Wang L, Jiao S, Jiang Y, Yan H, Su D, Sun G, Yan X and Sun L (2013) Genetic diversity in parent lines of sweet sorghum based on agronomical traits and SSR markers. Field Crops Res 149: 11-19.

Yousaf Z, Wang Y and Baydoun E (2013) Phytochemistry and pharmacological studies on Solanum torvum Swartz. J Appl Pharma Sci 3(4): 152-160.