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Dissection of genetic diversity present in eggplant populations using simple sequence repeat markers

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ABSTRACT

Eggplant (*Solanum melongena* L.) is the third most important *solanaceous* vegetable and most diversified within species spread across the world-geographical area. A study was conducted to assess the genetic diversity among the selected fifty-four eggplant genotypes (sub-categorized into five sub-population) using twenty-three SSR markers. The Analysis of Molecular Variance among the five sub-population of eggplant revealed the existence of 90.67% variation within populations and 9.34% variation among populations. The SSR markers analysis revealed important locus-wise information like mean Observed-Heterozygosity (0.216), mean Expected-Heterozygosity (0.496), Shannon's Information Index (0.879), mean number of different alleles (3.209), mean number of effective alleles (2.535), Fixation-Index (0.649). Further, Phylogenetic-analysis clearly categorize genetically distinct individuals in which the most diversified clusters was cluster-1 (C1) out of total of five clusters and especially, wild cultivars were grouped into cluster-5 (C5). The obtained results can be used in eggplant breeding and germplasm conservation in a resourceful manner.

KEYWORDS: DNA, Genetic diversity, SSR markers, AMOVA, GenAlEx, PCA

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INTRODUCTION

Eggplant (*Solanum melongena* L.), is one of the important Solanaceous vegetables grown in tropical and subtropical regions of the world including India. It is a warm season herbaceous perennial but grown as an annual crop for commercial purposes and is popularly called as Brinjal in India. Eggplant is rich in carbohydrates, proteins, fat, dietary fibres, vitamins and minerals properties (Somawathi *et al.*, 2014).

The assessment of genetic features is a vital process for breeders to produce a new genetic line or to improve an existing one further. India as being major diversity hub for eggplant the occurrence of variation for various traits is high. Since there is a need to evaluate and characterize the eggplant basic material which is distributed in multiple areas. Earlier, genetic evaluation was mainly based on morphological, (Faizan *et al.*, 2021a) physiological (Faizan *et al.*, 2021b) and biochemical traits (isozymes and chromatography) (Weijun, 1992; Isshiki & Fujieda 1994). Presently, molecular markers

have massive and latent to discover genetic diversity by identifying polymorphisms. Molecular diversity analysis is one of the powerful tools for genome determination, genotype recognition, and studying the evolution pattern of crop plants and utilizing it in further breeding programmes. Several works related to molecular genetic diversity analysis in eggplant have been reported using different types of molecular markers viz., RAPD (Hu & Quiros, 1991; Tiwari *et al.*, 2009; Ali *et al.*, 2011), AFLP (Liao *et al.*, 2009), SCARs (Liao *et al.*, 2009), ISSR (Tiwari *et al.*, 2009) and SSR (Jellan *et al.*, 2016; Mikaela *et al.*, 2017).

During recent eras, SSR molecular markers are becoming more popular because of their co-dominant inheritance, high abundance, enormous extent of allelic diversity, ease of assessing SSR size variation through PCR and high reproducibility (Stagel *et al.*, 2008). The development of SSR markers derived from the SSR-enriched genomic library of eggplant was reported by Nunome *et al.* (2003, 2009).

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The goal of the current investigation was to characterize eggplant genotypes and to study the relationship between the genotypes which fall under different subgroups using SSR molecular marker. Valuation of genetic diversity is imperative for breeding purposes, and the exploitation of molecular markers helps fast-track the evaluation progression.

MATERIAL AND METHODS

Plant Material

Around fifty-four eggplant genotypes were obtained from different sources and categorised into different sub-groups based on their type or kind viz ^alocal cultivars (9 genotypes), ^bcommercial hybrids (2 genotypes) ^creleased varieties (15 genotypes), ^dadvanced breeding lines (24 genotypes), and ^ewild relatives/related species of eggplant (4 species) (Table 1).

Molecular Characterization

DNA extraction

DNA samples from fresh fully opened leaf tissue of each genotype were extracted using the CTAB method (Doyle & Doyle, 1987). DNA quality and quantity were assessed on a 0.8 % agarose gel stained with ethidium bromide and also by using a NanoDrop® ND-1000 spectrophotometer respectively.

SSR Analysis

Twenty-three SSRs markers were selected to the evaluate genetic diversity present among eggplant germplasm. The selection of eggplant SSRs was based on their high polymorphism information content and the quality scores reported by Nunome *et al.* (2009), Jellan *et al.* (2016) and Mikaela *et al.* (2017) (Table 2).

The polymerase chain reaction (PCR) mixture confined with >80 ng DNA, 5 pmol of each primer, PCR master mix Ampliqon® and nuclease free water, total PCR mixture composed of 10 µl.

PCR amplification was achieved using the Eppendorf® PCR System. The amplification conditions involved an initial step of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55-69°C and 1 min at 72°C, final extension at 72°C for 10 min and final withhold temperature with 4°C. The obtained PCR products were gel electrophorized using 2.5% agarose gel and the PCR profile image was captured using a gel documentation system (Syngene Pvt. Ltd., USA).

The scoring of the PCR profile was done by using the software GeneTool (Syngene Pvt. Ltd., USA). A 100 bp ladder was used as a standard molecular weight size marker for each gel alongside the DNA samples. The bands were scored based on the weightage of DNA fragments. The analysis was repeated at least twice to confirm the reproducibility of the results and the variation for each band was observed around 5 to 10 bp.

Table 1: List of genotypes used in the present investigation

Name of the genotype	Sub-group	Sources of Collection
PUSA UPKAR	c	IIVR, Varanasi, Uttar Pradesh
ARKA KRANTI	c	
BHAGYAMATI	c	
PUSA ANKUR	c	
PUSA BINDU	c	
PUNJAB SADABAHAR	c	
ARUNA	c	
SHOBHA	c	
SWARNA MANJARI	c	
CH-215	d	
JAWAHAR BRINJAL-8	c	V.R.S. Kalyanpur, Uttar Pradesh
JAWAHAR BRINJAL-69	c	
R-2580	d	
R-2594	d	
R-2591	d	
MALAPUR LOCAL	d	
L-2232	d	
L-3272	d	
R-2581	d	
L-2230	d	
L-3268	d	COH, Mudigere
M4	d	
M21	d	
M23	d	
M17	d	
MATTIGULLA	a	
RAMDURGA	a	
MELAVANKI	a	
M6	d	
M19	d	
VERY GREEN LONG	a	ZRS, Chianky, Palamu, Jharkhand
IIHR-322	d	
PANT SAMRAT	c	
IIHR-7	d	
LONG GREEN	a	
SWARNA PRATIBHA	c	
SWARNA MANI	c	
EARLY ROUND MARKET	a	
RAMPUR LOCAL	a	
HEBBAL GULLA	a	
ROUND GREEN	a	NBPGR, New Delhi
IC354140	d	
IC90785	d	
IC99676- LONG	d	
IC99676- ROUND	d	
IC90691	d	
IC354597-ROUND	d	
SUVARNA GP098	b	
VIJAYA ARBH98	b	
CO-2	c	
<i>Solanum macrocarpon</i>	e	COH, Bangalore
<i>Solanum indicum</i>	e	
<i>Solanum torvum</i>	e	
<i>Solanum mammosum</i>	e	

COH- College of Horticulture, VRS- Vegetable Research Station, ZRS- Zonal Research Station, IIVR- Indian Institute of Vegetable Research, NBPGR- National Bureau of Plant Genetic Resources, TNAU- Tamil Nadu Agricultural University

Molecular Analysis

The molecular analysis was done using GenAlEx V.6.0 software for PCA derivation and parameter calculation of the number

Table 2: Nucleotide sequence of Single Sequence Repeat (SSR) primer pairs used for genetic diversity in the eggplant germplasm

Si. No.	Primer Name	Nucleotide Sequence (5' - 3')	Tm (°C)	Reference
1	SM104 (F)	TGGATCTGCAAAGAAAAGGAGAAAG	55.0-56.0	Dept. of Biotechnology & Crop Improvement, College of Horticulture, Bengaluru.
	SM104 (R)	CGCAAATCGGGTAGACTTTTCGAT		
2	SM107 (F)	GGCCCTAGAC TGAGCTGAAATGTT		
	SM107 (R)	TGCTACAACCAACACAACCCCTCAA		
3	SM114 (F)	AGCCTAAACTTGGTTGGTTTTTGC		
	SM114 (R)	GAAGCTTTAAGAGCCTTCTATGCAG		
4	SM116 (F)	TTAGAAATTTTCGGAACAAAGAGA		
	SM116 (R)	CCACATGAAACTTGGACCAATGAG		
5	SM117 (F)	GATCATCACTGGTTTGGGCTACAA		
	SM117 (R)	AGGGGAGAGGAAACTTGATTGGAC		
6	SM119 (F)	CCCCACCCCATTTGTGTATGTT		
	SM119 (R)	ACCCGAGAGCTATGGAGTGTCTG		
7	SM120 (F)	GGATCAACTGAAGAGCTGGTGGTT		
	SM120 (R)	CAGAGCTTCAATGTTCCATTICACA		
8	SM126 (F)	GCATAGCTTATGAGTCAGGTGGCTTT		
	SM126 (R)	GCTCATCAAACCATCACATTCAAG		
9	SM127 (F)	CAGACACAACCTGCTGAGCCAAAAT		
	SM127 (R)	CGGTTTAATCATAGCGGTGACCTT		
10	SM128 (F)	TAGCGGTGCTAGGTCATCATCTCA		
	SM128 (R)	TTCTCAAGAAGTTGCTCCAAGGA		
11	eme01G19(F)	AATTAAGGCTGAGAGGGGAAGACG	65.2	Jellam et al., 2016
	eme01G19(R)	AAAGGAGGAAAGGGAAAGGGAAAG	63.6	
12	emj04E17(F)	TCACGCTGCTGAAATAGTTTCTTAG	62.5	
	emj04E17(R)	CGAGTTATGCTGAGAGCAGTGTGA	65.2	
13	emf01G17(F)	AAGGCAACTGATAATGCAGACGTG	63.6	
	emf01G17(R)	CTCTCACTCTTACATGTGGCTGGC	66.9	
14	emg01D17(F)	GCTAATAGCCAAAACACCCACAA	63.6	
	emg01D17(R)	TAGTATGTGACCTTGGCTGGAAA	63.6	
15	emg21I10(F)	ATCCTTGTCTTCTGACGGGACTTG	63.6	
	emg21I10 (R)	TGGTCTCTTTGGTTTGTAGTGG	63.6	
16	emi02E20(F)	TTAGTTCCCTGAGTCCCATTTGA	63.6	
	emi02E20 (R)	CAAGACTGAAGGACCACAAATCACA	64.1	
17	emf11L21(F)	GAAGCCTAGGTAACGTACCCCTCG	68.5	
	emf11L21(R)	GGCTCTATTTCTGGGCTTTTCAT	63.6	
18	EM144(F)	AGTGCATTTCTCAAATCAAAGGG	60.3	Mikaela et al., 2017
	EM144(R)	GTTTCAATTTACAGGCTCCTGCATTA	65.3	
19	EM145(F)	ATTGTGTCGATGAGATTTTGGTCA	60.3	
	EM145(R)	GTTTAGCTACGTTGGTTTGGTGCTGAA	66.6	
20	EM146(F)	GATCATCACTGGTTTGGGCTACAA	63.6	
	EM146(R)	AGGGGAGAGGAAACTTGATTGGAC	65.2	
21	EM147(F)	TTAGTGCCAGCAAAAATTGG	54.3	
	EM147(R)	TTTAAAGCTTTAGCGCTCTCC	57.5	
22	EM150(F)	AGCAAACATTACAAAAGCAGTT	54.7	
	EM150 (R)	TCAGGCATCAGTATCACCAC	58.4	
23	EM148(F)	ACGGTATCGAAGAGAGTGAATGCCT	65.8	
	EM148 (R)	GTTTCCCATTTTCATCTGAAAATCCAC	65.6	

of different alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I), Observed Heterozygosity (Ho), Expected Heterozygosity (He), number of migrants (Nm), Polymorphic Information Content (PIC) value, Fixation Index (F) and F-statistics. The analysis of molecular variance was determined from Arlequin V3.0 (Excoffier & Lischer, 2010). A Neighbour- Joining (NJ) tree (Figure 3) was constructed across the samples to analyze the genetic relationship among the individuals and populations using MEGA V5.0 software.

RESULTS

Analysis of Molecular Variance (AMOVA)

Analysis of Molecular Variance (AMOVA) with a fixation index value of 0.649 specified significant differences among the

individuals used in the present study. The *per cent* variation found among the sub-populations was 9.34 and within sub-populations was 90.66 *per cent* (Table 3).

Statistics of SSR Genetic Marker

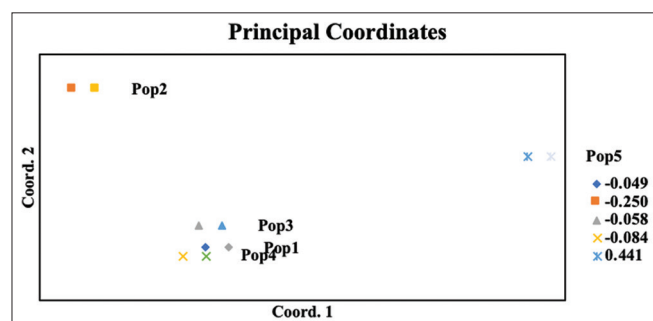
Around twenty-three SSR markers were used for genotyping in which twenty-two were polymorphic with mean value of 0.858 ranged from 0.0 for marker SM104 to 0.968 for EM147. The number of different alleles (Na) among the SSR loci ranged between 1.00 and 7.44 (SM114) with mean of 3.209. The effective number of alleles (Ne) was high about 5.643 (SM114) with the highest Shannon's Information Index (I) of 1.825 (SM114). The Observed Heterozygosity (Ho) ranged from 0.00 to 0.992 (SM128) and whereas the Expected Heterozygosity (He) was about 0.00 to 0.818 (SM114). The fixation index for

twenty-three SSR was ranged from -0.362 (SM128) to 1.000 (Table 4).

In the sub-population, POP4 recorded the highest number of distinct alleles (4.17) followed by POP3 (3.91) and POP1 (3.61) while the minimum number of distinct alleles was recorded in POP2 (1.826). Ne value was maximum for the sub-population, POP4 (3.02) and the POP2 chronicled minimal value of 1.78. The Expected heterozygosity (H_e) values for sub-populations ranged between 0.332 (POP2) and 0.577 (POP3 and POP4). Whereas, observed heterozygosity (H_o) values

Table 3: Summary of analysis of molecular variance (AMOVA) among the five sub-populations of eggplant

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage variation
Among populations	4	78.007	0.69	9.34
Within populations	53	687.01	6.69	90.67
Total	57	765.01	7.38	



Pop1- Local cultivars, Pop2- Hybrids, Pop3- Registered varieties, Pop4- Advanced breeding lines and Pop5- Eggplant species

Figure 1: Principal coordinate analysis (PCA) using pair-wise genetic distance matrix of five eggplant sub-populations.

for sub-populations ranged between 0.185 (POP5) and 0.238 (POP3). The percentage polymorphism observed maximum for POP1, POP3 and POP4 (95.65%) and its least value was observed in the case of POP2 (60.87%) with a mean percentage polymorphism of 85.22 (Table 5).

F Co-efficient and Genetic Distance

The major components of F-statistics are F_{IS} , F_{IT} and F_{ST} ; where, 'I' represent individuals, 'S' represents sub-population and 'T' represents the total population. The F_{IS} mean value across all the loci and populations was 0.684. In the present study, the mean F_{IT} value observed was 0.721. However, the observed F_{ST} mean value was 0.238. Among the pair-wise population F_{ST} the values ranged from a minimum of 0.021 between POP1 and POP3 and a maximum of 0.348 between POP2 and POP5. The pair-wise F_{ST} values indicated the presence of significant genetic differentiation among the sub-populations of eggplant working collection (Table 6). Considering the SSR markers, the F_{ST} values ranged between 0.00 (SM104) and 0.473 (SM127) indicating the presence of significant genetic differentiation among the accessions of eggplant for individual SSR loci studied (Table 4). Pair-wise genetic distance across sub-populations ranged from 0.053 (POP1 and POP3) to 1.03 (POP2 and POP5) (Table 7).

Principal Coordinate Analysis

The Principal Coordinate Analysis (PCA) was done to understand the relationships between the sampled accessions based on genetic distance. The relative position of the fifty-four genotypes is illustrated in Figure 2. However, there was no distinct categorization of eggplant genotypes were observed except the genotypes of POP5 (Wild/related species of eggplant) are situated in quadrant C. Whereas, in Figure 1 it was observed that the sub-population POP5

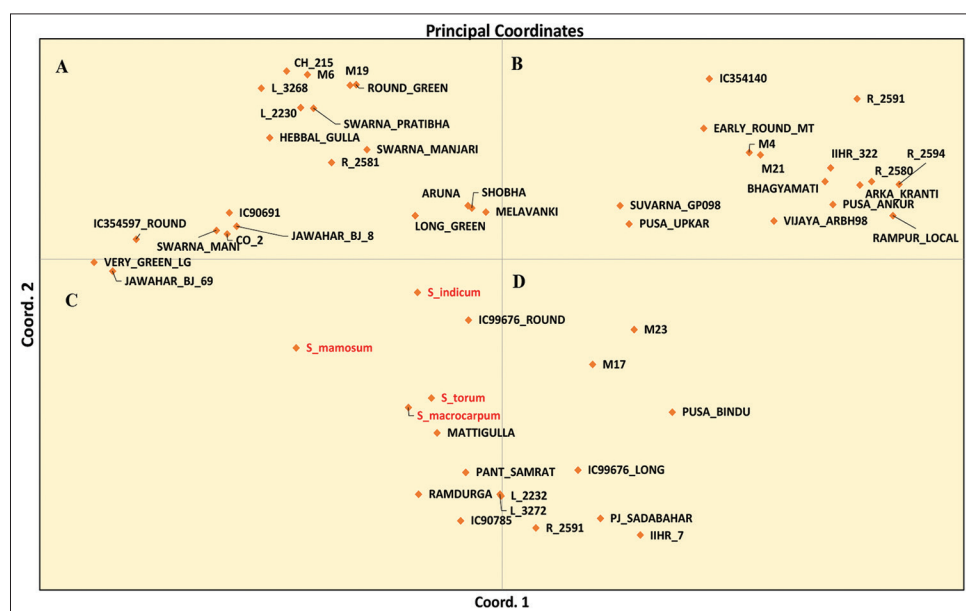
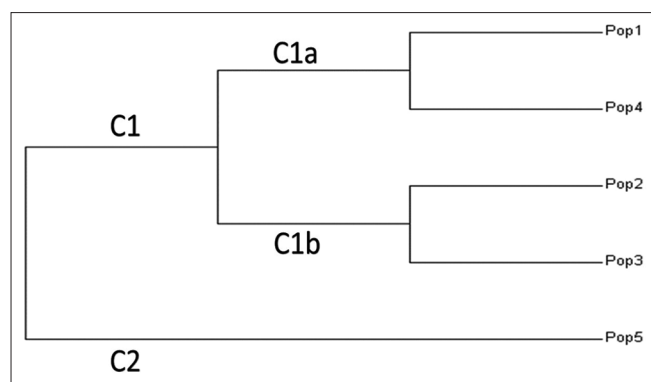


Figure 2: Principal coordinate analysis (PCA) using pair-wise genetic distance matrix of 54 eggplant accessions



Pop1- Local cultivars, Pop2- Hybrids, Pop3- Released varieties, Pop4- Advanced breeding lines and Pop5- Wild/related species of eggplant
Figure 3: Neighbor joining (NJ) tree constructed using MEGA V.7.0 based on pair-wise genetic distance across five sub-populations of eggplant.

(wild/related species of eggplant) while the lower right quadrant consisted of no sub-populations. The upper left quadrant consisted of sub-population POP2 (Hybrids) and lower left quadrant entailed sub-populations POP3 (Registered varieties), POP1 (Local cultivars) and POP4 (advanced breeding lines).

Phylogenetic Analysis

The phylogenetic analysis for 54 accessions of eggplant across five sub-populations resulted in the formation of five major clusters (Figure 4). The accessions from POP4, POP3, POP1 sub-populations are dispersed across all major clusters (all 5 major clusters), possibly due to a high rate of gene flow or genetic drift. Cluster 1 included the individuals of POP1, POP2, POP3 and POP4. The second cluster consisted of individuals of POP1, POP3 and POP4 whereas, cluster 3 entailed POP1, POP3 and POP4. Cluster 4 comprised of POP1, POP3 and POP4 though cluster 5 entailed of POP3, POP4 and POP5. The individual accessions of POP5 which consisted of wild species of eggplant have formed a separate sub cluster under the major cluster 5.

DISCUSSION

Eggplant is an important Solanaceous vegetable which has luxurious genetic diversity for various traits including drought tolerance (Jellan *et al.*, 2016). This was manifested from the results of genetic diversity analysis of 54 accessions of eggplant in the present study.

Analysis of Molecular Variance (AMOVA) with a mean fixation index value of 0.649 for eggplant indicated significant differences among the individuals, among the sub-populations and also indicates the presence of gene flow among the sub-populations. The AMOVA fallouts show a very high level of intra-population diversity and low nevertheless significant genetic differentiation in interpopulation diversity of eggplant in the present study. Similarly, Gramazio *et al.* (2017) also reported that, the AMOVA showed significant genetic discrepancy among forty-eight eggplant accessions in the genetic diversity analysis of eggplant.

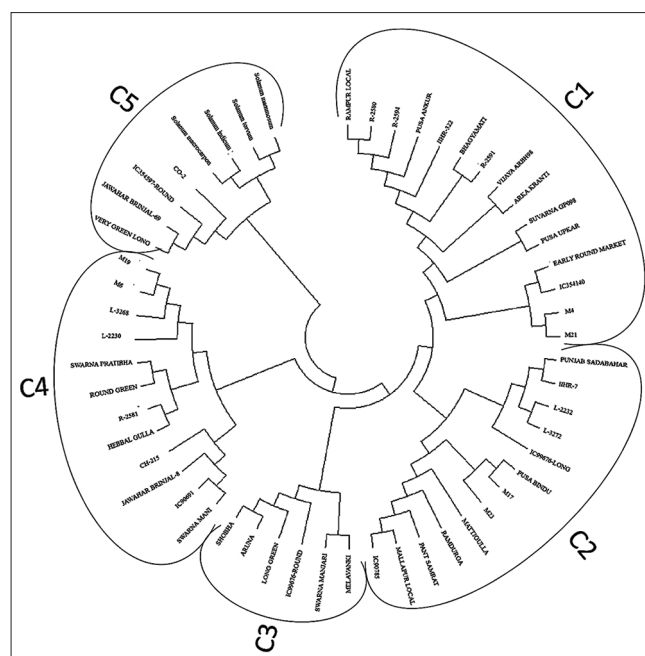
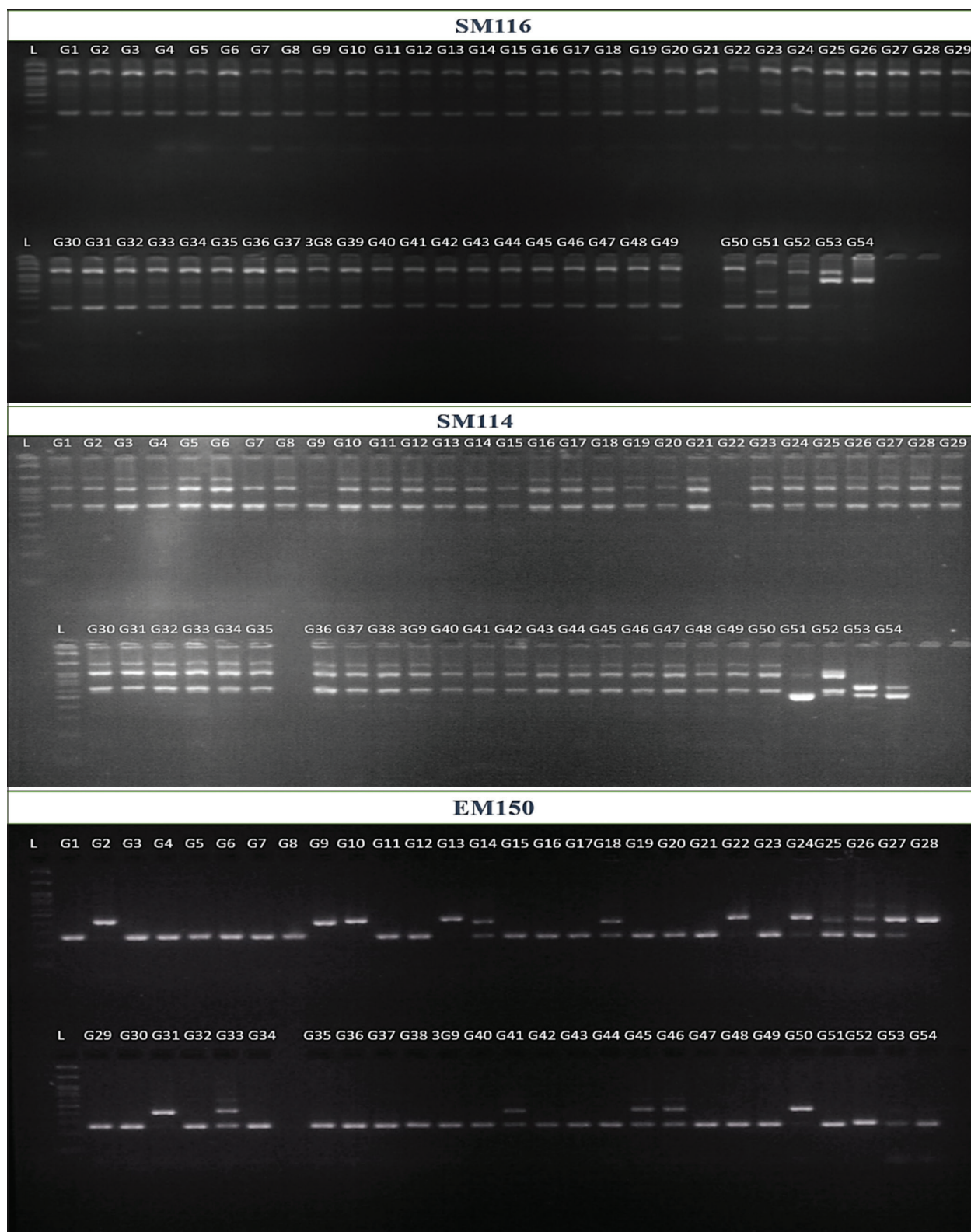


Figure 4: Neighbor joining (NJ) tree constructed using MEGA V.7.0 based on pair-wise genetic distance across 54 accessions of eggplant

In eggplant, allelic diversity present across the sub-populations of eggplant may have occurred due to natural genetic variation like cross pollination between the sub-populations or use of species as a parent for hybrid development or varietal development programme which shows gene flow within and among the sub-populations. Miyatake *et al.* (2019) reported allelic frequency between five sub-populations of eggplant which was about 0.79 for whole population collection.

The effective number of alleles (N_e) and Shannon's Information Index provides an effective measure of genetic variation in accordance with sub-population size. The N_e was for the sub-population POP4 (Advanced breeding lines) (3.024) followed by POP3 (Registered varieties) (2.822). Similarly, Ge *et al.* (2013) derived N_e value for ninety-two eggplant accessions. The overall mean Shannon's Information Index was 0.879 among all the five sub-populations. The grand mean of observed heterozygosity (H_o) was 0.216 whereas grand mean of expected heterozygosity (H_e) was 0.496. The mean heterozygosity values for sub-populations ranged between 0.332 (POP2) to 0.558 (POP1). Similar findings were reported by Vilanova *et al.* (2014) in 30 eggplant accessions using 19 SSRs.

The mean N_m value (1.026) indicated an overall number of migrants and possible divergence among the microsatellite loci used in the working collection of the eggplant. The maximum expected and observed heterozygosity among sub-populations is also one of the parameters which indicated that sub-populations were genetically diverse. These parameters provide the basis for occurrence of vast genetic divergence in eggplant for drought tolerance. Similarly, the significant



G1-Suvarna Gp098, G2-Pusa Upkar, G3-Vijaya Arbh98, G4-Arka Kranti, G5-Bhagyamati, G6-IIHR322, G7-Rampur Local, G8-Pusa Ankur, G9-R2580, G10-R2594, G11-R-2591, G12-Ic354140, G13-Early Round Market, G14-M4, G15-M21, G16-M23, G17-M17, G18-Pusa Bindu, G19-Mattigulla, G20-Ramdurga, G21-Malapur Local, G22-Pant Samrat, G23-Ic90785, G24-Ic99676 Long, G25-IIHR7, G26-Punjab Sadabahar, G27-L2232, G28-L3272, G29-IC99676 Round, G30-Aruna, G31-Shobha, G32-Long Green, G33-Swarna Manjari, G34-Melavanki, G35-Hebbal Gulla, G36-R-2581, G37-Swarna Pratibha, G38-Round Green, G39-L2230, G40-L3268, G41-M6, G42-M19, G43-CH215, G44-Jawahar Brinjal-8, G45-Swarna Mani, G46-IC90691, G47-Jawahar Brinjal-69, G48-Very Green Long, G49-IC354597 Round, G50-CO 2, G51-*Solanum macrocarpon*, G52-*Solanum indicum*, G53-*Solanum torvum* and G54-*Solanum mammosum*. G- Genotypes; L- Endogenous control 100bp Ladder

Figure 5: SSR banding pattern of 54 accessions of eggplant obtained by different microsatellite markers.

Table 4: Locus-wise information on the number of different alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I), Observed Heterozygosity (Ho), Expected Heterozygosity (He), number of migrants (Nm), Polymorphic Information Content (PIC) value, Fixation Index (F) and F-statistics across populations of eggplant

Marker Name	Na		Ne		I		Ho		He		F		PIC value	Nm	F _{IS}	F _{IT}	F _{ST}
	Mean	S.E.m. ±	Mean	S.E.m. ±	Mean	S.E.m. ±	Mean	S.E.m. ±	Mean	S.E.m. ±	Mean	S.E.m. ±					
SM104	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.00	0.00	0.00	0.00
SM107	3.800	0.970	3.136	0.687	1.060	0.311	0.000	0.000	0.558	0.149	1.000	0.000	0.952	0.821	1.000	1.000	0.234
SM114	7.400	0.927	5.643	0.423	1.825	0.111	0.987	0.013	0.818	0.017	-0.209	0.035	0.956	3.130	-0.207	-0.117	0.074
SM116	5.000	0.837	4.185	0.601	1.439	0.193	0.950	0.050	0.728	0.058	-0.353	0.170	0.942	1.393	-0.304	-0.106	0.152
SM117	2.400	0.400	1.901	0.283	0.672	0.182	0.000	0.000	0.410	0.111	1.000	0.000	0.850	1.390	1.000	1.000	0.152
SM119	2.400	0.510	1.997	0.377	0.682	0.213	0.000	0.000	0.414	0.120	1.000	0.000	0.906	0.713	1.000	1.000	0.260
SM120	1.800	0.200	1.711	0.193	0.526	0.134	0.000	0.000	0.373	0.096	1.000	0.000	0.749	1.217	1.000	1.000	0.170
SM126	5.400	1.030	3.649	0.409	1.413	0.151	0.950	0.050	0.712	0.032	-0.340	0.076	0.898	1.785	-0.334	-0.171	0.123
SM127	2.400	0.400	1.539	0.197	0.505	0.148	0.000	0.000	0.301	0.097	1.000	0.000	0.804	0.279	1.000	1.000	0.473
SM128	4.800	0.490	3.884	0.328	1.420	0.097	0.992	0.008	0.733	0.028	-0.362	0.060	0.911	2.473	-0.353	-0.228	0.092
eme01G19	2.800	0.583	2.329	0.444	0.811	0.235	0.000	0.000	0.481	0.127	1.000	0.000	0.919	0.461	1.000	1.000	0.351
emj04E17	3.200	0.583	2.427	0.339	0.951	0.133	0.000	0.000	0.563	0.046	1.000	0.000	0.939	0.934	1.000	1.000	0.211
emf01G17	2.400	0.245	1.998	0.173	0.752	0.075	0.000	0.000	0.487	0.037	1.000	0.000	0.909	0.759	1.000	1.000	0.248
EM147	4.000	0.894	3.357	0.677	1.191	0.228	0.000	0.000	0.644	0.074	1.000	0.000	0.968	1.076	1.000	1.000	0.188
EM146	5.000	0.707	3.902	0.449	1.428	0.124	0.928	0.049	0.730	0.031	-0.281	0.094	0.912	1.869	-0.271	-0.121	0.118
EM145	2.400	0.245	1.862	0.161	0.686	0.070	0.000	0.000	0.446	0.050	1.000	0.000	0.907	0.552	1.000	1.000	0.312
EM144	2.800	0.490	2.383	0.346	0.856	0.215	0.000	0.000	0.507	0.127	1.000	0.000	0.934	0.711	1.000	1.000	0.260
emf11L21	2.800	0.200	2.502	0.159	0.958	0.070	0.000	0.000	0.593	0.027	1.000	0.000	0.937	1.123	1.000	1.000	0.182
emi02E20	2.600	0.510	2.150	0.348	0.755	0.209	0.000	0.000	0.462	0.119	1.000	0.000	0.930	0.647	1.000	1.000	0.279
emg21I10	2.200	0.374	1.757	0.196	0.589	0.152	0.000	0.000	0.388	0.098	1.000	0.000	0.913	0.301	1.000	1.000	0.454
emg01D17	1.600	0.245	1.521	0.214	0.394	0.161	0.000	0.000	0.279	0.114	1.000	0.000	0.750	0.412	1.000	1.000	0.378
EM148	2.600	0.245	1.889	0.073	0.746	0.053	0.000	0.000	0.467	0.023	1.000	0.000	0.889	0.522	1.000	1.000	0.324
EM150	3.000	0.837	1.581	0.194	0.567	0.173	0.157	0.106	0.321	0.096	0.572	0.245	0.869	1.031	0.511	0.607	0.195
GRAND	3.209	0.174	2.535	0.122	0.879	0.049	0.216	0.037	0.496	0.023	0.649	0.055	0.858	1.026	0.684	0.721	0.238

Table 5: Population-wise information on the number of alleles (N), number of different alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I), Observed Heterozygosity (Ho), Expected Heterozygosity (He), Fixation Index (F) and percentage polymorphism (% P) observed across 23 microsatellite loci

Populations	N	Na	Ne	I	Ho	He	F	P (%)
Pop1	Mean	9.000	3.609	2.757	1.020	0.213	0.558	0.709
	S.E.m. ±	0.000	0.349	0.264	0.097	0.086	0.042	0.115
Pop2	Mean	2.000	1.826	1.783	0.497	0.217	0.332	0.419
	S.E.m. ±	0.000	0.174	0.162	0.092	0.088	0.058	0.171
Pop3	Mean	14.957	3.913	2.822	1.052	0.238	0.577	0.661
	S.E.m. ±	0.043	0.421	0.267	0.095	0.087	0.038	0.119
Pop4	Mean	23.957	4.174	3.024	1.096	0.226	0.577	0.692
	S.E.m. ±	0.043	0.420	0.312	0.107	0.087	0.046	0.111
Pop5	Mean	3.957	2.522	2.289	0.732	0.185	0.438	0.686
	S.E.m. ±	0.043	0.314	0.275	0.111	0.076	0.056	0.109
GRAND	Mean	10.774	3.209	2.535	0.879	0.216	0.496	0.649
	S.E.m. ±	0.747	0.174	0.122	0.049	0.037	0.023	0.055

Table 6: Pair-wise F_{ST} values for five sub-populations of eggplant germplasm

POP1	POP2	POP3	POP4	POP5
0.000				POP1
0.211	0.000			POP2
0.021	0.181	0.000		POP3
0.029	0.215	0.029	0.000	POP4
0.201	0.348	0.190	0.221	0.000

expanse and organisation of genetic diversity was indicated by allelic frequency (Na, Nm, Ne, He and Ho) found in eggplant (Tumbilen *et al.*, 2011; Ge *et al.*, 2013; Vilanova *et al.*, 2014).

Table 7: Pair-wise genetic distance for five sub-populations of eggplant germplasm

POP1	POP2	POP3	POP4	POP5
0.000				POP1
0.509	0.000			POP2
0.053	0.398	0.000		POP3
0.077	0.525	0.077	0.000	POP4
0.640	1.030	0.597	0.731	0.000

Percent polymorphism observed across the 23 SSR markers for five sub-populations represents the amount of diversity present in intra-population and inter-population. The PIC values obtained in the present study were ranged from 0.0 for marker SM104 to 0.968 for EM147, with a mean PIC value of 0.85 (Figure 5). The PIC value has shown to be influenced by the occurrence of variants per locus as well as relative distribution of the alleles (Botstein *et al.*, 1980). The maximum range of PIC value indicates that the markers were quite informative showing high replicability and reliability, covering the entire genome, evenly distributed on chromosomes and having high potentiality for multiplexing and high throughput genotyping. Similarly, mean PIC values of 0.574 and 0.50 were obtained by Hurtado *et al.* (2012) and Vilanova *et al.* (2014) while assessing 52 and 19 accessions, respectively.

The significant mean values, F_{IS} (0.684), F_{IT} (0.721) and F_{ST} (0.238) for five sub-populations of eggplant indicated the level of clustering across the sub-populations which, clearly revealed the existence of distinct genetic clustering at each hierarchy of the population *i.e.*, individual, sub-population and total population. In quantitative genetics, F-statistics describe the

statistically expected level of heterozygosity in a population; more specifically the expected degree of a reduction in heterozygosity when compared to Hardy–Weinberg expectation.

Pair-wise genetic distance across sub-populations indicated the presence of significant genetic differentiation among the populations than among the individuals within a population which may have occurred due to genetic contamination of subpopulations. The genetic distance of eggplant population was illuminated by Behera *et al.* (2005) and Gramazio *et al.* (2017) where, genetic distance between sub-populations more than pair-wise F_{ST} value indicated different populations of eggplant being more genetically diverse than individuals within a population.

In eggplant genetic diversity analysis, PCA uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables (principal components). The Principal Coordinate Analysis obtained in the present study separated the five sub-populations of eggplant as well as 54 individuals of five sub-populations which is possibly due to high degree of diversity between different populations than within a particular population (Figure 2) as that of Peakall and Smouse, 2012. Similar results for PCA analysis were obtained by Hurtado *et al.* (2012) in which, eggplant accessions of the different origins were admixed from China and Sri Lanka were mostly distributed in different areas of the plot (Hurtado *et al.*, 2012).

Phylogenetic analysis clearly distinguishes a genetically distinct individual or a population from the rest (Yang *et al.*, 2015). In *Solanum* species, analysis of the phylogeny of populations is an important step in understanding the relationships among the populations and their evolution.

Based on the results of Neighbour joining tree method, two major clusters on the basis of genetic similarity were formed for five eggplant sub-populations wherein, Cluster 1 includes POP1, POP4, POP2 and POP3. On the other point, the cluster C2 entailed of sub-population POP5 which, show clear dissimilarity between the sub-populations of two clusters. Further, the accessions from POP4, POP3, POP1 sub-populations are dispersed across all major clusters (Figure 4), possibly due to high rate of gene flow or genetic drift. The individual accessions of POP5 which consisted of wild species of eggplant have formed a separate sub cluster under the major Cluster 5. The admixture of different individuals of five sub-populations of eggplant was possibly due to drastic effect of genetic flow or genetic drift. Similarly, these types of genetically admixed clusters were obtained by Demir *et al.* (2010) and Jellan *et al.* (2016) during the phylogenetic assessment of twenty eggplant genotypes each.

The high degree of genetic variation within populations could be due to high rate of gene flow. Further, the contribution of allelic variation to the difference between populations is less compared to their contribution to the variation within population. Eggplant is modified with different style of flower that leads to crosspollination in nature which expectedly, may result in genetic contamination of eggplant accessions.

CONCLUSION

The eggplant or brinjal which is a most acceptable and commercially grown solanaceous vegetable after tomato, potato and chilli. Due to high abiotic as well as biotic stress incidence, the productivity of crop decreasing day by day. In order to enhance the yield potentiality of crop breeder needs to study the presence of genetic variation and diversity in a gene pool. There is a need of broaden the genetic base for development of adversely acclimatized eggplant hybrids for Indian seed markets. The obtained analytical results can be utilised for grouping of individual based on genetic similarities and dissimilarities. Such information aids in the selection of diverse parents for obtaining superior allele combinations in the hybrid or varietal development programmes.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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