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Genetic diversity in eggplant (*Solanum melongena* L.) germplasm from three secondary geographical origins of diversity using SSR markers

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Abstract: Indo-Burmese region was the primary center of eggplant diversity from where the crop extended to several secondary origins of diversity. In this study, the genetic diversity among fifty-six eggplant accessions collected from three countries was assessed using sixteen polymorphic SSR markers to determine suitable parents for heterotic hybridization. The estimation of genetic diversity among the population of three countries (Bangladesh, Malaysia, and Thailand) varied from 0.57 to 0.74, with Shannon's index value of 0.65. The mean value of expected heterozygosity and Nei's index was 0.49, with an average PIC value of 0.83. A dendrogram was constructed based on UPGMA (unweighted pair group method with arithmetic mean), and the dendrogram categorized all accessions into six groups. The AMOVA (analysis of molecular variance) revealed a 77% total variation within the population from three different countries and 23% total variation among the populations. The result revealed a high genetic differentiation among the eggplant germplasms while the accessions that are farther from each other show a high level of diversity; thus, they can be recommended as parental in breeding programs. Hence, accessions, EB12, ET11, ET13, ET15, ET16, and ET17 could be crossed with accessions EM3, EB34, and EB3 for improvement in the future breeding program.

Introduction

Solanum melongena L., also known as eggplant, belongs to the family Solanaceae and is ranked as one of the beneficial vegetables worldwide. The crop ranks among high-valued vegetables with the highest antioxidant activity and nutritional value (Liu *et al.*, 2018). The Indo-Burma region is considered the primary origin and center of eggplant's domestication, where the highest diversity of this crop is found (Augustinos *et al.*, 2016). The crop enjoys extensive cultivation throughout the tropics and warm temperate regions, especially in Southern USA and Mediterranean regions (Liu *et al.*, 2018). Despite its profitability and nutritional value, the breeding attempts for this vegetable are limited compared to other members of the Solanaceae family, such as potato and tomato (Hurtado *et al.*, 2012). The study of genetic diversity is vital in breeding programs because it provides useful utilization of germplasm in the advancement of closely related species (Jasim *et al.*, 2018). It is essential to assess polymorphisms among existing cultivars and select parents for hybridization. Generally, morphological characterization is considered the first step towards exploring genetic variation in eggplant (Sulaiman *et al.*, 2020). However, morphological characters have particular limitations in distinguishing homozygous from heterozygous. Aside from this, morphological characters cannot define the exact level of diversity among existing germplasm due to additive gene action of disclosure of economically important traits (Jasim *et al.*, 2018). Molecular markers are not environmentally controlled and can reveal the genotypic difference at the DNA level.

Hence, molecular markers play a crucial role in analyzing plant genealogy, gene mapping, construction of genetic maps, evolution, germplasm characterization, selection for characters, diversity study, and the determination of genome organization (Zuki *et al.*, 2020; Sarif *et al.*, 2020). Microsatellites, also known as simple sequence repeats (SSR), have become one of the most prevalent genetic markers due to their co-dominance inheritance, multi-allelic nature, reproducibility, high genome coverage, abundance

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and are polymerase chain reaction (PCR)-based (Demir *et al.*, 2010; Chukwu *et al.*, 2020). The first set SSR marker for eggplant was developed from the screening of small insert of di- and trinucleotide repeats of genomic libraries (Nunome *et al.*, 2003). Subsequently, a set of small SSR markers from genic DNA sequence developed by Stàgel *et al.* (2008) developed was lodged in public databases. Similarly, over 1,000 SSR markers were identified by Nunome *et al.* (2009) while screening enriched cDNA and gDNA libraries. Barchi *et al.* (2011) isolated a wide-range of approximately 2,000 putative eggplant SSRs markers from restriction-site associated DNA tags out of which a subset exhibited polymorphism among the mapping population parents. A wide range of SSR markers is publicly obtainable for eggplant, either from genomic SSRs (genomic libraries of SSR enriched) or EST-SSR (genic libraries). Genomic SSRs are usually related to non-coding parts, but EST-SSRs are derived from expressed regions of the genome (Muñoz-Falcón *et al.*, 2011). Meanwhile, EST-SSRs are less polymorphic compared to genomic SSRs (Muñoz-Falcón *et al.*, 2011). The main objective of this study was to evaluate genetic diversity among collected materials using both genomic SSR and EST-SSR polymorphic markers and examine suitable parents for heterotic hybridization in future breeding programs. This study will be useful in the germplasm conservation and characterization for future breeding programs of eggplant resources.

Materials and Methods

Planting materials

Fifty-six accessions of eggplant (*S. melongena*) which formed three populations, of which 33 from Bangladesh (EB), 15 from Thailand (ET), and 8 from Malaysia (EM), were used for this study (Tab. 1). The materials were selected to represent the genetic diversity of local materials for each country (Fig. 1).

Markers selection

A total of 102 markers were selected for analyzing diversity, among which 16 markers (Nunome *et al.*, 2003; Stàgel *et al.*, 2008; Muñoz-Falcón *et al.*, 2011; Tümbilen *et al.*, 2011; Vilanova *et al.*, 2012; Cericola *et al.*, 2013) were found to be polymorphic with a clear band among the 56 accessions as shown in Tab. 2.

DNA extraction, genotyping and electrophoresis

Young leaves from individual accessions (approximately 100 mg) were used for the extraction of genomic DNA following a slight modification on the CTAB procedure (Oladosu *et al.*, 2015). The DNA extraction was diluted to 50 ng/μL using a TE buffer and stored at −20°C until PCR amplification. The DNA concentration and purity were quantified using a Nanodrop 2000 spectrophotometer machine (ND 1000). The extracted DNA purity for individual samples was measured at an absorbance ratio from 1.95 to 2.0 of 260 nm divided by 280 nm. The PCR was conducted using a 15 μL-reagent containing: 7.5 μL 2× Taq DNA polymerase master mix (Thermo Scientific, USA), 4.5 μL nucleus free water, 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), and 1 μL DNA template (50 ng/μL). The PCR was run on a PCR machine using a touchdown protocol which was optimized for eggplant with initial denaturation of 94°C for 3 min followed by 10 cycles at 94°C for 30 s (decrease 1°C per cycle for denaturation), 55–65°C for 1 min and 72°C for 30 s followed by 30 cycles at 94°C for 30 s, annealing at 55°C for 1 min, 72°C for 1 min and final extension at 72°C for 5 min, followed by rapid cooling at 4°C prior to analysis. Annealing temperature depends on primers. For the DNA fragments amplification, 5 μL of PCR product loaded on the on 2% MetaPhor™ agarose (Lonza Rockland, Inc., USA) with 1X TBE buffer (0.05 M Tris, 0.05 M boric acid, 1 mM EDTA; pH 8.0) that was

TABLE 1

List of germplasm and their origin

No.	Code	Status of materials	Source name	Country of collection	No.	Code	Status of materials	Source name	Country of collection
1	EB1	Hybrid	China3	Bangladesh	29	EB32	Inbred	317	Bangladesh
2	EB3	Hybrid	MuktaJhuri	Bangladesh	30	EB33	Inbred	346	Bangladesh
3	EB4	Inbred	MuktaKeshi	Bangladesh	31	EB34	Inbred	350	Bangladesh
4	EB5	Hybrid	Chinese Macra	Bangladesh	32	EB35	Inbred	262	Bangladesh
5	EB6	Hybrid	BARI Eggplant 2	Bangladesh	33	EB36	Inbred	357	Bangladesh
6	EB7	Hybrid	Tal Begun	Bangladesh	34	EM3	Hybrid	214	Malaysia
7	EB8	Hybrid	Pahuja seed co.	Bangladesh	35	EM4	Hybrid	311	Malaysia
8	EB9	Inbred	Pahuja seed co.	Bangladesh	36	EM5	Hybrid	330	Malaysia
9	EB10	Inbred	Laskar seed	Bangladesh	37	EM6	Hybrid	418	Malaysia
10	EB11	Inbred	Singhnath	Bangladesh	38	EM7	Hybrid	428	Malaysia
11	EB12	Inbred	BARI Eggplant1	Bangladesh	39	EM8	Inbred	313	Malaysia
12	EB13	Inbred	BARI Eggplant4	Bangladesh	40	EM9	Hybrid	312	Malaysia

(Continued)

Table 1 (continued).

No.	Code	Status of materials	Source name	Country of collection	No.	Code	Status of materials	Source name	Country of collection
13	EB14	Inbred	BARI Eggplant5	Bangladesh	41	EM10	Inbred	MTe2	Malaysia
14	EB15	Inbred	BARI Eggplant6	Bangladesh	42	ET1	Hybrid	636/2559	Thailand
15	EB16	Inbred	BARI Eggplant7	Bangladesh	43	ET2	Inbred	01387/2552	Thailand
16	EB17	Inbred	BARI Eggplant8	Bangladesh	44	ET3	Inbred	1845/2338	Thailand
17	EB18	Inbred	BARI Eggplant9	Bangladesh	45	ET4	Inbred	00558/2551	Thailand
18	EB19	Inbred	BARI Eggplant10	Bangladesh	46	ET5	Hybrid	Parquy	Thailand
19	EB20	Inbred	220	Bangladesh	47	ET6	Inbred	969/2560	Thailand
20	EB21	Inbred	217	Bangladesh	48	ET7	Inbred	01451/2551	Thailand
21	EB22	Inbred	253	Bangladesh	49	ET8	Inbred	914/2558	Thailand
22	EB23	Inbred	222	Bangladesh	50	ET9	Inbred	01450/2551	Thailand
23	EB24	Inbred	275	Bangladesh	51	ET10	Inbred	01166/2551	Thailand
24	EB26	Inbred	288	Bangladesh	52	ET11	Inbred	762/2556	Thailand
25	EB27	Inbred	291	Bangladesh	53	ET13	Inbred	1745/2560	Thailand
26	EB28	Inbred	311	Bangladesh	54	ET15	Inbred	548/2558	Thailand
27	EB30	Inbred	330	Bangladesh	55	ET16	Inbred	01200/2553	Thailand
28	EB31	Inbred	338	Bangladesh	56	ET17	Inbred	548/2556	Thailand

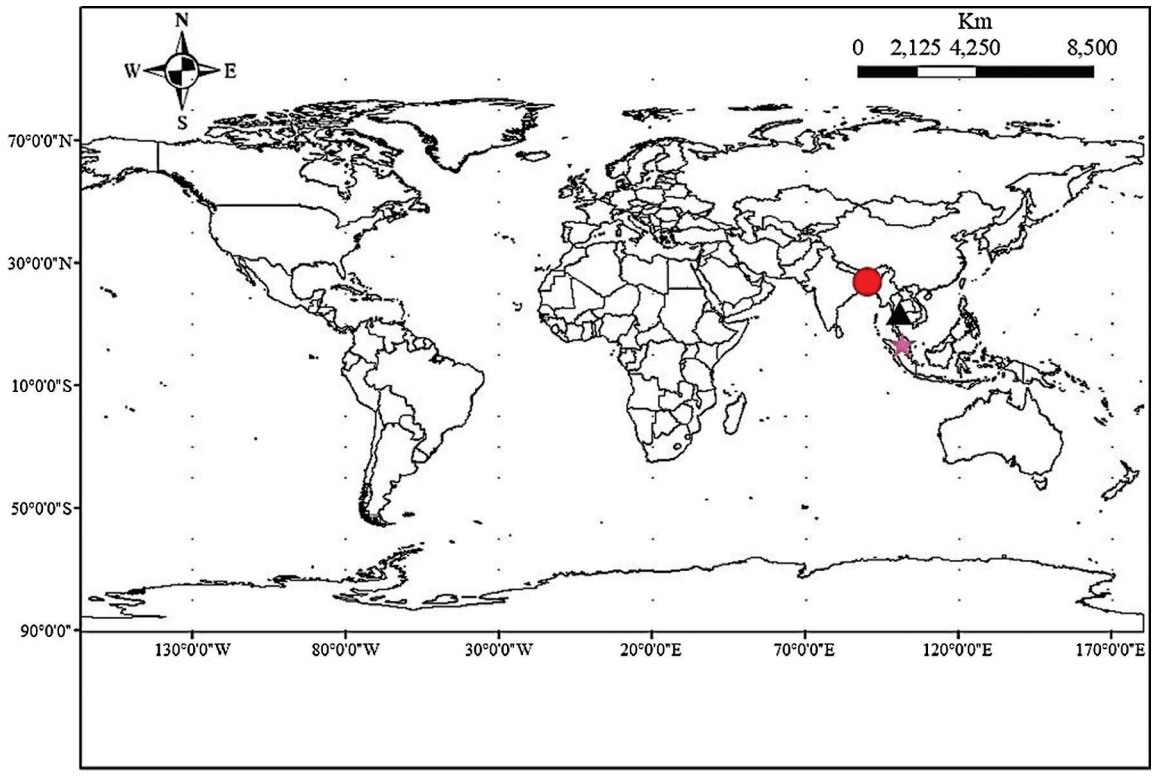

FIGURE 1. Map showing sample collection site (Circular mark = Gazipur, Bangladesh; Triangle = Bangkok, Thailand and Star = Selangor, Malaysia).

pre-stained with Midori Green Nucleic Acid Staining Solution (1:100,000) and run at 80 V for 60 min. Then the gels were documented using Molecular Imager® (GelDoc™ XR, Bio-Rad). Exactly 100 bp (GeneDirex) DNA ladder was utilized to score the band.

Data analysis

The data were transformed into binary data using the UVIDoc software. NTSys software was used for clustering and PCA, with number 1 indicating present, and 0 showing (0) for each locus. The data was then analyzed using the Popgen software Version 1.32, reported by Yeh (1997). Polymorphic Information Content (PIC) values were computed using the following formula:

$$\text{PIC} = 1 - \sum p_{ij}^2 \quad (1)$$

Here, p_{ij} is the frequency of the j-th allele for the i-th marker and summed over N alleles (Anderson *et al.*, 1993).

TABLE 2

List of polymorphic markers (SSR)

Code	Sequence of marker (5'-3')	Motif	Size Range (bp)	Anneal. Temp.
Genomic-SSR				
CSM27	F: TGTTTGGAGGTGAGGGAAAG	(GA)23	206	60
	R: TCCAACTCACCGGAAAAATC			
CSM29	F: GGATGAAATGAAGGCTTAGGG	(AG)17	236	60.1
	R: GCCATCCTCATCTTTGATGG			
CSM31	F: CAACCGATATGCTCAGATGC	(AG)28	259	60
	R: GCCCTATGGTCATGTTTTGC			
CSM44	F: CGTCGTTGTAACCCATCATC	(AG)14	249	58.7
	R: TTGCCAAATTCCTTGTGTTC			
CSM54	F: ATGTGCCTCCATTCTGCAAG	(GA)19	227	61.1
	R: TGGGTGGGATGCTGAGTAAG			
CSM73	F: TTCAACATAGCCTGGACCATT	(CT)22	209	60
	R: AATGCAGGGTTTGGACTTCA			
BSMSSR1	F: CTCCACGCTACTTAGGGGACTCAA	–	217	55
	R: AGACCACACTTGGCATGTCTTGAA			
EM131	F: TCTGGGACACCAAGTGAAAAATCA	(AT)5(AC)3A(AC)14(AT)7GTA(TG)5(TA)3	213	60
	R: TGCGTTTTTGGCTCCTCTATGAAT			
EST-SSR				
smSSR1	F: GTGACTACGGTTTCACTGGT	(ATT)21	310	60
	R: GATGACGACGACGATAATAGA			
smSSR3	F: ATTGAAAGTTGCTCTGCTTC	(TA)9 (GA)8	145	60
	R: GATCGAACCCACATCATC			
smSSR11	F: AAACAAACTGAAACCCATGT	(AGC)6	126	58
	R: AAGTTTGCTGTTGCTGCT			
smSSR35	F: CACCACCAAAGAATTCCTAA	(ATG)5	269	60
	R: TTGCTAGAAATAGCAAAGGG			
EEMS18	F: GGAGAAACTGAAAAATTTGTAGAGAG	(AG)7	187	62
	R: GAGGAGTTTCCGACATGAGC			
EEMS20	F: AACATCAGCCAGGGTGTTTC	(AT)8	215	62
	R: TACGGCTGAGATTCATTTGC			
EEMS37	F: CCCTTCCTACCCACACTTCA	(TCC)5	117	61.5
	R: GTTTTGCACCTTTCCATCGT			
ecm001	F: ACCTTACGCAATTTACACTTCCCC	(TC)17	229	62
	R: GTTTCAATGGCGTCACCTCTCTCTCT			

Note: Muñoz-Falcón *et al.*, (2011); Vilanova *et al.*, (2012); Tümbilen *et al.*, (2011); Stàgel *et al.*, (2008); Nunome *et al.*, (2003); Cericola *et al.*, (2013).

All the data were analyzed using the NTSYS Pc software Version 2.20 for multivariate analysis. First, the data were standardized to remove the effects of different measurements using the STAND function. The distance coefficient was then worked out using the DICE similarity index by utilizing the transformed data and the information was exemplified in dendrogram following unweighted pair group method with arithmetic average (UPGMA), and SHAN (sequential, hierarchical, and nested clustering) methods in NTSYS Pc. 2.20. The adjustment between the dendrogram and dissimilarity matrix was estimated by the cophenetic correlation coefficient (r) according to Rohlf (1998). The average genetic distance was then used as a cut-off value to define genotype clusters. Principal component analysis (PCA) was calculated using DECENTRE, EIGEN, and GRAPHICS as described by Rohlf (1998) to complement cluster analysis. The distribution of genetic variation within and between families from different countries was determined using the analysis of molecular variance (AMOVA) was calculated using the Gene Alex 6.502 software (Peakall and Smouse, 2006). The test for significance of the estimated parameters was conducted based on 10,000 bootstrap resamples.

Results

Polymorphism analysis with SSR (Simple Sequence Marker)

From the studied 102 markers, the 16 which showed polymorphism bands were selected for genetic diversity analysis. The expected heterozygosity ranged from 0 to 0.756, with an average of 0.493 (Tab. 3). The observed heterozygosity was zero (0) for seven markers to 0.446 for marker EM131, with an average value of 0.040. The PIC value ranged from 0.660 (EEMS 37) to 0.966 for CSM 27, with an average value of 0.830. This indicates that the most informative marker was CSM27, which differentiated the highest number of accessions; whereas, the least informative marker was EEMS 37, which separated the least number of accessions. The effective number of alleles ranged from 0.609 (SMSSR 35) to 3.971 (CSM27), with an average of 2.208. The highest numbers of alleles (6) were observed from 'marker CSM27 and smSSR1', while the lowest numbers of alleles (2) were observed from 'markers BSMSSR1, smSSR3, smSSR11, EEMS20, EEMS37, and ECM001 with an average value of 3.250. The Nei's index varied from 0.147 (EEMS 37) to 0.748 for CSM 27 with a mean value of 0.488. The observed Shanon's index varied from 0.278 to 1.505, with an average value of 0.849. The summary of the SSR marker with their genetic diversity-related traits is presented in Tab. 3. The observed heterozygosity (H_o) estimate values were exhibited at 0.040 (average value).

Genetic diversity within populations

The percentage of average polymorphic loci was 91.67% among populations (Tab. 4). Thailand's accessions exhibited the highest genetic diversity, i.e., 100%, whereas Malaysia's accessions showed 81.25%, which was the lowest, while Bangladesh revealed a 93.75% level of diversity. Moreover, among these populations, the observed number of alleles ranged from 2.187 (Malaysia) to 2.687 (Bangladesh), with a mean value of 2.458. The effective number of alleles (ne) varied from 0.018 (Thailand) to 1.767 (Bangladesh), with an average value of 1.184. Shanon's information index varied from 0.569 (Malaysia) to 0.745 (Thailand), with an average value of 0.650.

Clustering using SSR markers

The selected sixteen SSR marker data were analyzed for clustering using the NTSYS software. Clustering was conducted to group all accessions into the dendrogram. The similarity coefficient is ranged from 0.23 to 0.88. All accessions were classified into six groups, with a threshold level of 0.33 (Fig. 2). The first cluster consisted of 31 accessions (30 from Bangladesh and one from Malaysia). In contrast, the second Cluster had 5 accessions from Thailand and Bangladesh, while Cluster III consisted of only one accession from Bangladesh, Clusters IV and V had 6 accessions each, and Cluster VI consisted of seven accessions from Thailand. The result of the PCA is presented in Fig. 3. Accessions such as EB12, ET11, ET13, ET15, ET16, and ET17

TABLE 3

Different traits of genetic diversity among 56 accessions based on 16 SSR primers

No.	Marker	na	H_o	H_e	Ne	PIC	Nei's Index	I
Genomic- SSR								
1	CSM27	6	0	0.756	3.971	0.966	0.748	1.505
2	CSM29	4	0	0.687	3.121	0.945	0.679	1.229
3	CSM31	5	0.24	0.631	2.662	0.917	0.624	1.161
4	CSM44	3	0.018	0.518	2.053	0.837	0.513	0.827
5	CSM54	3	0	0.4	1.655	0.819	0.396	0.708
6	CSM73	3	0.021	0.612	2.533	0.907	0.605	1.01
7	BSMSSR1	2	0.318	0	1.459	0.768	0.315	0.494
8	EM131	3	0.446	0	1.791	0.852	0.442	0.784
	Mean	3.625	0.13	0.45	2.406	0.876	0.54	0.965
EST-SSR								
9	smSSR1	6	0.167	0.726	3.569	0.943	0.719	1.378
10	smSSR3	2	0.152	0.305	1.431	0.709	0.301	0.479
11	smSSR11	2	0	0.383	1.612	0.7	0.379	0.567
12	smSSR35	3	0.019	0.609	2.518	0.88	0.603	1.008
13	EEMS18	4	0	0.627	2.638	0.915	0.621	1.076
14	EEMS20	2	0	0.318	1.46	0.713	0.315	0.495
15	EEMS37	2	0	0.149	1.173	0.66	0.147	0.278
16	ECM001	2	0.02	0.411	1.686	0.752	0.407	0.597
	Mean	2.875	0.045	0.441	2.011	0.784	0.436	0.735
	Total Mean	3.25	0.04	0.493	2.208	0.83	0.488	0.849

Note: na, Observed number of alleles; Ho, observed Heterozygosity; He, expected Heterozygosity; ne, effective number of alleles; PIC, Polymorphic Information Content; Nei's, Nei's index; I, Shanon's Information Index.

were farthest from the center. Meanwhile, accessions like EM3, EB34, and EB3 were located near the center.

Analysis of molecular variance (AMOVA) using SSR markers

The SSR profiles of the eggplant genotypes in this research were analyzed using AMOVA to determine the inter-population genetic variances. The inter-genetic variances and intra-genetic variances among the populations were 23% and 77%, respectively (Tab. 5). Moreover, the AMOVA analysis showed highly significant ($p \leq 0.01$) genetic differences among populations (Bangladesh, Malaysia, and Thailand) and within populations. Total genetic variation within populations was 77% in 56 eggplant genotypes; whereas, genetic variation among populations was 23% in three regions. This indicated that high genetic dissimilarity existed within-population compared to among-population.

TABLE 4

Estimation of genetic diversity among the accessions of eggplant population

Population	% P	Na	Ne	I
Bangladesh	93.75	2.687	1.767	0.636
Malaysia	81.25	2.187	1.767	0.569
Thailand	100	2.500	0.018	0.745
Mean	91.67	2.458	1.184	0.650

Note: %P, Percentage of polymorphic loci; na, Observed number of alleles; ne, effective number of alleles; I, Shanon's Information Index.

Discussion

This research revealed the level of genetic diversity among the available germplasm of eggplant collected from three different countries using the SSR marker (Fig. 2). The determination of genetic variation among germplasm is vital in the breeding and conservation of genetic resources. It is also important in genetic improvement and exploitation of genes for tolerance against abiotic stress. The detection of polymorphism within germplasm is important in breeding. There are reports (Nunome *et al.*, 2003; Stàgel *et al.*, 2008; Demir *et al.*, 2010) of low polymorphism frequency within intraspecific lines and cultivars among crops of Solanaceae family, and this is possibly due to their autogamous nature. Eggplant is an autogamous crop, and most of the materials are commercial varieties, so low heterozygosity is not unexpected (Cericola *et al.*, 2013). The more or less similar value of H_o (0.038) was also reported by Augustinos *et al.* (2016). The low value of H_o was also observed by Liu *et al.* (2018) and

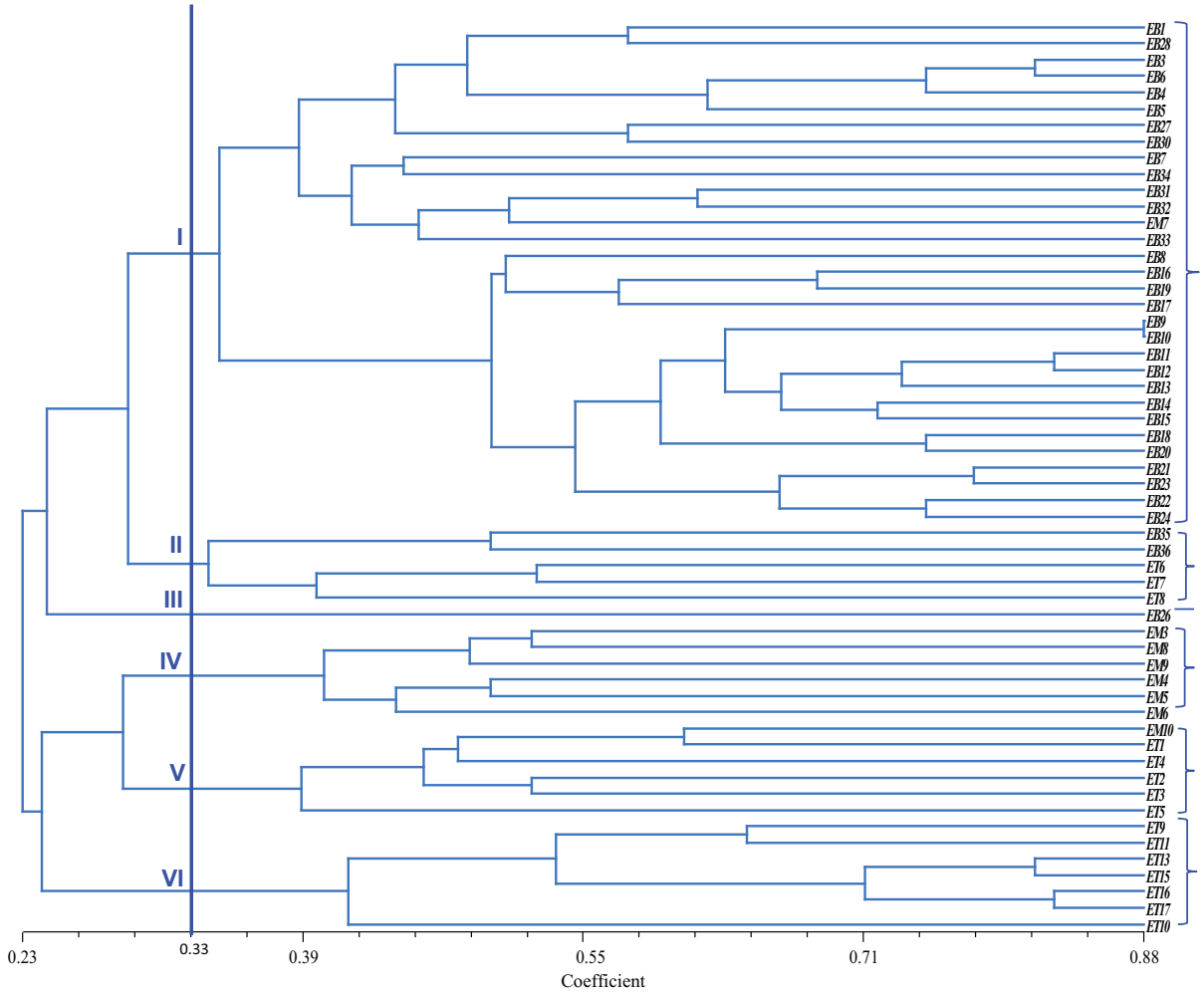

FIGURE 2. Clustering of 56 eggplant genotypes based on polymorphic SSR markers regardless of origin using Jaccard's coefficient of similarity and UPGMA clustering.

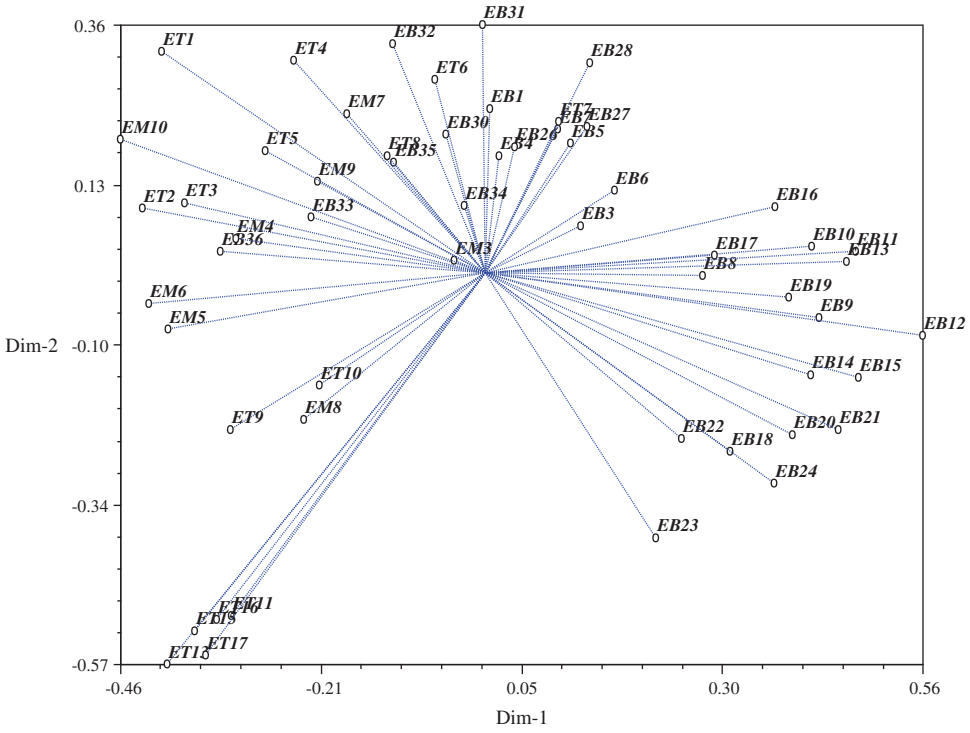

FIGURE 3. Principal Component analysis (PCA) of relationship among all accessions in two dimensional graph.

TABLE 5

AMOVA (Analysis of molecular variance) among 56 eggplant genotypes

SV	Df	SS	MS	Est. Var.	% of variation
Among Populations	2	79.41	39.705	2.08	23%
Within Populations	53	371.02	7	7	77%
Total	55	450.429			100%

Note: SV, Sources of Variation; Df, Degree of freedom; SS, Sum of Squares; MS, Means of Sum Squares.

Vilanova *et al.* (2012), who reported 0.03 and 0.06 in their respective study. The high value of homozygosity indicates that pure lines can be found from the selection of individuals among this germplasm (Vilanova *et al.*, 2014; Gramazio *et al.*, 2019). Although molecular diversity depends on the number of markers, types of markers, and the tested accessions (Augustinos *et al.*, 2016; Tümbilen *et al.*, 2011; Gramazio *et al.*, 2019). The high average value of PIC, the high mean number of alleles per locus, and high levels of observed heterozygosity in this research are comparable with other studies, thus indicating that great diversity exists among the collected germplasm. The study reported by Nunome *et al.* (2003) showed an average value of the observed number of alleles per locus was 3.1, and the expected heterozygosity value was 0.38 when an evaluation was conducted within 11 germplasm of *S. melongena* using 16 polymorphic dinucleotide genomic microsatellites. Thirty-eight *S. melongena* accessions were evaluated using 11 EST-SSR polymorphic markers, the mean value of alleles per locus was 3.1 and the average PIC value was 0.38, according to a report by Stàgel *et al.* (2008). From their study, it was observed that genomic SSR markers are more polymorphic compared to EST-SSRs. This was in tandem with reports by Kalia *et al.* (2011), Vilanova *et al.* (2012), and Nunome *et al.* (2009). Five eggplants SSRs, developed by Nunome *et al.* (2009), chosen for the high value of PIC, showed an average of 5alleles/locus. Considering the five SSRs with the highest value of PIC, the average number of alleles per locus in this study was 5, which is greater than the value reported by Demir *et al.* (2010). This indicates a wide diversity exists with different origins and types.

SSR marker is useful for the analysis of genetic diversity in eggplant. For this study, the PIC value range from 0.660 to 0.966, with an average value of 0.830. This value was higher than the mean PIC value of 0.401, 0.47, and 0.507, reported by Vilanova *et al.* (2012), Muñoz-Falcón *et al.* (2011), and Liu *et al.* (2018), respectively. A PIC value greater than 0.5 indicates a highly polymorphic locus. A PIC value of 0.25–0.50 shows an intermediate polymorphic locus, while a PIC of lower than 0.25 indicates a low polymorphic locus (Gramazio *et al.*, 2019; Kalia *et al.*, 2011; Nunome *et al.*, 2009; Ge *et al.*, 2013). In this research, the average value of PIC was 0.830, indicating a high level of polymorphism in the loci. Genetic diversity level measured in eggplants varies in different literature studies. Hurtado *et al.* (2012) recorded a high genetic diversity study for some Chinese accessions, i.e., He = 0.494, and some Sri Lankan accessions, i.e., He = 0.540, which is similar to that observed in this study

(He = 0.493). This research showed Nei's index and Shanon's information index values of 0.488 and 0.849, respectively, which were higher than the result recorded by Ge *et al.* (2013), where Nei's index value & Shannon's index value 0.323 and 0.570, respectively. This may be due to different materials, sample sizes, and different types of markers used.

The dendrogram coefficient range varied from 3.51 to 12.89, indicating a high amount of variation present among existing materials. Higher diversity was observed among genotypes of Groups I to VI due to their different morphological characters. The accessions were admixtured from different countries, indicating that these accessions had a common origin or more or less the same morphological characters. On the other hand, the accessions which were distant from one another, meaning that these accessions had different agronomical traits or distinct origin. The accessions from different clusters but different origins suggest an exchange of genetic materials by plant breeders from different geographical locations. Dissimilarities among accessions could be due to environmental influence occurring over a long period of time.

The AMOVA showed highly significant genetic differences within populations. This result indicates that high genetic dissimilarities existed among the accessions within the population (77%), while there are low significant differences among the populations (23%), showing the presence of low genetic dissimilarities among the population. This research results were similar to previous research by Mazid *et al.* (2013), in which 67% variation was present within groups of 41 rice genotypes while there was 33% variation among the 41 rice genotypes.

Conclusion

The microsatellite markers are valuable tools in determining the genetic relationship among eggplant accessions such as those from three different countries (Bangladesh, Malaysia, and Thailand) used in this study. These markers also helped to reveal a high level of polymorphism within the population and a low polymorphism level among populations. Through the improvement of eggplant accessions and widening of their genetic base, the population which has the least genetic similarities could be selected as parental materials. Therefore, hybridization should be conducted using two distant populations like any accessions of Cluster I with Cluster V. Hence, accessions EB12, ET11, ET13, ET15, ET16, and ET17 could be crossed with accessions EM3, EB34, and EB3 for improvement in the future breeding program. The molecular variance analysis showed that 77% of total genetic variations were due to differences within populations, whereas 23% genetic variation was exhibited among populations.

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