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ASSESSMENT OF GENETIC DIVERSITY AMONG SIXTY FIVE BRINJAL GENOTYPES USING SSR MARKERS

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Abstract

The present study was conducted at the Biotechnology Laboratory and Horticulture Field Laboratory of the Bangladesh Institute of Nuclear Agriculture (BINA) to study the genetic diversity among 65 brinjal genotypes using single sequence repeats (SSR) markers. Molecular characterization of the collected genotypes was performed using six SSR markers. The selected primers, namely EM107, EM114, EM133, EM139, EM140 and EM145 showed polymorphism. The values of pair-wise comparison of Nei's genetic distance (D) between genotypes computed from combined data for the 6 primers ranged from 0.17 to 1.00. The highest genetic dissimilarity (100%) was observed among various combinations, such as Islampuri (S31), Thapara (S32), Mentar (S33), Iribegun (S35), Eye-red (S36), Deembegun (S37), Comilla-L (S38) and Chega (S39) with Dohazari G (S10), Borka (S11), Khatkhatia B (S12), Khatkhatia BAU (S13), ISD-006 (S2) and Laffa-M (S3). The UPGMA cluster based on Nei's genetic distance analysis led to the grouping of the 65 brinjal genotypes into three major clusters with nine sub-clusters.

Key words: Brinjal, SSR marker, molecular characterization, genetic diversity, dendogram

Introduction

Brinjal (*Solanum melongena* Linn.), also known as eggplant or aubergine, is an important solanaceous vegetable crop grown widely in the Central, South and South-East Asian countries, and in a number of African countries of the world (Kalloo, 1993; Kumar *et al.*, 2003; Shaukat *et al*., 2009). Brinjal is believed to have been originated in India, as the people of this subcontinent were reported to grow brinjal since last 4000 years (Dunlop, 2006). Brinjal occupies a distinct place in the realm of vegetable crops globally. The current global production of brinjal is estimated as 54.08 million tons of which 93% is contributed by the Asian countries (FAO, 2020). In terms of production, China ranked the top (45% of world output) followed by India (24% of world output) (FAO, 2020). Brinjal is one of the most nutritious and culturally important vegetables and is a good source of minerals and vitamins. It is also one of the most common, popular and principal vegetables grown both in summer and winter seasons in Bangladesh. To meet domestic demand, Bangladesh produces substantial amounts of brinjal every year. During 2018-19, 531 thousand metric tons of brinjal were produced from 53 thousand ha of land (BBS, 2019).

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The identification of crop plant has become increasingly important for the documentation of genetic resource and to protect breeders' interests. For recipes and food industries, this is especially important for different varieties of brinjal having wide range of morphological, eating and marketing qualities. Farmers need varietal identity for protection of their proprietary rights. Identification process must assure varietal identity and free from mixture. Examination of morphological characters was used to be the common method of identification, but not all of them can be distinguished on morphological basis only.

Molecular characterization of genotypes is considered a powerful and efficient technique for assessment of genetic variability required for successful breeding programme. Molecular markers are used now-a-days as recent innovation for characterization and evaluation of genetic diversity within and between species and population. These markers can provide significant information which may enhance the scope of diversified use of genotypes in crop improvement programme. Characterization at molecular level reveals the extent of relationship among the genotypes and in the estimation of genetic diversity or relatedness. SSR marker was first developed for brinjal by Nunome *et al.* (2003), where they confirmed the usefulness of these markers for genetic analysis and found useful for agronomically-important traits in brinjal that could facilitate marker assisted breeding. Despite widespread cultivation, and nutritional, cultural and economic importance, brinjal genome has not yet been extensively evaluated as compared to those of other solanaceous vegetables, namely tomato, potato, pepper and so on. Few studies have been conducted to determine genetic diversity of brinjal using RAPD (Nunome *et al*., 2001), AFLP (Mace *et al*., 1999), RAPD and AFLP (Nunome *et al*., 2001), SSR (Nunome *et al*., 2003; Stagel *et al*., 2008; Nunome *et al*., 2009) and ISSR (Isshiki *et al*., 2008) markers.

In Bangladesh, information on molecular characterization using SSR marker is scanty and for brinjal it is a new attempt. Very few studies with smaller number of genotypes were conducted and reported in Bangladesh. However, reports of in-depth investigation with most of the available genotypes in Bangladesh and potential exotic lines are hardly found available in the in Bangladesh. Therefore, the main objectives of the present investigation were to precisely describe the genetic variation, genetic distance and genetic dissimilarities among the 65 brinjal genotypes sourced from home and abroad using SSR markers in Bangladesh.

Materials and Methods

The experiment was conducted at the Field Laboratory of BINA with 65 brinjal genotypes primarily obtained from IPM Lab, BAU; BARI, Gazipur and BINA, Myemnsingh. The collected genotypes were assigned to accession numbers starting from S1-S65. The seedlings were grown in the pot up to 25-30 days. To carry out the analysis, a fresh young leaf from each of the brinjal genotypes was collected and held in zip-lock plastic bags and preserved in icebox. Then the samples in icebox were brought to the laboratory and cut into 2-3 cm pieces for extraction of genomic DNA. Then 800 µl DNA

extraction buffer was added and samples were grounded with mortar and pestle and was taken into eppendrof (2 ml) tube and vortex well to suspend the powder. Then the eppendrof tubes were placed in 65^{o}C water bath in a tube holder for 20 minutes (after 10 minutes solution were mixes by inverting and returned to the water bath). Then the tube were removed, mixed inverting and bring to a chemical fume hood. Later 800 µl chloroform mix (Phenol:Chloroform:Isomyl alcohol=24:25:1) was added. The tightly closed tubes were placed in a tube rack covered with paper towels and hold a second tube rack against the top of the tubes and invert repeatedly for three minutes. Then the tubes were centrifuge for eight minutes at 11,000 rpm in a micro centrifuge. Then 500 µl of upper aqueous layer was removed then to a new 1.5 ml tube being careful not to pipette near the dirty layer. 1000 µl of cold 100% ethanol was added and mixed by inverting. Then the samples were centrifuged for 12 minutes at maximum speed (13,200 rpm), a small pellet was visible. Later the supernatant was decanted carefully by pouring into a beaker. The DNA pellet was washed with cold 70% ethanol and allowed to air dry until they were completely dried using a concentrator for maximum 10 min. The DNA pellets were re-suspended into 100 µl of 10×TE buffer, and was dissolved the pellet. After the pellet was dissolved, it was stored at -20 ^{o}C. DNA quality were assessed on a 0.80% agarose gel at 120 volt for one and half an hour stained with ethium bromide andvisualization under UV light using Gel Doc system (Biometra, Japan). The qauntity of DNA concentration was measured by NanoDrop Spectrophotometer (Thermo Scientific NanoDrop^{TM} 1000) by taking UV absorbance at 260 nm.

SSR-PCR analysis

Twenty primers were used for survey with 27 randomly selected genotypes (Table 1). Among them, 5 polymorphic micro satellite markers were selected based on intensity of bands, PCR product size (bp), annealing temperature, presence of smearing and potential of population discrimination, to evaluate the collected 65 brinjal genotypes for molecular characterization. The polymerase Chain Reaction (PCR) mixture contained 2 µl genomic DNA, Primer forward 1.00 µl, Primer reverse 1.00 µl, 10x buffer 1.00 µl, dNTPs 1.00 µl, $MgCl_2$ 0.60 µl, Taq polymerase 0.20 µl, ddH_2O 3.20 µl in a total volume of 10 µl. PCR amplification was performed using Biometra T3 thermal cycler. The amplification conditions involved initial denaturation of 5 min at 94 ^{o}C, followed by 35 cycles of 30 sec at 94^{o}C, 1 min at 65^{o}C and 2 min at 72^{o}C, with a final extension at 72^{o}C for 7 min. The PCR product were separated on 8% polyacrylamide gel in 1x TBE buffer with 90 volts and 500 mA for 1.5-2 h and visualized under ultraviolet light (Whatman Biometra gel Documentation System, prod nr: 1603209) after staining in ethidium bromide and capture image of each gel and saved in a computer as a JPEG file. The 100 bp DNA ladder plus molecular weight was used to compare the molecular weight of amplified product (Fig. 1-4). The six SSR primers were previously selected for assortment of genetic diversity of brinjal genotypes.

Data analysis

The size (in nucleotide base pairs) of the amplified band for each microsatellite marker was determined based on its migration relative to molecular weight size marker (100 bp DNA Ladder) with the help of Alpha VIEW (Version 3.2.8) software. Data of each SSR locus, the number of alleles, allele size, major frequency allele, genetic diversity and polymorphism information content (PIC) ware determined using the POWER MARKER version 3.23 (Liu and Muse, 2005). The score were obtained in the form of matrix with '1' and '0', which indicate presence and absence of bands in each variety, respectively. This observation was further analysed with NTSYS-pc version 2.2 (Rohlf, 2000). NTSYS-pc was used to construct a UPGMA (Unweighted Pair Group method with Arithmetic Mean Averages) dendogram showing the distance based on interrelationship among the genotypes.

Result and Discussion

Selection of primers

Primers were selected on the basis of band resolution, intensity, presence of smearing, consistency within individuals and potential for population discrimination. All the selected primers (EM107, EM114, EM133, EM139, EM140 and EM145) showed clear polymorphism which were used for further analysis. The properties of the selected primers are furnished in Table 2.

Overall allelic diversity

Among the six SSR motifs used in the present study five SSR motifs were clearly polymorphic, and produced varying number of alleles with different size ranges (Table 3). A total of 26 alleles were detected among the 65 brinjal genotypes. The number of allele locus^{-1} ranged from 3.0 to 6.0 with an average of 4.33 alleles locus^{-1}. The primers EM107 and EM114 had the highest number of alleles (6) and EM139 and EM145 had the lowest number of alleles (3). However EM140 had high genetic diversity (0.7730), while EM133 had lowest genetic diversity (0.5960) with a mean diversity of (0.6746) (Table 3). The overall size of amplified products ranged from 100 bp in locus EM107 to 340 bp in locus EM139. On an average, 41.03% of the 65 brinjal genotypes shared a common major allele ranging from 30.77% (EM107) to 46.15% (EM114) common allele at each locus (Table 3). These results revealed that markers EM140 (0.7730) would be the best in screening the collected brinjal genotypes followed by the markers EM107 (0.7115), EM139 (0.6627), EM114 (0.6551), EM145 (0.6495) and EM133 (0.5960).

PIC (Polymorphism Information Content)

The PIC value is a reflection of allele diversity and frequency among genotypes. PIC value of each marker can be evaluated on the basis of alleles. In the present investigation, PIC varied markedly for all the tested SSR loci. Polymorphism among the 65 studied genotypes was evaluated by calculating PIC values for each of the six SSR loci, which

ranged from 0.5120 (EM133) to 0.7387 (EM140) with an average of 0.6128 locus^{-1} (Table 3). Reports on molecular characterization of available brinjal genotypes using SSR markers are meager in Bangladesh. However, Demir *et al*. (2010) conducted molecular characterization of eggplant genotypes collected from different geographical regions in Turkey using both SSR and RAPD markers. They found that with amplification of five SSR loci, the number of alleles per microsatellite locus ranged from 2 to 10, with a total of 24 alleles. The greatest number of alleles was found at the emf21H22 locus (10 alleles) followed by emh11O01 (5 alleles) and emf21C11 (4 alleles). The average number of alleles locus^{-1} was 4.8. Adeniji *et al.* (2012) also reported that the proportion of polymorphic alleles for EM 114 and 145, and these SSR markers are adequate for detecting genetic diversity among seven *Solanum* species.

Pair-wise genetic dissimilarity

A dissimilarity matrix was used to determine the level of relatedness among the 65 genotypes studied. The pair-wise genetic dissimilarity co-efficient was measured among the test entries. The values of pair-wise comparison of Nei's (1973) genetic distance (D) between plants were computed from combine data for the 6 selected primers ranged from 0.17 to 1.00. The pair-wise genetic dissimilarity indices indicated that the highest genetic dissimilarity (100%) was observed among various combinations of the genotypes. For instance, the highest similarity was observed among Islampuri (S31), Eye-red (S36), Deembegun (S37), Comilla-L (S38) and Chega (S39) with Dohazari G (S10), Borka (S11), Khatkhatia BAU (S13), ISD-006 (S2) and Laffa-M (S3). The highest genetic dissimilarity also existed among Marich Begun-E (S58) with ISD-006 (S2), Shingnath-S (S29), Longla Talbegun (S30) and Islampuri (S31). The lowest genetic dissimilarity (17%) among the collected brinjal genotypes was observed as: Dohazari G (S10) and Borka (S11) with Zhumki (S1), Khatkhatia-B (S12) and Khatkhatia-BAU (S13). Similarly, Kaikka-G (S15) and Islampuri-BADC (S16) with Dohazari G(S10), Borka (S11), Khatkhatia B (S12), Khatkhatia BAU (S13) and Kaikka-N (S14) and Jessore L(S17). There were a number of combinations of genotypes where no genetic dissimilarity (0%) existed such as: Borka (S11) with Dohazari G (S10); Khatkhatia BAU(S13) with Khatkhatia B (S12); Islampuri, BADC (S16) with Kaikka-G (S15); Jessore L(S17) with Kaikka-N (S14); China oblong (S24) Ishurdi-WS (S25) and Putabegun (S27); Pahuza-2 (S50) and Magura Local (S51) and Long Lived High Plant (S52), Bholanath (S46), BAU Begun-1 (S47) and Pahuza-1 (S49); Purple Long (S53) with Laffa BAU (S55) and Katabegun WS (S56); Natore Local (Round) (S61) with Marich Begun-E (S58). Similar research was conducted by Khorsheduzzaman *et al.* (2008) with very small number of genotypes (5) using SSR markers. Behera *et. al*. (2006) found broader genetic diversity in 92 South Asian brinjal accessions (genetic similarity between 0.37 and 0.90) using microsatellite markers. The genotypes showed considerable variation in respect of morphological, anatomical and biochemical aspects. For study of relatedness, plant genomic DNA was extracted using 11 randomly selected primers which developed 22 bands through PCR amplification, and out of which 15 from 3 primers were

polymorphic. The similarity value ranged from 0.83 to 1.00 which indicated the presence of narrow range of genetic diversity at molecular level but have still a possibility of crossing among the genotypes of two clusters.

Construction of phylogenetic tree

A phylogenetic tree was constructed based on the Nei's genetic distance using 6 SSR markers among 65 brinjal genotypes (Nei, 1973). This is also called UPGMA cluster dendrogram. UPGMA based dendrogram was obtained from DNA profiles of the analyzed 65 brinjal genotypes (Fig 5). The UPGMA cluster based on the Nei'sgenetic distance led to the grouping of the collected 65 brinjal genotypes into three major clusters with nine sub-clusters. The Cluster-I led to three Sub-clusters. The Sub-cluster-I comprised 4 genotypes, namely S63, S65, S62 and S64. The Sub-cluster-II led to 13 genotypes, namely S48, S56, S53, S55, S51, S52, S46, S47, S49, S50, S60, S58 and S61, and the Sub-Cluster-III comprised 3 genotypes, namely S54, S57 and S59. Altogether, Cluster-I consisted of 20 out of 65 genotypes (Table 4). The Cluster-II led to three Sub-clusters. Sub-Cluster-I comprised 5 genotypes, namely S6, S8, S2, S12 and S13. The Sub-Cluster-II under Cluster-II consisted of 8 genotypes, namely S1, S9, S10, S11, S3, S4, S5 and S7. The Sub-Cluster-III accommodated 6 genotypes such as S14, S17, S15, S16, S18 and S19. Altogether, Cluster-II comprised 19 out of 65 genotypes (Table 4). The Cluster-III led to three Sub-clusters. The Sub-cluster-I comprised 10 genotypes (S43, S44, S45, S42, S40, S41, S36, S37, S38 and S39). The Sub-cluster II consisted of 11 genotypes, namely S28, S29, S30, S24, S25, S27, S21, S23, S20, S22 and S26. On the other hand, the Sub-cluster III consisted of 5 genotypes (S31, S32, S34, S33 and S35). Altogether, the Cluster-III consisted of 26 genotypes (Table 4). The tolerant and moderately tolerant genotypes were observed to be located across all three clusters. For example, the Cluster-I comprised 5 tolerant to moderately tolerant genotypes (S8, S9, S11, S13 and S17). Cluster-II consisted of 9 tolerant to moderately tolerant genotypes (S26, S43, S22, S23, S24, S30, S35, S38, S39) and the Cluster-III contained 8 tolerant and moderately tolerant genotypes (namely S57, S47, S55, S58, S61, S62, S63 and S65) (Table 4). In a similar study with 5 genotypes a dendrogram generated two clusters and they were clearly distinct and separated from each other (Khorsheduzzaman *et al.* 2008).

Table 1. List of SSR markers used in molecular characterization of 65 brinjal genotypes

Sl. No	Name of the primers	Primer sequence (5′-3′)	Repeat motif	Motif length	Annealing Temperature (^{0}C)	Product size (reference)	Expected product size
1	EM 107	F- GGC CCT AGA CTG AGC CTG AAATGT T	$(AC)_{13}$ $(AT)_{13}$	25	65	214	100-240
		R-TGG TAC AAC CAA CAC AAC CCT CAA		24			
2	EM 114	F-AGC CTA AAC TTG GTT GGT TTT TGC	$(AC)_{13}$	24	65	221	159-250
		R-GAA GCT TTA AGA GCC TTC TAT GCA G		25			
3	EM 116	F-TTA GAA ATT TGC GAA CAA AGA GA	$(AC)_{12}$ $(AT)_{8}$	23	65	246	150-230
		R-CCA CAT GAA ACT TGG ACC AAT GAG		24			
4	EM 117	F-GAT CAT CAC TGG TTT GGG CTA CAA	$(AC)_{19}$ $(AT)_{11}$	24	65	160	120-220
		R-AGG GGA GAG GAA ACT TGATTG GAC		24			
5	EM 119	F-CCC CAC CCC ATT TGT GTT ATG TT	$(GGAGG)_5(AT)_8$	23	65	210	100-210
		R-ACC CGA GAG CTA TGG AGT GTT CTG		24			
6	EM 120a	F-GGA TCA ACT GAA GAG CTG GTG GTT	$(AC)_{16}$	24	65	160	100-218
		R-CAG AGC TTC AAT GTT CCA TTT CAC A		25			
7	EM 120b	F-CAA AAG ATA AAA AGC TGC CGC ATG	$(AC)_{16}$	24	65	248	80-240
		R-CATGCG TGA GTT TTG GAG AGA GAG		24			
8	EM 127	F-CAG ACA CAA TGC TGA GCC AAA AT	$(AC)_{13}$ $(AT)_{13}$	23	65	200	150-230
		R-CGG TTT AAT CAT AGC GGT GAC CTT		24			
9	EM 128	F- TAG CGG TGC TAG GTC CAT CAT CTC A	$(CA)_{26}$ $(TA)_{19}$	25	60	295	100-230
		R-TTC TCA AGA AGT TGC TCC AAA GGA		24			
10	EM 131	F-TCT GCA ACA CCA AGT GAA AAA TCA	$(AT)_5(AC)_3A(AC)_{14}$	24	65	213	120-220
		R-TGC GTT TTT GGC TCC TCT ATG AAT	$(AT)_7GTA(TG)_5(TA)_3$	24			
11	EM 133	F-GCG GAT CAC CTG CAGTTA CAT TAC	$(AC)_{13}$ $(AT)_6$	24	65	177	120-220
		R-TCC TTT GAC CTA TAG TGG CAC GTA GT		26			
12	EM 134	F-AGT AAG GGA AAG TGC TGA CGA AGG	$(GT)_2GC(GT)_6$	24	65	168	120-300
		R-CAG AGT CAT CGT TAT GGG GAG GTT		24			
13	EM 135	F-ATC CTG TTG CTGCTC ATT TTC CTC	$(CA)_{11}(GA)_{20}$		65		256-260
		R-AGG AGG ATC CAAAGA GGT TTGTTGA					
14	EM 139	F-TGC TAA GTC GTC ATC CAACAA GAA	$(AC)_3AT(AC)_{11}(AT)_{10}$	24	65	258	130-340
		R-GAT TTT GGC TCC TTG ACC ATT TTG		24			
15	EM 140	F-CCA AAA CAA TTT CCA GTG ACT GTG C	$(AC)_4GC$ $(AC)_5T(AC)_3$	25	65	268	150-300
		R-GAC CAG AAT GCC CCT CAA ATT AAA	$ATGC(AC)_4AT(AC)_6$ $(AT)_6G(TA)_{13}$	24			
16	EM 141	F-TCT GCA TCG AAT GTC TAC ACC AAA	$(AT)_{16}(GT)_{19}$	24	65	228	100-260
		R-AAA AGC GCT TGC ACT ACA CCT GAA T		25			
17	EM 145	F-TGA TTT GGC CCT TAA GCC TAA GTA TG	$(TG)_3TA(TG)_8(TA)_8$	26	65	165	145-220
		R-GAC TCC TCA AGC CTT TAC CTC CAA		24			
18	EM 146	F-GGA CCA AAG CGA AAT TTT CAC AAC	$(AC)_{19}(AT)_{11}AC(AT)_2$	24	63	288	120-350
		R-TTG CAC CAATTG GGA AGT AAC ACA		24			
19	EM 155	F-CAA AAG ATA AAA AGC TGC CGG ATG	$(CT)_{38}$		65	248	256-260
		R-CAT GCG TGA GTT TTG GAG AGA GAG					
20	EM 104a	F-TGG ATC GTC GTC ATC CAACAA GAA	$(TC)_9(AC)_{38}(AT)_{19}$	25	60	246	230-350
		R-GAT TTT GGC TCC TTG ACC ATT TTG		23			

Table 2. Properties of the six selected primers

Sl. No.	Name of primers	Primer sequence (5′-3′)	Repeat motif	Motif length	Annealing Temperature (^{o}C)	Product size (reference)	Expected product size
1	EM 107	F- GGC CCT AGA CTG AGC CTG AAATGT T R-TGG TAC AAC CAA CAC AAC CCT CAA	$(AC)_{13}$ $(AT)_{13}$	25 24	65	214	100-240
2	EM 114	F-AGC CTA AAC TTG GTT GGT TTT TGC R-GAA GCT TTA AGA GCC TTC TAT GCA G	$(AC)_{13}$	24 25	65	221	159-250
3	EM 133	F-GCG GAT CAC CTG CAGTTA CAT TAC R-TCC TTT GAC CTA TAG TGG CAC GTA GT	$(AC)_{13}$ $(AT)_{6}$	24 26	65	177	120-220
4	EM 139	F-TGC TAA GTC GTC ATC CAACAA GAA R-GAT TTT GGC TCC TTG ACC ATT TTG	$(AC)_3AT(AC)_{11}(AT)_{10}$	24 24	65	258	130-340
5	EM 140	F-CCA AAA CAA TTT CCA GTG ACT GTG C R-GAC CAG AAT GCC CCT CAA ATT AAA	$(AC)_4GC$ $(AC)_5T(AC)_3$ $ATGC(AC)_4AT(AC)_6$ $(AT)_6G(TA)_{13}$	25 24	65	268	150-300
6	EM 145	F-TGA TTT GGC CCT TAA GCC TAA GTA TG R-GAC TCC TCA AGC CTT TAC CTC CAA	$(TG)_3TA(TG)_8(TA)_8$	26 24	65	165	145-220

Table 3. Name of the marker, sample size, allele no., major allele frequency, genetic diversity and polymorphism information content (PIC) for six markers

Name of the marker	Sample size	Allele No.	Major allele frequency	Genetic diversity	PIC
EM107	65.0	6.0	0.4308	0.7115	0.6688
EM114	65.0	4.0	0.4615	0.6551	0.5931
EM133	65.0	4.0	0.4462	0.5960	0.5120
EM139	65.0	3.0	0.3846	0.6627	0.5888
EM140	65.0	6.0	0.3077	0.7730	0.7387
EM145	65.0	3.0	0.4308	0.6495	0.5756
Mean	65.0	4.33	0.4103	0.6746	0.6128

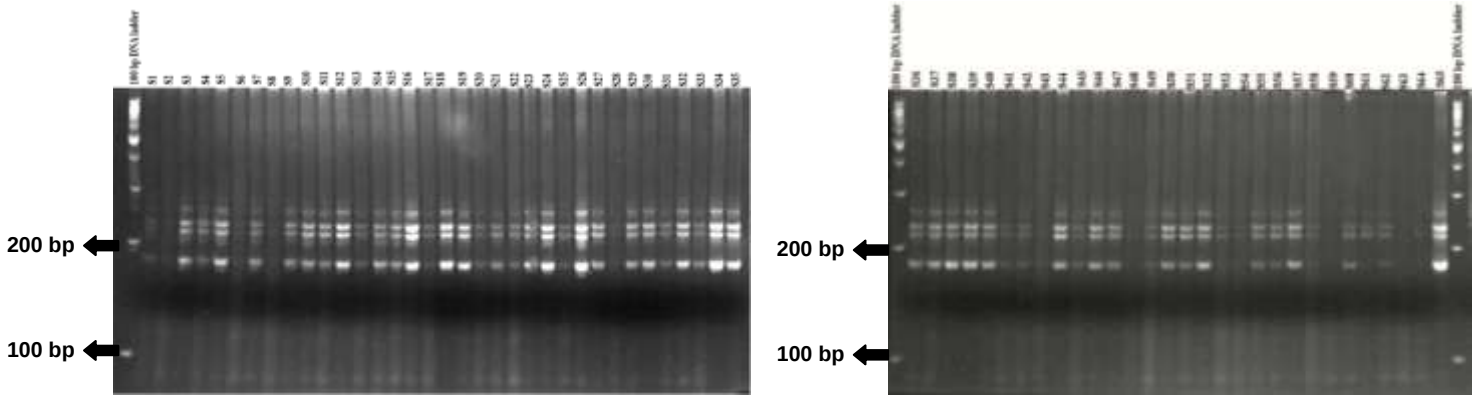

Fig. 1. DNA profile of the 65 brinjal genotypes (A-35 and B-30) using the primer EM107.

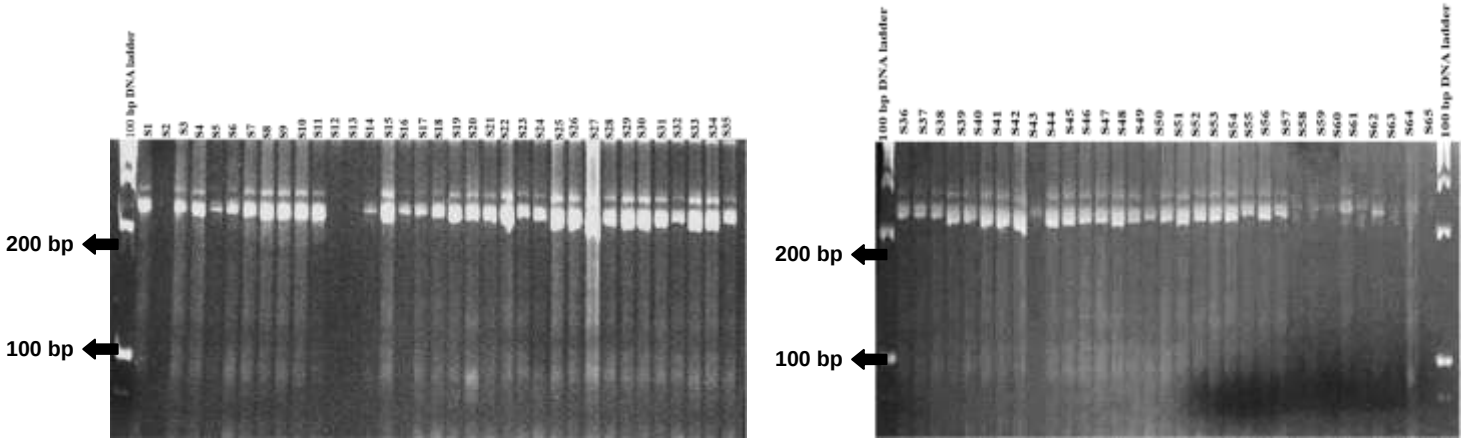

Fig. 2. DNA profile of the 65 brinjal genotypes (A- 35 and B-30) using the primer EM114.

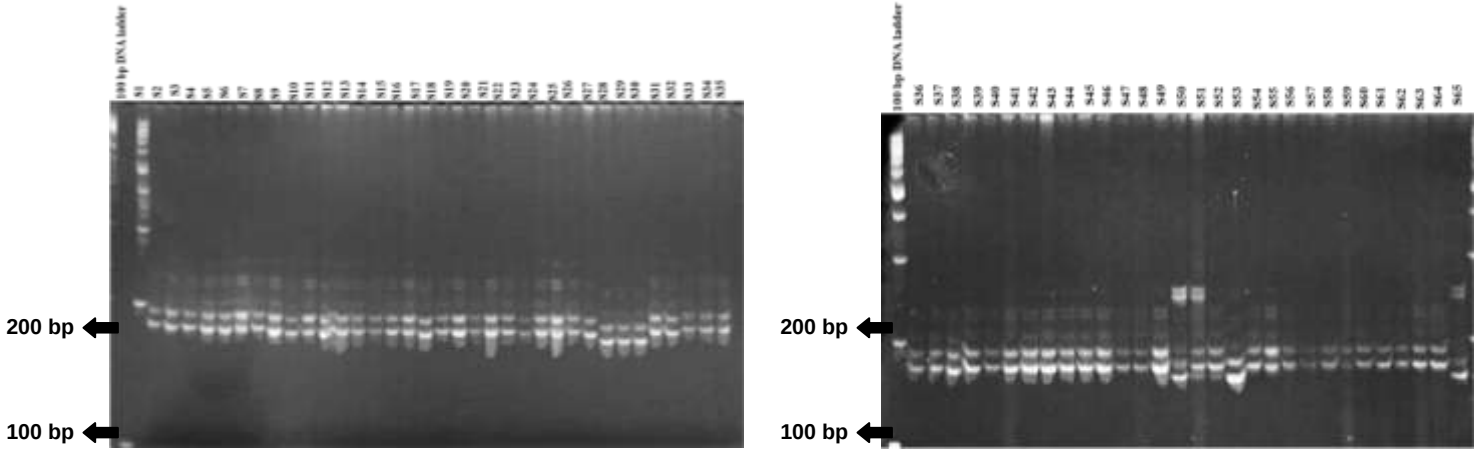

Fig. 3. DNA profile of the 65 brinjal genotypes (A- 35 and B-30) using the primer EM133.

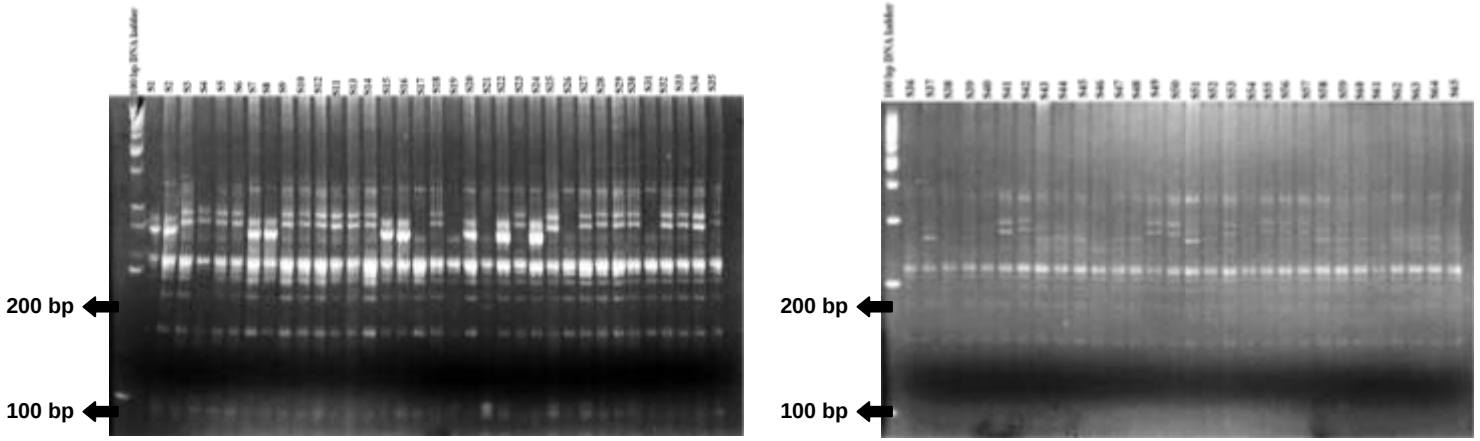

Fig. 4. DNA profile of the 65 brinjal genotypes (A- 35 and B- 30) using the primer EM140.

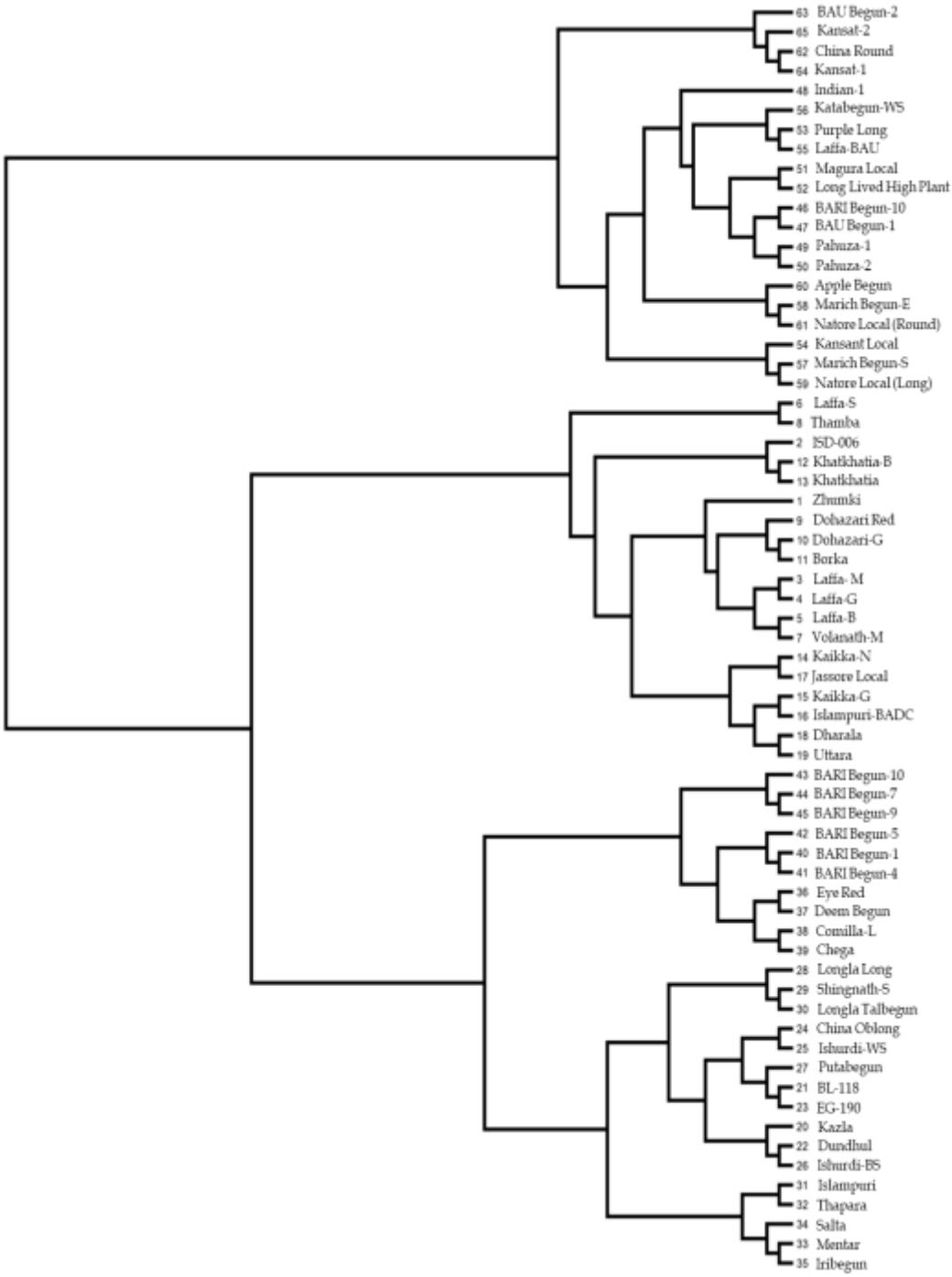

Fig. 5. UPGMA cluster dendrogram obtained from DNA profiles of 65 brinjal genotypes.

Table 4. Clusters and sub-clusters of the 65 brinjal genotypes based on the Nei's (1973) genetic distance using 6 SSR primers

Cluster	Sub-Cluster	Genotypes
I	I	S63, S65, S62, S64
	II	S48, S56, S53, S55, S51, S52, S46, S47, S49, S50, S60, S58, S61
	III	S54, S57, S59
II	I	S6, S8, S2, S12, S13
	II	S1, S9, S10, S11, S3, S4, S5, S7
	III	S14, S17, S15, S16, S18, S19
III	I	S43, S44, S45, S42, S40, S41, S36, S37, S38, S39
	II	S28, S29, S30, S24, S25, S27, S21, S23, S20, S22, S26
	III	S31, S32, S34, S33, S35

Conclusion

The present investigation provided new information about the genetic diversity and relationship among brinjal (*Solanum*) genotypes using microsatellite markers. To perform molecular characterization using SSR markers for identifying genetic diversity and relatedness among the genotypes at molecular level. Molecular analysis using SSR markers led to the grouping of the 65 genotypes into 3 major clusters with 9 sub-clusters. The relatedness among the 65 genotypes was also estimated.

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