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Molecular characterization of some brinjal genotypes (*Solanum melongena* L) using simple sequence repeat (SSR) markers

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To analyze genetic variability of 48 brinjal genotypes by Simple Sequence Repeat (SSR) markers, four primers named smSSR01, smSSR03, smSSR11 and smSSR14 were selected for analyze the data. Four primers generated clear bands and a total of 44 alleles were detected among the 48 brinjal genotypes (9.0 to 14.0 alleles per locus with a mean of 11.0 alleles per locus). According to Nei's (1973), the highest level of gene diversity value 0.8672 was observed in loci smSSR11 and the lowest level of gene diversity value 0.6580 was observed in the loci smSSR14 with a mean diversity of 0.7847. As a measure of the informativeness of microsatellites, the PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660. The genetic distance (GD) between genotypes was computed from combined data for the four primers, ranging from 0.250 to 1.000. The highest genetic distance (1.000) was observed between Morich Begun (Small) vs. Laffa (Long, Violet), Morich Begun (Small) vs. BARI Begun -1 and many other genotype pairs and also the lowest genetic distance (0.250) was found in Morich Begun (Small) vs. Pahoza-2, Morich Begun (Small) vs. BAU Begun-1, Singnath (Long, Violet) vs. Laffa BAU (Long, Violet), Borka (Long, Green) vs. Purple Long and many other genotype pairs. The Unweighed Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis lead to the grouping of the 48 genotypes into seven major clusters and the genotypes that are derivatives of genetically similar type form cluster together. Maximum brinjal genotypes include cluster IV. The SSR markers used in the present study were able to differentiate forty eight brinjal genotypes genetically. The results of the study suggested that SSR can be used as a suitable genetic marker to identify the brinjal genotypes. However, further studies involving large number of genotypes and primers need to be conducted to get more precise information and help enhance the knowledge of students and researchers relating to molecular characterization of other solanaceious crops.

Key words: Brinjal, SSR, Genetic Diversity

INTRODUCTION

Brinjal (*Solanum melongena* L.), also known as eggplant or Aubergine (French name) is one of the most important, inexpensive and popular vegetable crops grown in Bangladesh.

Brinjal is grown on 17,28,271 ha with a total production of 4,31,73,989 tons (FAO, 2012). Asia has the largest

eggplant production which comprises more than 90% of the world production area and 87% of the world production. It is cultivated and available year-round in Bangladesh with annual production areas of 124,214 ha and the total production was 450,136 tons in the year 2014-2015 (BBS, 2016).

As a popular vegetable in Bangladesh brinjal has a great potentiality. But due to limitation of land it is not possible to increase the area of the crop. Brinjal exhibits extensive variation in morphological and biochemical traits (Arivalagan et al., 2013). The study of genetic diversity and relationships of collections of local genotypes provides information of relevance for the breeding programmes. DNA marker technology and molecular characterization are immensely helpful in selective breeding from diverse parents to widen the breeding gene pool (Fu, 2006). Several molecular studies (Cericola et al., 2013; Prohens et al., 2005; Tumbilen et al., 2011) have shown that eggplant cultivar groups are genetically diverse. SSR markers indicated a strong genetic affinity of eggplant (*Solanum viarum, Solanum melongena* and *Solanum aethiopicum*; *Aculeatum* group) and also assayed informative for the potential to serve as perfect markers for studying variation (Adeniji et al., 2012). Genetic diversity and relatedness may be informative for the varietal identification and genetic improvement of brinjal (Sultana et al., 2018).

However, despite its widespread cultivation and nutritional and economic importance, the eggplant genome has not yet been extensively evaluated as for the other solanaceous vegetables such as tomato, potato and pepper, all of which have high density linkage maps (Barchi et al., 2007; Jacobs et al., 2004; Tanksley et al., 1992). Morphological, molecular and combined trait analyses consistently recognized the main groups of eggplants (cultivars and land races). It also exhibited higher variation compared to the landraces and cultivars. For eggplants landraces, morphological variability was moderately high but low diversity was observed on SSRs and combined data analyses (Caguiat and Hautea, 2014).

High degree of diversity of brinjal cultivars may be attributable to genetic improvement programme based on the molecular clustering patterns. It also provides support for selection of crossing combinations from parental genotypes and for broadening the genetic basis of breeding programs. Due to having some medicinal values particularly against cancer, diabetes and cardiovascular disease, marker assisted breeding and production of eggplant may contribute to enrich diets and bring health benefits (Sultana et al., 2018). The use of molecular markers in eggplant breeding has been limited compared to other relevant crops of the same family (Barone et al., 2009; Jo et al., 2010; Danan et al., 2006). A number of SSR markers have been identified in Solanaceae (Yi et al., 2006; Bindler et al., 2007), but the numbers are less in eggplant. Portis et al. (2018) developed an “Eggplant Microsatellite DataBase” (*EgMiDB*) which permits identification of SSR markers in terms of their location on the genome, type of repeat (perfect vs. imperfect), motif type, sequence, repeat number and genomic/gene context. It also suggests forward and reverse primers and also employed an *in silico* PCR analysis to validate these SSR markers, using as templates two CDS sets and three assembled transcriptomes obtained from diverse eggplant accessions.

The aim of the present study was to characterize eggplant genotypes collected from different geographical regions of Bangladesh using SSR markers and to assess the genetic diversity within these genotypes. Assessment of genetic diversity is important for breeding purposes, and the utilization of molecular markers helps accelerate the evaluation process. Therefore, the present study was conducted at molecular level with the following objectives: (i) to estimate the level of genetic diversity of brinjal genotypes by means of SSR markers; and (ii) to estimate the relationship among some brinjal genotypes of particular location or geographic origin.

MATERIALS AND METHODS

Forty eight brinjal genotypes were used in the study. In order to carryout SSR analysis, young leaves from each of the forty eight genotypes were collected randomly, which were used as the source of genomic DNA (Table 1).

After preparation of lands the seeds of brinjal were sown in lines to get uniform and healthy seedlings during July 2016. The seeds of Indian genotype, local exotic Zumka and Islampuri brinjal were exposed to gamma irradiation (^{60}Co) at different doses. The M_1 and M_2 generations were grown in two consecutive years and then selected mutants as $IndD_{150}L_8P_4$, $IndD_{150}L_1P_{27}$, $IndD_{300}L_{13}P_{11}$, $IndD_{300}L_{12}P_{12}$, $ZumD_{150}L_1P_{22}$, $ZumD_{150}L_{13}P_{21}$, $ZumD_{300}L_{12}P_5$, $ZumD_{300}L_{13}P_{12}$, $IslD_{150}L_{13}P_{22}$, $IslD_{150}L_{13}P_{14}$, $IslD_{300}L_5P_3$, $IslD_{150}L_{14}P_{23}$, $IslD_{150}L_2P_{25}$ and $IslD_{300}L_{12}P_{19}$. The M_3 populations were used for molecular characterization using SSR markers. For genomic DNA the seedlings were grown in the field of Horticulture Division, Bangladesh Institute of Nuclear Agriculture, Bangladesh Agricultural University Campus, Mymensingh. The uniform, viable, healthy, disease and insect free seeds of 48 brinjal genotypes were used for growing seedlings. Young, vigorously growing fresh leaf samples were collected for the SSR analysis from 20 days old seedling of each of the germplasm. Modified CTAB mini-prep method was followed to extract DNA from leaf samples of brinjal. PCR reactions were followed by Adeniji et al. (2012). After completion of PCR, the gel image (agarose and PAGE) resolution was adjusted using the camera setting. The gel was exposed to UV light and save the gel image as a tiff or jpeg file. Five primers of Simple Sequence

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Table 1. Accession number of 48 brinjal genotypes.

S/N	Name of genotype	S/N	Name of genotype
01	Morich Begun (Small)	25	$IndD_{300}L_{13}P_{11}$
02	$IndD_{150}L_{8}P_{4}$	26	Purple Long
03	BARI Begun -1	27	$IndD_{300}L_{12}P_{12}$
04	Thama	28	Kaikka-N (Violet)
05	BARI Begun-7	29	BAU Begun-1
06	Thapa	30	$ZumD_{150}L_{1}P_{22}$
07	Indian-1	31	Darata (Round, Green)
08	Morich Begun	32	Dohazari (Long, Violet)
09	Khatkhatia Long (Green)	33	Laffa Long Violet-G
10	Uttara (Violet)	34	$ZumD_{150}L_{13}P_{21}$
11	Singnath (Long, Violet)	35	$ZumD_{300}L_{12}P_{5}$
12	Borka (Long, Green)	36	$ZumD_{300}L_{13}P_{12}$
13	China Long	37	$IslD_{150}L_{13}P_{22}$
14	Laffa (Long, Violet)	38	$IslD_{150}L_{13}P_{14}$
15	Kailkkah (Long, Green)	39	Dohazari (Green)
16	Laffa BAU (Long, Violet)	40	Pahoza-1
17	Islampuri BS	41	Salta
18	Pahoza-2	42	Apple Begun (Round, Violet)
19	Puta Begun	43	$IslD_{300}L_{5}P_{3}$
20	Longla Tal Begun	44	$IslD_{150}L_{14}P_{23}$
21	Irri Begun (Round, Green)	45	Jessore Local
22	Dhondol Begun (Long, Violet)	46	$IslD_{150}L_{2}P_{25}$
23	Khatkhetia Long (Violet)	47	Zumka
24	$IndD_{150}L_{1}P_{27}$	48	$IslD_{300}L_{12}P_{19}$

Table 2. Summary of microsatellite markers used for diversity study.

Primer name	Sequence		Product size (bp)	Annealing temperature (°C)
smSSR01	For. Rev.	GTGACTACGGTTTCACTGGT GATGACGACGACGATAATAGA	310	54.8
smSSR03	For. Rev.	ATTGAAAGTTGCTCTGCTTC GATCGAACCCACATCATC	145	54.8
smSSR04	For. Rev.	CTCTGCTTCACCTCTGTGTT CCATGAAAGAGAAGATCGAG	320	54.8
smSSR11	For. Rev.	AAACAAACTGAAACCCATGT AAGTTTGCTGTTGCTGCT	126	54.8
smSSR14	For. Rev.	ATACCACATCAATCCAAAGC CATCATCATCTTCACAGTGG	241	54.8

Repeat were screened on a sub sample of five randomly chosen brinjal genotypes, to test their suitability for amplifying brinjal SSRs that could be accurately scored. To confirm the reproducibility of SSR markers, the selected primers were screened five times on the same sample. The details of the primers are shown in Table 2.

Molecular weight for each amplified alleles was measured in base pair using Alpha Ease PC 4.0 software. The allele frequency data from Power Marker Version 3.25 (Liu and Muse, 2005) was used to export the data in binary format (allele presence=1 and allele absence=0) for analysis with NTSYS-PC Version 2.2 (Rohlf, 2002).

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, and Polymorphism Information Content (PIC) values were determining using Power Marker Version 3.25 (Liu and Muse, 2005). Alleles frequencies were calculated directly from the observed genotypes. Allelic variations and fit to Hardy-Weinberg proportions were estimated by the software POPGENE version 1.31 (Yeh et al., 1999). Expected (*He*) and observed heterozygosity (*Ho*) were also calculated (Nei, 1973) using the following formula and with the help of POPGENE version 1.31 (Yeh et al., 1999) computer package program. For the unrooted polygenic tree, genetic distance was calculated using the "CS Chord 1967" distance, Cavalli-Sforza and Fdwards (1967) followed by phylogeny reconstruction using neighbor-joining as implemented in Power Marker with tree viewed using the Tree view. The allele frequency dated from Power Marker was used to export the data in binary format (allele presence = 1 and allele absence = 0) for analysis with NTSYS-PC Version 2.2 (Rohlf, 2002). A similarity matrix was calculated with Simqual Subprogram using the Dice coefficient, followed by cluster analysis with the SAHN Subprogram using the UPGMA clustering methods implemented in NTSYS-PC used to construct a dendogram showing relationship among the genotypes. The similarity matrix was also used for principal coordinate analysis (PC_OA) with Dcenter, Eigen, Output and MX plat subprogram in computer program Numerical Taxonomy and Multivariate analysis system (NTSYS-PC). Nei's (1973) genetic distance value was computed using the formula as described in the POPGENE (version 1.31) software using manual (Yeh et al., 1999).

RESULTS

The analysis of genetic diversity is very important for brinjal improvement that can be obtained through DNA fingerprinting techniques. In this study, 48 genotypes of brinjal were analyzed using 4 primer pairs (smSSR01, smSSR03, smSSR11 and smSSR14). Amplified microsatellite loci were analyzed for polymorphism using Polyacrylamide Gel Electrophoresis (PAGE) and the results revealed that all the primer pairs detected polymorphism among the brinjal genotypes analyzed. The microsatellite loci were also multi-allelic (9.0 to 14.0 alleles per locus with a mean of 11.0 alleles per locus in the present study) and the alleles were co-dominant suggesting their relative superiority in detecting DNA polymorphism over some other markers.

Using 4 SSR markers, a total of 44 alleles were detected among the 48 brinjal germplasm. The average number of allele per locus was 11, with a range of 9 (smSSR03) to as many as 14 (smSSR01) (Table 3).

Comparing microsatellite markers with the different repeat motifs, those with high number of AGC repeats has the highest genetic diversity 0.8672, while those with high number of ATT, AT/GA and ACCAA repeats had the lower number of genetic diversity (0.8472, 0.7665 and 0.6580, respectively). According to Nei's (1973), the highest level of gene diversity value 0.8672 was observed in loci smSSR11 and the lowest level of gene diversity value 0.6580 was observed in the loci smSSR14 with a mean diversity of 0.7847. The value of pair-wise comparisons of Nei's (1973) genetic distance (GD) between genotypes were computed from combined data for the four primers, ranging from 0.250 to 1.000. SSR markers with ATT motifs show the maximum variation in allele size. As a measure of the informativeness of microsatellites, the PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660 (Table 3). The means of genetic distances between genotypes were used to evaluate the genetic diversity of different brinjal genotypes.

The number of alleles, their size range and allele frequency of different brinjal germplasm are shown in Table 4.

From the difference between the highest and lowest genetic distance value it was revealed that there were wide variability's among 48 brinjal genotypes. High genetic variability within genotypes and significant difference between genotypes indicate rich genetic material of a species. This study indicated genotypes that showed the highest genetic variation can be used as parental source for breeding line to improve brinjal varieties.

A dendogram was constructed based on the Nei's genetic distance calculated from the 44 SSR alleles generated from the 48 brinjal genotypes (Figure 1). All 48 brinjal genotypes could be easily distinguished. The Unweighed Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis leads to the grouping of the 48 germplasm into seven major clusters, three genotypes Thama, Kaikka-N (Violet) and BARI Begun-1 formed cluster I. BARI Begun-7, Kailkkah (Long, Green), Khatkhatia Long (Green), $IslD_{300}L_{12}P_{19}$, Indian-1, Singnath (Long, Violet) and Laffa BAU (Long, Violet) were grouped in cluster II in which sub cluster-1 includes BARI Begun-7 and Kailkkah (Long, Green) and sub cluster-2 includes $IslD_{300}L_{12}P_{19}$, Indian-1, Singnath (Long, Violet) and Laffa BAU (Long, Violet). Cluster-III includes $IslD_{300}L_5P_3$ and Jessore Local brinjal genotypes. In cluster IV $ZumD_{150}L_{13}P_{21}$, $IndD_{150}L_8P_4$ and Darata (Round, Green) formed sub cluster-1; Thapa, Morich Begun, BAU Begun-1, $ZumD_{150}L_1P_{22}$, Puta Begun, Laffa Long Violet-G, Morich Begun (Small), Borka (Long, Green), Pahoza-2, Purple Long, $ZumD_{300}L_{13}P_{12}$ and Zumka formed sub cluster 2 and $IslD_{150}L_{14}P_{23,}$ $IslD_{150}L_{13}P_{22}$ and Apple Begun (Round, Violet). It is clearly observed that there is no genetic difference between Laffa Long Violet-G, Morich Begun (Small) and Borka (Long, Green). Cluster V includes Irri Begun (Round, Green), Laffa (Long, Violet) and Khatkhatia Long (Violet) genotypes. $IndD_{300}L_{12}P_{12}$, Dohazari (Long, Violet), $IslD_{150}L_{13}P_{14}$ and Dohazari (Green) formed cluster VI. In cluster VII, Dhondol Begun (Long, Violet), Laffa (Long, Violet), $ZumD_{300}L_{12}P_5$, Salta and China Long formed sub cluster-1; Pahoza-1, Islampuri BS, Longla Tal Begun, Uttara (Violet), $IndD_{150}L_1P_{27}$, $IndD_{300}L_{13}P_{11}$ and $IslD_{150}L_2P_{25}$ formed sub cluster-II. From this study, the dendrogram revealed that the genotypes that are derivatives of genetically similar type form cluster together. Maximum brinjal genotypes

Table 3. Data on the number of alleles, gene diversity, highest frequency allele and polymorphism information content (PIC).

Marker	Repeat motifs	Allele no.	Size range (bp)	Gene diversity	Highest frequency allele		PIC Value	Average PIC value
					Size (bp)	Frequency (%)		
smSSR01	(ATT)21	14	263-315	0.8472	293	14	0.8341	
smSSR03	(TA)9 (GA)8	09	144-156	0.7665	149	21	0.7350	
smSSR11	(AGC)6	10	124-133	0.8672	126	23	0.8536	0.7660
smSSR14	(ACCAA)3	11	187-268	0.6580	193	10	0.6413	
Mean	-	11	-	0.7847	-	-	0.7660	

Table 4. Size and frequency of alleles at 4 SSR loci of 48 brinjal genotypes.

Locus	Allele size (bp)	Allele frequency	Genotypes
smSSR 01	263	0.0208	Khatkhatia Long (Green)
	268	0.0208	Laffa (Long, Violet)
	269	0.0208	Indian-1
	286	0.0208	Kailkkah (Long, Green)
	288	0.0417	Uttara (Violet), IndD150L1P27
	290	0.0625	BARI Begun-7, Singnath (Long, Violet), Laffa BAU (Long, Violet)
	292	0.0625	Islampuri BS, IndD300L12P12, Dohazari (Green)
	293	0.1458	Thama, China Long, Dhondol Begun (Long, Violet), ZumD300L12P5, Pahoza-1, Salta, Apple Begun (Round, Violet)
	295	0.1042	ZumD150L1P22, Dohazari (Long, Violet), IslD150L13P14 IslD150L14P23, Jessore Local
	297	0.0208	BARI Begun-1
	298	0.0833	Irri Begun (Round, Green), IndD300L13P11, IslD150L13P22, IslD150L2P25
	300	0.0625	Longla Tal Begun, Kaikka-N (Violet), ZumD150L13P21
	315	0.0208	IslD300L12P19.
smSSR 03	144	0.0208	BARI Begun-7
	147	0.0417	IslD300L5P3, Jessore Local
	148	0.1875	Uttara (Violet), Irri Begun (Round, Green), Dhondol Begun (Long, Violet), Khatkhatia Long (Violet), IndD300L12P12, Dohazari (Long, Violet), ZumD300L12P5, IslD150L13P14, Dohazari (Green)
	149	0.2083	China Long, Laffa (Long, Violet), Islampuri BS, Islampuri BS, IndD150L1P27, IndD300L13P11, Kaikka-N (Violet), Pahoza-1, Salta , IslD150L2P25,
	151	0.0208	Laffa BAU (Long, Violet)
	152	0.1042	Indian-1, Khatkhatia Long (Green), Singnath (Long, Violet), Kailkkah (Long, Green), IslD300L12P19
	155	0.0208	BARI Begun -1
	156	0.0208	Thama
smSSR 11	124	0.0417	China Long, Pahoza-2

Table 4. Contd.

	125	0.1250	Laffa (Long, Violet), Islampuri BS, Longla Tal Begun, Irri Begun (Round, Green), Dhondol Begun (Long, Violet), Khatkhatia Long (Violet)
	126	0.2292	BARI Begun-7, Indian-1, Morich Begun, Khatkhatia Long (Green), Uttara (Violet), Singnath (Long, Violet), Kailkkah (Long, Green), Laffa BAU (Long, Violet), IndD150L1P27, IndD300L13P11, Purple Long
	127	0.1042	Thapa, IndD300L12 P12, BAU Begun-1, ZumD150L1P22, Darata (Round, Green)
	128	0.0833	BARI Begun -1, Thama, Kaikka-N (Violet), Dohazari (Long, Violet)
	129	0.1667	IndD150L8P4, ZumD150L13P21, ZumD300L12P5, ZumD300L13P12, IslD150L13P14, Dohazari (Green), Pahoza-1, Salta
	130	0.0417	IslD150L13P22, Apple Begun (Round, Violet)
	131	0.0417	IslD300L5P3, Jessore Local
	133	0.0833	IslD150L14P23, IslD150L2P25, Zumka, IslD300L12P19
smSSR 14	187	0.0417	Thama, BARI Begun-7
	189	0.0417	Thapa, Morich Begun
	190	0.0417	Khatkhatia Long (Green), Kailkkah (Long, Green)
	192	0.0208	Puta Begun
	193	0.1042	IndD150L8P4, BARI Begun -1, Irri Begun (Round, Green), Darata (Round, Green), ZumD150L13P21
	194	0.0625	Laffa (Long, Violet), Khatkhatia Long (Violet), Kaikka-N (Violet)
	258	0.0208	IslD150L13P14
	263	0.0208	Dohazari (Green)
	267	0.0208	Salta
	268	0.0625	Apple Begun (Round, Violet), IslD300L5P3, IslD150L14P23

include cluster IV.

DISCUSSION

The previous knowledge about the genetic relationships among breeding materials is documentary for the effective use of the genotypes in a breeding programme. Brinjal is an important vegetable in Bangladesh, but little information is available on genetic structure of brinjal genotypes. There are no specific markers found to remark different genotypes accurately, the SSR technique stated some degree of polymorphisms for investigating the genetic relationship among different brinjal genotypes.

Using 4 SSR markers, a total of 44 alleles were detected among the 48 brinjal genotypes. The average number of allele per locus was 11, with a range of 9 (smSSR03) to as many as 14 (smSSR01) which is supported by Vilanova et al. (2014), they found 2 to 11 alleles/locus by using 19 genomic SSRs for the molecular characterization of 30 eggplant accessions; the number of alleles ranged from 2 to 8 with a mean of 4.3 alleles per marker recorded by Caguiat and Hautea (2014), Adeniji et al. (2012) observed a total of 417 alleles amplified with the number of alleles ranging from 5 (EM 141) to 38 (EM 120 b), Verma et al. (2012) recorded the number of alleles per primer ranging from 2 to 10, with a mean of 4.67; Sunseri et al. (2010) found the number of alleles ranging from 2 to 7 with an average of 4.5. Nearly similar observation was found by Li et al. (2003) where they ranged from 2 alleles to 11 alleles with an average of 6.61 alleles per locus in chilli.

Comparing microsatellite markers with the different repeat motifs, those with high number of AGC repeats has the highest genetic diversity 0.8672, while those with high number of ATT, AT/GA and ACCAA repeats had the lower number of genetic diversity (0.8472, 0.7665 and 0.6580, respectively). This genetic diversity based on repeat motifs are compared from some previous analysis in brinjal such as Nunone et al. (2003a) who recorded that the AT repeat was the most common, representing 8.3% of the total SSRs

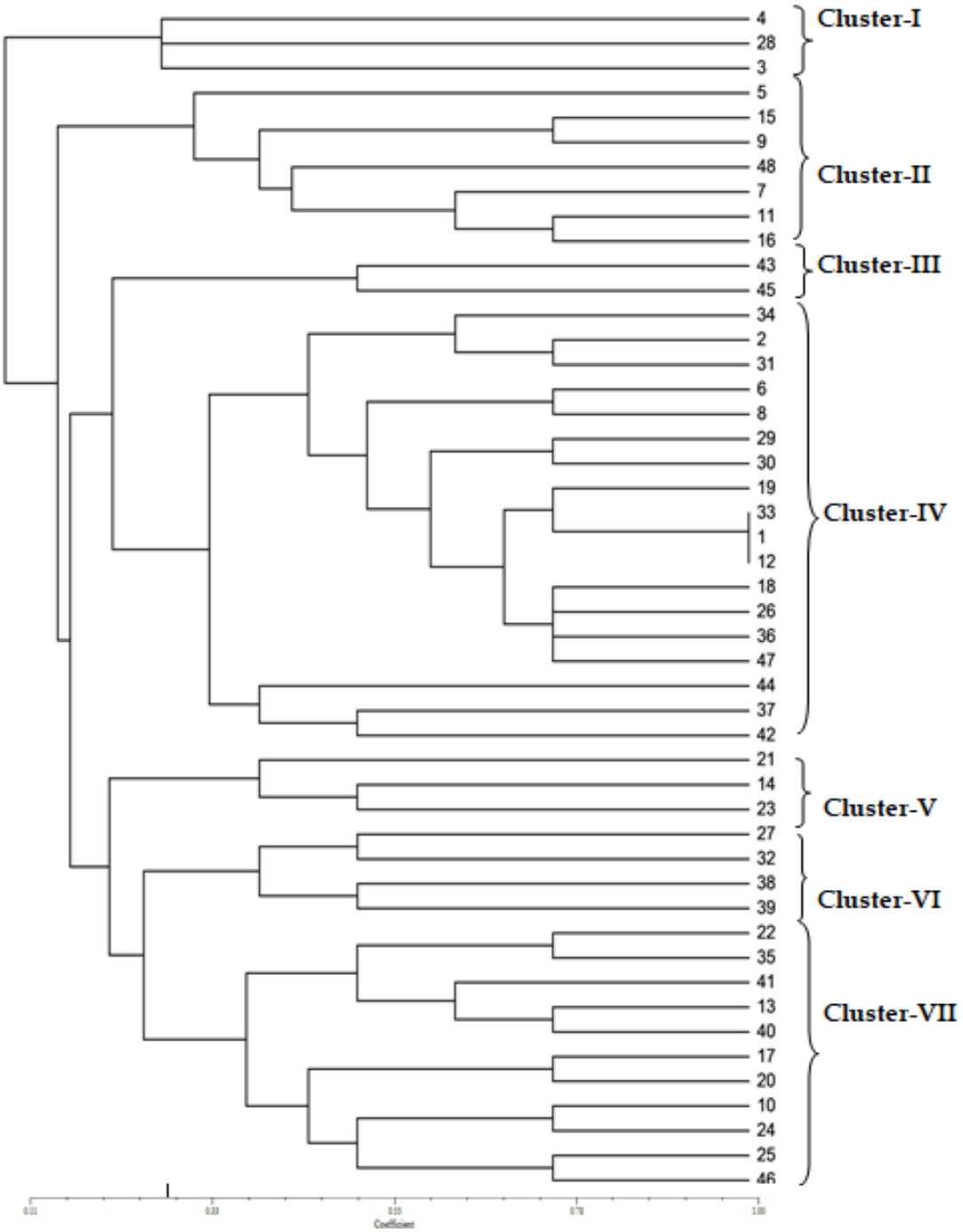

Figure 1. A UPGMA cluster dendrogram showing the genetic relationships among 48 brinjal genotypes detected by SSR markers. 01 = Morich Begun (Small), 02 = $IndD_{150}L_8P_4$, 03 = BARI Begun-1, 04 = Thama, 05 = BARI Begun-7, 06 = Thapa, 07 = Indian-1, 08 = Morich Begun, 09 = Khatkhatia Long (Green), 10 = Uttara (Violet), 11 = Singnath (Long, Violet), 12 = Borka (Long, Green), 13 = China Long, 14 = Laffa (Long, Violet), 15 = Kailkkah (Long, Green), 16 = Laffa BAU (Long, Violet), 17 = Islampuri BS, 18 = Pahoza-2, 19 = Puta Begun, 20 = Longla Tal Begun, 21 = Irri Begun (Round, Green), 22 = Dhondol Begun (Long, Violet), 23 = Khatkhatia Long (Violet), 24 = $IndD_{150}L_1P_{27}$, 25 = $IndD_{300}L_{13}P_{11}$, 26 = Purple Long, 27 = $IndD_{300}L_{12}P_{12}$, 28 = Kaikka-N (Violet), 29 = BAU Begun-1, 30 = $ZumD_{150}L_1P_{22}$, 31 = Darata (Round, Green), 32 = Dohazari (Long, Violet), 33 = Laffa Long Violet-G, 34 = $ZumD_{150}L_{13}P_{21}$, 35 = $ZumD_{300}L_{12}P_5$, 36 = $ZumD_{300}L_{13}P_{12}$, 37 = $IslD_{150}L_{13}P_{22}$, 38 = $IslD_{150}L_{13}P_{14}$, 39 = Dohazari (Green), 40 = Pahoza-1, 41 = Salta, 42 = Apple Begun (Round, Violet), 43 = $IslD_{300}L_5P_3$, 44 = $IslD_{150}L_{14}P_{23}$, 45 = Jessore Local, 46 = $IslD_{150}L_2P_{25}$, 47 = Zumka, 48 = $IslD_{300}L_{12}P_{19}$.

identified. The AT repeat has also been frequently identified in other genic and genomic SSRs in eggplant. Herrera et al. (2008) recorded SSR markers with ATT, GA, TAA and GTT motifs showed the maximum variation in allele size; James et al. (2000) observed that ATT motifs showed the maximum variation in allele size. Stagel et al. (2008) found that CCG/CGG and AGG/CCT are the most common monocotyledonous EST-SSR motifs (18,24,29) and were under-represented in dicotyledonous species as well as in the present dataset, which is less polymorphic than other motif. Adeniji et al. (2012) investigated polymorphism was fairly high (0.05 to 0.92) among SSR markers with high number of repeats.

According to Nei's (1973), the highest level of gene diversity value 0.8672 was observed in loci smSSR11 and the lowest level of gene diversity value 0.6580 was observed in the loci smSSR14 with a mean diversity of 0.7847. It was observed that there is minimum difference in loci smSSR11 and smSSR01 in terms of gene diversity. The maximum allele size range is 315 bp, which is in locus smSSR01 and showed the second highest genetic diversity. Considering the genetic diversity a number of SSR markers have been identified in Solanaceae (Yi et al., 2006; Bindler et al., 2007), but the numbers are less in eggplant. The development of SSR markers derived from SSR-enriched genomic library of eggplant has been reported by Nunome et al. (2003b, 2009). SSR markers have been used in determination of genetic diversity in eggplant (Nunome et al., 2003a, 2003b; Stagel et al., 2008; Nunome et al., 2009).

As a measure of the informativeness of microsatellites, the PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660 (Table 3). The results of the study supported by Vilanova et al. (2014), recorded the polymorphism information content (PIC) of SSR markers ranging from 0.07 to 0.77, with an average value of PIC=0.50. Also SSRs was selected by Caguiat and Hautea (2014) based on their high polymorphism information content (PIC) and the high quality of bands.

The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. Basically this value is an indication of their genetic dissimilarity. Genotype pair with higher value is more dissimilar than a pair with lower value. The value of pair-wise comparisons of Nei's (1973) genetic distance (GD) between genotypes were computed from combined data for the four primers, ranging from 0.250 to 1.000.

Considering the genetic distance values the result indicates that the genotypes of brinjal were used in the present study genetically different from each other. This proportion of genetic distance are compared from some previous analysis in brinjal such as Verma et al. (2012) recorded the maximum genetic distance of 1 was found between Pusa Bhairav and Green Long, Green Long and KS-224, Green Long and SL-195, Green Long and KS331 and between Pusa Kranti and SL-195 followed by 0.85 between Pusa Kranti and KS-224, and NDB-25 and Pusa Kranti. Demir et al. (2010) recorded that the genetic similarity according to SSR data was scaled between 0.15 and 1, suggesting the potential of SSR markers in discriminating among plants of close or distant genetic backgrounds.

From this study, the dendrogram revealed that the genotypes that are derivatives of genetically similar type form cluster together. Maximum brinjal genotypes includes cluster IV.

Considering the genetic similarity the result supports the previous findings in brinjal such as Ansari and Singh (2015) who recorded six cluster groups using SAHN cluster analysis UPGMA method which revealed that morphological characters *viz.*, shape, size and peel colour of fruits and plant type showed a positive relationship with the DNA based molecular analysis through SSR markers; Demir et al. (2010) found that UPGMA dendrograms were used to examine the genetic relatedness of the genotypes. Khorsheduzzaman et al. (2008) estimated genetic similarities of SSR profiles based on Jaccard's coefficient value. The dendrogram generated two clusters and they were clearly distinct and separated from each other. Cluster-I consisted of genotypes TURBO and BL009; and cluster-II comprised genotypes EG058, EG075 and ISD006. Genotype TURBO and BL009 were identified as the diverse genotype and showed a maximum of 17% dissimilarity from EG058, EG075 and ISD006. The similarity value ranged from 0.83 to 1.00 which indicated the presence of narrow range of genetic diversity at molecular level. It has been reported that SSR markers were ideal markers for constructing high resolution genetic maps in order to identify similarity between different species within a single genus (Provan et al., 1999). Nunome et al. (2003a) evaluated primer SSR for *S. melongena* and its related species and found them most suitable for brinjal. SSRs used in this study provided important information about the genetic diversity and relationships among brinjal genotypes.

Conclusion

Five random primers were initially screened and finally four primers smSSR01, smSSR03, smSSR11 and smSSR14 were selected for the analysis. The microsatellite loci were also multi-allelic (9.0 to 14.0 alleles per locus with a mean of 11.0 alleles per locus in the present study) and the alleles were co-dominant suggesting their relative superiority in detecting DNA polymorphism over some other markers. According to Nei's (1973), the highest level of gene diversity value 0.8672 was observed in loci smSSR11 and the lowest level of gene diversity value 0.6580 was observed in the loci smSSR14 with a mean diversity of 0.7847. As a measure of the informativeness of microsatellites, the

PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660. As a measure of the informativeness of microsatellites, the PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660. All 48 brinjal genotypes could be easily distinguished. From this study, the dendrogram revealed that the genotypes that are derivatives of genetically similar type form cluster together. Maximum brinjal genotypes include cluster IV. These genotypes can be of a potential value for the breeders with wider genetic base that increase the present eggplant collection and to widen the genetic diversity of currently cultivated eggplant varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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