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DNA fingerprinting and molecular diversity analysis for the improvement of brinjal (*Solanum melongena* L.) cultivars

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ABSTRACT: An investigation was conducted to assess genetic diversity of Bangladesh Agricultural Research Institute (BARI) released 11 varieties of brinjal (BARI begun 1, BARI begun 4-10, BARI hybrid begun 1, 3 and 4) using PCR-based randomly amplified polymorphic DNA (RAPD) markers. A total of 44 distinct DNA amplified bands were observed for five primers (OPB-04, OPB-08, OPD-02, OPP-13 and OPW-08) with an average of 5.6 polymorphic bands per primer. The overall gene diversity was detected 0.216 and level of polymorphism was found 63.64%. The pair wise inter-variety similarity indices ranged from 67.03% to 97.61%. The UPGMA dendrogram segregated 11 genotypes of brinjal into two main clusters. The first major cluster had only one genotype (BARI begun 6) and the second major cluster had rest of ten genotypes. BARI begun 6 vs BARI begun 1 showed the highest Nei's genetic distance (0.5261) as they are released from different parental origin. On the other hand, BARI begun 9 vs BARI begun 7 varietal pair showed lowest genetic distance (0.0465) as they are released from same parental origin. The experiment reveals genetic diversity and relatedness among 11 brinjal genotypes which may be informative for the future varietal identification and genetic improvement of this vegetable crop.

KEYWORDS: DNA fingerprinting, *Solanum melongena*, Genetic diversity

INTRODUCTION

Brinjal (*Solanum melongena* L.) is an economically important Solanaceous vegetable, widely consumed in Asia, Europe, Africa and America [1; 2; 3]. It has the early European name 'eggplant" locally known as "Begun" in Bangladesh. It is a self-pollinated, diploid (2n=24) annual crop belongs to the family *Solanaceae*. Brinjal is largely cultivated in almost all districts of Bangladesh. The total area of eggplant cultivation is 76370 acres with total annual production of 310354 metric tons with an average yield of 4064 kg per acre in 2014-2015 [4]. Globally, the eggplant is ranked as third most important crop from *Solanaceae* family after potato and tomato with an annual production of more than $41x10^6$ tons [5].

Brinjal is nutritious vegetable and has got multifarious use as a dish item. It is a valuable source of minerals (particularly iron) and vitamins. The fruit and other parts of the plant are used in traditional medicine [6]. Eggplant is ranked as one of the top ten vegetables due to its high oxygen radical scavenging capacity [7]. Research studies have shown that phenolic compounds of eggplant have antioxidant properties preventing oxidation and diabetes complications [8; 9]. Additionally, the eggplant peel is rich in anthocyanins having therapeutic potential against hyperlipidemia and cardiovascular diseases by inhibiting lipid peroxidation [9; 10].

Genetic resources management of brinjal cultivars is co-related with the sustainable agricultural and economic development of the country. The yield potential of this eggplant in Bangladesh is very low compared to other countries due to the incidence of insect pests and diseases [11; 12]. Molecular characterization of this eggplant may provide an avenue to screen the germplasm resources for any selective breeding and genetic improvement.

Molecular markers are reliable tools to characterize the DNA profile of plant genotypes to study the genetic diversity. In Bangladesh, genetic data on brinjal is not rich enough. So genetic status of this important crop is needed to be established and documented by using DNA markers which may provide valuable information for further breeding programme. Randomly amplified polymorphic DNA (RAPD) markers are effective for the evaluation of genetic diversity due to easy application and relatively low expensive compared to other molecular markers [13; 14]. Molecular characterization by RAPD markers is easy and rapid. RAPD markers are being used for the identification of genetic relationship among cultivars [15; 16].

The present investigation was formulated with the objective of studying genetic variation among the different released brinjal cultivars through RAPD markers, molecular characterization by DNA profiling and **t**o reveal phylogenetic relatedness and genetic distance among the brinjal genotypes.

MATERIALS AND METHODS

Experimental plant materials and their sources

The plant materials for this study consisted of 11 brinjal varieties namely BARI begun 1, BARI begun 4-10, F1 hybrids (BARI hybrid begun 1, 3 and 4). All the varieties were collected from Horticulture Research Centre (HRC) of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Seedlings were grown at the Laboratory of Department of Biotechnology at Sher-e-Bangla Agricultural University, Dhaka in earthen tubs containing soils collected from nursery.

Genomic DNA isolation

Genomic DNA of the brinjal seedlings was isolated following protocol described by Saghai-Maroof and co-workers [17] as well as Islam and co-workers [18; 19] with some modifications. Approximately 200 mg of young leaves of 30 days old plants were cut into minuscule pieces, homogenized and digested with extraction buffer (pH= 8.0): 50 mM Tris-HCl, 25 mM EDTA (Ethylene diamine tetraacetic acid), 300 mM NaCl and 1% SDS (Sodium Dodecyl Sulfate). Following incubation for 20 minutes at 65°C with intermittent shaking, the mixture was vortexed with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v) for 20 seconds. The emulsified mixture was centrifuged at 13000 rpm for 10 minutes. The supernatant was used for precipitation with 2/3 volume of 100% alcohol in presence of 0.3 M sodium acetate and DNA was pelleted by centrifugation. The DNA pellets were then washed with 70% ethanol. The air dried DNA pellets were resuspended in an appropriate volume of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH=8.0) and finally treated with RNAse and stored at -20°C. The quality and quantity of DNA were checked by electrophoresis and spectrophotometer, respectively.

Screening and selection of RAPD primers

Initially, nine decamer primers namely OPA-18, OPB-04, OPB-06, OPB-08, OPD-02, OPF-08, OPG-19, OPP-13 and OPW-08 (Operon Technologies, Inc. Alameda, California, USA) were selected and screened by polymerase chain reaction (PCR) for their ability to produce polymorphic patterns in brinjal varieties and five primers (OPB-04, OPB-08, OPD-02, OPP-13 and OPW-08) which gave reproducible and distinct polymorphic amplified products were selected finally for RAPD analysis.

Polymerase chain reaction

PCR reactions were performed for each DNA sample in a 25 µL reaction mix containing 12.5 µL 2X Taq Master mix (Gene ON, Germany), 2.5 µL of RAPD primer (10 pM), 7.5 µL of sterile de-ionized water and 2.5 µL of genomic DNA (25ng/ µL). DNA amplification was performed in an oil-free thermal cycler by setting a thermal profile as pre-heating at 95^{o} C for 5 min followed by 33 cycles of 45 s denaturation at 95^{o} C, 30 s annealing at 30^{o} C and extension at 72^{o} C for 1 min. After the last cycle, a final step of 5 min at 72^{o} C was added to allow complete extension of all amplified fragments.

Visualization of PCR products

PCR products were confirmed by electrophoresis on 1.5 % agarose gel containing 1 µL ethidium bromide (10 mg/L) in 1X TBE buffer at 85 V for 50 min. After electrophoresis, the gel was taken out carefully from the gel chamber and was placed on high-performance ultraviolet light box (UV trans-illuminator) of gel documentation for checking the RAPD bands and photographed by a Gel Cam Polaroid camera.

RAPD data analysis

Following electrophoresis, the sizes of the PCR products were estimated by comparisons of distance travelled by each fragment with distance travelled by known size fragments of the DNA molecular weight markers (100 bp DNA ladder, Genei, India or 1 Kb DNA ladder, BRIC, Korea). All distinct bands or fragments (RAPD markers)

were thereby given identification numbers according to size and scored visually on the basis of their presence (1) or absence (0), separately for each variety for each primer. The scores recorded for all RAPD primers were then pooled to create a single data matrix. This was used to compare the frequencies of all polymorphic RAPD markers among populations with 1000 simulated samples using POPGENE (version 1.31) [20] computer program. The size of the RAPD markers were estimated by using the software DNA frag, version 3.03 [21]. Nei's [22] genetic distance values were calculated based on the frequencies of polymorphic markers using the POPGENE (Version 1.31) computer package [20]. For each population group, Nei's gene diversity (h) was calculated for each locus and then averaged over all loci.

These parameters were performed using POPGENE software [20] in the formula: $h=1-\sum pi^2$, $I= \sum pi \log pi$, where pi = the frequency of the allele i in the population. A value indicates small genetic distances, 0.10-0.15 indicates moderate genetic distances, 0.15-0.2 indicates high genetic distances and >0.2 indicates very large genetic distances [23]. The dendrogram was constructed using POPGENE (Version 1.31) [20]. Genetic similarity values were calculated manually based on the formula, Similarity Index (SI) = $2N_{XY}/(N_X + N_Y)$; where, N_{XY} is the number of RAPD bands shared by individuals X and Y, respectively, and N_X and N_Y are the number of bands in individuals X and Y, respectively [24; 25].

RESULTS

Profiling of RAPD bands and their polymorphisms

A total of 44 distinct and different PCR based RAPD bands were produced with 8.8 per primer. The size of the bands ranges from 198 to 2898 bp (Table 1)). The highest number of bands (12) was generated by primer OPD-02. Besides, the primer OPB-04, OPB-08, OPP-13 and OPW-08 generated 8 scorable bands each. The RAPD banding profile is shown in Figure 1. The highest proportion of polymorphic bands (87.5%) was detected by primer OPW-08 while primer OPB-04 detected the least proportion (37.5%) and overall level of polymorphism was 63.64% (Table 1). The average values of Nei's gene diversity for the RAPD primers ranged from 0.083 to 0.388 with an overall value of 0.216 (Table 1).

Table 1. RAPD primers with corresponding number and size range of bands together with percentage of polymorphic loci and Nei's gene diversity values observed in 11 brinjal varieties

Name of RAPD primer	Sequence of the primer	GC content (%)	No. of bands scored	Size ranges (bp) observed	No. of polymorphic bands	Percentage of polymorphic loci	Nei's gene diversity (average)
OPB-04	GGACTGGAGT	60%	8	206-2836	3	37.5	0.169
OPB-08	GTCCACACGG	70%	8	198-1157	5	62.5	0.198
OPD-02	GGACCCAACC	70%	12	419-3898	9	75	0.242
OPP-13	GGAGTGCCTC	70%	8	222-870	4	50	0.083
OPW-08	GACTGCCTCT	60%	8	267-897	7	87.5	0.388

Inter-variety similarity indices

Inter-varietal similarity indices (Sij) ranged from 67.03% to 97.61% (Data are not shown due to large sized table). The highest similarity indices (97.61%) were found between BARI begun 7 and BARI begun 9. BARI begun 6 and BARI begun 10 showed least inter-variety similarity indices 67.03%. All the 55 varietal pairs were not homogenous at different number of loci.

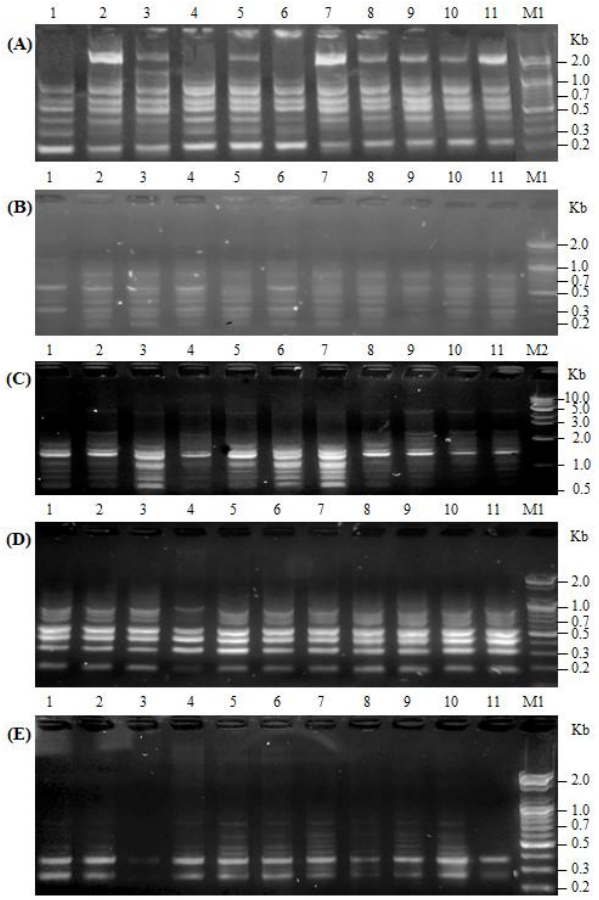

Figure 1.DNA fingerprinting profile of 11 brinjal varieties based on five RAPD primers, (A) OPB-04, (B) OPB-08, (C) OPD-02, (D) OPP-13 and (E) OPW-08. Lane 1: BARI begun 1, lane 2: BARI begun 4, lane 3: BARI begun 5, lane 4: BARI begun 6, lane 5: BARI begun 7, lane 6: BARI begun 8, lane 7: BARI begun 9, lane 8: BARI begun 10, lane 9: BARI hybrid begun 1, lane 10: BARI hybrid begun 3, lane 11: BARI hybrid begun 4. Ms: M1=100 bp DNA ladder and M2: 1 kb DNA ladder

Genetic identity and genetic distance

The highest Nei's genetic identity (0.9545) was observed in BARI begun 9 vs BARI begun 7 varietal pairs whereas the lowest genetic identity (0.6136) was estimated in BARI begun 6 vs BARI begun 5 varietal pair (Table 2).

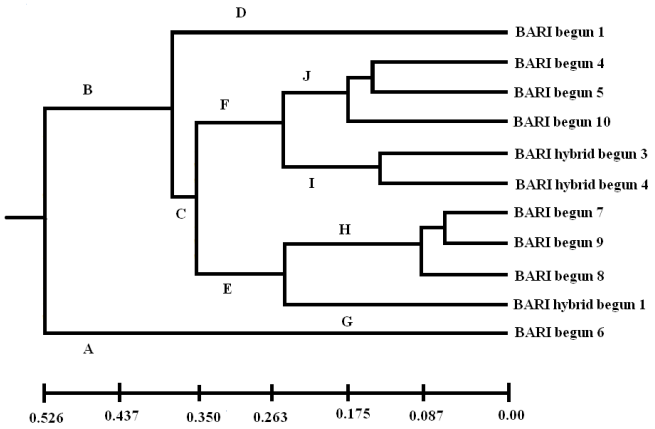

Figure 2. The UPGMA (Unweighted pair group method of arithmetic mean) dendrogram based on Nei's (1972) genetic distance within 11 brinjal varieties according to RAPD analysis

The result indicates that the low or high level genetic distance exists between varieties with their same or different origins. BARI begun 6 vs BARI begun 1 showed highest Nei's genetic distance (0.5261) as they are released from different parental origin. On the other hand BARI begun 9 vs BARI begun 8 varietal pair showedlowest genetic distance (0.0706) as they are released from same parental origin. This variation might be created due to geographical origin.

Phylogenetic relatedness and construction of dendrogram

Phylogenetic dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated the segregation of 11 genotypes of brinjal into two main clusters, 'A' and 'B' (Figure 2). The first major cluster 'A' had only one genotype (BARI begun 6) and the second major cluster 'B' had rest of ten genotypes. The second major cluster subdivided into two minor clusters (C & D) in which one cluster 'D' had only one genotype (BARI begun 1) and the other cluster 'C' had rest of the nine varieties. This minor cluster further subdivided into two clusters (E & F). In which cluster E divided into two sub clusters (G and H). BARI hybrid begun 1 formed cluster 'G' and BARI begun 7, BARI begun 8 and BARI begun 9 grouped in cluster 'H'. Cluster 'I' and 'J' were the subdivision of cluster 'F'. BARI hybrid begun 3 and BARI hybrid begun 4 formed cluster 'I' and BARI begun 4, BARI begun 5 and BARI begun 10 were grouped in cluster 'J'.

DISCUSSION

With advancement of DNA marker technology, molecular characterization plays a vital role in managing and utilizing plant genetic resources [26]. It is immensely helpful in selective breeding from diverse parents to widen the breeding gene pool [27]. Thereby, DNA markers are increasingly adopted as an effective and

Table 2. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values between 11 brinjal varieties .

Acc. No.	BARI begun 1	BARI begun 4	BARI begun 5	BARI begun 6	BARI begun 7	BARI begun 8	BARI begun 9	BARI begun 10	BARI hybrid begun 1	BARI hybrid begun 3	BARI hybrid begun 4
BARI begun 1	****	0.795	0.750	0.590	0.681	0.750	0.681	0.750	0.750	0.681	0.750
BARI begun 4	0.228	****	0.863	0.659	0.750	0.727	0.795	0.863	0.772	0.795	0.863
BARI begun 5	0.287	0.146	****	0.613	0.795	0.727	0.795	0.863	0.681	0.750	0.863
BARI begun 6	0.526	0.416	0.488	****	0.681	0.659	0.636	0.659	0.659	0.681	0.659
BARI begun 7	0.383	0.287	0.228	0.383	****	0.931	0.954	0.704	0.795	0.818	0.704
BARI begun 8	0.287	0.318	0.318	0.416	0.070	****	0.931	0.681	0.818	0.750	0.636
BARI begun 9	0.383	0.228	0.228	0.452	0.046	0.070	****	0.750	0.840	0.818	0.704
BARI begun 10	0.287	0.146	0.146	0.416	0.350	0.383	0.287	****	0.818	0.750	0.863
BARI hybrid begun 1	0.287	0.257	0.383	0.416	0.228	0.200	0.173	0.200	****	0.840	0.772
BARI hybrid begun 3	0.383	0.228	0.287	0.383	0.200	0.287	0.200	0.287	0.173	****	0.886
BARI hybrid begun 4	0.287	0.146	0.146	0.416	0.350	0.452	0.350	0.146	0.257	0.120	****

appropriate tool for basic and applied studies addressing biological components in agricultural production systems [28].

In the present study, the overall level of polymorphism (63.64%) indicated the effectiveness of RAPD technique to investigate genetic diversity among the different genotypes of brinjal. Number of polymorphic bands ranged from 3-9 with an average of 5.6. Verma and co-workers [29] reported 5.58 bands per primer and Islam and co-workers [30] found 5.67 bands per primer in brinjal varieties which are mostly consistent with the present study. Almost similar level of polymorphisms was reported in different experiment with brinjal and other eggplants, for instance 57.89% [31], 57.58% [32]. In contrast, higher level of polymorphism in brinjal germplasm was obtained by Aguoru and co-workers [33] (83.33%) as well as by Islam and coworkers [30] (70.59%). The value of overall gene diversity was 0.216 in this study which is very close to the findings reported by Biswas and co-workers [31], where they observed overall gene diversity value of 0.23 among 10 promising brinjal varieties with RAPD markers.

Karihaloo and co-workers [34] reported the highest similarity (0.947) between *S. insanum* and *S. melongena* while analyzing genetic similarity of 52 accessions of *Solanum*. The present study indicates a high level of genetic variation within some brinjal varieties which were perceived from the data of inter-variety similarity indices and genetic distance value. Phylogenetic clustering patterns based on genetic distance values also indicated diverse relationship that occurred might be due to geographical origin of the parental varieties.

Finally it can be concluded that high degree of diversity of the studied brinjal cultivars may be attributable to genetic improvement programme based on the clustering patterns. It will also provide 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 this important eggplant may contribute to enrich diets and bring health benefits.

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AUTHOR CONTRIBUTIONS

SS performed the experiment; MNI and MEH conceived the study; SS and MNI analyzed the data; SS and MNI wrote the paper.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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