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Assessment of genetic diversity among sweet potato varieties through RAPD markers in the Southern coastal region of Bangladesh

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Abstract. *Saimon AH, Sultana S, Mannan MA, Mamun AA. 2023. Assessment of genetic diversity among sweet potato varieties through RAPD markers in the Southern coastal region of Bangladesh. Asian J Agric 7: 116-121.* Sweet potato (*Ipomoea batatas* (L.) Lam.) is the sixth most significant food crop. Evaluating this crop's genetic diversity is crucial for food security and preserving agricultural genetic resources. Bangladesh is South Asia's second-largest sweet potato producer, but little is known about the genetic diversity of this crop there. The study aimed to assess the genetic diversity among six sweet potato varieties (Five BARI-released sweet potato varieties: BARI Misty Alu-10, BARI Misty Alu-11, BARI Misty Alu-12, BARI Misty Alu-14, BARI Misty Alu-15, and a local cultivar) using RAPD marker in the Southern coastal region of Bangladesh. Six primers were utilized to determine the polymorphic and monomorphic bands. Data was analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram and Principal Component Analysis (PCA). Thirty-seven polymorphic bands were found, with an average of 3 polymorphic and 3.17 monomorphic bands. Primer OPM-02 showed the highest polymorphic bands (6). The results showed that BARI Misty Alu-10 and BARI Misty Alu-15 had the most genetic diversity, at 37%. The average polymorphism percentage was 44.88%. The dendrogram featured two distinct clusters that showed BARI Misty Alu-12, the most distant variety. The clustering pattern corresponded with PCA, demonstrating that BARI Misty Alu-12 had the most genetic variation (81.36%).

Keywords: Genetic diversity, molecular marker, polymorphism, RAPD, sweet potato

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is considered one of the most significant root crops in tropical and subtropical areas belonging to the Convolvulaceae family. It became sixth among the world's primary food crops (CIP 2019). It is considered one of the food security crops for its several resilient, vital features such as versatility, vigorous growth, drought and salt tolerance, cold tolerance, high productivity, and high adaptability with minimum inputs. (Lebot 2010; Sun et al. 2014; Ahmed et al. 2015). According to FAOSTAT (2020), Bangladesh is the second largest producer of sweet potatoes (27%) after India (68%) in South Asia. Sweet potato is a rich superfood with nutrients, carbohydrates, vitamins, and minerals, low fat and protein (Mahmud et al. 2021). It contains vitamin A, which helps alleviate vitamin A deficiency in children. Consuming sweet potatoes can also provide essential vitamins, minerals, and fibers, making it an ideal meal based on calories (Bovell-Benjamin 2007; Lebot 2010; Sun et al. 2019).

Genetic diversity is crucial for species continuity and adaptation to biotic and abiotic stress (Muhammed et al. 2012). It measures genetic variance and is a primary source of biodiversity (Hughes et al. 2008). Crop improvement programs require sufficient genetic diversity for successful hybridization. Primary and secondary centers of gene diversity occur in domesticated and introduced areas, with understanding this diversity essential for developing effective measures for plant genetic resources (Roullier et al. 2013).

Agronomic, morphological, molecular, biochemical, physiological, and other traits can be used to assess genetic diversity. Among them, molecular markers manifest the presence of more polymorphic loci, making it possible to recognize separate accessions that might have identical morphological and agronomical characteristics (Goncalves et al. 2008); molecular markers aid in clarifying the crops' genetic framework, which supports successful breeding programs. Random Amplified Polymorphic DNA (RAPD) markers are frequently employed to determine the genetic connection between sweet potato cultivars (Tosti and Negri 2002), because the primers are in an arbitrary sequence, RAPDs can be created relatively quickly and easily (Verma et al. 2017). For assessing diversity and identifying germplasm in various plant species, RAPD is reasonably effective at detecting genetic variants (Moghaieb et al. 2017). On the other hand, the RAPD marker has some limitations, such as band profiles cannot be explained in terms of loci and alleles since the markers are not locus-specific (Tikendra et al. 2021). RAPDs have low reproducibility; hence, highly standardized laboratory techniques and purified DNA are essential (Reshma and Das 2021).

Many studies have assessed the genetic variability among different sweet potato varieties. Samiyarsih et al. (2020) investigated the genetic diversity among eight sweet potato cultivars in Central Java, Indonesia, and reported a

range of genetic similarities (37-93%) among the cultivars by RAPD markers. Shah et al. (2018) observed genetic distinctiveness among 92 sweet potato accessions collected from different locations in Malaysia and Indonesia through RAPD markers. However, no similar study of sweet potato varieties in Bangladesh's coastal region prioritized this experiment. Among the 17 sweet potato varieties released by Bangladesh Agricultural Research Institute (BARI), 5 sweet potato varieties recommended for saline areas (BARI Misty Alu -10, BARI Misty Alu -11, BARI Misty Alu -12, BARI Misty Alu -14, and BARI Misty Alu -15) and a local cultivar were used in this study. The study aimed to assess the genetic diversity among the 5 sweet potato-released varieties of BARI and a local cultivar through RAPD markers.

MATERIALS AND METHODS

Research site

The experiment was conducted at the Plant Protection Laboratory, Molecular, and Horticulture Laboratory of Agrotechnology Discipline, Khulna University, Khulna District, Bangladesh, from January 2022 to June 2022.

Plant materials

Moreover, 6 sweet potato varieties leaf samples were collected from two locations in the Khulna region: Germplasm Center of Khulna University and farmer's fields in Batiaghata Upazila of Khulna District (Table 1). The zone is within AEZ-11, which stands for 'High Ganga River Flood Plain. The maximum annual mean temperature of the region is 35.5°C, and the minimum is 12.5°C, whereas the mean annual rainfall is 1,710 mm (Rashid et al. 2014).

Procedures

DNA isolation

Six varieties of sweet potato leaf samples (each 3 g) were powdered with liquid nitrogen with a sterilized mortar and pestle and were taken in a 1.5 mL Eppendorf tube. Then, those were stored at -86ºC ultra-low freezer (Thermo Scientific, USA). DNA was isolated using GeneDireX, Inc. Plant genomic DNA extraction kit followed the manufacturer's protocol. The genomic DNA of six varieties was extracted, and the Eppendorf tube's DNA solution was stored at -20ºC. An electrophoresis test in 1% agarose gel was run to confirm the presence of DNA materials. The Gel documentation system showed a picture of DNA residues under UV light. Multiskin GO Microplate Spectrophotometer (Thermo Fisher Scientific, Germany) was used to quantify purified DNA at a wavelength of 260 nm. The final concentration of the template DNA for PCR was adjusted to 50 ng $(\mu L)^{-1}$ and stored at -20ºC (Kandan et al. 2013).

PCR amplification and electrophoresis

A Polymerase Chain Reaction (PCR) was performed in a 0.2 mL thin-walled PCR tube. Amplifications were performed in a thermos cycler (Biometra 4, Germany) utilizing the protocols: initial denaturation at 95°C for 4 min, 40 cycles of denaturation at 94°C for 30 s, following annealing at 55°C for 60 s, and the final extension at 72°C for 5 min. Then cool down to 8ºC. Six Primers were utilized to screen out the DNA banding pattern (OPM-02, OPM-03, OPM-04, OPM-05, OPM-10, and OPM-12). The product amplifications were separated by electrophoresis using 1% agarose gels with TAE buffer and ethidium bromide (at 0.5 μg mL^{-1}) for staining. Then, high-voltage electricity (90 V) was supplied to the Tris Acetate EDTA buffer solution (1.0 X TAE) for 45 minutes.

A Bioneer gel electrophoresis machine was used. The electrophoresis gels were scanned, and photographs were taken with Biodoc Analyze Computer software related to the Gel documentation system, version 2.2 (Biometra gel documentation, A Biodoc Analyse 2.2 version, Germany). Molecular weight indicator 1kbp (Direct load, Sigma Aldrich, USA) was used to size the amplicons.

Data analysis

RAPD-PCR bands were detected using the gel documentation technique. Bands were scored according to absence (0) or presence (1). All scored band was considered as single allele/locus. The band sizes were determined using a standard 1kb ladder/marker. Some parameters such as polymorphism percentage, polymorphic information content, resolving power of the marker (Rp), and marker index were calculated to evaluate the RAPD primers utilized in this study's informativeness and discriminating power. Then binary data were subjected to similarity correlation analysis by simple matching coefficient, and then an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was constructed.

The UPGMA is a clustering method used to analyze and represent the genetic relationships between different samples based on their genetic data. Moreover, Principal Component Analysis was conducted to calculate the eigenvalue and eigenvector, and then a 3D plot was made using Numerical Taxonomy and Multivariate Analysis System version 2.01e (NTSYSpc) portable software (Rohlf 2002). The Eigenvalues are used to determine how many factors to retain. The sum of the Eigenvalues usually equals the number of variables (Bhanupriya et al. 2014).

Table 1. Sweet potato variety name, label, and collecting site

Variety	Sample Labeling	Collecting Site
BARI Misty Alu-12	SP1	Germplasm Center of Khulna University
BARI Misty Alu-10	SP2	Germplasm Center of Khulna University
BARI Misty Alu-11	SP3	Germplasm Center of Khulna University
BARI Misty Alu-14	SP4	Germplasm Center of Khulna University
BARI Misty Alu-15	SP5	Germplasm Center of Khulna University
Local cultivar	SP6	Farmer's field in Batiaghata Upazila of Khulna District

RESULTS AND DISCUSSION

Genetic variation generated by RAPD markers:

Genetic variation was detected among six sweet potato varieties using RAPD markers. Polymorphic and monomorphic bands were detected among the amplified DNA products. Six RAPD primers produced 37 bands. Among them, 18 were polymorphic bands, and 19 were monomorphic bands. Primer OPM-2 generated the highest polymorphism percentage (75%). Primers OPM-03, OPM-04, OPM-05, OPM-10, and OPM-12 showed 40%, 20%, 20%, 71.42%, and 42.86% polymorphism percentage, respectively, with an average of 44.88% polymorphism percentage per primer (Table 2).

RAPD patterns of the amplified bands with sizes between 500 to 3000 bp for all primers are shown in Figure 1.

Table 2. RAPD primer sequences with the number of polymorphic and monomorphic bands

Primer	Primer Sequence (5' to 3')	GC Content (%)	NPB	NMB	PP (%)
OPM-02	ACAACGCCTC	60	6	2	75
OPM-03	GGGGGATGAG	70	2	3	40
OPM-04	GGCGGTTGTC	70	1	4	20
OPM-05	GGGAACGTGT	60	1	4	20
OPM-10	TCTGGCGCAC	70	5	2	71.42
OPM-12	GGGACGTTGG	70	3	4	42.86
Total number of amplified bands			18	19	
Average amplified bands			3	3.17	
Total Number of amplified bands (Monomorphic and Polymorphic)				37	
Average polymorphism percentage				44.88	

Note: NPB: Number of Polymorphic Bands; NMB: Number of Monomorphic Bands; PP: Polymorphism Percentage

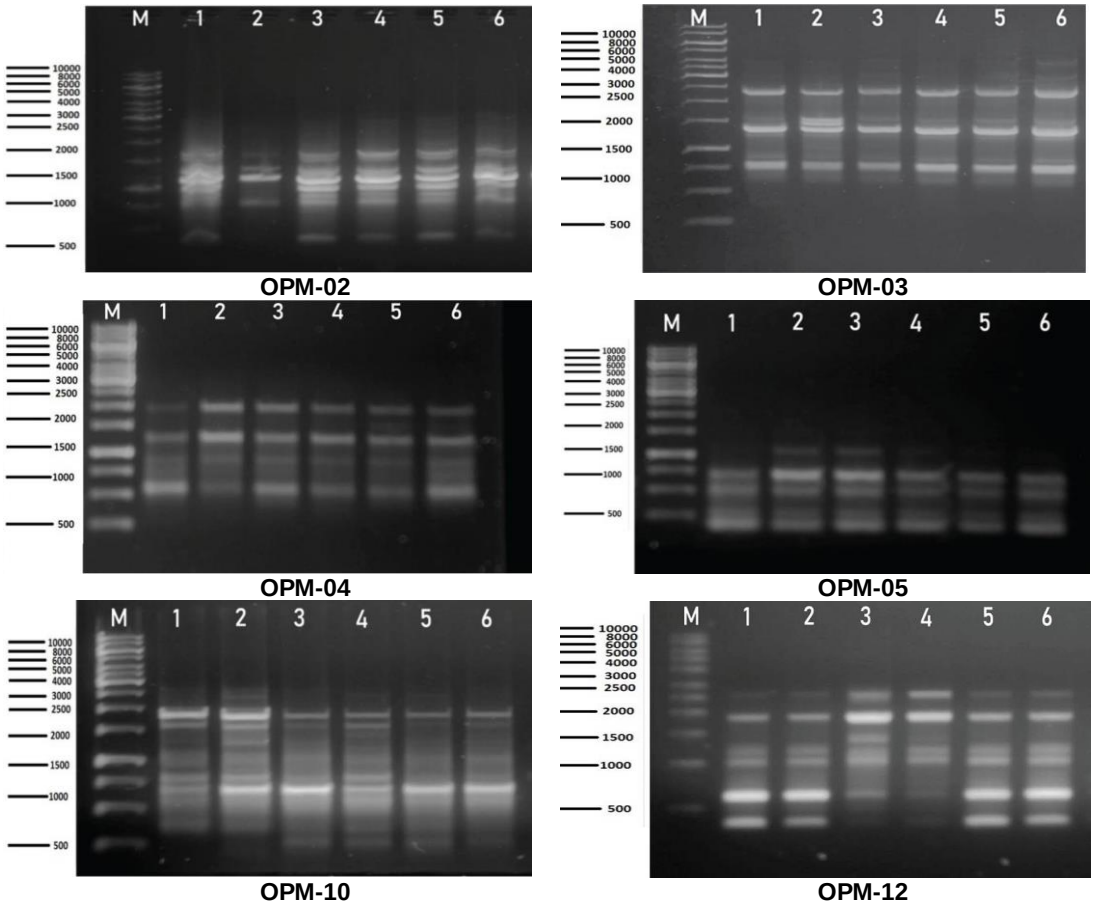

Figure 1. RAPD banding patterns of six sweet potato varieties generated by: A. OPM-02, B. OPM-03, C. OPM-04, D. OPM-05, E. OPM-10, F. OPM-12. Note: M: Ladder, 1: SP1, 2: SP2, 3: SP3, 4: SP4, 5: SP5, 6: SP6

Genetic similarity among varieties of sweet potato

The genetic similarity among six sweet potato varieties is shown in Table 3. According to Jaccard's coefficient, the genetic similarity of each variety ranged from 0.63 to 0.90. The SP5 and SP6 varieties showed the highest genetic similarity of 0.9, whereas SP2 and SP5 showed the lowest genetic similarity of 0.63.

Cluster analysis

The UPGMA cluster analysis constructed a dendrogram among six sweet potato varieties presented in Figure 2. All varieties studied were separated into two distinct clusters. Cluster I contained only one variety, BARI Misty Alu-10 (SP2), indicating its distant characteristic. Cluster II was divided into two subclusters (II A and II B), where subcluster IIA had only one variety, BARI Misty Alu-12 (SP1), and subcluster II B constituted SP3, SP4, SP5, and SP6 varieties.

Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) mostly confirms the cluster analysis. The initial step is to compute eigenvalues, which specify how much overall variation is shown on the Principal Component (PC) axes. The first PC describes most of the variability in the original data compared to the subsequent PCs. Furthermore, the second PC explains most of the variability not described by the first PC and is unrelated to the first, and so on (Jolliffe 1986). Here, the first PC (SP2) accounted for about 81.36% of the total variation for all genetic traits. In comparison, the second PC (SP1) accounted for 7.85% of the total variation (excluding SP1), and so on, indicating genetic variability among the six sweet potato varieties (Table 4).

Based on the three-dimensional plot of PCA, the most genetically identical varieties were discovered in BARI Misty Alu-15 (SP5) and local cultivars (SP6). The distant relationship among the BARI Misty Alu-10 (SP2) and BARI Misty Alu-15 (SP5) was detected (Figure 3). SP2 exhibited a distant connection with the other varieties.

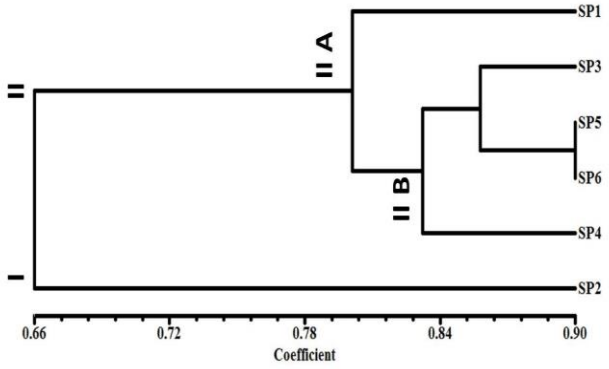

Figure 2. A dendrogram among six varieties of sweet potato generated by the UPGMA cluster analysis

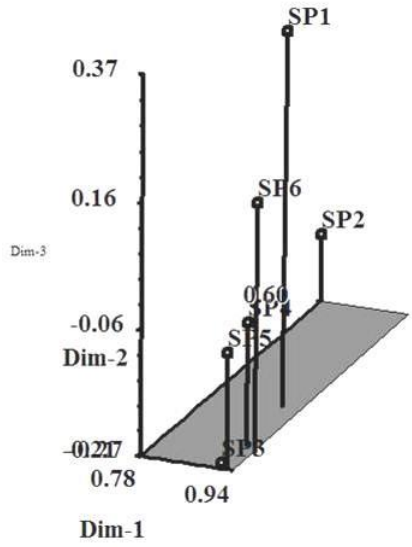

Figure 3. Three-dimensional plot of PCA showing relationships between six sweet potato varieties using genetic traits

Table 3. Genetic similarity among six sweet potato varieties

Variety	SP1	SP2	SP3	SP4	SP5	SP6
SP1	1.00					
SP2	0.71	1.00				
SP3	0.72	0.64	1.00			
SP4	0.81	0.66	0.84	1.00		
SP5	0.87	0.63	0.87	0.84	1.00	
SP6	0.87	0.66	0.84	0.81	0.90	1.00

Table 4. PCA analysis of the six (sweet potato varieties) principal components

PC components	Eigenvalues	Percent of variation	Cumulative variation
SP2	4.88	81.36	81.36
SP1	0.47	7.85	89.22
SP3	0.26	4.47	93.69
SP4	0.19	3.28	96.98
SP5	0.11	1.93	98.91
SP6	0.06	1.08	100.00
Sum of Eigenvalue= 06			

Discussion

Six primers amplified 37 polymorphic (18) and monomorphic (19) bands. Primer OPM-2 generated the highest number of amplified bands (8) and polymorphism percentage (75%) (Table 2). However, polymorphic bands generated by primer OPM-2 are lower than in the study conducted by Nusifera and Alia (2019). They reported that primer OPM-2 generated 13 polymorphic bands on the cinnamon plant. Based on our results, the average polymorphism percentage was 44.88% (Table 2). These findings demonstrated the applicability of random PCR primers to characterize and evaluate intra-specific polymorphisms among sweet potato varieties. The higher the proportion of polymorphism, the more likely two or

more traits will be located on a single gene. Moulin et al. (2012) found a 42.8% polymorphism percentage compared to other studies using Egyptian sweet potato germplasm when tested on Brazilian sweet potato landraces. Lee et al. (2019) studied Korean sweet potatoes and found that the highest polymorphism percentage was 83.3%. The RAPD marker's high level of polymorphism detection and the ability to screen a larger number of anonymous loci suggest that this marker can effectively discover sweet potato germplasm.

Understanding the genetic diversity within and between populations is critical to creating efficient and cost-effective conservation methods for plant genetic resources (Cadima et al. 2017). Based on our study, the highest genetic similarity presented by BARI Misty Alu-15 and Local cultivar varieties is 0.90. This similarity indicates that they had a common parent, and their traits are remarkably similar. At the same time, BARI Misty Alu-10 (SP2) and BARI Misty Alu-15 (SP5) had the lowest genetic similarity of 0.63. This finding suggests that sweet potato germplasm represents diversity in genetic characters. This distinctiveness will help with environmental adaptation to different circumstances. As a result, such variety may provide helpful features to overcome the problems with food security (Onda and Mochida 2016). Soegianto et al. (2011) observed 0.78 genetic similarity between the *Biru Ungu* and *Bestak* clones utilizing RAPD markers. Veasey et al. (2008) investigated the genetic differences of 78 Brazilian sweet potatoes and revealed that 0.418 similarity was spread in households.

Cluster analysis was done by UPGMA dendrogram using the TREE program. Six sweet potato varieties were clustered into two main clusters using a cutoff point at a distance of 0.06. Cluster I featured only one variety, BARI Misty Alu-10 (SP2), while cluster II had the remaining varieties, indicating a distant relationship between SP2 and the other varieties (Figure 2). Because of the creation of these two clusters, the distribution pattern may contain two gene pools. Singh et al. (2017) and Bhadauriya et al. (2018) also reported a distant relationship during the clustering of sweet potato varieties.

PCA demonstrated the genotypic relationship among the varieties. Cluster analysis corresponds with PCA, indicating comparable results. According to PCA, the first four PCs comprised 96.96% of the total variation, whereas the first PC (SP2) accounted for 81.36% of the total variation and exhibited a high distant correlation among the varieties analyzed. There was a clear distribution pattern of sweet potato varieties based on their genetic variation. A possible explanation for the distribution pattern in this study would be farmer's preferences and genetic adaptation. Farmers often determine their variety selection by taste, texture or color, and yield. A farmer's decision to select a particular cultivar is based on a combination of technical and socioeconomic factors (Sthapit et al. 2008). Crop adaptation may involve natural processes like mutation and continuous outcrossing between genotypes (Flint-Garcia 2013). Thus, these processes may create variations in sweet potato germplasm. Special further assessment should be focused to characterize these varieties.

In conclusion**,** the study showed that genetic diversity among six sweet potato varieties was detected by RAPD markers in the Southern coastal region of Bangladesh. All varieties studied were separated into two distinct clusters based on cluster analysis. Cluster I contained only one variety, BARI Misty Alu-10 (SP2), indicating its distant characteristic. Cluster II was divided into two subclusters (II A and II B), where subcluster IIA had only one variety, BARI Misty Alu-12 (SP1), and subcluster II B constituted SP3, SP4, SP5, and SP6 varieties. Our result was confirmed by Principal Component Analysis (PCA).

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