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MOLECULAR CHARACTERIZATION AND DIVERSITY ANALYSIS OF SOME LOCAL POTATO (*Solanum tuberosum* L.) GENOTYPES OF BANGLADESH BY RAPD MARKERS

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ABSTRACT

Genetic identification of cultivars and varieties is useful in preserving endangered potato germplasm and planning for new breeding programs. Molecular characterization and genetic diversity of 10 local potato genotypes of Bangladesh were investigated with five RAPD primers, namely OPA-18, OPB-06, OPC-01, OPD-02 and OPW. Genomic DNA was extracted from young leaves and PCR reactions were performed with selected primers. The selected primers generated 33 distinct and differential amplified bands (size ranged from 131-1188 bp), out of which 28 were polymorphic. The percentage of polymorphic loci was valued from 33.33% to 100%. Gene frequency was ranged from 0.300 to 1 and gene diversity was from 0 to 0.7. The inter-varietal similarity indices was ranged from 42.45% to 92.67%. Pair-wise comparisons of Nei's genetic identity value was from 0.4848 to 0.9394. The highest Nei's genetic identity (0.9394) was observed in Sheel Bilati and SAU Promising Genotype-5 genotype pair. Nei's (1972) genetic distance was from 0.0625 to 0.7239. Dendrogram based on Nei's genetic distance using Unweighted Pair Group Method with Arithmetic Means (UPGMA) indicated the segregation of studied potato genotypes into two main clusters. It was found that RAPD markers are sensitive enough to identify genetic variation in a variety of germplasm, which will be helpful for choosing genetically unique germplasm in potato breeding programs.

Keywords: DNA fingerprinting; Dendrogram; Genetic distance; Molecular marker; PCR

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INTRODUCTION

Potato, popularly known as 'The king of vegetables', is the most important vegetable crop of Bangladesh as it contributes to about 63% of the total annual vegetable production of the country (Somana et al., 2021). In Bangladesh, it is a substantial crop in terms of both production and demand, and over the past few decades, it has become more and more popular to cultivate (Somana et al., 2021). Total area under potato cultivation is 468,689 hectares and total potato production has been estimated 98,87,242 metric tons. Average yield of potato has been estimated 21.096 metric tons per hectare (BBS, 2021).

Broadly speaking, local and high-yielding varieties of potato are grown in Bangladesh. The local genotypes are completely indigenous and were introduced to this subcontinent in the past, but due to lack of varietal improvement, they exhibit poor yield performance (Somana et al., 2021). Despite poor yield, some of the local varieties are still being cultivated because of their taste and cooking qualities. In different regions of Bangladesh, about 27 native potato types are grown (Islam, 2009; Khalil et al., 2013). These potato genotypes are thin in stem size, have less branching, narrow leaf size, are low yielder and have small to medium tuber size (Somana et al., 2021).

Though per unit yield of local potato genotypes (11.49 tha^{-1}) is lower than the modern varieties (22.76 tha^{-1}), they possess many desirable qualities, viz. storage properties and cooking and culinary qualities (BBS, 2021; Somana et al., 2021). Moreover, they are a good source of diseases and pests' resistances germplasm (Somana et al., 2021). Therefore, increasing the yield per unit area of local potato varieties is necessary.

The selection and improvement of local varieties having other desirable characteristics is the appropriate solution to overcome these problems (Anoumaa et al., 2017). It is, therefore, essential for the breeders to know the genetic background of these local varieties (Yasmin et al., 2006).

Earlier, morphological and biochemical markers were used to characterize and study genetic divergence among target genotypes. But nowadays, these are considered inadequate tools as they are laborious, time-consuming and greatly influenced by environmental factors (Ahmad et al., 2019). Consequently, Different molecular markers are consistently used in the assessment of genetic diversity, characterization of germplasm, identification of hybrids and phylogenetic studies (Tiwari et al., 2015; Ahmad, et al., 2019).

However, the PCR-based RAPD (random amplified polymorphic DNA) marker technique has various precedence because these are generally dominant in nature, can be carried out efficiently with a small amount of DNA, and can also demonstrate polymorphism to a high degree. The method does not need radioactive labeling or previous genomic information (Shamsuzzaman et al., 2021). In addition to that, it is a reliable and comparatively inexpensive technique too (Tiwari et al., 2015). Furthermore, RAPD primers are species-neutral and applicable to all crops. Therefore,

it is possible to construct the RAPD primer without any prior knowledge of the sequence, DNA probes, or hybridization. The RAPD technique has been successfully used by a number of researchers to characterize and analyze the diversity of various plant species, including the chili pepper (Hossain et al., 2003), ginger (Gavande, et al., 2018), potato (Afrasiab and Iqbal, 2010, 2012, Tiwari et al., 2015), and tomato (Soniya et al., 2001).

Molecular data on the local potato of Bangladesh is inadequate. Therefore, the current study has been undertaken to characterize and estimate the genetic diversity among some local potato genotypes of Bangladesh.

MATERIALS AND METHODS

Name and Source of study materials

Ten potato genotypes were studied, among these, Comilla Local, Jham Alu, Deshi Pakri, Sindur Kota, Indur Kani, Fata Pakri, Ishwardi Local and Sheel Bilati are well-known local potato genotypes in Bangladesh. These were collected from Bangladesh Agricultural Research Institute, Gazipur, Regional Agricultural Research Center, Jashore, Ishurdi and Rangpur. At the same time, SAU Promising genotype-4 and SAU Promising genotype-5 lines were developed by the Department of Biotechnology, Sher-e-Bangla Agricultural University (SAU) through the introduction and selection of some local potato genotypes of Bangladesh.

Genomic DNA Extraction, Confirmation and Quantification

For isolation of genomic DNA, vigorous, young, actively growing fresh leaf tissues were collected from potato genotypes at the 3-4 leaf stage. Total genomic DNA was extracted following the protocol described by Saghai-Maroof et al. (1984) with some modifications. Approximately 200 mg of leaf tissues were cut into small pieces and taken in morter. Then, homogenized and digested with extraction buffer (pH=8.0) containing 50 mM Tris-HCl, 25 mM EDTA (Ethylenediaminetetraacetic acid), 300 mM NaCl, 1% SDS (Sodium Dodecyl Sulfate) and deionized water and incubated at 65°C for 20 minutes in a hot water bath. The mixture was vortexed for 20 seconds with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v). The blended mixture was centrifuged for 10 minutes at 13000 rpm. The supernatant was collected, precipitated using two-volume of absolute alcohol in the presence of 0.3M sodium acetate and pelleted by centrifugation. The DNA pellet was then purified with 70% ethanol and air-dried for 2-3 hours. It was then dissolved in an appropriate volume (30-40 mL) of TE buffer (pH=8.0), treated with 3 µL of RNAase for removing RNA and finally, stored in the freezer at -20°C. The quality of extracted DNA was confirmed using electrophoresis after loading DNA from each sample on 1% agarose gel and placing the gel in the gel chamber (Continental Lab product. Inc.) containing 1X TBE buffer. Better quality band amplifying DNA samples were taken for quantification using a spectrophotometer.

Primer selection

Seven decamer primers of random sequence viz. OPA-17, OPA-18, OPB-06, OPB-09, OPC-01, OPF-08 and OPW-01 (Operon Technologies, Inc., Alameda, California, USA; Pattanayak et al., 2004; Shamsuzzaman et al., 2021) were initially screened on 10 potato genotypes and the best five (OPA-18, OPB-06, OPC-01, OPD-02 and OPW-01) were selected based on their ability to produce polymorphic DNA band.

PCR Reaction and Amplified PCR Products Visualization

Polymerase chain reactions were conducted for every sample with 12.5 µL of 2X Taq Mastermix (GeneON, Germany), 2.5 µL of RAPD primer, 7.5 µL of sterile deionized water and 2.5 µL of Genomic DNA (25 ng/ µl); total reaction mixture volume was 25 µL. An oil-free thermal cycler (Esco Technologies Swift™ Mini Thermal Cyclers) was used to perform DNA amplification following the thermal profile described by Shamsuzzaman et al., (2021).

PCR products of each sample were visualized and confirmed with gel electrophoresis at 85V for 50 minutes on 1.5% agarose gel (containing 1 µL of 10 mg/L ethidium bromide) in 1X TBE buffer. Two molecular weight markers: 100 bp (BIONEER, Cat. No. D-1030, South Korea) and 1kb (BIONEER, Cat. No. D-1040, South Korea) DNA ladder was also loaded on the left and right side of the gel, respectively. The gel was then gently removed from the gel chamber and put on a high-performance ultraviolet lightbox (UV transilluminator) to check the DNA amplification (observed as a band). The photograph was taken with a 'Gel Cam Polaroid' camera.

RAPD Bands Scoring and Data Analysis

The visualized RAPD bands were scored manually. For the presence of the band, one (1) decimal number and for the absence of the band, zero (0) was scored. DNAfrag (version 3.03) computer program was used to calculate the sizes of the band length. A data matrix was created pooling all the scores obtained from each RAPD primer. This data matrix was used to estimate genetic distance (GD), polymorphic loci, Nie's gene diversity, frequencies of polymorphism and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among genotypes investigated, with the help of a computer software namely POPGENE (version 1.31). The same program was used to estimate the homogeneity in different locus between the tested genotype pairs.

The genetic similarity values were calculated manually using the formula described by Lynch (1990): Similarity index (SI) $= \frac{2Nxy}{\mathrm{Nx+Ny}}$

Here, Nx and Ny are the number of bands in individuals x and y, respectively

RESULTS AND DISCUSSION

RAPD Band Pattern, Their Size and Polymorphism

Five (5) RAPD primers were selected (Fig. 1) from 7 pre-tested primers for further amplification based on their ability to amplify polymorphic patterns.

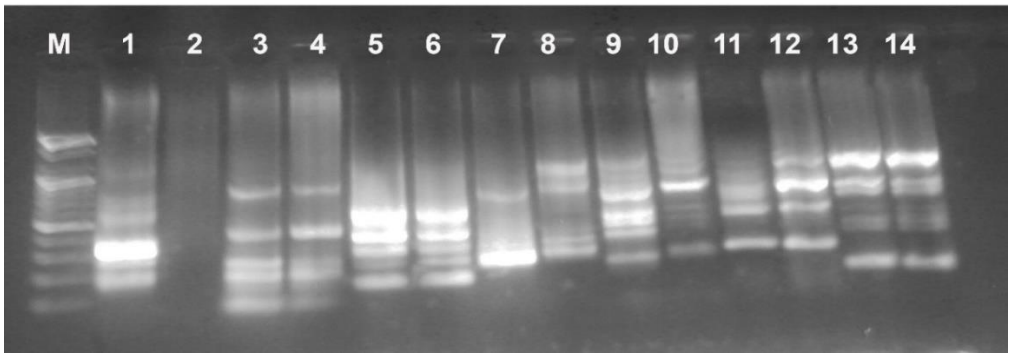
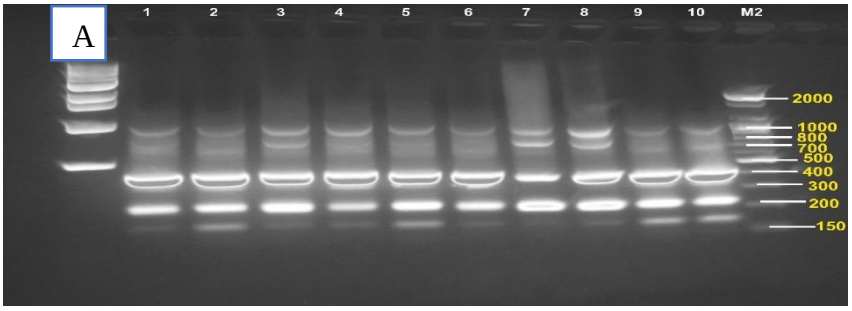


Figure 1. Amplified PCR products of 7 decamer RAPD primers using DNA of randomly selected two genotypes

(SL# 1-2: OPA-17; SL# 3-4: OPA-18; SL# 5-6: OPB-06; SL# 7-8: OPB-09; SL# 9-10: OPC-01; SL# 11-12: OPF-08 and SL# 13-14: OPW-01 (M = 100bp ladder; BIONEER, South Korea).

Each of the selected primer produced a comparatively maximum high-intensity band with minimal smearing, good technical resolution and sufficient variation among different genotypes (Table 1 and Fig. 2). They generated 33 distinct and differential amplified bands, out of which 28 were polymorphic. The average of total bands and polymorphic bands per primer is 6.6 and 5.6, respectively. The size of the bands ranged from 131-1188 bp. The highest number (10) of bands was generated by primer OPC-01 whereas, primer OPB-06 and OPF-08 produced the lowest number (5) of bands. Here, the percentage of polymorphic loci was ranged from 33.33% (by primer OPA-18) to 100% (by primer OPF-08 and OPW-01).



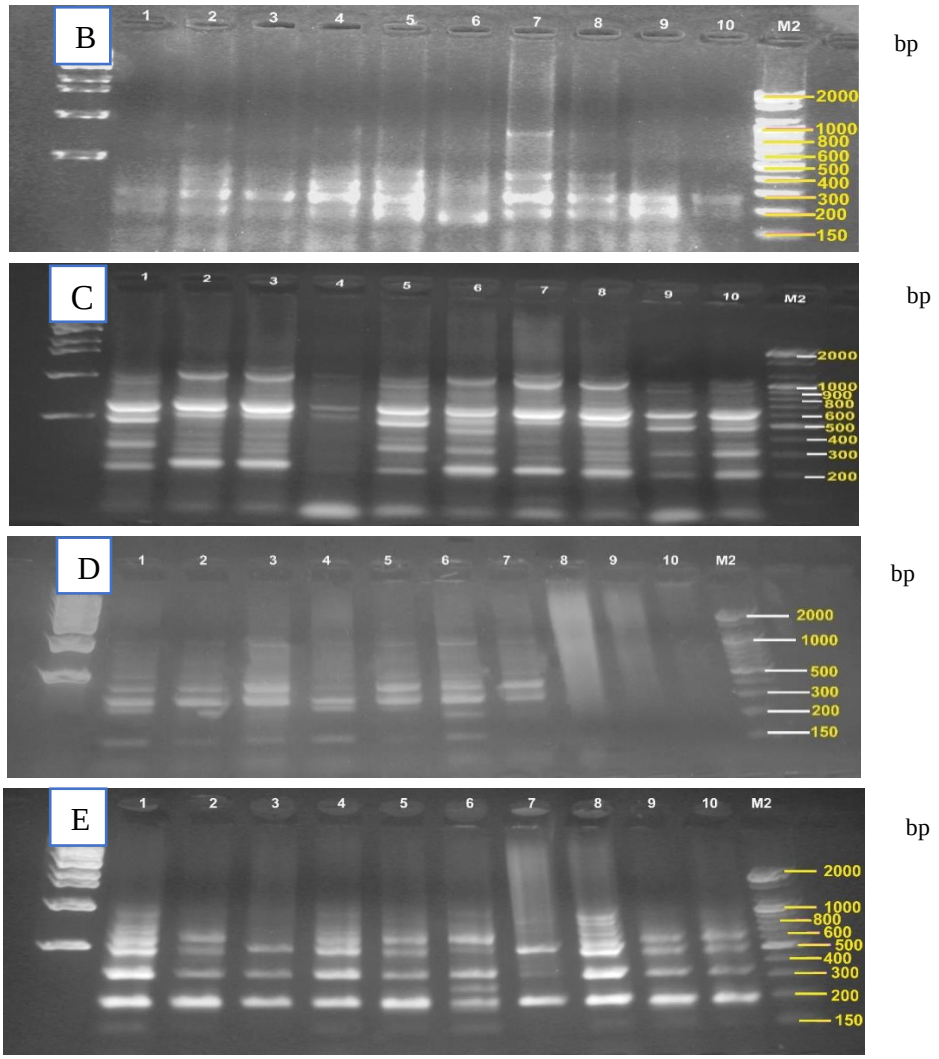


Figure 2. Amplified DNA pattern of 10 potato genotypes generated by A) OPA-18, B) OPB-06, C) OPC-01, D) OPF-08, E) OPW-01

(SL# 1: Comilla Local*; SL# 2:* Jham Alu*; SL# 3:* Deshi Pakri*; SL# 4:* Sindur Kota*; SL# 5:* Indur Kani*; SL# 6:* SAU Promising Genotype -4*; SL# 7:* Fata Pakri*; SL# 8:* Ishwardi Local*; SL# 9:* Sheel Bilati*; SL# 10:* SAU Promising Genotype -5. M1 and M2: Molecular weight marker (1 kb and 100 bp, respectively; BIONEER, South Korea)

Table 1. Features of selected RAPD primers and their amplification result in ten potato genotypes

Primer name	Sequences (5′- 3′)	(G+C) %	The Total no of bands scored	Size ranges (bp)	Number of polymorphic bands	The proportion of polymorphic loci (%)
OPA-18	AGGTGACCGT	60	06	131-796	02	33.33
OPB-06	TGCTCTGCCC	70	05	184-787	05	100
OPC-01	TTCGAGCCAG	60	10	209-1188	09	90
OPF-08	GGGATATCGG	60	05	174-904	05	100
OPW-01	CTCAGTGTCC	60	07	114-813	07	100
Total	-	310	33		28	
Average	-	62	6.6		5.60	84.85

AL-Salihy et al. (2014) screened 6 RAPD primers on four potato genotypes and successfully studied genetic diversity with five primers. ‘Band size range’ proximate to this analysis was found by Pattanayak et al. (2004) and Ahmad et al. (2013). Moreover, Verma and Singh (2019) reported 1.9 bands per potato cultivar and 4.55 bands per primer, which were lower than the present study regarding bands per genotypes and bands per primer. Besides, Tiwari et al. (2015) detected 2.25 bands per genotype and 7.71 bands per primer. They scored fewer bands per genotype but more bands per primer. The current study's average level of polymorphism (~85%) indicates that the genetic variation level among the studied genotypes is high. Similar polymorphism percentage (~82%) was detected by Onamu et al. (2016). In contrast, a lower level of average polymorphism (~53) was found by Salem and Hassanein (2017) in three potato genotypes with 11 RAPD primers. These variations may be due to the changes in used primers and genotypes. The primer sequence determines the size and number of DNA fragments. Various factors, viz., the primer sequence, quality and quantity of template and polymerase concentration, influenced the reproducibility of the RAPD technique (Afrasiab and Iqbal, 2012).

Gene frequency and gene diversity

The studied five primers showed a different level of gene diversity and different gene frequencies (Table 2). Gene frequency was ranged from 0.3 to 1. At the same time, gene diversity was valued from 0 to 0.7.

The highest gene frequency (1) and lowest gene diversity (0) were shown by the primer OPA-18 (at 1020, 661, 397 and 230 bp), OPC-01 (at 606 bp), and OPW-01 (at 196 bp). While the lowest gene frequency (0), i.e., the highest gene diversity (0.70), was shown by the primer OPC-01 (at 446 bp), OPF-08 (at 221 bp) and OPW-01 (at 813 bp). Khatab and El-Banna (2011) reported the gene frequency range and the frequency of polymorphic loci from 0.056 to 1 and 0 to 0.944, respectively. Their result was

resembling to the outcome of this investigation. In comparison, a lower gene frequency range (0.02 to 0.55) was reported by Paez et al. (2005).

Table 2. Molecular sizes of the loci, their diversities and frequencies among ten local potato genotypes

RAPD primer	Locus No.	Locus Size (bp)	Gene Frequency	RAPD primer	Locus No.	Locus Size (bp)	Gene Frequency
OPA-18	1	1020	1.0000	OPC- 01	6	446	0.3000
	2	796	0.7000		7	375	0.5000
	3	661	1.0000		8	317	0.7000
	4	397	1.0000		9	251	0.7000
	5	230	1.0000		10	209	0.6000
	6	189	0.5000	OPF-08	1	904	0.6000
OPB-06	1	787	0.4000		2	407	0.7000
	2	430	0.5000		3	354	0.7000
	3	317	0.9000		4	221	0.3000
	4	225	0.9000		5	174	0.6000
	5	184	0.4000	OPW-01	1	813	0.3000
OPC- 01	1	1188	0.6000		2	594	0.7000
	2	1018	0.5000		3	477	0.6000
	3	737	0.8000		4	389	0.7000
	4	606	1.0000		5	275	0.9000
	5	508	0.5000		6	196	0.1000

Inter-genotype Similarity Indices (Sij)

Inter-genotype means of the pair-wise similarity indices (Sij) was ranged from 42.45% - 92.67% (due to large-sized table, data are not presented here). The highest similarity indices of 92.67% were found between Jham Alu vs. Indur Kani genotype pair. So, the genetic distance was lower between that pair than the other genotype pairs. On the other hand, SAU Promising Genotype-4 vs. SAU Promising Genotype -5 pair showed the least Inter-variety similarity indices, 42.45%; therefore, the genetic distance was higher between that pair than the rest of the genotype pairs.

Similarity values close to the present investigation were found by Isenegger et al. (2001) and Das et al. (2010). On the other hand, Gauchan et al. (2012) reported a lower (55.2% to 69%) similarity value among 4 Nepali local potato cultivars with 10 RAPD primers. It may happen due to using fewer cultivars and primers.

Nei's (1972) genetic distance and genetic identity

Pair-wise comparisons of Nei's (1972) genetic identity among 10 potato cultivars were ranged from 0.485 to 0.939 (Table 3). The highest Nei's genetic identity (0.939) was observed in Sheel Bilati and SAU Promising Genotype-5 genotype pair. In comparison, the lowest genetic identity (0.485) was estimated in Deshi Pakri vs. Sheel Bilati, Deshi Pakri vs. SAU Promising Genotype-5, Sindur Kota vs. SAU Promising Genotype-4, Indur Kani vs. Sheel Bilati, Indur Kani vs. SAU Promising Genotype-5 and Fata Pakri vs. SAU Promising Genotype-5 genotype pairs.

Table 3. Summary of Genetic identity (above diagonal) and Nei's (1972) genetic distance (below diagonal) values among 10 local potato genotypes

Genotypes	Comilla Local	Jham Alu	Deshi Pakri	Sindur Kota	Indur Kani	SAU-4	Fata Pakri	Ishwardi Local	Sheel Bilati	SAU-5
Comilla Local	****	0.727	0.727	0.758	0.667	0.546	0.576	0.576	0.515	0.5152
Jham Alu	0.319	****	0.818	0.788	0.879	0.576	0.606	0.606	0.546	0.5455
Deshi Pakri	0.319	0.201	****	0.667	0.758	0.697	0.606	0.546	0.485	0.4848
Sindur Kota	0.278	0.238	0.406	****	0.727	0.485	0.636	0.636	0.515	0.5152
Indur Kani	0.406	0.129	0.278	0.319	****	0.636	0.667	0.606	0.485	0.4848
Fata Pakri	0.606	0.552	0.361	0.724	0.452	****	0.546	0.606	0.546	0.4848
SAU-4	0.552	0.501	0.501	0.452	0.406	0.606	****	0.636	0.576	0.5152
Ishwardi Local	0.552	0.501	0.606	0.452	0.501	0.5001	0.45	****	0.758	0.697
Sheel Bilati	0.663	0.606	0.724	0.663	0.724	0.606	0.552	0.278	****	0.939
SAU-5	0.663	0.606	0.724	0.663	0.724	0.7234	0.663	0.361	0.063	****

Nei's (1972) genetic distance among 10 potato cultivars was from 0.063 to 0.724. The highest Nei's genetic distance (0.724) was observed in Deshi Pakri vs. Sheel Bilati, Deshi Pakri vs. SAU Promising Genotype-5, and Sindur Kota vs. SAU Promising Genotype 4, Indur Kani vs. Sheel Bilati, Indur Kani vs. SAU Promising Genotype 5 and Fata Pakri vs. SAU Promising Genotype -5 genotype pairs. At the same time, the lowest genetic distance (0.063) was estimated in Sheel Bilati and Promising Genotype-5 genotype pair.

Yasmin et al. (2006) reported Nei's (1972) genetic identity from 0.6530 to 0.8674 and genetic distance from 0.154 to 0.558 among six potato cultivars. Again, Hoque et al. (2019) reported significantly higher genetic variation (0.55 to 1) among eight potato genotypes. They analyzed both indigenous and high-yielding varieties, which might be the reason for their higher genetic variation.

UPGMA Dendrogram

Dendrogram, based on Nei's (1972) genetic distance using Unweighted Pair Group Method with Arithmetic Means (UPGMA), indicated the segregation of 10 potato genotypes into two main clusters viz., 1 and 2 (Fig. 3). Cluster-1 contained seven potato genotypes and cluster-2 contained three genotypes.

Again, both clusters are divided into sub-clusters. The cluster-1 had 2 sub-clusters SI (SAU-4) and SII (Comilla Local, Jham Alu, Deshi Pakri, Sindur Kota, Indur Kani, and Fata Pakri). Cluster-2 was split into two sub-clusters where Ishwardi Local was in one sub-cluster and Sheel Bilati, and SAU Promising Genotype 5 in the other sub-cluster. Here, Sheel Bilati and SAU Promising Genotype-5 genotype pairs are more closely related than other genotype pairs. Likewise, Brenna (2004) and Shamsuzzaman et al. (2021) showed the genetic relationship among 12 potatogenotypes with similar dendrogram.

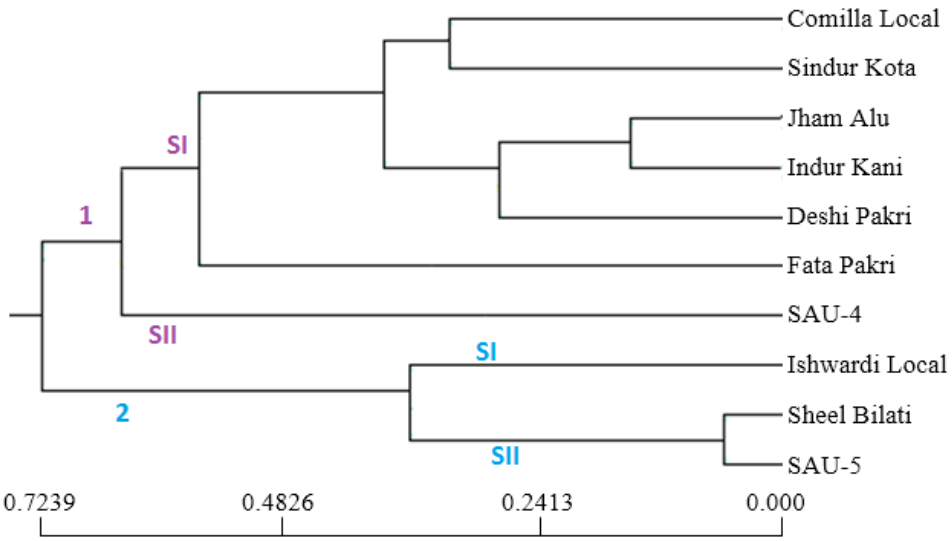


Figure 3. UPGMA dendrogram displaying the phylogenetic relationship among 10 potato genotypes based on Nei's (1972) genetic distance

CONCLUSION

The present study clearly indicates variations in the investigated potato genotypes. It also shows the effectiveness of the RAPD technique in molecular characterization and in studying a substantial number of polymorphisms or diversity among the different local potato genotypes. Although more primers should be screened to provide conclusive data on Bangladeshi 'local potato' genetic diversity, Sheel Bilati and SAU Promising Genotype-5 might be used in future potato improvement programs.

Therefore, the findings could be the guideline for prospective fingerprinting and genetic diversity research of potato. Moreover, using this basic information, it may be possible to identify genetic diversity of Bangladeshi potato cultivars which can be used as a complementary tool for potato breeding. Further investigation can now be done using this set of useful primers on major cultivated varieties and local potato cultivars of Bangladesh. However, it is suggested that more molecular markers are required to have a better understanding of the presence of genetic variability in potato germplasm and, consequently, a more efficient utilization of this exciting variability for improvement of the potato crop in Bangladesh.

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