Assessment of genetic diversity among tomato varieties using RAPD markers

Abstract

The aim of this study was to assessment the genetic diversity of collected tomato germplasm using RAPD markers.The relationship between genetic diversity estimates and germplasm collections is important to facilitate effective germplasm collection, evaluation and utilization.RAPD markers are also more effective and efficient than morphological markers in determining recombination variability in genetic diversity assessment. The experiment was conducted at the Laboratory of Molecular Horticulture in Agrotechnology Discipline, Khulna University, Khulna, Bangladesh from October to December 2022. Sixteen isolates were amplified three times with eight primers. RAPD fragments were identified by the name of the primer and the size of the amplified bands. Only well-defined fragments were rated as having two alleles (present and absent) of the putative locus. All tomato Varieties were in two distinct clusters, Cluster I consisted of 6 varieties and cluster II contained the remaining 10 varieties. BARI Tomato-2 and Suraksa were most distinctly related and Ruma-19 and Guli were most closely related.

Keywords: RAPD markers, germplasm, morphological markers, genetic analysis

Introduction

Tomato is an important and remunerative vegetable, ranking first in world vegetable production (Statista, 2021). There are more than 7500 tomato varieties which are successfully bred and grown worldwide. Genetic analysis of tomato is essential for the enhancement of the genetic yield potential and maximum utilization of the desirable characters for synthesis of any ideal genotypes (Kumar *et al.*, 2003).

The relationship between genetic diversity estimates and germplasm collections is important to facilitate effective germplasm collection, evaluation and utilization (Oladosu et al., 2021). Genetic diversity information is the first step in crop improvement (Poczai et al., 2011 and Herison et al., 2018).

The diversity of tomatoes can be assessed by morphological and molecular characters. The use of molecular markers is a modern and appropriate method for varietal identification as it is faster and cost effective (Korir et al., 2012).

RAPD is an effective method for tomato varietal identification, polymorphism study, genetic mapping, biodiversity, genetic map construction, hybridization and

phylogenetic relationships (Alam et al, 2012; Salunke et al. Singh et al. 2014 and Rai et al. 2016).

RAPD markers are also more effective and efficient than morphological markers in determining recombination variability in genetic diversity assessment (Biswas et al., 2009; Paul et al., 2010). Moreover, the main advantages of RAPD over other molecular methods are the low sample DNA requirements, high frequency of detectable polymorphic DNA bands and independent from the effects of environmental factors (Kuras *et al.*, 2004).

BARI releases and other available varieties should be evaluated based on their genomic information (Alam et al., 2012). Moreover, private companies release various varieties under different trade names without indicating their origin.

Therefore, it is necessary to characterize and analyze the genetic diversity of tomato varieties before establishing a program to improve them. The aim of this study was to assessment the genetic diversity of collected tomato germplasm using RAPD markers.

Materials and Methods

The experiment was conducted at the Laboratory of Molecular Horticulture in Agrotechnology Discipline, Khulna University, Khulna, Bangladesh from October to December 2022. The experimental material was 16 tomato varietiesincluding eight improved varieties developed by Bangladesh Agricultural Research Institute (BARI), namely BARI Tomato 2, BARI Tomato 8, BARI Tomato14, BARI Tomato15, BARI tomato16, BARI tomato17, BARI tomato18, BARI tomato19 and eight cultivars collected from farmers field of Khulna region namely Bonkim Ruby, Pusa Ruby. Suraksa, Patharkuchi, Ruma VF, Ruma 19, Guli and Paltola.

Selection of primers

Primers were selected based on GC content, band intensity, the presence of smearing, intra-individual consistency and population identification potential. The primers with GC content above 60% were suitable for RAPD analysis.

Table 1. Primers and their corresponding sequences

Sl. No.	Primer Code	Primer Sequence (5´- 3´)	(GC) content (%)
1	OPA-11	CAA TCG CCG T	60%

2	OPA-20	GTT GCG ATC C	60%
3	OPB-15	GCA GGG TGT T	60%
4	OPK-3	CCA GCT TAG G	60%
5	OPK-5	TCT GTC GAG G	60%
6	OPK-18	CCT AGT CGA G	60%
7	OPM-8	TCT GTT CCC C	60%
8	OPM-18	CAC CAT CCG T	60%

Sample collection and preparation

About 5 g of leaves (10-15 days old) were washed with distilled water, wiped with 70% (v/v) ethanol, and air-dried, of which 0.1 g was weighed and used for DNA extraction. The samples were then freeze-dried in an ultra-low temperature refrigerator (Thermo Scientific, USA) at -86ºC to freeze-dry the samples. The remaining leaves were stored in sealed plastic bags at -80ºC for further use.

DNA extraction, purification andquantification

The freeze-dried (lyophilized) leaves were finely ground in liquid nitrogen and the final powder of the leaves was found. Two hundred (200) mg of leaf powder was taken in a sterile 1.5 mL eppendorf tube. DNA extraction was performed using Pure Link Leaf DNA Purification Kit (Invitrogen by life technologies, USA) according to the manufacturer's protocol. Genomic DNA from 16 tomato cultivars was extracted and stored at -20°C. DNA extracts were subjected to electrophoresis in a 1% agarose gel to confirm DNA content.

Purified DNA was quantified by using a spectrophotometer (Multiskan GO Microplate, Thermo Fisher Scientific, Germany) at 260 nm. The final concentration of template DNA was adjusted to 50 ng μL^{-1} for PCR (Kandan, 2013) and stored at -20°C.

Polymerase chain reaction (PCR) and amplification

Amplification reaction mixture for RAPD analysis: polymerase chain reaction was performed for each isolate in 0.2 mL thin-walled PCR tubes in a reaction volume consisting of the following components of total 20 µL (Table 2).

Table 2. Polymerase chain reaction chemicals for RAPD analysis

Sl. No.	PCR reaction chemicals	Concentrations	Quantity (µL)
1.	Genomic DNA	50 ng μL^{-1}	1.0
2.	Reaction buffer without $MgCl_2$	10 X	1.0

3.	dNTPs	10 mM	0.2
4.	Random primer	5 pM μL^{-1}	1.0
5.	Taq DNA polymerase	5 U μL^{-1}	0.2
6.	$MgCl_2$	25 mM	1.2
7.	Molecular water	-	15.4
	Total volume of reaction mixture		20.0

Amplification was performed in a programmable thermal cycler (Biometra, Germany). The amplification protocol was optimized to include the following steps for successful amplification of PCR products.

Table 3. Thermal profile for RAPD analysis

Thermal profile		Motives
Temperature (ºC)	Duration (minute)	
94.0	02	Hot start
94.0	0.5	Initial denaturation
30.0	01	Primer annealing
72.0	01	Primer extension
Repeated for 40 cycles		
72.0	05	Final extension
4.0	-	Hold

Agarose gel electrophoresis

The amplified products were resolved by electrophoresis on 1% agarose gels stained with ethidium bromide (at 0.5 µg mL^{-1}) run at 60 volts in Tris Acetate EDTA buffer (1.0 × TAE) for one hour. The amplicon profiles of all primers isolated were visualized with a computer program (Biometra Gel Recording, A Biodoc Analyze version 2.2, Germany) under a UV Tech gel recording system. A molecular weight marker of 1 kbp (Direct load, Sigma Aldrich, USA) was used to determine the size of the amplicons.

PCR product analysis

To study genetic diversity, each isolate was manually scored for the presence of a specific amplification product. Sixteen isolates were amplified three times with eight primers. RAPD fragments were identified by the name of the primer and the size of the amplified bands. Only well-defined fragments were rated as having two alleles (present and absent) of the putative locus. Each feature of each primer was compared based on binary data of presence (1) versus absence (0) of RAPD products (bands) of the same length. Genetic relationships among the 16 tomato varieties were analyzed from the 0/1 matrix data of the random primer mapping using the PAUP (Pasimony) version 4.0 computer program and fragment sizes were estimated according to standard 1kb DNA markers. The matrices were analyzed using pairwise distance coefficients. The dendrograms were constructed according to the unweighted pairwise group method (UPGMA) using arithmetic averaging (Sneath and Sokal, 1973) using pairwise distance matrices. The data were also used for cluster analysis by the neighbor-joining (NJ) method (Saitou and Nei, 1987) to estimate the evolutionary relationships between isolates. DNA migration distances in gels were analyzed with the PyEIph, version 1.4 computer program, and DNA molecular weights were calculated with the Excel-2010 computer program. Polymorphism percentage is the ratio of polymorphic bands to the total number of bands for the respective primers.

Results and Discussion

DNA fingerprint analysis

Genetic variation was detected among the sixteen (16) tomato varieties by following RAPD technique. Eight random primers were used in this study and distinct and reproducible bands were amplified (Table 4). Eight primers amplified up to 35 number of DNA fragments. The RAPD experiments yielded a total of 35 bands, 29 of them showed polymorphism. The percentage of polymorphic loci was 81.87%thatindicating a high level of polymorphism. Primers OPB-11 and OPM-18 produced maximum amplified fragments (6) and gave significant amplification results. Moonmoon (2006); Biswas et al. (2009) reported the level of polymorphism in different crops (Tomatoand egg plants respectively). The average number of bands primer^{-1} was 4.37%, while Shah et al., 2015 reported an average of 5.1% bands primer^{-1}using 20 RAPD markers in 21 tomato genotypes, whereas Paul and Saha

(2018) observed 5 bands using 3 RAPD markers in 28 tomato genotypes. El-Hady et al. (2010) also observed 11.57 bands using 7 different primers and Tabassum et al. (2013) found 29.2 bands/primer using 20 primers with the highest number of polymorphic bands being 47 and the lowest being 13.

Table 4. Amplified and polymorphic bands of tomato germplasm generated by 8 primers

Sl. No.	Primer Code	Maximum number of amplified bands	Number of Polymorphic bands	Number of Monomorphic bands	Polymorphism (%)
1	OPA-11	06	06	0.0	100
2	OPA-20	04	03	01	75
3	OPB-15	04	04	0.0	100
4	OPK-3	04	02	02	50
5	OPK-5	03	03	0.0	100
6	OPK-18	05	04	01	80
7	OPM-8	03	02	01	66.66
8	OPM-18	06	05	01	83.33
Total		35	29	06	
Average		4.37	3.62	0.75	81.87

Polymorphism in 16 tomato genotypes

DNA polymorphisms are detected based on the presence and absence of bands. The absence of bands may be due to the inability of primers to anneal due to the different nucleotide sequences of some individuals, or insertions or deletions between primer sites (Clarck and Lanigan, 1993). The frequency of polymorphic bands found varied depending on the primer (Table 4).

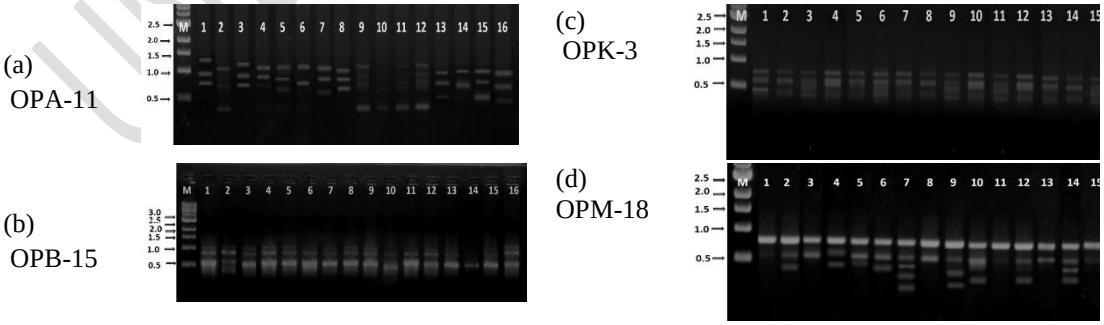

Figure 1. RAPD fragment profiles of 16 selected tomato varieties generated by using OPA-11, OPB-15, OPK-3 and OPM-18 primers

BARI Tomato-2, 2. BARI Tomato-8, 3. BARI Tomato-14, 4. BARI Tomato-15, 5.BARI Tomato-16, 6. BARI Tomato-17, 7. BARI Tomato-18, 8. BARI Tomato-19, 9. Bonkim Ruby, 10. Pusa Ruby and 11. Suraksa, 12. Patharkuchi, 13. Ruma VF, 14. Ruma 19, 15. Paltola and 16. Guli (M- Kilo base molecular weight ladder)

The percentage of polymorphisms ranged from 50% to 100%, with an overall mean of 81.87%. The high level of polymorphism shown by the percentage of polymorphic loci (100%) suggests that RAPD markers can be considered as an effective tool for estimating genetic diversity in diversevarieties of tomato. From this study, a total of 81.87% of polymorphic fragments were established. The PCR amplification products using RAPD primers are shown in Figure 1 (a b, c and d). From Figure 1 (d), the 0.8 Kb size monomorphic band of primerwere found by using OPM-18 primer. In this study most of the primers produced ≥80% polymorphic fragments.

Cluster analysis

A dendrogram was constructed from the data generated by amplifying 16 tomato varieties with eight primers, showing two distinct clusters (Figure 2). Cluster I consisted of 6 varieties and cluster II contained the remaining 10 varieties. Cluster I was further divided into 2 sub clusters; I A and IB. Similarly, cluster II was divided into 2 sub clusters; II A and II B. In sub cluster IA, V10 (Pusa Ruby) and V11 (Suraksa) were grouped together. In subgroup IB, V4 (BARI Tomato-15), V5 (BARI Tomato-16), V9 (Bonkim Ruby) and V12 (Patharkuchi) were grouped together. In subgroup IIA, V2. BARI Tomato-8, V6 (BARI Tomato-17) and V13 (Ruma VF) were grouped together. The remaining seven varieties (V1. BARI Tomato-2, V3. BARI Tomato-14, V7. BARI Tomato-18, V8. BARI Tomato-19, V14. Ruma 19, V15. Paltola and V16. Guli) were grouped together in subgroup IIB. Genetically similar genotypes were gathered in the same cluster (Figure 2). Elham et al. (2010) reported more or less similar results for UPGMA method using seven RAPD primers to classify eight Egyptian tomato varieties into three different clusters. Naz et al. (2013) also reported on the phylogenetic relationship and diversity of 25 tomato varieties by using 15 RAPD primers

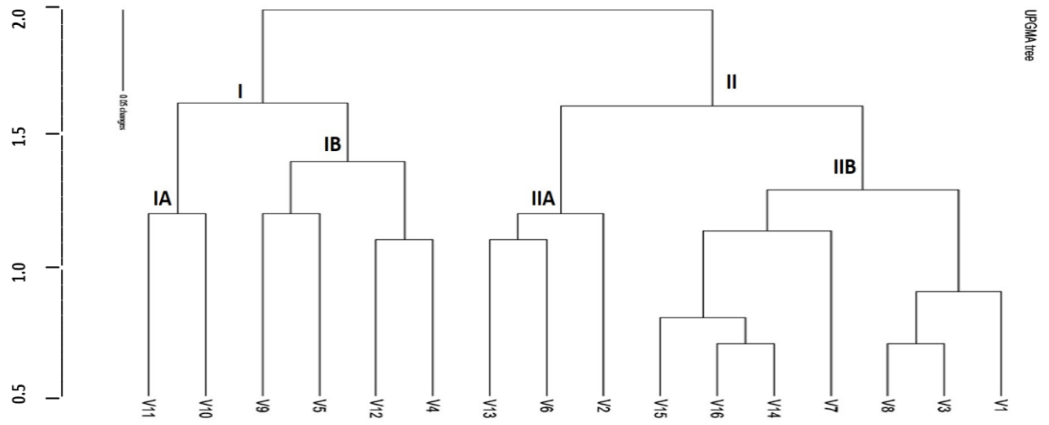

Figure 2. Dendrogram constructed based on combined data obtained from using eight primers in RAPD analysis against sixteen tomato varieties

V1. BARI Tomato-2, V2. BARI Tomato-8, V3. BARI Tomato-14, V4. BARI Tomato-15, V5.BARI Tomato-16, V6. BARI Tomato-17, V7. BARI Tomato-18, V8.BARI Tomato-19, V9.Bonkim Ruby, V10.Pusa Ruby and V11. Suraksa, V12. Patharkuchi, V13. Ruma VF, V14. Ruma 19, 15. Paltola and 16. Guli

Evolutionary relationship among the varieties

Neighbor-joining (NJ) trees show the evolutionary relationships among the varieties. To construct the NJ tree, BARI Tomato-2 (V1) was used as the common emergence point (ancestor) (Figure 3). Two distinct populations were found here. Cluster I contain only two isolates, both varieties released by BARI, and the remaining isolates belong to cluster II. Cluster II was divided into two groups, one containing only Paltola (V15), and the other into two groups.

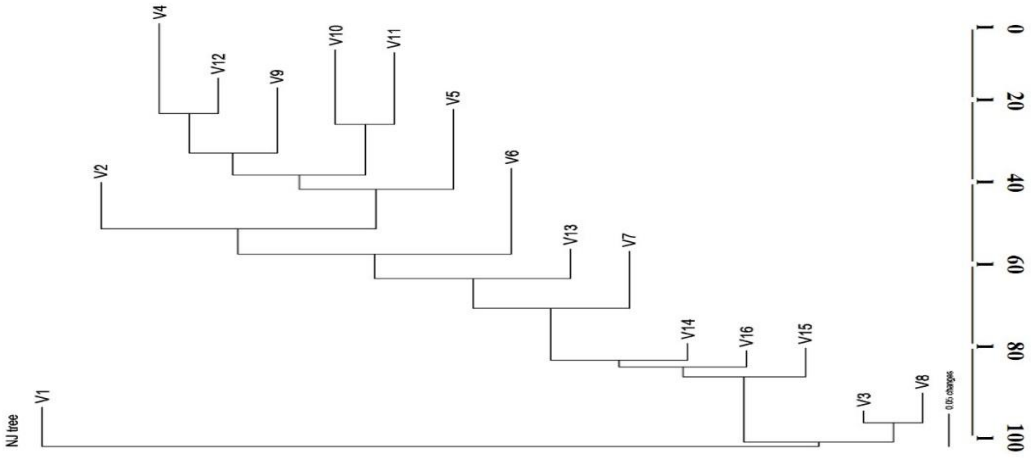

Figure 3. Evolutionary relationships among tomato varieties through neighbor-joining method

V1. BARI Tomato-2, V2. BARI Tomato-8, V3. BARI Tomato-14, V4. BARI Tomato-15, V5.BARI Tomato-16, V6. BARI Tomato-17, V7. BARI Tomato-18, V8.BARI Tomato-19, V9.Bonkim Ruby, V10.Pusa Ruby and V11. Suraksa, V12. Patharkuchi, V13. Ruma VF, V14. Ruma 19, 15. Paltola and 16. Guli

Pair wise genetic distance

By analyzing the matrix constructed from the amplification data, the largest heterogeneity coefficient of 67% was observed between varieties BARI Tomato-2 and Suraksa; one of them is a re-marketed variety of BARI and the other is a local cultivar from the Khulna region. The minimum pairwise distance of 6% was observed between two varieties, Ruma-19 and Guli, both from the Khulna region (Table 5). The greater difference between the lowest and highest genetic distance indicate a wide range of variability among the 16 accessions of tomato.

Table 5. Pair wise genetic distance of 16 tomato varieties based on DNA polymorphism

Sam	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
V1	-															
V2	0.48	-														
V3	0.12	0.48	-													
V4	0.58	0.29	0.64	-												
V5	0.51	0.29	0.45	0.32	-											
V6	0.51	0.22	0.51	0.45	0.45	-										
V7	0.38	0.22	0.38	0.51	0.32	0.32	-									
V8	0.12	0.54	0.06	0.64	0.45	0.51	0.38	-								
V9	0.48	0.25	0.48	0.29	0.22	0.41	0.41	0.54	-							
V10	0.51	0.35	0.38	0.51	0.45	0.38	0.51	0.45	0.35	-						
V11	**0.67**	0.32	0.54	0.41	0.29	0.29	0.48	0.54	0.32	0.22	-					
V12	0.58	0.22	0.51	0.19	0.32	0.32	0.45	0.58	0.22	0.32	0.22	-				
V13	0.38	0.22	0.38	0.51	0.25	0.19	0.19	0.38	0.41	0.51	0.41	0.45	-			
V14	0.19	0.29	0.19	0.58	0.45	0.32	0.19	0.25	0.48	0.45	0.61	0.51	0.19	-		
V15	0.16	0.32	0.22	0.61	0.48	0.35	0.22	0.29	0.45	0.54	0.64	0.54	0.22	0.09	-	
V16	0.19	0.35	0.19	0.64	0.45	0.32	0.19	0.19	0.54	0.51	0.61	0.58	0.19	**0.06**	0.09	-

V1. BARI Tomato-2, V2. BARI Tomato-8, V3. BARI Tomato-14, V4. BARI Tomato-15, V5. BARI Tomato-16, V6. BARI Tomato-17, V7. BARI Tomato-18, V8.BARI Tomato-19, V9.Bonkim Ruby, V10.Pusa Ruby and V11. Suraksa, V12. Patharkuchi, V13. Ruma VF, V14. Ruma 19, V15. Paltola and V16. Guli

Conclusion

The selected varieties were genetically distinct from each other. All tomato Varieties were in two distinct clusters, Cluster I consisted of 6 varieties and cluster II contained the remaining 10 varieties. BARI Tomato-2 and Suraksa were most distinctly related and Ruma-19 and Guli were most closely related.

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