Progressive Agriculture 29 (4): 276-283, 2018 **ISSN: 1017 - 8139**

Progressive Agriculture
Journal homepage:http://www.banglajol.info/index.php/PA

Assessment of genetic diversity of different tomato genotypes using RAPD markers

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Abstract

The present investigation was carried out for assessment of genetic diversity among the 28 tomato genotypes though three random amplified polymorphic DNA (RAPD) markers. A total of 15 distinct DNA fragments ranging form 100-1000 bp were amplified by using three selected primers of which 5.00 polymorphic bands per primer and over all polymorphic loci was 100 percent. The extent of genetic diversity among these genotypes was computed through parameters of genetic diversity and Shanon's information indices. The highest genetic distance was observed among the accession Cl-3d-0-99 (V93) vs. F_1 (G X V12), F_1 (G X V17), F_1 (G X V29) and Durch fuegel (G) vs. F_1 (G X V17) and F_1 (G X V93) vs. F_1 (G X V12), F_1 (G X V17), while the lowest genetic distance was observed among the accessions Fut. Wed Abrid (V94) vs. Sunlight pole (V67) and F_1 (V67 X VG) vs. F_1 (V17 X VG), F_1 (V93 X VG) and F_1 (V93 X V17) vs. F_1 (V17 X VG), F_1 (V67 X VG), F_1 (V93 X VG). The Unweighted Pair Group Method of Arithmetic Mean (UPGMA) dendrogram based on Nei's genetic distance divided the genotypes into two main clusters: A & B. Cluster 'A' consists of 19 accessions and cluster 'B' consists of 9 accessions. The information generated from this study could be useful in gene mapping and marker assisted breeding for future tomato breeding programs.

Key words: Tomato genotypes, genetic diversity, RAPD markers, cluster

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Introduction

The tomato (*Lycopersicon esculentum* Mill.), belongs to the family *Solanaceae* having somatic chromosome number 2n= 24, is a major vegetable crop that has achieved tremendous popularity for fresh consumption and processed products. Intense breeding programs worldwide have resulted in tomato being the second most important vegetable in production in the world (FAOSTAT, 2014). In 2016, the worldwide production of tomatoes totaled 179 million tons (FAOSTAT, 2017). In Bangladesh, the total production of tomato was 0.37 million tons in the year 2016 (FAOSTAT, 2017). Due to increasing the popularity and consumption of fresh and processed tomato products, the crop has become promising in Bangladesh. Development of high yielding varieties with wider adaptability and disease resistance is necessary to enhance total production for meeting the domestic demand and earning foreign exchange.

Genetic diversity is an important factor in crop improvement programme and the analysis of genetic variability within and among breeding materials is of fundamental concern for plant breeders (Chakravarthi and Naravaneni, 2006). More diverse the parents,

greater are the chances of obtaining high heterotic F_1 and broad-spectrum variability in segregation generations (Arunachalam, 1991). In order to develop desired tomato cultivars, it is important to catalogue the genetic diversity within the germplasm (Islam *et al.,* 2004). Genetic diversity may be assessed by phenotypic markers or descriptors due to their omnipresence and easy availability (Paterson *et al.,* 1996). However, the approach has the limitation in terms of authenticity due to having greater environmental effect thus cannot be used as an unambiguous marker. Molecular marker is the powerful tool to overcome this limitation to evaluate the degree of genetic diversity (Ferreira, 2006). Among the several molecular markers, random amplified polymorphic DNA (RAPD) was found simple and efficient (Welsh and McClelland, 1990) and it did not require any kind of sequence information (Karp *et al*., 1997). Advantages of RAPD marker include suitability for work in anonymous genomes; applicability to work where limited DNA is available, efficiency and low expense and is useful in distinguishing individuals, cultivars or accessions (Hadrys *et al.,* 1992; Karp *et al.,* 1996). In tomato, RAPD methods have been allowed as fast and effective approaches for detecting polymorphism at the DNA level (Kochieva *et al*., 2002) and have been widely used in variety identification (Peteira *et al*., 1999), genetic relatedness (Noli *et al*., 1999), and genetic diversity analysis (Zhao *et al*., 2002; Zhu *et al*., 2004). Therefore, the present investigation was conducted to find out the genetic diversity and genetic relationship within the selected tomato genotypes using three RAPD markers.

Materials and Methods

The study was conducted in the molecular laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. Twenty eight tomato genotypes were used in this study (Table 1). In order to carryout RAPD analysis, fresh leaf samples of tomato were collected from plants grown in earthen pots as a source of DNA.

***Extraction of genomic DNA*:** DNA was extracted from fresh leaves by following the cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). Total genomic DNA was isolated from young actively growing fresh leaf tissues following SDS extraction, phenol: chloroform: isoamyl alcohol purification and ethanol precipitation method. Approximately 25 mg of leaf tissues was cut into small pieces, homogenized and digested with extraction buffer [50 mM Tris-HCl, 25 mM EDTA (Ethylene Diamine Tetra Acetic acid), 300 mM NaCl and 1% sodium dodecyl sulphate (SDS)] at 65ºC for 15 minutes. DNA was purified by extraction with phenol: chloroform: isoamyl alcohol (25: 24: 1, v:v:v). DNA was precipitated using absolute alcohol in the presence of 0.3M sodium acetate and pelleted by centrifugation. The pellets were then washed with 70% ethanol, air dried and resuspended in an appropriate volume of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 8.0). DNA quality was checked by electrophoresis in a mini-gel. DNA samples were then quantified using a UV-spectrophotometer. Genomic DNA concentrations, absorbance reading of the DNA samples were recorded at 260 nm using a spectrophotometer UV lamp. The DNA samples were evaluated both quantitatively and qualitatively using 1.0 % agarose gel.

Primer selection: Initially, 15 primers of random sequence were screened on a sub sample of two randomly chosen individuals from two different varieties to evaluate their suitability for amplification of the DNA sequences, which could be scored accurately. Primers were evaluated based on intensity of bands, consistency within individual, presence of smearing, and potential for population discrimination. A final subset of three primers exhibiting good quality banding patterns and sufficient variability were selected for further analysis.

PCR amplification: Conditions for RAPD amplification reactions were based on Williams *et al.* (1990) with some modifications. PCR reactions were performed for each DNA sample in a 24 µl reaction

mix containing 4 µl of 10X Taq DNA polymerase buffer, 2 µl of 10 µM primer, 0.5 µl of 250 µM dNTPs, 0.3 µl of 0.25 unit *Taq* DNA polymerase, 2 µl of genomic DNA and the rest amount of sterile deionized water to prepare 24 µl reaction mixtures. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94°C for 4 minutes followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 35°C for 1 minute and extension at 72°C for 2 minutes. After the last cycle, a final step of 7 minutes at 72°C was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4°C.

Table 1. The name and sources of 28 genotypes of tomato used in the experiment

SL. No.	Genotype code	Parents and F_1s	Sources of collection
1	(V12)	World champion (V12)	BARI,Gazipur
2	(V17)	Flobidal (V17)	BARI,Gazipur
3	(V29)	Big cherry (V29)	BARI,Gazipur
4	(V67)	Sunlight pole (V67)	BARI,Gazipur
5	(V93)	Cl-3d-0-99 (V93)	BARI,Gazipur
6	(V94)	Fut. Wed Abrid (V94)	BARI,Gazipur
7	(V230)	TC0020-10-41-28-3-0=12 (V230)	BAU
8	(G)	Durch fuegel (wild) (G)	Germany
9	V12 X G	World champion X Durch fuegel	Developed by crossing
10	V17 X G	Flobidal X Durch fuegel	Developed by crossing
11	V29 X G	Big cherry X Durch fuegel	Developed by crossing
12	V67 X G	Sunlight pole X Durch fuegel	Developed by crossing
13	V93 X G	Cl-3d-0-99 X Durch fuegel	Developed by crossing
14	V94 X G	Fut. Wed Abrid X Durch fuegel	Developed by crossing
15	V230 X G	TC0020-10-41-28-3-0=12 X Durch fuegel	Developed by crossing
16	G X V12	Durch fuegel X World champion	Developed by crossing
17	G X V17	Durch fuegel X Flobidal	Developed by crossing
18	G X V29	Durch fuegel X Big cherry	Developed by crossing
19	G X V67	Durch fuegel X Sunlight pole	Developed by crossing
20	G X V93	Durch fuegel X Cl-3d-0-99	Developed by crossing
21	G X V94	Durch fuegel X Fut. Wed Abrid	Developed by crossing
22	V93 X V17	Cl-3d-0-99 X Flobidal	Developed by crossing
23	V93 X V67	Cl-3d-0-99 X Sunlight pole	Developed by crossing
24	V93 X V230	Cl-3d-0-99 X TC0020-10-41-28-3-0=12	Developed by crossing
25	V17 X V93	Flobidal X Cl-3d-0-99	Developed by crossing
26	V230 X V93	TC0020-10-41-28-3-0=12 X Cl-3d-0-99	Developed by crossing
27	V94 X V93	Fut. Wed Abrid X Cl-3d-0-99	Developed by crossing
28	V230 X V94	TC0020-10-41-28-3-0=12 X Fut. Wed Abrid	Developed by crossing

Agarose gel electrophoresis: The amplified products were separated electrophoretically on 1.5% agarose gel containing ethidium bromide. The gel was prepared using 2.25 g agarose powder, 100 ml 1X TBE buffer and 8 µl ethidium bromide. Electrophoresis was conducted in 1X TBE buffer at 90 V for 72 minutes. A

molecular weight marker DNA (100 bp ladder) was also electrophoresed alongside the PCR products. Bands were observed under UV Transilluminator and photographed by Image Documentation System.

***Data analysis*:** The RAPD markers were scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. For more accuracy, band scoring was performed by two independent persons. Bands not identified by the two readers were considered as non-scorable. The scores obtained using all primers in the RAPD analysis were then pooled for constructing a single data matrix. This was used for estimating polymorphic loci, Nei (1972) gene diversity, genetic distance and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram using computer program POPGENE (Version 1.31) (Yeh *et al.,* 1999).

Results and Discussion

***RAPD profile*:** Among the 15 primers initially tested, 3 primers, OPG-01, OPG-02, and GLA-05 produced comparatively maximum number of high intensity bands with minimal smearing (Figure 1-3). Selected 3 primers generated 15 bands with size ranging from 300-1000 bp ranging from 2 to 9 bands (Table 2). In our study, the average bands per primer was 5 bands compared with (Shah *et al*., 2015) who reported an average of 5.1 bands per primer using 20 RAPD markers in 21 tomato genotypes. Out of 15 bands, all the bands (100%) were found to be polymorphic (Table 2). Among the three primers, the primer OPG-01 produced the highest number of polymorphic bands (9) which indicated a high level of polymorphism. On the other hand, the primers GLA-05 and OPG-02 generated 2 and 4 bands, respectively (Table 2).

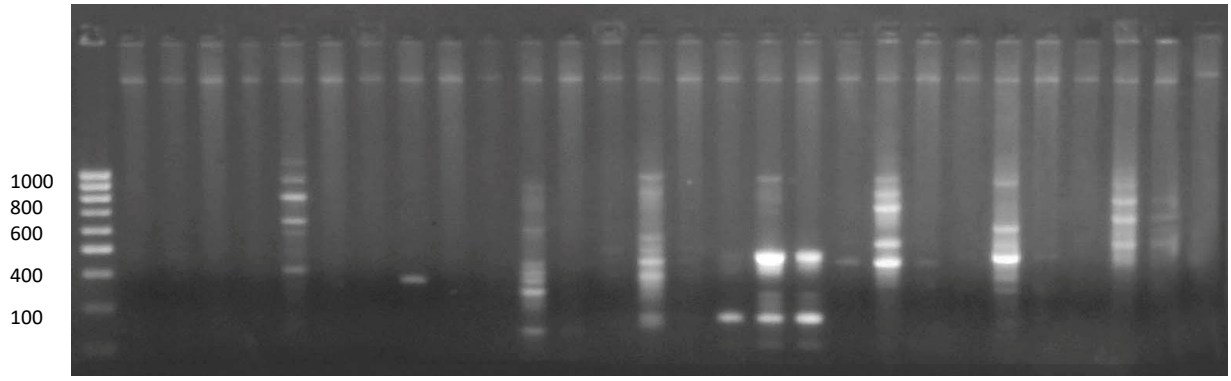

Figure 1. RAPD profiles of 28 tomato genotypes using OPG- 01 primer

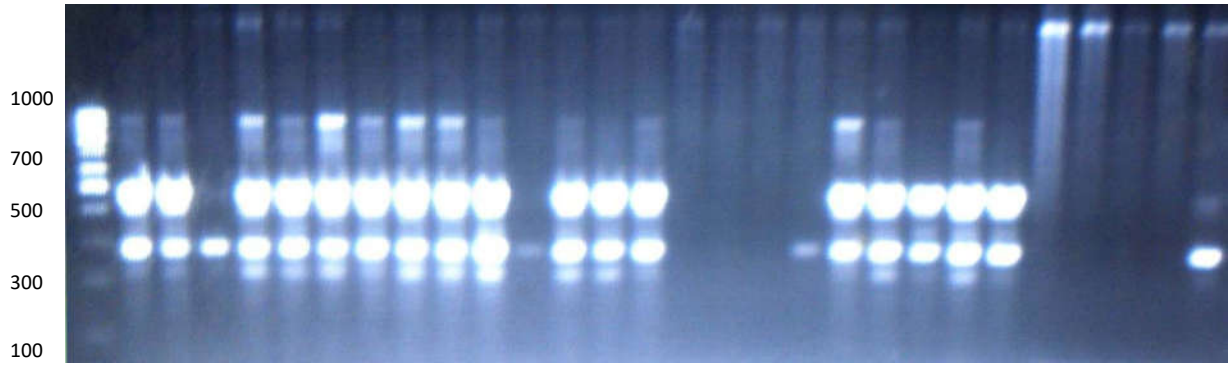

Figure 2. RAPD profiles of 28 tomato genotypes using OPG-02 Primer

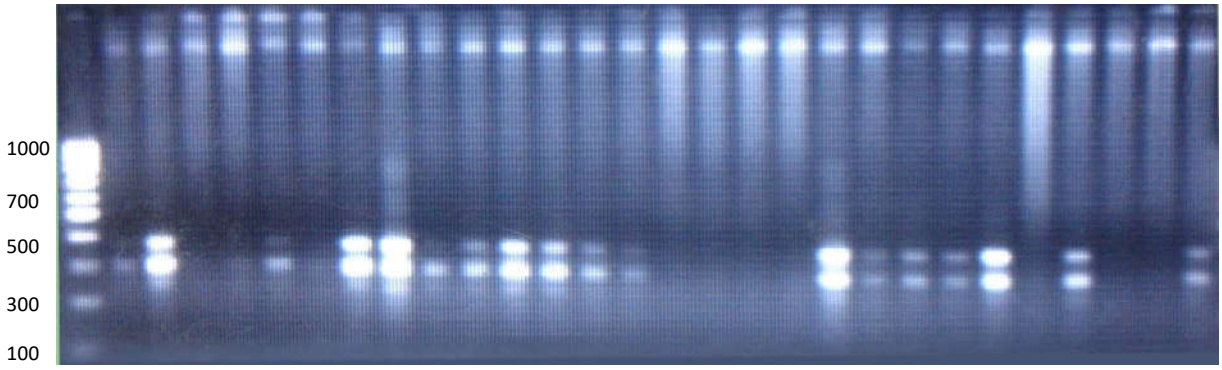

Figure 3. RAPD profiles of 28 tomato genotypes using GLA-05 primer

Table 2. RAPD primers with corresponding bands score and their size range together with polymorphic bands observed in 28 tomato accessions.

Primer code	Sequences (5′-3′)	Total number of bands scored	Size ranges (bp)	Number of polymorphic bands	Proportion of polymorphic loci (%)
GLA-05	**5′**-AGG GGT CTT G **-3′**	2	300-400	2	100
OPG-01	**5′**-TGC CGA GCT G**-3′**	9	150-1000	9	100
OPG-02	**5′**-AGT CAG CCA C**-3′**	4	350-1000	4	100
Total		15		15	300
Average		5.00		5.00	100

bp= Base pair

***Polymorphism in 28 tomato genotypes*:** The DNA polymorphisms were detected according to presence and absence of bands. Absence of band may be caused by failure of primer to anneal a site in some individuals due to nucleotide sequences difference or by insertions or deletions between primer sites (Clark and Lanigan, 1993). The frequencies of polymorphic band obtained varied from primer to primer (Table 3). Frequencies of maximum number of polymorphic loci were found to be high with the exception of OPG 01-8 and OPG 01-3 (0.0714) (Table 3). Though no accession-specific marker has been scored in the present study, the high level of polymorphism revealed by the proportion of polymorphic loci (100%) indicated that RAPD markers could be considered as effective tools for estimating genetic diversity in different accessions of tomato (Table 2).

***Genetic diversity*:** The values of Nei (1972) gene diversity and Shannon's information index for different accessions of tomato across all loci are shown in Table 3. The mean estimate of Nei (1972) genetic diversity for entire accessions of tomato was 0.3388 and Shannon's information index was 0.5141 which supports the existence of high level of genetic variation among the studied genotypes (Table 3). A high level of genetic variation was also present from the view point of proportion of polymorphic loci. The highest gene diversity value (0.4898) was found in locus GLA 05-2 and Shannon's information index (0.6829) was found in locus GLA 05-1 whereas the lowest gene diversity value (0.1327) was found in locus OPG 01-1 and OPG 01-6 and Shannon's information index (0.2573) was found in locus OPG 01-5 (Table 3).

Genetic distance: Genetic distance was calculated among the studied tomato genotypes. Genetic distance between the genotypes ranged from 0.0000 to 1.3218. The highest genetic distance (1.3218) was observed between the accession Cl-3d-0-99 (V93) vs. F_1 (G X V12), F_1 (G X V17), F_1 (G X V29) and Durch fuegel (G) vs. F_1 (G X V17) and F1 (G X V93) vs. F_1 (G X V12), F_1 (G X V17), while the lowest genetic distance 0.000 was observed among the accessions Fut. Wed Abrid (V94) vs. Sunlight pole (V67) and F_1 (V67 X VG) vs. F_1 (V17 X VG), F_1 (V93 X VG) and F_1 (V93 X V17) vs. F_1 (V17 X VG), F_1 (V67 X VG), F_1 (V93 X VG). The greater difference between the highest and lowest genetic distance indicated the presence of wide variability among the 28 accessions of tomato.

Table 3. Summary of gene frequency, gene diversity and Shanon's information index for all loci

Loci	Gene frequency	Gene diversity	Shanon's information index
OPG 01-1	0.6429	0.1327	0.4101
OPG 01-2	0.5714	0.2449	0.5623
OPG 01-3	0.0714	0.3750	0.5983
OPG 01-4	0.1429	0.4082	0.5196
OPG01-5	0.2500	0.3367	0.2573
OPG01-6	0.2857	0.1327	0.4101
OPG01-7	0.2143	0.2449	0.5983
OPG01-8	0.0714	0.4082	0.4101
OPG01-9	0.1429	0.2449	0.6518
OPG02-1	0.2857	0.4592	0.5623
OPG02-2	0.1429	0.3750	0.6700
OPG02-3	0.3571	0.4770	0.4692
OPG02-4	0.7500	0.2934	0.6518
GLA05-1	0.6071	0.4592	0.6829
GLA05-2	0.1786	0.4898	0.4101
Mean	**0.3142**	**0.3388**	**0.5141**

***Dendogram*:** The UPGMA dendrogram based on Nei's genetic distance indicated segregation of 28 tomato genotypes into two main clusters: A & B. Cluster 'A' consists of 19 accessions and cluster 'B' consists of 9 accessions. Cluster 'A' again divided into 5 sub-clusters; A- I, A- II, A- III, A- IV and A- V (Figure 4).

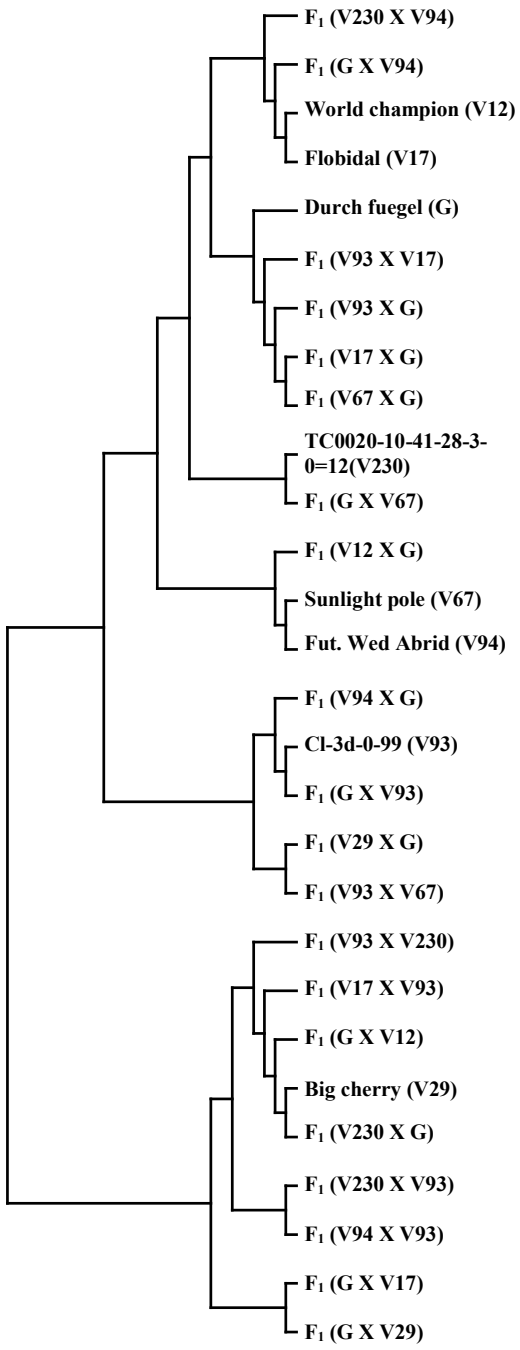

Figure 4. Unweighted Pair Group Method of Arithmetic Mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation among 28 tomato accessions according to RAPD analysis.

In sub-cluster A- I, F_1 (V230 X V94), F_1 (G X V94), World champion (V12)**,** Flobidal (V17) were grouped together. In sub-cluster A- II, Durch fuegel (G), F_1 (V93 X V17), F_1 (V93 X G), F_1 (V17 X G)**,** F_1 (V67 X G) grouped together. In sub-cluster A- III, TC0020-10-41-28-3-0= 12 (V230) and F_1 (G X V67) grouped together. (V12 X G)**,** Sunlight pole (V67), Fut. Wed Abrid (V94) grouped together in sub-cluster A- IV and F_1 (V94 X G), Cl-3d-0-99 (V93), F_1 (G X V93), F_1 (V29 X G)**,** F_1 (V93 X V67) grouped together in sub-cluster A- V. The genotypes that are genetically similar grouped together in the same cluster (Fig. 4). More or less similar results were reported by Elham *et al.* (2010) where UPGMA analysis divided the eight Egyptian tomato varieties into three distinct clusters using 7 RAPD markers. Naz *et al*. (2013) also reported the phylogenetic diversity and relationships of 25 tomato accessions by using 15 RAPD primers.

Conclusion

Over all, RAPD markers provided a fast, efficient technique for variability assessment that complements methods currently being used in genetic resources management and in plant biosystematics to confirm morphological differences among population and/or to differentiate apparently similar populations. The results of this study can be used as a baseline of relationships for future diversity assessment and genetic analysis of tomato genotypes in Bangladesh. Moreover, the polymorphism detected among the accessions can be used in future tomato breeding programs for appropriate parent selection as well as to maximize the use of genetic resources.

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