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Analysis of Genetic Diversity in Eleven Tomato (*Lycopersicon esculentum* Mill.) Varieties using RAPD Markers

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Abstract

Genetic diversity of 11 tomato (*Lycopersicon esculentum* Mill.) varieties was assessed through RAPD analysis. Twenty arbitrary oligonucleotide primers used in the RAPD-PCR produced a total of 584 different marker bands with an average of 29.2 bands per primer. Based on the banding pattern 94.168% polymorphism observed among the tomato varieties. Size range of amplified DNA bands varied from 0.1 - 10 kb. A total of 15 unique bands were amplified from the genome of the 11 tomato varieties. The values of pair-wise genetic distances ranged from 0.1838 - 0.9049, indicating the presence of wide genetic diversity. The dendogram constructed based on phylogenetic relationship analysis revealed that the highest genetic diversity (0.9049) found between the variety BARI Tomato 8 and RATAN whereas the lowest (0.1838) between the variety BARI Tomato 9 and MADHURI. The dendogram has segregated the 11 tomato varieties into two major clusters. RATAN and SPECIAL-OP formed cluster 1 and rest 9 varieties *viz*., ROMA-VF, MARGLOBE, MADHURY and BARI Tomato 9, 2, 6, 14, 8 and 3 have constituted the second cluster.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops of both tropics and sub-tropics of the world. It is an excellent source of lycopene, a powerful antioxidant and reduces the risk of prostate cancer (Hossain et al. 2004). A number of germplasms on the basis of phenotypic characters like color, size, taste etc. are available in tomato. In conventional breeding program, wild tomato species are widely used as sources of genes

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conferring resistance/tolerance to biotic or abiotic stresses (Kochieva et al. 2002). The scientists of Bangladesh Agricultural Research Institute (BARI) have been trying to develop better variety by incorporating desirable traits. As a consequence they were able to release several tomato varieties through selection or hybridization which were named or classified solely on their un-reliable morphological feature. These varieties are now available in the local markets. Due to phenotypic plasticity germplasm may show different morphology (Goodrich et al. 1985). Unfortunately no genetic information about these varieties is available although this is a stable character.

However, knowledge on genetic information obtained through the analysis of genetic diversity and relatedness between or within different species, population and individuals is a pre-requisite towards effective utilization and conservation of plant genetic resources (Chaudhuri et al. 1976, Weising et al. 1995). Therefore, characterization and analysis of genetic affinity among the tomato varieties are necessary before setting any program for their improvement. Moreover, several private commercial companies released various tomato varieties with different trade names. Due to non-availability of the sources and parents of these varieties a lot of confusions are created regarding the authenticity of these tomato germplasms. To prevent trade piracy, BARI released varieties and other commercially available varieties need to be judged on the basis of their genomic information (Alam et al. 2012).

Different molecular markers have been proved as useful tools for characterizing agricultural crops based on genetic diversity. Researchers have studied genetic variation in tomato landrace and cultivars using various molecular techniques, *viz*., RFLP, AFLP, RAPD and SSR (Miller and Tanksley 1990, Rus-Kortekaas et al. 1994, Bredemeijer et al. 1998, Villand et al. 1998, Mazzucato et al. 2003, Carelli et al. 2006 and Garcia-Martinez et al. 2006).

RAPD is a powerful tool for identification and monitoring pedigree breeding record of inbred parents or varieties (Baird et al. 1992, Echt et al. 1992, Struss et al. 1992) and determining genetic relationships among genotypes (Alam et al. 2012). It is an updated plant varietal identification method independent of restriction sites employing in the detection of polymorphisms by using the PCR technology (Welsh and McCleland 1990). This technique uses specific oligonucleotide primers, which are highly sensitive in mapping traits and fingerprinting of individuals for crop improvement (Carlson et al. 1991, Klein-Lankhorst et al. 1991, Rafalsik et al. 1991, Rajput et al. 2006 and Waugh and Powell 1992). 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).

In this study, RAPD analysis was conducted with five commercial and six BARI released varieties of tomato. The aim was to (i) characterize each variety with RAPD markers and (ii) elucidate phylogenetic relationship among 11 tomato varieties.

Materials and Methods

Eleven tomato (*Lycopersicon esculentum* Mill.) varieties *viz.* RATAN, ROMA-VF, SPECIAL-OP, MARGLOBE, MADHURI, and BARI Tomato 2, 3, 6, 8, 9 and 14 were used for RAPD analysis. The seeds of first five varieties were purchased from local market and those of BARI varieties collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur. Gazipur. DNAs used in RAPD-PCR experiment were isolated from the leaf of the plants. Tender leaves were used to extract total genomic DNA by using modified CTAB method (Doyle and Doyle 1987). DNA concentration was quantified through spectrophotometer (Analylikjena, Specord 50, Germany). The A260/280 readings for DNA samples were 1.6 - 1.8. The PCR reaction mixture for 25 µl containing template DNA (25 ng) 2 µl, de-ionized distilled water 18.8 µl and Taq buffer A 10× (10 mM Tris-HCl with 1.5 mM $MgCl_2$) 2.5 µl, primer (10 µM) 1.0 µl, dNTP mix (10 mM) 0.5 µl, Taq DNA polymerase (5U/µl) 0.2 µl. PCR amplification was done in an oil-free thermal cycler (Biometra UNOII, Germany) for 46 cycles after initial denaturation at 94ºC for 5 min and at 94ºC for 1 min, annealing at 36ºC for 30 sec, extension at 72ºC for 3 min and final extension at 72ºC for 5 min.

Twenty random primers used in the present study which showed reproducible results: OPA-1 (CAG GCC CTTC), OPA-2 (TGC CGA GCTC), OPA-3 (AGT CAG CCA C), OPA-4 (AAT CGG GCT G), OPA-5 (AGG GGT CTT G), OPA-6 (GGT CCC TGA C), OPA-7 (GAA ACG GGT G), OPA-8 (GTG ACG TAG G), OPA-9 (GGGTAA CGC C), OPA-10 (GTC ATC GCA G), A 15 (TTC CGA ACC C), A 09 (GG TAA CGC C), D 02 (GGA CCC AAC C), C 02 (GTG AGG CGT C), B 06 (TGC TCT GCC C), B 14 (TCC GCT CTG G), D 01 (ACC GCG AAG G), A 03 (AGT CAG CCA C), A 08 (GTG ACG TAG G), C 01 (TTC GAG CCA G) (Table 1).

Amplified products from the RAPD reactions were separated by horizontal gel electrophoresis using 1.2% agarose gel containing 0.5 µg/ml ethidium bromide in TAE buffer at 50 volts for 1.5 hrs. The gel was visualized by UV-transilluminator to examine the banding patterns and photographed by gel documentation system (CSL-Microdoc Sytem, Cleaver Scientific Ltd., USA). Each PCR reaction was repeated twice to ensure the consistence of RAPD banding

patterns and only reproducible stable products scored. The photographs of the RAPD gel analysis were critically discussed on the basis of presence (1) or absence (0) of bands, size of bands and overall polymorphism of bands. 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, genetic diversity, genetic distance (D) and constructing a Unweighted pair group method of arithmetic means (UPGMA) dendogram among the varieties using computer program "POPGENE" (Version 1.31) (Yeh et al. 1999).

Results and Discussion

All the 20 random primers produced distinguishable polymorphic bands in each of the DNA sample. The RAPD experiments produced a total of 584 bands of which 574 were polymorphic. The percentage of polymorphic loci was (94.168) indicating a higher level of polymorphism. A diverse level of polymorphism in different crops has been reported in Tomato (Moonmoon 2006), egg plants (Biswas et al. 2009) and chili (Paran et al. 1998). The RAPD profiles of the amplified products of six representative primers are shown in Fig. 1a-f. The data set produced in this experiment was sufficient to categorize all the 11 varieties (Table 1). The band size ranged from 100 - 10,000 bp. The average number of bands per primer was 29.2, whereas 11.57 bands/primer were reported using seven different primers (El-Hady et al. 2010). Highest number of polymorphic bands (47) was produced by the primer A 15 and lowest (13) by the primer OPA-4.

Most of the varieties had one or more novel sequences which were not found in others. These bands can successfully be used as genetic markers for identification of these varieties. Primers OPA-2, OPA-5, OPA-6, OPA-9, A 03, A 08, A 15, B 14, C 01 and C 02 were found to be the most effective in generating unique bands. Ten primers produced a total of 15 unique bands in 8 cultivars (Table 1). These unique bands are variety specific and thus useful to differentiate specific variety from each other.

The values of pair-wise Nei's (1972) genetic distance ranged from 0.1838 - 0.9049. The highest genetic distances (0.9049) were found between variety BARI Tomato 8 and RATAN, and the lowest (0.1838) between the variety BARI Tomato 9 and MADHURI. The difference between the highest and the lowest value of genetic distance revealed the wide range of variability persisting among the 11 tomato varieties (Table 2).

The dendogram constructed based on Nei's (1972) genetic distance segregated the 11 tomato varieties into two major clusters (Fig. 2). Varieties RATAN and SPECIAL-OP formed cluster 1 on the other hand, varieties ROMA-VF, MARGLOBE, MADHURI, BARI Tomato 9, 2, 6, 14, 8, and 3 formed cluster 2. In cluster 1, RATAN and SPECIAL-OP formed group 1. Cluster 2 was divided into two sub-clusters. ROMA-VF alone formed a sub-cluster 1 and group 2. MARGLOBE, MADHURI, BARI Tomato 9, 2, 6, 14, 8, and 3 were included into

Table 1. RAPD finger printing with twenty primers in eleven tomato varieties.

Primer code	Sequence	Total bands obtained	bp size range	No. of unique bands with variety and bp size	Poly-morphic bands
OPA-1	CAGGCC CTT C	20	2500 - 1000	-	20
OPA-2	TGC CGA GCT C	19	2500 - 1000	RATAN: 1(1000)	19
OPA-3	AGT CAG CCA C	20	10000 - 500	-	20
OPA-4	AAT CGG GCT G	13	2000 - 1200	-	13
OPA-5	AGG GGT CTT G	31	1400 - 250	RATAN: 1(250)	31
OPA-6	GGT CCC TGA C	47	2000 - 200	BARI Tomato 9: 1(200)	40
OPA-7	GAA ACG GGT G	17	750 - 200	-	17
OPA-8	GTG ACG TAG G	28	1500 - 200	-	28
OPA-9	GGG TAA GC C	33	1600 - 200	BARI Tomato 6: 1(1600)	33
OPA-10	GTC ATC GCA G	45	1000 - 250	-	45
A 15	TTC CGA ACC C	47	2000 - 4000	SPECIAL-OP : 1(900)	47
A 09	GGGTAA CGC C	48	2200 - 250	-	47
D 02	GGA CCC AAC C	40	1500 - 400	-	40
C 02	GTG AGG CGT C	41	2000 - 750	ROMA-VF: 1(2000)	41
B 06	TGC TCT GCC C	08	750 - 100	-	08
B 14	TCC GCT CTG G	20	1800 - 300	MARGLOBE: 1(1000) BARI Tomato 9: 2(300,1300)	20
D 01	ACC GCG AAG G	17	2000 - 1500	-	17
A 03	AGT CAG CCA C	42	1800 - 400	ROMA-VF: 1(1800) BARI Tomato 14: 1(1200)	41
A 08	GTG ACG TAG G	17	1200 - 200	MARGLOBE: 1(400) BARI Tomato 2: 1(5000	16
C 01	TTC GAG CCA G	31	3000 - 500	RATAN: 1(500) SPECIAL-OP: 1(1200)	31
Total = 20 primers		Total = 584		Total = 15	Total = 574

- indicates absence of polymorphic bands.

sub-cluster 2 and group 3. Sub-cluster 2 of cluster 2 was again divided into more sub-clusters forming sub-sub-clusters. From the cluster analysis it was found that MADHURI and BARI Tomato 9 were closely related according to phylogenetic relationship because they maintained lowest genetic distances than other members in this group. BARI Tomato 9 also maintained close relationship with BARI Tomato 2. However, BARI Tomato 2 is linked with both BARI Tomato 9

and MADHURI because of having equal genetic distances (Table 2). BARI Tomato 6 and BARI Tomato 2 were also closely related. Close relationships were found among the following pairs BARI Tomato 6 and 8; 6 and 14, 8 and 14. Though placed in the same group BARI Tomato 3 is maintaining distant relation with the variety BARI Tomato 2. Similar finding was reported by Alam et al. (2012). This indicates that the markers used in this experiment were stable and reproducible. From the dendogram it was clear that the trading variety RATAN is placed far away from BARI Tomato 2 in separate cluster. This was not expected

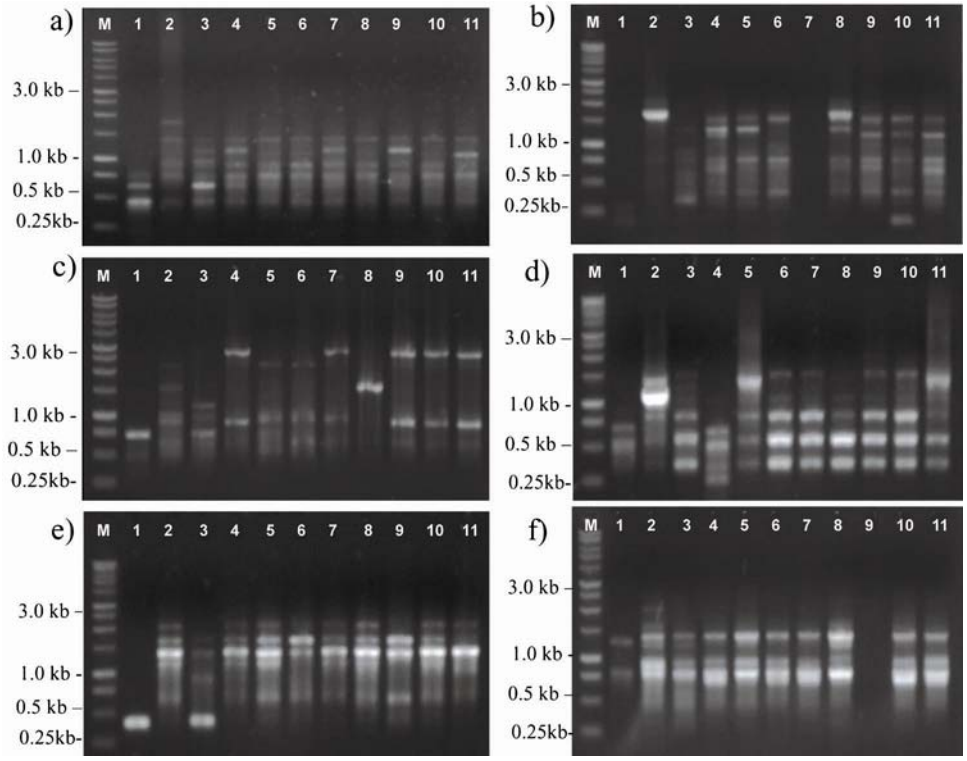

Fig. 1. RAPD profile obtained from 11 tomato varieties DNA with different primers. (a) primer A 03, (b) OPA-6, (c) primer C 01, (d) primer OPA-10, (e) primer A 15, and (f) primer C 02. Lane M- 1.0 Kb DNA ladder, Lane 1-RATAN, Lane 2- ROMA-VF, Lane 3-SPECIAL-OP, Lane 4- MARGLOBE, Lane 5- MADHURI, Lane 6- BARI Tomato 6, Lane 7- BARI Tomato 2, Lane 8- BARI Tomato 3, Lane 9- BARI Tomato 8, Lane 10- BARI Tomato 9, Lane 11- BARI Tomato 14 .

because RATAN is the commercial form of BARI Tomato 2 variety. The reason might be either i) the seed traders are not maintaining genetic purity of RATAN or ii) mixed with others through natural crossing. Results indicating that RAPD markers developed in this study can be effectively used for the determination of hybrid seed purity of tomato as was reported by Singh et al. (2007). From this

investigation it is revealed that each of the 11 tomato varieties possessed specific RAPD finger printing profile which could be used for their authentic identification.

Table 2. Inter-variety similarity indices (%) (above diagonal) and pair-wise genetic distances (below diagonal) in different tomato varieties.

Variety ID	1	2	3	4	5	6	7	8	9	10	11
1	****	0.4962	0.6412	0.5573	0.5344	0.4962	0.5191	0.4809	0.4046	0.5191	0.4656
2	0.7008	****	0.6260	0.6183	0.7023	0.6336	0.6870	0.6183	0.5725	0.6260	0.6336
3	0.4444	0.4685	****	0.6412	0.6336	0.5649	0.6489	0.5496	0.5038	0.6031	0.5191
4	0.5847	0.4807	0.4444	****	0.7939	0.6794	0.7328	0.6489	0.6336	0.7328	0.6947
5	0.6267	0.3534	0.4564	0.2308	****	0.8092	0.8168	0.7786	0.7328	0.8321	0.7939
6	0.7008	0.4564	0.5711	0.3866	0.2118	****	0.7939	0.6794	0.7405	0.7939	0.7405
7	0.6557	0.3754	0.4325	0.3108	0.2024	0.2308	****	0.7328	0.7176	0.8168	0.7634
8	0.7321	0.4807	0.5985	0.4325	0.2502	0.3866	0.3108	****	0.6794	0.7328	0.6641
9	0.9049	0.5577	0.6855	0.4564	0.3108	0.3005	0.3319	0.3866	****	0.7634	0.7405
10	**0.6557**	0.4685	0.5057	0.3108	0.1838	0.2308	0.2024	0.3108	0.2700	****	0.7939
11	0.7643	0.4564	0.6557	0.3643	**0.2308**	0.3005	0.2700	0.4093	0.3005	0.2308	****

1 = RATAN, 2 = ROMA-VF, 3 = SPECIAL-OP, 4 = MARGLOBE, 5 = MADHURI, 6 = BARI-6, 7 = BARI-2, 8 = BARI-3, 9 = BARI-8, 10 = BARI-9 and 11 = BARI-14.

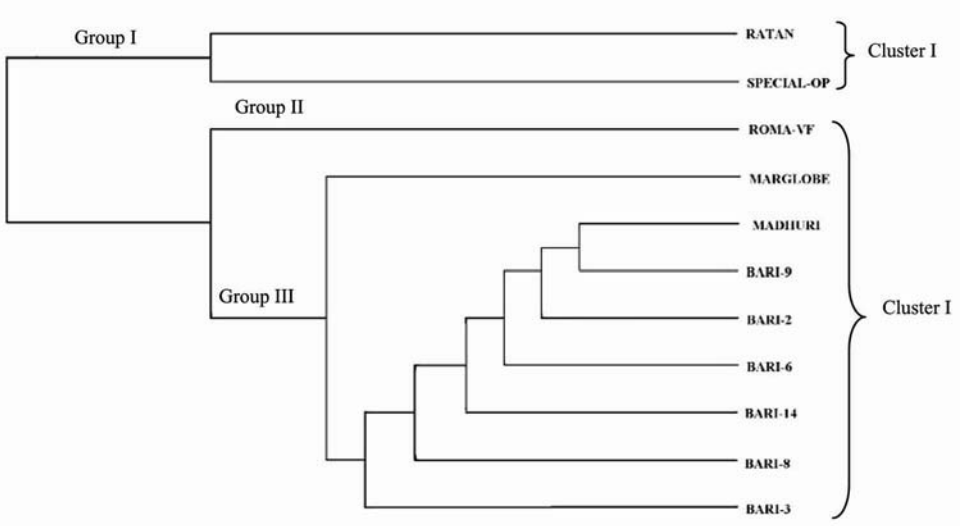

Fig. 2. A dendogram based on Nei's (1972) genetic distance summarizing the data on differentiation among 11 varieties, according to RAPD analysis.

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