Journal of Horticultural Research 2013, vol. 21(1): 83-89
DOI: 10.2478/johr-2013-0012

ASSESSMENT OF GENETIC DIVERSITY IN CULTIVATED TOMATO (*SOLANUM LYCOPERSICUM* L.) GENOTYPES USING RAPD PRIMERS

Saida SHARIFOVA[1,3*], Sabina MEHDIYEVA[2,3], Konstantinos THEODORIKAS[3], Konstantinos ROUBOS[3]
[1]Department of Vegetable and Melon Plants
[2]Department of Cytogenetics, Genetic Resources
Institute of Azerbaijan National Academy of Sciences, Baku, Azerbaijan
[3]Laboratory of Plant Molecular Biology and Plant Propagation,
Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece

Received: April 24, 2013; Accepted: June 17, 2013

ABSTRACT

Random Amplified Polymorphic DNA (RAPD) analysis was carried out on 19 Azerbaijan tomato genotypes, both cultivars and local populations. A total of 26 amplified products were revealed by 6 primers. The genetic similarity among evaluated genotypes ranged from 0.188 to 1.000. The lowest similarity was observed between cultivars 'Azerbaijan' and 'Shakar' (0.188), while the highest between 'Elnur' and 'Garatag' (1.000). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis based on Jaccard's similarity coefficient divided genotypes into four main groups. The first group was the largest and consisted of 12 genotypes, while the fourth group was the smallest consisted of 1 genotype only. The most polymorphic primer was OPB-18 that presented a genetic diversity index of 0.823, while the least informative was primer OPG-17 with an index of 0.349. The average genetic diversity calculated from RAPD data was 0.665.

Key words: Azerbaijan; breeding; polymorphism; RAPD; similarity; tomato

INTRODUCTION

The cultivated tomato (*Solanum lycopersicum* L.) is economically one of the most important and widely grown plants of the Solanaceae family. It is estimated that over 62 800 accessions of the cultivated and wild species of tomato are maintained in gene banks around the world, including those at the Asian Vegetable Research and Development Center (AVRDC) in Tainan, Taiwan (http://www.avrdc.org), the Plant Genetic Resources Unit (PGRU) in New York, USA (http://www.usda.gov), and at the CM Rick Tomato Genetics Resource Center (TGRC), University of California in Davis, USA (http://tgrc.ucdavis.edu). The European Cooperative' Programme for Plant Genetic Resources (ECPGR) tomato database contains passport information of more than 20 000 accessions of several tomato species (http://documents.plant.wur.nl/cgn/pgr/tomato).

The tomato core collection of European Solanaceae database is composed of about 7 000 domesticated (*S. lycopersicum* L.) lines, along with representatives of wild species (www.eu-sol.wur.nl). The cultivated tomato is a well-studied species in terms of genetics, genomics, and breeding (Foolad 2007). It has been one of the first crop plants for which a genetic linkage map was constructed (Rick 1975; Tanksley and Rick 1980; Bernatzky and Tanksley 1986; Tanksley *et al*. 1992). Currently, there are several molecular maps based on crosses between the cultivated and wild species of tomato (Grandillo and Tanksley 1996; Bernacchi and Tanksley 1997; Chen and Foolad 1999; Frary *et al*. 2004).

DNA fingerprinting is a convenient tool for assessing genetic diversity (Park *et al*. 2004; Semagn *et al*. 2006; Mondini *et al*. 2009). The characterization of various plant genetic resources with molecular markers offers a unique opportunity to define significant marker-trait associations of biological and agronomic interest (Parmar 2010).

Cultivated tomato (*Solanum lycopersicum* L.) is a species in which biochemical and molecular markers such as isozymes and RFLPs yielded limited amount of information due to the lack of vari-

Corresponding author:
e-mail: saidasharifzade@yahoo.com

ability, as a consequence of self-pollination in combination with the narrow genetic base of the modern cultivars (Miller and Tanksley 1990; Breto *et al*. 1993; Alvarez *et al*. 2001). Nevertheless, different types of molecular markers such as RFLPs, AFLPs, SSRs, CAPS, and ESTs have been developed and mapped onto the 12 tomato chromosomes (Broun and Tanksley 1996; Saliba-Colombani *et al*. 2000; Suliman-Pollatschek *et al*. 2002; Frary *et al*. 2005). Since the successful construction of RAPD markers gene mapping in tomato by Klein-Lankhorst *et al*. (1991), the application of the RAPD technique in varietal identification of tomato has been well explored (Noli *et al*. 1999; Rajput *et al*. 2006; Singh *et al*. 2007).

The main collections of vegetable plants in Azerbaijan are conserved in the National Gene Bank in Genetic Resources Institute (GRI), in the Scientific Research Institute of Vegetable Growing, and in the Azerbaijan State Agricultural Academy (Sharifova 2012). The study of genetic diversity is necessary for efficient utilization, conservation and management of genetic resources deposited in gene banks. Since the morphological characterization does not provide accurate information necessary to distinguish different genotypes, further assessment of collected germplasms at the molecular level is required (Carmen de Vicente *et al*. 2006; Ferreira 2006). The present study was conducted in order to examine the genetic diversity of the local tomato genotypes collected at GRI using RAPD markers.

MATERIALS AND METHODS

Plant material consisted of 19 different genotypes (cvs 'Garatag', 'Elnur', 'Shakar', 'Nuru', 'Gurman', 'Ilkin', 'Zafar', 'Azerbaijan', 'Leyla', 'Zarrabi' and local populations 'Sabirabad', 'Saatly', 'Nakhchivan', Absheron-1, Absheron-2, Absheron-3, AG-1222, AG-1223, and AG-1224) of cultivated tomato (*S. lycopersicum* L.). All samples were obtained from the Gene Bank of the GRI of the Azerbaijan National Academy of Sciences (ANAS) (http://www.genres.az).

DNA was extracted from fresh leaves of seedlings according to Roubos *et al*. (2010). The quality and quantity of nucleic acids was determined on the basis of UV spectrum using Nanodrop ND-1000 spectrophotometer. Ten RAPD primers: OPA-14, OPA-15, OPB-17, OPB-18, OPC-08, OPC-09, OPG-17, OPU-03, OPU-14, and OPV-19 (Operon Technologies Inc., USA) were used for amplification. Polymerase chain reactions (PCRs) were performed in 25 mm^3 of reaction mixture containing 1× PCR buffer (Invitrogen), 0.2 mM of each dNTP (Invitrogen), 1.5 mM $MgCl_2$, 0.4 mM of a primer (Invitrogen), 50 ng genomic DNA, and 1 U Taq Polymerase (Invitrogen). Amplification was performed in a Mastercycler® thermal cycler (Eppendorf). The cycling programs for DNA amplification consisted of denaturation for 2 min at 94 °C followed by 38 cycles of 60 s at 94 °C for denaturation, 60 s at 40 °C for primer annealing and 60 s at 72 °C for extension. A final extension was at 72 °C for 7 min. PCR products were separated by electrophoresis in 1.5% agarose gel (Invitrogen) at 100 V for 1.5 h. A 100-bp ladder (Invitrogen) was used as a size standard. Gels were visualized and photographed under UV light with the Molecular Imager® Gel Doc™ XR system (Bio-Rad) and then analysed with the Quantity 1.0 software (Bio-Rad).

Amplified fragments were scored for presence (1) or absence (0) of the respective bands in all the genotypes tested. After identification of the polymorphic bands, different patterns were identified among genotypes. The genetic diversity index was calculated for each primer and each pattern frequency using the formula:

$$H = 1 - \Sigma Pi^2$$

where: H is genetic diversity index and Pi is pattern's frequency (Nei 1973).

Jaccard's similarity coefficients matrix and dendrogram were constructed using the unweighted pair group method with arithmetic mean (UPGMA) module of SPSS v.12.0 computer package (SPSS 2003).

RESULTS AND DISCUSSION

Four of the primers used (OPA-15, OPU-03, OPU-14 and OPA-14) did not provide any evaluable bands. The polymorphic bands obtained with the other primers: OPC-08, OPC-09, OPB-17, OPB-18, OPV-19 and OPG-17, were scored as 1 for presence or 0 for absence and imported into SPSS. A band was considered polymorphic if it was present or absent in at least 3 of the 19 accessions tested. A total of 26 scorable bands were obtained from 19 cultivated tomato accessions (Table 1).

A total of 65.3% of the produced bands showed polymorphism (Table 1). Primer OPV-19 and OPB-18 produced 4 polymorphic bands, while OPG-17 1 band only. Polymorphism percentage

ranged from 50 to 100%. Primer OPB-18 generated the greatest diversity index with a value of 0.823, while primer OPG-17 showed the smallest diversity with an index of 0.349. The average genetic diversity index was 0.665 (Table 1).

Table 1. List of primers, numbers of polymorphic and monomorphic bands, and genetic diversity index obtained with tomato genotypes

Primers	Sequences (5' - 3')	Number of bands	Number of polymorphic bands	Polymorphism ratio, percentage (%)	Genetic diversity index
OPC-08	5'-TGGACCGGTG-3'	4	2	50.0	0.681
OPC-09	5'-CTCACCGTCC-3'	3	3	100.0	0.722
OPB-17	5'-AGGGAACGAG-3'	4	3	75.0	0.662
OPB-18	5'-CCACAGCAGT-3'	5	4	80.0	0.823
OPV-19	5'-GGGTGTGCAG-3'	8	4	50.0	0.752
OPG-17	5'-ACGACCGACA-3'	2	1	50.0	0.349
Total		26	17	65.3	0.665 (aver.)

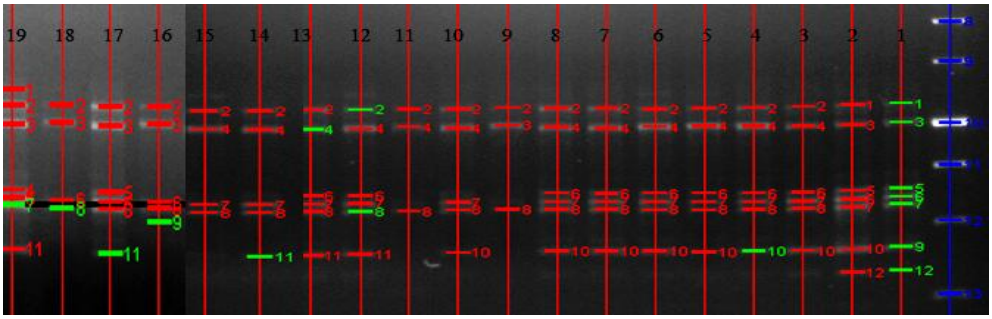

Figure 1. RAPD-PCR analysis with use of primer OPV-19 on 19 tomato genotypes. Shown is a gel picture after scoring with Quantity 1.0

The primers that were used in our study have been applied successfully for assessing different plant genotypes (Teixeira-Cabral *et al*. 2002; Tardin *et al*. 2003; Rana *et al*. 2005; Badjakov *et al*. 2006; Kawar *et al*. 2009; Butiuc-Keul *et al*. 2010). OPC-09 was used for coffee tree genotypes grouping analysis and amplified polymorphic markers (Teixeira-Cabral *et al*. 2002). Moreover, OPC-09 generated 17 bands, 13 of which were polymorphic in Indian cotton (*Gossypium* spp.) accessions (Rana *et al*. 2005). OPC-08 and OPG-17 were used successfully for assessing genetic diversity in sugarcane cultivars (Kawar *et al*. 2009). OPB-17 was used by Butiuc-Keul *et al*. (2010) for analysing the genetic polymorphism in several cultivars of grapevine and 5 polymorphic fragments were obtained with *Feteasca alba* cultivars. OPB-19 and OPC-08 were used in assessing the genetic diversity of Bulgarian raspberry germplasms and generated high number of polymorphic bands in different lines (Badjakov *et al*. 2006). OPV-19 was used in research on twenty lettuce (*Lactuca sativa* L.) accessions and generated 2 polymorphic bands (Tardin *et al*. 2003). Ezekiel *et al*. (2011) have used 10 RAPD primers for characterization of Nigerian tomato cultivars and suggested that RAPD markers are efficient in characterization of tomato genotypes. In the above mentioned study, the primer OPB-18 recorded the highest percentage of polymorphism (83.3%), as it revealed 5 polymorphic bands in 6 amplified fragments. Additionally, OPG-17 was one of the most effective fragment amplifiers (yielding up to 9 fragments), while the OPC-09 was the least effective with 2 fragments only. Four primers: OPA-15, OPU-03, OPU-14 and OPA-14, which did not generate any bands with our tomato accessions, amplified polymorphic products in Nigerian tomato cultivars, thus revealing the differences between Nigerian and Azerbaijan tomato genotypes.

According to Jaccard`s similarity index, the lowest similarity of 0.188 and 0.2 was found between 'Azerbaijan' and 'Shakar' and between 'Shakar' and 'Saatly' cultivars, respectively. Similarity coefficient between 'Elnur' and 'Garatag' reached 1.000. The 'Leyla' and 'Zarrabi' genotypes

gave the second highest ratio of 0.944. High similarity index of 0.917 was also observed between AG-1223 and 'Nuru' genotypes.

Ten genotypes in this study were known cultivars ('Garatag', 'Elnur', 'Shakar', 'Nuru', 'Gurman', 'Ilkin', 'Zafar', 'Azerbaijan', 'Leyla' and 'Zarrabi') while other 6 genotypes ('Sabirabad', 'Saatly', 'Nakhchivan', 'Absheron-1', 'Absheron-2', 'Absheron-3') were collected from different regions of the country and included into the gene bank. Another three genotypes (AG-1222, AG-1223 and AG-1224) were registered as local genotypes in the gene bank, but there was no information about their origin(s). The last 9 accessions were registered as local population samples.

UPGMA average gene cluster analysis based on the Jaccard's similarity coefficient grouped the genotypes into four main clusters (Fig. 2). The first cluster represented 7 of 9 so-called local population samples. Three of these were collected from the Absheron region (Absheron-1, Absheron-2, and Absheron-3) and joined in the upper cluster, while another two ('Sabirabad' and 'Saatly') were local populations grown at the Aran region (Fig. 3). Yet another genotype represented in the first cluster ('Nakhchivan') was collected from the Nakhchivan Autonomous Republic of Azerbaijan. Moreover, the above cluster included five cultivars, of which 'Leyla' and 'Zarrabi' were mainly cultivated at the Lankaran region, 'Elnur' at the Lankaran and Guba-Khachmaz region, 'Azerbaijan' at the Ganja-Gazakh region, while 'Garatag' is an old cultivar that is not cultivated anymore and is threatened to extinct (Fig. 3). 'Nuru' cultivar that is located in the second cluster (Fig. 2), and is cultivated at the Lankaran and Guba-Khachmaz region, while the other cultivar of this cluster ('Gurman') can be found in different regions of the country (Absheron, Ganja-Gazakh). Cv. 'Gurman' is also threatened to extinct. Concerning the third cluster, 'Ilkin' cultivar can be found in all the vegetable producing regions shown in Fig. 3. 'Zafar' (third cluster) and 'Shakar' (fourth cluster) cultivars are also cultivated mainly at the Lankaran and Guba-Khachmaz region. Concerning the genotypes with no specific information about their collecting sites, AG-1224 is located in the first cluster, AG-1223 in the second cluster and AG-1222 in the third cluster (Fig. 2).

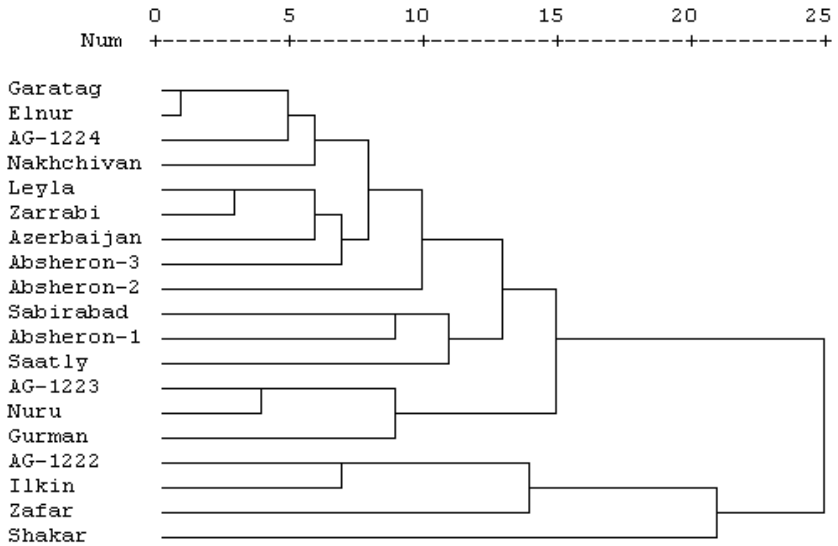

Figure 2. Dendrogram of 19 tomato genotypes revealed by UPGMA cluster analysis based on Jaccard`s similarity coefficients generated from RAPD markers

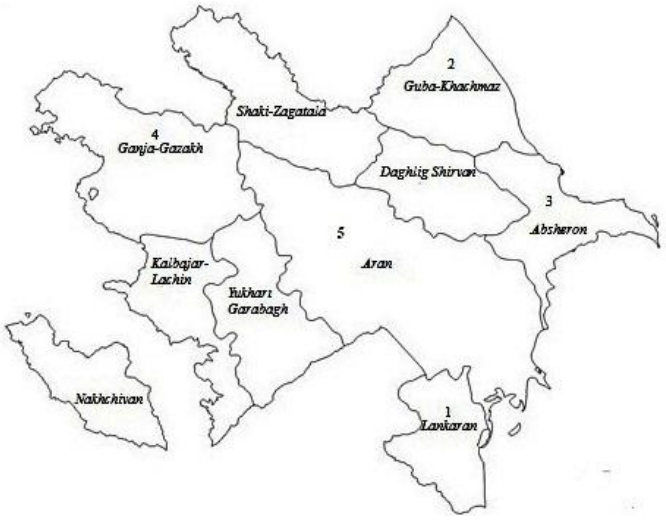

Figure 3. Main vegetable producing economical regions of Azerbaijan

Although accessions with the same or adjacent geographic origin have the tendency to cluster together, accessions from different regions were also found to be closely related regardless of their geographic origin. This suggests that selection of parent genotypes for breeding should not be based on geographical origin only because this is not always an accurate indicator of genetic diversity (Keneni *et al*. 2005; Zvingila *et al*. 2005; Gashaw *et al*. 2007; Celka *et al*. 2010). The tendency of grouping genotypes with different geographical origins into the same cluster did not allow us also to make an assumption about the geographical origin of AG-1222, AG-1223, and AG-1224 genotypes.

In our experiment, two cultivars: 'Garatag' and 'Elnur' showed 100% similarity level based on 6 primers used (Fig. 2). This suggests that they belong to the same genotype. Nevertheless, 'Garatag' is the old cultivar whereas 'Elnur' is a breeding variety obtained by crossing 'Mayak 12/20' and 'Azerbaijan' cultivars. Both of them are known genotypes and have been collected from the same city (Ganja). Therefore, we can suppose that a mistake occurred during either collecting or entering data into the database. Nevertheless, further studies with the use of morphological and molecular markers are required in order to come to more precise conclusions.

The results of the study allows to conclude that RAPD markers are effective in assessing and discriminating local tomato genotypes conserved at the gene banks in Azerbaijan. This is the first study on DNA fingerprinting of Azerbaijan tomato genotypes. Although the major cultivated local tomato genotypes in Azerbaijan have been described, further study is needed in order to elucidate the genetic structure of the local cultivars, populations, landraces, hybrids, introduced accessions, and all other tomato accessions deposited in the gene banks. Such studies should be useful both for identification of duplicate accessions and establishment of core collection in the gene banks, as well as for sustainable conservation of the genotypes collected. Precise molecular characterization of conserved collections will allow for more efficient management and utilization of genotypes in the breeding programs.

Acknowledgements

Research supported by the Erasmus Mundus Action 2 Program. We acknowledge the contributions of Professor Athanassios K. Roubos and Dr. Zeynal I. Akparov.

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