Genetic Diversity and Population Structure of Asian Tomato Accessions Based on Simple-Sequence Repeats

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ABSTRACT Tomato (*Solanum lycopersicum* L.) is one of the most economically important plants in the family *Solanaceae*. Understanding its genetic diversity of accessions is vital for additional collection of tomato germplasms. The objective of this study was to determine the genetic diversity and population structure of 355 tomato accessions from Asia using 18 simple-sequence repeats (SSRs). A total of 176 alleles were detected at an average of ten alleles per SSR locus. The average major allele frequency and polymorphic information content were 0.69 and 0.39, respectively. Model-based structure analysis revealed two subpopulations (88%), including admixtures (11%) in the 355 Asian tomato accessions, consistent with clustering results based on genetic distance. The overall F_{ST} value was 0.135, indicating a moderate differentiation between the inferred subpopulations. Analysis of molecular variance showed that the genetic variance among geographical groups was less than 6%, in contrast to 86% of genetic variance among individuals. The results from this study will provide important information for future germplasm conservation and improvement programs for tomato.

Keywords Tomato, Genetic diversity, Population structure, Simple sequence repeats

INTRODUCTION

Tomato (*Solanum lycopersicum*) belongs to the family of *Solanaceae*. It ranks the first in the world of vegetables, accounts for 14% of world vegetable production (over 100 million metric tons/year) (Food and Agriculture Organisation [FAO] 2010). Tomato is a rich source of micro-nutrients for human diet. It has multipurpose uses in both fresh and processed food industries. Sustainable cultivation, conservation, and utilization of tomato germplasms are imperative due to its agronomic importance. However, cultivated tomatoes have several bottlenecks due to their Latin American origin and related domestication, leading to drastic reduction of their genetic diversities. Identification of variabilities among Genbank accessions is vital for the maintenance and utilization of their germplasms (Lee *et al.* 2015).

Molecular markers have been used to genetically characterize tomato germplasms. Recent technological advancements have accelerated studies on whole genomes and genetic loci of interest. Molecular markers include random amplified polymorphic DNA (RAPD) (Klein-Lankhorst *et al.* 1991; Rajput *et al.* 2006; Singh *et al.* 2007; Sharifova *et al.* 2013), amplified fragment length polymorphisms (AFLP) (Suliman-Pollatschek *et al.* 2002), and restriction fragment length polymorphisms (RFLP) (Saliba-Colombani *et al.* 2000) have been studied on tomatoes. Simple-sequence repeats (SSRs) are the most frequently used markers for genotyping (Tautz 1989). Several studies have described the use of SSRs to reveal polymorphisms in tomato (He *et al.* 2003; Ohyama *et al.* 2009; Geethanjali *et al.* 2010; Geethanjali *et al.* 2011).

In 1998, the Human Genome Project introduced the use of single nucleotide polymorphisms (SNPs) as markers

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(Wang *et al.* 2008). Since then, SNPs have become increasingly important as genetic markers for studying the evolutionary history of populations (Gupta *et al.* 2012; Singh *et al.* 2015). The release of the tomato genome sequence has expedited the characterization of SNPs, allowing SNPs to be the most effective and useful genetic markers in modern tomato breeding programs (Tomato Genome Consortium 2012). Aflitos *et al.* (2014) have employed the whole-genome sequencing strategy to explore the genetic variations in tomatoes by using SNPs. Numerous verified SNPs have been successfully used to develop tomato cultivars for practical use (Labate and Baldo 2005; Shirasawa *et al.* 2010). However, this technology remains in the laboratories because it needs special equipment and financial support. In contrast, SSR marker is simple to use. It has been used widely in genetic analysis of many crop species. The present study was to evaluate the genetic diversity (GD) and population structures of tomato accessions collected from Asian countries using polymorphic SSR markers.

MATERIALS AND METHODS

Plant materials

A total of 355 Asian tomato accessions (Table 1, Supplement Table 1) were used in this study. These accessions were collected from six different Asian geographical origins. Seeds were obtained from the National Agrobiodiversity Center at the Rural Development Administration, Republic of Korea.

Table 1. Summary of accessions used in the study.

Geographical regions	Number of countries	Number of accessions
Central Asia	4	38
East Asia	5	118
South Asia	5	52
South West Asia	2	18
South East Asia	7	120
West Asia	2	9
Total	25	355

SSR genotyping

DNA was extracted from fresh leaves of each accession using Qiagen DNA extraction kit (Qiagen Korea, Seoul, Korea). The purity and concentration of extracted DNA were estimated using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). The final concentration of each DNA sample was adjusted to 20 ng/μl. For SSR assays, a total of 18 polymorphic SSR markers were selected from SOL Genomics Network (<https://solgenomics.net>) and those reported previously (Kwon *et al.* 2009; Geethanjali *et al.* 2010; Geethanjali *et al.* 2011). SSR alleles were amplified with fluorescent labelled primers, resolved on ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA) using GeneScan 3.7 software (Applied Biosystems), and sized precisely using GeneScan 500 ROX (6-carbon-X-rhodamine) molecular size standards (35-500 bp) and Genotyper 3.7 software (Applied Biosystems).

Data analysis

Genetic analysis package PowerMarker ver. 3.23 (Liu and Muse 2005) was used to determine the variability at each locus, including the number of alleles (NA), major allele frequency (M_{AF}), number of genotypes (NG), observed heterozygosity (H_O), GD, and polymorphic information content (PIC). Neighbor-joining method based on genetic distance matrix was used to construct a phylogram using MEGA4 software (Tamura *et al.* 2007).

Possible population structures were analyzed using model-based software program Structure 2.3.3 (Pritchard *et al.* 2000; Falush *et al.* 2003) for the 355 Asian tomato accessions and a model allowing admixture and correlated allele frequencies. Three runs of Structure were performed with the number of clusters (K) set from 1 to 10. The average likelihood value, $L(K)$, across all runs was calculated for each K . In this model, several subpopulations (K) were assumed to be present, each of which was characterized by a set of allele frequencies for each locus. Individuals in the sample were assigned to subpopulations or jointly to two or more subpopulations if their genotypes were admixed. The model choice criterion to detect the

most likely value of K was ΔK , an ad hoc quantity related to the second-order change in the log probability of data ($\text{LnP}[D]$) with respect to the number of clusters inferred by Structure (Evanno *et al.* 2005). An individual was assigned to a subpopulation group if >80% of its genome fraction value was derived from that group.

In addition, analysis of molecular variance (AMOVA) was performed using GENALEX 6.5 (Peakall and Smouse

2012) to estimate the genetic structures between and among geographical groups. The significance of variance component was tested by permuting DNA marker data 999 times. Principal component analysis (PCA) was applied to show the distribution of individual accessions in scatter diagram. Two-dimension PCA graph was drawn using the GENALEX 6.5 package.

Table 2. Total number of alleles and the genetic diversity index for 18 simple sequence repeat (SSR) loci in the 355 Asian Tomato accessions.

SSR marker	Reference	Primers	NA ²⁾	M _{AF}	NG	H _o	GD	PIC
14F	Tomato-EXPEN 2000	F: TCTGCATCTGGTGAAGCAAG R: CTGGATTGCCTGGTTGATTT	4.0	0.84	5.0	0.02	0.28	0.24
20N	Tomato-EXPEN 2000	F: GAGGACGACAACAACAACGA R: GACATGCCACTTAGATCCACAA	3.0	0.69	5.0	0.05	0.43	0.34
565H	Tomato-EXPEN 2000	F: GAGGATGATGAGAACTCGCC R: TCAGAGGCTTCTGGGTCAGT	3.0	0.86	4.0	0.00	0.24	0.22
37F	Tomato-EXPEN 2000	F: ATTGAAGACCGAAACGGTTG R: CTGATAAACC CGCAAGACT	3.0	0.91	4.0	0.02	0.16	0.15
214N	Tomato-EXPEN 2000	F: AAATTCCCAACACTTGCCAC R: CCCACCACTATCCAAACCC	2.0	0.97	3.0	0.01	0.05	0.05
22F	Tomato-EXPEN 2000	F: GATCGGCAGTAGGTGCTCTC R: CAAGAAACACCCATATCCGC	3.0	0.88	5.0	0.01	0.23	0.21
99H	Tomato-EXPEN 2000	F: GCCTCGGATTCAATAGCATT R: CACAAAGAAGCAAACAACCTCA	3.0	0.95	4.0	0.03	0.10	0.09
26F	Tomato-EXPEN 2000	F: CGCCTATCGATACCACT R: ATTGATCCGTTTGTTCTGC	2.0	0.96	3.0	0.01	0.08	0.08
593F	Tomato-EXPEN 2000	F: TGGCATGAACAACAACCAAT R: AGGAAGTTGCATTAGGCCAT	4.0	0.73	7.0	0.05	0.4	0.32
SSR63f	Kwon <i>et al.</i> 2009	F: CCACAAACAATTCCATCTCA R: GCTTCCGCCATACTGATACG	20.0	0.51	31.0	0.10	0.68	0.64
SSR99f	Kwon <i>et al.</i> 2009	F: GCCTCGGATTCAATAGCATT R: CACAAAGAAGCAAACAACCTCA	4.0	0.96	5.0	0.05	0.08	0.08
SLM12-12f	Geethanjali <i>et al.</i> 2011	F: AATTGCCACGTGGATTGACT R: TGCAAGCTGTTCTTTTCAGA	16.0	0.37	28.0	0.07	0.77	0.73
SLM12-31f	Geethanjali <i>et al.</i> 2011	F: TCGTAGCTTCTTTCACGTTGT R: CCGAATGAAAAGGACAAGGA	19.0	0.26	32.0	0.07	0.86	0.84
SLM12-33f	Geethanjali <i>et al.</i> 2011	F: GGACACATTTATGTCATAGCGTAG R: CGATTGTCTGCATATCGGAAG	22.0	0.42	30.0	0.04	0.76	0.74
SLM12-34h	Geethanjali <i>et al.</i> 2011	F: ATCCTCTGGTCTTTGCCAAC R: TCATCCTGAACCACATGTCC	4.0	0.92	7.0	0.03	0.15	0.15
SLM6-5h	Geethanjali <i>et al.</i> 2010	F: ATGCACGCAAAGGTTATTCC R: AGTCGAAGTTGGCTTGACCA	25.0	0.61	32.0	0.05	0.61	0.59
SLM6-14h	Geethanjali <i>et al.</i> 2010	F: TCCGTAATAAGTTGAGGAACCA R: TCACAAGAATATTTGCCGTCAT	21.0	0.30	23.0	0.01	0.81	0.79
SLM6-15h	Geethanjali <i>et al.</i> 2010	F: GGATTTCAGCTGCCTACTGAG R: TTCGGAACATAATAGGGGTTT	18.0	0.36	25.0	0.03	0.74	0.70
Total			176		253			
Mean			9.8	0.69	14.1	0.04	0.41	0.39

²⁾NA: number of alleles, M_{AF}: major allele frequency, NG: number of genotypes, H_o: observed heterozygosity, GD: genetic diversity, PIC: polymorphic information content.

RESULTS

SSR polymorphism in the entire accession

SSR polymorphisms in these 355 Asian tomato accessions were measured in terms of numbers of alleles, gene diversity, and PIC using the PowerMarker software ver. 3.23 (Liu and Muse 2005). The 18 SSR markers revealed 176 alleles among these Asian tomato accessions representing 25 countries (Table 1). SSR loci diversity data are summarized in Table 2. Allelic richness per locus varied widely among markers, ranging from 2 to 25 (SLM6-5h), with an average of nine alleles per locus. The frequency of major alleles per locus ranged from 0.26 (SLM12-31f) to 0.97 (214 N), with an average value of 0.69 per marker. Of the 18 SSR markers, the overall PIC value ranged from 0.05 (214 N) to 0.84 (SLM12-31f), with an average value of 0.39.

Population structures and GD

The population structure of the 355 Asian tomato accessions was inferred by using Structure V2.3.3 based on 18 SSR markers. At this level, individual proportions of membership in each group were estimated using multi-allele data set and the results suggested the existence of some population structure. However, the distribution of $L(K)$ did not indicate a clear mode for the true K (Fig. 1A). Thus, ad hoc quantity (ΔK) was used to overcome the difficulty of interpreting the real K values (Evanno *et al.* 2005). The true value of K was determined by illustration of the peak based on ΔK . A highest peak of ΔK for the 355

accessions was found for $K=2$ (Fig. 1B), indicating that the entire population could be grouped into two subpopulations (Pop1 and Pop2).

Based on membership fractions, accessions with the probability of $\geq 88\%$ were assigned to corresponding subpopulation (pop1 and pop2) while the others were categorized as admixture (Fig. 2). In addition, accessions under different subpopulations were categorized as pure or admixture using structure analysis for categorization purpose. Accessions with more than 0.80 score were considered as pure while those with less than 0.80 score were considered as admixture. The pop1, pop2, and admix categories included 211, 103, and 41 accessions, respectively (Fig. 2). The mean genetic diversities for each SSR locus in pop1 and pop2 of Asian tomato were 0.32 and 0.50, respectively. The mean PIC values for each SSR locus in pop1 and pop2 were 0.30 and 0.46, respectively (Table 3). Comparative study showed that the accessions from pop2 (GD=0.50, PIC=0.46) possessed greater genetic diversities than pop1 (GD=0.32, PIC=0.30). Similarly, the average allele number in pop2 (7.6) was higher than that in pop1 (6.0), with an average of 6.8 for the two inferred clusters (Table 3).

Clustering analysis based on unrooted neighbor-joining tree revealed a similar trend to that based on genetic similarity analysis using model-based Structure (Fig. 3). East Asian geographical group was mainly appertained in pop1. PCoA was used to characterize subgroups of the populations. A two-dimensional scatter plot involving all 355 accessions showed that the first two PCA axes

Fig. 1. Estimated (A) $\ln P(D)$ and (B) ΔK of the 355 Asian tomato accessions over five runs for each K -value.

Fig. 2. Estimated population structure of Asian tomato accessions assessed by STRUCTURE. Each individual is represented by a thin vertical bar, partitioned into up to k colored segments.

Table 3. Diversity information and F_{ST} values in the inferred subpopulations.

Inferred group	Diversity ²⁾					F_{ST} *		
	n	N_A	GD	H	PIC	1	2	Overall
1	211	6.0	0.32	0.02	0.30	0.000	-	-
2	103	7.6	0.50	0.05	0.46	0.135	0.000	-
Average		6.8	0.41	0.03	0.38	-	-	0.135

*For AMOVA-based estimates, $P < 0.005$ for 100 permutations for all population comparisons.

²⁾n: the number of accession, N_A : average number of allele, GD: gene diversity, H: heterozygosity, PIC: polymorphic information content.

accounted for 23.9% and 18.55% of the genetic variations among geographical groups (Fig. 4).

The distribution of molecular variance among and within clusters was estimated using AMOVA. The results revealed that 6% of the total variation was among geographical groups while 86% of the variations were among individuals within population clusters (Table 4). Determination of F_{ST} for the polymorphic loci across all six geographical groups showed a F_{ST} of 0.064, indicating high

genetic variations (data not included). The pairwise F_{ST} estimate value was 0.135 between the inferred clusters 1 and 2, indicating significantly different clusters between the two (Table 3).

DISCUSSION

Molecular markers have great potential to identify the

Fig. 3. Unrooted neighbor-joining tree (UPGMA) based on Nei's genetic distance matrix (shared allele frequency) among 355 Asian tomato accessions. Colors correspond to those of the model-based populations.

Fig. 4. Principal component analysis of the 355 Asian tomato accessions collected from Asian geographical regions. Each symbol represents one variety from one of the six studied regions.

Table 4. Analysis of molecular variance of a number of populations.

Source	df ^{a)}	SS	MS	Est. Var.	%Tv	P-value
Among geographical groups	5	183.9	36.8	0.3	6%	0.001
Among individuals	349	2713.8	7.8	3.7	86%	0.001
Within individuals	355	109.0	0.3	0.3	7%	0.001
Total	709	3006.7		4.3	100%	

^{a)}df: degree of freedom, SS: sum of square, MS: mean square, Est. Var.: estimate variance, %Tv: percentage of total variation.

structure and GD of accessions. GD analysis is important for collections, conservation, and sustainable utilization of Genbank accessions (Suresh *et al.* 2014). Various types of DNA markers studies have been carried out to estimate the GD and phylogenetic relationship among tomato genotypes (Klein-Lankhorst *et al.* 1991; Kwon *et al.* 2009; Geethanjali *et al.* 2011). Various methods have been used for surveying population structures. The model-based approach implemented in the Structure software might be the most frequently used method (Falush *et al.* 2003).

In this study, 18 SSR markers produced 176 alleles and the number of detected alleles over all loci across population ranged from 2 to 25 (SLM6-5h), with an average of 9 alleles per locus (Table 2). In the present study, the NA detected and the mean NA per locus were higher than those reported earlier. For example, Bredemeijer *et al.* (2002) have reported a mean number of 4.7 (range, 2-8) alleles per locus in 521 tomato accessions. Todorovska *et al.* (2014) have reported a mean number of 1.8 (range, 1-6) alleles per locus in eight Bulgarian tomato accessions. Benor *et al.* (2008) have reported an average of 4.3 alleles per locus in 39 determinant and indeterminant tomato inbred lines. Smulders *et al.* (1997) have detected 3 alleles per locus on average for 7 inbred lines of tomato. He *et al.* (2003) have identified 2.7 alleles per locus on average among 17 varieties and 2 parental lines of tomatoes. We used diverse tomato genetic resources preserved in the National Agrobiodiversity Center (RDA, Republic of Korea) and applied the 18 SSR markers which revealed high alleles and polymorphism based on the previous studies (Kwon *et al.* 2009; Geethanjali *et al.* 2010; Geethanjali *et al.* 2011). These factors might cause relatively high average NA per locus in this study.

To compare the genetic variabilities among population,

we calculated PIC and GD values of SSR primers used. The average PIC and GD were 0.39 and 0.41, respectively. Todorovska *et al.* (2014) have reported an average PIC and GD value of 0.196 and 0.22, respectively, for Bulgarian tomato accessions. Bredemeijer *et al.* (2002) have reported that the GD values of the tomato sequence-tagged micro-satellite (STMS) markers are from 0.01 to 0.70, which are relatively low when compared to the results of the present study. Similarly, Benor *et al.* (2008) have observed a separation of inbred lines at a genetic similarity value of 0.85, an evidence for the low level of GD in the tomato germplasm studied. Compared to these reports, our result revealed higher level of mean PIC (0.39) and GD value (0.41). Therefore, our results will be useful for further genetic studies on tomato species.

Pairwise comparisons of Nei's genetic distance (D) between geographical groups were computed from combined data for the 18 primers (Nei 1973). Nei's standard genetic distances (D) between pairs of geographical groups with SSR markers varied from 0.028 to 0.153 (Supplement Table 2). The smallest distance was found between Central Asia and East Asia while the largest distance was between Southeast Asia and West Asia. The genetic distance-based results observed in the unrooted neighbor-joining tree revealed a similar trend to the genetic similarity analysis, revealing two possible subpopulations using model-based Structure (Fig. 3). PCoA analysis was also consistent with the clustering results of the structure analysis (Fig. 2, 4).

In conclusion, 176 alleles were detected with an average of 9.8 alleles per SSR locus among the 355 Asian tomato accessions. Model-based structure analysis revealed the presence of two subpopulations, which was essentially consistent with clustering results based on GD. Our study

showed a high level of GD in the tested 355 accessions. Therefore, the accessions of tomatoes with diverse geographical regions used in this study might be valuable gene pools for allele mining and association mapping for future improvement of tomato crop.

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