

SSR-BASED ASSESSMENT OF GENETIC DIVERSITY IN TOMATO CULTIVARS AND LINES GROWN IN KAZAKHSTAN

Genievskaya Yuliya A.¹, Jantsov Serik K.², Nurbayeva Elmira A.³, Turuspekov Yerlan K.^{1,4*}

¹ Institute of Plant Biology and Biotechnology, Almaty 050040, Kazakhstan

² Kazakh National Agrarian Research University, Almaty 050010, Kazakhstan

³ Kazakh Research Institute of Fruit and Vegetable Growing, Almaty 050060, Kazakhstan

⁴ al-Farabi Kazakh National University, Almaty 050040, Kazakhstan

* yerlant@yahoo.com

ABSTRACT

Tomato (*Solanum lycopersicum* L.) is a versatile crop known for its nutritional value and health benefits, thriving in diverse climates worldwide. Nevertheless, regional variations persist in tomato yield, with Kazakhstan serving as an example of lower productivity in contrast to global averages. Closing this disparity requires comprehensive genetic studies and breeding efforts. This study is focused on the genetic diversity of 49 tomato cultivars and hybrids sourced from Kazakh Research Institute of Fruit and Vegetable Growing (Almaty), employing 10 SSR markers associated with important agronomic traits. SSR genotyping unveiled polymorphisms across 6 markers with variable allele numbers. Genetic diversity metrics highlighted significant genetic diversity within both outdoor and greenhouse tomato cultivars and lines. Bayesian clustering, Neighbor-joining (NJ) clustering, and principal coordinate analysis (PCoA) delineated genetic differentiation between outdoor and greenhouse tomatoes with small admixture, indicating distinct breeding directions for these two types. Highly polymorphic SSRs (PIC > 0.5) associated with essential fruit traits emerge as promising targets for marker-assisted selection (MAS) that can be used to enhance tomato breeding efficiency in Kazakhstan. According to 8 SSRs, 22 out of 30 outdoor accessions and 8 greenhouse tomato accessions were genetically uniform. This study offers comprehensive insights into the genetic diversity and population structure of tomato cultivars and lines in Kazakhstan, laying the foundation for informed breeding endeavors aimed at bolstering yield and resilience in tomato crops.

Key words: *Solanum lycopersicum* L., microsatellites, population structure, outdoor tomato, greenhouse tomato

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) thrive in diverse climates and habitats for growth, boasting a rich supply of dietary fiber, vitamins A and C, minerals, and lycopene and exhibiting anticancer properties [1]. In December 2022, the Food and Agriculture Organization (FAO) [2] released updated data on global tomato production for 2021. The report revealed that total production, encompassing tomatoes for processing and fresh consumption, reached 189.1 million metric tonnes. In 2021, China, India, and Turkey were the leading countries in tomato production (67.5, 21.2, and 13.1 million metric tonnes, respectively) [3]. In Kazakhstan, in 2022, the harvested area of tomatoes was 30.3 thousand hectares, yielding 26.4 tonnes per ha and a total production of 801 thousand tonnes of tomatoes in the country [4]. This is Kazakhstan's third most cultivated vegetable crop after potato and onion. However, tomato's yield in Kazakhstan is 10.7 t/ha lower than the average in the World (37.1 t/ha) and almost twenty times lower than in the Netherlands (486.6 t/ha) – the best performance in the World [2]. The urgency of introducing new tomato germplasm with diverse genetic traits and enhancing existing local cultivars through breeding programs becomes evident.

Utilizing commercial varieties to foster diversity is widespread in numerous public tomato breeding programs [5]. However, limited data on the breeding potential of commercial tomato varieties restricts pedigree selection for generating new lines. Introducing genetically distant germplasm can significantly improve local tomato cultivars' adaptability, yield, and fruit quality. Unlocking the potential of tomato genetic diversity through breeding and biotechnology holds immense promise for the tomato-producing industry. Genetic studies

empower us to conserve existing cultivars and breed superior ones, ensuring a sustainable future for tomatoes. Different approaches to measuring the genetic variations in the crop collections include pedigree information morphological and molecular markers [6]. However, morphological traits cannot be used to measure the genotypic variations since these can be affected by the environment [7]. Therefore, one of the most effective ways of searching for new germplasm for breeding is an assessment of genetic diversity using various DNA markers [8, 9]. Information about genetic diversity is also important for germplasm conservation and diversification [10]. The genetic diversity of the world tomato germplasm was studied using amplified fragment length polymorphism (AFLP) [11], sequence characterized amplified region (SCAR) [12], single nucleotide polymorphism (SNP) [13, 14], inter simple sequence repeats (ISSR) [15], and, one of the most common marker types, simple sequence repeats (SSR) [16, 17, 18]. Among them, SSR-type markers offer several advantages over other types of genetic markers. SSRs are highly polymorphic, codominant, multi-allelic, abundant throughout the genome, and are often found in non-coding regions, making them less likely to be affected by selection or other evolutionary pressures [19, 20, 21]. In addition, SSRs can be easily and efficiently analyzed using PCR, making them affordable and effective simultaneously.

In breeding studies, the selection efficiency in tomatoes is also improved by using DNA markers [22]. Tomato is one of the first plants with genetic maps based on high-density DNA markers [23], facilitating molecular studies including genotyping, quantitative trait locus (QTL) analysis, and marker-assisted selection (MAS) approach in research and breeding pro-

grams [24]. The associations between economically valuable traits of tomato and SSRs were identified for cold tolerance [25, 26], fruit quality [26], fruit nutritional and flavor components [27], plant adaptation and yield-related traits [28], etc.

Previously, Kazakhstan's tomato cultivars and lines were studied using molecular markers for the resistance to different tomato pathogens: tomato mosaic virus, tomato spot wilt virus, tomato yellow leaf curl virus, fungus *Fusarium oxysporum*, and oomycete *Phytophthora infestans*. [29, 30]. These researches demonstrated a lack of well-known genetic resistance factors and high genetic similarity among local tomato germplasm.

The current study was focused on a comprehensive assessment of genetic diversity in a collection of 49 tomato cultivars and hybrids provided by the Kazakh Research Institute of Fruit and Vegetable Growing (Almaty) using SSR markers associated with various agronomic traits of tomatoes.

MATERIALS AND METHODS

Tomato collection and DNA extraction

The tomato collection used in the study included 30 cultivars and hybrids for outdoor production and 19 cultivars and hybrids for the greenhouse (Table 1).

The DNA was extracted from 8-10 days of seedlings of Table 1. The list of tomato accessions used in the study

No.	Accession name	Growth type	No.	Accession name	Growth type
1	Somalday	outdoor	26	No 33 BSS-335 x Lider	outdoor
2	Tansholpan	outdoor	27	No 5 Avicena x G-205	outdoor
3	Luchezarniy	outdoor	28	No 2 G-2005	outdoor
4	Ayan	outdoor	29	No 35 Roza Vostoka x G-205	outdoor
5	Ogonek-777	outdoor	30	No 3 Gloria x G-2001	outdoor
6	Rassvet	outdoor	31	Alua F1	greenhouse
7	Mechta	outdoor	32	Dias	greenhouse
8	Zarya Vostoka	outdoor	33	Nurai F1	greenhouse
9	Meruert	outdoor	34	Zhalyn	greenhouse
10	Korkem	outdoor	35	Zolotaya businka (2017D)	greenhouse
11	Chudesniy	outdoor	36	Keremet (2413D)	greenhouse
12	Yantar	outdoor	37	Solnechnaya zhemchuzhina	greenhouse
13	Lider	outdoor	38	Luna F7	greenhouse
14	Umit	outdoor	39	Luna F5	greenhouse
15	Venera	outdoor	40	Daniela F6	greenhouse
16	Vostorg	outdoor	41	I-4 chY	greenhouse
17	Daryn	outdoor	42	I-1 chR	greenhouse
18	Plamya	outdoor	43	12-2 (27-2)	greenhouse
19	Surpriz	outdoor	44	C-27-31DK	greenhouse
20	Narttay	outdoor	45	C-274	greenhouse
21	G-10	outdoor	46	C-27-3	greenhouse
22	No 31 Avicena x TMK	outdoor	47	C-27-31 DR	greenhouse
23	No 37 L-91-95-2	outdoor	48	C-27-31 BK	greenhouse
24	No 36 Lider x Luchezarniy	outdoor	49	Gondola F5	greenhouse
25	No 32 Gloria x BSS-335	outdoor			

tomato accessions in three replicates of each accession using a modified CTAB method [31]. The quality and quantity of the DNA were checked on NanoDrop One spectrophotometer (Thermo Fisher Scientific Inc., USA) and 1% agarose gel electrophoresis.

SSR genotyping

SSR-genotyping of tomato collection was performed using 10 markers (Table 2). These SSRs were selected due to their association with the economically valuable traits of tomato. PCR conditions were optimized in order to provide high efficiency and accuracy for each marker (Table 2). The PCR was performed in a total volume of 20 μ L including 20 ng of genomic DNA, 1 U of Taq polymerase, 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), 10 pM of each primer, 1.5 mM of magnesium chloride ($MgCl_2$), and a standardized 1× Taq buffer solution. The amplification has comprised of 1 cycle of 3 min at 94 °C, 30 cycles of 30 secs at 94 °C, 30 secs at Ta °C (Table 2), 30 secs at 72 °C, and final 1 cycle of 7 mins at 72 °C.

The PCR products were separated on a QIAxcel Connect System for capillary electrophoresis (QIAGEN, Germany) using a QIAxcel DNA High-Resolution Kit and QX Alignment Marker (15 bp/3 kb). The standard OH500 method was used to run the samples with an injection time of 20 s.

Table 2. SSR-markers used in the analysis

SSR	Chr.	Ta, °C	F (5'-3')	R (5'-3')	Traits associated
TMS7	12	60	CCTTGCAGTTGAGGTGAATT	TCAAGCACCTACAATCAATCA	Fruits per plant [28]
TMS23	12	55	GGATTGTAGAGGTGTTGTTGG	TTTGTAAATTGACTTTGTCGATG	Yield per plant [28]
TC11	4	55	TCAACACAGAGAAAATAGGCA	CAGCTTGCTCAGCCAGC	Days to fruit maturity [28]
TMS37	5	64	CCTTGCAGTTGAGGTGAATT	TCAAGCACCTACAATCAATCA	Fruits per plant [28]
TMS42	11	55	AGAATTTTTCATGAAATTGTCC	TATTGCGTTCCACTCCCTCT	Fruit weight and/or shape [32]
TMS43	9	48	TTGGCCTAATCCTTGTCA	AACAATGTGACGTCTTATAAGGG	Leaf length [28]
TMS52	12	60	TTCTATCTCATTGGCTTCTTC	TTACCTTGAGAATGGCCTTG	Fruit weight [32], plant height [33]
Tom59-60	3	48	TAACACATGAACATTAGTTGA	CACGTAAAATAAAGAAGGAAT	Fruit weight and/or shape [32]
TMS63	1	60	GCAGGTACGCACGCATATAT	GCTCCGTCAGGAATTCTCTC	Fruit color [32]
TES856	1	55	GAAACAAAACCCGAAACGAA	AACCACCACTCTCATCACCC	Chilling index [25]

RESULTS

SSR genotyping

A total of 10 SSR markers associated with various agronomic traits of tomato (Table 2) were used to analyze the genetic diversity in 49 tomato genotypes. Out of 10 SSR markers, 6 were observed to be polymorphic, 2 SSRs were monomorphic, and the remaining 2 SSRs did not amplify (Table 3). These 6 polymorphic SSRs were only used to analyze genetic diversity and population structure.

The number of alleles per polymorphic locus varied from 2 to 7, with an average of 2.6 ± 2.16 alleles per SSR (Table 3). All markers' expected and observed allele sizes did not match, demonstrating wider ranges.

Genetic diversity of tomato collection

Genetic diversity indices were calculated separately for outdoor and greenhouse types of tomato accessions and the whole collection (Table 4).

Table 3. Results of SSR-genotyping

SSR	Chromosome	Expected sizes (bp)	Actual sizes (bp)	Na	Genotyping result
TMS7	12	170 [28]	160, 163, 167, 170, 174, 179, 185	7	Polymorphic
TC11	4	91 [28]	95, 105	2	Polymorphic
TMS23	12	412 [28]	-	-	Did not amplify
TMS37	5	160 [28]	134	1	Monomorphic
TMS42	11	272-283 [32]	-	-	Did not amplify
TMS43	9	323 [28]	260, 335	2	Polymorphic
TMS52	12	152-174 [32, 33]	166, 170, 174, 176, 183	5	Polymorphic
Tom59-60	3	113-122 [32]	185, 179	2	Polymorphic
TMS63	1	130-150 [32]	167, 189	2	Polymorphic
TES856	1	-	216	1	Monomorphic
Na – number of alleles					

Table 4. Genetic diversity among tomato accessions of outdoor type, greenhouse type and within the whole collection

Type	Locus	N	Na	Ne	I	h	PIC	%P	
Outdoor	TMS7	90	3	2.271	0.892	0.566	0.560	50.0	
	TC11		2	1.976	0.687	0.499	0.494		
	TMS37		1	Monomorphic					
	TMS43		1	Monomorphic					
	TMS52		1	Monomorphic					
	Tom59-60		3	2.677	1.039	0.633	0.626		
	TMS63		1	Monomorphic					
	TES856		2	1.442	0.485	0.310	0.306		
	Mean*		2.50	2.092	0.776	0.502	0.497		
	SE*		0.58	0.520	0.242	0.139	0.138		
Greenhouse	TMS7	57	7	4.622	1.745	0.798	0.784	75.0	
	TC11		2	1.191	0.297	0.163	0.160		
	TMS37		1	Monomorphic					
	TMS43		2	1.999	0.693	0.509	0.500		
	TMS52		2	1.633	0.576	0.395	0.388		
	Tom59-60		5	3.957	1.482	0.761	0.747		
	TMS63		1	Monomorphic					
	TES856		2	1.150	0.254	0.133	0.131		
	Mean*		3.33	2.425	0.841	0.460	0.452		
	SE*		2.16	1.492	0.626	0.285	0.280		
Whole collection	TMS7	147	7	3.148	1.413	0.687	0.682	75.0	
	TC11		2	1.739	0.616	0.428	0.425		
	TMS37		1	Monomorphic					
	TMS43		2	1.464	0.497	0.319	0.317		
	TMS52		2	1.224	0.330	0.185	0.183		
	Tom59-60		5	3.253	1.308	0.697	0.693		
	TMS63		1	Monomorphic					
	TES856		2	1.324	0.410	0.247	0.245		
	Mean*		2.60	1.801	0.632	0.375	0.373		
	SE*		2.16	0.927	0.474	0.221	0.219		

* – only polymorphic loci were used for mean and SD values; N – number of samples per type; Na – number of alleles; Ne – number of effective alleles; I – Shannon's information index; h – Nei's genetic diversity index; PIC – polymorphic information content; %P – percentage of polymorphic loci per type; SE – standard error

The values of Shannon's information index (*I*) for polymorphic loci in the outdoor type group ranged from 0.485 to 1.039 (mean *I* = 0.776 ± 0.242); in the greenhouse type – from 0.254 to 1.745 (mean *I* = 0.841 ± 0.626); and for the whole collection from 0.330 to 1.413 (mean *I* = 0.632 ± 0.474) (Table 4). Nei's genetic diversity index (*h*) varied from 0.310 to 0.633 (mean *h* = 0.502 ± 0.139) for the outdoor type group, from 0.133 to 0.798 (mean *h* = 0.460 ± 0.285) for the greenhouse group, and from 0.85 to 0.697 (mean *h* = 0.375 ± 0.221) for the whole collection (Table 4). As for the PIC values, in the outdoor type group, they ranged from 0.306 to 0.626 (mean PIC = 0.497 ± 0.138); in the greenhouse type group – from 0.131 to 0.748 (mean PIC = 0.452 ± 0.280); and in the whole collection – from 0.183 to 0.693 (mean PIC = 0.373 ± 0.219) (Table 4).

The largest polymorphism was observed for SSRs TMS7 on chromosome 12 and Tom59-60 on chromosome 3, with 7 and 5 alleles for the whole tomato collection (Table 4).

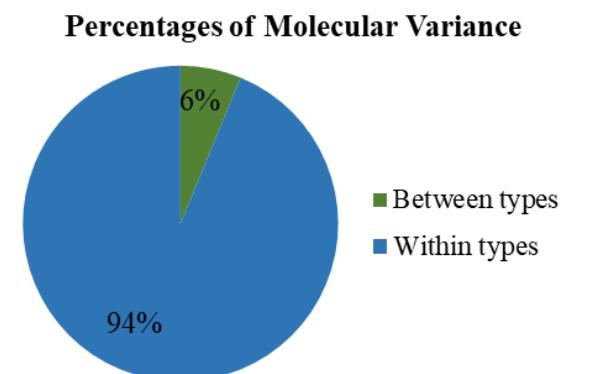


Figure 1 – Results of AMOVA between and among growth types in the studied tomato collection

The results of SSR-genotyping demonstrated a relatively small number of polymorphic loci in the outdoor type tomato group: 50 % of polymorphic loci in the outdoor type vs. 75 % in the greenhouse type and 75 % in the whole collection (Table 4). The genetic diversity indices based on polymorphic loci were greater for outdoor and greenhouse types than for the whole collection: means of *I*, *h*, and PIC values were higher for these two groups compared to the whole collection

(Table 4). Results of AMOVA also revealed higher percentages of molecular variance within two tomato types than between them (Figure 1).

Thus, the general genetic diversity in outdoor-type and greenhouse-type tomato groups was similar but higher than in the whole collection. However, genetic diversity was assessed for tomato types and SSRs within each tomato accession used in the study (Table 5).

Table 5. Genetic diversity of studied tomato accessions

Outdoor type						
ID	N	Na	Ne	I	h	%P
TOM_01	3	1.000	1.0	0.000	0.000	0.0
TOM_02	3	1.000	1.0	0.000	0.000	0.0
TOM_03	3	1.000	1.0	0.000	0.000	0.0
TOM_04	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_05	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_06	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_07	3	1.375 ± 0.183	1.3 ± 0.2	0.239 ± 0.116	0.167 ± 0.081	37.5
TOM_08	3	1.000	1.0	0.000	0.000	0.0
TOM_09	3	1.000	1.0	0.000	0.000	0.0
TOM_10	3	1.000	1.0	0.000	0.000	0.0
TOM_11	3	1.000	1.0	0.000	0.000	0.0
TOM_12	3	1.000	1.0	0.000	0.000	0.0
TOM_13	3	1.000	1.0	0.000	0.000	0.0
TOM_14	3	1.000	1.0	0.000	0.000	0.0
TOM_15	3	1.000	1.0	0.000	0.000	0.0
TOM_16	3	1.000	1.0	0.000	0.000	0.0
TOM_17	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_18	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_19	3	1.000	1.0	0.000	0.000	0.0
TOM_20	3	1.000	1.0	0.000	0.000	0.0
TOM_21	3	1.000	1.0	0.000	0.000	0.0
TOM_22	3	1.000	1.0	0.000	0.000	0.0
TOM_23	3	1.000	1.0	0.000	0.000	0.0
TOM_24	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_25	3	1.000	1.0	0.000	0.000	0.0
TOM_26	3	1.000	1.0	0.000	0.000	0.0
TOM_27	3	1.000	1.0	0.000	0.000	0.0
TOM_28	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_29	3	1.000	1.0	0.000	0.000	0.0
TOM_30	3	1.000	1.0	0.000	0.000	0.0
MEAN	3	1.041	1.033	0.027	0.019	4.2
SE	0	0.035	0.030	0.022	0.015	12.5
Greenhouse type						
ID	N	Na	Ne	I	h	%P
TOM_31	3	1.625 ± 0.263	1.6 ± 0.3	0.376 ± 0.152	0.250 ± 0.098	50.0
TOM_32	3	1.625 ± 0.183	1.5 ± 0.2	0.398 ± 0.116	0.278 ± 0.081	62.5
TOM_33	3	1.250 ± 0.164	1.2 ± 0.1	0.159 ± 0.104	0.111 ± 0.073	25.0
TOM_34	3	1.875 ± 0.295	1.8 ± 0.3	0.513 ± 0.165	0.333 ± 0.103	62.5

TOM_35	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_36	3	1.000	1.0	0.000	0.000	0.0
TOM_37	3	1.000	1.0	0.000	0.000	0.0
TOM_38	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_39	3	1.000	1.0	0.000	0.000	0.0
TOM_40	3	1.250 ± 0.164	1.2 ± 0.1	0.159 ± 0.104	0.111 ± 0.073	25.0
TOM_41	3	1.000	1.0	0.000	0.000	0.0
TOM_42	3	1.000	1.0	0.000	0.000	0.0
TOM_43	3	1.375 ± 0.183	1.3 ± 0.2	0.239 ± 0.116	0.167 ± 0.081	37.5
TOM_44	3	1.125 ± 0.125	1.1 ± 0.1	0.080 ± 0.080	0.056 ± 0.056	12.5
TOM_45	3	1.500 ± 0.189	1.4 ± 0.2	0.318 ± 0.120	0.222 ± 0.084	50.0
TOM_46	3	1.000	1.0	0.000	0.000	0.0
TOM_47	3	1.000	1.0	0.000	0.000	0.0
TOM_48	3	1.000	1.0	0.000	0.000	0.0
TOM_49	3	1.500 ± 0.267	1.5 ± 0.3	0.296 ± 0.153	0.194 ± 0.098	37.5
MEAN	3	1.230	0.779	0.130	0.097	18.6
SE	0	0.110	0.105	0.067	0.045	22.9
Whole collection						
ID	N	<i>Na</i>	<i>Ne</i>	<i>I</i>	<i>h</i>	%P
MEAN	3	1.136	1.812	0.079	0.058	11.4
SE	0	0.073	0.068	0.045	0.030	17.7

N – number of samples per accession; Na – number of alleles; Ne – number of effective alleles; I – Shannon's information index; h – Nei's genetic diversity index; %P – percentage of polymorphic loci per accession; SE – standard error

Based on the results of SSR genotyping, 30 out of 49 tomato cultivars and lines demonstrated genetic uniformity with zero diversity per accession, including 22 outdoor accessions and 8 greenhouse accessions (Table 5). The percentage of polymorphic loci per accession varied from 0 to 37.5 % for outdoor-type tomatoes and from 0 to 62.5 % for the greenhouse type, with average values of 4.2 ± 12.5 % and 18.6 ± 22.9 %, respectively (Table 5). Thus, tomatoes of the outdoor type used in the study were generally more genetically uni-

form than tomatoes of the greenhouse type.

Population structure in studied tomato collection

For the assessment of population structure in the studied tomato collection, 3 methods were used: Bayesian clustering approach with MCMC and DeltaK estimation in STRUCTURE, NJ clustering, and PCoA. Results of the STRUCTURE analysis estimated that K = 3 was the optimum for the studied population, with 3 clusters formed (Figure 2A).

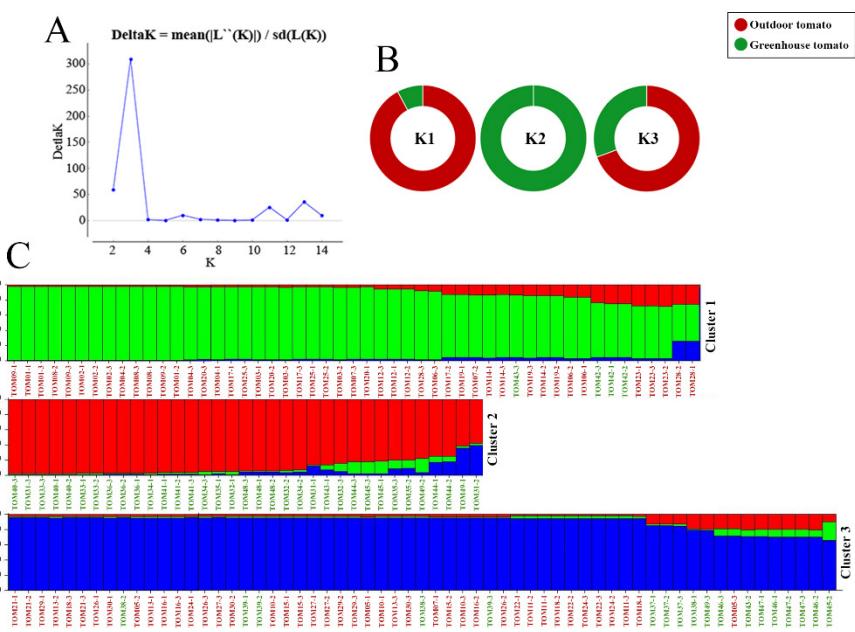


Figure 2. Number of clusters and population structure according to STRUCTURE

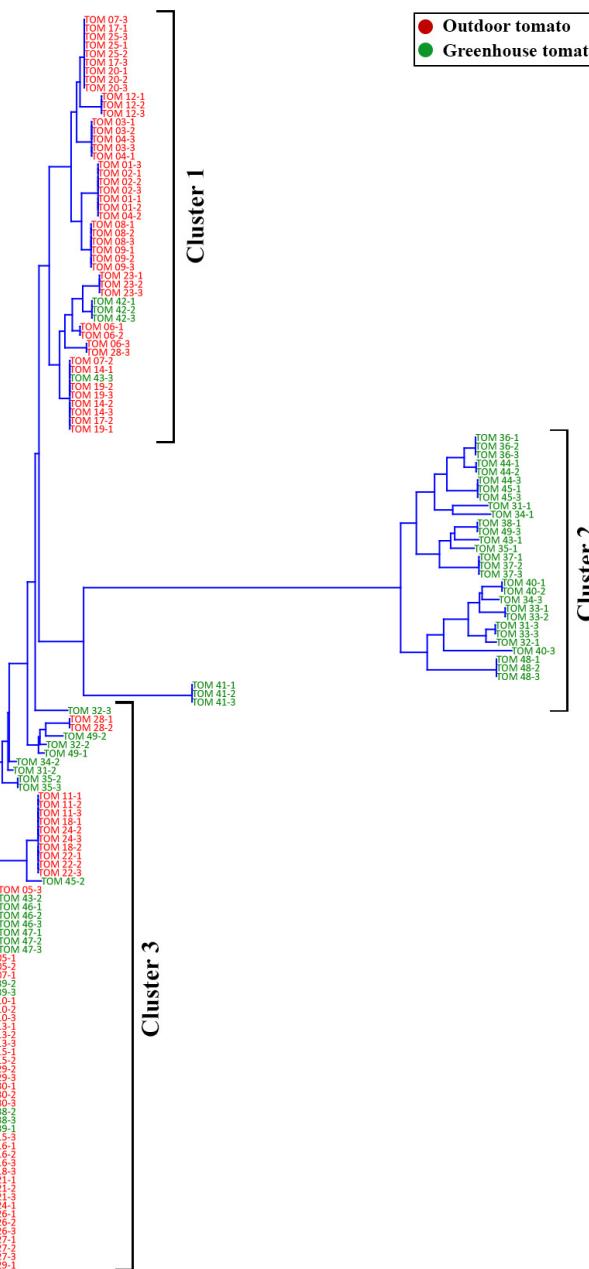


Figure 3. Caurterization of tomato accessions via Neighbor-joining (NJ) method

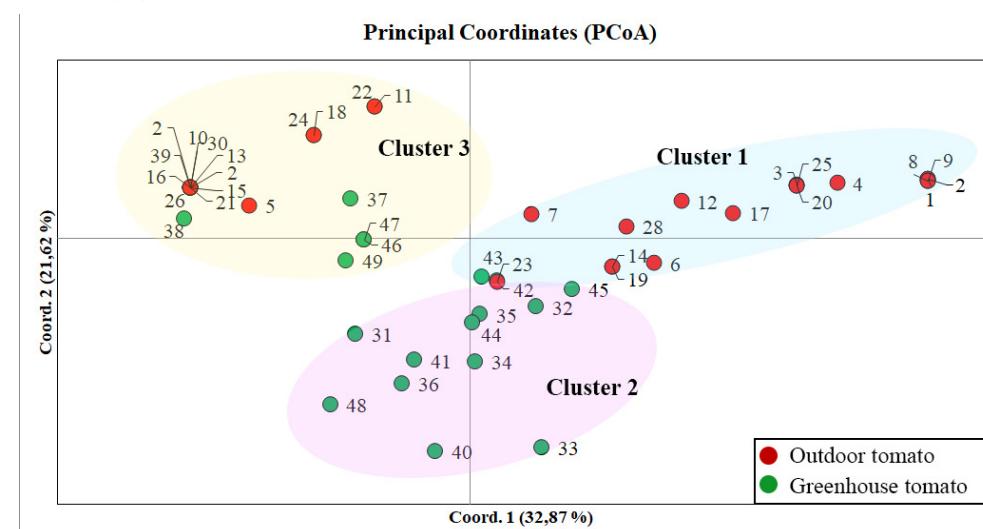


Figure 4. Principal coordinate analysis (PCoA)

The first cluster, K1, included 51 out of 147 samples (49 accessions in 3 replications), with 47 outdoor samples and 4 greenhouse samples (Figures 2B, 2C). The second cluster, K2, included 35 greenhouse samples only, while the largest cluster, K3, included 61 samples (18 greenhouse samples and 43 outdoor samples) (Figures 2B, 2C). Thus, according to STRUCTURE results, outdoor tomatoes are subdivided into two clusters occupying 92.2 % of K1 and 70.5 % of K3 (Figures 2B, 2C). Greenhouse tomatoes fully formed cluster K2 and partially were found in K1 and K3 (Figures 2B, 2C). Results of NJ clustering (Figure 3) and PCoA (Figure 4) confirmed the presence of 3 clusters in the studied tomato collection with the same distribution of samples among clusters.

Thus, 147 tomato samples (49 accessions in 3 replications) were grouped into 3 clusters according to the Bayesian clustering approach with MCMC and DeltaK estimation in STRUCTURE, NJ clustering, and PCoA based on SSR-genotyping results. The 30 outdoor tomato accessions formed two clusters: 16 accessions in Cluster 1 and 14 accessions in Cluster 3 (Figures 2, 3, and 4). Among 19 greenhouse tomato accessions, 11 accessions formed a separate cluster 2, while the remaining 8 greenhouse accessions were grouped with outdoor tomatoes in clusters 1 and 3 (Figures 2, 3, and 4).

DISCUSSION

Previously, genetic assessment of tomato breeding collection in Kazakhstan using SSR, SCAR, and CAPS markers showed low diversity and weak genetic structure of tomato cultivars [30]. On the contrary, the present study, using SSR-markers, Bayesian clustering, NJ clustering, and PCoA, demonstrated genetic differentiation of outdoor-type tomatoes from greenhouse ones (Figures 2, 3, and 4) with a large molecular variability within two types (Figure 1). These findings suggest the generally parallel breeding of these two tomato types. However, the presence of greenhouse accessions in clusters of the outdoor type may suggest the involvement of some outdoor tomato germplasm in the breeding of these greenhouse cultivars and lines (or vice versa) used in the study.

As for the genetic diversity, the total genetic diversity of greenhouse accessions was almost two times higher than

outdoor ones' (Table 4). Three SSRs (*TMS7*, *Tom59-60*, and *TMS43*) demonstrated high information content (PIC ≥ 0.5 for codominant markers [34]) among greenhouse tomatoes (Table 4). These markers were associated with the number of fruits per plant [28], fruit weight [32], and leaf length [28], respectively (Table 2). *TMS7* and *Tom59-60* were also highly polymorphic among outdoor types, and the whole tomato collection had PIC > 0.5 (Table 2). Two SSRs (*TC11* and *TES856*) associated with the number of days to fruit maturity [28] and frost tolerance [25], respectively (Table 2), showed moderate polymorphism levels ($0.25 \leq \text{PIC} < 0.5$ [34]) for outdoor tomatoes (Table 4). Moderate polymorphism was also observed for *TMS52* (fruit weight [32], plant height [33]) among greenhouse tomatoes and *TC11*, *TMS43* (leaf length [28]), and *TES856* in the whole collection (Table 4). Two SSRs (*TMS37* and *TMS63*) associated with the number of fruits per plant [28] and fruit color [32], respectively (Table 2), were monomorphic for the whole tomato collection (Table 4). Thus, highly polymorphic SSRs *TMS7* and *Tom59-60* associated with the number and weight of tomato fruits are good candidates for the MAS and genetic diversity analysis of tomatoes of both types in Kazakhstan. Markers *TC11* and *TES856*, associated with the number of days to fruit maturity and frost tolerance, respectively, are promising for the MAS and the analysis of the genetic diversity of outdoor tomatoes. Finally, markers *TMS52* and *TMS43* associated with fruit weight/plant height and leaf length can be used in genetic diversity analysis and MAS of greenhouse tomatoes. Generally, the mean genetic diversity based on polymorphic SSR markers in the whole tomato collection under the study was moderate (PIC = 0.373 ± 0.219) (Table 4).

Outdoor tomatoes generally demonstrated a lower number of polymorphic loci (50 %) than greenhouse ones (75 %) (Table 4). Based on the SSR-genotyping results, 22 out of 30 outdoor accessions were genetically uniform, 7 accessions showed 12.5 % of polymorphic loci within the accession, and only one outdoor tomato cultivar «Mechta» contained 37.5 % of polymorphic loci (Table 5). Among greenhouse types, only 8 accessions were genetically uniform (Table 5). The remaining 11 accessions contained from 12.5 % to 62.5 % of polymorphic loci. The largest polymorphism was observed in cultivars «Dias» and «Zhalyn». Genetic uniformity is very important for crop breeding. For example, pedigree selection stands out as an effective strategy for developing inbred tomato lines with enhanced yield. This breeding method suggests the accumulation of positive alleles through successive generations via natural self-fertilization processes [5]. At the same time, genetic uniformity helps to predict the results of other common tomato breeding methods – heterosis [39]. For both methods, ensuring genetic uniformity among tomato cultivars holds significant importance.

CONCLUSION

The collection of 49 tomato cultivars and lines used in the study was characterized by moderate polymorphism with two highly polymorphic SSR markers associated with important agronomic traits of tomato. The result of population structure analysis based on Bayesian clustering, NJ clustering, and PCoA demonstrated the differentiation of outdoor-type tomatoes from greenhouse ones with a small admixture between

the two types. Thus, 8 SSR markers used in the current study have a potential for both MAS for the future development of tomato breeding in Kazakhstan and genetic diversity analysis.

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ОЦЕНКА ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ СОРТОВ И ЛИНИИ ТОМАТА В КАЗАХСТАНЕ С ИСПОЛЬЗОВАНИЕМ SSR-МАРКЕРОВ

Гениевская Юлия А.¹, Джантасов Серик К.², Нурбаева Эльмира А.³, Туруспеков Ерлан К.^{1,4*}¹ Институт биологии и биотехнологии растений, Алматы 050040, Казахстан² Казахский национальный аграрный исследовательский университет, Алматы 050010, Казахстан³ Казахский научно-исследовательский институт плодоовощеводства, Алматы 050060, Казахстан⁴ Казахский национальный университет им. аль-Фараби, Алматы 050040, Казахстан

*yerlant@yahoo.com

АННОТАЦИЯ

Томат (*Solanum lycopersicum* L.) – это универсальная овощная культура, известная своей питательной ценностью и пользой для здоровья, произрастающая в различных климатических условиях по всему миру. Несмотря на это, в урожайности томатов сохраняются региональные различия, и Казахстан служит примером более низкой урожайности по сравнению со средними мировыми показателями. Для улучшения данной ситуации требуются как усилия селекционеров, так и комплексные генетические исследования. Данная работа представляет собой результаты оценки генетического разнообразия 49 сортов и гибридов томата, полученных из Казахского научно-исследовательского института плодоовощеводства (Алматы), с использованием 10 SSR-маркеров, связанных с важными агрономическими признаками. SSR-генотипирование выявило полиморфизм для 6 маркеров с различным числом аллелей. Индексы генетического разнообразия позволили выявить присутствие значительного генетического разнообразия как среди сортов и линий томатов для открытого грунта, так и среди томатов для теплиц. Байесовская кластеризация, кластеризация по методу присоединения соседей (NJ) и анализ главных координат (PCoA) позволили определить генетическую дифференциацию между томатами для открытого грунта и тепличными сортами и линиями с небольшой примесью, указывая на параллельные направления селекции для этих двух типов. Высокополиморфные SSR-маркеры (PIC > 0,5), ассоциированные с основными признаками продуктивности томата, являются многообещающими объектами для маркерной селекции (MAS), которая может быть использована для повышения эффективности селекционных программ в Казахстане. По данным генотипирования по 8 SSR-маркерам, 22 из 30 образцов открытого грунта и 8 тепличных образцов томата оказались генетически однородными. Это исследование дает всестороннее представление о генетическом разнообразии и популяционной структуре сортов и линий томата в Казахстане, закладывая основу для селекционной работы, направленной на повышение урожайности и разнообразия данной культуры.

Ключевые слова: *Solanum lycopersicum* L., микросателлитные маркеры, структура популяции, томаты открытого грунта, тепличные томаты

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ҚАЗАҚСТАНДАҒЫ ҚЫЗАНАҚ СОРТТАРЫНЫҢ ГЕНЕТИКАЛЫҚ АЛУАНТҮРЛІЛІГІN SSR-МАРКЕРЛЕРИМЕН БАҒАЛАУ

Гениевская Юлия А.¹, Джантасов Серик К.², Нурбаева Эльмира А.³, Туруспеков Ерлан К.^{1,4*}¹ Өсімдіктер биологиясы және биотехнологиясы институты, Алматы 050040, Қазақстан² Қазақ ұлттық аграрлық зерттеу университеті, Алматы 050010, Қазақстан³ Қазақ бау-бақша шаруашылығы ғылыми-зерттеу институты, Алматы 050060, Қазақстан⁴ әл-Фараби атындағы Қазақ ұлттық университеті, Алматы 050040, Қазақстан

*yerlant@yahoo.com

ТҮЙІН

Қызанак (*Solanum lycopersicum* L.) – дүние жүзінің әр түрлі климаттық аймақтарында өссетін тағамдық күндылығымен және денсаулыққа пайдасымен танымал жан-жакты қөкеніс дақылы. Осыған қарамастан, қызанактың өнімділігінде аймақтық айырмашылықтар сақталады және Қазақстан орташа әлемдік қөрсеткіштермен салыстырылғанда төмен өнімділіктің үлгісі болып табылады. Бұл жағдайды жақсарту үшін селекционерлердің қүш-жігері де, жан-жакты генетикалық зерттеулер де қажет. Бұл жұмыс маңызды агрономиялық белгілермен байланысты 10 SSR-маркерлерді пайдалана отырып, Қазақ бау-бақша шаруашылығы ғылыми-зерттеу институтынан (Алматы) алынған қызанактың 49 сорты мен будандарының генетикалық алутантүрлілігін бағалау нәтижелері болып табылады. SSR-генотиптеу әртүрлі аллель саны бар 6 полиморфты маркерді анықтады. Генетикалық алутантүрлілік индекстері ашық грунттағы қызанак сорттары мен линиялары арасында да, жылыштайтындағы қызанактары арасында да айттарлықтай генетикалық алутантүрліліктің болуын анықтады. Байес кластері, көршілес қосу әдісі (NJ) кластері және негізгі координаталық талдау (PCoA) ашық грунт қызанактары мен жылыштайтын сорттары және аз аралас линиялар арасындағы генетикалық дифферен-

циацияны анықтауға мүмкіндік берді, бұл екі түрге параллель селекция бағыттарын қөрсетеді. Қызанак өнімділігінің негізгі белгілерімен байланысты жоғары полиморфты SSR-маркерлері (PIC > 0,5) Қазақстанда селекциялық бағдарламалардың тиімділігін арттыру үшін пайдаланылуы мүмкін маркерлік селекция (MAS) үшін перспектиналы объектилер болып табылады. 8 SSR-маркерлері бойынша генотиптеу деректері бойынша 30 ашық грунт үлгілерінің 22-сі және 8 жылыштайтын қызанак үлгілері генетикалық жағынан біртекті болып шықты. Бұл зерттеу Қазақстандағы қызанак сорттары мен линияларының генетикалық алутантүрлілігі мен популяциялық құрылымы туралы жан-жакты түсінік береді, осы дақылдың өнімділігі мен алутантүрлілігін арттыруға бағытталған селекциялық жұмыстың негізін қалайды.

Түйін сөздер: *Solanum lycopersicum* L., микросателлитті маркерлер, популяция құрылымы, ашық грунт қызанактары, жылыштайтын қызанактары