Genetic diversity of the breeding collection of tomato varieties in Kazakhstan assessed using PCR based markers

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Tomato is one of the most prominent crops in global horticulture and an important vegetable crop in Kazakhstan. Despite its importance, tomato breeding tomato breeding remains relatively underdeveloped in the country. This study aimed to perform an initial evaluation of the breeding collection of tomato varieties from the point of view of their genetic structure and pathogen resistance based on molecular markers. The use of 13 SSR markers revealed a weak genetic structure in the samples of varieties including local cultivars and, predominantly, varieties from Russia and other ex-USSR countries. The screening for a set of SCAR and CAPS markers of resistance against five pathogens revealed a common occurrence of the resistance locus *I* against *Fusarium oxysporum* and only an occasional presence of resistant alleles of other markers. The obtained results reflect the lack of attention that has been paid to tomato breeding in Kazakhstan since its independence. Further development of tomato breeding in the country would require the re-establishment of selection processes involving the diversification of source germplasm and the use of molecular data, especially paying attention to genetic factors of resistance to pathogens and other biotic and abiotic stresses.

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Abstract

Tomato is one of the most prominent crops in global horticulture and an important vegetable crop in Kazakhstan. Despite its importance, tomato breeding tomato breeding remains relatively underdeveloped in the country. This study aimed to perform an initial evaluation of the breeding collection of tomato varieties from the point of view of their genetic structure and pathogen resistance based on molecular markers. The use of 13 SSR markers revealed a weak genetic structure in the samples of varieties including local cultivars and, predominantly, varieties from Russia and other ex-USSR countries. The screening for a set of SCAR and CAPS markers of resistance against five pathogens revealed a common occurrence of the resistance locus *I* against *Fusarium oxysporum* and only an occasional presence of resistant alleles of other markers. The obtained results reflect the lack of attention that has been paid to tomato breeding in Kazakhstan since its independence. Further development of tomato breeding in the country would require the re-establishment of selection processes involving the diversification of source germplasm and the use of molecular data, especially paying attention to genetic factors of resistance to pathogens and other biotic and abiotic stresses.

Keywords: *Solanum lycopersicum,* SSR, SCAR, CAPS, resistance, *Phytophtora infestans, Fusarium oxysporum,* tomato mosaic virus, tomato spotted wilt virus, tomato yellow curly leaf virus

Introduction

Tomato *Solanum lycopersicum* L. is a representative plant species of the Solanaceae family which includes a number of important vegetable and technical crops. Tomato is one of the most popular vegetable crops all over the world, alongside other species of the family such as potato *Solanum tuberosum* L., eggplant *Solanum melongena* L., peppers *Capsicum annuum* L., and *Capsicum chinense* Jacq.

Tomatoes comprise an important part of overall vegetable production in Kazakhstan, with 788,760 tons harvested from 30.2 thousand hectares in 2022. Tomato production has been developed in the country extensively rather than intensively; the growing area has doubled but the yield per hectare volume has stagnated in the last 30 years (Food and Agriculture Organization of the United Nations, 2021). Among the tomato varieties approved for cultivation in the country, the foreign cultivars prevail with a significant share of varieties from Russia and other ex-USSR countries (The Ministry of Agriculture of the Republic of Kazakhstan, 2009). Such a dependence on imported planting material poses various risks for food security, the most concerning of which is the possible importation of dangerous pests (Chalam et al., 2021), weeds (Wilson et al., 2016) , and pathogens (Elmer, 2001; Rodoni, 2009). Thus, it is important for the domestic market of agricultural crops to adopt a wider use of old and newly obtained varieties which are bred locally, and it should be associated with comprehensive plant epidemiological controls. To confront potentially deleterious plant pathogens, it is not only necessary to detect and eradicate infected plants in a timely manner, but also to increase the resistance potential of cultivated crops against disease by breeding and selecting varieties with genetic factors of resistance. Modern practices require the extensive utilization of molecular methods to solve both these problems. The development of DNA-based methods has enabled the detection of pathogens with a high sensitivity and reliability (McCartney et al., 2003; López et al., 2009); moreover,

66 modern systems have been moving towards prioritizing portability and time efficiency, helping
67 to perform analyses directly in the field (Donoso & Valenzuela, 2018). Molecular markers
68 associated with disease resistance in plants play a crucial role in modern breeding programs since
69 their use in marker assistant selection (MAS) helps to significantly reduce the time and labor
70 required for developing new resistant varieties (Collard & Mackill, 2008; Miedaner, 2016).
71 However, in Kazakhstan, the implementation of such advanced breeding practices for tomato is
72 limited by relatively low economic and scientific interests. Indeed, to date, no systematic efforts
73 have been made to lay the molecular genetic basis for selection programs for tomato crops. In
74 contrast, the molecular genetics of wheat, the crop playing a prominent role in both the country's
75 domestic food marker and international trade, have received significant research attention for
76 years (Kokhmetova et al., 2017; Anuarbek, Abugalieva & Turuspekov, 2018; Genievskaya et al.,
77 2022).

78 The objective of this work was to investigate genetic structure of the collection of tomato
79 varieties deposited in the Fruit and Vegetable Research Institute (Almaty, Kazakhstan). The
80 collection included established local cultivars along with varieties from abroad, predominantly
81 from Russia and other ex-USSR countries. Most of them have not been included into the state
82 register of crop varieties recommended for use (The Ministry of Agriculture of the Republic of
83 Kazakhstan, 2009) and thus require extensive investigations of their genetic compositions,
84 immunity, physiological features under local growth conditions, etc. Along with previously
85 published data on the genetic markers of resistance against three common viruses (Pozharskiy et
86 al., 2022), this work presents the results of the first molecular genetic study of tomato varieties in
87 Kazakhstan. A set of simple sequence repeats (SSR), sequence characterized amplified region
88 (SCAR), and cleavage amplified polymorphic sequences (CAPS) markers was used to evaluate

the relations between selected cultivars and identify varieties bearing known loci of resistance to common tomato pathogens: oomycete *Phytophtora infestans,* fungus *Fusarium oxysporum,* Tomato mosaic virus (ToMV), Tomato spotted wilt virus (TSWV), and Tomato yellow curly leaf virus (TYLCV). The obtained results will lay a basis for an initial inventory of tomato plant materials to be used both in agriculture and in breeding programs in Kazakhstan.

Materials and methods

A selection of tomato varieties was obtained from the collection of the Fruit and Vegetable Research Institute (FVRI; Almaty, Kazakhstan) (Table 1). Seed materials were grown and DNA was isolated as previously described in (Pozharskiy et al., 2022).

SSR genotyping was conducted using known markers (Table 2) (Smulders et al., 1997; Areshchenkova & Ganal, 2002). Forward primers labeled with either FAM or HEX fluorescent dye were used for all markers. The PCR conditions were set in accordance with the corresponding published protocols. The PCR products were first checked for yield and specificity by agarose gel electrophoresis, then 20-fold diluted and combined into groups for multiplex fragment reading. Three groups were considered based on the expected fragment size ranges and used primer labels, to avoid overlaps between markers and ensure their independent detection. The diluted PCR mixes were added to high-purity formamide (1 µl PCR mix, 0.15 µl LIZ(-500) Size Standard (Applied Biosystems, Thermo Fisher Scientific, USA), 8.85 µl formamide), denatured at 95°C for 4 min, cooled on ice for 5 min, and loaded into a 3500 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA). Genotypes were determined using GeneMapper software and analyzed using a Bayesian approach implemented in MrBayes (Ronquist et al., 2012) and STRUCTURE (Pritchard, Stephens & Donnelly, 2000)

112 software. R language (R Core Team, 2019) and adegenet (Jombart & Ahmed, 2011), ape
113 (Paradis, Claude & Strimmer, 2004), and ggtree (Yu et al., 2017) packages were used for general
114 data handling and visualization. The genotyping data was encoded using an additive pseudo-
115 haploid scheme where each observed allele was represented as a single digit value: 0 for absence,
116 1 for heterozygous state, 2 for homozygous state.
117 MrBayes was run for 50,000,000 generations with the Dirichlet distribution model for
118 standard data; each 2000th generation was sampled and used for diagnostics by the average
119 standard deviation of tree probabilities in two parallel runs. The parameters of the run were
120 monitored using built-in MrBayes statistics and Tracer (Rambaut et al., 2018). The summary tree
121 was generated using a burn-in threshold of 50%.
122 STRUCTURE was run for expected numbers of clusters *K* from 1 to 10 using the
123 standard admixture model with 50,000 burn-in and 100,000 MCMC iterations. To find the
124 optimal *K,* ten replicates were calculated for each value, and CLUMPAK web-server (Kopelman
125 et al., 2015) was used to estimate Δ*K* following Evanno's method (Evanno, Regnaut & Goudet,
126 2005).
127 PCR was performed for previously known markers of resistance against pathogens in
128 accordance with published protocols (Table 3). All PCR products were checked using agarose
129 gel electrophoresis. Markers requiring restriction (CAPS) were digested by corresponding
130 enzymes in a 20 µl mix containing 5 µl of the PCR mix, 0.5 µl of enzymes, and 2 µl of the
131 appropriate restriction buffer, according to the manufacturer's recommendations. Restriction was
132 performed overnight with the regular enzyme or for an hour with the enzymes of the
133 FastDigest™ product series (Thermo Fisher Scientific, USA). The results of the restriction were
134 evaluated by agarose gel electrophoresis. All results of the genotyping by resistance markers

were interpreted in accordance with the results reported in the source publications. For 31 specimens, previously published data on ToMV, TSWV, and TYCLV were used for comparison (Pozharskiy et al., 2022), as indicated in Table 1.

For all individual PCR reactions, both for SSR and resistance markers, the samples failing to produce a result were re-processed at least twice. If no results were obtained in any replicate, the genotype was reported missing.

Results

A total of 68 tomato varieties were used in the study, including 13 cultivars of domestic origin. Most of these varieties represent a pool of tomato genotypes used in ongoing breeding programs. The local cultivars ‘Meruert’, ‘Vostorg’, ‘Luchezarnyi’, and ‘Samaladay’, as well as Russian cultivars ‘Novichok’ and ‘Rassvet 362’, have also been approved for commercial use in Kazakhstan (The Ministry of Agriculture of the Republic of Kazakhstan, 2009).

According to the results of the SSR genotyping, four markers – LEPRP4, LESODB, LECHSOD, and LEMDDNb – were revealed to be monomorphic across all tomato varieties (Table 4). LEPRP4 also had the highest missing genotype rate among all markers (11.76%). Markers LELE25, LELEUZIP, and LECHSOD were amplified in all studied samples. None of the other markers exceeded a missing rate of 7.35%, corresponding to five missing samples of 68. Among the polymorphic markers, LEATRACAb, LPHSF24, and TMS58 had levels of observed heterozygosity not significantly differing from the expected values. The LEMDDNA marker had a slightly higher observed heterozygosity (p-value 0.0003; significance level 0.001); the other five markers had significantly lower observed values compared to expected values (p-values near zero). Considering the nature of the studied samples, which comprised a

158 heterogeneous set of specimens of different varieties rather than a single population, we did not
159 expect the samples to follow Hardy-Weinberg equilibrium, and thus deviations between the
160 expected and observed levels of heterozygosity were not surprising. Although the volume and
161 heterogeneity of the samples limited any possible genetic inferences of the population, it could
162 be speculated that the LEATRACAb, LPHSF24, and TMS58 markers were neutral with respect
163 to the selection of tomato varieties.

164 The genetic heterogeneity of the studied samples was revealed by a Bayesian cluster
165 analysis (Fig. 1, a, b). The results obtained using two algorithms implemented in MrBayes and
166 STRUCTURE software were compared to acquire a more detailed picture of the genetic
167 structure of the samples. According to the MrBayes results, most of the studied tomato varieties
168 formed a large subtree with weak sub-structure. The results obtained with STRUCTURE
169 produced a data partition into five clusters, in accordance with the best Evanno's ΔK value (Fig.
170 1, d). The first cluster (shown pale green) was the most distinct group representing a compact
171 sub-group at the tree; the highest probabilities were assigned to the 'Lipen' (Ukraine),
172 'Yablochnyi [Apple-like]' (Uzbekistan), 'Choportula' (Georgia), and 'Shalun [Varmint]'
173 (Russia) cultivars, which had identical genotypes. The local variant of the 'Yablochnyi [Apple-
174 like]' cultivar was the only variety from Kazakhstan included in this cluster; however, it was
175 located apart from the rest in the tree and differed from its Uzbekistani relatives in two markers,
176 LE21085 and TMS58. Another distinct cluster (shown in yellow) included two small subclusters
177 in the tree; the typical members of this group were the 'Ayan' (Kazakhstan), 'Ruzha' (Belarus),
178 'Nicola' (Russia), and 'Pyatnica [Friday]' (local breeding line based on Russian cultivar)
179 cultivars. The other three clusters (shown in red, blue, and purple) appeared as a mixed set of
180 subgroups and intermediate genotypes within the main subtree.

Fifteen tomato varieties are the results of breeding efforts established in Kazakhstan. All local varieties yielded a high genetic similarity according to SSR markers used (Fig. 1, e; Additional file 1). Across all 11 polymorphic markers, only three markers demonstrated genotype variations within the local cultivars: LEMDDNA with a set of detected alleles 211, 213, 227, 233; LELEUZIP with alleles 102, 105, 106; and TMS58 with alleles 226, 228, 230. The LELE25, LEATRACAb, and TM63 markers had only two differing genotypes across 15 local varieties, and marker LE20592 had the only differing genotype in the 'Sladkoyezhka' cultivar. This cultivar was the most distinct one across all local varieties. The 'Yantar [Amber]', 'Leader', 'Luchezarnyi [Shiny]', 'Meruert', 'Vostorg [Delight]', and 'Mechta [Dream]' varieties formed a group of similar genotypes (purple color in Fig. 1, b), along with Russian cultivars 'Novichok [Newcomer]', 'Korolek [Kinglet]', 'Rassvet 365 [Sunrise 365]', and '33 bogatyrya [33 heroes]'. The breeding line of the 'Samaladay' cultivar (specimen T634) also belonged to this group, however, the finally established line for commercial use (specimen T625) differed in the LELEUZIP (genotype 102/102) and LEATRACAb (184/186) markers. The LEPRP4 and TMS58 markers were characterized by a notably high occurrence of missing genotypes in this group. All these local varieties were obtained by the breeding programs of the former Research Institute of Potato and Vegetable Breeding (now part of the Fruit and Vegetable Research Institute, Almaty, Kazakhstan) (Kurganskaya & Dzhantasova). Other local varieties were more diverse, with relation to various foreign cultivars.

The analysis of SCAR and CAPS markers associated with a resistance against infections revealed the prevailing presence of resistance loci to fungus *Fusarium oxysporum* and oomycete *Phytophtora infestans,* compared to viruses (Table 5, Fig. 1, c; Additional file 2). The most commonly occurring marker was At2, associated with the resistance locus *I* against *F.*

204 *oxysporum*: half of all 64 successfully genotyped samples were positive for resistance. Another
205 resistance marker against *F. oxysporum,* Z1063, associated with *I2* resistance genes, was
206 observed in six specimens, including the local 'Meruert' cultivar. Both these markers are
207 dominant SCAR markers linked with the corresponding resistance loci introduced to tomatoes
208 from *Solanum pimpinellifolium (Arens et al., 2010).* Two codominant markers, Ph3-gsm and
209 TG328, have been linked with *Ph-3* locus conferring resistance to *P. infestans* (Robbins et al.,
210 2010; Wang et al., 2016). Two local cultivars, 'Meruert' and 'Leader', had the resistant allele of
211 Ph3-gsm; the only specimen with the resistant variant of TG328 was the Russian cultivar
212 'Korolek [Kinglet]'. Only two cultivars had the resistant allele of marker PrRuG086-151
213 associated with locus *Tm-2* conferring resistance to ToMV (Lanfermeijer, Warmink & Hille,
214 2005), Russian cultivar 'Zhiraf [Giraffe]' and Armenian 'Sunnik', as waspreviously revealed in
215 (Pozharskiy et al., 2022). Almost no markers associated with the resistant locus *Sw-5* against
216 TSWV (Dianese et al., 2010; Kim et al., 2020) were detected, with a single exception of marker
217 Sw5-2 in a Russian 'Super exotic' variety. For TYCLV, markers associated with resistance loci
218 *Ty-2* and *Ty-3* were tested (Kim et al., 2020). No resistant allele of marker Ty2-UpInDel was
219 revealed. Three markers associated with the resistant variant of *Ty-3* were previously identified
220 in Russian cultivars (Pozharskiy et al., 2022).

221 **Discussions**
222
223 The results of this study reflect the history and current state of tomato breeding in
224 Kazakhstan. As highlighted by Amirov (2012), no breeding programs for vegetable crops were
225 established in Kazakhstan until 1946, following World War II. The collapse of the Soviet Union
226 in 1991 cut the country from the all-Union system of vegetable crop breeding and seed

227 production. The development of vegetable breeding and seed production has remained stagnant
228 in the independent Kazakhstan due to a shortage of funding and highly qualified experts
229 (Amirov, 2012). The seven local varieties developed in the only systematic tomato breeding
230 program in the country for years demonstrated low genetic diversity in the study. An overview
231 of the studied collection of varieties, as well as the list of approved cultivars (The Ministry of
232 Agriculture of the Republic of Kazakhstan, 2009), reflects a high dependence of the local tomato
233 market on Russian seed materials. Such dependence not only make the local horticulture more
234 vulnerable to political and economic factors, but also decreases the diversity of the genetic
235 resources available for cultivation. Hopes for the future development of horticulture are related
236 with the plans of the Republic to join the International Union for the Protection of New Varieties
237 of Plants (UPOV), to stimulate the development of plant breeding through the management and
238 protection of the intellectual property of breeders (Amirov, 2012). The access to this
239 international system will help broaden the spectrum of potentially used plant varieties from
240 throughout the world, and thus increase the diversity of available food products and improve
241 food safety in the country.

242 Despite the role of the former Research Institute of Potato and Vegetable Breeding, in
243 general, the development of tomato breeding in Kazakhstan has been led in a poorly organized
244 and sporadic manner. Because of the losses of information resulting from outdated
245 infrastructures and insufficient funding since the early years of the country's independence, the
246 origin and the subsequent selection of local tomato varieties cannot be traced. The re-
247 establishment of tomato selection in the country on the contemporary level will require joined
248 efforts from the government, farming businesses, and research institutions. Methods involving
249 molecular genetics are essential for modern breeding practices in order to, on the one hand,

identify, classify, and evaluate the genetic diversity of plant germplasm, and, on the other hand, to help provide a fast and reliable assessment of genetic factors conditioning important phenotypic traits (Amiteye, 2021). The results of this study have highlighted the need for extensive works on the inventorying and systematization of tomato varieties used in local breeding, and DNA-based analyses should play a central role in the former. The history of the selection of local cultivars could be restored using molecular methods, however, this would require (a) a sufficient number of markers covering most parts of the tomato genome; (b) a wider range of available tomato germplasm from throughout the world, or available data on their diversity and compatibility with used marker sets. As was shown, a low number of SSR markers and the limited diversity of analyzed genetic sources used in this study limited the conclusions that could be drawn about the relationships of local varieties with foreign germplasm.

A set of SCAR and CAPS markers of resistance to five diseases revealed a low abundance of corresponding resistant factors not only in the local cultivars, but in all those studied here. The most common marker, At2, associated with the resistance locus *I* against *F. oxysporum,* had an equal proportion of resistant and susceptible variants across all varieties; approximately the same ratio, 8:7, was observed in the group of local cultivars. However, this marker displayed no strong genotype distribution pattern in relation to SSR data. Another *F. oxysporum* resistance marker, Z1063 (locus *I2*), had the allele associated with resistance in one local cultivar, ‘Meruert’. Four local cultivars had a resistant genotype in marker Ph3-gsm to *P. infestans,* and no local varieties had resistance markers against three of the considered viruses. These results indicate that no systematic approaches have so far been developed to work with resistance factors in breeding; the observed markers appeared occasionally and without a strong relation to the overall genetic structure.

The obtained results demonstrated that further studies with expanded sets of markers and varieties are required. A promising path to this is the use of microarray based techniques allowing the simultaneous genotyping of thousands of single nucleotide polymorphisms (SNPs). This method has a high replicability, allowing the successful combination and comparison of novel genotyping results with data from external sources to look at the wider genetic landscape, as has previously been applied to local apples (Gritsenko et al., 2022). However, this technique is expensive and demanding for required technical infrastructures and staff proficiency in laboratories. Particular attention should be paid to the evaluation of a wider range of markers associated with resistance to various diseases and other biotic and abiotic stress factors, supplementing experimental tests. The plant disease monitoring of tomatoes in Kazakhstan lacks the involvement of modern techniques; the evaluation of pathogens is usually performed in a traditional manner involving descriptive phytopathology (Babayeva et al., 2021). Insufficient data on the distribution of tomato pathogens in the country can potentially lead to massive disease outbreaks and subsequent economical losses when the centers of infection are not being identified in a timely manner. The development of new resistant varieties and their introduction for wide scale commercial usage will increase the sustainability of the tomato market in Kazakhstan and, thus, help strengthen food safety in the republic. Marker-assisted selection should therefore play a key role in breeding in order to achieve this goal.

Conclusions

Future advances in the molecular breeding of tomatoes in Kazakhstan depend on the overall development of agrarian science. This study has presented the results of a pilot study on local tomato cultivars and foreign varieties used for selection with the application of molecular markers for evaluating their genetic structure and the detection of pathogen-resistant genotypes.

296 Overall, the results have indicated the low genetic diversity of local tomato varieties and low
297 occurrence of the considered genetic markers of resistance. Further studies employing a wider
298 range of markers and involving more diverse tomato genotypes will be important for the future
299 development of tomato breeding in Kazakhstan.

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Figure 1

Results of the genotyping of tomato varieties with SSR markers and markers associated with disease resistance

(A) Bayesian tree of varieties based on SSR markers. (B) STRUCTURE plot for five cluster configurations based on SSR markers. (C) tomato genotypes in markers of resistance against *Phytophtora infestans* (1), ToMV (2), *Fusarium oxysporum* (3), TSWV (4), and TYCLV (5). (D) Evanno's Δ*K* plot indicating the optimal *K.* (E) variations of SSR genotypes in tomato varieties of Kazakhstani origin.

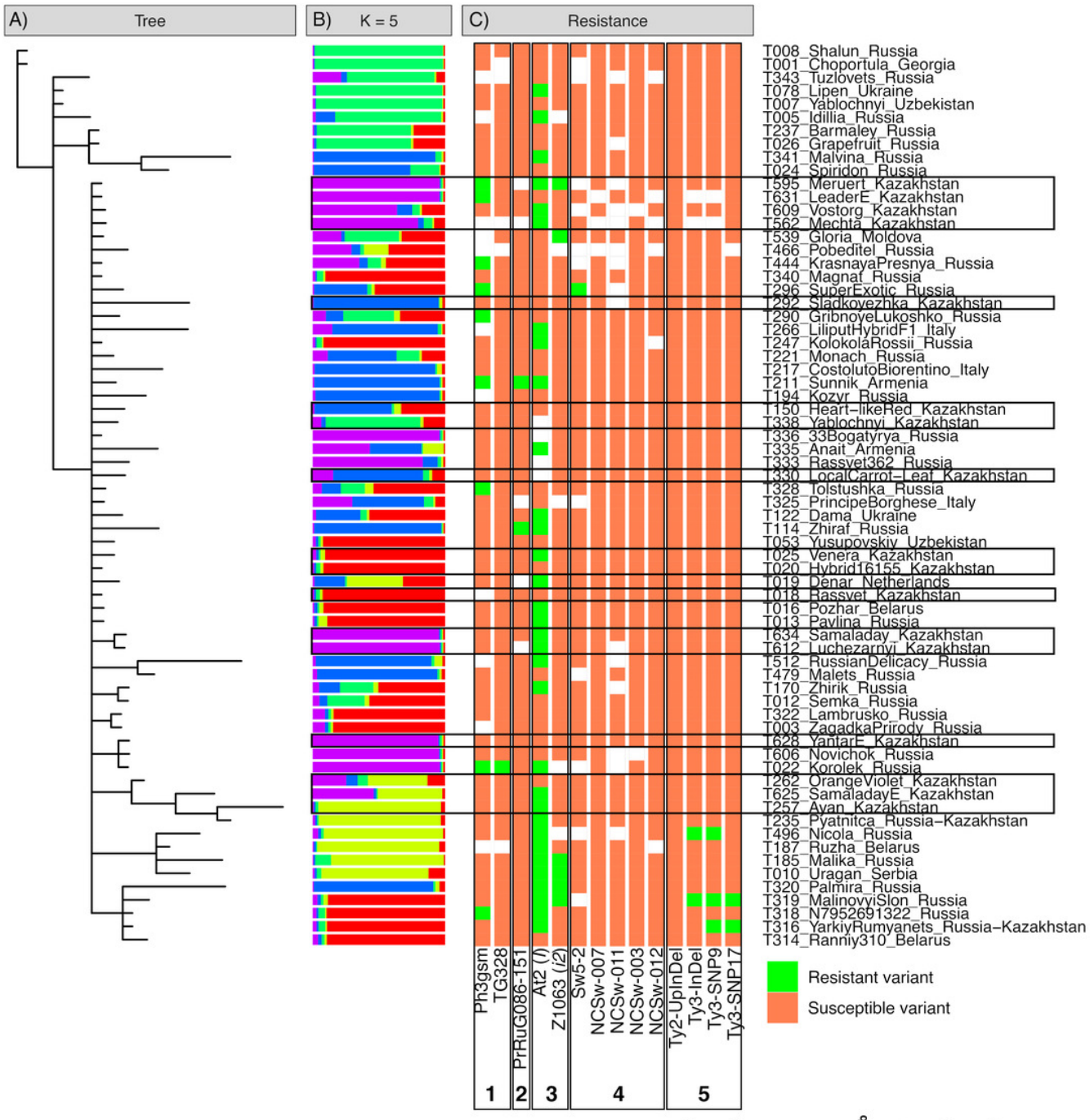
A)
Tree
B)
K = 5
C)
Resistance
T008_Shalun_Russia
T001_Choportula_Georgia
T343_Tuzlovets_Russia
T078_Lipen_Ukraine
T007_Yablochnyi_Uzbekistan
T005_Idillia_Russia
T237_Barmaley_Russia
T026_Grapefruit_Russia
T341_Malvina_Russia
T024_Spiridon_Russia
T595_Meruert_Kazakhstan
T631_LeaderE_Kazakhstan
T609_Vostorg_Kazakhstan
T562_Mechta_Kazakhstan
T539_Gloria_Moldova
T466_Pobeditel_Russia
T444_KrasnayaPresnya_Russia
T340_Magnat_Russia
T296_SuperExotic_Russia
T292_Sladkoyezhka_Kazakhstan
T290_GribnoyeLukoshko_Russia
T266_LiliputHybridF1_Italy
T247_KolokolaRossii_Russia
T221_Monach_Russia
T217_CostolutoBiorentino_Italy
T211_Sunnik_Armenia
T194_Kozyr_Russia
T150_Heart-likeRed_Kazakhstan
T338_Yablochnyi_Kazakhstan
T336_33Bogatyrya_Russia
T335_Anait_Armenia
T333_Rassvet362_Russia
T330_LocalCarrot-Leaf_Kazakhstan
T328_Tolstushka_Russia
T325_PrincipeBorghese_Italy
T122_Dama_Ukraine
T114_Zhiraf_Russia
T053_Yusupovskiy_Uzbekistan
T025_Venera_Kazakhstan
T020_Hybrid16155_Kazakhstan
T019_Denar_Netherlands
T018_Rassvet_Kazakhstan
T016_Pozhar_Belarus
T013_Pavlina_Russia
T634_Samaladay_Kazakhstan
T612_Luchezarnyi_Kazakhstan
T512_RussianDelicacy_Russia
T479_Malets_Russia
T170_Zhirik_Russia
T012_Semka_Russia
T322_Lambrusko_Russia
T003_ZagadkaPrirody_Russia
T628_YantarE_Kazakhstan
T606_Novichok_Russia
T022_Korolek_Russia
T262_OrangeViolet_Kazakhstan
T625_SamaladayE_Kazakhstan
T257_Ayan_Kazakhstan
T235_Pyatnitca_Russia-Kazakhstan
T496_Nicola_Russia
T187_Ruzha_Belarus
T185_Malika_Russia
T010_Uragan_Serbia
T320_Palmira_Russia
T319_MalinovyiSlon_Russia
T318_N7952691322_Russia
T316_YarkiyRumyanets_Russia-Kazakhstan
T314_Ranniy310_Belarus
Ph3gsm
TG328
PrRuG086-151
At2 (/)
Z1063 (/2)
Sw5-2
NCSw-007
NCSw-011
NCSw-003
NCSw-012
Ty2-UpInDel
Ty3-InDel
Ty3-SNP9
Ty3-SNP17
1
2
3
4
5
Resistant variant
Susceptible variant
D)
DeltaK
0
5
10
15
20
2
3
4
5
6
7
8
9
E)
LE20592
LE21085
LELE25
LELEUZIP
LEMDDNA
LEPRP4
LESODB
LEATRACAb
LPHSF24
LECHSOD
LEMDDNb
TMS63
TMS58
T018_Rassvet 167/167 103/103 220/220 106/106 211/233 201/201 207/207 184/184 158/158 195/195 277/277 184/184 226/228
T020_Hybrid16155 167/167 103/103 220/220 106/106 211/233 201/201 207/207 184/184 158/158 195/195 277/277 184/184 226/228
T025_Venera 167/167 103/103 220/220 106/106 227/233 201/201 207/207 184/184 158/158 195/195 277/277 184/184 228/228
T330_LocalCarrot-Leaf 167/167 103/103 218/218 105/105 213/233 201/201 207/207 184/184 158/158 195/195 277/277 184/184 228/228
T338_Yablochnyi 167/167 103/103 220/220 105/105 211/233 201/201 207/207 184/184 158/158 195/195 277/277 184/184 228/230
T150_Heart-likeRed 167/167 103/103 220/220 106/106 213/233 201/201 207/207 184/184 158/158 195/195 277/277 158/184 226/228
T262_OrangeViolet 167/167 103/103 220/220 105/105 213/233 201/201 207/207 184/186 158/158 195/195 277/277 184/184 228/228
T292_Sladkoyezhka 170/170 103/103 218/218 106/106 213/233 201/201 207/207 184/184 158/158 195/195 277/277 158/158 226/228
T562_Mechta 167/167 103/103 220/220 105/105 NA NA 207/207 184/184 158/158 195/195 277/277 NA NA
T609_Vostorg 167/167 103/103 220/220 105/105 233/233 201/201 NA 184/184 158/158 195/195 277/277 NA NA
T612_Luchezarnyi 167/167 103/103 220/220 105/105 213/213 NA 207/207 184/184 158/158 195/195 277/277 184/184 228/228
T625_SamaladayE 167/167 103/103 220/220 102/102 213/213 NA 207/207 184/186 158/158 195/195 277/277 184/184 NA
T628_YantarE NA NA 220/220 105/105 227/227 201/201 NA 184/184 NA 195/195 277/277 184/184 228/228
T631_LeaderE 167/167 103/103 220/220 105/105 211/211 NA 207/207 184/184 158/158 195/195 277/277 184/184 228/228
T634_Samaladay 167/167 103/103 220/220 105/105 213/213 NA 207/207 184/184 158/158 195/195 277/277 184/184 NA
T595_Meruert 167/167 103/103 220/220 105/105 211/211 NA 207/207 184/184 158/158 195/195 277/277 184/184 228/228

Table 1(on next page)

List of studied tomato varieties

1 **Table 1. List of studied tomato varieties.**

Sample ID	Variety name		Country of origin	Included to the State Register	Sample ID	Variety name		Country of origin	Included to the State Register
T001	Choportula*		Georgia		T290	Gribnoye Lukoshko		Russia	
T003	Zagadka Prirody*	[Enigma of Nature]****	Russia		T292	Sladkoyezhka	[Sweet-tooth]	Kazakhstan	
T005	Idillia*	[Idyll]	Russia		T296	Super Exotic		Russia	
T007	Yablochnyi*	[Apple-like]	Uzbekistan		T314	Ranniy310*	[Early 310]	Belarus	
T008	Shalun*	[Varmint]	Russia		T316	Yarkiy Rumyanets*	[Bright Blush]	Russia-Kazakhstan**	
T010	Uragan*	[Hurricane]	Serbia		T317	N7952691322*		Russia	
T012	Semka*	[Seed]	Russia		T319	Malinovyi Slon*	[Crimson Elephant]	Russia	
T013	Pavlina*		Russia		T320	Palmira*		Russia	
T016	Pozhar*	[Fire]	Belarus		T322	Lambrusko*		Russia	
T018	Rassvet*	[Sunrise]	Kazakhstan	+	T325	Principe Borghese		Italy	
T019	Denar*		Netherlands		T328	Tolstushka*	[Fatty]	Russia	
T020	Hybrid16155		Kazakhstan		T330	Local with Carrot- Leaf		Kazakhstan	
T022	Korolek	[Kinglet]	Russia		T333	Rassvet362*	[Sunrise 362]	Russia	+
T024	Spiridon*		Russia		T335	Anait		Armenia	
T025	Venera*	[Venus]	Kazakhstan		T336	33 Bogatyrya	[33 Heroes]	Russia	
T026	Grapefruit		Russia		T338	Yablochnyi*	[Apple-like]	Kazakhstan	
T053	Yusupovskiy		Uzbekistan		T340	Magnat		Russia	
T078	Lipen*		Ukraine		T341	Malvina		Russia	
T114	Zhiraf*	[Giraffe]	Russia		T343	Tuzlovets		Russia	
T122	Dama*	[Dame]	Ukraine		T444	Krasnaya Presnya	[Red Presnya]	Russia	
T150	Heart-likeRed		Kazakhstan		T466	Pobeditel	[Winner]	Russia	
T170	Zhirik		Russia		T479	Malets	[Small Boy]	Russia	
T185	Malika*		Russia		T496	Nicola*		Russia	
T187	Ruzha*		Belarus		T512	Russian Delicacy		Russia	
T194	Kozyr*	[Trump]	Russia		T539	Gloria		Moldova	
T211	Sunnik*		Armenia		T562	Mechta	[Dream]	Kazakhstan	
T217	Costoluto Biorentino*		Italy		T595	Meruert		Kazakhstan	+
T221	Monach*	[Monk]	Russia		T606	Novichok	[Newcomer]	Russia	+
T235	Pyatnitca	[Friday]	Russia-Kazakhstan**		T609	Vostorg	[Delight]	Kazakhstan	+
T237	Barmaley		Russia		T612	Luchezarnyi	[Shiny]	Kazakhstan	+
T247	Kolokola Rossii	[Russian Bells]	Russia		T625	Samaladay		Kazakhstan	+
T257	Ayan		Kazakhstan		T628	Yantarnyi	[Amber]	Kazakhstan	
T262	Orange-Violet		Kazakhstan		T631	Leader		Kazakhstan	+
T266	Lilliput Hybrid		Italy		T634	Samaladay***		Kazakhstan	+

	F1							

* Data on resistance markers against ToMV, TSWV, TYCLV taken from (Pozharskiy et al., 2022)
** Local breeding line based on Russian cultivars
*** Intermediate breeding line
**** Translations of the Russian names of cultivars

2

Table 2(on next page)

Tomato SSR markers used for genotyping

Table 2. Tomato SSR markers used for genotyping

Marker name	PCR primers	Repeating pattern*	Expected allele range	Multiplex group	Source
LE20592	F: 5'-**FAM**-CTGTTTACTTCAAGAAGGCTG R: 5'-ACTTTAACTTTATTATTGCCACG	$(TAT)_{15\text{-}1}(TGT)_4$	165–172	1	(Smulders et al., 1997)
LE21085	F: 5'-**FAM**-CATTTTATCATTTATTTGTGTCTTG R: 5'-ACAAAAAAAGGTGACGATACA	$(TA)_2(TAT)_{9\text{-}1}$	103–119	1	
LELE25	F: 5'-**FAM**-TTCTTCCGTATGAGTGAGT R: 5'-CTCTATTACTTATTATTATCG	$(TA)_{13\text{-}1}$	222–225	2	
LELEUZIP	F: 5'-**HEX**-GGTGATAATTTGGGAGGTTAC R: 5'-CGTAACAGGATGTGCTATAGG	$(AAG)_{6\text{-}1}TT$	101-105	2	
LEMDDNA	F: 5'-**HEX**-ATTCAAGGAACTTTTAGCTCC R: 5'-TGCATTAAGGTTCATAAATGA	$(TA)_9$	210-226	3	
LEPRP4	F: 5'-**HEX**-TTCATTTCTTGCAACTACGAT R: 5'-CATACTAGCAACATCAAAGGG	$(TAT)_3(TGT)_5$	108-112	3	
LESODB	F: 5'-**FAM**-TTATCAATTCATCATTGTGGC R: 5'-AGTAAGGGGTTTAGGGGTAGT	$(TTC)_6$	208–212	1	
LEATRACAb	F: 5'-**FAM**-GTATGTCAAATCTCTCTTGCG R: 5'-ACTCTCCATCGTCTCTTTCAC	$(GA)_7$	184–186	2	
LPHSF24	F: 5'-**HEX**-TTGGATTTACAAGTTCGATGT R: 5'-GCATTTGACTTGATAGCAGTC	$(TA)_6$	156–158	1	
LECHSOD	F: 5'-**FAM**-TTATCAATTCATCATTGTGGC R: 5'-AGGGGTAGTGACAGCATAAAG	$(CTT)_6$	196–198	3	
LEMDDNb	F: 5'-**FAM**-TAAATACAAAAGCAGGAGTCG R: 5'-GAGTTGACAGATCCTTCAATG	$(TG)_4(TA)_4$	278–280	2	
TMS63	F: 5'-**HEX**-GCAGGTACGCACGCATATAT R: 5'-GCTCCGTCAGGAATTCTCTC	$(AT)_4(GT)_{18}(AT)_9$	130–150**	2	(Areshchenkova & Ganal, 2002)
TMS58	F: 5'-**HEX**-CATTTGTTGTATGGCATCGC R: 5'-CAGTGACCTCTCGCACAAAA	$(TA)_{15}(TG)_{17}$	223–226**	3	

* (-1) at the subscript indicates the presence of an imperfect repeat

** According to (Mazzucato et al., 2008); otherwise according to (Castellana et al., 2020)

Table 3(on next page)

Tomato SCAR and CAPS markers associated with resistance to pathogens

Table 3. Tomato SCAR and CAPS markers associated with resistance to pathogens

Pathogen	Resistance locus	Linked marker	PCR primers	Restriction enzyme	Source
Phytophtora infestans	*Ph-3*	CAPS Ph3.gsm	F: 5'-TAGTATGGTCAAACATATGCAG R: 5'-CTTCAAGTTGCAGAAAGCTATC	FD *Hin*cII	(Wang et al., 2016)
		CAPS TG328	F: 5'-GGTGATCTGCTTATAGACTTGGG R: 5'-AAGGTCTAAAGAAGGCTGGTGC	FD *Mva*I (*Bst*NI) ***	(Robbins et al., 2010)
Fusarium oxysporum	*I*	SCAR At2	F: 5'-CGAATCTGTATATTACATCCGTCGT R: 5'-GGTGAATACCGATCATAGTCGAG + control (LAT): F: 5'-AGACCACGAGAACGATATTTGC R: 5'-TTCTTGCCTTTTCATATCCAGACA	-	(Arens et al., 2010)
	I2	SCAR Z1063	F: 5'-ATTTGAAAGCGTGGTATTGC R: 5'-CTTAAACTCACCATTAAATC + control (Rubisco): F: 5'-ATGTCACCACAAACAGAGAC R: 5'-CTCACAAGCAGCAGCTAG	-	
Tomato mosaic virus (ToMV)	*Tm2*	CAPS PrRuG086-151	F: 5'-GAGTTCTTCCGTTCAAATCCTAAGCTTGAGAAG R: 5'-CTACTACACTCACGTTGCTGTGATGCAC	*Ksp*AI (*Hpa*I) ***	(Lanfermeijer, Warmink & Hille, 2005)
Tomato spotted wilt virus (TSWV)	*Sw-5*	SCAR NCSw-003	F: 5'-TCTCGTTATCCAATTTCACC R: 5'-GCAATTTTGTTTCTTGGTCT	-	(Panthee & Ibrahem, 2013)
		SCAR NCSw-012	F: 5'-ATGGTCAACTCGATCAGAAC R: 5'-TTTGGTGAGGATCTGATTTC	-	
		CAPS NCSw-007	F: 5'-GTTGCTAACTCGACTCGTTC R: 5'-TCACTCACGTCCTATTGACA	FD *Hin*fI	
		CAPS NCSw-011	F: 5'-TATCATCCTCATACCCCTTG R: 5'-GGATTTTCTCATCATCTCCA	*Hpy*F3I (*Dde*I) ***	
		SCAR Sw5-2	F: 5'-AATTAGGTTCTTGAAGCCCATCT R: 5'-TTCCGCATCAGCCAATAGTGT	-	(Dianese et al., 2010)
Tomato yellow curly leaf virus (TYLCV)	*Ty-2*	SCAR Ty2-UpInDel	F: 5'-ACCCCAAAAACATTTCTGAAATCCT R: 5'-TGGCTATTTTGTGAAAATTCTCACT	-	(Kim et al., 2020)
	Ty-3	CAPS Ty3-InDel/SNP9	F: 5'-CCTATCCTCAGTGTTTCGGTCA R: 5'-GGCGAAAGACTTTGTGTACACA	*Bst*1107I (*Bst*Z17I) / *Mun*I (*Mfe*I)***	
		CAPS Ty3-SNP17	F: 5'-TCTCAGGTGATGCTGAGCAC R: 5'-AGAGAACGAAAACGAAATTTCAAACA	*Rsa*I	

* Gene ID and genomic positions according *S. lycopersicum* genome assembly SL3.0;
** Marker positions in *S. lycopersicum* genome assembly SL3.0;
*** Isoschizomers used in the work and by the original authors (in parentheses);
FD – FastDigest™ restriction enzyme product series (Thermo Fisher Scientific, USA)

2

Table 4(on next page)

Summary of SSR genotyping of 68 tomato varieties

1 **Table 4. Summary of SSR genotyping of 68 tomato varieties**

Marker name	*N*	Detected alleles	Missing genotype rate	MAF	H_e	H_o	H_e vs. H_o (χ^2 test *p*-value)
LE20592	3	164,167,170	0.0147	0.1045	0.3206	0.0149	0
LE21085	2	103,117	0.0441	0.1769	0.2912	0.0154	2.2315×10^{-14}
LELE25	3	218,220,222	0	0.0735	0.3334	0.2059	6.7279×10^{-14}
LELEUZIP	4	102,104,105,106	0	0.3088	0.5978	0	0
LEMDDNA	5	211,213,219,227,233	0.0147	0.2463	0.6788	0.7164	0.0003
LEPRP4	1	201	0.1176	-	-	-	-
LESODB	1	207	0.0294	-	-	-	-
LEATRACAb	2	184,186	0.0294	0.0303	0.0588	0.0606	0.7995
LPHSF24	2	158,164	0.0147	0.0298	0.0579	0.0597	0.8011
LECHSOD	1	195	0	-	-	-	-
LEMDDNb	1	277	0.0147	-	-	-	-
TMS63	4	158,184,188,202	0.0735	0.2222	0.3818	0.0793	0
TMS58	3	226,228,230	0.0735	0.1667	0.3287	0.3333	0.8085

N – number of detected alleles;
MAF – minor allele frequency;
H_e – expected heterozygosity;
H_o – observed heterozygosity

2

Table 5(on next page)

Summary of the genotyping results of 68 tomato varieties with SCAR and CAPS markers of resistance against infectious diseases.

Table 5. Summary of the genotyping results of 68 tomato varieties with SCAR and CAPS markers of resistance against infectious diseases.

Pathogen	Marker	Marker type	Number of genotypes		
			Susceptible	Resistant	Missing
Phytophtora infestans	Ph3.gsm	CAPS	48	9	11
	TG328	CAPS	62	1	5
Fusarium oxysporum	At2	SCAR	32	32	4
	Z1063	SCAR	57	6	5
Tomato mosaic virus (ToMV)	PrRuG086-151*	CAPS	61	2	5
Tomato spotted wilt virus (TSWV)	NCSw-003*	SCAR	66	0	2
	NCSw-012*	SCAR	62	0	6
	NCSw-007*	CAPS	65	0	3
	NCSw-011*	CAPS	53	0	15
	Sw5-2*	SCAR	56	1	11
Tomato yellow curly leaf virus (TYLCV)	Ty2-UpInDel*	SCAR	68	0	0
	Ty3-InDel*	CAPS	64	2	2
	Ty3-SNP9*	CAPS	63	3	2
	Ty3-SNP17*	CAPS	65	2	1
* Including data from (Pozharskiy et al., 2022), as indicated in Table 1					