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Research article

# Screening a collection of local and foreign varieties of *Solanum lycopersicum* L. in Kazakhstan for genetic markers of resistance against three tomato viruses

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ABSTRACT

The tomato is one of the most important vegetable crops. The successful development of tomato cultures in Kazakhstan depends on the implementation of intensive agricultural methods, including breeding and selecting for new tomato varieties resistant to plant pathogens. Common tomato viruses, although not detected in our country to date, may potentially have a deleterious impact on agriculture if allowed to spread. The implementation of tomato breeding programs based on molecular markers of resistance is therefore an important preventive measure for protecting the agriculture and food safety of Kazakhstan. In the present work, we used nine molecular markers associated with resistance to three tomato viruses, i.e., tomato mosaic virus (ToMV), tomato spot wilt virus (TSWV), and tomato yellow leaf curl virus (TYLCV), to test the local breeding collection for the presence of genetic resistance factors. Two tomato varieties, 'Zhiraf' (Russia) and 'Sunnik' (Armenia), were revealed to possess the resistant allele marker PrRuG86-151 against ToMV; three hybrid forms had the same allele in the heterozygous state. One hybrid, based on the 'Mirsini' F1 variety from the Netherlands, had resistance to TSWV, which was confirmed by four markers: NCSw003, NCSw007, NCSw011, and NCSw012. Two cultivars, 'Nicola' and 'Malinovyi Slon' (Russia), and the local hybrid based on 'Yarkiy Rumyanets' had two to three resistant alleles of markers based on locus *Tm-3* of resistance to TYLCV. The obtained results have demonstrated that the collection of tomato varieties involved in breeding programs in Kazakhstan lacks well-known genetic resistance factors to the considered tomato viruses. Thus, the prospective breeding programs require introduction of known resistant genetic resources to establish resistance to viruses using marker-assisted selection.

## 1. Introduction

Tomato *Solanum lycopersicum* L. is one of the most important vegetable crops in the world. According to data from FAOSTAT [1], global tomato production has expanded both extensively and intensively, as the total harvested area increased from 1.68 million to 5.05 million hectares from 1961 to 2020, while the average yield increased from 16.4 tons to 37 tons per hectare. However, while the total harvested area doubled during the beginning of independence in Kazakhstan and reached 30.2 thousand hectares in 2020, the average yield has not exceeded this level since 1992 and has been estimated as 26.1 tons per hectare. The growth in total annual tomato yield from 400 000 tons in 1992 to 788 760 tons in 2020 [1] was therefore probably due to the extensive development of tomato production through the expansion of open planting and greenhouse areas rather than the implementation of new methods and techniques increasing the production efficiency of existing grounds. The land resources suitable for tomato cultivation have natural limits related to the plant's demands for growth conditions such as temperature [2], humidity [3], water supply, and salinity [4]. The development and implementation of intensive agricultural methods are therefore crucial for further expansion of tomato production in the country to satisfy the demands of the domestic market and to increase the potential for export.

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According to data by the State Revenue Committee of the Ministry of Finance of the Republic of Kazakhstan, a significant share of the local tomato market belongs to imports. In 2020, approximately 58 636 tons of tomatoes were imported, mainly from Central Asian countries [5], which corresponds to circa 7.4% of the local production volume. Domestic tomato farms also rely on the import of seed material from abroad (57.3 tons of vegetable crop seeds were imported in 2020, although the particular crop was not accounted for). Importation possesses the risk of introducing and transmitting new deleterious infections. Various pathogens (i.e., bacteria, fungi, viruses, and oomycetes) may lead to enormous yield losses and can be very difficult to manage when spread.

Tomato mosaic virus (ToMV), tomato spot wilt virus (TSWV), and tomato yellow leaf curl virus (TYLCV) are among the most dangerous pathogens affecting tomatoes and other crops. ToMV causes approximately 20% of the losses in world tomato production [6], and TSWV and TYLCV can potentially cause as much damage as the total yield loss [7, 8]. These viruses are capable of infecting a broad range of potential host species. ToMV can infect various species of the Solanaceae family [9], TSWV has a potential host range of more than 1000 species of monocotyledons and dicotyledons [10], and TYLCV has been detected and described in at least nine plant families of dicotyledons [11]. Such diverse specialization makes infection control more difficult and increases the potential impact of virus spread. No data are available concerning the presence of tomato viruses in Kazakhstan to date, as no studies have been conducted. However, ToMV, TSWV, TYLCV, and their corresponding vector insects have been included in the list of quarantine objects, invasive species, and dangerous organisms by the Ministry of Agriculture of the Republic of Kazakhstan [12]. Biological control of the imported plant material cannot be the only protective measure against the spread of viruses when considering the potentially deleterious consequences of a quarantine breach, not only for tomatoes but also for many other crops in Kazakhstan. Moreover, global climate change may alter which areas have favorable conditions for the spread of pathogens and their vectors, leading to the natural introduction of diseases to new locations [13, 14, 15]. Thus, the additional line of protective measures is important for maintaining plant health in agriculture and to ensure food safety in the country.

The most efficient strategy against plant infections, especially viruses, is the development of new plant varieties that are resistant to particular pathogens. The modern practices of targeted breeding involve the extensive use of molecular markers associated with traits of interest, such as resistance against pathogens (marker-assisted selection [MAS]) [16, 17, 18]. The molecular markers for MAS in tomatoes have been developed and mapped for more than three decades, and a representative set of resistance markers is currently available [19].

The set of molecular markers used in the present study is based on known tomato loci conferring qualitative resistance to viruses. Gene *Tm-2* has two alleles conferring resistance to ToMV: *Tm-2* and the more durable *Tm-2*$^2$, introgressed from the wild tomato *S. peruvianum* [20, 21]. Cleavage amplified polymorphic site (CAPS) marker PrRuG86-151 uses restriction to differentiate these alleles from the recessive susceptible variant *tm-2*; however, discrimination between *Tm-2* and *Tm-2*$^2$ requires the use of additional markers [22]. Resistance to TSWV in tomato is associated with a series of loci found mostly in the following wild tomato species: *Sw-1a, Sw-1b, sw-2, sw-3, sw-4, Sw-5, Sw-6, and Sw-7* [23]. Locus *Sw-5,* expressing the highest level of resistance, was introgressed into domestic tomato varieties [24]. A series of four markers showing close association with the locus were developed and recommended for use in MAS, including the sequence characterized amplified region (SCAR) markers NCSw03 and NCSw12 and CAPS markers NCSw07 and NCSw11 [25]. Loci *Ty-2* and *Ty-3* were described and mapped as the resistance genes against TYLCV, introgressed from the wild tomato species [26, 27]. The series of SCAR and CAPS markers were developed to describe sequence variations differentiating between susceptible and resistant allelic variants [28].

The scope of the present study is to use the described molecular markers of resistance to ToMV, TSWV, and TYLCV to test the collection of local and foreign tomato cultivars. The results will help estimate the potential of local and important genotypes when breeding for virus resistance and aid in discovering the possibilities of implementing MAS of tomato in our country.

## 2. Materials and methods

### 2.1. Collection and storage of plant material

The seeds of 54 tomato varieties were retrieved from the collection of the Fruit and Vegetable Research Institute (FVRI), Almaty, Kazakhstan. All varieties were classified according to their breeding status: 'cultivar' – cultivars with known origin and stable inheritance; 'specimen' – accessions from the breeding (experimental) populations or with unknown origin; and 'hybrid' – crosses obtained by the FVRI (Table 1). Seeds were planted in universal turf ground and grown using a 12-hour light/day period and irrigation three times a week. Tomato seedlings were grown for 3–5 weeks, depending on the development speed of each variety. Each seedling reaching 10–15 cm in height and developing mature leaves was examined for the absence of abnormal phenotypic traits and placed in storage at -80 °C until further use.

### 2.2. DNA extraction

DNA was extracted from 100 mg of frozen plant material, including the fragments of leaves and young shoots. The samples were ground with a mortar in 1 mL of preheated (approximately 60–65 °C) solution containing 2% hexadecyltrimethylammonium bromide (CTAB), 2% polyvinylpyrrolidone (PVP), 1.4 M sodium chloride, 20 mM sodium ethylenediaminetetraacetate (EDTA) pH 8.0, 100 mM Tris-HCl pH 8.0, and 0.2% 2-mercaptoethanol. Samples were incubated in 2 mL microcentrifuge tubes at 60 °C for 30 min with regular shaking. After incubation, samples were cooled on ice, mixed with an equal volume of the pre-chilled chloroform (4 °C), and centrifuged at 4 °C at 12 000g for 10 min. The aqueous phase (circa 1 mL) was sampled into clean 2-mL tubes and divided in two equal aliquots (approximately 500 μL). Each aliquot was mixed with 200 μL of sodium chloride solution (5 M) and pre-chilled 96% ethanol (−20 °C) to the final volume of 2 mL. A single aliquot of each sample was then centrifuged at 4 °C at 13 000g for 15 min. The liquid was then discarded, and the content of the second aliquot was transferred into the same tube and centrifuged with the same conditions. After removing the final liquid, the pellet was washed with 1 mL of 70% ethanol by centrifugation (10 min, 10 000g), then air dried and dissolved in 100 μL of MilliQ water, containing 0.5% of RNAse A solution (Thermo Fisher Scientific, USA). Finally, the DNA samples were incubated for 1 h at 37 °C and overnight at 4 °C. The quantity and purity of the isolated DNA were tested using a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific, USA). The integrity of the isolated DNA was checked by electrophoresis in 1% agarose gel.

### 2.3. PCR and restriction

PCR for SCAR and CAPS resistance markers was performed in accordance with the published protocols [21, 25, 28], with volume adjustment to 20 μL of the final PCR mix, including 50 ng of DNA as the template. PCR was performed using regular recombinant *Taq* DNA polymerase (Thermo Fisher Scientific, USA) in the standard ammonium buffer. The primer sequences are listed in Table 2. Amplification results were checked by electrophoresis in 1.5% agarose gel in TAE buffer.

CAPS marker restriction was performed using the enzymes of regular and FastDigest (FD) product series by Thermo Fisher Scientific. Restriction reactions were prepared in 20 μL containing 5 μL of successful PCR mix, 2 μL of the restriction buffer recommended by the manufacturer, and 0.4 μL of the corresponding enzyme solution. The CAPS markers Ty3-InDel and Ty3-SNP9 were obtained by the independent digestions of a single PCR product with two enzymes. Restriction was carried out

**Table 1.** Tomato varieties screened for resistance markers against viruses.

| Sample ID | Accession name | Country of origin | Breeding status | Sample ID | Accession name | Country of origin | Breeding status |
|---|---|---|---|---|---|---|---|
| T109 | Anait | Armenia | Cultivar | T021 | Gribnoe Lukoshko [Mushroom Basket] | Russia | Cultivar |
| T211 | Sunnik | Armenia | Cultivar | T024 | Spiridon | Russia | Cultivar |
| T016 | Pozhar [Fire] | Belarus | Cultivar | T114 | Zhiraf [Giraffe] | Russia | Cultivar |
| T121 | Dochodnyi [Profitable] | Belarus | Cultivar | T319 | Malinovyi Slon [Crimson Elephant] | Russia | Cultivar |
| T314 | Ranniy 310 [Early 310] | Belarus | Cultivar | T320 | Palmira | Russia | Cultivar |
| T187 | Ruzha | Belarus | Cultivar | T322 | Lambrusko | Russia | Cultivar |
| T001 | Choportula | Georgia | Cultivar | T328 | Tolstushka [Fatty] | Russia | Cultivar |
| T217 | Costoluto fiorentino | Italy | Cultivar | T329 | Cherry Lisa | Russia | Cultivar |
| T002 | K2501 | Kazakhstan | Specimen | T333 | Rassvet 362 [Sunrise 362] | Russia | Cultivar |
| T006 | #1051 | Kazakhstan | Hybrid | T337 | Yula | Russia | Cultivar |
| T011 | Mestnyi 806 [Local 806] | Kazakhstan | Specimen | T339 | Krasnaya Presnya | Russia | Cultivar |
| T017 | Orange | Kazakhstan | Specimen | T143 | Malinovka | Russia | Cultivar |
| T018 | Rassvet [Sunrise] | Kazakhstan | Cultivar | T194 | Kozyr [Trump] | Russia | Cultivar |
| T025 | Venera [Venus] | Kazakhstan | Cultivar | T221 | Monach [Monk] | Russia | Cultivar |
| T103 | Mestnyi 3284 [Local 3284] | Kazakhstan | Specimen | T496 | Nicola | Russia | Cultivar |
| T338 | Yablochnyi [Apple-like] | Kazakhstan | Specimen | T316 | Yarkiy Rumyanets [Bright Blush] | Russia-Kazakhstan | Hybrid |
| T135 | #332 | Kazakhstan | Hybrid | T324 | Russkoe Lakomstvo [Russian Delicacy] | Russia-Kazakhstan | Hybrid |
| T317 | 7952691322 | Kazakhstan | Hybrid | T010 | Uragan [Hurricane] | Serbia | Hybrid |
| T185 | Malika | Kazakhstan | Cultivar | T078 | Lipen | Ukraine | Cultivar |
| T014 | Gloria | Moldova | Cultivar | T122 | Dama [Dame] | Ukraine | Cultivar |
| T019 | Denar | Netherlands | Cultivar | T004 | Chetwertoe Iyulya [July The Fourth] | Unknown | Cultivar |
| T009 | Mirsini F2 | Netherlands-Kazakhstan | Hybrid | T073 | Heart-shaped red | Unknown | Specimen |
| T003 | Zagadka Prirody [Enigma of Nature] | Russia | Cultivar | T116 | Orange 86 | Unknown | Specimen |
| T005 | Idillia [Idyll] | Russia | Cultivar | T332 | Krupnyi [Big] | Unknown | Specimen |
| T008 | Shalun | Russia | Cultivar | T132 | Egg shaped | Unknown | Specimen |
| T012 | Semka [Seed] | Russia | Cultivar | T007 | Yablochnyi [Apple-like] | Uzbekistan | Specimen |
| T013 | Pavlina | Russia | Cultivar | T053 | Jusupovskiy | Uzbekistan | Cultivar |

overnight for the regular enzymes and for 1 h for the FD enzyme at 37 °C. The restriction results were checked by electrophoresis in 1.5% agarose gel in TAE buffer. The patterns of DNA fragments were identified as susceptible or resistant allelic variants in accordance with the original publication.

## 3. Results and discussion

The collection of analyzed tomato accessions included 11 specimens of Kazakhstan origin – three were registered cultivars ('Rassvet' [T018], 'Venera' [T025], and 'Malika' [T185]), five belonged to the breeding population with uncertain origin (T002, T011, T017, T103, and T338), and three were experimental hybrids (#1051 [T006], #332 [T135], and 7952691322 [T317]). Additionally, two new hybrids based on Russian varieties ('Yarkiy Rumyanets' [T316] and 'Russkoe Lakomstvo' [T324]) and a second-generation hybrid based on the cultivar 'Mirsini' [T009] (the Netherlands) were present, which were obtained by the FVRI. The majority of the collection comprised of varieties originating from countries of the former USSR, mostly from Russia (22 varieties); four cultivars had European origins ('Mirsini' [T009] and 'Denar' [T019] from the Netherlands, 'Costoluto Biorentino' [T217] from Italy, and 'Uragan' [T010] from Serbia). All obtained samples were efficiently processed with the DNA extraction protocol based on use of CTAB buffer. The DNA extraction using CTAB buffer has a long history, and numerous improved modifications of this method were reported for a wide range of experimental cases [32]. The version of the protocol described here has resulted in an average DNA yield of circa 75 μg per 100 mg of plant material, without significant protein contamination and DNA fragmentation. The obtained DNA samples diluted to 50 ng/μL have shown excellent performance in PCR with all markers. The small number of unsuccessful amplification reactions were re-analyzed, to test whether the absence of the results was due to biological reasons (e.g., probable null alleles) rather than technical errors.

The analysis results of nine resistance markers are shown in Table 3. The genotype-based resistance status was determined in accordance with the data of the corresponding original studies. The marker PrRuG086-150, conferring resistance to ToMV [16], shows cleavage with *Ksp*AI (*Hpa*I) in the resistant alleles *Tm-2* or *Tm-2*$^2$, producing two fragments of approximate sizes of 700 bp and 400 bp, respectively; non-resistant allele *tm-2* produces a single uncleaved fragment with an approximate size of 1100 bp (Figure 1), in accordance with the original study [21]. The presence of all three fragments indicates a heterozygous genotype on the marker. Two tomato cultivars, 'Zhiraf' (T114) and 'Sunnik' (T211), which originated from Russia and Armenia, respectively, had homozygous resistant genotypes. Two specimens of unclear status, 'Chetwertoe Iyulya' (T004) and 'Orange 86' (T116), and a hybrid based on the Russian variety 'Russian Lakomstrvo' (T342) had heterozygous genotypes, indicating the involvement of artificial cross-pollination in the development of these specimens.

Four markers of resistance against TSWV, i.e., NCSw-007, NCSw-011, NCSw-003, and NCSw-012, are known to be linked with the locus *Sw-5*. Allele variants associated with resistance or susceptibility to TSMV are known to have specific sizes [25]. NCSw003 is a codominant SCAR marker with allele sizes of 600 bp and 680 bp, corresponding to

**Table 2.** Molecular markers of tomato resistance against tomato mosaic, tomato spot wilt, and tomato yellow curl leaf viruses.

| Virus | Resistance locus* | Linked marker** | Primer pair | Restriction enzymes | Reference |
|---|---|---|---|---|---|
| ToMV | Tm2 (Solyc09g018220; 9: 13,619,029-13,621,615) | CAPS PrRuG086-151 (9:13,618,982-13,620,039) | F: 5′-GAGTTCTTCCGTTCAAATCCTAAGCTTGAGAAG R: 5′-CTACTACACTCACGTTGCTGTGATGCAC | *Ksp*AI (*Hpa*I) | [22] |
| TSWV | Sw-5 (Solyc09g098130; 9: 72,527,604-72,533,377) | SCAR NCSw-003 (9:72,290,589-72,291,168) | F: 5′-TCTCGTTATCCAATTTCACC R: 5′-GCAATTTTGTTTCTTGGTCT | - | [25] |
| | | SCAR NCSw-012 (9:72,525,649-72,526,628) | F: 5′-ATGGTCAACTCGATCAGAAC R: 5′-TTTGGTGAGGATCTGATTTC | - | |
| | | CAPS NCSw-007 (9:72,418,093-72,418,673) | F: 5′-GTTGCTAACTCGACTCGTTC R: 5′-TCACTCACGTCCTATTGACA | FD *Hin*fI | |
| | | CAPS NCSw-011 (9:72,501,343-72,502,416) | F: 5′-TATCATCCTCATACCCCTTG R: 5′-GGATTTTCTCATCATCTCCA | *Hpy*F3I (*Dde*I) | |
| TYLCV | Ty-2 (chromosome 11, no position data available) | SCAR Ty2-UpInDel (11:54,815,602-54,815,797) | F: 5′-ACCCCAAAAACATTTCTGAAATCCT R: 5′-TGGCTATTTTGTGAAAATTCTCACT | - | [28] |
| | Ty-3 (chromosome 6, no position data available) | CAPS Ty3-InDel/SNP9 (6:34,472,415-34,473,062) | F: 5′-CCTATCCTCAGTGTTTCGGTCA R: 5′-GGCGAAAGACTTTGTGTACACA | *Bst*1107I (*Bst*Z17I)/ *Mun*I (*Mfe*I) | |
| | | CAPS Ty3-SNP17 (6:34,468,873-34,469,660) | F: 5′-TCTCAGGTGATGCTGAGCAC R: 5′-AGAGAACGAAAACGAAATTTCAAACA | *Rsa*I | |

* Gene ID and genomic positions according to *S. lycopersicum* genome assembly SL3.0 [29,30].
** Marker positions in *S. lycopersicum* genome assembly SL3.0 [29] including primer sequences using the FastPCR software [31].

susceptible and resistant genotypes, respectively. NCSw-012 is a dominant SCAR marker producing a PCR fragment with a size of approximately 1000 bp only in susceptible genotypes. The markers NCSw-007 and NCSw-011 are codominant CAPS markers. The cleavage of the NCSw-007 PCR product with a size of 480 bp by the *Hin*fI enzyme produces two equal fragments in susceptible genotypes, whereas the resistant allele is not affected. The NCSw-011 PCR product digested with the HpyF3I (*Dde*I) enzyme results in two fragments with sizes of 380 bp and 600 bp in susceptible genotypes and three fragments of sizes 200 bp, 380 bp, and 420 bp in resistant genotypes (Figure 2). All four markers were shown to belong to various parts of the *Sw-2* locus [25] and to provide more reliable identification of the resistant allelic variants for the needs of MAS. In our results, only one tomato accession, T009 (a local second-generation hybrid based on 'Mirsini' F1, the Netherlands) demonstrated resistant genotypes for all four markers. The dominant marker NCSw-012 was difficult to interpret, so we treated all empty results as missing, unless resistant genotypes were found in three other markers.

Four markers based on loci *Ty-2* and *Ty-3* of the tomato genome are known to be associated with tomato resistance to TYLCV. No tomato varieties showing the resistant allele of the SCAR marker Ty2-UpInDel were revealed – all samples produced a PCR fragment with a size of 200 bp, whereas the resistant allele should correspond to a fragment with a size of 120 bp [28]. The markers Ty3-InDel4 and Ty3-SNP9 presented variants of the same PCR fragment, produced by two restriction enzymes, *Bst*1107I (*Bst*Z17I) and *Mun*I (*Mfe*I), correspondingly. Ty3-InDel4 produced a single fragment with a size of 669 bp in susceptible genotypes and two close fragments with sizes of 325 bp and 353 bp in resistant genotypes; Ty3-SNP9 resulted in two fragments with sizes of 114 bp and 555 bp in susceptible genotypes and a single fragment with a size of 678 bp in resistant genotypes [28]. The PCR product with the marker Ty3-SNP17 digested with the *Rsa*I enzyme reportedly demonstrates complex restriction patterns for both allelic variants: 51+52+148+562 bp in susceptible genotypes and 51+52+65+78+497 bp in resistant genotypes [28]. We considered the largest fragment, 562/497 bp, to be primary for genotype determination, as its size and variation are suitable for easy fragment resolution in agarose gel (Figure 3). Three *Ty-3* markers covering different gene variations can reportedly confer resistance to TYLCV independently, although their possible interactions with other resistance loci are not clear [28]. In our results, the Russian cultivar 'Malinovyi slon' (T319) had resistant genotypes in all three *Ty-3* markers and 'Nicola' (T496) had resistance in Ty3-InDel4/SNP9. The hybrid based on the Russian cultivar 'Yarkiy rumyanets' (T326) had resistant genotypes in markers Ty3-SNP9 and Ty3-SNP17. Additionally, the local hybrid #332 (T135) demonstrated a resistant allele of Ty3-SNP9, and the variety of unknown origin 'Orange 86' (T116) was heterozygous in the same marker.

The results of the present work indicate that the genetic factors of resistance to common tomato viruses are currently out of scope of tomato breeding in Kazakhstan. Only a small number of tomato genotypes of foreign origin were found to possess the allelic variants associated with resistance. The possible presence of potentially deteriorating tomato viruses in the country remains unclear, as no detailed studies on this matter have been reported to date, although tomato virus symptoms were detected in Kazakhstan years ago [33]. The measures taken to prevent the import of infected plant material, both agricultural products and seed material [34], are only one line of defense. Taking into account the potential damage to horticulture that can be caused by the possible quarantine breach in our country, selecting for varieties bearing genetic resistance factors of resistance to viruses and other pathogens should be implemented as a necessary part of tomato breeding programs [35, 36]. Modern MAS techniques are a powerful tool that greatly facilitates the breeding process, reducing the time and labor required [37]. MAS is widely used for developing new tomato varieties resistant to viruses and other pathogens, with a growing list of known markers associated with both qualitative and quantitative resistance loci [19]. The markers used in the present article were initially developed for use in MAS and have a high potential in the breeding application, along with other known markers [38, 39, 40]. Foreign tomato varieties with reported resistance to viruses can be a valuable genetic resource in the breeding directed by MAS.

## 4. Conclusion

The present study used a set of known molecular markers of tomato resistance to three common viruses, ToMV, TSWV and TYLCV, for

**Table 3.** Results of the screening of 54 tomato accessions for nine markers of resistance to three viruses. R – resistant genotype; H – resistant allele in heterozygous state; S – susceptible genotype; 0 – absence of the amplification product.

| Sample | Variety | TMV | TSWV | | | | TYLCV | | | | Sample | Variety | TMV | TSWV | | | | TYLCV | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PrRuG 086-151 | NCSw-007 | NCSw-011 | NCSw-003 | NCSw-012 | Ty2-UpInDel | Ty3-InDel | Ty3-SNP9 | Ty3-SNP17 | | | PrRuG 086-151 | NCSw-007 | NCSw-011 | NCSw-003 | NCSw-012 | Ty2-UpInDel | Ty3-InDel | Ty3-SNP9 | Ty3-SNP17 |
| T001 | Choportula | S | S | S | S | S | S | S | S | S | T116 | Orange 86 | ***H*** | S | S | S | S | S | S | ***H*** | S |
| T002 | K2501 | S | S | S | S | S | S | S | S | S | T121 | Dochodnyi | S | S | S | S | S | S | S | S | S |
| T003 | Zagadka Prirody | S | S | S | S | S | S | S | S | S | T122 | Dama | S | S | S | S | S | S | S | S | S |
| T004 | Chetwertoe Iyulya | ***H*** | S | S | S | S | S | S | S | S | T314 | Ranniy 310 | S | S | S | S | S | S | S | S | S |
| T005 | Idillia | S | S | S | S | 0 | S | S | S | S | T316 | Yarkiy Rumyanets | S | S | S | S | S | S | S | **R** | **R** |
| T006 | #1051 | S | S | S | S | S | S | S | S | S | T317 | 7952691322 | S | S | S | S | S | S | S | S | S |
| T007 | Yablochnyi | S | S | S | S | S | S | S | S | S | T319 | Malinovyi Slon | S | S | S | S | S | S | **R** | **R** | **R** |
| T008 | Shalun | S | S | S | S | S | S | S | S | S | T320 | Palmira | S | S | S | S | S | S | S | S | S |
| T009 | Mirsini F2 | S | **R** | **R** | **R** | **R** | S | S | S | S | T322 | Lambrusko | S | S | S | S | S | S | S | S | S |
| T010 | Uragan | S | S | S | S | S | S | S | S | S | T324 | Russkoe Lakomstvo | ***H*** | S | S | S | S | S | S | S | S |
| T011 | Mestnyi 806 | S | S | S | S | S | S | S | S | S | T328 | Tolstushka | S | S | S | S | S | S | S | S | S |
| T012 | Semka | S | S | S | S | S | S | S | S | S | T329 | Cherry Lisa | S | S | S | **R** | S | S | S | S | S |
| T013 | Pavlina | S | S | S | S | S | S | S | S | S | T332 | Krupnyi | S | S | S | S | S | S | S | S | S |
| T014 | Gloria | S | S | S | S | S | S | S | S | S | T333 | Rassvet 362 | S | S | S | S | S | S | S | S | S |
| T016 | Pozhar | S | S | S | S | S | S | S | S | S | T337 | Yula | S | S | S | S | S | S | S | S | S |
| T017 | Orange | S | S | S | S | S | S | S | S | S | T338 | Yablochnyi | S | S | S | S | S | S | S | S | S |
| T018 | Rassvet | S | S | S | S | S | S | S | S | S | T339 | Krasnaya Presnya | S | S | S | S | S | S | S | S | S |
| T019 | Denar | 0 | S | S | S | S | S | S | S | S | T132 | Egg shaped | S | S | S | S | S | S | S | S | S |
| T021 | Gribnoe Lukoshko | S | S | S | S | S | S | S | S | S | T135 | #332 | S | S | S | S | S | S | S | **R** | S |
| T024 | Spiridon | S | S | S | S | S | S | S | S | S | T143 | Malinovka | S | S | S | S | S | S | S | S | S |
| T025 | Venera | S | S | S | S | S | S | S | S | S | T185 | Malika | S | S | S | S | S | S | S | S | S |
| T053 | Jusupovskiy | S | S | S | S | S | S | S | S | S | T187 | Ruzha | S | S | S | S | 0 | S | S | S | S |
| T073 | Heart-shaped red | S | S | S | S | S | S | S | S | S | T194 | Kozyr | S | S | S | S | S | S | S | S | S |
| T078 | Lipen | S | S | S | S | S | S | S | S | S | T211 | Sunnik | **R** | S | S | S | S | S | S | S | S |
| T103 | Mestnyi 3284 | S | S | S | S | S | S | S | S | S | T217 | Costoluto biorentino | S | S | S | S | S | S | S | S | S |
| T109 | Anait | S | S | S | S | S | S | S | S | S | T221 | Monach | S | S | S | S | S | S | S | S | S |
| T114 | Zhiraf | **R** | S | S | S | S | S | S | S | S | T496 | Nicola | S | S | 0 | S | S | S | **R** | **R** | S |

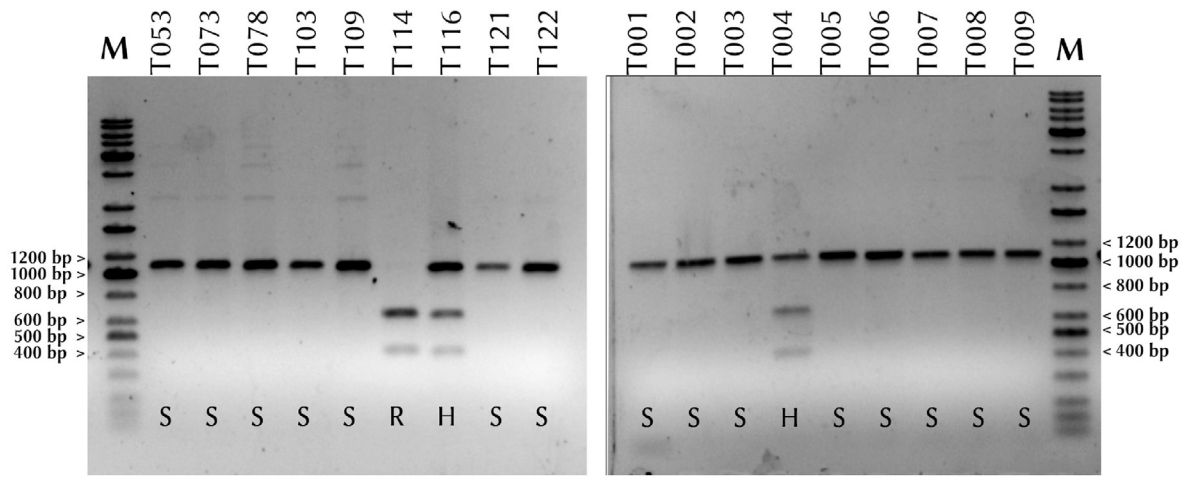


**Figure 1.** PCR products obtained using marker PrRuG186-151 associated with resistance to tomato mosaic virus (ToMV): S – susceptible genotype, R – resistant genotype [22], H – heterozygous genotype; M – DNA size marker KAPA™ Universal Ladder (KAPA Biosystems). The original images are available as the supplementary materials.

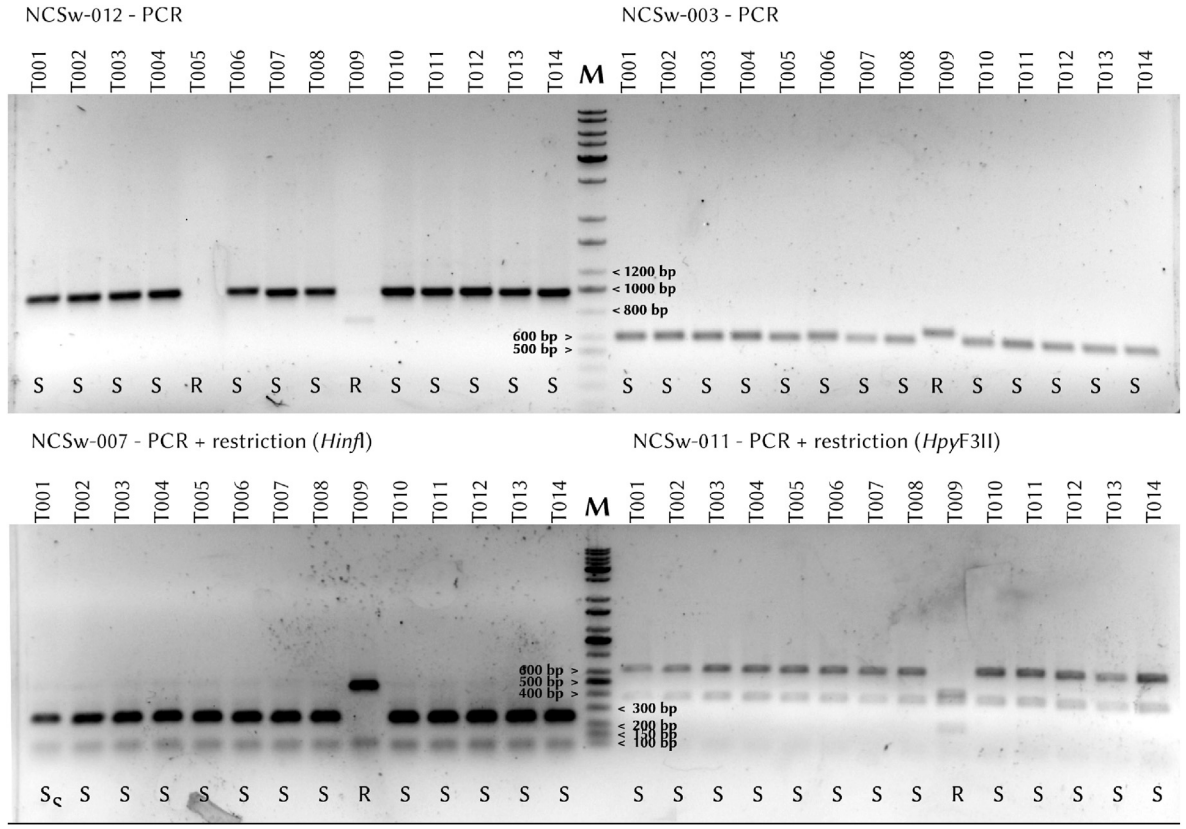


**Figure 2.** PCR products obtained using four markers associated with resistance to tomato spot wilt virus (TSWV): S – susceptible genotype, R – resistant genotype, H – heterozygous genotype [25], M – DNA size marker KAPA™ Universal Ladder (KAPA Biosystems). The original images are available as the supplementary materials.

screening the collection of tomato varieties used in the breeding programs of the FVRI. Only five tomato varieties had markers of resistance to ToMV in a homozygous or heterozygous state. One hybrid, based on the 'Mirsini' F1 variety, revealed a resistant genotype against TSWV. Four tomato genotypes had 1-3 markers associated with resistance to the *Ty-3* locus of TYLCV. The obtained results revealed that genetic factors of tomato resistance against common viruses are not currently present in the genetic pools of tomato breeding programs in Kazakhstan. Further

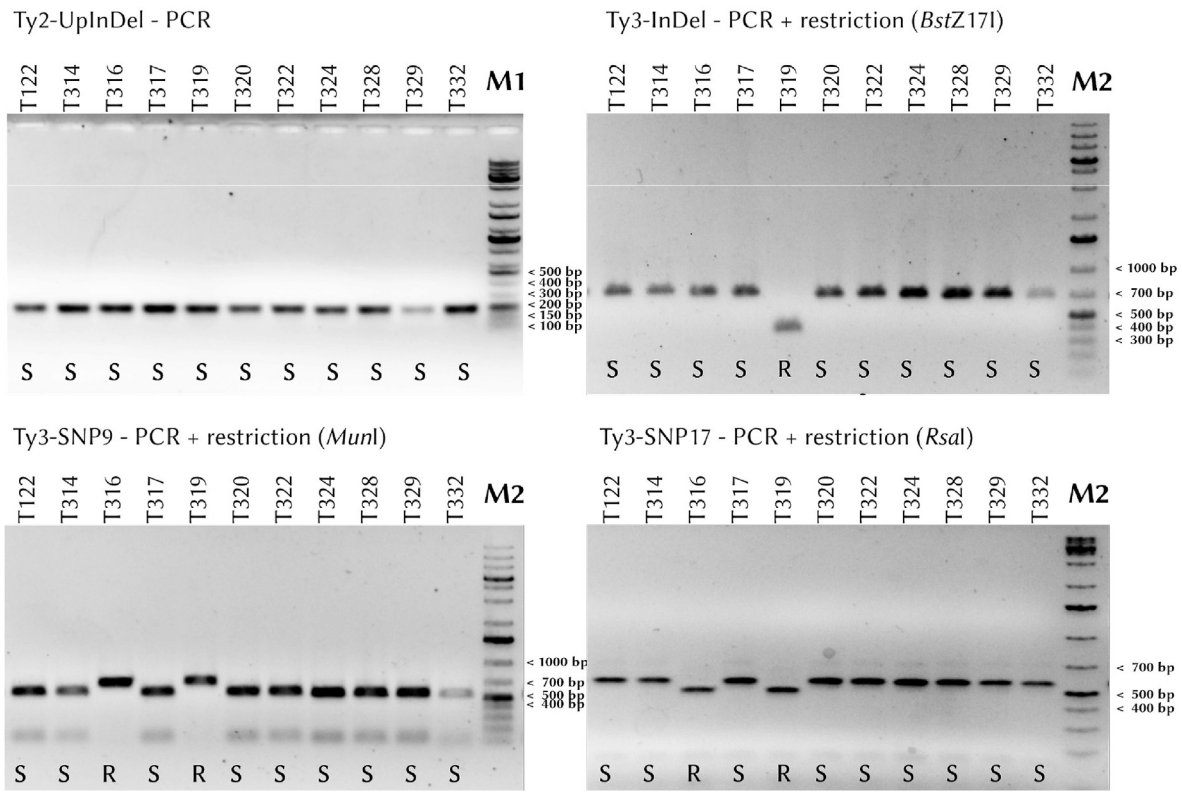


**Figure 3.** PCR products obtained using four markers associated with resistance to tomato yellow leaf curl virus (TYLCV): S – susceptible genotype, R – resistant genotype [28]; M1 – DNA size marker KAPA Universal DNA Ladder (KAPA Biosystems); M2 – DNA size marker GeneRuler 1kb Plus DNA Ladder (Thermo Fisher Scientific). The original images are available as the supplementary materials.

work on introducing resistance factors from foreign tomato varieties is important as a preventive measure against potential tomato virus outbreaks.

## Declarations

### *Author contribution statement*

Alexandr Pozharskiy: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Valeriya Kostyukova, Aisha Taskuzhina, Gulnaz Nizamdinova: Performed the experiments.

Nina Kisselyova: Contributed reagents, materials, analysis tools or data.

Ruslan Kalendar: Analyzed and interpreted the data.

Nurlybek Karimov: Contributed reagents, materials, analysis tools or data.

Dilyara Gritsenko: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### *Data availability statement*

Data included in article/supp. material/referenced in article.

### *Declaration of interests statement*

The authors declare no conflict of interest.

### *Additional information*

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e10095.

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