SCIENTIFIC NOTE

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# Screening of wild tomato species and interspecific hybrids for resistance/tolerance to *Tomato brown rugose fruit virus* (ToBRFV)

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## ABSTRACT

Tomato (*Solanum* spp.) is the second most-consumed vegetable after potato and grown all over the world. *Tomato brown rugose fruit virus* (ToBRFV) was first identified in 2014 on tomato plants, since then it has been reported in many countries. It is a significant threat to tomato production. This work aimed to identify the disease resistance source(s). To achieve this aim, a total of 44 tomato materials including 28 accessions of eight wild species, two accessions of *Solanum arcanum* Peralta, *S. pennellii* Correll, and *S. sitiens* I.M. Johnst., seven accessions of *S. chilense* (Dunal) Reiche, five accessions of *S. pimpinellifolium* L., four accessions of *S. habrochaites* S. Knapp & D.M. Spooner, three accessions of *S. peruvianum* L., one accession of *S. chmielewskii* (C.M. Rick et al.) D.M. Spooner et al. and *S. huaylasense* Peralta, 5 cultivated tomatoes (*S. lycopersicum* L.) and 11 interspecific $F_1$ hybrids derived from *S. habrochaites* and *S. pennellii* were tested with ToBRFV isolates by using the biological testing method. Mechanical inoculation method was used for biological testing. ToBRFV was inoculated to 10 plants with 2-3 true leaves two replicates for each genotype. As a result, *S. pimpinellifolium* (LA1651), *S. penellii* (LA0716), and *S. chilense* (LA4117A, LA2747) were found tolerant to ToBRFV with the lowest disease severity index (DSI) with 19.6%, 28.3% and 35.0%, respectively. Also, molecular genetic analysis of the plant material by using molecular markers revealed that there was no interaction between other virus resistance genes (*Tm-2*$^2$ and *Tm-1*) and ToBRFV resistance. These wild tomato species identified in the present study are valuable genetic resources to develop new resistance cultivars for ToBRFV resistance in tomato breeding programs.

**Key words:** Resistance, ToBRFV, tomato, wild species.

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most economically essential vegetables in the world. The world tomato production is over 180 million tons from an area of 5.03 million hectares (FAO, 2021). Tomato production is affected by biotic stress due to the susceptibility of cultivated tomato to more than 200 diseases, including fungi, nematodes, bacteria, and viruses that can cause significant economic losses (Singh et al., 2017). Although natural resistance genes originating from wild tomato species are used to improve the disease resistance of cultivated tomatoes, newly evolved biotic factors can overcome the resistance provided by the resistance genes. A freshly discovered *Tobamovirus* in Jordan and Israel called *Tomato brown rugose fruit virus* (ToBRFV) endangers the production of tomatoes by overcoming the resistance of *Tm-2*$^2$ resistance gene and confer resistance to *Tomato mosaic virus* (Levitzky et al., 2019). The disease has spread throughout the Middle East, Europe, America, and China (Salem et al., 2016; Luria et al., 2017; Fidan et al., 2019; Beris et al., 2020). ToBRFV has since been reported in several countries on tomato in China, UK, USA, Germany, Turkey, Spain, Egypt, Mexico (Cambrón-Crisantos et al., 2019; Fidan et al., 2019; Ling et al., 2019; Menzel et al., 2019; Skelton

et al., 2019; Yan et al., 2019; Alfaro-Fernández et al., 2020; Amer and Mahmoud, 2020) and on pepper in Italy and Jordan (Panno et al., 2020a; Salem et al., 2020) and is likely to spread to other countries.

ToBRFV has local and systemic symptoms and mild to severe mosaic on leaves with occasional narrowing of leaves. The fruit affected by this virus has yellow spots, necrotic and brown areas which result in a non-marketable product (Salem et al., 2016; Luria et al., 2017; Fidan et al., 2021). ToBRFV was expected to cause a total 30%-70% reduction in marketable tomato fruit production in Florida, resulting in an annual economic effect of USD 262 million (Klap et al., 2020). However, studies estimating gross tomato fruit production losses are still to be completed (Jones, 2021).

ToBRFV transmission is mainly mechanical, but it can also be transmitted via contaminated seeds or fruits over long distances and bumblebees in a greenhouse (Levitzky et al., 2019; Panno et al., 2020b). The source of inoculum may continue to stay on soil, greenhouse equipment, and the human body (Oladokun et al., 2019). Natural resistance genes are known as the best methods for virus-based disease management, several virus resistance genes such as *Tm-2*, *Sw-5*, *Ty-1* and *Ty-3* that confer resistance to *Tomato mosaic virus* (ToMV), *Tomato spotted wild virus* (TSWV) and *Tomato yellow leaf curl virus* (TYLCV), respectively, were identified in wild tomato species and cultivated tomato (Ji et al., 2007; Pérez de Castro et al., 2007; Dianese et al., 2010; Shi et al., 2011). Thus, identification of new resistance sources has become even more critical because ToBRFV outbreaks have been observed in a number of countries throughout the world (Chanda et al., 2021). Thus, this study's main objective was to screen wild species, cultivated tomato genotypes, and interspecific hybrids for ToBRFV resistance to determine the resistance source used in tomato breeding programs.

## MATERIALS AND METHODS

### Plant material

A total of 44 tomato (*Solanum* spp.) materials including 28 wild species (two accessions of *S. arcanum* Peralta, *S. pennellii* Corell and *S. sitiens* I.M. Johnst., seven accessions of *S. chilense* (Dunal) Reiche, five accessions of *S. pimpinellifolium* L., four accessions of *S. habrochaites* S. Knapp & D.M. Spooner, three accessions of *S. peruvianum* L., one accession of *S. chmielewskii* (C.M. Rick et al.) D.M. Spooner et al. and *S. huaylasense* Peralta, five cultivated tomatoes (*S. lycopersicum* L.) and 11 interspecific $F_1$ hybrids derived from *S. habrochaites* and *S. pennellii* were used as plant materials (Table 1). Entire genome of virus isolate (MT107885.1 TBRFV-Ant-Tom) used in the study was registered in the National Center for Biotechnology Information (NCBI, Bethesda, Maryland, USA).

**Table 1. *Solanum* species, accession number and origin of the genotypes used in the study.**

| Name of species | Accession number | Origin | Name of species | Accession number | Origin |
|---|---|---|---|---|---|
| *Solanum arcanum* Peralta | LA2151 | TGRC | *S. peruvianum* L. | LA1337 | TGRC |
| *S. arcanum* | LA2157 | TGRC | *S. peruvianum* | LA2744 | TGRC |
| *S. chilense* (Dunal) Reiche | LA4117A | TGRC | *S. peruvianum* | LA0462 | TGRC |
| *S. chilense* | LA2748 | TGRC | *S. pimpinellifolium* L. | LA2656 | TGRC |
| *S. chilense* | LA2880 | TGRC | *S. pimpinellifolium* | LA2093 | TGRC |
| *S. chilense* | LA2931 | TGRC | *S. pimpinellifolium* | LA1651 | TGRC |
| *S. chilense* | LA1932 | TGRC | *S. pimpinellifolium* | LA0442 | TGRC |
| *S. chilense* | LA1971 | TGRC | *S. pimpinellifolium* | LA1579 | TGRC |
| *S. chilense* | LA2747 | TGRC | *S. sitiens* I.M. Johnst. | LA4110 | TGRC |
| *S. chmielewskii* (C.M. Rick et al.) D.M. Spooner et al. | LA1318 | TGRC | *S. sitiens* | LA4331 | TGRC |
| *S. habrochaites* S. Knapp & D.M. Spooner | LA1393 | TGRC | Other | LA4135 | TGRC |
| *S. habrochaites* | LA1777 | TGRC | *S. lycopersicum* × *S. habrochaites* | AKT11 | AKD |
| *S. habrochaites* | LA0407 | TGRC | *S. lycopersicum* × *S. habrochaites* | AKT4 | AKD |
| *S. habrochaites* | LA1778 | TGRC | *S. lycopersicum* × *S. habrochaites* | AKT5 | AKD |
| *S. huaylasense* Peralta | LA1982 | TGRC | *S. lycopersicum* × *S. habrochaites* | AKT6 | AKD |
| *S. lycopersicum* L. | AKT44 | AKD | *S. lycopersicum* × *S. habrochaites* | AKT8 | AKD |
| *S. lycopersicum* | AKT45 | AKD | *S. lycopersicum* × *S. habrochaites* | AKT9 | AKD |
| *S. lycopersicum* | Ayaş | Local variety | *S. lycopersicum* × *S. habrochaites* | AKT10 | AKD |
| *S. lycopersicum* | Lice | Local variety | *S. lycopersicum* × *S. habrochaites* | AKT11 | AKD |
| *S. lycopersicum* | Torry $F_1$ | Sygenta | *S. lycopersicum* × *S. penellii* | AKT13 | AKD |
| *S. pennellii* Correll | LA0716 | TGRC | *S. lycopersicum* × *S. penellii* | AKT14 | AKD |
| *S. pennellii* | LA1940 | TGRC | *S. lycopersicum* × *S. penellii* | AKT16 | AKD |

TGRC: Tomato Genetic Resource Center; AKD: Akdeniz University Manavgat Vocational School Tomato Gene Pool.

**Growth conditions and inoculum preparation**

The test plants for inoculation were in an equal volume of steam-sterilized perlite: peat mix. The experiment was conducted in a completely randomized block with two replicates. Each replicate tested 10 plants, and non-inoculated plants from each tomato material were used as control plants. The origin of the *Tomato brown rugose fruit virus* (ToBRFV) was greenhouse tomato plants grown in Antalya, reported by Fidan et al. (2019). Details of molecular validation of ToBRFV were described in the respective publication. The inoculum was prepared from the collected symptomatic fruit and leaf samples which were individually homogenized in 0.01 mol $L^{-1}$ phosphate buffer (0.8 mol $L^{-1}$ $KH_2PO_4$, 0.1 mol $L^{-1}$ $Na_2HPO_4$, pH 7.0). A sponge was dipped into the inoculum and rubbed across healthy, immature leaves of the test plants. This process created micro-abrasions that served as entry points for virus infection after inoculation test plants were grown in a growth chamber with a photoperiod of 16:8 h and a target air temperature set at 28 °C/20 °C day/night (Fidan et al., 2021).

**Disease severity index (DSI)**

The symptoms of ToBRFV were evaluated according to disease severity index (DSI) after 30 d of the mechanical inoculation and using the 0 to 3 DSI modified by Zinger et al. (2021) given in Table 2; where: 0 indicates no ToBRFV symptoms, and 3 indicates severe symptoms (Figure 1). The tested plants were scored using a scale of 0-3 as specified, and whether all plants were infected with ToBRFV. The DSI values were calculated as follows (Chiang et al., 2017):

$$\text{DSI}\,(\%) = \frac{\Sigma\,(\text{Class frequency} \times \text{Score of rating class}}{(\text{Total number of observations}) \times (\text{Maximal disease index})} \times 100$$

**Molecular markers and PCR amplifications**

Genomic DNA of the genotypes was isolated from the fresh leaves according to CTAB method (Doyle and Doyle, 1990). The presence of the resistance genes *Tm-1* and *Tm-2²* was investigated essentially, using sequence characterized amplified region (SCAR) markers (Ohmori et al., 1996) and tetra-primer amplification refractory mutation system (ARMS) (Lanfermeijer et al., 2003) primers, respectively.

**Table 2. Symptom severity classes and disease reaction of tomato genotypes against *Tomato brown rugose fruit virus* (ToBRFV).**

| Classes | Symptoms | Disease classes interval | Disease reaction |
|---|---|---|---|
| 0 | No visible symptoms | 0 | Resistant |
| 1 | Very slight chlorosis, mosaic form on apical leaf | 0.01-1.4 | Tolerant |
| 2 | Severe mosaic form and blistering on the leaf surface | 1.5-2.4 | Susceptible |
| 3 | Very severe blistering on the leaf and leaf narrowing, wilt, and death of complete plants | 2.5-3.0 | Highly susceptible |

**Figure 1. *Tomato brown rugose fruit virus* (ToBRFV) symptoms of the tomato leaves: 0 indicates no symptoms and 3 indicates severe symptoms.**

## RESULTS AND DISCUSSIONS

**Screening for tomato genotypes resistant or tolerant to ToBRFV**

We have inoculated 44 tomato genotypes, including 28 wild genotypes, 5 cultivated tomatoes, and 11 interspecific hybrids with a mechanical inoculation technique. As a result, *S. pimpinellifolium* (LA1651), *S. penellii* (LA0716), and *S. chilense* (LA4117A, LA2747) were found to be tolerant to ToBRFV due to the lowest disease severity index (DSI) with 19.6%, 28.3%, 35.0% and 35.2%, respectively (Table 3). These lines also had lowest symptom severity classes based on Table 2 (0.6, 0.9, and 1.1, respectively) (Figure 2).

On the contrary, Torry $F_1$ *S. pimpinellifolium* $F_1$, Lice, *S. pimpinellifolium* (LA2656), *S. pimpinellifolium* (LA2093) (Figure 3), Ayaş, AKT45, and AKT10 were evaluated as highly susceptible to ToBRFV with 100% (DSI) (Table 3). The severity index value of these genotypes was evaluated 3 (Figure 1). The incidence of ToBRFV disease reached 100% in some commercially grown tomato cultivars planted in greenhouse environments (Samarah et al., 2021). For the first time, the tolerant genotypes are presented here to different wild genotypes.

**Table 3. Disease severity index (DSI) of tomato plants infected with *Tomato brown rugose fruit virus* (ToBRFV).**

| Accession number | Disease severity (%) | Accession number | Disease severity (%) |
|---|---|---|---|
| LA1940 | 51.7 | LA1337 | 54.2 |
| LA0716 | 28.3 | LA1318 | 44.4 |
| LA4117A | 35.0 | LA1982 | 46.7 |
| LA1971 | 74.1 | LA2151 | 57.6 |
| LA2747 | 35.2 | LA2157 | 63.3 |
| LA2748 | 70.0 | LA4135 | 61.5 |
| LA2880 | 52.4 | AKT44 | 81.5 |
| LA2931 | 47.9 | AKT45 | 100.0 |
| LA1932 | 61.1 | Ayaş | 100.0 |
| LA0407 | 41.7 | Lice | 100.0 |
| LA1778 | 40.0 | Torry $F_1$ | 100.0 |
| LA1393 | 60.0 | AKT1 | 66.7 |
| LA1777 | 41.7 | AKT4 | 80.0 |
| LA4110 | 59.6 | AKT5 | 83.3 |
| LA4331 | 42.9 | AKT6 | 71.4 |
| LA2656 | 100.0 | AKT8 | 77.8 |
| LA0442 | 60.0 | AKT9 | 66.7 |
| LA1579 | 38.9 | AKT10 | 100.0 |
| LA2093 | 100.0 | AKT11 | 81.0 |
| LA1651 | 19.6 | AKT13 | 54.2 |
| LA2744 | 68.5 | AKT14 | 69.4 |
| LA0462 | 68.5 | AKT16 | 75.8 |

**Figure 2. Symptom severity classes of tested tomato material.**

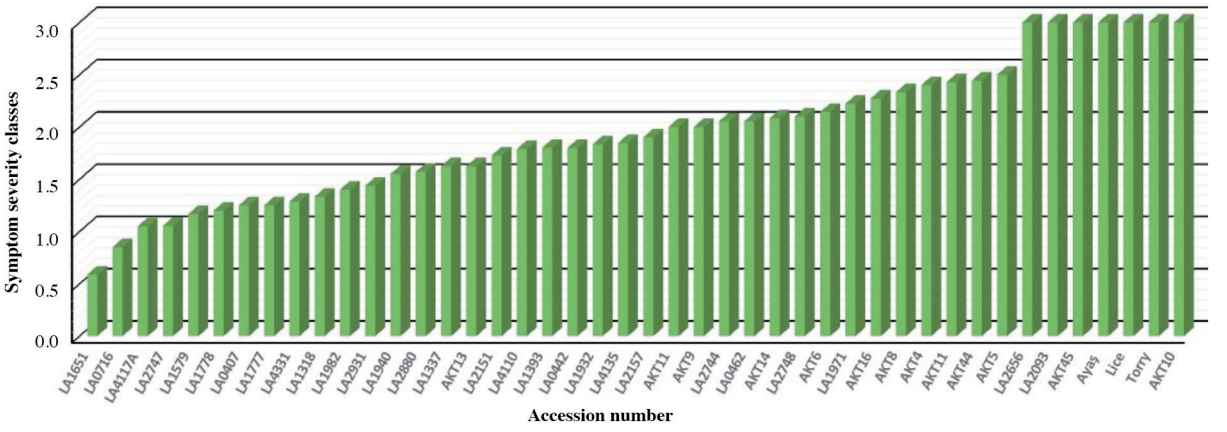


**Figure 3. Torry F1, LA2656, and LA2093 are highly susceptible tomato plants to *Tomato brown rugose fruit virus* (ToBRFV). The diseased leaves have severe blistering and narrowing.**


Resistant or tolerant variety is the most important variety to control this disease, so the breeders need resistance sources to improve the new variety. The resistance source of ToBRFV is not clear, but Zinger et al. (2021) determined that 160 genotypes were tested in a greenhouse with ToBRFV and 29 (18.1%) which consist of nine (31.0%) belong to *S. pimpinellifolium* and eight (27.6%) were cultivated lines or hybrids. Similar to the present study results, they reported that tolerance of *S. pimpinellifolium* (LA1651, LA1579), other accessions LA0442, LA2656, and LA2093 were susceptible and enhanced susceptible respectively. Many investigations reported that the response of accessions in the same tomato species could show different results to biotic stress. Foolad et al. (2014) reported that 16 out of 67 accessions of the wild tomato species, *S. pimpinellifolium*, were identified with strong late blight resistance in both field and greenhouse experiments.

Our study supports that *S. pimpinellifolium* (LA1651) could be used as a resistance source. Additionally, *S. penellii* (LA0716) and *S. chilense* (LA4117A, LA2747) could be another potential source to ToBRFV resistance (Figure 4). To our knowledge, this is the first time that a potential resistance source (different tomato species especially wild and cultivated) to ToBRFV was determined with pathogenicity tests.

**Screening for tomato genotypes *Tm-1* and *Tm-2²* locus**

In the present study, the presence of the resistance genes *Tm-1* and *Tm-2²* were determined using SCN20F, SCN20R (Ohmori et al., 1996), and Outer primer TM2-748F, TM2-1256R, TM2-SNP901misR, and TM2-SNP901misF (Lanfermeijer et al., 2003). Primers are shown in Table 4. The results of genotypes are given in Table 5. Among 36 out of 44 tomato genotypes had *Tm-2²* gene, 17 out of 44 genotypes had *Tm-1* gene, and 16 tomato genotypes had two genes respectively (Figures 5a and 5b). The *Tm-1* gene is a dominant gene found in *S. habrochaites*; while *Tm-2²* is determined in *S. peruvianum* (Pfitzner, 2006). Our result showed that *Tm-1* and *Tm-2²* genes were not associated with ToBRFV. Plants carrying both *Tm-1* and *Tm-2²* in a homozygous state were highly susceptible to ToBRFV (Zinger et al., 2021). Tomato cultivars containing the *Tm-2²* gene were not resistant to ToBRFV, but were resistant to *Tomato mosaic virus* (ToMV) and *Tomato mottle mosaic virus* (ToMMV), according to a comparative examination of disease resistance across tomato cultivars to three tobamoviruses (Chanda et al., 2021). The result of the present study was similar in terms of breaking the *Tm-2²* resistance to results of Zinger et al. (2021) and Chanda et al. (2021). Therefore, results were different because Zinger et al. (2021) reported that resistance gene in chromosome T11 had interaction with *Tm-1* gene on chromosome T2. Comprehensive study is needed to find novel gene or loci confering resistance to ToBRFV.

**Figure 4. Symptoms of tomato plants against *Tomato brown rugose fruit virus* (ToBRFV). Genotype LA1651 is disease tolerant and shows very slight chlorosis, mosaic forms on leaf (1) (a); LA0716 and LA2747 genotypes have different leaf types and disease tolerance too (b-c).**


**Table 4. Resistance genes and their primers used for sequencing.**

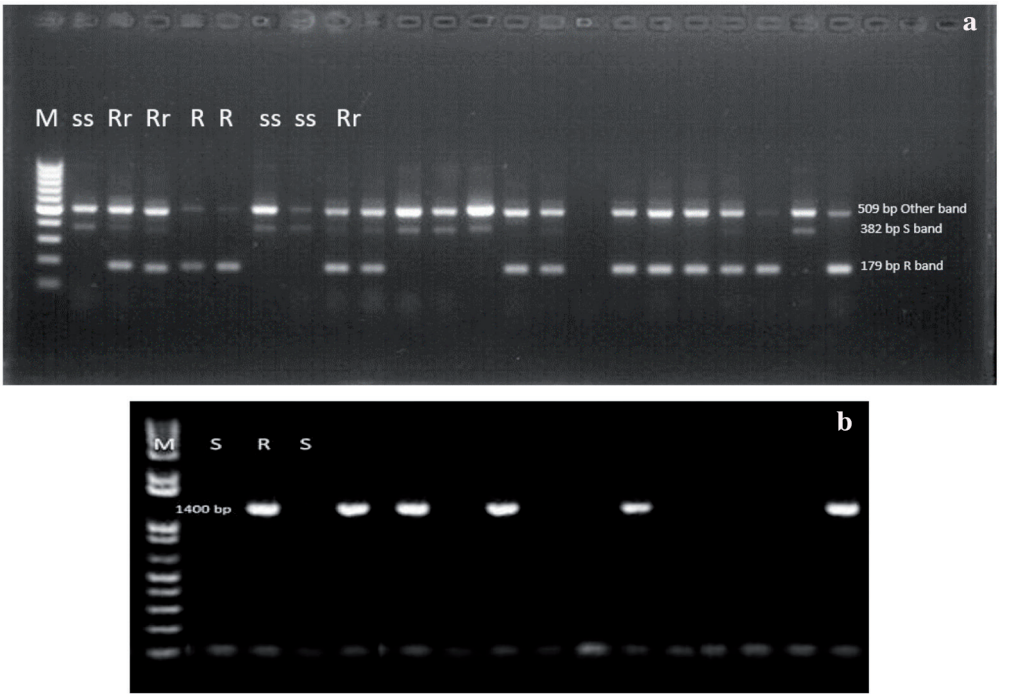
| Gene | Primer | Sequence | Resistance/ susceptible alleles | References |
|---|---|---|---|---|
| *Tm-1* | SCN20F | GGTGCTCCGTCGATGCAAAGTGCA | 1400 R | Ohmori et al., 1996 |
| | SCN20R | GGTGCTCCGTAGACATAAAATCTA | | |
| *Tm-2²* | Outer primer TM2-748F | CGGTCTGGGGAAAACAACTCT | 179 R/382 S/509 other | Lanfermeijer et al., 2003 |
| | Outer primer TM2-1256R | CTAGCGGTATACCTCCACATCTCC | | |
| | TM2-SNP901misR | GCAGGTTGTCCTCCAAATTTTCCATC | | |
| | TM2-SNP901misF | CAAATTGGACTGACGGAACAGAAAGTT | | |

**Table 5. Genotypes of tomato accessions to *Tm-2²* and *Tm-1* resistance genes determined by polymerase chain reaction analyses.**

| Accession number | *Tm-2²* | *Tm-1* | Accession number | *Tm-2²* | *Tm-1* |
|---|---|---|---|---|---|
| LA1940 | RR | S | LA1337 | S | R |
| LA0716 | RR | S | LA1318 | RR | S |
| LA4117A | RR | S | LA1982 | RR | S |
| LA1971 | RR | S | LA2151 | RR | S |
| LA2747 | RR | R | LA2157 | RR | S |
| LA2748 | RR | R | LA4135 | RR | S |
| LA2880 | RR | R | AKT44 | S | S |
| LA2931 | RR | R | AKT45 | S | S |
| LA1932 | RR | S | Ayaş | S | S |
| LA0407 | RR | R | Lice | RR | S |
| LA1778 | RR | R | Torry $F_1$ | RR | S |
| LA1393 | RR | R | AKT1 | RR | R |
| LA1777 | RR | R | AKT4 | RR | S |
| LA4110 | RR | S | AKT5 | RR | R |
| LA4331 | S | S | AKT6 | RR | S |
| LA2656 | RR | R | AKT8 | RR | R |
| LA0442 | S | S | AKT9 | RR | R |
| LA1579 | RR | S | AKT10 | RR | R |
| LA2093 | S | S | AKT11 | RR | R |
| LA1651 | S | S | AKT13 | RR | S |
| LA2744 | RR | S | AKT14 | RR | S |
| LA0462 | RR | S | AKT16 | RR | R |

RR: Homozygote resistance; R: resistance; S: susceptible.

**Figure 5. (a) Tetra specific primers were used to amplify *Tm-2²* locus and its product were run and visualized. (b) SCN20F and SCN20R specific primers were used to amplify *Tm1* locus and its product were run and visualized. ss: Susceptible, Rr: heterozygous resistant, R: resistant 179bp R band 382 S band and 509 other band.**




## CONCLUSIONS

*Tomato brown rugose fruit virus* (ToBRFV) is the main *Tobamovirus* that can be spread very rapidly by mechanical wounding, seeds, and human activities in tomato production areas. The development of resistant cultivars is the most effective approach of ToBRFV control. The present study initiated such approach by screening eight wild tomato species. As result, three wild tomato species (*Solanum pimpinellifolium* LA1651, *S. penellii* LA0716, and *S. chilense* LA4117A, LA2747) were found to be tolerant to ToBRFV based on morphological evaluation and disease severity index. Also, the study revealed that other virus resistance genes (*Tm-2²* and *Tm-1*) were independent for resistance to ToBRFV. The current study results will be invaluable to develop new resistant tomato lines or hybrids plants.

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