Hungarian University of Agriculture and Life Sciences

Screening *Solanum* germplasm for resistance to tomato brown rugose fruit virus (ToBRFV) and molecular characterization of a resistance breaking mutant of ToBRFV

Doctoral (Ph.D.) dissertation

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Gödöllő

2022

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LIST OF ABBREVIATIONS

+ssRNA: positive-sense single-stranded RNA

µL: microliter

aa: amino acid

Amp: ampicillin

Arg: Arginine

Asn: Asparagine

Avr: avirulence

AVRDC: Asian Vegetable Research and Development Center

BME: β-Mercaptoethanol

bp: base pair

CC: coiled-coil

cDNA: complementary DNA

CMV: Cucumber mosaic virus

CNL: Coiled-coil-Nucleotide-binding site -leucine-rich repeat

CP: Coat protein

Cys: Cysteine

ddNTPs: dideoxynucleotides

DNA: Deoxyribonucleic acid

DNase: Deoxyribonuclease

DNTP: Deoxyribose Nucleotide Triphosphate

dpi: days post-inoculation

DSI: Disease Severity Index

EDTA: Ethylenediamine tetraacetic acid

EFα: Elongation Factor Alpha

ELISA: Enzyme-Linked ImmunoSorbent Assay

GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase

Gln: Glutamine

Glu: Glutamic acid

His: Histidine

HR: Hypersensitive response or reaction

IPTG: Isopropyl β-D-1-thiogalactopyranoside

Kb: Kilobase

KDa: Kilodalton

LB: Luria Broth

LRR: Leucine-Rich Repeats

Lys: Lysine

MAMPs/PAMPs: Microbe- or Pathogen-Associated Molecular Patterns

MATE: Hungarian University of Agriculture and Life Sciences

mg: milligram

ml: milliliter

MP: Movement protein

MQ: Milli-Q water

MS: Murashige and Skoog

N: Nicotiana
NB: Nucleotide-binding
NB-LRR: Nucleotide-Binding, Leucine-Rich Repeat
NBS: Nucleotide-Binding site
NBS-LRR: Nucleotide-Binding site, Leucine-Rich Repeats
NCBI: National Center for Biotechnology Information
NPK: Nitrogen, Phosphorus, and Potassium
nt: nucleotide
nv: netted-virescent
ObPV: Obuda pepper virus
ORF: Open reading frame
PCR: Polymerase chain reaction
PepMV: Pepino mosaic virus
PGRU: Plant Genetic Resources Unit at Geneva
Phe : Phenylalanine
PM: plasma membrane
PRRs: Pathogen or Pattern Recognition Receptors
R: Resistance
RdRP: RNA-dependent RNA polymerase
ReMV: Rehmannia mosaic virus
Rep: Replicase
RLK: Receptor-Like Kinase
RLP: Receptor-Like Protein
RNA: Ribonucleic acid
Rpm: rotations per minute
RT-PCR: Reverse transcription-polymerase chain reaction
RT-qPCR: Reverse-transcription quantitative polymerase chain reaction
S: *Solanum*
SDS: Sodium dodecyl sulfate
Ser: Serine
TBE: Tris-Borate-EDTA
TGRC: Tomato Genetics Resource Center
TIM barrel: Triose-phosphate isomerase
TMV: Tobacco mosaic virus
TNL: Toll/interleukin-1 receptor- Nucleotide-binding site -leucine-rich
ToBRFV: Tomato brown rugose fruit virus
ToMMV: Tomato mottle mosaic virus
ToMV: Tomato mosaic virus
Tyr: Tyrosine
USA: United States of America
USDA: United States Department of Agriculture
wpi: weeks post-inoculation
X-Gal: 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside

1. INTRODUCTION

The *Solanaceae* family has about 3500 species, including very important, food plants like potato, tomato, pepper and eggplant, which rank at the top of vegetable crops in consumption (GEBHARDT, 2016). However, this crop is confronted with several biotic and abiotic stresses that reduce the yield's quality and quantity. Virus diseases are among the major biotic factors that influence tomato production (HANSSEN et al. 2010). The tomato is susceptible to a wide range of plant viruses, including tobamoviruses (ADAMS et al. 2009). The most important tomato pathogenic tobamoviruses are tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), tomato mild mottle virus (ToMMV) as well as the recently described tomato brown rugose fruit virus (ToBRFV) (LI et al. 2013; JONES et al. 2016; SALEM et al. 2015).

Because of the high stability and infectivity of viral particles can be transmitted by contact with the plants and mechanical injuries caused by workers as well as by pollen, seeds and bumblebees (DOMBROVSKY and SMITH 2017; LEVITZKY et al. 2019). The control of tobamoviruses using preventive measures is difficult. Therefore, breeding tobamovirus-resistant cultivars and hybrids are highly important in tomato production worldwide (RAZDAN and MATTOO 2006). In the past 60 years, three resistance genes marked *Tm1*, *Tm2* and *Tm2²* have been discovered in wild relatives of tomato and successfully incorporated into the cultivated *Solanum lycopersicum* (KOLE 2011). Out of these genes, the *Tm2²* proved durable for decades as long as the new tobamovirus species ToBRFV suddenly appeared in Jordan (SALEM et al. 2015). Although rare mutants of TMV and ToMV overcoming the *Tm* resistance genes have been isolated before, ToBRFV became known to break all known tobamovirus resistance in tomato (LURIA et al. 2017).

Symptoms caused by ToBRFV vary depending on varieties and genotypes. Foliar symptoms include chlorosis, mosaic and mottling with occasional leaf narrowing and fruit showing yellow or brown spots, with wrinkled (rugose) symptoms rendering them unmarketable. Disease incidence was close to 100% (OLADOKUN et al. 2019; SALEM et al. 2015). In addition, ToBRFV is distributed rapidly in Europe and later on all continents (VAN DE VOSSENBERG et al. 2020; EPPO 2022a). It appeared more recently also in Hungary (KRIZBAI et al. 2022). ToBRFV was mentioned in the alert list of the EPPO and categorized as an A2 pest (EPPO 2022b). It is a dangerous plant virus because, besides tomato, it also became known to infect pepper in many countries (PANNO et al. 2020; SALEM et al. 2020).

Overcoming the resistance genes, high stability, and fast geographical distribution rendered ToBRFV in the focus of tomato pathology and urges plant breeders and pathologists to

continuously search for effective novel sources of resistance in gene pools of the wild tomato, hybrid tomato and introgressed tomato lines. Therefore, our study started three years ago, intending to screen wild tomato (*Solanum*) germplasm and their relatives to find and evaluate accessions that can be utilized as sources of resistance to ToBRFV.

2. OBJECTIVES

2.1 Screening of *Solanum* germplasm for reactions to the ToBRFV

We aimed first to screen a wide range of wild tomato species and their relatives for reaction to mechanical inoculation with a Jordanian isolate of ToBRFV. During this work, we aimed to focus on the characterization of symptoms caused by ToBRFV in plants of different *Solanum* accessions and to classify them according to a disease severity index. The inoculated plants which remained symptomless were studied for the presence or absence of the virus to discriminate between the tolerant and resistant genotypes.

2.2 Demonstration and characterization of resistance in *Solanum habrochaites* and *S. peruvianum* to ToBRFV

In the course of screening, we found several accessions of *S. habrochaites* and *S. peruvianum* having resistant individuals. The aim of our further work was to characterize the type of resistances in these accessions under different conditions i.e. under high inoculation pressure, elevated temperature and after grafting. Comparative analyses of DNA sequences prepared from some resistant plants were also carried out to clear the molecular genomic background of the resistance.

2.3 Isolation and molecular characterization of a resistance breaking mutant of ToBRFV

In the course of mass inoculation of vegetatively propagated resistant *S. habrochaites* plants with ToBRFV Jordanian isolate, a single plant was unexpectedly observed showing mosaic symptoms. Therefore, we suspected the appearance of a spontaneous mutant of ToBRFV, which was able to overcome the newly discovered resistance in *S. habrochaites*. Our objectives were to isolate the presumed ToBRFV mutant and evaluate its pathogenicity to different tomato genotypes. In addition, with the aim to determine the nucleotide and amino acid changes potentially responsible for the altered pathological character, we sequenced the mutant and the parent viruses and compared their genomic sequences with each other.

3. LITERATURE REVIEW

3.1 Origin and classification of tomatoes

The tomato originated from South America (Andean region), growing in parts of Ecuador, Peru, Chile, Colombia and Bolivia (RAZDAN and MATTOO 2006).

Tomatoes are members of the family *Solanaceae* (nightshade family), genus *Solanum*, section *Lycopersicon*. The *Solanaceae* family also includes other important vegetable crops such as hot and sweet peppers (*Capsicum annuum*), potato (*S. tuberosum*), aubergine (*S. melongena*), tree tomato (*S. betaceum*) and tobacco (*Nicotiana tabacum*) (KNAPP 2002). The plant group *Solanum* sect. *Lycopersicon* include 13 closely related species or subspecies: cultivated tomato (*Solanum lycopersicum),* which also include cherry tomato (S. *lycopersicum 'cerasiforme'),* and wild species *S. arcanum, S. cheesmaniae, S. chilense, S. chmielewskii, S. corneliomulleri, S. galapagense, S. habrochaites, S. huaylasense, S. neorickii, S. pennellii, S. peruvianum, S. pimpinellifolium* and two groups of sibling species, *S. ochranthum* and *S. juglandifolium* in sect. *Juglandifolium* and *S. lycopersicoides* and *S. sitiens* in sect. *Lycopersicon* subsect. *Lycopersicoides* (Table 1) (PERALTA et al. 2008).

Table 1. Classification of *Solanum* sect *Lycopersicon* and allied species

Section	Species group	Species
Section *Lycopersicoides*	–	*Solanum lycopersicoides*
–	–	*Solanum sitiens*
Section *Juglandifolia*	–	*Solanum juglandifolium*
–	–	*Solanum ochranthum*
Section *Lycopersicon*	"Neolycopersicon"	*Solanum pennellii*
–	"Eriopersicon"	*Solanum chilense*
–	–	*Solanum corneliomulleri*
–	–	*Solanum habrochaites*
–	–	*Solanum huaylasense*
–	–	*Solanum peruvianum*
–	"Arcanum"	*Solanum arcanum*
–	–	*Solanum chmielewskii*
–	–	*Solanum neorickii*
–	"Lycopersicon"	*Solanum cheesmaniae*
–	–	*Solanum galapagense*
–	–	*Solanum lycopersicum*
–	–	*Solanum pimpinellifolium*

Species within each group are in alphabetical order

3.2 Importance, breeding and production methods of tomato

Tomato is the second most-consumed vegetable in the world after potato worldwide (EL-MANSY et al. 2021). More than 100 million metric tons were produced in 2001, with the top 15 countries (in descending order) being China, the United States, India, Turkey, Egypt, Italy, Spain, Brazil, the Islamic Republic of Iran, Mexico, Greece, the Russian Federation, Ukraine, Chile, and Uzbekistan (FAO 2018; Figure 1). Tomatoes are produced as fresh or processed into different forms as purees, pastes and juices. Tomatoes contain high vitamins A and C sources, minerals (iron and phosphorus), water, Beta-carotene, lycopene and have a low-calorie count (WILLCOX et al. 2003).
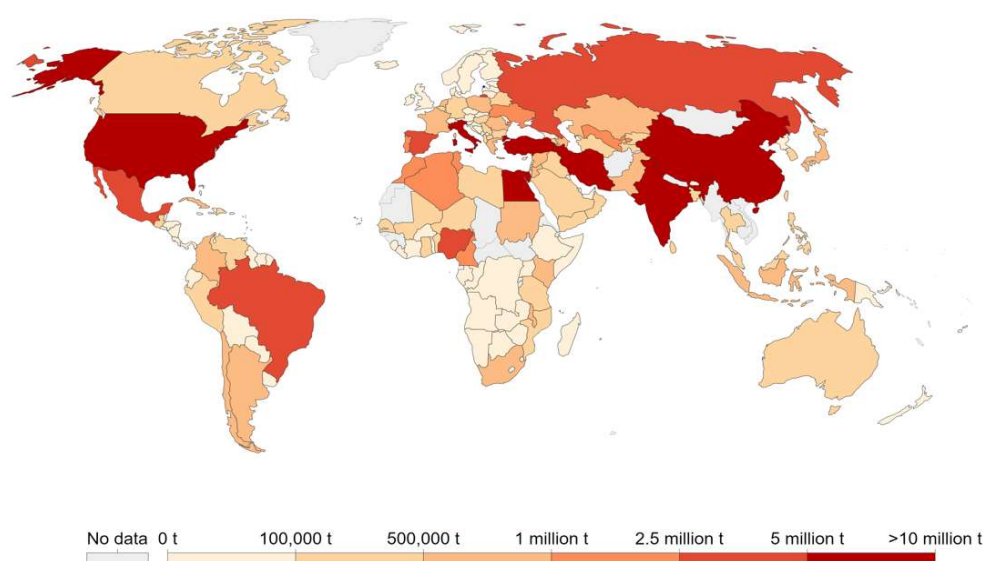
Figure 1. Tomato production map measured in tonnes (FAO 2018).

Tomato is a rapidly growing crop with a 92 to 140 days ripening period. The day length of the tomato plant considers neutral. The optimum temperature for growth is 17 to 26ºC, with night temperatures between 9 and 21ºC. Fluctuations in temperature between day and night adversely affect yield. Tomato considers very sensitive to frost. Dry climates are preferred for tomato production. On the other hand, high humidity leads to the spread of pests and diseases like fruit rotting. This crop can be grown on almost any moderately well-drained soil type. Tomato has two kinds of plant growth: indeterminate and determinate (HEUVELINK 2018).

The production of tomato is influenced by different biotic and abiotic factors (such as fungi, viruses, bacteria, salt, cold, heat, etc. (JONES et al. 2016). There are many methods to control plant

disease, but the best way is to use resistant varieties containing highly resistant genes or genes by tomato-breeding programs. Breeders started introducing disease-resistant cultivars in the early 1940s to tomato using closely related wild species *Solanum* sect. *Lycopersicon* depends on the type of use. Different breeding objectives encompass sensory and nutritional quality, improved yield, adaptation to biotic and abiotic stresses, and other important traits (KOLE 2011).

Tomato breeding and research can depend on a wide range of germplasm resources, including large collections of wild tomato forms and their derivatives. The cultivated and wild species of tomato estimated that have over 62 800 accessions (mostly *S. lycopersicum* accessions), which are maintained in gene banks around the world, including those in the Asian Vegetable Research and Development Center (AVRDC), United States Department of Agriculture (USDA), Plant Genetic Resources Unit at Geneva (PGRU), and Tomato Genetics Resource Center (TGRC). The TGRC (http://tgrc.ucdavis.edu) is known to maintain the largest collection of wild tomato species, while PGRU has a large collection of open-pollinated cultivars. Furthermore, big collections of tomato germplasm are also maintained in the Netherlands (IVT), Russia (VIR), Japan (NIAS), Peru (DHUNA), and Cuba (INIFAT) (FOOLAD 2007).

Wild tomatoes have been an excellent model system for basic and applied plant research. This has been due to many reasons; one of them is those wild tomatoes have a large genetic diversity. They have been utilized as the source of resistance to all tomato diseases (fungi, bacteria, viruses, or nematodes) (KOLE 2011). Resistance resources have been identified in most related wild species of tomato, in particular *S. pimpinellifolium*, *S. peruvianum*, and *S. habrochaites* (syn.: *L. hirsutum*). For example, in the cases of some tomato diseases such as bacterial (bacterial wilt, bacterial spot and tomato pith necrosis) and fungal (anthracnose, leaf mold, septoria leaf spot, verticillium wilt, and phytophthora root rot), the sources of resistance come from *S. pimpinellifolium* and *S. lycopersicum var cerasiforme* (KOLE 2011). In addition, resources of resistance or tolerance to tomato viruses were found in accessions of *S. pimpinellifolium, S. peruvianum, S. habrochaites, S. cheesmanii*, S. *chilense* and *S. lycopersicoides*. For instance, the *Sw-5* gene responsible for resistance to tomato spotted wilt virus (TSWV) originated from *S. peruvianum* and the tomato yellow leaf curl virus (TYLCV) resistance alleles *Ty-1* and *Ty2* were introgressed from *S. lycopersicum* and *S. chilense* (RAZDAN and MATTOO 2006). Furthermore, three dominant tobamovirus resistance genes named *Tm-1, Tm-2*, and *Tm-2² (Tm-2ᵃ)* have been incorporated to *S. lycopersicum* from *S. habrochaites* (accession number PI 126445) and *S. peruvianum* (PI 126926, PI 128650) to TMV and ToMV, respectively (HOLMES 1954; PELHAM, 1972; ALEXANDER 1963; SCHROEDER et al. 1967; LATERROT and PECAUT 1969).

Crosses between wild tomatoes and the cultivated tomato *S. lycopersicum* are possible, despite varying degrees of difficulty. For example, crossing *S. ochranthum* with *S. lycopersicum* was not yet utilized in tomato-improvement programs. That is because the *S. ochranthum* is sexually incompatible and seems genetically isolated from *S. lycopersicum* and other tomato species in all combinations tested (Figure 2). However, the cross is possible by using somatic hybridization, although with varying degrees of difficulty (RICK 1979; RICK and CHETELAT 1995; PERTUZÉ et al. 2002; WIDHOLM 2005; KOLE 2011). For instance, somatic hybrids between *S. ochranthum* and *S. lycopersicum*, have been obtained through protoplast fusion, nevertheless, they are highly sterile and have not yet provided a pathway for gene transfer (KOLE 2011). However, backcrosses between *S. ochranthum* + tomato somatic hybrids and tomato, combined with embryo rescue, may result in the desired progeny and facilitate further recombination between these species (KOBAYASHI et al. 1996).
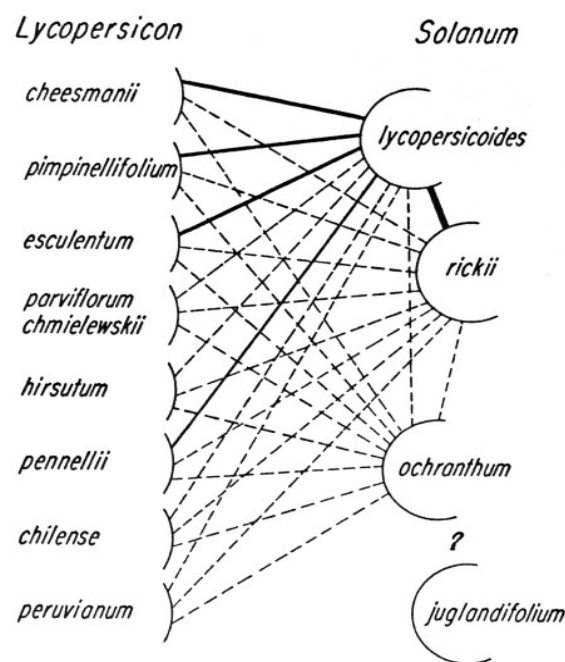
Figure 2. Diagram of cross ability relations among wild tomatoes and outgroups used by RICK (1979). Diagram to support the separation of *Lycopersicon* and *Solanum*. *S. ochranthum* by failed crossability in every combination; flowering. Solid lines indicate compatible combinations and dashed lines cross failures (PERALTA et al. 2008).

The cultivated tomato (*S. lycopersicum*) is considered a diploid species with 2n = 24 chromosomes. However, only two cases of naturally occurring tetraploidy in *S. chilense* have been reported (RICK

1990). The studies of chromosome morphology revealed new evidence of rearrangements and structural differences among the wild species based on light microscopy, higher resolution genetic, physical maps, and improved cytological methods (KOLE 2011). For example, *S. ochranthum* or *S. juglandifolium* has reciprocal whole arm translocation in chromosomes 8 and 12. Furthermore, both *S. ochranthum* and *S. juglandifolium* have inverted orientation in chromosome 10, suggesting they are more closely related to the tomatoes (ALBRECHT and CHETELAT 2009) than are members of the sect. *Lycopersicoides*, but contrasts with the evidence from crossing relationships, which suggests sect. *Lycopersicoides* is more tomato-like. These findings may explain the cross difficulty of *S. ochranthum* or other sect members. *Lycopersicoides* (KOLE 2011).

3.3 Tobamoviruses and the tomato brown rugose fruit virus (ToBRFV)

3.3.1 General characterization of the *Tobamovirus* genus

Tobamoviruses are a group of related virus species classified as a genus within the family *Virgaviridae*. The group name tobamovirus is derived from the name Tobacco mosaic virus, which is the type species of the genus. There are currently 37 species within the genus *Tobamovirus* (ICTV, virus taxonomy: 2020 release, https://talk.ictvonline.org/taxonomy).

Many families of plants, including *Apocynaceae, Brassicaceae, Solanaceae, Cucurbitaceae, Cactaceae, Malvaceae, Passifloraceae, Fabaceae, Cannabaceae* and *Orchidaceae,* serve as natural hosts of tobamoviruses. There are informal subgroups within this genus which include viruses specialized mainly to *Solanaceous*, cucurbits, malvaceous, brassicas and cactaceous plants. The main differences between these groups are their genome sequences and the respective range of host plants (MIN et al. 2006; GIBBS et al. 2008). However, there is strong evidence that these viruses likely co-evolved and co-diverged with their hosts (LEFEUVRE et al. 2019). The taxonomy of tobamoviruses have continuously and basically changed in the past 60-70 years (BAWDEN 1950; GIBBS et al. 1999; REGENMORTEL 1999). Currently, molecular genomic data play the most critical role in the differentiation of tobamovirus species (BAMFORD and ZUCKERMAN 2021).

The virions of tobamoviruses are about 18 nm in diameter with a length of 300–310 nm. The virus particles are non-enveloped, rigid helical rods with a helical symmetry encapsidating a positive-sense single-stranded RNA (+ssRNA) genome (LEFKOWITZ et al. 2018) (Figure 3). Virions

generally form large crystalline arrays in cells that can be seen under a light microscope (STEERE and WILLIAMS 1953).

The genome of tobamoviruses is 6.3–6.6 kb in size, an approximately 70 nucleotide (nt) long 5′ untranslated region (UTR) contains many A, C repeats and few or no G nucleotides. The 3- UTR is ∼200 nucleotides in length and contains sequences that can be folded into pseudoknots followed by 3′-terminal sequences that can be folded into a transfer RNA (tRNA)-like, amino acid-accepting structure. In infected cells, the subgenomic mRNAs transcribed also have a 5′-terminal cap and 3′-tRNA-like structure (Figure 4) (ISHIBASHI and ISHIKAWA 2016).
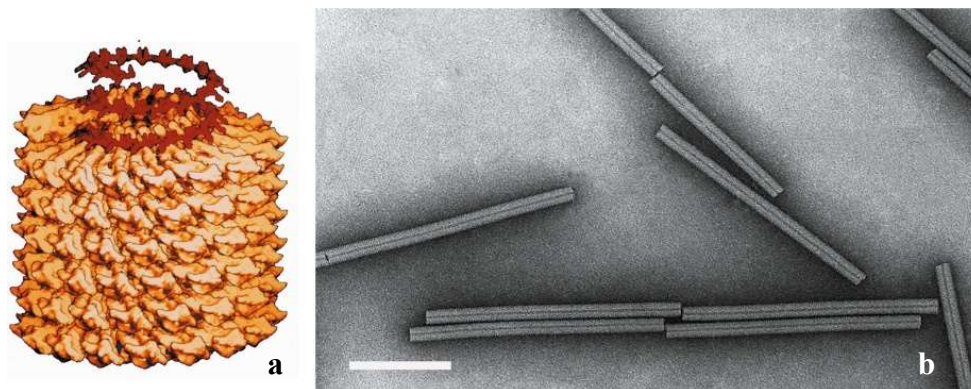
Figure 3. Model of TMV particle with the RNA genome (a). Electron micrograph of TMV particles (b) (Adapted from ICTV, https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/virgaviridae/672/genus-tobamovirus).

The genome encodes at least four proteins: a 126 and 183-kDa protein are translated directly from the 5' proximal open reading frames (ORF) of the genomic RNA. The 126 kDa replicase (Rep) protein contains the methyltransferase (Mtr) and helicase (Hel) domains. The 183-kDa Rep protein additionally contains the polymerase (POL) domain or RNA-dependent RNA polymerase (RdRP), synthesized by occasional readthrough of the leaky termination codon of the 126 kDa protein encoding ORF. The 30-kDa is a movement protein (MP) that participates in cell-to-cell and long-distance movement in viral spread from infected cells to neighboring uninfected cells, which localized to the plasmodesmata in host plant tissues. The 17.5-kDa is a coat protein (CP) not required for cell-to-cell movement but has a role in vascular tissue-dependent virus accumulation (ISHIBASHI and ISHIKAWA 2016). The MP and CP proteins are synthesized from their respective subgenomic mRNAs. They are dispensable for viral RNA replication (Figure 4)

(HUNTER et al. 1976; MESHI et al. 1987). In some species, the MP 30-kDa overlaps both 183-kDa protein and CP 17.5-kDa ORFs, while in other species, it does not overlap (STOBBE et al. 2012).
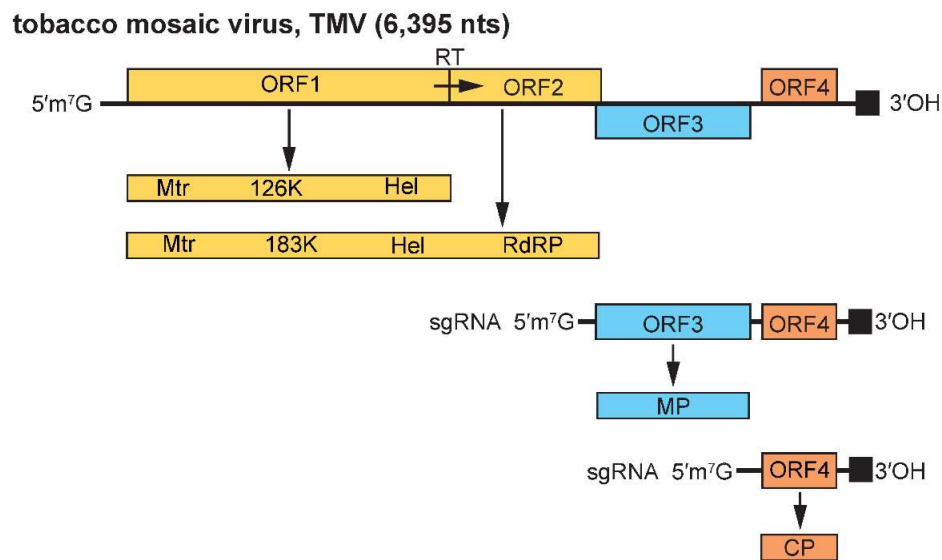
Figure 4. Genome organization and expression of TMV as a model of tobamovirus genome. Yellow box sign to replication proteins 126 and 183 kDa. The blue and orange boxes are MP and CP, expressed from separate 3′ co-terminal sgRNAs. In the dark square is the tRNA structure motif at the 3′-end of the RNA (Adapted from ICTV, https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/virgaviridae/672/genus-tobamovirus).

Tobamoviruses have no specific animal vectors, but they are easily transmit by plant sap and vegetative propagation of infected plants. In addition, infected seeds and pollens often carried by bees and bumblebees may play an important role in the distribution (DOMBROVSKY and SMITH 2017).

Tobamoviruses cause different types of symptoms in infected hosts, mainly mosaic, leaf distortion, dwarfing of the plant, chlorotic, misshapen and discolored fruits. These symptoms generally affect both the quantity and quality of the yield of vegetables and ornamentals (MATTHEWS and HULL 2002).

The most important tobamovirus species known to infect tomato plants are tobacco mosaic virus (TMV) (MAYER et al. 1942; PANNO et al. 2021), tomato mosaic virus (ToMV) (BROADBENT 1976; PANNO et al. 2021), tomato mild mottle virus (ToMMV) (LI et al. 2013) and new tobamovirus tomato brown rugose fruit virus (ToBRFV) (SALEM et al. 2015).

3.3.2 Tomato pathogenic tobamoviruses and tomato brown rugose virus (TBRFV)

3.3.2.1 Tobacco mosaic virus (TMV)

TMV was the first virus discovered over a century ago (IVANOWSKI 1892), and it was also the first virus to be purified (CREAGER et al., 1999). It has since revealed fascinating details about how viruses infect their hosts (SCHOLTHOF et al. 2011). TMV has been maintained as a model plant virus for more than 110 years. Many scientific studies have been initiated to understand how to control TMV-induced disease on tobacco (SCHOLTHOF 2004).

TMV research has also resulted in significant Nobel Prize-winning discoveries about general life principles. It is considered the first RNA plant virus sequenced, first defined movement protein (MP) and the first molecular evidence of a gene-for-gene resistance interaction (BAKER et al. 1997; SCHOLTHOF et al. 1999; CREAGER 2002; KLUG 2010; SCHOLTHOF et al. 2011).

TMV is known to infect plants in nine families and at least 125 individual species, especially tobacco, tomato, pepper and other *Solanaceae* members (SCHOLTHOF 2004). The virions of TMV are very stable and easily transferred by direct contact, contaminated tools and workers' hands who become contaminated with TMV after smoking cigarettes. A wounded plant cell provides a site of entry for TMV. In addition, the virus can be transmitted by an insect (Bumblebees) (OKADA et al. 2000). The seeds consider a primary source of infection in which contaminated seed coats by the virus could germinate and produce an infected plant (ZAITLIN 1998).

TMV symptoms vary depending on the host plant genetic background, TMV strains, environmental conditions and age of the infected plant. The symptoms on plants and leaves include mosaic, mottling, leaf curling, yellowing, necrosis and stunting (Figure 5), while on fruits causing distorted fruits, nonuniform fruit color and delayed fruit ripening (HARRISON and WILSON 1999).

Figure 5. Typical mosaic symptoms caused by TMV on tobacco leaves (SCHOLTHOF 2008).

3.3.2.2 Tomato Mosaic Virus (ToMV)

ToMV is the second economically important tomato virus within the genus *Tobamovirus* (PANNO et al. 2021). ToMV was often considered a strain of TMV (VAN REGENMORTEL 1975; FRASER and LOUGHLIN 1980; FRASER et al. 1980; BURGYAN and GABORJANYI 1984). However, because the two are easily distinguished by differences in serological affinities, host range and protein compositions, ToMV has been increasingly recognized as a distinct virus over the last 15 years (HARRISON et al. 1971; FENNER and MAURIN 1976; GIBBS 1986; PANNO et al. 2021). ToMV can infect several plant species, but the main hosts are in the family *Solanaceae*, mainly tomato and pepper plants, where the yield can reduce between 25–71% (MOHAMED 2010).

The virus particles are very stable and can contaminate surfaces, objects, soil and other substrates, particularly in leaves and roots residues, which remain infectious for many years. It can be found in all plant organs, including the seeds (probably it can be found in small quantities in the endosperm but not found in embryos) and pollen (BROADBENT 1965). Moreover, ToMV can be easily transmitted from infected plants to healthy plants during cultivation operations.

ToMV symptoms can appear at any growth stage, and the plant can be infected in any part (PANNO et al. 2021). ToMV symptoms on the leaves are mosaic or yellow mosaic. In summer in glasshouses, younger leaves show light and darker green mosaic leaf mottle and deformation (Figure 6). Symptoms on immature and ripe fruit appear as spot discolorations that maybe

associated with necrosis and the fruits become pitted. Diagnostic fruit symptoms are probably confused with a physiological disorder known as blotchy ripening (JONES et al. 2016).

Figure 6. ToMV infection on young tomato leaf showing mottling and blistering symptoms (BLANCARD 2012).

Some mutant strains of ToMV named as mild strains have been reported to infect tomato without symptoms (MUNDRY and GIERER 1958; RAST 1972). Based on the cross-protection mechanism found by (MCKINNEY 1929), these mild strains were used for "immunization" of greenhouse plants in the 70th (RAST 1972). However, the tomato mild strains proved extremely severe in pepper and as a result, the immunization of tomatoes was stopped. Nevertheless, using cross-protection of ToMV mild strains was eventually replaced by introducing new resistant cultivars.

3.3.2.3 Tomato brown rugose fruit virus (ToBRFV)

Appearance and distribution

In 2015 a new tobamovirus named tomato brown rugose fruit virus (ToBRFV) was detected in tobamovirus-resistant greenhouse tomatoes in Jordan (SALEM et al. 2015) and described subsequently also in Israel (LURIA et al. 2017). In South America, the virus was detected for the first time in Mexico, in the state of Michoacán (MANUEL et al. 2019) and later in the state of Baja California (CAMACHO-BELTRÁN et al. 2019). In the United States, it was recorded in commercial greenhouses in California and Florida (LING et al. 2019; DEY et al. 2021). In Europe, there were reports in Germany (MENZEL et al. 2019), United Kingdom (SKELTON et al. 2019),

Italy (PANNO et al. 2019a), Greece (BERIS et al. 2020), Netherlands (VAN DE VOSSENBERG et al. 2020), Spain (ALFARO-FERNÁNDEZ et al. 2020) and Hungary (KRIZBAI et al. 2022). On the other hand, in the Mediterranean region, the ToBRFV was reported in Palestine (ALKOWNI et al. 2019), Turkey (FIDAN et al. 2019), Iran (GHORBANI et al. 2021), Saudi Arabia (SABRA et al. 2021), Syria (HASAN et al. 2021) and Egypt (AMER and MAHMOUD 2020). The virus has also occurred in China (YAN et al. 2019) and suspected cases have been reported but still not officially confirmed in Chile, Ethiopia and Sudan (OLADOKUN et al. 2019).

Transmission and dispersal

The ToBRFV is very stable, like other members of the genus tobamovirus, and can be mechanically transmitted. The virus increases the risks of its spread through various cultural practices such as pruning, tools and harvesting; direct contact from an infected plant to a healthy plant. Viral particles can enter plant cells through small wounds and the virus replicates in the cytoplasm of cells using cellular components from its host. Its structural stability allows it to survive for long periods of time without losing its infective capacity on various surfaces such as plant residues, nutrient solutions or soil (OLADOKUN et al. 2019; PANNO et al. 2021)

ToBRFV is considered a seed-borne virus, and the infected seeds serve as a primary source of infection, increasing the risk of introduction into other areas where the virus is not yet present (DOMBROVSKY and SMITH 2017). Klap et al. (2020) showed that mesocarp, exocarp, fruit juice and seeds facing the mesocarp of the symptomatic fruits were infected by ToBRFV. Later, SALEM et al. (2021) and DAVINO et al. (2020) proved that the ToBRFV is a seed-borne virus and the virions are located externally on the tomato seed coat (testa) and not in the internal seed tissues embryo, but sometimes in the endosperm. LEVITZKY et al. (2019) revealed that bumblebees (*Bombus terrestris* L.) contributed to the spread of ToBRFV in tomatoes..

Molecular properties

The ToBRFV genome has been described as typical of the genus *Tobamovirus*. Viral particles have rigid rod morphology and their genome is +ssRNA. Its genetic material comprises four open reading frames (ORF): ORF1a and ORF1b encode protein complexes related to the Rep process whose proteins have 126 and 183 kDa, respectively; the ORF2 encoding MP of 30 kDa and ORF3 encodes a CP of 17.5 kDa (MAAYAN et al. 2018). The genomic analysis of this virus has revealed that one strain or typical isolates have a genome of approximately 6391 to 6393 base pair (bp) nucleotides and all reported isolates are genetically interrelated (OLADOKUN et al. 2019).

Phylogenetic analysis has revealed that the genomic sequence of ToBRFV differs from ToMV and TMV by 18%. However, the sudden appearance of ToBRFV in countries where it is now present is unknown. There has been some report about the potential pathways that led to the emergence of this new virus. MAAYAN et al. (2018) revealed from comprehensive phylogenetic analysis and genomic comparison of different tobamoviruses that a host-shifting event (jumping) of the ToBRFV variant occurred with a relatively low mutation rate within a very short time. It is also thought that ToBRFV emerged as a result of recombination. An earlier analysis of the ToBRFV genome used seven detection algorithms to identify a recombination event in a 314-nucleotide segment of the replication gene that identified ToMMV as the potential minor parent and TMV strain Ohio V as the major parent (SALEM et al. 2015).

Host range and symptomatology

Tomato (*Solanum lycopersicum*) and sweet pepper (*C. annuum*) are the two main natural hosts of ToBRFV (SALEM et al. 2015; LURIA et al. 2017; OLADOKUN et al. 2019; PANNO et al. 2020; SALEM et al. 2020; CHANDA et al. 2021a). Other natural hosts identified as potential reservoirs of the virus, include the weeds *Chenopodium murale, C. quinoa, Petunia hybrida,* and *S. nigrum* (SALEM et al. 2015; LURIA et al. 2017; CHANDA et al. 2021a; FIDAN et al. 2021). Experimentally inoculated species of *Nicotiana*, demonstrating hypersensitivity responses and systemic symptoms expression (Table 2) (SALEM et al. 2015; LURIA et al. 2017; OLADOKUN et al. 2019; CHANDA et al. 2021a; FIDAN et al. 2021; YAN et al. 2021a; ZINGER et al. 2021).

ToBRFV symptoms in tomato plants vary greatly depending on the cultivar and the environmental conditions. The symptoms variations may generally correspond to the temperature, photoperiod, and plant age at the time of infection (PANNO et al. 2021). The foliar symptoms usually appear as mosaic patterns, chlorosis and mottling occasionally associated with leaf narrowing (Figure 7a). Necrotic symptoms may appear on sepals, pedicles, calyces, petioles, and longitudinal stem (Figure 7b). Symptoms on fruits of diseased plants are marbling, deformations, yellow spots or brown wrinkled (rugose) patches, rendering them unmarketable (Figure 7c-d). The disease incidence often reaches to 100% in infected crops, which could have a significant economic impact (SALEM et al. 2015; LURIA et al. 2017).

Table 2. Susceptibility and reactions of plants inoculated with ToBRFV

Plant family and species	Symptoms	
	L	S
Amaranthaceae		
Chenopodium murale	CLL (++)	NS
Chenopodium quinoa	CLL (++)	NS
Cucurbitaceae		
Cucumis sativus	NS (--)	NS (--)
Cucurbita pepo	NS (--)	NS (--)
Solanaceae		
Capsicum annuum (L^+)	NS	M (++)
Capsicum (L^1)	NLL (+)	NS
Capsicum (L^2)	NLL (+)	NS
Capsicum (L^3)	NLL (+)	NS
Capsicum (L^4)	NLL (+)	NS
Solanum melongena	NS	NS (-)
Nicotiana benthamiana	NS	M (++)
Nicotiana clevelandii	NS	LY (+)
Nicotiana glutinosa	NLL (+)	NS
Nicotiana megalosiphon	NLL	M (++)
Nicotiana sylvestris	CLL (+)	NS
Nicotiana tabacum var. Samsun	NS	M (+)
Nicotiana tabacum var. Xanthi. Nc	NLL (+)	NS
Solanum lycopersicum (tm-1)	NS	VSM (+)
Solanum lycopersicum(Tm-1)	NS	VSM (+)
Solanum lycopersicum (Tm-2)	NS	VSM (+)
Solanum lycopersicum ($Tm-2^2$)	NS	SM (+)
Solanum nigrum	NS	MM (+)
Petunia hybrida	NS	NS (+)
Solanum tuberosum	NS	NS (-)

Abbreviations- L: Local symptoms developed on the inoculated leaf at 4–7 days post-inoculation (dpi) and S: Systemic symptoms developed on the inoculated leaf at 10–14 dpi, CLL = Chlorotic local lesions, NS= no symptoms, LY: Leaf yellowing, NLL= necrotic local lesion, M = mosaic, MM: mild mottling, SM: severe mosaic, VSM: very severe mosaic, (++) = virus detected on RT-PCR (reverse transcriptase-polymerase chain reaction), (--) = virus not detected on RT-PCR, (+) = virus detected on ELISA (enzyme-linked immunosorbent assay), (-) = virus not detected on ELISA.

Figure 7. Typical symptoms caused by ToBRFV in tomato. a: Severe mosaic and deformations, b: Necrosis of the sepals on young tomato fruit, c-d: Tomato fruits showing marbling and decolorations (c), brown wrinkled (rugose) patches (d) (EPPO 2022c).

Methods for detection of ToBRFV

Several diagnostic techniques have been developed and used to detect tobamoviruses. First biological test such as bioassay using *N. tabacum* reacting by local lesions (indexing) was applied to detect the presence of different tobamoviruses and this method proved useful for ToBRFV, too (ISHI-VEG 2019). Modern serological techniques such as direct or indirect ELISA could also be applied, but the disadvantage of this method probably could cross-react with other tobamoviruses. Molecular techniques, including polymerase chain reaction (PCR) and sequencing, are specific, efficient, and reliable to detect and identify viruses (Singh and Singh 1995). Most of these techniques have also been used for the detection of ToBRFV, such as RT-PCR (RODRÍGUEZ-MENDOZA et al. 2019), real-time RT-PCR (PANNO et al. 2019b), loop-mediated isothermal amplification (LAMP) (SARKES et al. 2020; RIZZO et al. 2021), quadruplex RT-PCR (YAN et al. 2021a), CRISPR/Cas technology (ALON et al. 2021). Moreover, new technologies such as

next-generation sequencing (NGS) and the single-molecule sequencing platform of Oxford Nanopore could be used for the detection of low titer of ToBRFV (LURIA et al. 2017; CHALUPOWICZ et al. 2019; VAN DE VOSSENBERG et al. 2020)

Management strategies

To control ToBRFV, it is necessary to use seed treatment using 2% hydrochloric acid (HCl), 10% trisodium phosphate or sodium hypochlorite, which can inactivate ToBRFV (DAVINO et al. 2020; SAMARAH et al. 2021). Furthermore, using disinfected materials, certified pathogen-free propagation material, sterilize cutting tools during cultural and manipulation operations, sanitation, crop rotation, elimination of infected plants, removal of crop residues, weed control and grafting the plant on virus-resistant rootstock could lead to preventing the spread of the disease (OLADOKUN et al. 2019; SPANÒ et al. 2020; CHANDA et al. 2021b; PANNO et al. 2021).

The use of resistant or tolerant cultivars would be the best way to control ToBRFV. However, new intermediate resistant or resistant commercial cultivars breeded by different companies will be available soon in the market but they are still under investigation. More recently, KABAS et al. (2022) reported results on testing 44 wild tomato accessions and hybrids to ToBRFV. Although they published tolerance in some accessions of *S. pimpinellifolium, S. penellii* and *S. chilense*, the resistant plants could not be found against ToBRFV. However, HAMELINK et al . (2019), ASHKENAZI et al. (2020) and YKEMA et al. (2020) claims they found resistance to ToBRFV in some genotypes of *S. pimpinellifolium*, *S. lycopersicum* and *S. habrochaites*, respectively and tolerance in genotypes of *S. lycopersicum* and *S. pimpinellifolium* (ASHKENAZI et al. 2018; ZINGER et al. 2021). YKEMA et al. (2020) identified a genomic sequence or a locus introgressed from *S. habrochaites* that could induce NBS-LRR protein coding by a resistance gene to ToBRFV located on chromosome 8. Moreover, Zinger et al. (2021) developed a DNA marker linked to the resistance gene. They speculated that the *Tm-1* gene located at chromosome 2, which interacts with the locus discovered on chromosome 11, is the main cause for resistance to ToBRFV.

3.4 Resistance of tomato to tobamoviruses – types and genes of resistance

3.4.1 Resistance to TMV and ToMV in tomato

Many breeder and breeding programs have been started to find sources of resistance against TMV and ToMV. So far, three dominant resistance genes have been found in wild tomato (*Solanum*) species and introgressed into commercial tomato (*S. lycopersicum*) genotypes: *Tm-1* (from *S. habrochaites*), *Tm-2* and *Tm-2²* (both from *S. peruvianum*) (ALEXANDER 1963; PELHAM 1972; HALL 1980).

3.4.1.1 The *Tm-1* gene

The *Tm-1* gene was discovered in plants of *S. habrochaites* (PI 126445) grown from seeds collected in South America. The TMV infected plants showed no symptoms and low levels of virus titers were detected in their tissues (PORTE et al. 1939). Holmes (1954) used the back cross method to transfer this resistance to a susceptible *S. lycopersicum* variety. Later, *Tm-1* homozygous line was generated and the resistance gene was mapped on chromosome 2 (PELHAM, 1972).

The *Tm-1* gene is incompletely dominant and suppresses virus replication and symptom development (HOLMES 1954; FRASER et al. 1980). Moreover, MOTOYOSHI and OSHIMA (1979) demonstrated by inoculation of *Tm-1* homozygous tomato plants with ToMV RNA that this resistance is efficient against infection with RNA inocula. This suggested that the *Tm-1* resistance somehow interferes with ToMV RNA replication rather than virus uncoating. These results were confirmed by FRASER and LOUGHLIN (1980) and FRASER et al. (1980).

Fraser and colleagues revealed that inhibition of virus replication by the *Tm-1* gene is gene dose-dependent. As a result, in homozygous *Tm-1* tomato plants, virus RNA accumulation was reduced compared to heterozygous and susceptible tomato lines. Furthermore, CIRULLI and CICCARESE (1975) and FRASER and LOUGHLIN (1982) reported that the effectivity of *Tm-1* associated resistance was temperature-dependent, as it was broken at high temperatures (28-35°C).

3.4.1.2 The *Tm-2* and *Tm-2²* genes

The genes *Tm-2 and Tm-2²* are dominant for resistance to TMV and ToMV and confer a higher level of resistance than *Tm-1* by preventing cell-to-cell movement the viruses (SOOST 1963; LATERROT and PECAUT 1969). They were discovered in *S. peruvianum* (PI 126926 and PI 128650) and were allocated to chromosome 9 (ALEXANDER 1963; SCHROEDER et al. 1967). In the beginning, transfer of *Tm-2* from the breeding lines was unsuccessful because undesirable recessive genes caused stunting and yellowing (netted-virescent (*nv*)) in the homozygous condition was found to be tightly linked to it (CLAYBERG 1959). Later new source of *Tm-2* was found in *S. peruvianum,* which did not contain the *nv* gene (LATERROT and PECAUT 1969). The *Tm-2* and *Tm-2²* are considered allelic and located on the same locus or extremely closely linked (PÉCAUT 1965; SCHROEDER et al. 1967).

The resistance response of *Tm-2* and *Tm-2²* to common strains of TMV and ToMV are based on necrotic reactions and localization of the virus. There are two types of necrotic reactions called local necrotic lesions or systemic necrosis. The local necrotic lesions appeared on inoculated leaves within five days of inoculation and are regarded as a hypersensitive reaction. The systemic reaction shown slightly by *Tm-2* and particularly by the *Tm-2²* genotype at higher temperatures (PFITZNER 2006). The development of the necrotic phenotype varies depending on the gene dose. For example, ToMV produces necrosis at 30°C on *Tm-2*/+ plants but no necrosis at any temperature on *Tm-2/Tm-2* (PELHAM 1966; PFITZNER 2006).

3.4.2 Resistance breaking TMV and ToMV strains

Resistance-breaking strains of TMV and ToMV have been found for decades (MCRITCHIE and ALEXANDER 1963). These strains are known to overcome the *Tm-1*, *Tm-2* and *Tm-2²* genes, but they did not spread widely in tomato crops until now. The names of the four ToMV strains (Tm-0, Tm-1, Tm-2, and Tm-2²) currently recognized in tomato are based on the introgressed resistance genes *Tm-1*, *Tm-2* and *Tm-2²* from related wild species (Table 3) (LOEBENSTEIN and CARR 2006).

Table 3. ToMV strains and expected reactions on tomato genotypes (PFITZNER 2006).

ToMV strain	Tomato genotypes			
	Tm⁺	*Tm-1*	*Tm-2*	*Tm-2²*
0	S	R/IR	R	R
1	S	S	R	R
2	S	R/IR	S	R
1.2	S	S	S	R
2²	S	R/IR	R	S

S = Susceptible; IR = Intermediately resistant, R = Resistant

Several *Tm* gene breaking strains of TMV and ToMV were cloned and sequenced to determine the molecular basis of the *Tm* genes resistance-breaking phenotype (MESHI et al. 1988; MESHI et al. 1989; CALDER and PALUKAITIS 1992; BETTI et al. 1997; STRASSER 2002). Comparing the nucleotide sequences of all of these virus strains and the deduced amino acid sequences of the respective viral proteins revealed that all *Tm-1* breaking ToMV strains had amino acid changes in the overlapping open reading frames of the 130 kDa /180 kDa replication proteins. Mutation analysis of these ToMV strains revealed that all amino acid changes are found in a small region at the C-terminus of the 130 kDa protein, where at least two amino acid (aa) changes (aa 979 Gln > Glu and aa 984 His > Tyr) are responsible to overcome the *Tm-1* resistance (Figure 8) (MESHI et al. 1988).

```
aa-Pos.   940                       970 971                      990
130.0     INRVTGFPYPA--//--RCP ADVTHFLNQRYEGHVMCTSS
130.1     INRVIGSPYPA--//--RCP ADVTHFLNERYEGYVMCTSS
130.Lta1  INRVTGFPYPA--//--RCP ADVTHFLNERYEGYVMCTSS
```

Figure 8. Sequence of amino acid at the C-terminus of the 130 kDa protein of wild type ToMV (130.0)(OHNO et al. 1984), and of two *Tm-1* breaking ToMV strains (130.1 and 130.Lta1) (MESHI et al. 1988; STRASSER 2002). Bold letters indicate amino acid changes.

Sequence analyses of molecular interaction between the *Tm-2 and Tm-2²* resistance genes and ToMV strains were compared by different *Tm-2* breaking strains from Italy (STRASSER 2002), Japan (MESHI et al. 1989) and the Netherlands (CALDER and PALUKAITIS 1992). All these ToMV strains contained amino acid substitutions in the ORF coding for the 30 kDa MP compared to the parent isolate (ToMV-0). A further example, Meshi et al. (1988) reported that two amino

acid substitutions at position 68 (Cys > Phe) or 133 (Glu > Lys) (Glu > Lys) of the MP caused virus mutants, which could fully overcome the *Tm-2* resistance (Figure 9). Weber and Pfitzner (1998) revealed that both amino acid substitutions (aa 238 Ser > Arg, aa 244 Lys > Glu) in the C-terminus of the MP are required for overcoming the resistance *Tm-2²* gene.

```
aa-Pos.   36                    55 56                    75
30.0      VSKVDKIMVHENESLSEVNL LKGVKLIEGGYVCLVGLVVS
30.2      VSKVDKIMVHENESLSEVNL LKGVKLIEGGYVCLVGLVVS
30.Ltb1   VSKVDKIMVHENESLSEVNL LKGVKLIEGGYVFLVGLVVS
          76                   126 127                  146
30.0      GEWNL----//----VPNYG ITTKDAEKNIWQVLVNIKNV
30.2      GEWNL----//----VPNYG ITTKDAKKSIWQVLVNIKNV
30.Ltb1   GEWNL----//----VPNYG ITTKDAKKNIWQVLVNIKNV
```

Figure 9. The amino acid sequence of the 30 kDa protein of wild type ToMV (30.0) (OHNO et al. 1984), and of two *Tm-2* breaking ToMV strains (30.2 and 30. Ltb1) (MESHI et al. 1988; STRASSER 2002). Bold letters indicate amino acid changes.

3.4.3 ToBRFV overcome the resistance genes *Tm-1*, *Tm-2* and *Tm-2²*

For decades, cultivating tomatoes was achieved via the genotypes of the elite tomato varieties harboring the resistance genes *Tm-1*, *Tm-2* and *Tm-2²*. However, the new tobamovirus ToBRFV causes systemic infection of all tomato genotypes harboring the *Tm-1*, *Tm-2* and *Tm-2²* genes, respectively (LURIA et al. 2017). Similarly, peppers (*Capsicum*) without tobamovirus resistance gene(s) are highly susceptible to the virus, while pepper plants harboring the *L* resistance genes L^1, L^2, L^3 and L^4 are resistant to ToBRFV under normal temperature (24-26°C) but became susceptible at 32°C or above (LURIA et al. 2017; PANNO et al. 2020; SALEM et al. 2020; ABOU KUBAA et al. 2021; FIDAN et al. 2021).

Many studies focused to understanding the evolutionary path leading to the emergence of the resistance breaker ToBRFV. MAAYAN et al. (2018) carried out sequence analysis to map the mutations responsible for overcoming the *Tm-2²* resistance. Compared with tobamoviruses pathogenic to tomato (TMV, ToMV and Rehmannia mosaic virus (ReMV), they identified 21 potential mutations that are probably responsible for the resistance-breaking property. Twelve mutations are found in the MP and nine in the Rep proteins of ToBRFV. Some of these mutations resembled resistance-breaking TMV and ToMV, which led to overcoming the *Tm-2²* gene. Furthermore, HAK and SPIEGELMAN (2021) revealed that replacing the MP of ToMV with MP

of ToBRFV resulted in a recombinant virus that could evade the *Tm-2²* resistance. In addition, transient expression of ToBRFV MP failed to induce the *Tm-2²* resistance response. Indeed, YAN et al. (2021b) also proved that ToBRFV MP had six residues located in the central 60–186 amino acids of the MP (H^{67}, N^{125}, K^{129}, A^{134}, I^{147}, and I^{168}) that were necessary for ToBRFV to overcome *Tm-2²* resistance in transgenic *N. benthamiana* and tomato cv. Jinpeng plants. It means that the MP of ToBRFV is responsible for breaking *Tm-2²*.

3.5 Host-virus interactions (plant defense)

3.5.1 Disease resistance genes in plants

Plant responds to pathogens by passive and active defense mechanisms. Passive mechanisms are barriers present before contact with the pathogen, such as physical (cuticle, stomatal aperture and cell wall) or chemical (inhibitory compounds or the absence of stimulatory compounds required for pathogen development) (VAN OOIJEN et al. 2007). Active defense is activated only after pathogen recognition. Active defense depends on specialized receptors divided into two groups: the Pathogen or Pattern Recognition Receptors (PRRs) and the Resistance (R) proteins. PRRs recognize Microbe or Pathogen-Associated Molecular Patterns (MAMPs/PAMPs) using a limited set of receptors (JONES and DANGL 2006). While R proteins respond to molecules (called avirulence proteins or elicitors) that are encoded by large gene families, numbering several hundreds of genes per genome (ZHANG et al. 2013).

R genes are coding proteins that recognize specific pathogen effectors known as avirulence proteins (*Avr*) in a specific gene-for-gene model (FLOR 1971). R protein domains can be classified into four classes (VAN OOIJEN et al. 2007). The first two classes are Receptor-Like Protein (RLP) and the Receptor-Like Kinase (RLK), which span the plasma membrane (PM) and contain an extracellular Leucine-Rich Repeat (LRR) domain (Figure 10). While the other two classes of R proteins are coiled-coil (CC)-Nucleotide-binding site (NBS)-leucine-rich (LRR) (CNL) and Toll/interleukin-1 receptor (TIR)-NB-LRR (TNL) classes, located intracellularly (cytoplasmic, nuclear, or membrane-bound) (Figure 10). They contain a central NB-ARC domain (consisting of NB, ARC1 and ARC2 subdomains) coupled to an LRR domain. C-terminal to the NB-ARC domain occupies a leucine-rich repeat (LRR) domain (VAN OOIJEN et al. 2007; COLLIER and MOFFETT 2009). Therefore, this group is collectively referred to as NB-LRR proteins. The majority of *Solanaceous* R genes encode NB-LRR proteins, which make up one of the largest and most variable gene families found in plants (MOFFETT 2009).
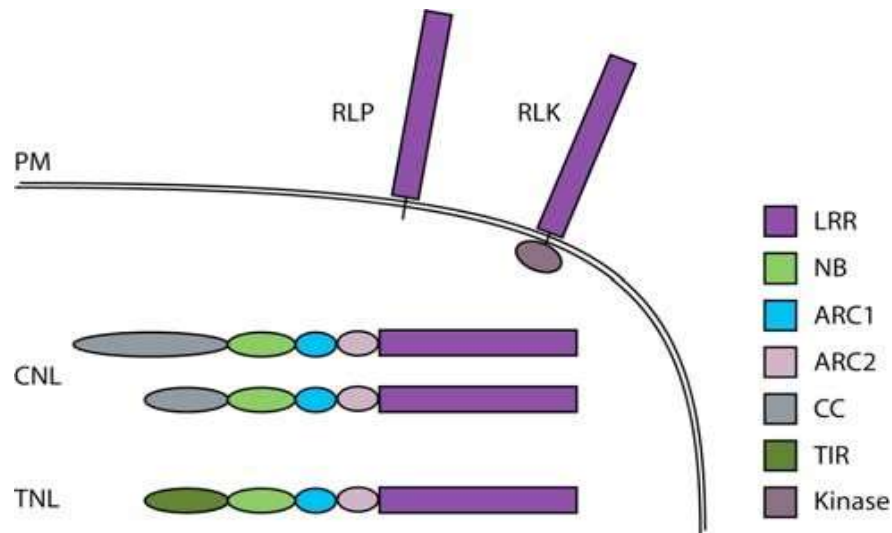
Figure 10. Schematic diagram of the proteins encoded by disease resistance genes (VAN OOIJEN et al., 2007).

The first plant virus gene to be identified as an *Avr* gene was the helicase domain of the TMV 126/183-kDa replicase protein. This protein triggers a hypersensitive response (HR) in tobacco (*N. glutinosa*) plants that carry the *N* gene (HOLMES 1938; LES ERICKSON et al. 1999). The *N* gene codes for a TIR-NB-LRR class protein mediates the resistance accompanied by an HR phenotype consisting of necrotic spots (necrotic local lesions) on inoculated leaves (WHITHAM et al., 1994; DINESH-KUMAR and BAKER 2000). Furthermore, two other R genes *Tm-2* and *Tm-2²* have been found in the wild tomato species *S. peruvianum* and introgressed into the commercial tomato varieties used extensively for resistance to TMV and ToMV. They encode proteins belonging to the CC-NBS-LRR class of proteins and induce HR (DE RONDE et al. 2014). Another type of a distinct R gene is *Tm-1*, found in *S. habrochaites*, which encodes a protein containing a TIM-barrel. This barrel binds the replication proteins of ToMV and therefore inhibits RNA replication. In this *Tm-1* gene, no typical NB-LRR type-associated response, such as HR, is induced (ISHIBASHI et al. 2007).

Producing cultivars harboring R genes are the most effective method to control virus replication, spread, or symptom induction. The deployment of R genes requires no special equipment and is simple. As a result, when R genes are available, they are usually present as the most labor-saving, cost-effective, and environmentally friendly approaches to virus disease control. The identification of resistance genes to plant viruses and their properties have been reported by many authors (FRASER 1990; PENNAZIO et al. 1999; TAKKEN and JOOSTEN, 2000; BARKER et al. 2001).

3.5.2 Types of responses of plants to virus inoculation

When a plant is inoculated with a virus, there are two possible outcomes: either infection occurs or not. Cooper and Jones (1983) suggested and described terms for the various kinds of responses made by plants to inoculation with a virus as below:

1- Immune (non-host): Virus does not replicate in protoplasts, nor in cells of the intact plant, even in initially inoculated cells. Inoculum virus may be uncoated, but no progeny viral genomes are produced.

2- Infectible (host): Virus can infect and replicate in protoplasts and divided into three situations:

A- Resistant (extreme hypersensitivity): Virus multiplication is limited to initially infected cells because of an ineffectual virus-coded movement protein, giving rise to subliminal infection. Plants are field resistant.

B- Resistant (hypersensitivity): Infection limited by a host response to a zone of cells around the initially infected cell, usually with the formation of visible necrotic local lesions. Plants are field resistant.

C- Susceptible (systemic movement and replication):

    1- Sensitive: Plants react with more or less severe disease symptoms.

    2- Tolerant: There is little or no apparent effect on the plant, giving rise to latent infection.

There are many methods that could be used to reveal the resistance gene's mode of action. For example, tobamoviruses consider mechanically transmissible viruses. Therefore, it is possible to detect the virus by using rub-inoculation on leaves and then waiting for symptoms to appear on inoculated and top leaves. In addition, other methods could be used, such as inoculating virus via protoplasts (NASU et al. 1996), grafting (SPANÒ et al. 2020), tissue printing (SALEM et al. 2021), and reporter gene through expressing green fluorescent protein (GFP) (HAK and SPIEGELMAN 2021).

3.5.3 Antiviral RNA silencing

Gene silencing is an important antiviral defense mechanism in plants. Gene silencing target viral RNA for translational repression or degradation (LOPEZ-GOMOLLON and BAULCOMBE 2022). As a result, virus replication and movement are restricted, and the plant recover from

symptoms caused by the virus (GHOSHAL and SANFAÇON 2015; KORNER et al., 2018). All viruses activate their genes and/or replicate their genome during an RNA intermediate (AHLQUIST 2006). The great majority of plant viruses have a +ssRNA viruses genome that is replicated through double-stranded RNA (dsRNA) intermediates (TUSCHL et al., 1999). Once recognized by the plant cell surveillance machinery, viral dsRNAs are cleaved by dicer-like (DCL) RNase III enzymes into 21 to 24 nt small interfering RNAs (siRNAs). The siRNAs led to RNA-induced silencing complexes (RISCs) into the target RNA in a sequence-specific manner. The target RNA is then cleaved by argonaute (AGO) proteins, which are RNase H-like enzymes and are linked with the RISCs (BUCHER and PRINS 2006).

Gene silencing is not enough to restrict virus infection. That is because of the inhibitory activity of virus-encoded gene silencing suppressors. Suppressors increase susceptibility, promote virus replication and movement as well as promote symptom development via interfering with endogenous and antiviral gene silencing (BURGYÁN and HAVELDA 2011; GARCIA-RUIZ, 2019). The mechanisms of silencing suppression comprise triggering the degradation of an essential component of gene silencing such as DCL, RDR6, AGO and suppressor of gene silencing 3 (SGS3) proteins, and also binding of both virus-derived and cellular siRNAs including micro-RNAs (miRNAs) (BURGYÁN and HAVELDA 2011). However, VOGLER et al. (2008) revealed that virus with suppressor deficits is shown to be more tendency for silencing with MP than without MP, indicating that MP enhances antiviral silencing during infection.

The earliest experimental evidence for a correlation between RNA silencing and an antiviral defense mechanism derived from the characterization of recovery phenotypes observed in some natural virus infections or in transgenic plants developed for viral resistance (GHOSHAL and SANFAÇON 2015). Recovery was initially described by WINGARD (1928) and it is characterized by an initial symptomatic infection followed by symptom elimination or reduction in newly emerging leaves. The recovery phenomenon was reported with nepoviruses, for example, tomato black ring virus (TBRV) in *Nicotiana clevelandii* associated with a decreased viral RNA concentration. In contrast, tomato ringspot virus (ToRSV) in *N. clevelandii*, *N. benthamiana* and *Cucumis sativus* was not accompanied by a commensurate reduction in viral RNA levels, which is often this phenomenon consequence of RNA silencing (Figure 11) (RATCLIFF et al. 1997; JOVEL et al. 2007). It was also shown that sequence-specific resistance to further virus infection exists or secondary infection is triggered prior to recovery and may be responsible for the reduced virus accumulation and surveillance phenotype, which is also linked to RNA silencing (GHOSHAL and SANFAÇON 2015; SANTOVITO et al. 2014). Additionally, incubating plants at a higher

temperature leads to increased RNA silencing activity, reduced viral accumulation, and attenuation in symptom development or the induction of recovery phenotypes (GHOSHAL and SANFAÇON 2015).

Figure 11. Symptom recovery of ToRSV-infected *N. benthamiana* plant (GHOSHAL and SANFAÇON 2015)

3.5.4 Virus and host factors are determinants of infection

Plant–virus interactions could be either incompatible or compatible. Compatible interactions between a virus and a susceptible host are defined by the establishment of virus infection and the presence of proviral cellular components and resources required for virus infection and movement(OTULAK-KOZIEŁ et al. 2018). In contrast, incompatible interactions occur when a virus interacts with a non-host plant and are defined by the absence of virus infection. They can be explained by a lack of cellular components required for the virus to replicate or spread, antiviral defense, or a combination of these factors (JAUBERT et al. 2011).

Various genetic studies have revealed that the result of plant–virus interactions is genetically governed by viral factors, host factors, and their interaction (PANAVAS et al. 2005). RNA translation, genome replication, virion movement and formation and gene silencing suppressors are considered viral factors that determine the extent of infection and disease severity (NELSON and CITOVSKY 2005). Host factors are considered an antiviral defense that could target viral nucleic acids or proteins by multiple mechanisms such as proteasome degradation, autophagy,

RNA decay and gene silencing (GARCIA-RUIZ 2019). Furthermore, host genes also play as host factors against virus activity. For example, the *Tm-2²* gene in tomato encodes a leucine-rich protein that interacts with the movement protein and confers resistance to tobamoviruses which appear as a result of hypersensitive response and localized cell death (CHEN et al. 2017).

4. MATERIALS AND METHODS

4.1 Origin, growing and handling of experimental plants

The seeds of *Solanum* species were kindly supplied by the United States Department of Agriculture (USDA, Beltsville, Maryland), Tomato Genetic Resources Centre (University of California, Davis) and MATE (Hungarian University of Agriculture and Life Sciences). Seeds of the tobacco species *N. glutinosa*, *N. tabacum* cv. Samsun and Xanthi-nc were from the collection of the Department of Genetics and Microbiology of MATE.

The seeds were washed with distilled water for 3 hours, sterilized in calcium hypochlorite (10%) for 20 minutes, and washed five times with distilled water for 10 minutes each time. The *Solanum* seeds were germinated in sterilized wetted tissue and sowed in peat soil (Klasmann Traysubstrate) in pots. Tobacco seeds were sowed in soil and individual seedlings were transplanted into pots. The growing plants were regularly fertilized with Volldunger Linz fertilizer (NPK) and sprayed with different pesticides (Vertimec, Actara, Mospilan, Amistar Top) weekly regularly. The plants were maintained in an insect-proof glasshouse at $24 \pm 2°C$, 50– 70% relative humidity and 14/10 h photoperiod.

A total of 809 accessions belonging to 16 *Solanum* species (sections *Lycopersicon* and *Juglandifolia*) were studied (Table 4) in two independent screening experiments. In the first one, 636 *Solanum* accessions (denoted Group A plants) were investigated. Plants of *S. habrochaites* PI 126445 (the original source of the *Tm-1* gene), *S. peruvianum* PI 126926 (source of the *Tm-2* gene) and PI 128650 (source of the *Tm-2²* gene), *S. lycopersicum* LA1221 (carrying the introgressed *Tm-2²* gene), and the susceptible cultivar *S. lycopersicum cv.* Ceglédi (genotype +/+) were used as controls. In the second one (denoted Group B plants) a total of 81 accessions of *S. peruvianum* and 92 accessions of *S. habrochaites* were evaluated. In this experiment *S. lycopersicum* GCR26-Craigella (*tm-1*[CRG26]), GCR237-LA3269 (*Tm-1*); LA2088 (*Tm-2*), LA3471-Moneymaker (*Tm-2²*) and Ceglédi (*Tm+*) plants carrying known resistance genes were used as controls.

Table 4. *Solanum* species and the number of accessions used for screening.

Number	Plant species	Number of accessions
1	*S. arcanum*	9
2	*S. cheesmaniae*	21
3	*S. chilense*	99
4	*S. chmielewskii*	10
5	*S. corneliomulleri*	26
6	*S. galapagense*	11
7	*S. habrochaites*	114
8	*S. huaylasense*	9
9	*S. juglandifolium*	3
10	*S. lycopersicum*	81
11	*S. neoricki*	16
12	*S. ochranthum*	5
13	*S. pennellii*	18
14	*S. peruvianum*	124
15	*S. pimpinellifolium*	256
16	*S. sitiens*	7

4.2 Virus isolates and preparation of inocula

Three tobamovirus isolates were used in this work: a Jordanian isolate of ToBRFV marked -Tom2-Jo (GenBank acc.no. MZ323110), the ToMV-DH and TMV-U1 isolates maintained in the plant virus collection of Hungarian University of Agriculture and Life Sciences (MATE) kindly provided by Dr. Pál Salamon. All of the tobamoviruses were transmitted through single local lesions from *N. glutinosa* and propagated in *N. tabacum* cv. Samsun. Inocula were prepared by grinding systemically infected "Samsun" tobacco leaves in sterile porcelain mortar adding sterile phosphate buffer 0.01 M, pH 7.0 (1:5 w/v). The sap was then filtered through cheesecloth, and the extract was preserved in aliquots 5 mL at $-20\ \mathrm{C}^0$ for inoculation.

4.3 Plant inoculation

For the inoculation of plants, the mechanical transmission was used. Virus inoculum was gently rubbed onto the carborundum dusted lower leaves of young tomato and tobacco test plants using a sterile glass spatula. After inoculation, the plants were rinsed with tap water. The infectivity of inocula was always assayed using *N. tabacum* cv. Xanthi- nc and/or *N. glutinosa* local lesion test plants.

4.4 Screening of *Solanum* germplasm for reactions to the ToBRFV

For screening the susceptibility and resistance, 3-10 (group A) and 15 (group B) individual seedlings as well as the controls, respectively, were inoculated at 3-4 true leaf stage on the 2nd and 3rd leaves with the frozen and thawed inocula of ToBRFV. Local and systemic symptoms were evaluated 1–5 weeks post-inoculation (wpi). For disease assessments, symptom severity classes were established for the two experiments as listed in Table 5 (Group A plants) and Table 6 (Group B plants), respectively.

Table 5. Symptom severity classes on newly developed top leaves of inoculated plants (group A).

Classes	Symptoms
0	No symptoms
1	Mild mosaic or mottling, followed by recovery
2	Mild mosaic or mottling with leaf deformation
3	Moderate mosaic or mottling and leaf deformation followed by rolling
4	Severe mosaic or mottling, and leaf deformity
5	Severe mosaic or mottling, leaf deformity, shoestring

Table 6. Symptom severity classes on newly developed top leaves of inoculated plants (group B).

Classes	Symptoms
0	No symptoms
1	Mild mosaic or mottling
2	Mosaic
3	Mosaic and leaf deformation
4	Mosaic and leaf deformation, shoestring
5	Severe mosaic, leaf deformation, rolling, shoestring, stunting

The disease symptoms caused by ToBRFV was assessed in each inoculated plant 2–3 wpi according to symptom classes listed in Table 5 and Table 6 and the disease severity index (DSI) were calculated by the formula developed by (CAMARA et al. 2013):

$$DSI(\%) = \sum_{e=0}^{4} \frac{e\text{Re} \times 100}{5N}$$

Where DSI = disease severity index; e = class; Re = number of plants in class (e); N = total number of plants.

To study the nature of resistance following the evaluations of symptoms, our studies were focused on the symptomless plants (class 0) plants. The presence or absence of viruses in leaf samples of symptomless plants was assayed using bioassays, RT-PCR and RT-qPCR. The virus-free plants expected to be resistant were later investigated in more detail.

4.5 Detection methods of viruses

4.5.1 Bioassay

For biological tests, samples were collected at 2–3 wpi from newly developed top leaves of inoculated donor tomato plants. In the cases of resistant plants, inoculated leaves were also assayed at 1 wpi. To avoid surface virus contamination, the assayed leaf samples were immersed for 10 seconds in a 2% NaOH solution and then exhaustively washed with tap water. Inocula were prepared from the NaOH-treated the leaves and *N. glutinosa* assay plants were inoculated as described detail in **Chapter 4.3**. The assay plants were inspected for appearing local lesions within 3-5 days, parallelly with the control *N. glutinosa* plant inoculated with the same virus for comparison (ISHI-VEG 2019).

4.5.2 Reverse transcription-polymerase chain reaction (RT-PCR)

RNA Extraction

RNA extraction was done using Promega SV (USA) total RNA extraction kit, following manufacturer instructions. Samples were taken from inoculated leaves at 1 wpi and newly developed top leaves at 2–3 wpi from tomato plant. The assayed leaves were treated with 2% NaOH and then with tap water to avoid virus contamination. Leaf samples were cut and placed in sterilized 2 ml Eppendorf tubes, then immersed in liquid nitrogen. The samples were ground to powder in a homogenizer while freezing in liquid nitrogen to prevent thawing. This was done very fast to minimize RNA degradation.

175 µl of RNA Lysis Buffer with β-Mercaptoethanol (BME) was added to each sample and mixed by inversion. 350 µl of RNA Dilution Buffer (blue) was added to each tube and the contents were mixed by inversion. They were then placed in a heating block at 70°C for only 3 minutes and then centrifuged for 10 minutes at 13,000 rpm (rotations per minute). The cleared lysate solutions (supernatant) were transferred to 1.5 ml Eppendorf tubes by pipetting, with care not to disturb the pelleted debris. 200 µl of 95% ethanol was added to the cleared lysate and mixed by pipetting. The mixtures were transferred to spin columns and centrifuged at 13,000 rpm for 1 minute and the flow-through was discarded. 600 µl RNA Wash Solution (diluted with ethanol) was added and centrifuged at 13,000 rpm for 1 minute and the flow-through was discarded.

For each isolation to be performed, the DNase incubation mix was freshly prepared by combining 40 µl yellow Core Buffer, 5 µl 0.09 M MnCl$_2$ and 5 µl of DNase I enzyme per sample in sterile tubes (in this order) and mixed by gentle pipetting (no vortexing). 50 µl of the DNase incubation mix was applied directly to the membrane. The Spin Baskets were incubated for 15 minutes at room temperature. 200 µl of DNase Stop Solution (with added ethanol) was added to the Spin Basket and centrifuged at 13,000 rpm for 1 minute. Washing was done using 600 µl RNA Wash Solution and centrifugation at 13,000 rpm for 1 minute. This was repeated using 250 µl RNA Wash Solution. The samples were centrifuged at 13,000 rpm for 2 minutes to get rid of all the liquid. The spin columns were transferred to elution tubes. 100 µl nuclease-free water was added to the membranes and centrifuged at 13,000 rpm for 1 minute.

The concentration of the purified RNA was measured in a nanodrop spectrophotometer. RNA products were detected by electrophoresis in 1.5% agarose gel containing 10 mg/ml ethidium bromide in 0.5 X TBE (Tris-Borate-EDTA) buffer. The purified RNA was stored at –80°C.

Complementary DNA (cDNA) synthesis

The cDNA synthesis was done using RevertAid® First Strand cDNA Synthesis Kit (Thermo Scientific, USA); Manufacturer instructions on avoiding ribonuclease contamination have been strictly adhered. RNA was used from each sample according to their concentration. It was mixed with nuclease-free water to a volume of 10 µl. 2 µl specific primer from the select virus were added and incubated at 65°C for 5 minutes. The samples were then put in ice for 2 minutes. 4 µl of 5x Reaction buffer and 1 µl of RevertAid® premium enzyme and 2 µl of 10 mM dNTP mix and 1 µl of RiboLock RNase Inhibitor (20 U/µL) were added to the samples to a final volume of 20 µl. They were incubated at 42°C for 60 minutes and then at 70°C for 5 minutes in a thermocycler. The cDNA samples were then stored at -20°C.

Designing Primers for tobamoviruses

Primer3 web version 4.0.0 computer software was used to design the PCR primers required for amplification of the coat protein gene segment of the target virus, using the ToBRFV (KT383474), ToMV (MH507165) and TMV (FR878069) reference virus genomes (Table 7).

Table 7. Primer sequences are used to amplify CP gene segments of tobamovirus species.

Name	Sequences (5′ to 3′)	Region	Fragment size (bp)	Annealing. temperature
ToBRFV-Jo	GTT CCA AAC ACA ACA AGC TAG A	F-5894, CP		
ToBRFV-Jo	AAA GTG CAT CCG GTT TAC AAT G	R-6250, CP	355	58ºC
ToMV	GTT TCA AAC ACA GCA AGC AAG A	F-5894, CP		
ToMV	CAG ACC AAC CCA GAC ATA CTT T	R-6250, CP	355	60ºC
TMV	CTC CAT CTC AGT TCG TGT TCT TG	F-5809, CP		
TMV	CAA ACC AAA CCA GAA GAG CTC T	R-6250, CP	400	60ºC

F= Forward direction; R= Reverse direction

PCR amplification of coat protein gene segments

The melting temperature of specific primers was optimized using the thermal gradient PCR feature with a gradient of 11.0 ranging from 55°C to 66°C. The PCR mixture was composed of the following: 2.5 μl 10x long PCR buffer with $MgCl_2$, 2.5 μl dNTP mix 2 mM each, 2.5 μl forward primer, 2.5 μl reverse primer, 0.25 μl long PCR enzyme mix, 2.5 μl template cDNA and Milli-Q (MQ) water to a final volume of 25 μl. The PCR cycling conditions were used, shown in Table 8.

The PCR products were then electrophoresed using 0.5 x TBE buffer and 2% agarose gel stained with 10 mg/ml ethidium bromide for 1 hour and the results were recorded.

Table 8. PCR cycling conditions for using RT-PCR

Step	Temperature	Time	Number of cycles
Initial Denaturation	94°C	4 min	
Denaturation	94°C	1 min	
Primer Annealing	58 or 60°C	1 min	35
Extension	72°C	1 min	
Final Extension	72°C	7 min	

4.5.3 Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

The RT-qPCR method was carried out for a molecular demonstration of the presence of tobamoviruses in resistant, twice inoculated as well as in vegetatively propagated progenies of resistant plants inoculated with different tobamoviruses.

RNA extraction

RNA extraction was done using Trizolate (TRI) reagent RNA extraction kit (UD-GenoMed, Debrecen, Hungary) following manufacturer instructions. The sample leaves or assayed leaves were prepared from inoculated and top leaves as described (**Chapter 4.5.2 *RNA extraction***). Leaf samples were cut and placed in sterilized 2 ml Eppendorf tubes, then immersed in liquid nitrogen. Next, the samples were ground to powder in a homogenizer while freezing in liquid nitrogen to prevent thawing. This was done very fast to minimize RNA degradation.

1 ml of TRI reagent was added to each sample and mixed by inversion. Samples were then incubated for 5 min at room temperature (RT). 200 ul of chloroform was added and mixed by vortex (shake it) for 15 sec to promote phase separation and purification. The samples were incubated for 10 min in RT and then centrifuged for 15 minutes at 13,000 rpm (4°C). The cleared lysate solutions (supernatant) were transferred to 1.5 ml Eppendorf tubes by pipetting, careful not to disturb the pelleted debris. 0,5 ml of isopropanol was added and then incubated for 10 min in RT to precipitate RNA. Pour off by pipette the fluid from the precipitated RNA. Washing was done using 500 ul 70% ethanol alcohol (EtOH) and centrifugation at 7,500 rpm for 1 minute. This was repeated using 250 μl 70% EtOH and the flow-through was discarded. Dry the pellet on RT or in a vacuum centrifuge. 30 μl nuclease-free water was added to dissolve the pellet.

The concentration of the purified RNA was measured in a nanodrop spectrophotometer. RNA products were detected by electrophoresis in 1.5% agarose gel containing 10 mg/ml ethidium bromide in 0.5X TBE buffer. The purified RNA was stored at –80°C.

qPCR amplification of coat protein gene segments

The extracted RNA was utilized as a template for one-step RT-qPCR using the qPCRBIO SyGreen 1-Step Detect Kit, which was performed following the manufacturer's guidelines (PCR Biosystems, London, UK). The ToBRFV, ToMV and TMV specific primers used in this work were the same as described (**Chapter 4.5.2 RT-PCR**).

The PCR mixture was composed of the following: 10 μl of 2x qPCRBIO SyGreen 1-Step Mix, 1μl of forward primer, 1μl of reverse primer, 1μl of 20x RTase Go (contains RNase inhibitor), 1μl

template RNA and MQ water to a final volume of 20 µl. The PCR cycling conditions were used, shown in Table 9.

The PCR products were then electrophoresed using 0.5 x TBE buffer and 2% agarose gel stained with 10 mg/ml ethidium bromide for 1 hour and the results were recorded. The data was analyzed using the LightCycler® 96 detection system software.

Table 9. PCR cycling conditions for using RT-qPCR

Step	Temperature	Time	Number of cycles
Reverse transcription	45°C	10 min	1
Preincubation (For polymerase activation)	95°C	2 min	1
Denaturation, Anneal and Extension	95°C and then 60°C	5 sec and then 30 sec	25
Melt analysis	95°C	60 sec	1
	40°C	60 sec	
	65°C	1 sec	
	97°C	1 sec	

Internal control gene primer and amplification

Two candidate reference genes, *EFα* and *GAPDH* (Table 10) were selected to check RNA samples' quality and expression levels. The primer sequences of candidate reference genes were obtained from EXPÓSITO-RODRÍGUEZ et al. 2008. The internal control genes were amplified in all selected samples using RT-qPCR.

Table 10. Primer sequences used for the Internal control gene.

Name of gene	Oligo Sequence Forward/Reverse	Fragment size (bp)	Annealing. temperature
GAPDH	GGCTGCAATCAAGGAGGAA/AAATCAATCACACGGGAACTG	207	60°C
EFα	TACTGGTGGTTTTGAAGCTG/AACTTCCTTCACGATTTCATCATA	166	

4.6 Selection of resistant *Solanum ochranthum*, *S. habrochaites* and *S. peruvianum* plants and demonstration of their resistance to ToBRFV, TMV and ToMV

After the first inoculation with ToBRFV, the symptomless and virus-free plants that were expected to be resistant were inoculated once again. After the second inoculation, the plants that remained symptomless were decapitated to induce lateral shoots. Two weeks later, two leaves of a lateral shoot in each plant were inoculated again with ToBRFV, and another lateral shoot of each plant was cut-off and rooted in Murashige and Skoog media (MS, Figure 12) for four weeks. Around 10–12 plants from each rooted shoot were propagated by stem cuttings and transferred to pots for further experiments. Three to four vegetatively propagated plants were inoculated with ToBRFV-Tom2-Jo, ToMV-DH and TMV-U1, respectively. They were evaluated for symptoms and the presence or absence of tobamoviruses both in the inoculated (10 dpi) and top leaves (40 dpi) using bioassays and RT-PCR for *S. ochranthum* and RT-qPCR for *S. habrochaites* and *S. peruvianum*. All the greenhouse and laboratory experiments were carried out under quarantine conditions.

Figure 12. Plantlets of resistance regenerated in rooting MS medium

4.7 Evaluation of resistance to ToBRFV under high temperature

Six resistant plants from each accession were propagated vegetatively on MS media, were inoculated with ToBRFV-Tom2-Jo. Three inoculated plants from each accession were maintained in a Sanyo environment plant growth chamber at a constant temperature of 33°C (light intensity 50 Wm^{-2}, day length 14 h) for 14 days. For comparison, three sister plants were grown as a control in

a greenhouse at 24 ± 2°C. The symptomatological evaluation was carried out in both the "Sanyo" population and the greenhouse population at the same time. Bioassays on *N. glutinosa* and RT-qPCR were conducted on each plant, regardless of symptoms were appeared. Three weeks after inoculation, plants from the chamber were transported to the greenhouse and maintained there for weeks to evaluate the symptoms and examine the presence of the virus in newly developed leaves.

4.8 Cleft grafting

For the cleft grafting, four-week-old *S. lycopersicum* cv. Ceglédi plants infected with ToBRFV were used as rootstocks, and a side shoot from the resistant *S. habrochaites* LA1739 plants at the same age was utilized as a scion. Rootstocks were decapitated above two basal leaves and the stems were cut vertically 1–2 cm deep at the center of the stem. Scions (3–5 cm) were prepared by removing the lower leaves, trimming the top leaves, and cutting the stem wedge-shaped into the split rootstock. The rootstock and scion junction were wrapped with Parafilm. The grafted plants were covered with plastic bags to keep humidity until the graft was complete. The presence of ToBRFV was conducted by using bioassays.

4.9 Cloning, sequencing and sequence analysis of putative ToBRFV resistance gene in *Solanum habrochaites* and *S. peruvianum*

To our knowledge, YKEMA et al. (2020) described a gene in *S. habrochaites* (LYC4943) responsible for resistance against ToBRFV. Analyzing the sequence published by YKEMA et al. (2020), we identified the *S. lycopersicum* gene Solyc08g075630 (Solgenomics) using BLASTN and BLASTP. We compared the protein sequences of our symptomless plants in *S. habrochaites* and *S. peruvianum* accessions LA1738, LA1739, LA2171, LA2541, LA 2812, PI 308181, PI 308182, PI 379012, PI 379014 and PI 390659), with those of Solyc08g075630 locus and the data of YKEMA et al. (2020).

DNA extraction

Total genomic DNA was extracted from fresh leaves of a selected symptomless plant of each accession to obtain their sequence and compare them with sequence resistance gene to ToBRFV published by YKEMA et al. (2020).

For DNA extraction, ZenoGene DNA extraction kit (ZenonBio, Szeged, Hungary) was used according to manufacturer protocol. A fresh leaf section was cut from each plant, put in a 1.5 ml Eppendorf tube, and homogenized for 2 minutes with 260 µl NA + NC solution (lysis buffer). They

were then heated at 65°C for 15 minutes in a heating block and put in ice. 80 μl ND solution (contains acetic acid) was added and mixed gently by inversion. They were kept in ice for 15 minutes and centrifuged for 15 minutes at 13,000 rpm. The supernatant was carefully transferred to 400 μl NF solution (96% ethanol), mixed by pipetting, and then transferred to 2 ml Eppendorf tube containing a filter membrane. The mixture was centrifuged at 2000 rpm for 5 minutes, followed by 5000 rpm for 2 minutes and then at 13,000 rpm for 1 minute. The flow-through was discarded and the samples were washed twice with 500 μl 70% ethanol and centrifuged for 1 minute at 5000 rpm discarding the flow-through each time. The samples were centrifuged for 2 minutes at 13,000 rpm and then transferred to elution tubes. The samples were kept at room temperature for 15 minutes to allow ethanol to evaporate. 80 μl of E solution (elution buffer, heated to 65°C) was added to the samples. The samples were then incubated for 5 minutes at 65°C in the heating block to enhance DNA dissolution. The tubes were centrifuged for 1 minute at 3000 rpm and then for 1 minute at 13,000 rpm to elute DNA. The concentration of the purified DNA was measured in a nanodrop spectrophotometer. DNA products were detected by electrophoresis in 1.5% agarose gel containing 10 mg/ml ethidium bromide in 0.5 X TBE buffer. The DNA was then stored at -20°C.

Designing primers for Solyc08g075630 loci

Primer3 web version 4.0.0 computer software was used to design the PCR primers required for amplification 3500 bp genomic segment of the NBS-LRR gene (resistance gene to ToBRFV) based on sequence published by YKEMA et al. (2020), which is ortholog with the sequences of *S. lycopersicon* (SOLgenomics) Solyc08g075630 loci (Table 11).

Table 11. Primer sequences used for the amplification resistance gene to ToBRFV.

Name	Sequences (5′ to 3′)	Fragment size (bp)	Annealing. temperature
Solyc08-F	ATGGCTGAAGCTTTCCTTCA		
Solyc08-R	GGTTACAAATAGTTGATTTGTTTCC	3500	58 °C

F= Forward direction; R= Reverse direction

Purification of amplified DNA

PCR fragments were purified using the GFX PCR DNA and Gel Band Purification Kit following the manufacturer's protocols. Sample capture from agarose gel: using a clean scalpel, long-wavelength (365 nm) ultraviolet light and minimal exposure time, the agarose band containing the sample of interest was carefully cut out and placed into a 1.5 ml Eppendorf tube. 500 μl binding buffer was added to the gel slice. Mixing was done by inversion and the tubes were incubated at 60°C for 15 minutes. Mixing was repeated every 3 minutes during incubation at 60°C and then the samples were centrifuged briefly. Sample capture from PCR product of DNA amplification 500 μl binding buffer was directly added to the PCR product of genomic DNA amplification. Sample binding was transferred into collection tubes with filter membrane and incubated at room temperature for 1 minute. They were spun at 13,000 rpm for 1 minute. The flow-through was discarded. 500 μl of Wash buffer was added to the sample and spun at 13,000 rpm for 1 minute. The filter membranes were transferred to DNase-free 1.5 ml Eppendorf tubes. 50 μl of elution buffer was added to the membrane and incubated at room temperature for 1 minute, then spun at 13,000 rpm for 1 minute. The purified DNA was stored at -20°C.

Cloning of the PCR products for sequencing

The products of genomic DNA amplification were cloned using the pGEM®-T Easy cloning vector (Promega, Madison, USA), following manufacturer instructions as described below.

Ligation Using 2X Rapid Ligation Buffer

Ligation reactions were set up using 5 μl of 2X Rapid Ligation Buffer T4 DNA Ligase, 1 μl pGEM®-T Easy Vector (50 ng), 2 μl PCR product, 1 μl T4 DNA Ligase (3 Weiss units/μl), Deionized water to a final volume of 10 μl. The reaction was mixed by pipetting and then incubated overnight at 4°C.

Transformation of JM109 High-Efficiency Competent Cells

Prepared LB/IPTG/X-Gal/Amp plates were used. The ligation reactions were centrifuged briefly. The competent cells were placed in an ice bath until they were just thawed (10 minutes). 2 μl of the ligation reaction was carefully mixed with 100 μl competent cells and incubated in ice for 20 minutes. The cells were then heat-shocked for 45 seconds in a water bath at exactly 42°C. Without shaking, the tubes were immediately returned to the ice for 2 minutes and 500 μl LB medium was added. They were then incubated for 1.5 hours at 37°C with shaking at 150 rpm. 150 μl of each

transformation culture was plated onto an LB/IPTG/X-Gal/Amp plate. The plates were incubated overnight at 37°C. White colonies were selected.

Colony PCR of Transformation Products

Distinct white colonies were transferred to LB/IPTG/X-Gal/Amp plate and also into a 0.2 ml PCR tube containing 2.0 μl MQ water. The plates were incubated overnight at 37°C. Colony PCR master mix was prepared as follows:

1.2 μl 10x Dream Taq® Buffer with 20 mM MgCl2, 1.0 μl M13 forward primer, 1.0 μl M13 reverse primer, 0.3 μl 10 mM dNTPs mix, 0.5 μl Taq DNA polymerase, MQ water to a final volume of 12μl. 10 μl of the master mix was added to the PCR tube containing 2 μl template (bacterial colony). Colony PCR conditions were used, shown in Table 12.

Table 12. PCR cycling conditions for colony PCR

Step	Temperature	Time	Number of cycles
Initial Denaturation	94°C	4 min	
Denaturation	94°C	1 min	
Primer Annealing	55°C	1 min	35
Extension	72°C	1 min	
Final Extension	72°C	7 min	

Mini-Prep Liquid Bacterial Culture Using ZenoGene Kit

This was done following manufacturer instructions. Selected positive colonies were transferred to 2 ml LB medium containing 50 μg/μl ampicillin antibiotic and incubated overnight (~16 hours) in a 37ºC shaker at 250 rpm.

The bacterial cultures were centrifuged at 13,000 rpm for 3 minutes and the supernatant was discarded. 200 μl of PA (5 ml 1 M pH 8 Tris-HCl + 2 ml 0.5 M EDTA + 1 ml 10 mg/ml RNase + 92 ml MQ per 100 ml) solution was added and the pellet suspended then 200 μl PB (186 μl MQ + 10 μl 20% SDS + 4 μl 10 N NaOH) solution was also added. The samples were mixed by inversion and then left for 5 minutes at room temperature. 650 μl PCE buffer (380 g Guanidinium chloride + 100 ml 3 M pH 5.5 potassium acetate + MQ to make up 1000 ml + 576 ml absolute ethanol) was added and the samples were mixed by inversion. The samples were put in ice for 15 minutes and then centrifuged at 13,000 rpm for 15 minutes. Filter membranes were activated by adding 500 μl MQ water and centrifuging for 1 minute at 5000 rpm. 700 μl of the supernatant was carefully

transferred to the membrane and centrifuged three times as follows: 2000 rpm for 3 minutes, 5000 rpm for 3 minutes and 13,000 rpm for 1 minute. The flow-through was discarded each time. The membranes were then washed 3 times with 700 μl of 70% ethanol while centrifuging at 5000 rpm for 1 minute each time. The membranes were then centrifuged at 13,000 rpm for 2 minutes to get rid of the alcohol. The membranes were transferred to elution tubes and left open for 15 minutes at room temperature for any remaining alcohol to evaporate. 80 μl elution buffer pre-heated at 65°C was added to the membranes and then incubated in the block heater at 65°C for 5 minutes. The samples were then centrifuged twice: at 3000 rpm for 3 minutes and at 13,000 rpm for 2 minutes. The eluted plasmid DNA was then subjected to electrophoresis in 1% agarose gel containing 10 mg/ml ethidium bromide for 1 hour in 1x TBE Buffer. The remaining plasmids were stored at -20°C.

Positive plasmids that showed strong bands were selected. The isolated plasmids were sent to a DNA sequencing company called Biomi (Gödöllő, Hungary). They were sequenced using the Sanger sequencing technique using fluorescently labeled dideoxynucleotides (ddNTPs) and capillary electrophoresis.

Sequence analysis

BioEdit® sequence alignment software, together with DNAstar® software programs including Seqman and Editseq, were used to analyse the sequences. Furthermore, online databases used to compare the sequence results included Sol Genomics Network and National Center for Biotechnology Information (NCBI).

DNAstar® Seqman program was used to trim vector sequences from the DNA sequences by removing pGEM®-T Easy vector. The sequences were compared to the gene that was resistant to ToBRFV by YKEMA et al. (2020) and by assembling the sequences and aligning them together in the Seqman program. The DNA sequence alignment showed the coding (exons) and non-coding DNA sequences (introns), which were then separated by cutting off the intron regions. The sequences were realigned to generate consensus among them using the same program. They were also compared to Solyc08g075630 from the tomato genome database for reference.

Coding DNA sequences from the predicted transcription start codon (5'ATG) to the stop codon (3'TAA) were aligned using BioEdit® sequence alignment software and DNAstar® software. The aligned DNA sequences were then translated into amino acids in the same programs and changes in amino acids sequences were identified.

The coding DNA and protein sequences were searched for similarity using BLASTN and BLASTP

tools. The protein with the highest identity to our sequences was selected. Amino acid changes that occurred between the resistant varieties were detected and their groups identified.

4.10 Isolation, pathological tests and molecular characterization of a mutant of ToBRFV

Isolation and pathological tests

In the course of the symptomatological evaluation, a single individual of *S. habrochaites* LA1738 was observed showing unusual mosaic symptoms. From this plant, we made transmission to *N. glutinosa* from which a single local lesion subculture was transmitted to *N. tabacum* cv. Samsun for propagation. For further investigations, the inocula of this isolate marked Tom2M-Jo were prepared as described (**Chapter 4.2**). For pathological comparison with the original isolate Tom2-Jo. Plants of *S. lycopersicum* carrying known resistance genes, wild *Solanum* species insusceptible to ToBRFV and *Nicotiana* plants were inoculated with the Tom2-Jo and Tom2M-Jo isolates, respectively. After inoculations, symptoms were evaluated and the presence of the viruses in the top leaves of *Solanum* plants was checked using bioassays and RT-qPCR as described (**Chapters 4.5.1 and 4.5.3**). For RT-qPCR, we used the same protocol as described (**Chapter 4.5.3**), except that we applied Promega (USA) extraction kit for the total extraction of RNA.

Molecular characterization of ToBRFV Tom2-Jo and ToBRFV Tom2M-Jo isolates

Total RNA was extracted from mosaic-affected leaves of *N. tabacum* cv. Samsun inoculated with ToBRFV-Tom2-Jo and ToBRFV-Tom2M-Jo, respectively. For this purpose, SV total RNA extraction kit (Promega, USA) was used following the manufacturer's instructions (detailed in **Chapter 4.5.2**). Extracted RNA samples were used as a template for cDNA transcription oligonucleotide specific for ToBRFV (detailed in **Chapter 4.5.2**). Primer3 computer software (version 4.0.0) was used to design the specific PCR primers using the ToBRFV (KT383474) reference virus genomes (Table 13). To amplify cDNA 6.4 kb fragment of the virus, the CloneAmp™ high-fidelity (HiFi) PCR Premix (Takara Bio) was used. The PCR conditions were used, shown in table 14. The PCR mixture was composed of the following: 12.5 µl of CloneAmp HiFi PCR Premix, 1 µl forward primer, 1 µl reverse primer, 1 µl template cDNA and MQ water to a final volume of 25 µl. Amplified fragments were purified and then ligated into pJET1.2/blunt Cloning Vector by using CloneJET PCR Cloning Kit, cloned in *Escherichia coli* competent cells according to standard protocols (The steps described in detail in cloning part, **Chapter 4.9** with minor change), and sequenced with SANGER technology on ABI Prism (3130xl Genetic Analyzer) (Biomi Ltd, Gödöllő, Hungary) using primer walking on the ToBRFV genome (Table

15). The sequences for both isolates ToBRFV-Tom2-Jo and Tom2M-Jo were deposited in NCBI GenBank under accessions numbers MZ323110 and MZ438228, respectively.

Table 13. Primer sequences used for the amplification of whole genome of ToBRFV.

Name	Sequences 5′ to 3′	Frag.Size bp	Ann.Temp.(ºC)
ToBRFV-Fu, F	GTATTTTTGTTTTACAACATATACCAAC	~6400	60
ToBRFV-Fu, R	TGGGCCCCTACCGGGGGTTCCGGGGGA		

F= Forward direction; R= Reverse direction

Table 14. PCR cycling conditions using CloneAmp™ high-fidelity (HiFi)

Step	Temperature	Time	Number of cycles
Initial Denaturation	98°C	4 min	
Denaturation	98°C	10 sec	
Primer Annealing	60°C	30 sec	35
Extension	70°C	3 min	

Table 15. Primers used as primer walking for ToBRFV genome sequencing.

Primer name	Sequence 5'-3'	Region match
ToBRFV_F1	GTATTTTTGTTTTACAACATATACCAAC	1
ToBRFV_F2	TGAGCGGGGCAACAAAGT	532
ToBRFV_F3	AAGGACCCGCAAAGAAGTC	1234
ToBRFV_F4	TATCGACGAAGGTCTGATGTTGCACACTGG	2789
ToBRFV_F5	AGATGCAGGGACCCAATAGC	3410
ToBRFV_F6	GACGACGCTGTGAGTGAGGTCCATAAAA	4803
ToBRFV_F7	GGAAGAAGTCCCGATGTCTGTAAGGCTT	5504
ToBRFV_R1	GGTGTATGCTATTGCATTGCA	3973
ToBRFV_R2	CATCAACAGAGTATCCGGTTTTCCGTACCC	3890
ToBRFV_R3	CTTCCGTCTATGTTCACACCTG	4829
ToBRFV_R4	GAATGGAGAGAGCGGACGAG	4787
ToBRFV_R5	TCCCCCGGAACCCCCGGTAGGGGCCCA	6394

F= Forward direction; R= Reverse direction

Sequencing results were analyzed and aligned with DNAstar® software programs (Seqman and Editseq), Bioedit and Multalin software to compare the ToBRFV-Tom2-Jo and ToBRFV-Tom2M-Jo. Furthermore, the genomes of Tom2-Jo and Tom2M-Jo isolates of ToBRFV were compared with all other ToBRFV genome sequences deposited in NCBI Genbank and data listed in the nextstrain build (VAN DE VOSSENBERG et al. 2020). BLAST program (BLASTN, BLASTX and BLASTP) all-vs-all were used to compare ORFs sequences and amino acid sequences of Rep, MP and CP.

5. RESULTS

5.1 Reactions of *Solanum* germplasms to inoculation with ToBRFV

The inocula of ToBRFV-Tom2-Jo prepared from *N. tabacum* cv. Samsun leaves were highly infectious, causing a great number of necrotic local lesion in *N. glutinosa* and *N. tabacum* cv, Xanthi-nc assay plants in our study.

In the first screening experiments (plants of group A), a total of 636 *Solanum* accessions were inoculated with ToBRFV and evaluated for symptoms and DSI (Table 5, Appendix I). Plants of the control accessions, *S. lycopersicum* (LA1221; *Tm-2²*), *S. lycopersicum* (Ceglédi; +/+) and *S. peruvianum* (PI 126926; *Tm-2*, PI 128650; *Tm-2²*), showed severe symptoms with DSIs ranged between 80 to 100%, while *S. habrochaites* (PI 126445; *Tm-1*) plants showed mild mosaic symptoms with a DSI of 20%. Out of 636 accessions, all plants of 603 wild *Solanum* accessions expressed systemic disease symptoms on top leaves typical of virus infections. These plants were evaluated to be susceptible and sensitive to ToBRFV and belonged to the tomato species *S. pennellii*, *S. pimpinellifolium*, *S. arcanum*, *S. cheesmaniae*, *S. chilense*, *S. corneliomulleri*, *S. habrochaites*, *S. huaylasense*, *S. neoricki*, *S. peruvianum*, *S. galapagense*, *S. sitiens*, *S. juglandifolium*, *S. chmielewskii*, and *S. lycopersicum* var. *cerasiforme*.

The systemic symptoms varied greatly, with an average DSI of 20 - 100% (Figure 13, 14 and Appendix I). Moreover, 26 accessions from different tomato species showed no symptoms or mild mosaic (Figure 15a, Appendix I and II). These were 4 accessions of *S. lycopersicum* var. cerasiforme (LA1456, LA2675, LA2688, LA1385), 2 accessions of *S. habrochaites* (LA1559 and LA2174), 1 accession of *S. chilense* (LA1932) and 19 accessions of *S. pimpinellifolium* (LA1301, LA1375, LA1547, LA1579, LA1607, LA1611, LA1612, LA1630, LA1634, LA1661, LA1670, LA1676, LA1679, LA1685, LA1728, LA1924, LA2903, LA2904, LA2982) with average DSI between 0 and 20% (Appendix I). ToBRFV was demonstrated in the top leaves of all of these symptomless or almost symptomless plants using bioassays and RT-PCR (Figures 15b and 16), excluding *S. ochranthum*. The reactions of this species' plants were unusual, which are analyzed in detail in **chapter 5.2.1**.

Figure 13. Typical systemic symptoms caused by ToBRFV on infected wild tomato plants. (a) *S. pennellii* (mosaic, deformation), (b) *S. pimpinellifolium* (mosaic, deformation, rolling), (c) *S. cheesmaniae* (mosaic, shoesstring, rolling), (d) *S. chilense* (mosaic, deformation), (e) *S. chmielewskii* (shoesstring, mosaic, rolling), (f) *S. corneliomulleri* (mosaic, deformation, rolling), (g) *S. galapagense* (mosaic), (h) *S. habrochaites* (mosaic), (i) *S. huaylasense peralta* (mosaic, deformation), (j) *S. juglandifolium* (mosaic), (k) *S. lycopersicum* (mosaic, deformation) and (l) *S. neoricki* (shoesstring).

Figure 14. Typical systemic symptoms caused by ToBRFV on infected wild tomato plants. (a) *S. sitiens* (mosaic), (b) *S. arcanum* (mosaic, deformation) and (c) *S. peruvianum* (mosaic, deformation, rolling).
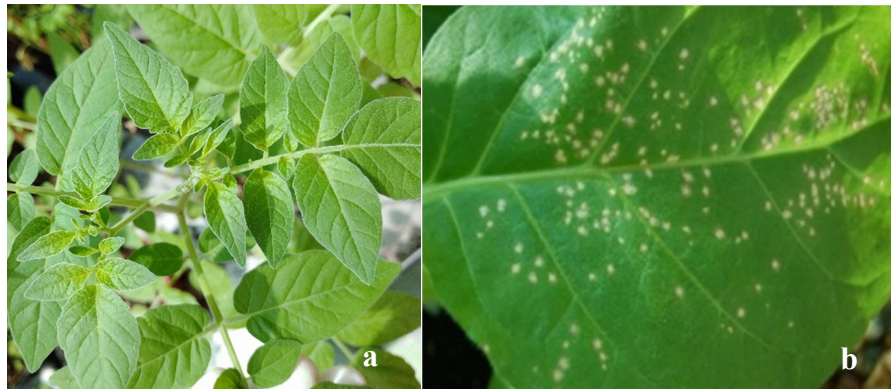
Figure 15. Symptomless plant of *S. pimpinellifolium* accession LA1924 inoculated with ToBRFV (a) and a leaf of *N. tabacum* var. Xanthi-nc plant inoculated with extract of its top leaves (b, note numerous necrotic lesions characteristic of ToBRFV).
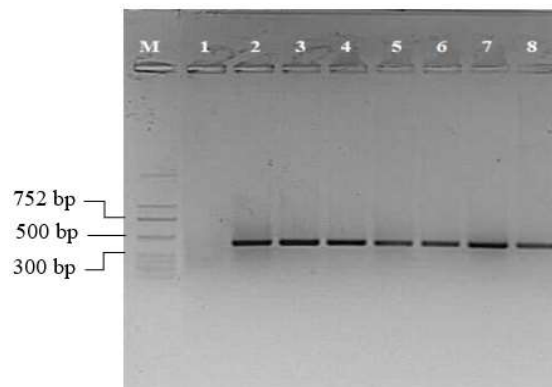
Figure 16. Detection of ToBRFV in selected symptomless *S. pimpinellifolium* LA1301, LA1375, LA1547, LA1924 (3-6) and *S. habrochaites* LA1559, LA2174 (7-8) plants by RT-PCR. M =

molecular marker, 1 = negative control, 2= positive control

Plants of Group B, covering 81 accessions of *S. peruvianum* and 92 accessions of *S. habrochaites* were inoculated with ToBRFV in parallel with the control tomatoes, including Ceglédi (*Tm+*), Craigella-GCR26 (*tm-1*[CRG26]), LA3269-GCR237 (*Tm-1*), LA2088 (*Tm-2*), and Moneymaker-LA3471 (*Tm-2²*). The great majority of the plants of these 173 accessions of *S. habrochaites* and *S. peruvianum* proved to be susceptible to ToBFRV-Tom2-Jo and showed a range of systemic symptoms with DSI of 20–100% (Appendix IV). The symptoms were mosaic, mottling, and sometimes deformation of top leaves, which started to appear at 10–14 dpi, and usually became characteristic as listed in Appendix IV and presented in Figure 17. Control tomatoes also became infected, showing severe viral symptoms.

In a single accession of *S. peruvianum* (PI 308181) and nine accessions of *S. habrochaites* (LA1738, LA1739, LA2171, LA2541, LA 2812, PI 308182, PI 379012, PI 379014 and PI 390659), the plant populations segregated for symptomatic and symptomless individuals at different frequencies (2-10 symptomless plants out of 15 inoculated) (Figure 18, Appendix IV and V). The symptomless plants were transplanted into pots for further investigations (**chapter 5.2.2**).
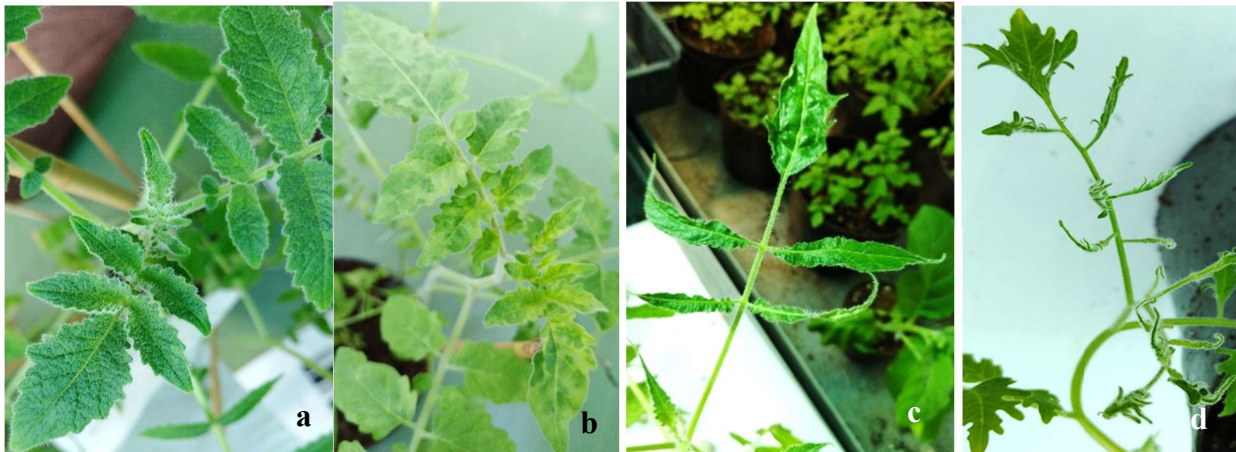
Figure 17. *S. habrochaites* (a, b and c) and *S. peruvianum* (d) plants susceptible to ToBRFV showed different classes of typical disease symptoms; a= mild mosaic, b = mosaic, c = mosaic and leaf deformations, d = mosaic, leaf deformation and shoestring

Figure 18. Plants of *S. habrochaites* PI 379012 segregate for symptomless (red arrow) and mosaic affected symptomatic (yellow arrow) individuals after repeated inoculation with ToBRFV.

5.2 Tobamovirus resistance in selected *Solanum ochranthum*, *S. habrochaites* and *S. peruvianum* plants

5.2.1 Resistance of *S. ochranthum* to tobamoviruses

In the course of screening of group A plants, five *S. ochranthum* accessions behaved unusually. Three of them (LA2160, LA2162, LA2166) remained symptomless after inoculation with ToBRFV (Figure 19, Appendix I and III), while two others (PI 473498 and PI 230519) showed mild systemic mosaic followed by total recovery (Appendix I).

Figure 19. Symptomless plant of *S. ochranthum* accession LA2166 inoculated with ToBRFV.

The inoculated and top leaves of *S. ochranthum* accessions, LA2160, LA2162 and LA2166, remained symptomless following the first, second and lateral shoot inoculation by ToBRFV. The presence of the virus has only been confirmed in inoculated leaves proved by bioassays. Similar reactions were detected on vegetatively propagated progenies of these accessions after inoculations with TMV and ToMV, respectively. Two of the other *S. ochranthum* accessions PI 230519 and PI 473498, responded differently to ToBRFV, ToMV, and TMV. They were both locally and systemically infected by ToBRFV but only locally by TMV and ToMV. Plants of the *S. ochranthum* accessions PI 473498 and PI 230519 had unexpected systemic reactions to ToBRFV. They initially, at 15 dpi showed mild systemic mosaic symptoms (DSI 20%) and contained an infective virus. Later, they recovered from the symptoms (Figure 20) and the virus could not be detected on their newly emerged symptomless leaves of the same plant (Table 16, Figure 21).
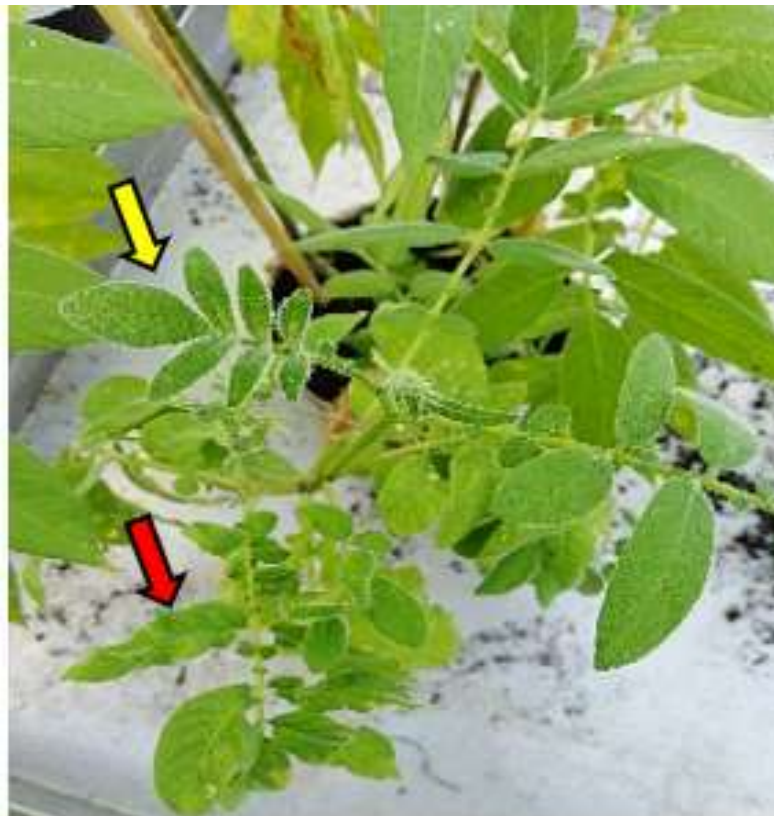
Figure 20. Mild mosaic symptoms (red arrow) followed by recovery (yellow arrow) on the newly developed top leaves of *S. ochranthum* PI 473498 inoculated with ToBRFV.

Table 16. Local and systemic reactions of *S. ochranthum* accessions to three tobamoviruses.

S. ochranthum accesions	ToBRFV		ToMV		TMV	
	Local	Systemic	Local	Systemic	Local	Systemic
LA2160	sl (+)[a]	sl (-)[b]	sl (+)[a]	sl (-)[b]	sl (+)[a]	sl (-)[b]
LA2162	sl (+)[a]	sl (-)[b]	sl (+)[a]	sl (-)[b]	sl (+)[a]	sl (-)[b]
LA2166	sl (+)[a]	sl (-)[b]	sl (+)[a]	sl (-)[b]	sl (+)[a]	sl (-)[b]
PI 473498	sl (+)[a]	mm (+)[a] →sl (-)[b]	sl (+)[a]	sl (-)[b]	sl (+)[a]	sl (-)[b]
PI 230519	sl (+)[a]	mm (+)[a] →sl (-)[b]	sl (+)[a]	sl (-)[b]	sl (+)[a]	sl (-)[b]

Abbreviations: sl = symptomless, mm = mild mosaic, (+)[a] = virus was detected by using bioassay, (-)[b] = virus was not detected by using bioassay and RT-PCR, → = became symptomless on top leaves.
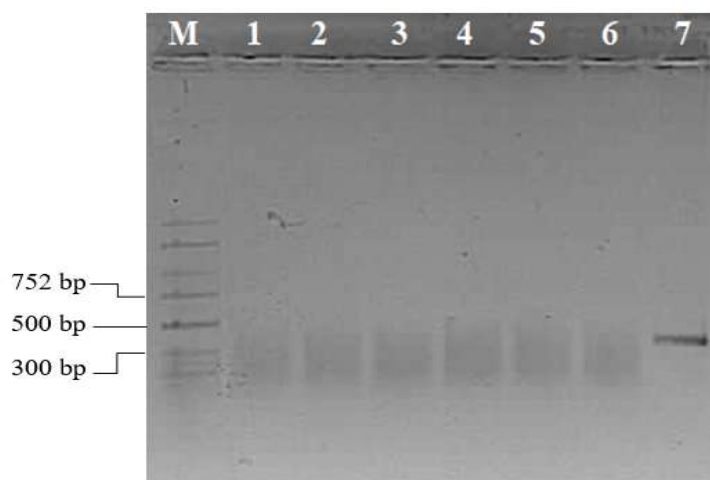
Figure 21. Detection of ToBRFV by RT-PCR in symptomless of *S. ochranthum* plants. M = molecular markers, 1 = negative control, 2 = LA2160, 3 = LA2162, 4 = LA2166, 5= PI 473498 and 6= PI 230519, 7= positive control. Plants 5-6 showed mild mosaic after inoculation, and according to the bioassays on *N. tabacum* var. Xanthi-nc contained the virus, but later they recovered, and no virus could be detected in their top leaves by bioassays and RT-PCR.

5.2.2 Resistance of *S. habrochaites* and *S. peruvianum* to tobamoviruses

5.2.2.1 Susceptibility and resistance of *S. peruvianum* and *S. habrochaites* to ToBRFV

After transplantation of symptomless individuals of *S. peruvianum* and *S. habrochaites* (**Chapter of 5.1.1**), the presence of ToBRFV was checked in their inoculated leaves at 10 dpi as well as the top leaves at 40 dpi by back inoculations to *N. glutinosa* local lesion test plants. No local lesions were detected, showing the absence of infective virus in the leaves of the donor plants (Figure 22a, left). Similarly, no virus was also demonstrated by RT-qPCR tests (Figure 23). Furthermore, the expression levels of the internal control genes of all samples were detected and shown in Figure 24. In contrast, numerous necrotic local lesions developed on leaves of *N. glutinosa* inoculated with the extract of symptomatic plants of *S. lycopersicum* controls (Figure 22a, right). *S. peruvianum* and *S. habrochaites* plants were then inoculated several times with ToBRFV. Systemic symptoms were never observed and the virus could never be detected either by bioassays or RT-qPCR in their inoculated and top leaves, not only in the original plants but also in their progenies (**Chapter 4.6 material and methods**).

To test the responses of the selected *S. peruvianum* and *S. habrochaites* plants to a broader range of tobamoviruses, young virus-free progenies of them were inoculated with TMV and ToMV, besides ToBRFV. *S. lycopersicum* cv. Ceglédi has used as susceptible sensitive control. Similar to ToBRFV, TMV and ToMV caused mosaic in susceptible control tomato and necrotic local lesions in *N. glutinosa* (Figure 22, b and c). No symptoms were induced in the selected *S. peruvianum* and *S. habrochaites* plants and viruses could not be detected in their inoculated and top leaves by bioassays or RT-qPCR.

Figure 22. No symptoms and necrotic local lesions on *N. glutinosa* assay plants inoculated with the extract of top leaf of *S. habrochaites* (LA1739, left side) and with extract of ToBRFV infected *S. lycopersicum* (control plants, right side), respectively (a). Local lesions caused by ToMV (b) and TMV (c) transmitted from infected leaves of *S. lycopersicum* susceptible control.
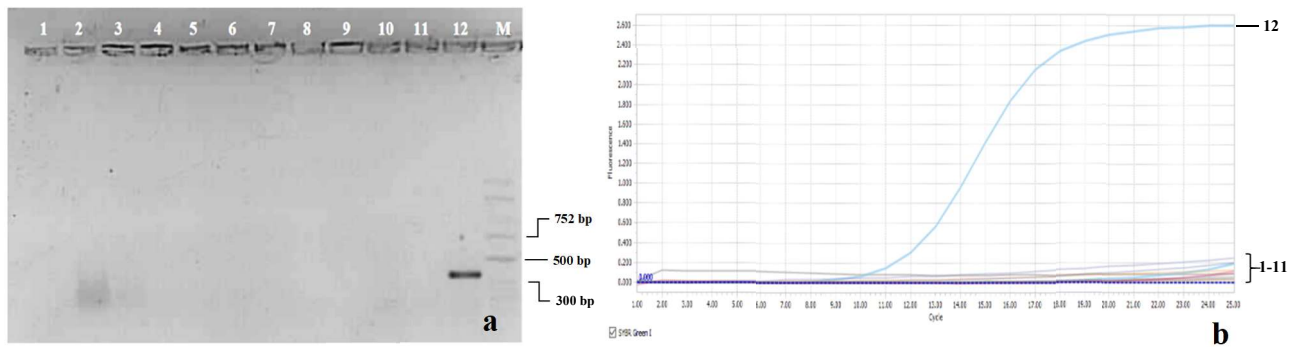
Figure 23. Electrophoretic detection of PCR product of symptomless *S. peruvianum and S. habrochaites* (2-11) and susceptible (12) tomatoes infected with ToBRFV (a). Amplification curves of PCR products using RT-qPCR (b). M = Molecular marker; Numbering of accessions: 1 = Negative control; 2 = LA1738; 3 = LA1739; 4 = LA2171; 5 = LA2541; 6 = LA2812; 7= PI 308182; 8 = PI 379012; 9 = PI 379014; 10 = PI 390659; 11 = PI 308181; 12= positive control. The expected PCR product size was 350 bp.
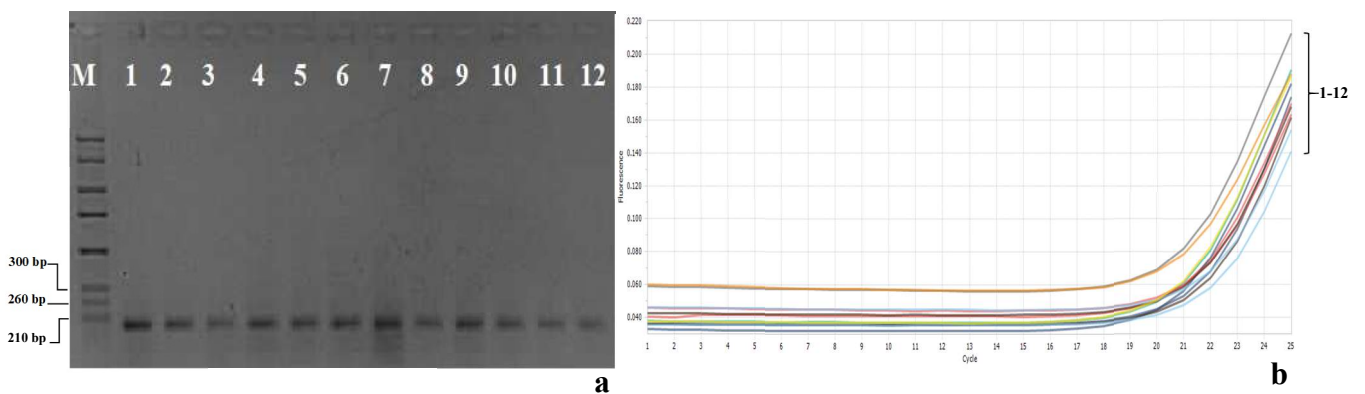
Figure 24. Electrophoretic detection of PCR product of internal control gene (*GAPDH*) of symptomless *S. peruvianum, S. habrochaites* and susceptible samples (a). Amplification curves of PCR products using RT-qPCR (b). M = Molecular marker; Numbering of accessions: 1 = Negative control; 2 = LA1738; 3 = LA1739; 4 = LA2171; 5 = LA2541; 6 = LA2812; 7= PI 308182; 8 = PI 379012; 9 = PI 379014; 10 = PI 390659; 11 = PI 308181; 12= positive control. The expected PCR product size was 200 bp.

5.2.2.2 Evaluation of resistance to ToBRFV under high temperature

To evaluate if the responses of the selected *S. peruvianum* and *S. habrochaites* plants ToBRFV-Tom2-Jo are influenced by the temperature, we maintained inoculated young plants at 33°C for 14 days. Plant of all accessions became diseased at 10-14 dpi showing mosaic, leaf deformation and stunting symptoms (Figure 25a). ToBRFV was detected in their symptomatic top leaves by bioassays and RT-qPCR (Figure 26, Figure 27). In contrast, the inoculated sister plants grown in a greenhouse at 24°C were symptomless and proved virus-free as examined by RT-qPCR and bioassay (Figure 25a, Figure 27). The resistant plants that displayed severe symptoms at 33°C, developed new symptomless leaves after being kept in the greenhouse at 24°C (Figure 25b). Unexpectedly, these newly developed leaves were also proved free from the virus, when assessed by RT-qPCR and bioassay (Figure 27).
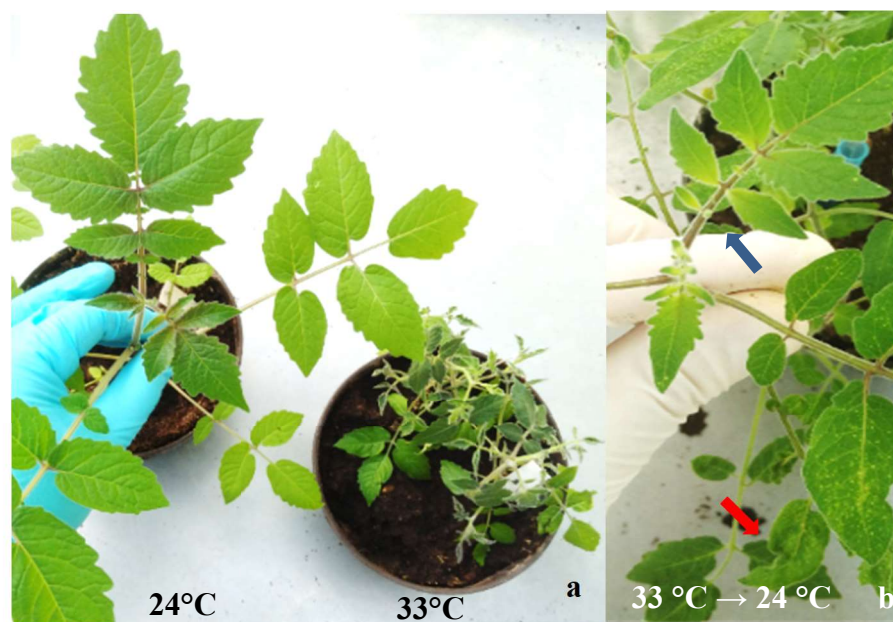
Figure 25. The effect of temperature on the development of symptoms on *S. habrochaites* PI 390659 inoculated with ToBRFV-Tom2-Jo. The plant remained symptomless and virus-free at 24°C (left) but showed mosaic, deformation and stunting and contained virus at 33°C after 15-20 dpi (right) (a). Recovery of symptomatic plants three weeks after transfer from 33°C to the greenhouse. Symptomatic leaves (red arrow) and recovered leaves (blue arrow) on the same plant (b).

Figure 26. Necrotic local lesions on N. *glutinosa* assay plant inoculated with the extract of top leaf of *S. habrochaites* PI 390659 ToBRFV (a) and with extract of ToBRFV infected *S. lycopersicum* positive control (b), respectively at 33°C.
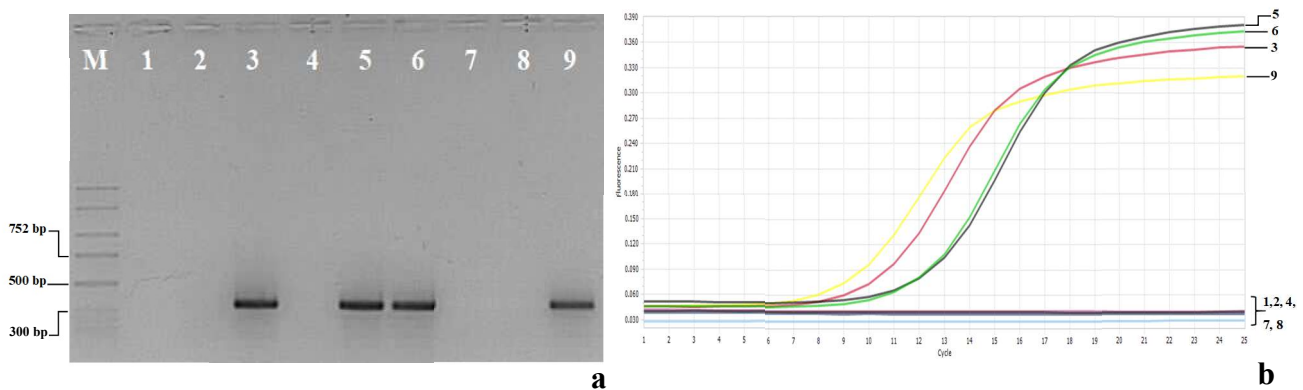
Figure 27. Electrophoretic detection PCR product of effect temperature on the development of symptoms on resistant *S. habrochaites* PI 390659 inoculated with ToBRFV-Tom2-Jo (a). Amplification curves of PCR products using RT-qPCR (b). M = Molecular marker, at 24°C: 1 = Negative control; 2 = Sample extracted top leaf; 3 = positive control, at 33°C: 4 = Negative control; 5 = Sample extracted top leaf; 6 = positive control, Recovery of resistant plants (at 24°C): 7 = Negative control; 8 = Sample extracted top leaf; 9 = positive control. The expected PCR product size was 350 bp.

5.2.2.3 Infection of plants following grafting

Five repetitions of cleft grafting *S. habrochaites* LA1739 as scions and ToBRFV infected *S. lycopersicum* cv. Ceglédi as rootstock were successful (Figure 28a). The scions started to show mosaic symptoms on the newly developed leaves 30 days after grafting on all five repetitions (Figure 28b). The virus was also detected in the diseased symptomatic leaves of the scions using *N. glutinosa* bioassays.

Figure 28. Cleft grafting was successful and the plant started to grow 15 days after grafting (a), mosaic symptoms (yellow arrow) expressed in resistant scion 30 days after grafting (b).

5.2.2.4 Molecular data analysis

Eight resistant plants selected from different accessions of *S. habrochaites* and one resistant plant of *S. peruvianum* revealed high heterogeneity. One resistant plant of *S. habrochaites* LA2812 was found harboring an allele almost identical (99.90%) to the resistance gene discovered in *S. habrochaites* LYC4943 by YKEMA et al. (2020). *S. habrochaites* LA2812 differed from LYC4943 in one nucleotide substitution resulting in a single amino acid (aa) change in the Solyc08g075630 gene. However, other sequences (PI 379012, PI 308181, LA1738 and PI 379014) present lower similarities (80-88%) compared to the resistance gene of Ykema, respectively. Moreover, five accessions, LA1739, LA2171, LA2541, PI 308182 and PI 390659, contained a truncated or putative version of the NBS-LRR gene (Appendix VI).

5.3 Isolation, pathological test and molecular characterization of a mutant of ToBRFV

5.3.1 Isolation of ToBRFV-Tom2M-Jo and its comparison with Tom2-Jo isolate

In the course of testing for responses to ToBRFV-Tom2-Jo, *S. habrochaites* and *S. peruvianum* plants found insusceptible to the virus before **(Chapter 5.2.2.1)** were propagated by rooting of their lateral shoots. Three young progenies of each plant were inoculated again. As it was expected, no symptoms were appeared in these plants, except for a single individual of *S. habrochaites* LA1738. This plant reacted to the inoculation with systemic mosaic symptoms, characteristic to tobamoviruses. With the extract of these symptomatic top leaves, *N. glutinosa* plants were inoculated in which necrotic lesions similar to those of characteristic to ToBRFV appeared. Transmission experiment from a single local lesion to *N. tabacum* cv. Samsun was successful, resulting in developing a strong systemic mosaic in this tobacco cultivar. The virus, a suspected mutant of the original Tom2-Jo, was marked as Tom2M-Jo and propagated in Samsun tobacco.

In a comparative inoculation experiment, ToBRFV-Tom2-Jo and ToBRFV-Tom2M-Jo were able to infect systemically the control tomato genotypes: GCR26-Craigella (*tm-1*[CRG26]), GCR237-LA3269 (*Tm-1*), LA2088 (*Tm-2*), LA3471-Moneymaker (*Tm-2²*) and Ceglédi (*Tm+*), which expressed severe mosaic, deformation, leaf narrowing symptoms. No phenotypic (symptomatological) differences between the two isolates were established.

Three vegetatively propagated individuals of the selected insusceptible *S. habrochaites* and *S. peruvianum* were then inoculated with the two isolates, respectively. As expected, ToBRFV-Tom2-Jo did not induce any symptoms (Figure 29a) and all plants were proved virus-free as assessed by bioassay and RT-qPCR (Figure 30). On the other hand, ToBRFV-Tom2M-Jo infected systemically each individual of *S. habrochaites* and *S. peruvianum*. At 14 dpi, the upper leaves of inoculated plants showed mosaic symptoms and virus propagation could be detected in their symptomatic top leaves using bioassays and RT-qPCR (Figure 29b and Figure 30).
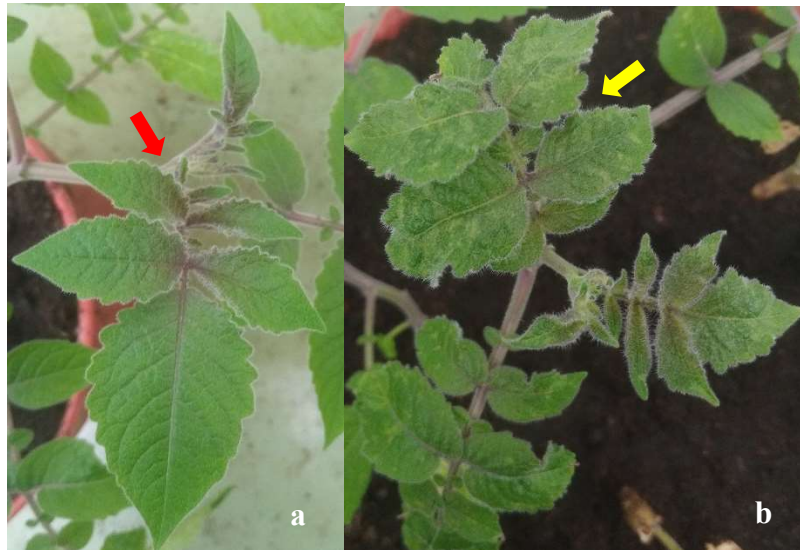
Figure 29. Comparing symptoms inoculated with ToBRFV-Tom2M-Jo and ToBRFV-Tom2-Jo on *S. habrochaites* LA 1738. The plant was inoculated with ToBRFV-Tom2-Jo and showed no symptoms (a, red arrow). The plant showing mosaic symptoms, was inoculated with ToBRFV-Tom2M-Jo (b, yellow arrow). (Both plants originated from lateral shoots of the same *S. habrochaites* LA 1738 plant).
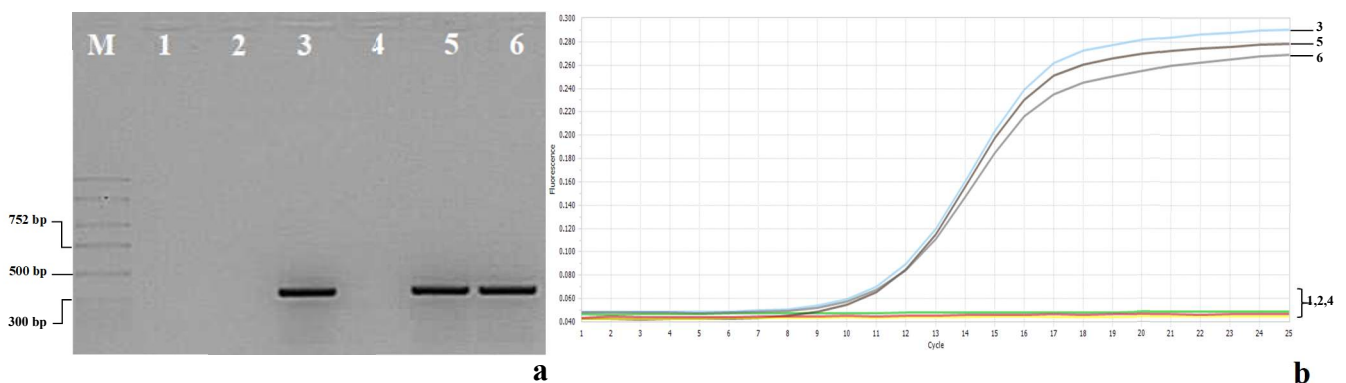
Figure 30. Electrophoretic detection of PCR product of *S. habrochaites* LA 1738 inoculated with ToBRFV-Tom2-Jo and ToBRFV-Tom2M-Jo (a). Amplification curves of PCR products using RT-qPCR (b). M = Molecular marker, 1 = Negative control Ceglédi (*Tm+*) not inoculated with ToBRFV-Tom2-Jo; 2 = Sample extracted from top leaf of *S. habrochaites* inoculated with ToBRFV -Tom2-Jo ; 3 = Positive control Ceglédi (*Tm+*) inoculated with ToBRFV-Tom2-Jo, 4= Negative control Ceglédi (*Tm+*) not inoculated with ToBRFV-Tom2M-Jo; 5 = Sample extracted top leaf of *S. habrochaites* inoculated with ToBRFV-Tom2M-Jo; 6 = Positive control Ceglédi (*Tm+*) inoculated with ToBRFV-Tom2M-Jo. The expected PCR product size was 350 bp

5.3.2 Comparing sequences of ToBRFV Tom2-Jo and Tom2M-Jo

The complete sequence of ToBRFV-Tom2M-Jo consists of 6,394 nucleotides and encodes four open reading frames (ORFs), which is typical to other ToBRFV and tobamoviruses genome sequences deposited in NCBI Genebank.

In comparing ToBRFV-Tom2M-Jo with the nucleotide sequence of ToBRFV-Tom2-Jo, the ToBRFV-Tom2M-Jo sequence has three synonymous nucleotides substitutions in the Rep region (C to T at nucleotide position 1018, 3622 and T to A at 3997). In addition, two nonsynonymous nucleotide substitutions in the MP (T to A at nucleotide position 4975, 5156) were detected (Figure 31), whereas, in CP, no change occurred.

A comparison of the amino acid sequence of both ToBRFV-Tom2-Jo and ToBRFV-Tom2M-Jo reveals that the ToBRFV-Tom2M-Jo has no change in Rep and CP protein parts but has changed two amino acid substitutions in the MP. ToBRFV-Tom2-Jo has a Phe at position 22 and Asn at position 82, while ToBRFV-Tom2M-Jo has a Tyr and Lys at the same positions from the MP, respectively (Figure 32).

Alignment sequences of nucleotide and amino acid of MP of ToBRFV-Tom2M-Jo with fifteen ToBRFV genome sequences isolated from different countries (MN549394 (Canada), MW314091 (China), MN882031 (Egypt), MK133095 (Germany), MN815773 (Greece), KX619418 (Israel), MN167466 (Italy), KT383474 (Jordan), MK319944 (Mexico), MN882011 (Netherland), MN013188 (Palestine), MW314111 (Peru), MT107885 (Turkey), MN182533 (United Kingdom), MT002973 (United States)) was also performed. The results also revealed that ToBRFV-Tom2M-Jo had only two unique nucleotides and amino acid changes in MP, that were not present in all other fifteen sequences (Figure 31 and Figure 32). Furthermore, it was also obtained the same alignment result with other 103 ToBRFV genome sequences in MP provided by data nextstrain build and NCBI genebank (VAN DE VOSSENBERG et al. 2020).

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              4910      4920      4930      4940      4950      4960      4970      4980      4990      5000
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
KT383474       TTGTTTATAGATGGCTCTTGTTAAGGGTAAAGTCAATATTAATGAGTTCATAGACTTGTCAAAATCAGAAAAATTTCTTCCGTCTATGTTCACACCTGTT
ToBRFV-Tom2-Jo  ...................................................................................................
ToBRFV-Tom2M-Jo ......................................................................[A]............................
MW314091        ...............................................G...................................................
MN882011        ...................................................................................................
MN167466        ...................................................................................................
MK133095        ...................................................................................................
MT002973        ...................................................................................................
MK319944        ...................................................................................................
MN549394        ...................................................................................................
MW314111        ...................................................................................................
MT107885        ...................................................................................................
KX619418        ...................................................................................................
MN013188        ...............................................G...................................................
MN182533        ...................................................................................................
MN815773        ...................................................................................................
MN882031        ...................................................................................................

              5110      5120      5130      5140      5150      5160      5170      5180      5190      5200
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
KT383474       GCTATGTACATCTTGCTGGTCTTGTGGTGACAGGTGAATGGAATTTGCCAGATAATTGTCGTGGTGGTGTCAGTGTCTGTTTGGTCGATAAGAGAATGGA
ToBRFV-Tom2-Jo  ..........C........................................................................................
ToBRFV-Tom2M-Jo ..........C...........................................[A].........................................
MW314091        ...................................................................................................
MN882011        ...................................................................................................
MN167466        ...................................................................................................
MK133095        ...................................................................................................
MT002973        ...................................................................................................
MK319944        ...................................................................................................
MN549394        ...................................................................................................
MW314111        .............................A.....................................................................
MT107885        ...................................................................................................
KX619418        ...................................................................................................
MN013188        ...................................................................................................
MN182533        ..............................................................................................A....
MN815773        ...................................................................................................
MN882031        ...................................................................................................
```

Figure 31. Alignment of the nucleotide sequences of the MP gene of ToBRFV Tom2-Jo, Tom2M-Jo and fifteen selected ToBRFV isolates from different countries. Dots indicate identical nucleotides. Differences between ToBRFV (Tom2-Jo) and ToBRFV (Tom2M-Jo) nucleotides were marked inside the box.

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              10        20        30        40        50        60        70        80        90       100
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ALP48478.1     MALVKGKVNINEFIDLSKSEKFLPSMFTPVKSVMISKVDKILVHEDESLSEVNLLKGVKLIDGGYVHLAGLVVTGEWNLPDNCRGGVSVCLVDKRMERAD
ToBRFV-Tom2-Jo  ..................................................................................................
ToBRFV-Tom2M-Jo ..................[Y].......................................................[K]....................
APB87945.1      ..................................................................................................
AZQ25022.1      ..................................................................................................
QCI56070.1      ..................................................................................................
QDM58713.1      ..................................................................................................
QEP52196.1      ..................................................................................................
QHD44458.1      ..........G.......................................................................................
QHW03636.1      ..................................................................................................
QHW03716.1      ..................................................................................................
QJQ51125.1      ..................................................................................................
QKF94941.1      ..................................................................................................
QKI31201.1      ..................................................................................................
QKY59644.1      ..................................................................................................
QWE91528.1      ..........S.......................................................................................
QWE91608.1      ..................................................................................................
```
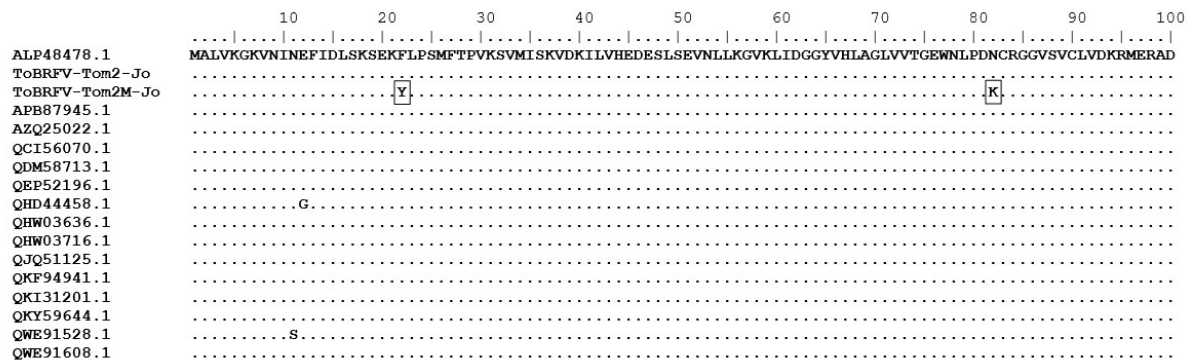
Figure 32. Compares ToBRFV Tom2-Jo and Tom2M-Jo amino acid sequences in the MP aligned with fifteen selected ToBRFV sequences from different countries. Dots indicate identical amino acids. The box marked two amino acid substitutions of Tom2-Jo and Tom2M-Jo isolates.

6. DISCUSSION

6.1 Screening of *Solanum* germplasm for reactions to the ToBRFV

Several members of the *Tobamovirus* genus like ToMV and TMV have been recognized for many years as dangerous pathogens of the tomato plant. These mechanically and seed-transmitted stable viruses are effectively managed by using resistant cultivars and hybrids harboring the well-known resistance genes *Tm-1, Tm-2,* and *Tm-2²* (SOOST 1963; ALEXANDER 1963; PFITZNER 2006). Although some TMV and ToMV mutants have been identified to break down the resistance conferred by these genes, they did not spread widely and no serious yield losses were reported (MESHI et al. 1989; CALDER and PALUKAITIS 1992; WEBER et al. 1993; BETTI et al. 1997; STRASSER and PFITZNER 2007; LI et al. 2013). However, ToBRFV, a newly discovered plant virus (SALEM et al. 2015), has been found to infect all tomato genotypes harbouring the characterized resistance genes, leading to widespread panic among seed companies and tomato producers (LURIA et al. 2017; DOMBROVSKY and SMITH 2017). Resistance to ToBRFV has been reported in several genotypes of *S. pimpinellifolium*, *S. lycopersicum*, and *S. habrochaites* (HAMELINK et al. 2019; ASHKENAZI et al. 2020; YKEMA et al. 2020), whereas tolerance to this virus, has been demonstrated in *S. lycopersicum* and *S. pimpinellifolium* (ASHKENAZI et al. 2018; ZINGER et al. 2021).

We found that a large number of 636 accessions from 16 different species were susceptible to ToBRFV, including the accessions of *S. arcanum*, *S. chmielewskii*, *S. huaylasense*, *S. juglandifolium*, *S. sitiens*, and *S. ochranthum* (Appendix I). To the best of our knowledge, the last-mentioned six *Solanum* species have never been evaluated as hosts or non-hosts of ToBRFV; hence they can be considered new experimental hosts of this virus.

The susceptible plants showed different types of symptoms such as mild mosaic, mosaic or mottling, leaf deformation followed by rolling and shoestring with an average DSI between 20 % to 100%, respectively (Appendix I). The severity of systemic symptoms varied between species and sometimes between accessions of the same species. Our observations connecting the leaf symptoms caused by ToBRFV did not differ remarkably from those described by SALEM et al. (2015), LURIA et al. (2017), FIDAN et al. (2021) and PANNO et al. (2021) and those described characteristics to the common strains of TMV and ToMV in susceptible tomatoes (BROADBENT 1964; RAST 1975).

Following inoculations, plants of several accessions were found to be infected by ToBRFV but did not display any systemic symptoms (Appendix I). These symptomless plants should be classified as tolerant, according to Cooper and Jones (1983). Similar tolerance has been found in *S. lycopersicum* and *S. pimpinellifolium* by ASHKENAZI et al. (2018) and ZINGER et al. (2021). However, in addition to *S. pimpinellifolium* and cultivated lines of *S. lycopersicum* var. cerasiforme, we also found tolerance in accessions of the wild tomato plants of *S. chilense* and S. *habrochaites*. In several cases, the accessions were segregated for susceptible and tolerant individuals (for example, *S. pimpinellifolium* LA1301 and LA1547), while others contained only tolerant plants (for example, *S. pimpinellifolium* LA1924 and LA1579) (Appendix 1). Tolerance to ToBRFV can be important in the production practice of tomato, but its genetic background is not yet determined.

The reaction of *S. ochranthum*, which is a close relative of the tomato, was extremely variable. Two accessions (PI 230519 and PI 473498) displayed transitional mild systemic mosaic symptoms followed by total recovery on the new apical leaves. While bioassays could detect ToBRFV in the mosaic affected leaves, no virus was present later in the newly developed symptomless top leaves of the same plant. This indicated that either the virus movement was arrested or the virus replication was strictly controlled. Interestingly, similar recovery from disease, including vanishing of symptoms and lack of detectable viruses, has been already reported in *S. ochranthum* when inoculated with the potexvirus, pepino mosaic virus (PepMV) (SOLER-ALEIXANDRE et al. 2007). Furthermore, the recovery phenomenon was also reported with nepoviruses, for example, tomato black ring virus (TBRV) in *Nicotiana clevelandii* associated with a decreased viral RNA concentration. In contrast, symptom attenuation of tomato ringspot virus (ToRSV) in *N. clevelandii*, *N. benthamiana* and *Cucumis sativus* was not accompanied by a commensurate reduction in viral RNA levels, which often appear as a consequence of RNA silencing (RATCLIFF et al. 1997; JOVEL et al. 2007). Other host factors such as proteasome degradation, autophagy, and RNA decay may play as antiviral defense through target viral nucleic acids or proteins, resulting in the plant recovery from symptoms (GARCIA-RUIZ 2019).

In contrast to the *S. ochranthum* PI 230519 and PI 473498 accessions, plants of the accessions LA2160, LA2162, and LA2166 inoculated with ToBRFV remained symptomless both locally and systemically and the presence of the virus could be confirmed only in their inoculated leaves. These results demonstrate that these plants had a high resistance level to ToBRFV. Similarly, we demonstrate high resistance of *S. ochranthum* also against TMV and ToMV, indicating that these plants may have the same genetic background for resistance to different tobamoviruses. Reactions of *S. ochranthum* have been investigated so far only to pepino mosaic virus (PepMV, member of

Potexvirus genus) and cucumber mosaic virus (CMV, member of *Cucumovirus* genus) (RICK 1988; SOLER-ALEIXANDRE et al. 2007). Consequently, we studied for the first time the reactions of *S. ochranthum* to tobamoviruses. It would be of special interest, whether *S. ochranthum* is resistant or susceptible to other important tobamoviruses pathogenic to solanaceous plants such as obuda pepper virus (ObPV) or tomato mild mottle virus (ToMMV).

The transfer of ToBRFV resistance from *S. ochranthum* to cultivated tomato is difficult because of the sexual incompatibility and seems to be genetically isolated from *S. lycopersicum* or other related tomato species. A potential alternative to surpass this genetic barrier can be the use of somatic hybridization among accessions of these species (RICK 1979; RICK and CHETELAT 1995; PERTUZÉ et al. 2002; KOLE 2011). For instance, somatic hybrids between *S. ochranthum* and *S. lycopersicum*, have been obtained through protoplast fusion; Nevertheless, they are highly sterile and have not yet provided a pathway for gene transfer (KOLE 2011). However, backcrosses between *S. ochranthum* + tomato somatic hybrids and tomato, combined with embryo rescue, may result in the desired progeny and facilitate further recombination between these species (KOBAYASHI et al., 1996).

6.2 Demonstration of resistance in *S. habrochaites* and *S. peruvianum* to ToBRFV

Our results discussed above, demonstrated only susceptible and tolerant plants in 636 accessions of 16 *Solanum* species, excluding *S. ochranthum*. To find truly resistant wild tomato plants, we followed the experiments with screening 173 accessions of *S. peruvianum* and *S. habrochaites* for responses to ToBRFV inoculation (Appendix IV). Although 163 accessions were found susceptible showing mosaic, mottling and sometimes deformation symptoms, we discovered nine accessions of *S. habrochaites* and one accession of *S. peruvianum,* which segregated to symptomless and symptomatic plants (Appendix IV and V). Surprisingly, in the leaves of symptomless individuals, ToBRFV could not be detected in the inoculated or newly developed leaves. Therefore, we categorized these plants as extremely resistant to ToBRFV. We can also predict that YKEMA et al. (2020) reported similar resistance in *S. habrochaites*. However, extreme resistance to ToBRFV in *S. peruvianum*, which we found in the accession PI 308181, has not yet been reported. ToBRFV resistant *S. habrochaites* and *S. peruvianum* also proved to be highly resistant to TMV and ToMV, indicating that the resistance of these plants covers a wide range of pathogenic tomato tobamoviruses.

Our results corroborated with TMV resistance data of five wild tomato accessions (PI 390658 /= LA1739/, PI 390659, PI 379012, PI 379014 and PI 308182) derived from the GRIN Plant Germplasm database (https://npgsweb.ars-grin.gov/gringlobal/descriptordetail?id=50145). We also demonstrated the same TMV resistance result in four other *S. habrochaites* and one *S. peruvianum* accession. It would be interesting to examine the reactions of these resistant plants to resistance breaking mutants of TMV, ToMV, Ohio V strain of TMV and ToMMV (MESHI et al. 1989; CALDER and PALUKAITIS 1992; WEBER et al. 1993; BETTI et al. 1997; STRASSER and PFITZNER 2007; LI et al. 2013).

Regarding the mechanism of resistance discovered in *S. habrochaites* and *S. peruvianum*, it is important to note that we were unable to detect ToBRFV either in the inoculated or the top (systemic) leaves even after repeated mechanical inoculations of resistant plants. Thus, a high inhibition capacity of virus replication and/or cell-to-cell movement can be assumed as the cause of the resistance. However, we also presume that ToBRFV starts to replicate in some locally infected cells of resistant plants, because at elevated temperatures at 33°C, the virus moves to the top of the plants and causes severe systemic disease symptoms. Interestingly, this resistance could also be characterized by restoration of function or activity at 24°C, because after transfer the infected plants from 33°C to 24°C, the newly developed leaves recovered from the symptoms as well as from the virus itself. Temperature-dependent virus multiplication was also reported by CIRULLI & CICCARESE (1975) and FRASER & LOUGHLIN (1982). The loss of resistance to ToBRFV in *S. habrochaites* line LA1739 was also observed by grafting onto the infected susceptible rootstock (*S. lycopersicum* cv. Ceglédi). Hence, the results demonstrated that ToBRFV could replicate and move in the extremely resistant *Solanum* plants under special conditions.

6.3 Molecular characterization of resistance genes in *S. habrochaites* and *S. peruvianum*

According to the results of ASHKENAZI et al. (2020), the *Tm-1* gene, in combination with QTL2 on chromosome 9 or QTL3 on chromosome 11, confers the highest resistance to ToBRFV. Furthermore, ZINGER et al. (2021) have also speculated that the *Tm-1* locus on chromosome 2, which interacted with the locus identified on chromosome 11, are responsible for symptom reduction and resistance. Thus, we cannot compare the molecular similarities of our resistant plants with those utilized by the above authors. However, we were able to make a molecular comparison with the *S. habrochaites* genotype LYC4943 characterized by YKEMA et al. (2021).

The results proved that the *S. lycopersicum* gene Solyc08g075630 is the ortholog locus (identity 90%) to the *S. habrochaites* LYC4943 resistance gene against ToBRFV. Besides *S. habrochaites* LA2812, which was very similar to those characterized by YKEMA et al. (2021), other resistant plants in our experiments showed high sequence variability on the NBS LRR locus (Appendix VI). Therefore, it could not be excluded, that *S. habrochaites* and *S. peruvianum* accessions may carry more than a single new resistance gene to ToBRFV.

The present study demonstrated that ten accessions of *S. habrochaites* and *S. peruvianum* carry a rich repository of ToBRFV resistance that has not been reported before. Therefore, these plants can be referred to as new resistance sources of this virus.

6.4 Isolation and molecular characterization of a resistance breaking mutant of ToBRFV

The ToBRFV resistant plants did not show any symptoms and the virus was not detectable either in their inoculated or in the top (systemic) leaves by using bioassays and RT-qPCR **(Chapter 5.2.2.1** and **5.3.1)**. However, when the inoculation was repeated several times, a single plant in *S. habrochaites* accession LA1738 became infected, showing systemic mosaic symptoms. Therefore, we assumed the appearance of a mutant virus that breaks the ToBRFV resistance of *S. habrochaites* discovered in our work. After transmission through a single local lesion and propagated in *N. tabacum* Samsun, this mutant isolate, called ToBRFV-Tom2M-Jo, was compared pathologically with ToBRFV-Tom2-Jo by inoculation all the control tomatoes and the ToBRFV resistant *S. habrochaites* and *S. peruvianum* genotypes. As a result, we established that ToBRFV-Tom2M-Jo differed from Tom2-Jo only by its pathogenicity to ToBRFV resistant wild tomato accessions. Besides the pathological indications, we also wanted to prove the "mutant" theory by molecular analysis.

The complete sequence of ToBRFV-Tom2M-Jo consists of 6,394 nucleotides and encodes four open reading frames (ORFs), which is typical to other ToBRFV and tobamoviruses genome sequences deposited in NCBI Genebank. Our isolates showed 99.73% identity with ToBRF-Tom1-Jo, the first Jordan isolate of the virus (SALEM et al. 2015).

To demonstrate the molecular background of the altered pathological behavior of the new strain, sequence comparison analysis between ToBRFV-Tom2-Jo and ToBRFV-Tom2M-Jo with other 118 genome accessions of the virus were aligned. The result revealed, two amino acid substitutions (Phe^{22} → Tyr and Asn^{82} → Lys) on the 30 kDa MP of the parent isolate ToBRFV-Tom2-Jo, respectively. The changed virus MP can be responsible for breaking the extreme resistance found

in S. *habrochaites* and *S. peruvianum*. Interestingly, molecular comparison of ToBRFV-Tom2M-Jo with 118 ToBRFV genome accessions resulted that ToBRFV-Tom2M-Jo also had two unique nucleotides and amino acid substitutions in MP.

Based on the results of pathological and molecular comparisons, we conclude that ToBRFV Tom2M-Jo is really a mutant strain of ToBRFV that breaks not only the resistance conferred by *Tm-1, Tm-2* and *Tm-2²* genes but also the resistance of all ten ToBRFV resistant accessions of *S. habrochaites* and *S. peruvianum*.

6.5 Resistance breaking mutant of a resistance breaking virus

These results resemble with former results described by MESHI et al. 1989; 1992; WEBER et al. 1993 and STRASSER and PFITZNER 2007, about the role of the MP of TMV and ToMV mutants as the target of resistance breaking of *Tm-2²*. Soon after discovering the ToBRFV, MAAYAN et al. (2018) carried out sequence analysis to identify the mutation map that led to breaking the *Tm-2²* resistance. They identified 21 potential resistance-breaking mutations by sequence analysis of ToBRFV. Compared with tobamoviruses pathogenic to tomato (ToMV, TMV and Rehmannia mosaic virus (ReMV)), they pointed to nine in Rep proteins and twelve changes in viral MP. Some of these mutations' substitutions resembled with resistance-breaking TMV and ToMV, which led to overcoming *Tm-2²*. Recently, HAK & SPIEGELMAN (2021) revealed that replacing the MP sequence of ToMV with the MP of ToBRFV resulted in a recombinant virus leading to break down the *Tm-2²* resistance. Furthermore, the vital role of MP to activate the resistance was confirmed by transient expression of ToBRFV MP in *N. benthamiana* and also in resistant tomato, where the MP gene of ToBRFV and the *Tm-2²* resistance gene of tomato were transiently co-expressed (HAK and SPIEGELMAN 2021). Interestingly, YAN et al. (2021b), using chimeric MP proteins of TMV and ToBRFV, proved that six residues located in the central region 60–186 of the ToBRFV MP (H^{67}, N^{125}, K^{129}, A^{134}, I^{147}, and I^{168}) were necessary for ToBRFV overcoming *Tm-2²* carrying in transgenic tomato plants and *N. benthamiana*. Hence, the MP of ToBRFV may be responsible for breaking the unknown resistance gene in our resistance *S. habrocheties* and *S. pervianum* accessions, which probably has similar mechanism interactions as in *Tm-2* and *Tm-2²* genes against ToBRFV.

The genetic relations between the resistances of *S. pimpinellifolium*, *S. lycopersicum* and *S. habrochaites* described by HAMELINK et al. 2019; ASHKENAZI et al. 2020; YKEMA et al. 2020 and ZINGER et al. 2021 and our resistant *S. peruvianum* and *S. habrochaites* genotypes are still

unknown. Thus, it can not predict whether the mutant Tom2M-Jo isolate will be able to break the resistances mentioned by the aforementioned authors. According to these results, Tom2M-Jo is a novel adaptive viral mutant that is capable of breaking the high ToBRFV resistance recently discovered in wild tomatoes.

The type of resistance is still unknown. The immunity can be excluded, due to the fact, that the resistant plants became infected at higher temperatures and after grafting to infected rootstock. It is important to note that the Tom2M-Jo mutant of ToBRFV **(Chapter 5.3** and **6.4)** that overcame the resistance found in *S. habrochaites* and *S. peruvianum* lines differed solely from the parent virus in the MP. These results strongly indicate that the resistance is an active process and the MP triggers a resistance gene similar to the Tm-2^2 against TMV and ToMV in tomatoes (PFITZNER 2006). We cannot exclude the possibility that a strong gene silencing mechanism is also involved in suppressing virus replication and/or movement in the resistant plants (BUCHER and PRINS 2006). Adapting viruses to new resistant hosts is a well-known phenomenon (HARRISON 2002) (GALLOIS et al. 2018), which we have witnessed in the case of ToBRFV Tom2M-Jo, a "resistance breaker mutant of a resistance breaker virus."

7. CONCLUSIONS and RECOMMENDATIONS

1- Based on the results of our screening experiments covering 809 accessions of 16 *Solanum* species (sections *Lycopersicon* and *Juglandifolia*), we can conclude that susceptibility and sensitivity were the common response of tomatoes to the inoculations with ToBRFV.

2- Based on its pathogenicity to TMV and ToMV resistant cultivated tomatoes (*S. lycopersicum*) carrying the resistance genes *Tm-1*, *Tm-2* and *Tm-2²*, our ToBRV-Tom2-Jo isolate did not differ from the typical isolates of this virus. The great majority of wild tomatoes including accessions of *S. arcanum*, *S. chmielewskii*, *S. huaylasense*, *S. juglandifolium*, and *S. sitiens* (Appendix I), were never investigated for the reactions to ToBRFV, which proved susceptible to ToBRFV-Tom2-Jo. ToBRFV shows a wide range of symptoms (mosaic, leaf deformations, mottling, shoestring, and stunting). Hence, we could establish that the five species mentioned above are new experimental host plants of ToBRFV.

3- A relatively few numbers of wild tomato accessions comprised plants that remained symptomless after inoculation with ToBRV-Tom2-Jo. In contrast to their healthy habit, plants in twenty-six accessions representing *S. chilense*, *S. habrochaites*, *S. pimpinellifolium* and *S. lycopersicum* var. cerasiforme were found infected by the virus. Consequently, we classified these plants as highly tolerant to the disease. Despite the symptomless appearance of these tolerant plants, we do not propose incorporating this property into cultivated tomatoes because the tolerant plants cause an epidemic hazard as they would be "brilliant" sources of ToBRFV. However, we think that the tolerance of wild tomatoes has a genetic background that needs to be analysed in the future.

4- *S. ochranthum*, a close relative to wild tomatoes (member of the sect. *Juglandifolia*), was not studied for reactions to tobamoviruses. In our work, three accessions of this species were demonstrated to be resistant not only to ToBRFV but also to TMV and ToMV. Following mechanical inoculation, the three tobamoviruses could be detected only in inoculated leaves in the accessions LA2160, LA2162, and LA2166, of which the top leaves remained symptomless. Consequently, we categorized these accessions as new locally susceptible hosts of the virus. Two other *S. ochranthum* accessions, PI 230519 and PI 473498, reacted unusually. They were demonstrated to be highly resistant to TMV and ToMV but proved transiently susceptible to ToBRFV showing mild systemic mosaic followed by total recovery from symptoms and the virus. This recovery phenomenon of the two accessions is unusual. Hence, further studies need to be clear its genetic and molecular mechanism. The practical use of the high resistance of *S. ochranthum* to

ToBRFV is difficult due to the sexual incompatibility between *S. ochranthum* and *S. lycopersicum* or other closely related tomato species. Somatic hybridization would be surpass this genetic barrier.

5- Breeding strategies that primarily focus on using genetic resistance have proved successful in combating viruses in tomato because resistant varieties are an effective, economical, and environment-friendly approach to managing plant diseases. However, we detected plants in nine accessions of *S. habrochaites* and one of *S. peruvianum* were found remaining symptomless and also proved virus-free after inoculation with ToBRFV-Tom2-Jo. Consequently, we classified these plants as highly resistant to the virus. It is important to note that the resistant individuals in presented accessions always showed segregation; 10-50% of the plants have resistance. The origin of this segregation is unknown, but it draws our attention to the use of at least 10-15 individuals of each accession for screening resistance. Resistant plants showed no symptoms at 22-24°C, and no virus could be detected in their inoculated and newly developed leaves using bioassays and RT-qPCR. ToBRFV-resistant plants were also resistant to TMV and ToMV. Therefore, these symptomless accessions can be considered as novel sources of ToBRFV resistance and can be use in the breeding program for ToBRFV resistance. Furthermore, It would be interesting to know their resistance to other tobamoviruses such as ToMMV, Ohio V strain of TMV or the resistance breaking mutants TMV and ToMV.

6- When resistant plants were inoculated with ToBRFV-Tom2-Jo and were incubated at a temperature of 33°C in a plant growth chamber, they displayed mosaic and deformation symptoms, indicating that the resistance was broken at elevated temperature. However, when these plants were transported to the greenhouse at 24°C, their newly emerged leaves showed no symptoms, and the virus could not be detected in the new leaves. Cleft grafting was conducted using scions from a resistant plant of *S. habrochaites* LA1739 into susceptible tomato rootstock infected with ToBRFV. The scions became infected and showed mosaic symptoms, indicating ineffective resistance after grafting. Therefore, the type of resistance is still unknown. The immunity can be excluded because the resistant plants became infected at elevated temperatures and after grafting to infected rootstock. Further investigations regarding the mechanism of resistance and behavior of other resistant accessions after grafting should be done in next future.

7- Comparison the sequences of nine resistant accessions at the Solyc08g075630 loci showed high heterogeneity. Only one resistant plant accession LA2812 of *S. habrochaites* carried an allele almost identical to the previously reported resistance gene. All other resistant plants may have probably an unknown gene(s) of resistance to ToBRFV. Therefore, it could not be excluded, that *S. habrochaites* and *S. peruvianum* accessions may carry more than a single new resistance gene

to ToBRFV or carry a rich repository of ToBRFV resistance, which need to be investigate and analysis in next future.

8- In this study, we found not only new resistance sources to ToBRFV among the wild tomatoes but also a tobamovirus that infects these new resistance sources. Our pathological and molecular studies revealed that the resistance-breaking tobamovirus could be identified as a spontaneous mutant strain of ToBRFV that evolved during the inoculation experiments in our greenhouse. Both the wild ToBRFV-Tom2-Jo and the mutant ToBRFV-Tom2M-Jo isolates were fully sequenced and compared to each other. Sequence analysis revealed five nucleotide substitutions in the ToBRFV-Tom2M-Jo genome compared to ToBRFV-Tom2-Jo. Two substitutions were located at the MP gene and resulted in amino acid changes in the 30-kDa (MP) (Phe^{22} → Asn and Tyr^{82} → Lys). Furthermore, molecular comparison of ToBRFV-Tom2M-Jo with all known ToBRFV isolates in the NCBI database, resulted that ToBRFV-Tom2M-Jo also had two unique nucleotides and amino acid substitutions in MP. No amino acid changes were found in the 126-kDa and the 183-kDa Rep and the 17.5-kDa CP. Our data strongly suggest that breaking the newly discovered resistance in wild tomatoes is associated with one or two mutations on the MP gene of ToBRFV. In addition, we presume that the resistance mechanism acts similarly to those directed by the *Tm-2* and *Tm-2²* alleles, because the resistance breaker ability of the mutant Tom2M-Jo is tightly connected with change(s) within the viral MP gene. Further investigations are needed to elucidate and prove the molecular mechanisms underlying these phenomena. For example, using transgenic plants that express the 30-kDa MP of the virus and using mutagenesis to generate ToBRFV isolates with altered MP genes.

8. NEW SCIENTIFIC RESULTS

1- This is the first report on such a large-scale screening (809 accessions) that has been performed to uncover ToBRFV resistance (symptomless) and susceptibility (symptomatic) in 16 different *Solanum* species (sections *Lycopersicon* and *Juglandifolia*) germplasm such as *S. arcanum*; *S. cheesmaniae*; *S. chilense*; *S. chmielewskii*; *S. corneliomulleri*; *S. galapagense*; *S. habrochaites*; *S. huaylasense*; *S. juglandifolium*; *S. lycopersicum*; *S. neoricki*; *S. ochranthum*; *S. pennellii*; *S. peruvianum*; *S. pimpinellifolium*; and *S. sitiens*.

2- We found that a large number of them were susceptible, including the accessions of *S. arcanum*, *S. chmielewskii*, *S. huaylasense*, *S. juglandifolium*, and *S. sitiens* (Appendix I). To the best of our knowledge, the last-mentioned five species are new experiments hosts of ToBRFV.

3- We demonstrated ToBRFV tolerance in S. *chilense* and S. *habrochaites* for the first time in the literature.

4- Our work is the first report in the science dealing with the reactions of *S. ochranthum* to tobamoviruses. High levels of resistance have been demonstrated in three accessions of *S. ochranthum* (LA2160, LA2162, and LA2166) not only to ToBRFV but also TMV and ToMV. However, two other *S. ochranthum* accessions, PI 473498 and PI 230519 proved transiently susceptible to ToBRFV followed by total recovery from symptoms and the virus, but highly resistant to TMV and ToMV.

5- We demonstrated for the first time a high level of resistance, probably extreme resistance, from nine accessions of *S. habrochaites* (LA1738, LA1739, LA2171, LA2541, LA 2812, PI 308182, PI 379012, PI 379014 and PI 390659) and one of *S. peruvianum* (PI 308181) against ToBRFV, ToMV and TMV. Those accessions numbers were not reported and tested before. Therefore, they are new resistance sources of three tobamoviruses.

6. Our work proved for the first time that ToBRFV resistance found in *S. habrochaites* LA1739 does not act at elevated temperature or after grafting the scions of resistant LA1739 onto infected tomato rootstock.

7. We demonstrated for the first time a sequences comparison of Solyc08g075630 loci of nine resistant accessions showed high heterogeneity. Only one resistant plant of *S. habrochaites* carried an allele almost identical to the resistance gene reported previously. All other resistant plants may have probably unknown gene(s) of resistance to ToBRFV.

8- We isolated for the first time a mutant strain marked Tom2M-Jo of ToBRFV, that breaks down

the ToBRFV resistance of *S. habrochaites* and *S. peruvianum*. Our data strongly suggest that two amino acid changes in the viral MP gene are responsible for the altered pathological property of ToBRFV-Tom2M-Jo.

9. SUMMARY

Tomato (*Solanum lycopersicum*) is one of the most significant vegetables grown and consumed worldwide. Tomato is susceptible to many viruses, including the tobamoviruses, TMV and ToMV, which are ranked as the most important tomato pathogen. To control them, three tobamovirus resistance genes (*Tm-1, Tm-2*, and *Tm-2²*) have been introgressed into *S. lycopersicum* via crossing with wild tomato species. Resistances based on these three resistance genes have been found overcome by mutant strains of ToMV or TMV, but fortunately, these strains did not distribute. In recent years, the appearance of ToBRFV, a new tobamovirus isolated first in Jordan, caused alarm because it overcame the resistance genes *Tm-1, Tm-2*, and *Tm-2²*. The particles of ToBRFV are very stable, highly infectious, and easily transmitted mechanically. All of these properties make the control of ToBRFV infections difficult. Because the known resistance genes are not active to ToBRFV, there is an urgent demand to find new sources of resistance.

The present study aimed to screen the susceptibility and resistance of 809 accessions of wild tomatoes *Solanum* and some of their relatives to ToBRFV. Furthermore, we aimed to characterize by pathological and molecular studies the high resistance that we found in accessions of *S. habrochaites* and *S. peruvianum*. In addition, we isolated and investigated a spontaneous mutant of ToBRFV that breaks down the resistance discovered in *S. habrochaites* and *S. peruvianum*.

In the screening experiments, 3-15 young plants of 809 wild tomato accessions were mechanically inoculated with the Jordanian isolate Tom2-Jo of ToBRFV. The local and systemic reactions of the inoculated plants were evaluated and based on symptom severities; we calculated disease severity indices (DSI) for each accession. The great majority of plants of wild tomato accessions became affected by a range of viral symptoms, but 31 accessions from different tomato plant species contained symptomless plants. Symptomless plants in 26 accessions in the species of *S. lycopersicum* var. cerasiforme, *S. habrochaites, S. chilense* and *S. pimpinellifolium* were demonstrated to be infected by ToBRFV. Therefore, we classified these plants as tolerant (= symptomless carriers of the virus). Plants of two accessions, PI 473498 and PI 230519 of *S. ochranthum*, a species distantly related to tomatoes reacted unusually to inoculation with ToBRFV. They initially showed mild systemic mosaic symptoms and contained an infective virus, but later recovered from the symptoms and the virus could not detected in the new top leaves. Three other *S. ochranthum* accessions, LA2160, LA2162 and LA2166 remained symptomless and demonstrated to be free in top leaves not only of ToBRFV but also of TMV and ToMV. Despite this high level of resistance, the use of *S. ochranthum* in breeding programs is difficult because of

the sexual incompatibility of this species to *S. lycopersicum* or other closely related tomato species.

Besides the tolerant wild tomatoes and the resistant *S. ochranthum*, we found numerous plants in nine accessions of *S. habrochaites* (LA1738, LA1739, LA2171, LA2541, LA 2812, PI 308182, PI 379012, PI 379014 and PI 390659) and one accession of *S. peruvianum* (PI 308181) showing high resistance to ToBRFV. These plants remained symptomless following three subsequent inoculations with ToBRFV and its inoculated and top leaves assayed with biotest and RT-qPCR proved virus-free during the experiments. These plants showed high resistance also to TMV and ToMV. However, when these resistant plants were inoculated with ToBRFV and incubated at 33°C became infected, showing severe systemic symptoms and containing a high amount of infective virus. Unexpectedly, when they transferred at 24°C, the same plants recovered from symptoms and no virus could be detected in their newly developed leaves. Cleft grafting with scions from a resistant plant of *S. habrochaites* LA1739 into ToBRFV-infected susceptible tomato rootstock, the scions became infected and expressed mosaic symptoms.

Sequences comparison revealed only one resistant plant of *S. habrochaites* carried an allele almost identical to the resistance gene reported previously. All other resistant plants may have the probably unknown gene(s) of resistance to ToBRFV. Therefore, these symptomless plants accessions can be considered as novel sources of ToBRFV resistance and can be using in breeding programs for ToBRFV resistance.

In the course of the inoculation experiments with ToBRFV-Tom2-Jo, we observed mosaic symptoms in a single inoculated *S. habrochaites* plant, in which the other vegetatively propagated sisters plants remained symptomless. We hypothesized that a new spontaneous mutant of ToBRFV appeared in this case, which breaks down the resistance of *S. habrochaites*. Pathological comparison between two isolates revealed that the suspected mutant isolate causes systemic mosaic symptoms on all plants resistant; In contrast, the parent isolate showed no symptoms and proved no virus by bioassay and RT-qPCR. For molecular analysis, we sequenced both the parent virus and its suspected mutant ToBRFV-Tom2M-Jo. Sequence analysis revealed five nucleotide substitutions in the mutant ToBRFV-Tom2M-Jo genome compared to parent ToBRFV-Tom2-Jo. Two unique substitutions were located at the MP gene and resulted in amino acid changes in the 30-kDa (MP) (Phe^{22}→Asn, and Tyr^{82} → Lys), which compared with all known ToBRFV isolates in the NCBI database. No amino acid changes were found in the 126-kDa and the 183-kDa Rep and 17.5-kDa CP. Because the breaker ability of the mutant Tom2M-Jo is tightly associated with change(s) within the viral MP gene, we presume that the resistance mechanism of high resistance to ToBRFV in wild tomatoes acts similarly to those directed by the *Tm-2* and *Tm-2²* alleles to

tobamoviruses. Further investigations and experiments are needed to prove the molecular mechanisms, such as using the generation of transgenic plants which express the 30-kDa movement proteins and mutagenesis on MP gene.

Reviewing our work, the most important results are:

We discovered high resistance to ToBRFV and two other tobamoviruses in *S. ochranthum*, *S. habrochaites* and *S. peruvianum*. These plants species carry a rich repository of ToBRFV resistance which were not reported before.

We isolated and characterized a new mutant of ToBRFV. It indicates that this virus can rapidly adapt to new resistant tomato genotypes; therefore, caution and monitoring should be taken to this new tobamovirus ToBRFV.

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11. ACKNOWLEDGEMENTS

First and foremost, I would like to thank God for letting me through all the difficulties. I have experienced your guidance day by day. You are the one who let me finish my degree. I will keep on trusting you for my future.

I am grateful to my family had to grudgingly learn to accept my separation from them and still gave me nothing but support, day after day, both emotionally and financially: my love and gratitude for them can hardly be expressed in words. I dedicate this thesis to my parents.

I am deeply grateful for the continuous support, insight and patience of my supervisors Dr. Zoltán Szabó (main supervisors) and Dr. Pál Salamon (scientific advisor): without their constant trust and, sometimes, gentle prodding, and immense knowledge this thesis would not have been completed.

I would also like to thank Dr. Nida Salem from the University of Jordan for her time, comments, and encouragement.

I am also grateful to the staff of the applied plant genomics group, Dr. Zoltán Tóth (Research Fellow), Edit Maszlag, (Laboratory assistant), Márta Csányi (Laboratory tissue culture), László Földesi (Farmer assistant), my fellows PhD students Francis Kiemo, Lilla Hajnik, Máté Tóth and Zoltán Tóth for their kind help during my work stages and in molecular work.

I am also grateful to the Stipendium Hungaricum Scholarship and the Tempus Public Foundation for providing the funding that allowed me to undertake my doctoral research

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13. Appendix I

Screening of *Solanum* (sections. *Lycopersicon* and *Juglandifolia*) germplasm to ToBRFV (Group A)

Number	Tomato species	Accession	Number of plants (Resistant or Tolerance/susceptible)	Symptoms	Reaction to ToBRFV	Disease severity Indexes (DSI)
1	*S. pennellii*	LA0716	0/10	Mosaic	Susceptible	40%
2	*S. pennellii*	LA0751	0/10	Mosaic, Deformation	Susceptible	60%
3	*S. pennellii*	LA1272	0/10	Mosaic, Deformation	Susceptible	60%
4	*S. pennellii*	LA1277	0/10	Mosaic, Deformation	Susceptible	60%
5	*S. pennellii*	LA1356	0/10	Mosaic, Deformation	Susceptible	60%
6	*S. pennellii*	LA1367	0/10	Mosaic, Deformation	Susceptible	60%
7	*S. pennellii*	LA1376	0/10	Mosaic, Deformation	Susceptible	60%
8	*S. pennellii*	LA1656	0/10	Mosaic, Deformation	Susceptible	60%
9	*S. pennellii*	LA1674	0/10	Mosaic, Deformation	Susceptible	60%
10	*S. pennellii*	LA1724	0/10	Mosaic, Deformation	Susceptible	60%
11	*S. pennellii*	LA1732	0/10	Mosaic, Deformation	Susceptible	60%
12	*S. pennellii*	LA1733	0/10	Mosaic, Deformation	Susceptible	60%
13	*S. pennellii*	LA1926	0/10	Mosaic, Deformation	Susceptible	60%
14	*S. pennellii*	LA1946	0/10	Mosaic, Deformation	Susceptible	60%
15	*S. pennellii*	LA2580	0/10	Mosaic, Deformation	Susceptible	60%
16	*S. pennellii*	LA2963	0/10	Mosaic, Deformation	Susceptible	60%
17	*S. pennellii*	PI 503516	0/10	Mosaic	Susceptible	40%
18	*S. pennellii*	PI 473464	0/10	Mosaic, Mild Mosaic	Susceptible	40%
19	*S.pimpinellifolium*	LA1478	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
20	*S.pimpinellifolium*	LA1521	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
21	*S.pimpinellifolium*	LA1576	0/10	Mosaic, Deformation, Rolling	Susceptible	80%
22	*S.pimpinellifolium*	LA1584	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
23	*S.pimpinellifolium*	LA1590	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
24	*S.pimpinellifolium*	LA2102	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
25	*S.pimpinellifolium*	LA2181	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
26	*S.pimpinellifolium*	LA2183	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
27	*S.pimpinellifolium*	LA2852	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
28	*S.pimpinellifolium*	LA1599	0/10	Mosaic, Deformation	Susceptible	60%
29	*S.pimpinellifolium*	LA2533	0/10	Mosaic, Deformation	Susceptible	60%
30	*S.pimpinellifolium*	LA1689	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
31	*S.pimpinellifolium*	LA1602	0/10	Mild Mosaic	Susceptible	20%
32	*S.pimpinellifolium*	LA2401	0/10	Mild Mosaic	Susceptible	20%
33	*S.pimpinellifolium*	LA0373	0/10	Mosaic	Susceptible	40%
34	*S.pimpinellifolium*	LA0400	0/10	Mosaic	Susceptible	40%
35	*S.pimpinellifolium*	LA0442	0/10	Mosaic	Susceptible	40%
36	*S.pimpinellifolium*	LA0722	0/10	Mosaic	Susceptible	40%
37	*S.pimpinellifolium*	LA1237	0/10	Mosaic	Susceptible	40%
38	*S.pimpinellifolium*	LA1245	0/10	Mosaic	Susceptible	40%

39	*S.pimpinellifolium*	LA1279	0/10	Mosaic	Susceptible	40%
40	*S.pimpinellifolium*	LA1578	0/10	Mosaic	Susceptible	40%
41	*S.pimpinellifolium*	LA1586	0/10	Mosaic	Susceptible	40%
42	*S.pimpinellifolium*	LA1593	0/10	Mosaic	Susceptible	20%
43	*S.pimpinellifolium*	LA1659	0/10	Mosaic	Susceptible	60%
44	*S.pimpinellifolium*	LA1936	0/10	Mosaic	Susceptible	60%
45	*S.pimpinellifolium*	LA1606	0/10	Mosaic, Rolling	Susceptible	60%
46	*S.pimpinellifolium*	LA1683	0/10	Mosaic, Rolling	Susceptible	60%
47	*S.pimpinellifolium*	LA0411	0/10	Mosaic, Deformation	Susceptible	60%
48	*S.pimpinellifolium*	LA1246	0/10	Mosaic, Deformation	Susceptible	60%
49	*S.pimpinellifolium*	LA1261	0/10	Mosaic, Deformation	Susceptible	60%
50	*S.pimpinellifolium*	LA1335	0/10	Mosaic, Deformation	Susceptible	60%
51	*S.pimpinellifolium*	LA1371	0/10	Mosaic, Deformation	Susceptible	60%
52	*S.pimpinellifolium*	LA1582	0/10	Mosaic, Deformation	Susceptible	60%
53	*S.pimpinellifolium*	LA1375	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
54	*S.pimpinellifolium*	LA1924	10/0	all No symptoms	all Tolerant	0%
55	*S.pimpinellifolium*	LA1547	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
56	*S.pimpinellifolium*	LA1301	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
57	*S.pimpinellifolium*	LA0417	0/10	Rolling , Deformation , Mosaic	Susceptible	60%
58	*S.pimpinellifolium*	LA1429	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
59	*S.pimpinellifolium*	LA1579	10/0	all No symptoms	all Tolerant	0%
60	*S.pimpinellifolium*	LA1580	0/10	Mild Mosaic	Susceptible	20%
61	*S.pimpinellifolium*	LA1598	0/10	Mosaic, Deformation	Susceptible	60%
62	*S.pimpinellifolium*	LA1600	0/10	Mosaic, Deformation	Susceptible	60%
63	*S.pimpinellifolium*	LA1603	0/10	Mosaic, Deformation	Susceptible	60%
64	*S.pimpinellifolium*	LA1605	0/10	Mild Mosaic	Susceptible	20%
65	*S.pimpinellifolium*	LA1607	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
66	*S.pimpinellifolium*	LA1608	0/10	Mosaic, Deformation	Susceptible	60%
67	*S.pimpinellifolium*	LA1611	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
68	*S.pimpinellifolium*	LA1612	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
69	*S.pimpinellifolium*	LA1613	0/10	Mosaic, Deformation	Susceptible	60%
70	*S.pimpinellifolium*	LA1615	0/10	Mosaic	Susceptible	40%
71	*S.pimpinellifolium*	LA1617	0/10	Mosaic, Deformation	Susceptible	60%
72	*S.pimpinellifolium*	LA1618	0/10	Mosaic, Deformation	Susceptible	60%
73	*S.pimpinellifolium*	LA1628	0/10	Mosaic, Deformation	Susceptible	60%
74	*S.pimpinellifolium*	LA1629	0/10	Mosaic	Susceptible	40%
75	*S.pimpinellifolium*	LA1630	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
76	*S.pimpinellifolium*	LA1631	0/10	Mosaic, Deformation	Susceptible	60%
77	*S.pimpinellifolium*	LA1634	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
78	*S.pimpinellifolium*	LA1635	0/10	Mosaic, Mild Mosaic	Susceptible	40%
79	*S.pimpinellifolium*	LA1636	0/10	Mosaic, Mild Mosaic	Susceptible	40%
80	*S.pimpinellifolium*	LA1637	0/10	Mosaic, Deformation	Susceptible	60%
81	*S.pimpinellifolium*	LA1638	0/10	Mosaic	Susceptible	60%
82	*S.pimpinellifolium*	LA1645	0/10	Mosaic	Susceptible	60%

83	*S.pimpinellifolium*	LA1651	0/10	Mosaic	Susceptible	60%
84	*S.pimpinellifolium*	LA1652	0/10	Mosaic	Susceptible	60%
85	*S.pimpinellifolium*	LA1660	0/10	Mosaic	Susceptible	60%
86	*S.pimpinellifolium*	LA1661	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
87	*S.pimpinellifolium*	LA1670	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
88	*S.pimpinellifolium*	LA1676	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
89	*S.pimpinellifolium*	LA1678	0/10	Mosaic, Mild Mosaic	Susceptible	40%
90	*S.pimpinellifolium*	LA1679	10/0	all No symptoms	all Tolerant	0%
91	*S.pimpinellifolium*	LA1680	0/10	Mosaic	Susceptible	60%
92	*S.pimpinellifolium*	LA1682	0/10	Mosaic	Susceptible	60%
93	*S.pimpinellifolium*	LA1684	0/10	Mild Mosaic	Susceptible	20%
94	*S.pimpinellifolium*	LA1685	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
95	*S.pimpinellifolium*	LA1686	0/10	Mosaic	Susceptible	60%
96	*S.pimpinellifolium*	LA1687	0/10	Mosaic	Susceptible	60%
97	*S.pimpinellifolium*	LA1688	0/10	Mosaic	Susceptible	60%
98	*S.pimpinellifolium*	LA1690	0/10	Mosaic	Susceptible	60%
99	*S.pimpinellifolium*	LA1697	0/10	Mosaic	Susceptible	60%
100	*S.pimpinellifolium*	LA1719	0/10	Mosaic	Susceptible	60%
101	*S.pimpinellifolium*	LA1720	0/10	Mosaic	Susceptible	60%
102	*S.pimpinellifolium*	LA1728	10/0	all No symptoms	all Tolerant	0%
103	*S.pimpinellifolium*	LA1729	0/10	Mosaic, Deformation	Susceptible	60%
104	*S.pimpinellifolium*	LA1742	0/10	Mosaic	Susceptible	40%
105	*S.pimpinellifolium*	LA1781	0/10	Mosaic, Deformation	Susceptible	60%
106	*S.pimpinellifolium*	LA1810	0/10	Mosaic, Deformation	Susceptible	60%
107	*S.pimpinellifolium*	LA2866	0/10	Mosaic, Deformation	Susceptible	60%
108	*S.pimpinellifolium*	LA2903	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
109	*S.pimpinellifolium*	LA2904	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
110	*S.pimpinellifolium*	LA2914A	0/10	Mosaic	Susceptible	60%
111	*S.pimpinellifolium*	LA2914B	0/10	Mosaic, Deformation	Susceptible	60%
112	*S.pimpinellifolium*	LA2915	0/10	Mosaic, Deformation	Susceptible	60%
113	*S.pimpinellifolium*	LA2933	0/10	Mosaic	Susceptible	40%
114	*S.pimpinellifolium*	LA2934	0/10	Mild Mosaic	Susceptible	20%
115	*S.pimpinellifolium*	LA2966	0/10	Mild Mosaic	Susceptible	20%
116	*S.pimpinellifolium*	LA2974	0/10	Mosaic	Susceptible	60%
117	*S.pimpinellifolium*	LA2982	10/0	all No symptoms	all Tolerant	0%
118	*S.pimpinellifolium*	LA2983	0/10	Mosaic, Deformation	Susceptible	60%
119	*S.pimpinellifolium*	LA3123	0/10	Mosaic, Deformation	Susceptible	60%
120	*S.pimpinellifolium*	LA3158	0/10	Mosaic, Deformation	Susceptible	60%
121	*S.pimpinellifolium*	LA3159	0/10	Mosaic, Deformation	Susceptible	60%
122	*S.pimpinellifolium*	LA3160	0/10	Mosaic, Deformation	Susceptible	60%
123	*S.pimpinellifolium*	LA3161	0/10	Mosaic, Deformation	Susceptible	60%
124	*S.pimpinellifolium*	LA3330	0/10	Mosaic, Deformation	Susceptible	60%
125	*S.pimpinellifolium*	LA3331	0/10	Mosaic, Deformation	Susceptible	60%
126	*S.pimpinellifolium*	LA1355	0/10	Mild Mosaic	Susceptible	20%

127	*S.pimpinellifolium*	PI 79532	0/10	Mild Mosaic	Susceptible	20%
128	*S.pimpinellifolium*	PI 110595	0/10	Mosaic	Susceptible	60%
129	*S.pimpinellifolium*	PI 124039	0/10	Mosaic, Deformation	Susceptible	60%
130	*S.pimpinellifolium*	PI 126932	0/10	Mosaic, Deformation	Susceptible	60%
131	*S.pimpinellifolium*	PI 126933	0/10	Mosaic	Susceptible	40%
132	*S.pimpinellifolium*	PI 127807	0/10	Mosaic, Deformation	Susceptible	60%
133	*S.pimpinellifolium*	PI 143524	0/10	Mosaic, Deformation	Susceptible	60%
134	*S.pimpinellifolium*	PI 143527	0/10	Mild Mosaic	Susceptible	20%
135	*S.pimpinellifolium*	PI 205009	0/10	Mosaic	Susceptible	60%
136	*S.pimpinellifolium*	PI 212409	0/10	Mosaic	Susceptible	40%
137	*S.pimpinellifolium*	PI 230327	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
138	*S.pimpinellifolium*	PI 251316	0/10	Mosaic	Susceptible	60%
139	*S.pimpinellifolium*	PI 263589	0/10	Mosaic, Deformation	Susceptible	60%
140	*S.pimpinellifolium*	PI 270444	0/10	Mosaic, Deformation	Susceptible	60%
141	*S.pimpinellifolium*	PI 270445	0/10	Mosaic, Deformation	Susceptible	60%
142	*S.pimpinellifolium*	PI 270446	0/10	Mosaic, Deformation	Susceptible	60%
143	*S.pimpinellifolium*	PI 270448	0/10	Mosaic, Deformation	Susceptible	60%
144	*S.pimpinellifolium*	PI 270449	0/10	Mosaic, Deformation	Susceptible	60%
145	*S.pimpinellifolium*	PI 303662	0/10	Mosaic, Deformation	Susceptible	60%
146	*S.pimpinellifolium*	PI 306216	0/10	Mosaic	Susceptible	40%
147	*S.pimpinellifolium*	PI 313943	0/10	Mild Mosaic	Susceptible	20%
148	*S.pimpinellifolium*	PI 340905	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
149	*S.pimpinellifolium*	PI 344103	0/10	Mosaic	Susceptible	40%
150	*S.pimpinellifolium*	PI 346340	0/10	Mosaic	Susceptible	60%
151	*S.pimpinellifolium*	PI 370093	0/10	Mosaic, Deformation	Susceptible	60%
152	*S.pimpinellifolium*	PI 375937	0/10	Mosaic, Deformation	Susceptible	60%
153	*S.pimpinellifolium*	PI 379058	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
154	*S.pimpinellifolium*	PI 390519	0/10	Mosaic	Susceptible	60%
155	*S.pimpinellifolium*	PI 390688	0/10	Mosaic, Deformation	Susceptible	60%
156	*S.pimpinellifolium*	PI 390689	0/10	Mosaic, Deformation	Susceptible	60%
157	*S.pimpinellifolium*	PI 390697	0/10	Mosaic	Susceptible	60%
158	*S.pimpinellifolium*	PI 390699	0/10	Mosaic, Deformation	Susceptible	60%
159	*S.pimpinellifolium*	PI 390700	0/10	Mosaic, Deformation	Susceptible	60%
160	*S.pimpinellifolium*	PI 390701	0/10	Mosaic, Deformation	Susceptible	60%
161	*S.pimpinellifolium*	PI 390702	0/10	Mosaic, Deformation	Susceptible	60%
162	*S.pimpinellifolium*	PI 390703	0/10	Mosaic, Deformation	Susceptible	60%
163	*S.pimpinellifolium*	PI 390704	0/10	Mosaic, Deformation	Susceptible	60%
164	*S.pimpinellifolium*	PI 390705	0/10	Mosaic, Deformation	Susceptible	60%
165	*S.pimpinellifolium*	PI 390706	0/10	Mosaic, Deformation	Susceptible	60%
166	*S.pimpinellifolium*	PI 390707	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
167	*S.pimpinellifolium*	PI 390709	0/10	Mosaic, Deformation	Susceptible	60%
168	*S.pimpinellifolium*	PI 390712	0/10	Mosaic	Susceptible	40%
169	*S.pimpinellifolium*	PI 390717	0/10	Mosaic	Susceptible	60%
170	*S.pimpinellifolium*	PI 390719	0/10	Mosaic, Deformation	Susceptible	60%

171	*S.pimpinellifolium*	PI 390720	0/10	Mosaic	Susceptible	60%
172	*S.pimpinellifolium*	PI 390721	0/10	Mosaic	Susceptible	60%
173	*S.pimpinellifolium*	PI 390722	0/10	Mosaic	Susceptible	60%
174	*S.pimpinellifolium*	PI 390723	0/10	Mosaic	Susceptible	60%
175	*S.pimpinellifolium*	PI 390731	0/10	Mosaic, Deformation, Rolling	Susceptible	80%
176	*S.pimpinellifolium*	PI 390732	0/10	Mosaic	Susceptible	60%
177	*S.pimpinellifolium*	PI 390733	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
178	*S.pimpinellifolium*	PI 390738	0/10	Mosaic, Deformation	Susceptible	60%
179	*S.pimpinellifolium*	PI 390739	0/10	Mosaic, Deformation	Susceptible	60%
180	*S.pimpinellifolium*	PI 390741	0/10	Mosaic, Deformation	Susceptible	60%
181	*S.pimpinellifolium*	PI 390742	0/10	Mosaic, Deformation	Susceptible	60%
182	*S.pimpinellifolium*	PI 390743	0/10	Mosaic, Deformation	Susceptible	60%
183	*S.pimpinellifolium*	PI 390745	0/10	Mosaic, Deformation	Susceptible	60%
184	*S.pimpinellifolium*	PI 390746	0/10	Mosaic, Deformation	Susceptible	60%
185	*S.pimpinellifolium*	PI 390747	0/10	Mosaic, Deformation	Susceptible	60%
186	*S.pimpinellifolium*	PI 390748	0/10	Mosaic, Deformation	Susceptible	60%
187	*S.pimpinellifolium*	PI 390749	0/10	Mosaic, Deformation	Susceptible	60%
188	*S.pimpinellifolium*	PI 390750	0/10	Mosaic, Deformation	Susceptible	60%
189	*S.pimpinellifolium*	PI 390751	0/10	Mosaic, Deformation	Susceptible	60%
190	*S.pimpinellifolium*	PI 390752	0/10	Mosaic, Deformation	Susceptible	60%
191	*S.pimpinellifolium*	PI 407535	0/10	Mosaic, Deformation	Susceptible	60%
192	*S.pimpinellifolium*	PI 407536	0/10	Mosaic, Deformation	Susceptible	60%
193	*S.pimpinellifolium*	PI 407538	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
194	*S.pimpinellifolium*	PI 407540	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	60%
195	*S.pimpinellifolium*	PI 407542	0/10	Mosaic, Deformation	Susceptible	60%
196	*S.pimpinellifolium*	PI 407543	0/10	Mosaic, Deformation	Susceptible	60%
197	*S.pimpinellifolium*	PI 407544	0/10	Mosaic, Deformation	Susceptible	60%
198	*S.pimpinellifolium*	PI 407547	0/10	Mosaic, Deformation	Susceptible	60%
199	*S.pimpinellifolium*	PI 407548	0/10	Mosaic, Deformation	Susceptible	60%
200	*S.pimpinellifolium*	PI 407550	0/10	Mosaic, Deformation	Susceptible	60%
201	*S.pimpinellifolium*	PI 407551	0/10	Mosaic, Deformation	Susceptible	60%
202	*S.pimpinellifolium*	PI 407552	0/10	Mosaic	Susceptible	60%
203	*S.pimpinellifolium*	PI 407553	0/10	Mosaic	Susceptible	60%
204	*S.pimpinellifolium*	PI 407555	0/10	Mosaic, Deformation	Susceptible	60%
205	*S.pimpinellifolium*	PI 407556	0/10	Mosaic, Deformation	Susceptible	60%
206	*S.pimpinellifolium*	PI 407558	0/10	Mosaic, Deformation	Susceptible	60%
207	*S.pimpinellifolium*	PI 438898	0/10	Mosaic, Deformation	Susceptible	60%
208	*S.pimpinellifolium*	PI 452284	0/10	Mosaic, Deformation	Susceptible	60%
209	*S.pimpinellifolium*	PI 503517	0/10	Mosaic, Deformation	Susceptible	60%
210	*S.pimpinellifolium*	PI 503521	0/10	Mosaic	Susceptible	60%
211	*S.pimpinellifolium*	PI 634844	0/10	Mosaic	Susceptible	60%
212	*S.pimpinellifolium*	LA1633	0/10	Rolling , Deformation , Mosaic	Susceptible	60%
213	*S.pimpinellifolium*	LA0397	0/10	Mosaic	Susceptible	40%
214	*S.pimpinellifolium*	LA1263	0/10	Mosaic, Deformation	Susceptible	60%

215	*S.pimpinellifolium*	LA1589	0/10	Mosaic	Susceptible	40%
216	*S.pimpinellifolium*	LA2093	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
217	*S.pimpinellifolium*	LA1614	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
218	*S.pimpinellifolium*	LA0100	0/10	Mosaic	Susceptible	40%
219	*S.pimpinellifolium*	LA0114	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
220	*S.pimpinellifolium*	LA0121	0/10	Mosaic	Susceptible	40%
221	*S.pimpinellifolium*	LA0122	0/10	Mosaic	Susceptible	40%
222	*S.pimpinellifolium*	LA0369	0/10	Mosaic, Deformation	Susceptible	60%
223	*S.pimpinellifolium*	LA0375	0/10	Mosaic, Deformation	Susceptible	60%
224	*S.pimpinellifolium*	LA0376	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
225	*S.pimpinellifolium*	LA0381	0/10	Mosaic, Deformation	Susceptible	60%
226	*S.pimpinellifolium*	LA0391	0/10	Mosaic	Susceptible	40%
227	*S.pimpinellifolium*	LA0398	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
228	*S.pimpinellifolium*	LA0412	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
229	*S.pimpinellifolium*	LA0413	0/10	Mosaic	Susceptible	40%
230	*S.pimpinellifolium*	LA0418	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
231	*S.pimpinellifolium*	LA0420	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
232	*S.pimpinellifolium*	LA0443	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
233	*S.pimpinellifolium*	LA0480	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
234	*S.pimpinellifolium*	LA0753	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
235	*S.pimpinellifolium*	LA1236	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
236	*S.pimpinellifolium*	LA1242	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
237	*S.pimpinellifolium*	LA1248	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
238	*S.pimpinellifolium*	LA1256	0/10	Mosaic	Susceptible	40%
239	*S.pimpinellifolium*	LA1258	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
240	*S.pimpinellifolium*	LA1259	0/10	Mosaic	Susceptible	40%
241	*S.pimpinellifolium*	LA1260	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
242	*S.pimpinellifolium*	LA1262	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
243	*S.pimpinellifolium*	LA1269	0/10	Mosaic, Deformation	Susceptible	60%
244	*S.pimpinellifolium*	LA1280	0/10	Mosaic, Deformation	Susceptible	60%
245	*S.pimpinellifolium*	LA1332	0/10	Mosaic	Susceptible	40%
246	*S.pimpinellifolium*	LA1341	0/10	Mosaic, Deformation	Susceptible	60%
247	*S.pimpinellifolium*	LA1342	0/10	Mosaic	Susceptible	40%
248	*S.pimpinellifolium*	LA1343	0/10	Mosaic, Deformation	Susceptible	60%
249	*S.pimpinellifolium*	LA1344	0/10	Mosaic	Susceptible	40%
250	*S.pimpinellifolium*	LA1345	0/10	Mosaic	Susceptible	40%
251	*S.pimpinellifolium*	LA1348	0/10	Mosaic	Susceptible	40%
252	*S.pimpinellifolium*	LA1349	0/10	Mosaic	Susceptible	40%
253	*S.pimpinellifolium*	LA1357	0/10	Mosaic	Susceptible	40%
254	*S.pimpinellifolium*	LA1359	0/10	Mosaic	Susceptible	40%
255	*S.pimpinellifolium*	LA1370	0/10	Mosaic	Susceptible	40%

256	*S.pimpinellifolium*	LA1374	0/10	Mosaic	Susceptible	40%
257	*S.pimpinellifolium*	LA1380	0/10	Mosaic, Deformation	Susceptible	60%
258	*S.pimpinellifolium*	LA1381	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
259	*S.pimpinellifolium*	LA1382	0/10	Mosaic	Susceptible	40%
260	*S.pimpinellifolium*	LA1383	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
261	*S.pimpinellifolium*	LA1384	0/10	Mosaic	Susceptible	40%
262	*S.pimpinellifolium*	LA1416	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
263	*S.pimpinellifolium*	LA1428	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
264	*S.pimpinellifolium*	LA1466	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
265	*S.pimpinellifolium*	LA1470	0/10	Mosaic, Deformation	Susceptible	60%
266	*S.pimpinellifolium*	LA1471	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
267	*S.pimpinellifolium*	LA1472	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
268	*S.pimpinellifolium*	LA1514	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
269	*S.pimpinellifolium*	LA1520	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
270	*S.pimpinellifolium*	LA1561	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
271	*S.pimpinellifolium*	LA1562	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
272	*S.pimpinellifolium*	LA1571	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
273	*S.pimpinellifolium*	LA1572	0/10	Mosaic	Susceptible	40%
274	*S.pimpinellifolium*	LA1573	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
275	*S. arcanum*	LA1351	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
276	*S. arcanum core*	LA2917	0/10	Deformation, Mosaic	Susceptible	80%
277	*S. arcanum core*	LA1346	0/10	Deformation, Mosaic	Susceptible	80%
278	*S. arcanum core*	LA1626	0/10	Deformation, Mosaic	Susceptible	80%
279	*S. arcanum core*	LA2172	0/10	Deformation, Mosaic	Susceptible	80%
280	*S. arcanum core*	LA2326	0/10	Deformation, Mosaic	Susceptible	80%
281	*S. arcanum core*	LA2152	0/10	Mosaic	Susceptible	40%
282	*S. arcanum core*	PI 127828	0/10	Mosaic, Deformation	Susceptible	60%
283	*S. arcanum core*	PI 127829	0/10	Mosaic, Deformation	Susceptible	60%
284	*S. cheesmaniae*	LA0166	0/10	ShoesString	Susceptible	80%
285	*S. cheesmaniae*	LA0421	0/10	ShoesString	Susceptible	80%
286	*S. cheesmaniae*	LA0426	0/10	ShoesString	Susceptible	80%
287	*S. cheesmaniae*	LA0434	0/10	ShoesString	Susceptible	80%
288	*S. cheesmaniae*	LA0437	0/10	ShoesString	Susceptible	80%
289	*S. cheesmaniae*	LA0521	0/10	ShoesString	Susceptible	80%
290	*S. cheesmaniae*	LA0522	0/10	Mosaic, Deformation	Susceptible	60%
291	*S. cheesmaniae*	LA0524	0/10	Mosaic, Deformation	Susceptible	60%
292	*S. cheesmaniae*	LA0528B	0/10	ShoesString	Susceptible	80%
293	*S. cheesmaniae*	PI 379035	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
294	*S. cheesmaniae core*	LA0428	0/10	Mosaic, ShoesString	Susceptible	80%
295	*S. cheesmaniae core*	LA0422	0/10	Mosaic, ShoesString, Rolling	Susceptible	80%
296	*S. cheesmaniae core*	LA0429	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%

297	*S. cheesmaniae core*	LA0531	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
298	*S. cheesmaniae core*	LA1039	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
299	*S. cheesmaniae core*	LA1041	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
300	*S. cheesmaniae core*	LA1406	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
301	*S. cheesmaniae core*	LA1407	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
302	*S. cheesmaniae core*	LA1412	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
303	*S. cheesmaniae core*	LA1450	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
304	*S. cheesmaniae core*	LA1409	0/10	Mosaic	Susceptible	40%
305	*S. chilense*	PI 251313	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
306	*S. chilense core*	LA2930	0/10	Mosaic	Susceptible	60%
307	*S. chilense core*	LA2946	0/10	Mosaic, Rolling	Susceptible	60%
308	*S. chilense core*	LA3114	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
309	*S. chilense core*	LA2884	0/10	Mosaic, ShoesString, Rolling	Susceptible	100%
310	*S. chilense core*	LA1930	0/10	Mild Mosaic	Susceptible	40%
311	*S. chilense core*	LA2750	0/10	Mosaic	Susceptible	20%
312	*S. chilense core*	LA1958	0/10	Mosaic, Deformation	Susceptible	60%
313	*S. chilense core*	LA1960	0/10	Mosaic, Deformation	Susceptible	60%
314	*S. chilense core*	LA1963	0/10	Mosaic, Deformation	Susceptible	60%
315	*S. chilense core*	LA1967	0/10	Mosaic, Deformation	Susceptible	60%
316	*S. chilense core*	LA1969	0/10	Mosaic, Deformation	Susceptible	60%
317	*S. chilense core*	LA2748	0/10	Mosaic, Deformation	Susceptible	60%
318	*S. chilense core*	LA2753	0/10	Mosaic, Deformation	Susceptible	60%
319	*S. chilense core*	LA2759	0/10	Mosaic, Deformation	Susceptible	60%
320	*S. chilense core*	LA2765	0/10	Mosaic, Deformation	Susceptible	60%
321	*S. chilense core*	LA2771	0/10	Mosaic, Deformation	Susceptible	60%
322	*S. chilense core*	LA2778	0/10	Mosaic, Deformation	Susceptible	60%
323	*S. chilense core*	LA2880	0/10	Mosaic, Deformation	Susceptible	60%
324	*S. chilense core*	LA1932	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
325	*S. chilense*	LA2779	0/10	Mosaic	Susceptible	40%
326	*S. chilense*	LA0458	0/10	Mosaic, Deformation	Susceptible	60%
327	*S. chilense*	LA0752	0/10	Mosaic, Deformation	Susceptible	60%
328	*S. chilense*	LA0130	0/10	Mosaic	Susceptible	60%
329	*S. chilense*	LA0294	0/10	ShoesString	Susceptible	80%
330	*S. chilense*	LA0456	0/10	Mosaic, Deformation	Susceptible	60%
331	*S. chilense*	LA0460	0/10	Mosaic	Susceptible	60%
332	*S. chilense*	LA1782	0/10	Mild Mosaic	Susceptible	20%
333	*S. chilense*	LA1917	0/10	Mosaic, Deformation	Susceptible	60%
334	*S. chilense*	LA1931	0/10	Mild Mosaic	Susceptible	20%
335	*S. chilense*	LA1938	0/10	Mosaic	Susceptible	40%
336	*S. chilense*	LA1959	0/10	Mosaic, Deformation	Susceptible	60%
337	*S. chilense*	LA1961	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
338	*S. chilense*	LA1962	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
339	*S. chilense*	LA1965	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%

340	*S. chilense*	LA1968	0/10	Mosaic	Susceptible	60%
341	*S. chilense*	LA1970	0/10	Mosaic, Deformation	Susceptible	60%
342	*S. chilense*	LA1972	0/10	Mosaic	Susceptible	60%
343	*S. chilense*	LA2405	0/10	Mosaic, Deformation	Susceptible	60%
344	*S. chilense*	LA2406	0/10	Mosaic, Deformation	Susceptible	60%
345	*S. chilense*	LA2729	0/10	Mosaic, Deformation	Susceptible	60%
346	*S. chilense*	LA2731	0/10	Mosaic, Deformation	Susceptible	60%
347	*S. chilense*	LA2737	0/10	Mosaic, Deformation	Susceptible	60%
348	*S. chilense*	LA2739	0/10	Mosaic, Deformation	Susceptible	60%
349	*S. chilense*	LA2743	0/10	Mosaic, Deformation	Susceptible	60%
350	*S. chilense*	LA2746	0/10	Mosaic, Deformation	Susceptible	60%
351	*S. chilense*	LA2749	0/10	Mosaic, Deformation	Susceptible	60%
352	*S. chilense*	LA2751	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
353	*S. chilense*	LA2754	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
354	*S. chilense*	LA2755	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
355	*S. chilense*	LA2757	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
356	*S. chilense*	LA2762	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
357	*S. chilense*	LA2764	0/10	Mosaic, Deformation	Susceptible	60%
358	*S. chilense*	LA2767	0/10	Mosaic, Deformation	Susceptible	60%
359	*S. chilense*	LA2768	0/10	Mosaic, Deformation	Susceptible	60%
360	*S. chilense*	LA2773	0/10	Mosaic, Deformation	Susceptible	60%
361	*S. chilense*	LA2774	0/10	Mosaic, Deformation	Susceptible	60%
362	*S. chilense*	LA2879	0/10	Mosaic, Deformation	Susceptible	60%
363	*S. chilense*	LA2881	0/10	Mosaic, Deformation	Susceptible	60%
364	*S. chilense*	LA2882	0/10	Mosaic, Deformation	Susceptible	60%
365	*S. chilense*	LA2887	0/10	Mosaic, Deformation	Susceptible	60%
366	*S. chilense*	LA0458	0/10	Mosaic	Susceptible	40%
367	*S. chilense*	LA2779	0/10	Mosaic, Deformation	Susceptible	60%
368	*S. chilense*	LA2931	0/10	Mosaic, Deformation	Susceptible	60%
369	*S. chilense*	LA2932	0/10	Mosaic, Deformation	Susceptible	60%
370	*S. chilense*	LA2947	0/10	Mosaic	Susceptible	40%
371	*S. chilense*	LA2952	0/10	Mosaic, Deformation	Susceptible	60%
372	*S. chilense*	LA2957	0/10	Mosaic, Deformation	Susceptible	60%
373	*S. chilense*	LA2965	0/10	Mosaic, Deformation	Susceptible	60%
374	*S. chilense*	LA2981A	0/10	Mosaic, Deformation	Susceptible	60%
375	*S. chilense*	LA3111	0/10	Mosaic, Deformation	Susceptible	60%
376	*S. chilense*	LA3112	0/10	Mosaic, Deformation	Susceptible	60%
377	*S. chilense*	LA3113	0/10	Mosaic, Deformation	Susceptible	60%
378	*S. chilense*	LA3115	0/10	Mosaic, Deformation	Susceptible	60%
379	*S. chilense*	LA3153	0/10	Mosaic, Deformation	Susceptible	60%
380	*S. chilense*	LA3356	0/10	Mosaic, Deformation	Susceptible	60%
381	*S. chilense*	LA4106	0/10	Mosaic, Deformation	Susceptible	60%
382	*S. chilense*	LA4107	0/10	Mosaic, Deformation	Susceptible	60%

383	*S. chilense*	LA4108	0/10	Mosaic	Susceptible	40%
384	*S. chilense*	LA4109	0/10	Mosaic, Deformation	Susceptible	60%
385	*S. chilense*	LA4118	0/10	Mosaic, Deformation	Susceptible	60%
386	*S. chilense*	LA4119	0/10	Mosaic, Deformation	Susceptible	60%
387	*S. chilense*	LA4122	0/10	Mosaic, Deformation	Susceptible	60%
388	*S. chilense*	LA4127	0/10	Mosaic, Deformation	Susceptible	60%
389	*S. chilense*	LA4129	0/10	Mosaic, Deformation	Susceptible	60%
390	*S. chilense*	LA4132	0/10	Mosaic	Susceptible	40%
391	*S. chilense*	LA4319	0/10	Mosaic, Deformation	Susceptible	60%
392	*S. chilense*	LA4321	0/10	Mosaic, Deformation	Susceptible	60%
393	*S. chilense*	LA4324	0/10	Mosaic, Deformation	Susceptible	60%
394	*S. chilense*	LA4327	0/10	Mosaic, Deformation	Susceptible	60%
395	*S. chilense*	LA4329	0/10	Mosaic, Deformation	Susceptible	60%
396	*S. chilense*	LA4330	0/10	Mosaic	Susceptible	40%
397	*S. chilense*	LA4332	0/10	Mosaic, Deformation	Susceptible	60%
398	*S. chilense*	LA4334	0/10	Mosaic, Deformation	Susceptible	60%
399	*S. chilense*	LA4335	0/10	Mosaic, Deformation	Susceptible	60%
400	*S. chilense*	LA4336	0/10	Mosaic, Deformation	Susceptible	60%
401	*S. chilense*	LA4337	0/10	Mosaic, Deformation	Susceptible	60%
402	*S. chilense*	LA4338	0/10	Mosaic, Deformation	Susceptible	60%
403	*S. chilense*	LA4339	0/10	Mosaic, Deformation	Susceptible	60%
404	*S. chmielewskii core*	LA1028	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
405	*S. chmielewskii core*	LA1306	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
406	*S. chmielewskii core*	LA1316	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
407	*S. chmielewskii core*	LA1317	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
408	*S. chmielewskii core*	LA1325	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
409	*S. chmielewskii core*	LA1330	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
410	*S. chmielewskii core*	LA2663	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
411	*S. chmielewskii core*	LA2677	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
412	*S. chmielewskii core*	LA2680	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
413	*S. chmielewskii core*	LA2695	0/10	ShoesString, Mosaic, Rolling	Susceptible	100%
414	*S. corneliomulleri*	PI 129144	0/10	Mosaic	Susceptible	40%
415	*S. corneliomulleri*	PI 308183	0/10	Mosaic	Susceptible	40%
416	*S. corneliomulleri*	PI 251301	0/10	Mosaic	Susceptible	40%
417	*S. corneliomulleri*	PI 365944	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
418	*S. corneliomulleri*	PI 126434	0/10	Mosaic, Deformation	Susceptible	60%
419	*S. corneliomulleri*	PI 126440	0/10	Mosaic, Deformation	Susceptible	60%
420	*S. corneliomulleri*	PI 126443	0/10	Mosaic, Deformation	Susceptible	60%
421	*S. corneliomulleri*	PI 199380	0/10	Mosaic, Deformation	Susceptible	60%
422	*S. corneliomulleri*	PI 365942	0/10	Mosaic, Deformation	Susceptible	60%
423	*S. corneliomulleri*	PI 365945	0/10	Mosaic, Deformation	Susceptible	60%
424	*S. corneliomulleri core*	LA0103	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
425	*S. corneliomulleri core*	LA0107	0/10	Mild Mosaic, Deformation, Rolling	Susceptible	80%
426	*S. corneliomulleri core*	LA1292	0/10	Mosaic, Deformation, Rolling	Susceptible	60%

427	*S. corneliomulleri core*	LA1305	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
428	*S. corneliomulleri core*	LA1331	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
429	*S. corneliomulleri core*	LA1339	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
430	*S. corneliomulleri core*	LA1647	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
431	*S. corneliomulleri core*	LA1677	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
432	*S. corneliomulleri core*	LA1910	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
433	*S. corneliomulleri core*	LA1937	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
434	*S. corneliomulleri core*	LA1945	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
435	*S. corneliomulleri core*	LA1973	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
436	*S. corneliomulleri*	LA2717	0/10	Mosaic	Susceptible	40%
437	*S. corneliomulleri*	LA2721	0/10	Mosaic, Deformation	Susceptible	60%
438	*S. corneliomulleri*	LA2724	0/10	Mosaic, Deformation	Susceptible	60%
439	*S. corneliomulleri*	LA1274	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	100%
440	*S. galapagense*	LA0317	0/10	Mosaic	Susceptible	60%
441	*S. galapagense*	LA0438	0/10	Mosaic	Susceptible	60%
442	*S. galapagense*	LA0483	0/10	Mosaic	Susceptible	60%
443	*S. galapagense*	LA0526	0/10	Mosaic	Susceptible	60%
444	*S. galapagense*	LA1136	0/10	Mosaic	Susceptible	60%
445	*S. galapagense*	LA1137	0/10	Mosaic	Susceptible	60%
446	*S. galapagense*	LA1141	0/10	Mosaic	Susceptible	60%
447	*S. galapagense*	LA1401	0/10	Mosaic	Susceptible	60%
448	*S. galapagense*	LA1410	0/10	Mosaic	Susceptible	60%
449	*S. galapagense*	PI 379042	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
450	*S. galapagense*	LA1400	0/10	Mosaic	Susceptible	20%
451	*S. habrochaites*	LA2409	0/10	Mosaic	Susceptible	60%
452	*S. habrochaites*	LA0407	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
453	*S. habrochaites*	LA1777	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
454	*S. habrochaites*	LA1928	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
455	*S. habrochaites*	LA1223	0/10	Mosaic	Susceptible	40%
456	*S. habrochaites*	LA1347	0/10	Mosaic	Susceptible	40%
457	*S. habrochaites*	LA1353	0/10	Mosaic	Susceptible	40%
458	*S. habrochaites*	LA1718	0/10	Mosaic	Susceptible	40%
459	*S. habrochaites*	LA1721	0/10	Mosaic	Susceptible	40%
460	*S. habrochaites*	LA1731	0/10	Mosaic	Susceptible	40%
461	*S. habrochaites*	LA1753	0/10	Mosaic	Susceptible	40%
462	*S. habrochaites*	LA2103	0/10	Mosaic	Susceptible	40%
463	*S. habrochaites*	LA2109	0/10	Mosaic	Susceptible	40%
464	*S. habrochaites*	LA2128	0/10	Mosaic	Susceptible	40%
465	*S. habrochaites*	LA2158	0/10	Mosaic	Susceptible	40%
466	*S. habrochaites*	LA2650	0/10	Mosaic	Susceptible	40%
467	*S. habrochaites*	LA2864	0/10	Mosaic	Susceptible	40%
468	*S. habrochaites*	LA2167	0/10	Mosaic, Mild Mosaic	Susceptible	40%
469	*S. habrochaites*	LA1559	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%

470	*S. habrochaites*	LA2174	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
471	*S. habrochaites L. hirsutum f. glabratum*	LA1252	0/10	Mosaic	Susceptible	40%
472	*S. habrochaites L. hirsutum f. glabratum*	LA1264	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
473	*S. huaylasense*	LA1365	0/10	Deformation, Mosaic	Susceptible	80%
474	*S. huaylasense*	LA2561	0/10	Mosaic, Deformation	Susceptible	60%
475	*S. huaylasense*	LA2563	0/10	Mosaic, Deformation	Susceptible	60%
476	*S. huaylasense*	LA2809	0/10	Mosaic, Deformation	Susceptible	60%
477	*S. huaylasense peralta (L.peruvianum)*	LA1358	0/10	Deformation, Mosaic	Susceptible	80%
478	*S. huaylasense peralta (L.peruvianum)*	LA1360	0/10	Deformation, Mosaic	Susceptible	80%
479	*S. huaylasense peralta (L.peruvianum)*	LA1979	0/10	Deformation, Mosaic	Susceptible	80%
480	*S. huaylasense peralta (L.peruvianum)*	LA1981	0/10	Deformation, Mosaic	Susceptible	80%
481	*S. huaylasense peralta (L.peruvianum)*	LA1983	0/10	Deformation, Mosaic	Susceptible	80%
482	*S. juglandifolium*	LA3322	0/10	Mild Mosaic	Susceptible	20%
483	*S. juglandifolium*	LA3325	0/10	Mild Mosaic	Susceptible	20%
484	*S. juglandifolium*	LA2134	0/10	Mosaic	Susceptible	40%
485	*S. lycopersicum*	PI 272219	0/10	Mosaic	Susceptible	40%
486	*S. lycopersicum*	PI 414772	0/10	Mosaic	Susceptible	40%
487	*S. lycopersicum*	PI 324708	0/10	Mosaic, Deformation	Susceptible	60%
488	*S. lycopersicum*	PI 560323	0/10	Mosaic, Deformation	Susceptible	60%
489	*S. lycopersicum*	PI 235673	0/10	Mosaic	Susceptible	60%
490	*S. lycopersicum*	PI 414160	0/10	Mosaic, Deformation	Susceptible	60%
491	*S. lycopersicum*	PI 414161	0/10	Mosaic, Deformation	Susceptible	60%
492	*S. lycopersicum core*	LA0473	0/10	Deformation, Mosaic, ShoesString	Susceptible	100%
493	*S. lycopersicum core*	LA1021	0/10	Deformation, Rolling, ShoesString	Susceptible	100%
494	*S. lycopersicum core*	LA1162	0/10	Deformation, Rolling, ShoesString	Susceptible	100%
495	*S. lycopersicum core*	LA0113	0/10	Mosaic	Susceptible	40%
496	*S. lycopersicum core*	LA0477	0/10	Mosaic	Susceptible	40%
497	*S. lycopersicum core*	LA1251	0/10	Mosaic	Susceptible	40%
498	*S. lycopersicum core*	LA2283	0/10	Mosaic	Susceptible	40%
499	*S. lycopersicum core*	LA0134C	0/10	Mosaic, some Deformation	Susceptible	60%
500	*S. lycopersicum core*	LA0146	0/10	Mosaic, some Deformation	Susceptible	60%
501	*S. lycopersicum core*	LA0147	0/10	Mosaic, some Deformation	Susceptible	60%
502	*S. lycopersicum core*	LA2285	0/10	Mosaic, some Deformation	Susceptible	60%
503	*S. lycopersicum core*	LA0126	0/10	Mosaic,Deformation	Susceptible	60%
504	*S. lycopersicum core*	LA0409	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
505	*S. lycopersicum core*	LA0468	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
506	*S. lycopersicum core*	LA2304	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
507	*S. lycopersicum core*	LA2307	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
508	*S. lycopersicum core*	LA0404	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	100%
509	*S. lycopersicum core*	LA0172	0/10	Mosaic,Deformation ,Rolling	Susceptible	60%
510	*S. lycopersicum core*	LA0358	0/10	Mosaic,Deformation ,Rolling	Susceptible	60%

511	*S. lycopersicum core*	LA0395	0/10	Mosaic,Deformation ,Rolling	Susceptible	60%
512	*S. lycopersicum core*	LA0466	0/10	Mosaic,Deformation ,Rolling	Susceptible	60%
513	*S. lycopersicum L. esculentum var. cerasiforme*	LA1230	0/10	Deformation, ShoesString, Rolling	Susceptible	80%
514	*S. lycopersicum var. cerasiforme*	LA2783	0/10	Mosaic	Susceptible	60%
515	*S. lycopersicum var. cerasiforme*	LA1511	0/10	Mosaic, Deformation	Susceptible	60%
516	*S. lycopersicum var. cerasiforme*	LA1228	0/10	Deformation, ShoesString, Rolling	Susceptible	80%
517	*S. lycopersicum var. cerasiforme*	LA1307	0/10	Deformation, ShoesString, Rolling	Susceptible	80%
518	*S. lycopersicum var. cerasiforme*	LA1312-2	0/10	Deformation, ShoesString, Rolling	Susceptible	80%
519	*S. lycopersicum var. cerasiforme*	LA1312-4	0/10	Deformation, ShoesString, Rolling	Susceptible	80%
520	*S. lycopersicum var. cerasiforme*	LA1314	0/10	Deformation, ShoesString, Rolling	Susceptible	80%
521	*S. lycopersicum var. cerasiforme*	LA1323	0/10	Deformation, ShoesString, Rolling	Susceptible	80%
522	*S. lycopersicum var. cerasiforme*	LA2709	0/10	Mild Mosaic	Susceptible	20%
523	*S. lycopersicum var. cerasiforme*	LA0292	0/10	Mosaic	Susceptible	40%
524	*S. lycopersicum var. cerasiforme*	LA1204	0/10	Mosaic	Susceptible	40%
525	*S. lycopersicum var. cerasiforme*	LA1231	0/10	Mosaic	Susceptible	40%
526	*S. lycopersicum var. cerasiforme*	LA1268	0/10	Mosaic	Susceptible	40%
527	*S. lycopersicum var. cerasiforme*	LA2670	0/10	Mosaic	Susceptible	40%
528	*S. lycopersicum var. cerasiforme*	LA1420	0/10	Mosaic,Deformation	Susceptible	60%
529	*S. lycopersicum var. cerasiforme*	LA1425	0/10	Mosaic,Deformation	Susceptible	60%
530	*S. lycopersicum var. cerasiforme*	LA1286	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
531	*S. lycopersicum var. cerasiforme*	LA1312-3	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	100%
532	*S. lycopersicum var. cerasiforme*	LA1338	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	100%
533	*S. lycopersicum var. cerasiforme*	LA1388	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	100%
534	*S. lycopersicum var. cerasiforme*	LA1453	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	100%
535	*S. lycopersicum var. cerasiforme*	LA2095	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
536	*S. lycopersicum var. cerasiforme*	LA2131	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
537	*S. lycopersicum var. cerasiforme*	LA2308	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
538	*S. lycopersicum var. cerasiforme*	LA1320	0/10	Mosaic, Deformation, ShoesString	Susceptible	100%
539	*S. lycopersicum var. cerasiforme*	LA1543	0/10	Mosaic, Deformation, ShoesString	Susceptible	100%
540	*S. lycopersicum var. cerasiforme*	LA1620	0/10	Mosaic, Deformation, ShoesString	Susceptible	100%
541	*S. lycopersicum var. cerasiforme*	LA2402	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
542	*S. lycopersicum var. cerasiforme*	LA2703	0/10	Mosaic, Deformation, ShoesString, Rolling	Susceptible	100%
543	*S. lycopersicum var. cerasiforme*	LA1461	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
544	*S. lycopersicum var. cerasiforme*	LA1464	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
545	*S. lycopersicum var. cerasiforme*	LA1482	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
546	*S. lycopersicum var. cerasiforme*	LA1483	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
547	*S. lycopersicum var. cerasiforme*	LA1509	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%

548	*S. lycopersicum var. cerasiforme*	LA1542	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
549	*S. lycopersicum var. cerasiforme*	LA1622	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
550	*S. lycopersicum var. cerasiforme*	LA2078	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
551	*S. lycopersicum var. cerasiforme*	LA2138A	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
552	*S. lycopersicum var. cerasiforme*	LA2392	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
553	*S. lycopersicum var. cerasiforme*	LA2621	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
554	*S. lycopersicum var. cerasiforme*	LA2710	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
555	*S. lycopersicum var. cerasiforme*	LA2845	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
556	*S. lycopersicum var. cerasiforme*	LA2871	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
557	*S. lycopersicum var. cerasiforme*	LA4133	0/10	Mosaic,Deformation , ShoesString ,Rolling	Susceptible	80%
558	*S. lycopersicum var. cerasiforme*	LA1206	0/10	Mosaic,Deformation ,Rolling	Susceptible	60%
559	*S. lycopersicum var. cerasiforme*	LA1385	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
560	*S. lycopersicum var. cerasiforme*	LA1456	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
561	*S. lycopersicum var. cerasiforme*	LA2675	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
562	*S. lycopersicum var. cerasiforme*	LA2688	5/5	No Symptoms / Mild Mosaic	Tolerant / Susceptible	0%/20%
563	*S. neoricki*	LA2641	0/10	ShoesString	Susceptible	80%
564	*S. neoricki*	LA0247	0/10	ShoesString	Susceptible	80%
565	*S. neoricki*	LA1319	0/10	ShoesString	Susceptible	80%
566	*S. neoricki*	LA1322	0/10	ShoesString	Susceptible	80%
567	*S. neoricki*	LA1626A	0/10	ShoesString	Susceptible	80%
568	*S. neoricki*	LA1716	0/10	ShoesString	Susceptible	80%
569	*S. neoricki*	LA2113	0/10	ShoesString	Susceptible	80%
570	*S. neoricki*	LA2133	0/10	ShoesString	Susceptible	80%
571	*S. neoricki*	LA2190	0/10	ShoesString	Susceptible	80%
572	*S. neoricki*	LA2198	0/10	ShoesString	Susceptible	80%
573	*S. neoricki*	LA2319	0/10	ShoesString	Susceptible	80%
574	*S. neoricki*	LA2325	0/10	ShoesString	Susceptible	80%
575	*S. neorickii*	PI 379030	0/10	Mosaic, Deformation	Susceptible	60%
576	*S. neorickii*	PI 379033	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
577	*S. neorickii L. parviflorum*	LA2193	0/10	ShoesString	Susceptible	80%
578	*S. neorickii L. parviflorum*	LA2194	0/10	ShoesString	Susceptible	80%
579	*S. ochranthum*	LA2160	8/0	All No Symptoms	All Resistant	0%
580	*S. ochranthum*	LA2162	3/0	All No Symptoms	All Resistant	0%
581	*S. ochranthum*	LA2166	7/0	All No Symptoms	All Resistant	0%
582	*S. ochranthum*	PI 473498	0/3	Mild Mosaic	Susceptible	20%
583	*S. ochranthum*	PI 230519	0/3	Mild Mosaic	Susceptible	20%
584	*S. peruvianum*	LA1474	0/10	Mosaic	Susceptible	40%
585	*S. peruvianum*	LA1537	0/10	Mosaic	Susceptible	40%
586	*S. peruvianum*	LA1336	0/10	Mosaic, Deformation	Susceptible	60%
587	*S. peruvianum*	LA1954	0/10	Mosaic, Deformation	Susceptible	60%
588	*S. peruvianum*	LA0371	0/10	Mosaic, Deformation	Susceptible	60%

589	*S. peruvianum*	LA2957B	0/10	Mosaic, Deformation	Susceptible	60%
590	*S. peruvianum*	LA4445	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
591	*S. peruvianum*	LA4446	0/10	Mosaic, Some Deformation	Susceptible	60%
592	*S. peruvianum*	LA0455	0/10	Mosaic	Susceptible	60%
593	*S. peruvianum*	LA0448	0/10	Mosaic, Rolling	Susceptible	60%
594	*S. peruvianum*	LA4325	0/10	Mosaic, Rolling	Susceptible	60%
595	*S. peruvianum*	LA2958	0/10	Mosaic, Deformation	Susceptible	60%
596	*S. peruvianum*	LA2964	0/10	Mosaic, Deformation	Susceptible	60%
597	*S. peruvianum*	LA0445	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
598	*S. peruvianum*	LA0453	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
599	*S. peruvianum*	LA0454	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
600	*S. peruvianum*	LA1161	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
601	*S. peruvianum*	LA1278	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
602	*S. peruvianum*	LA1333	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
603	*S. peruvianum*	LA1337	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
604	*S. peruvianum*	LA1368	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
605	*S. peruvianum*	LA1513	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
606	*S. peruvianum*	LA1517	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
607	*S. peruvianum*	LA1692	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
608	*S. peruvianum*	LA1759	0/10	Mosaic, Deformation, Rolling	Susceptible	60%
609	*S. peruvianum*	LA1913	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
610	*S. peruvianum*	LA1929	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
611	*S. peruvianum*	LA1935	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
612	*S. peruvianum*	LA1947	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
613	*S. peruvianum*	LA1949	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
614	*S. peruvianum*	LA1952	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
615	*S. peruvianum*	LA1955	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
616	*S. peruvianum*	LA1977	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
617	*S. peruvianum*	LA1989	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
618	*S. peruvianum*	LA3218	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
619	*S. peruvianum*	LA4125	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
620	*S. peruvianum*	LA4317	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
621	*S. peruvianum*	LA4318	0/10	Mosaic, Deformation, Rolling, ShoesString	Susceptible	80%
622	*S. peruvianum*	LA0446	0/10	ShoesString, Mosaic, Rolling, Deformation	Susceptible	100%
623	*S. peruvianum*	LA0370	0/10	ShoesString, Mosaic, Rolling, Deformation	Susceptible	100%
624	*S. peruvianum*	LA2581	0/10	Mosaic	Susceptible	40%
625	*S. peruvianum*	LA2834	0/10	Mosaic	Susceptible	40%
626	*S. peruvianum*	LA2770	0/10	Mosaic, Deformation	Susceptible	60%
627	*S. sitiens*	LA1974	0/10	Mosaic	Susceptible	40%
628	*S. sitiens*	LA2876	0/10	Mosaic	Susceptible	40%
629	*S. sitiens*	LA2877	0/10	Mosaic	Susceptible	40%

630	*S. sitiens*	LA2878	0/10	Mosaic	Susceptible	40%
631	*S. sitiens*	PI 558114	0/10	Mosaic	Susceptible	40%
632	*S. sitiens*	PI 558115	0/10	Mosaic	Susceptible	40%
633	*S. sitiens*	PI 498279	0/10	Mosaic	Susceptible	40%
634	*Solanum subsect. lycopersicon hybr.*	PI 306812	0/10	Mosaic	Susceptible	60%
635	*Solanum subsect. lycopersicon hybr.*	PI 306814	0/10	Mosaic, Deformation	Susceptible	60%
636	*Solanum subsect. lycopersicon hybr.*	PI 251296	0/10	Mosaic	Susceptible	60%

14. Appendix II

Symptomless plant (Tolerant) accession inoculated with ToBRFV. (1-4) *S. pimpinellifolium* LA1301, LA1375, LA1924 and LA1547; (5-6) *S. habrochaites* LA1559 and LA2174; (7) *S. chilense* LA1932; (8-11) *S. lycopersicum* var. cerasiforme LA1456, LA2675, LA2688 and LA1385.

15. Appendix III

Symptomless plants (Resistant) of the *S. ochranthum* (1) LA2160, (2) LA2162 and (3) LA2166.

16. Appendix IV

Screening of *S. habrochaites* and *S.peruvianum* accessions to reactions of ToBRFV (Group B)

Number	Tomato species	Accessions	Number of plants (Resistant/susceptible)	Symptoms	Reaction to ToBRFV	Disease severity index (DSI)
1	*Solanum habrochaites*	LA0094	0/15	Mosaic	Susceptible	40%
2	*Solanum habrochaites*	LA0361	0/15	Mosaic	Susceptible	40%
3	*Solanum habrochaites*	LA1033	0/15	Mild mosaic or mottling	Susceptible	20%
4	*Solanum habrochaites*	LA1252	0/15	Mosaic	Susceptible	40%
5	*Solanum habrochaites*	LA1255	0/15	Mosaic	Susceptible	40%
6	*Solanum habrochaites*	LA1295	0/15	Mosaic	Susceptible	40%
7	*Solanum habrochaites*	LA1298	0/15	Mosaic	Susceptible	40%
8	*Solanum habrochaites*	LA1352	0/15	Mosaic	Susceptible	40%
9	*Solanum habrochaites*	LA1354	0/15	Mosaic	Susceptible	40%
10	*Solanum habrochaites*	LA1362	0/15	Mosaic	Susceptible	40%
11	*Solanum habrochaites*	LA1378	0/15	Mosaic	Susceptible	40%
12	*Solanum habrochaites*	LA1557	0/15	Mosaic	Susceptible	40%
13	*Solanum habrochaites*	LA1648	0/15	Mosaic	Susceptible	40%
14	*Solanum habrochaites*	LA1681	0/15	Mosaic	Susceptible	40%
15	*Solanum habrochaites*	LA1691	0/15	Mosaic	Susceptible	40%
16	*Solanum habrochaites*	LA1695	0/15	Mosaic	Susceptible	40%
17	*Solanum habrochaites*	LA1696	0/15	Mosaic	Susceptible	40%
18	*Solanum habrochaites*	LA1736	0/15	Mosaic	Susceptible	40%
19	*Solanum habrochaites*	LA1737	0/15	Mosaic	Susceptible	40%
20	*Solanum habrochaites*	LA1738	3/12	Symptomless / Mosaic	Resistant / Susceptible	0%/40%
21	*Solanum habrochaites*	LA1739	2/13	Symptomless / Mosaic	Resistant / Susceptible	0%/40%
22	*Solanum habrochaites*	LA1761	0/15	Mosaic	Susceptible	40%
23	*Solanum habrochaites*	LA1764	0/15	Mosaic	Susceptible	40%
24	*Solanum habrochaites*	LA1772	0/15	Mosaic	Susceptible	40%
25	*Solanum habrochaites*	LA1775	0/15	Mosaic	Susceptible	40%
26	*Solanum habrochaites*	LA1778	0/15	Mosaic	Susceptible	40%
27	*Solanum habrochaites*	LA1779	0/15	Mosaic	Susceptible	40%
28	*Solanum habrochaites*	LA1927	0/15	Mosaic	Susceptible	40%
29	*Solanum habrochaites*	LA1978	0/15	Mosaic	Susceptible	40%
30	*Solanum habrochaites*	LA1986	0/15	Mosaic	Susceptible	40%
31	*Solanum habrochaites*	LA2106	0/15	Mosaic	Susceptible	40%
32	*Solanum habrochaites*	LA2108	0/15	Mosaic	Susceptible	40%
33	*Solanum habrochaites*	LA2114	0/15	Mosaic	Susceptible	40%
34	*Solanum habrochaites*	LA2115	0/15	Mosaic	Susceptible	40%
35	*Solanum habrochaites*	LA2116	0/15	Mosaic	Susceptible	40%
36	*Solanum habrochaites*	LA2124	0/15	Mosaic and leaf deformation	Susceptible	60%
37	*Solanum habrochaites*	LA2144	0/15	Mosaic and leaf deformation	Susceptible	60%
38	*Solanum habrochaites*	LA2156	0/15	Mosaic and leaf deformation	Susceptible	60%
39	*Solanum habrochaites*	LA2159	0/15	Mosaic and leaf deformation	Susceptible	60%
40	*Solanum habrochaites*	LA2171	5/10	Symptomless / Mosaic	Resistant / Susceptible	0%/40%

41	*Solanum habrochaites*	LA2196	0/15	Mosaic and leaf deformation	Susceptible	60%
42	*Solanum habrochaites*	LA2314	0/15	Mosaic	Susceptible	40%
43	*Solanum habrochaites*	LA2324	0/15	Mosaic	Susceptible	40%
44	*Solanum habrochaites*	LA2541	5/10	Symptomless / Mosaic	Resistant / Susceptible	0%/40%
45	*Solanum habrochaites*	LA2552	0/15	Mosaic	Susceptible	40%
46	*Solanum habrochaites*	LA2556	0/15	Mosaic	Susceptible	40%
47	*Solanum habrochaites*	LA2574	0/15	Mosaic	Susceptible	40%
48	*Solanum habrochaites*	LA2648	0/15	Mosaic	Susceptible	40%
49	*Solanum habrochaites*	LA2651	0/15	Mosaic and leaf deformation	Susceptible	60%
50	*Solanum habrochaites*	LA2723	0/15	Mosaic	Susceptible	40%
51	*Solanum habrochaites*	LA2728	0/15	Mosaic	Susceptible	40%
52	*Solanum habrochaites*	LA2812	5/10	Symptomless / Mosaic	Resistant / Susceptible	0%/40%
53	*Solanum habrochaites*	LA2855	0/15	Mosaic and leaf deformation	Susceptible	60%
54	*Solanum habrochaites*	LA2859	0/15	Mild mosaic or mottling	Susceptible	20%
55	*Solanum habrochaites*	LA2868	0/15	Mosaic	Susceptible	40%
56	*Solanum habrochaites*	LA2976	0/15	Mosaic and leaf deformation	Susceptible	60%
57	*Solanum habrochaites*	LA4654	0/15	Mosaic and leaf deformation	Susceptible	60%
58	*Solanum habrochaites*	LA4655	0/15	Mosaic and leaf deformation	Susceptible	60%
59	*Solanum habrochaites*	LA4656	0/15	Mosaic and leaf deformation	Susceptible	60%
60	*Solanum habrochaites*	PI 126445	0/15	Mosaic	Susceptible	40%
61	*Solanum habrochaites*	PI 126446	0/15	Mosaic and leaf deformation	Susceptible	60%
62	*Solanum habrochaites*	PI 126449	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
63	*Solanum habrochaites*	PI 127826	0/15	Mosaic	Susceptible	40%
64	*Solanum habrochaites*	PI 128644	0/15	Mosaic	Susceptible	40%
65	*Solanum habrochaites*	PI 129157	0/15	Mosaic and leaf deformation	Susceptible	60%
66	*Solanum habrochaites*	PI 134417	0/15	Mosaic and leaf deformation	Susceptible	60%
67	*Solanum habrochaites*	PI 134418	0/15	Mosaic and leaf deformation	Susceptible	60%
68	*Solanum habrochaites*	PI 209978	0/15	Mosaic	Susceptible	40%
69	*Solanum habrochaites*	PI 247087	0/15	Mosaic	Susceptible	40%
70	*Solanum habrochaites*	PI 251303	0/15	Mosaic	Susceptible	40%
71	*Solanum habrochaites*	PI 251305	0/15	Mosaic	Susceptible	40%
72	*Solanum habrochaites*	PI 308182	3/12	Symptomless / Mosaic	Resistant / Susceptible	0%/40%
73	*Solanum habrochaites*	PI 365904	0/15	Mosaic	Susceptible	40%
74	*Solanum habrochaites*	PI 365905	0/15	Mosaic	Susceptible	40%
75	*Solanum habrochaites*	PI 365907	0/15	Mosaic	Susceptible	40%
76	*Solanum habrochaites*	PI 365908	0/15	Mosaic	Susceptible	40%
77	*Solanum habrochaites*	PI 365936	0/15	Mosaic	Susceptible	40%
78	*Solanum habrochaites*	PI 379012	10/5	Symptomless / Mosaic	Resistant / Susceptible	0%/40%
79	*Solanum habrochaites*	PI 379013	0/15	Mosaic	Susceptible	40%
80	*Solanum habrochaites*	PI 379014	6/9	Symptomless / Mosaic	Resistant / Susceptible	0%/40%
81	*Solanum habrochaites*	PI 379056	0/15	Mosaic	Susceptible	40%
82	*Solanum habrochaites*	PI 390513	0/15	Mosaic	Susceptible	40%
83	*Solanum habrochaites*	PI 390514	0/15	Mosaic	Susceptible	40%
84	*Solanum habrochaites*	PI 390515	0/15	Mosaic and leaf deformation	Susceptible	60%
85	*Solanum habrochaites*	PI 390516	0/15	Mosaic	Susceptible	40%

86	*Solanum habrochaites*	PI 390517	0/15	Mosaic	Susceptible	40%
87	*Solanum habrochaites*	PI 390518	0/15	Mosaic	Susceptible	40%
88	*Solanum habrochaites*	PI 390659	4/11	No symptoms -Mosaic	Resistant / Susceptible	0%/40%
89	*Solanum habrochaites*	PI 390660	0/15	Mosaic	Susceptible	40%
90	*Solanum habrochaites*	PI 390661	0/15	Mosaic	Susceptible	40%
91	*Solanum habrochaites*	PI 390662	0/15	Mosaic	Susceptible	40%
92	*Solanum habrochaites*	PI 503515	0/15	Mosaic and leaf deformation	Susceptible	60%
93	*Solanum peruvianum*	PI 126431	0/15	Mosaic and leaf deformation	Susceptible	60%
94	*Solanum peruvianum*	PI 126435	0/15	Mosaic and leaf deformation	Susceptible	60%
95	*Solanum peruvianum*	PI 126439	0/15	Mosaic and leaf deformation	Susceptible	60%
96	*Solanum peruvianum*	PI 126441	0/15	Mosaic and leaf deformation	Susceptible	60%
97	*Solanum peruvianum*	PI 126444	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
98	*Solanum peruvianum*	PI 126926	0/15	Mosaic and leaf deformation	Susceptible	60%
99	*Solanum peruvianum*	PI 126928	0/15	Mosaic and leaf deformation	Susceptible	60%
100	*Solanum peruvianum*	PI 126929	0/15	Mosaic and leaf deformation	Susceptible	60%
101	*Solanum peruvianum*	PI 126930	0/15	Mosaic and leaf deformation	Susceptible	60%
102	*Solanum peruvianum*	PI 126935	0/15	Mosaic	Susceptible	40%
103	*Solanum peruvianum*	PI 126944	0/15	Mosaic	Susceptible	40%
104	*Solanum peruvianum*	PI 126945	0/15	Mosaic and leaf deformation	Susceptible	60%
105	*Solanum peruvianum*	PI 126946	0/15	Mosaic and leaf deformation	Susceptible	60%
106	*Solanum peruvianum*	PI 127830	0/15	Mosaic	Susceptible	40%
107	*Solanum peruvianum*	PI 127831	0/15	Mosaic and leaf deformation	Susceptible	60%
108	*Solanum peruvianum*	PI 127832	0/15	Mosaic and leaf deformation	Susceptible	60%
109	*Solanum peruvianum*	PI 128643	0/15	Mosaic	Susceptible	40%
110	*Solanum peruvianum*	PI 128645	0/15	Mosaic and leaf deformation	Susceptible	60%
111	*Solanum peruvianum*	PI 128646	0/15	Mosaic and leaf deformation	Susceptible	60%
112	*Solanum peruvianum*	PI 128647	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
113	*Solanum peruvianum*	PI 128648	0/15	Mosaic and leaf deformation	Susceptible	60%
114	*Solanum peruvianum*	PI 128649	0/15	Mosaic and leaf deformation	Susceptible	60%
115	*Solanum peruvianum*	PI 128651	0/15	Mosaic and leaf deformation	Susceptible	60%
116	*Solanum peruvianum*	PI 128652	0/15	Mosaic and leaf deformation	Susceptible	60%
117	*Solanum peruvianum*	PI 128653	0/15	Mosaic and leaf deformation	Susceptible	60%
118	*Solanum peruvianum*	PI 128654	0/15	Mosaic and leaf deformation	Susceptible	60%
119	*Solanum peruvianum*	PI 128655	0/15	Mosaic and leaf deformation	Susceptible	60%
120	*Solanum peruvianum*	PI 128656	0/15	Mosaic and leaf deformation	Susceptible	60%
121	*Solanum peruvianum*	PI 128657	0/15	Mosaic and leaf deformation	Susceptible	60%
122	*Solanum peruvianum*	PI 128658	0/15	Mosaic and leaf deformation	Susceptible	60%
123	*Solanum peruvianum*	PI 128659	0/15	Severe mosaic, leaf deformation, rolling, shoestring, stunting	Susceptible	100%
124	*Solanum peruvianum*	PI 128660	0/15	Mosaic and leaf deformation	Susceptible	60%
125	*Solanum peruvianum*	PI 128661	0/15	Mosaic and leaf deformation	Susceptible	60%
126	*Solanum peruvianum*	PI 128663	0/15	Severe mosaic, leaf deformation, rolling, shoestring, stunting	Susceptible	100%
127	*Solanum peruvianum*	PI 129145	0/15	Mosaic and leaf deformation	Susceptible	60%
128	*Solanum peruvianum*	PI 129146	0/15	Mosaic and leaf deformation	Susceptible	60%

129	*Solanum peruvianum*	PI 129147	0/15	Mosaic and leaf deformation	Susceptible	60%
130	*Solanum peruvianum*	PI 129149	0/15	Mosaic and leaf deformation	Susceptible	60%
131	*Solanum peruvianum*	PI 129152	0/15	Mosaic and leaf deformation	Susceptible	60%
132	*Solanum peruvianum*	PI 143679	0/15	Mosaic and leaf deformation	Susceptible	60%
133	*Solanum peruvianum*	PI 212407	0/15	Mosaic	Susceptible	40%
134	*Solanum peruvianum*	PI 246585	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
135	*Solanum peruvianum*	PI 246586	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
136	*Solanum peruvianum*	PI 251306	0/15	Mosaic and leaf deformation	Susceptible	60%
137	*Solanum peruvianum*	PI 251307	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
138	*Solanum peruvianum*	PI 251310	0/15	Mosaic and leaf deformation	Susceptible	60%
139	*Solanum peruvianum*	PI 251311	0/15	Mosaic and leaf deformation	Susceptible	60%
140	*Solanum peruvianum*	PI 251312	0/15	Mosaic and leaf deformation	Susceptible	60%
141	*Solanum peruvianum*	PI 251314	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
142	*Solanum peruvianum*	PI 266375	0/15	Mosaic and leaf deformation	Susceptible	60%
143	*Solanum peruvianum*	PI 266376	0/15	Mosaic and leaf deformation	Susceptible	60%
144	*Solanum peruvianum*	PI 270435	0/15	Mosaic and leaf deformation	Susceptible	60%
145	*Solanum peruvianum*	PI 303814	0/15	Mosaic and leaf deformation	Susceptible	60%
146	*Solanum peruvianum*	PI 306811	0/15	Mosaic and leaf deformation	Susceptible	60%
147	*Solanum peruvianum*	PI 308181	5/10	Symptomless / Mosaic	Resistant / Susceptible	0%/40%
148	*Solanum peruvianum*	PI 326173	0/15	Mosaic and leaf deformation	Susceptible	60%
149	*Solanum peruvianum*	PI 365938	0/15	Mosaic and leaf deformation	Susceptible	60%
150	*Solanum peruvianum*	PI 365939	0/15	Mosaic and leaf deformation	Susceptible	60%
151	*Solanum peruvianum*	PI 365943	0/15	Mosaic and leaf deformation	Susceptible	60%
152	*Solanum peruvianum*	PI 365955	0/15	Mosaic and leaf deformation	Susceptible	60%
153	*Solanum peruvianum*	PI 365956	0/15	Mosaic and leaf deformation	Susceptible	60%
154	*Solanum peruvianum*	PI 365969	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
155	*Solanum peruvianum*	PI 379018	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
156	*Solanum peruvianum*	PI 390664	0/15	Mosaic and leaf deformation	Susceptible	60%
157	*Solanum peruvianum*	PI 390665	0/15	Mosaic and leaf deformation	Susceptible	60%
158	*Solanum peruvianum*	PI 390666	0/15	Mosaic	Susceptible	40%
159	*Solanum peruvianum*	PI 390667	0/15	Mosaic and leaf deformation	Susceptible	60%
160	*Solanum peruvianum*	PI 390668	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
161	*Solanum peruvianum*	PI 390669	0/15	Mosaic and leaf deformation	Susceptible	60%
162	*Solanum peruvianum*	PI 390670	0/15	Mosaic	Susceptible	40%
163	*Solanum peruvianum*	PI 390671	0/15	Mosaic and leaf deformation	Susceptible	60%
164	*Solanum peruvianum*	PI 390672	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
165	*Solanum peruvianum*	PI 390673	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
166	*Solanum peruvianum*	PI 390676	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
167	*Solanum peruvianum*	PI 390678	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
168	*Solanum peruvianum*	PI 390679	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%
169	*Solanum peruvianum*	PI 390680	0/15	Mosaic and leaf deformation	Susceptible	60%
170	*Solanum peruvianum*	PI 390681	0/15	Mosaic and leaf deformation, shoestring	Susceptible	80%

171	*Solanum peruvianum*	PI 390682	0/15	Mosaic and leaf deformation	Susceptible	60%
172	*Solanum peruvianum*	PI 390684	0/15	Mosaic and leaf deformation	Susceptible	60%
173	*Solanum peruvianum*	PI 390685	0/15	Mosaic and leaf deformation	Susceptible	60%

17. Appendix V

Symptomless plant (Resistant) of *S. habrochaites* and *S. peruvianum* accessions inoculated with ToBRFV. *S. habrochaites* (1) PI 379012, (2), LA1738, (3) LA2171, (4) PI 379014, (5) LA 2812, (6) PI 308182, (7) LA2541, (8) PI 390659, (9) LA1739; *S. peruvianum* (10) PI 308181.

18. Appendix VI

Protein sequence alignment of the *S. lycopersicum* NBS-LRR gene Solyc08g075630 with the sequences published by YKEMA et al (2020) and our sequences from the resistant *S. habrochaites* and *S. peruvianum* lines

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                    10        20        30        40        50        60        70        80        90       100
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq  MAEAFLQIMLENLTCFIQGELGLILGFKDEFEKLQSTFTTIQAVVQDAQLKQLKDKAIENWLQKLNGAAYEADDILDECKTEAPIIQKKNKYGCYHPNVI
Ykema.seq           ................................................................................................
LA 2812.seq         ................................................................................................
PI 379014.seq       ................................................................................................
PI 379012.seq       ........L.K...S...E....FF.......N.K.........LE...E......PL.......V....V.......Q...ARLNQ.~...S...K..
LA 1738.seq         ........L.....S.......FF.......N.K.S.......LE...E......PL.......V....V.......Q...ARLNQ.~...S...K..
PI 308181.seq       ........L.....S.......FF.......N.K.S.......LE...E......PL.......V....V.......YQ...ARLNQ.~...S...R..
LA 1739.seq         ................................................................................................
PI 390659.seq       ........L.....S.......FF.......N.K.S.......LE...E......PL.......V....V.......Q...ARLNQ.~...S...K..
PI 308182.seq       ........L.....SS......FF.......N.K.........LE...E......PL.......V....V.......Q...ARLNQ.~.......K..
LA 2171.seq         ........L.K...S...E....FF.......N.K.........LE...E......PL.......V....V.......Q...ARLNQ.~.......K..
LA 2541.seq         .................................................T..............................................

                   110       120       130       140       150       160       170       180       190       200
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq  TFRHKIGKRMKKIMEKLDAIAAERIKFHLDERTIERQVATRQTGFVLNEPQVYGRDKDKDEIVKILINNAQTLSVLPILGMGGLGKTTLAQMVFNDQRVI
Ykema.seq           ...R............................................................................................
LA 2812.seq         ...R............................................................................................
PI 379014.seq       ...R............................................................................................
PI 379012.seq       A..YR......E...R.........S....EK..T..E...R.E.......EP.....EE...............Q................S........T
LA 1738.seq         A..Y.......E..K.........S...EK..T..E..R.......EL......EE...............Q................S........T
PI 308181.seq       A..Y.......E..K.........S...EK..T..E..R.......EL......EE...............Q................S........T
LA 1739.seq         ...........................L....................................................................
PI 390659.seq       A..Y.......E..K.........S...EK..T..E..R.......EL......EE...............Q................S........T
PI 308182.seq       A..Y.......E..K.........S...EK..T..E..R.......EP......EE...............Q................S........T
LA 2171.seq         A..Y.......E..K.........S...EK..T..E..R.......EP......EE...............Q................S........T
LA 2541.seq         ...............................................IWKRQR*

                   210       220       230       240       250       260       270       280       290       300
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq  EHFHPKIWICVSEDFNEKRLIKEIVESIEEKSLG~DMDLAPLQKKLQDLLNGKKYLLVLDDVWNEDQDKWAKLRQVLKAGASGAYVLTTTRLEKVGSIMG
Ykema.seq           ...........................K.........~..........................................................
LA 2812.seq         .....................................~..........................................................
PI 379014.seq       .....................................~..........................................................
PI 379012.seq       D...........D..K...A......GNP..DH.....P......M....R.F.........N.E..D.IKA..EV..R..S......K...A...
LA 1738.seq         D.....M........D..K...A......NP..D.........R....R.F.........N.E..D.IKA..EV..R..S......KR......
PI 308181.seq       D.....T........D..K...A......NP..D.........R....R.F.........N.E..D.IKA..EV..R..S......KR......
LA 1739.seq         .....................................~.........R................................................
PI 390659.seq       D.....M........D..K...A......NPP.D.........R....R.F.........N.E..D.IKA..EV..R..S......KR......
PI 308182.seq       D.....M........D..K...D......NP..D.........R....R.F.........N.E..D.IKA..EV..R..S......KR.....*
LA 2171.seq         D.....M........D..K...D......NP..D.........R....R.F.........N.E..D.IKA..EV..R..S......KR.....*
LA 2541.seq
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                         310       320       330       340       350       360       370       380       390       400
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq TLQPYELSNLSQEDCWLLFMQRAFGHQEEMNLNLVAIGKVIVKKCGGVPLAAKTLGGILRFKREERQWEHVRDX------------------------
Ykema.seq         ...................................C.........................................SEIWNLPQDESSILPALRLSYHHLPLD
LA 2812.seq       ...................................C.........................................SEIWNLPQDESSILPALRLSYHHLPLD
PI 379014.seq     ...................................C..............................L...VDQ..E......NEIRNLPQDESSILPALRLSYHHLPVD
PI 379012.seq     ....................K...ENR.KI.P.......E....S......L...VDQ..E......NEIWNLPQDESSILPALRLSYHHLPVD
LA 1738.seq       ....................K...EN..KI.P.......E....S......L...VDQ..E......NEIWNLPQDESSILPALRLSYHHLPVD
PI 308181.seq     ....................K...EN..KI.P.......E....S......L...VDQ..E......NEIWNLPQDESFILPALRLSYHHLPVD
LA 1739.seq       ...................................................................S*
PI 390659.seq     ...................K...EN..K*
PI 308182.seq
LA 2171.seq
LA 2541.seq

                         410       420       430       440       450       460       470       480       490       500
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq ---------XFPKDTKMEKENLISLWMAHSFLLSKGNLELEDVGNEVWNELYLRSFFQEIEVKYDQTYFKMHDLIHDLATSLFSASTSSSNIREINVEGY
Ykema.seq         LRQCFSYCAV.............................................Q..R.........................................
LA 2812.seq       LRQCFSYCAV.............................................Q..R.........................................
PI 379014.seq     LTQSFAYCAV.....V...G......................Q..................D.K.....................RA..N......KRN
PI 379012.seq     LTQSFAYCAV.....V...G......................Q..................D.K.....................RA..N......KRN
LA 1738.seq       LTQSFAYCAV.....V...G......................Q..................D.K.................L.RA.............
PI 308181.seq     LTQSFAYCAV.....V...G......................Q..................D.K.................L.RA.............
LA 1739.seq
PI 390659.seq
PI 308182.seq
LA 2171.seq
LA 2541.seq

                         510       520       530       540       550       560       570       580       590       600
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq LHMMSIGFAKVVSSYSPPHLQKFVSLRVLNLSSMGLKQLPSSIGDL------------------LCKLQNLQTLNVEYCWSLCCLPKETSKLGSLRNLL
Ykema.seq         .............................................VHLRYLNLSLNNMRTLPKQ...................F..............
LA 2812.seq       .............................................VHLRYLNLSLNNMRTLPKQ...................F..............
PI 379014.seq     P...............S............ELR..H......VHLRYLNLYRNNMRSLPKQ.........DLQ...L.S...NQ..Q.S.V....
PI 379012.seq     P...............S............ELR..H......VHLRYLNLYRNNMRSLPKQ.........DLQ...L.S...NQ..Q.S.V....
LA 1738.seq       P...............S............ELR..R......VHLRYLNLSGNNMRSLPKQ.........DLQ......G..NQ..QVS......
PI 308181.seq     P...............S............ELR..R......VHLRYLNLSRNNMRSLPKQ.........DLQ.........NQ..QVS......
LA 1739.seq
PI 390659.seq
PI 308182.seq
LA 2171.seq
LA 2541.seq
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                              610       620       630       640       650       660       670       680       690       700
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq       LDGCDGLDSMPPRIGSLTCLKTLSFFVIGERKDSLLGELRNLNLYGSIEITHLERVKNDRDAKEANLSAKENLHSLSM~~~TWKGRHRYESEEVEVLEAL
Ykema.seq                ..........................L.................................................~~~R.E.P...........S.
LA 2812.seq              ..........................L.................................................~~~R.E.P...........S.
PI 379014.seq            .H..YK.N..........N...C..V.RK.S.Q......Q......Q.....L....V..............~~~....P.................
PI 379012.seq            .H..YK.N..........N...C..V.RK.S.Q......Q......Q.....L....V..............~~~....P.................
LA 1738.seq              .H..HK.N.............C..V.RK.S.Q.............Q............V.............I.EWDDDERP.................
PI 308181.seq            .H..HK.N.............C..V.RK.S.Q.............Q............V.............I.EWDDDERP.................
LA 1739.seq
PI 390659.seq
PI 308182.seq
LA 2171.seq
LA 2541.seq

                              710       720       730       740       750       760       770       780       790       800
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq       KPHSNVTGLTITGFRGFRLPKWMNHSVLKNVVSIAIRGCENCSCLPPFGDLPCLESLELGDGSAELEYVEDSGFPTRRRFPSLRKLIIVNFDNLKGLLKE
Ykema.seq                .......C.........I...E.................................RS....V.....................M...T.E..........
LA 2812.seq              .......C.........I...E.................................RS....V.....................M...T.E..........
PI 379014.seq            .......C...H....I.F.E...............DV...K.........E....K..K.Q.....M.H.~.....A...........N...........
PI 379012.seq            .......C...H....I.F.E...............D....K.........E....K..K.Q.....M.H.~.................N...........
LA 1738.seq              .......C.K.YR...I...................R.................WS....V.....................D..............
PI 308181.seq            .......C.K.YR...I...E...............R.................WS....V....H................D..............
LA 1739.seq
PI 390659.seq
PI 308182.seq
LA 2171.seq
LA 2541.seq

                              810       820       830       840       850       860       870       880       890       900
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Solyc08g075630.seq       AGEEQFPVLEEMTISWCPVLVIPTLSSVKKLVVNRNMSDAIGLRSIYNLRALTSLNISHNLTATSLPEEMFKSLANLKYLEISFIFNLKELPNSLASLNA
Ykema.seq                ...........L..RC...F.............H..K....V................F..................A..S..............
LA 2812.seq              ...........L..RC...F.............H..K....V................F..................A..S..............
PI 379014.seq            ....P.......D.W....F...........L.HW.......S..S.......H.R..FI................K...FY.................
PI 379012.seq            ....P.......D.W....F...........L.HW.......S..S.......H.R..FI................K...FY.................
LA 1738.seq              ...........L.V.C..MF...........Y.....TV....S.....S......F..................A...FD.............
PI 308181.seq            ...........L...C...F...........Y.....TVF.............L.SI.................A...FD.............
LA 1739.seq
PI 390659.seq
PI 308182.seq
LA 2171.seq
LA 2541.seq
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                              910       920       930       940       950       960       970       980
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
Solyc08g075630.seq       LKHLKIEYCDALESLPEEGVKGLTSLTELSITNCKRLKCLPEGLQHLTNLSVRECPTLAKRCEKGIGQDWYKIAHIPHLLITNEM*
Ykema.seq                ....F.N..F........A........Q....Y.............Q......Y.........................D*
LA 2812.seq              ....F.N..F........A........Q....Y.............Q......Y.........................D*
PI 379014.seq            ....EMN..PK..T.............Q....Y..M.....E..Q....IKN...........................D*
PI 379012.seq            ....EMN..PK..T.............Q..V.Y..M.....E..Q....IKN...........................D*
LA 1738.seq              .......S.Y........A........Q...EY.EM........Q.....ITN..........................D*
PI 308181.seq            .......S.Y........A........Q...EY.EM........Q.....ITN..........................D*
LA 1739.seq
PI 390659.seq
PI 308182.seq
LA 2171.seq
LA 2541.seq
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