HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

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

DOI: 10.54598/002890

The Thesis of the PhD dissertation

Ahmad Abed Alfattah Jawdat Jewehan

Gödöllő

2022

The PhD School

Name: Doctoral School of Plant Sciences

Discipline: Crop and Horticultural Sciences

Head: **Prof. Dr. Lajos Helyes**
Director of institute
Hungarian University of Agricultural and Life Sciences, Gödöllő

Supervisor: **Dr. Zoltan Szabo**
Group leader
Hungarian University of Agricultural and Life Sciences, Gödöllő
Institute of Genetics and Biotechnology, Gödöllő
Applied Plant Genomics Group

....

Prof. Dr. Lajos Helyes
Approval of the School Leader

Dr. Zoltan Szabo
Approval of the Supervisor

1. BACKGROUND OF THE WORK

Tomato is one of the most important "protective foods" because of its high nutritional value, taste and versatile uses (PASSAM ET AL. 2007). However, this crop is confronted with several biotic and abiotic stresses contributing to the low production quality and yield than its potential. An example of biotic factors is viruses diseases, which are major constraints in tomato production (HANSSEN et al. 2010). The tomato is susceptible to many viruses, such as tobamoviruses (ADAMS et al. 2009). These viruses have very stable particles that are highly infectious and can be easily transmitted mechanically via wounds caused by workers or pollinating insects (OKADA et al. 2000; LEVITZKY et al. 2019). The most important tobamoviruses infecting tomato are tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), tomato mild mottle virus (ToMMV) as well as the recently characterized Tomato brown rugose fruit virus (ToBRFV)(LI et al. 2013; JONES et al. 2016; SALEM et al. 2015).

ToBRFV is a new tobamovirus initially isolated from tomato plants grown in greenhouses in Jordan (SALEM et al. 2015). ToBRFV is characterized by a typical rod-shaped particle (300 × 18 nm in size) morphology encapsulating a single-stranded RNA (+ssRNA) sense genome of 6.393 kb encoding four ORFs: two ORFs encoding replication-related proteins (Rep) (ORF1a and ORF1b) of 126 and 183 kDa; ORF2 encoding a movement protein (MP) of 30 kDa, and ORF3 encoding a coat protein (CP) of 17.5 kDa (MAAYAN et al. 2018). The virus infection can occur via seed transmission as primary inoculum and through pollen transmission of bumblebee (*Bombus terrestris*) (DOMBROVSKY and SMITH 2017; LEVITZKY et al. 2019).

ToBRFV Symptoms vary depending on the variety. 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. Besides tomato, ToBRFV can infect sweet pepper (*Capsicum annuum*) with symptoms that resemble descriptions of ToBRFV on tomato leaves

and fruit (OLADOKUN et al. 2019; PANNO et al. 2020; SALEM et al. 2020).

ToBRFV is of special concern because of the ability to overcome all known genetic resistances in tomato R genes *Tm-1*, *Tm-2* and *Tm-2*^{2}(*Tm-2*^{a}), which have been used to control tobamovirus in tomato for the last 60 years (LURIA et al. 2017). However, some mutant strains of TMV and four strains of ToMV (ToMV-0, ToMV-1, ToMV-2 and ToMV-2^2) have emerged as resistance overcome and recognized based on the introgressed resistance (*Tm1*, *Tm2* and *Tm2*^{2}) genes. On the other hand, resistance-breaking ToMV strains were not detected in nature (MESHI et al. 1989; CALDER and PALUKAITIS 1992; WEBER et al. 1993; BETTI et al. 1997; STRASSER and PFITZNER 2007). However, a new tobamovirus ToBRFV caused a "pandemic alert" in Europe and worldwide (VAN DE VOSSENBERG et al. 2020; EPPO, 2021a).

There are many ways to control ToBRFV, such as seed treatment, sanitation, disinfected material and grafting the plant on virus-resistant rootstock could limit damage through contaminated soil (DAVINO et al. 2020; SPANÒ et al. 2020; CHANDA et al. 2021; SAMARAH et al. 2021). Nevertheless, the best way is to use resistant varieties. For this purpose, wild tomatoes serve as excellent model systems for both basic and applied plant research. They have been utilized as a source of resistance to pathogens because of their genetic diversity (KOLE 2011). Resistance and tolerance to different viruses have been found in several wild tomato species, especially *Solanum pimpinellifolium*, *S. peruvianum* and *S. habrochaites* (syn: *Lycopersicon hirsutum*) (RAZDAN and MATTOO 2006).

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 the wild tomato gene pool. Therefore, our study started three years ago with the aim 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 resistance 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 aimed 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. MATERIALS AND METHODS

3.1 Plant materials

A total of 809 accessions belonging to 16 *Solanum* species (sections *Lycopersicon* and *Juglandifolia*) were studied (Table 1) in two independent screening experiments. In the first one, 636 *Solanum* accessions (denoted Group A plants) were investigated. Plant of *S. habrochaites* PI 126445 (original source of the *Tm-1* gene), *S. peruvianum* PI 126926 (source of the *Tm-2* gene) and PI 128650 (source of the *Tm-2^2 gene*), *S. lycopersicum* LA1221 (carrying the introgressed *Tm-2^2 gene*), and the susceptible cultivar *S. lycopersicum cv.* Ceglédi (genotype +/+) were used as controls.

Table 1. *Solanum* species and 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

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.

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 department of genetics and microbiology of MATE.

3.2 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 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 ºC for inoculation for future use.

3.3 Plant inoculation

For the inoculation of plants, mechanical transmission was used. Virus inoculum was gently rubbed using sterile glass spatula onto carborundum dusted on lower leaves of young tomato and tobacco test plants. 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.

3.4 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 2 (Group A plants) and Table 3 (Group B plants), respectively.

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

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

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 to the symptomless plants (class 0) plants. The virus-free plants expected to be resistant were later investigated in more detail.

3.5 Detection of viruses

The presence or absence of viruses in leaf samples of symptomless plants was assayed using bioassays, RT-PCR (reverse-transcription polymerase chain reaction) and RT-qPCR (reverse-transcription quantitative polymerase chain reaction). Samples were taken at 2-5 wpi from newly developed top leaves and from inoculated leaves at 1 wpi. The assayed leaves were rinsed with sodium hydroxide (2%) and then with tap water to avoid virus contamination. Bioassays were carried out by rubbing indicator plants (*N. glutinosa* and *N. tabacum* cv. Xanthi nc) with leaf extract prepared from donor tomato plants. PCR primers specific were used for ToBRFV, ToMV and TMV to amplify the coat protein gene segment of the target virus. Furthermore, two candidate reference genes, EFα1 and GAPDH, were selected to check RNA samples' quality and expression levels.

3.6 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 ones 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) 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 leaves at 10 days post-inoculation (dpi) and top leaves at 40 dpi using bioassays and RT-PCR for *S. ochranthum* and bioassays and RT-qPCR for *S. habrochaites* and *S. peruvianum*. All the greenhouse and laboratory experiments were carried out under quarantine conditions.

3.7 Evaluation of resistance to ToBRFV under high temperature

Six resistant plants from each accession propagated vegetatively on MS media were inoculated with ToBRFV-Tom2-Jo. Three inoculated plants from each accession were maintained into a Sanyo environment plant growth chamber at a constant temperature of 33 ºC (light intensity 50 Wm-2, day length 14 h). 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.

3.8 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. The presence of ToBRFV was conducted by using bioassays.

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

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). A 3500 bp genomic segment of the NBS-LRR gene was amplified by PCR using specific primers based on the gene sequence in *S. habrochaites* (LYC4943) responsible for resistance against ToBRFV, published by YKEMA et al. (2020), which is ortholog with the sequences of *S. lycopersicon* (SOLgenomics) Solyc08g075630 loci. The PCR fragments were cloned into pGEM®-T Easy plasmid (Promega, Madison, USA) according to standard protocols and sequenced with SANGER technology on ABI Prism (3130xl Genetic Analyzer (Biomi, Gödöllő, Hungary)). For genomic comparison, we used the programs DNASTAR Seqman, Bioedit, BLASTN, BLASTP and ClustalW.

3.10 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. 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 were checked using bioassays and RT-qPCR.

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. Extracted RNA samples were used as a template for complementary DNA (cDNA) transcription oligonucleotide specific for ToBRFV. Primer3 computer software (version 4.0.0) was used to design the PCR primers specific using the ToBRFV (KT383474) reference virus genomes. To amplify cDNA 6.4 kb fragment of the virus, the CloneAmp™ high-fidelity (HiFi) PCR Premix (Takara Bio) was used. 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 and sequenced with SANGER technology on ABI Prism (3130xl Genetic Analyzer) (Biomi Ltd, Gödöllő, Hungary) using primer walking on the ToBRFV genome. The sequences for both isolates ToBRFV-Tom2-Jo and Tom2M-Jo were deposited in NCBI GenBank under accessions numbers MZ323110 and MZ438228, respectively.

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.

4. RESULTS and DISCUSSION

4.1 Screening of *Solanum* germplasms for reactions to the ToBRFV

4.1.1 Evaluation of *Solanum* accessions for the responses to inoculation with ToBRFV

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 2). Plants of the control accessions, *S. lycopersicum* (LA1221; *Tm-2*^{2}), *S. lycopersicum* (Ceglédi; +/+) and *S. peruvianum* (PI 126926; *Tm-2*, PI 128650; *Tm-2*^{2}), 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%. Moreover, 26 accessions from different tomato species showed no symptoms or mild mosaic. 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%. ToBRFV was demonstrated in the top leaves of all of these symptomless or almost symptomless plants using bioassays and RT-PCR, excluding *S. ochranthum*. The reactions of this species' plants were unusual, which are analyzed in detail in chapter **4.2**.

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 ($Tm\text{-}2^2$.). 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%. The symptoms were mosaic, mottling, and sometimes deformation of top leaves, which started to appear at 10–14 dpi. 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). The symptomless plants were transplanted into pots for further investigations (**4.2.2**).

4.2 Investigations on tobamovirus resistance in selected *Solanum ochranthum*, *S. habrochaites* and *S. peruvianum* plants

4.2.1 Studies on tobamovirus resistance of *S. ochranthum*

In the course of screening group A plants, five *S. ochranthum* accessions behaved unusually. Three of them (LA2160, LA2162, LA2166) remained symptomless after inoculation with ToBRFV, while two (PI473498 and PI230519) showed mild

systemic mosaic followed by total recovery.

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. They initially, at 15 dpi showed mild systemic mosaic symptoms (DSI 20%) and contained an infective virus. Thereafter, they recovered from the symptoms and the virus could not be detected on their newly emerged symptomless leaves. 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).

4.2.2 Studies on tobamovirus resistance in *S. habrochaites* and *S. peruvianum*

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

After transplantation of symptomless individuals of *S. peruvianum* and *S. habrochaites* **(4.1.1)**, the presence of ToBRFV was checked in their inoculated at 10 dpi as well as 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. Similarly, no virus was also demonstrated by RT-qPCR tests. Furthermore, the expression levels of the internal control genes

of all samples were detected. In contrast, numerous necrotic local lesions developed on leaves of *N. glutinosa* inoculated with the extract of symptomatic plants of *S. lycopersicum* controls. *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 on their progenies.

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, besides ToBRFV with TMV and ToMV. *S. lycopersicum* cv. Ceglédi has used a susceptible sensitive control. Similar to ToBRFV, TMV and ToMV caused mosaic in susceptible control tomato and necrotic local lesions in *N. glutinosa*. 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.

4.2.2.2 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 days. Plant of all accessions became diseased at 10-14 dpi showing mosaic, leaf deformation and stunting symptoms. ToBRFV was detected in their symptomatic top leaves by bioassays and RT-qPCR. 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. The resistant plants that displayed severe symptoms at 33 °C, developed new symptomless leaves after being kept in the greenhouse at 24 °C. Unexpectedly, these newly developed leaves were also proved free from the virus, when assessed by RT-qPCR and bioassay. Hence, we presume that ToBRFV starts to replicate in some locally infected cells of resistant plants, because at elevated temperatures at

33 °C, the virus moved to the top of the plants and causes severe systemic disease symptoms. Interestingly, this resistance could also be characterized by restore in 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).

4.2.2.3 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. The scions started to show mosaic symptoms on the newly developed leaves at 30 days after grafting on all five repetitions. The virus was also detected in the diseased symptomatic leaves of the scions using *N. glutinosa* bioassays.

4.2.2.4 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.

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

4.3.1 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 **(4.2.2.1)** were propagated by rooting of their lateral shoots. Three young progenies of each plant were inoculated again. As 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 mosaic symptoms, characterized as tobamoviruses. The virus, a suspected mutant of the original Tom2-Jo, was marked after that as Tom2M-Jo and then propagated in Samsun tobacco.

In a comparative inoculation experiment, ToBRFV-Tom2-Jo and ToBRFV-Tom2M-Jo were able to infect systemically control tomato genotypes: GCR26-Craigella (*tm-1*CRG26), GCR237-LA3269 (*Tm-1*), LA2088 (*Tm-2*), LA3471-Moneymaker (*Tm-2*^{2}) and Ceglédi (*Tm*+), where showed severe symptoms such as mosaic, deformation, leaf narrowing. 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 and all plants were proved virus-free as assessed by bioassay and RT-qPCR. On the other hand, ToBRFV-Tom2M-Jo infected systemically in each individual of *S. habrochaites* and *S. peruvianum*. At 14 dpi, the upper leaves of infected plants showed obvious mosaic symptoms and virus propagation could be detected in their symptomatic top leaves using bioassays and RT-qPCR.

4.3.2 Comparing sequence of ToBRFV Tom2-Jo and Tom2M-Jo

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, 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.

The results of alignment sequences of nucleotide and amino acid on MP of ToBRFV-Tom2M-Jo with 118 ToBRFV genome sequences provided by data nextstrain build (VAN DE VOSSENBERG et al. 2020) and NCBI genebank revealed that ToBRFV-Tom2M-Jo had only two unique nucleotides and amino acid changes on MP, which were not found in all other 118 sequences.

These results resemble with former results described by MESHI et al. 1989; CALDER and PALUKAITIS 1992; WEBER et al. 1993; 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 mutations 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 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. (2021), 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*, which probably has similar mechanism interactions as in *Tm-2* and *Tm-2²* genes against ToBRFV.

5. 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 include accessions of *S. arcanum*, *S. chmielewskii*, *S. huaylasense*, *S. juglandifolium*, *S. sitiens*, 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 above-mentioned five species 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 analyse 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 always presented in accessions segregated for 10-50 % of resistant plants. 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 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) (Phe22 → Asn and Tyr82 → 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-2^2 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.

6. 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 species of wild tomato *Solanum* (sections *Lycopersicon* and *Juglandifolia*) germplasm.

2- We found that a large number of them were susceptible, including the accessions of *S. arcanum*, *S. chmielewskii*, *S. huaylasense*, *S. juglandifolium*, *S. sitiens*. 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 levels 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.

7. REFERENCES

1- ADAMS, M. J., ANTONIW, J. F., KREUZE, J. (2009): Virgaviridae: A new family of rod-shaped plant viruses, *Archives of Virology*, 154(12), 1967–1972.

2- BETTI, L., MARINI, F., MARANI, F., CUFFIANI, M., RABITI, A. L., CANOVA, A. (1997): A TMV strain overcoming both *Tm-2* and *Tm-2²* resistance genes in tomato, *Phytopathologia Mediterranea*, 36(1), 24–30.

3- CALDER, V. L., PALUKAITIS, P. (1992): Nucleotide sequence analysis of the movement genes of resistance breaking strains of tomato mosaic virus, *Journal of general virology*, 73(1), 165–168.

4- CAMARA, M., MBAYE, A. A., NOBA, K., SAMB, P. I., DIAO, S., CILAS, C. (2013): Field screening of tomato genotypes for resistance to Tomato yellow leaf curl virus (TYLCV) disease in Senegal, *Crop Protection*, 44(July 2020), 59–65.

5- CHANDA, B., SHAMIMUZZAMAN, M., GILLIARD, A., LING, K. S. (2021): Effectiveness of disinfectants against the spread of tobamoviruses: Tomato brown rugose fruit virus and Cucumber green mottle mosaic virus,

Virology Journal, 18(1), 1–12.

6- CIRULLI, M., CICCARESE, F. (1975): Interactions between TMV isolates, temperature, allelic condition and combination of the *Tm* resistance genes in Tomato, *Phytopathologia Mediterranea*, 14(2/3), 100–105.

7- DAVINO, S., CARUSO, A. G., BERTACCA, S., BARONE, S., PANNO, S. (2020): Tomato brown rugose fruit virus: Seed transmission rate and efficacy of different seed disinfection treatments, *Plants*, 9(11), 1615

8- DOMBROVSKY, A., SMITH, E. (2017): Seed transmission of tobamoviruses: aspects of global disease distribution. In: JIMENEZ-LOPEZ, J.C. (ed), *Seed biology,* IntechOpen, London, pp 234–260.

9- EPPO (2022a): *Tomato brown rugose fruit virus (TOBRFV) distribution*. Available at: https://gd.eppo.int/taxon/TOBRFV/distribution

10- FRASER, R. S. S. and LOUGHLIN, S. A. R. (1982): Effects of temperature on the *Tm-1* gene for resistance to tobacco mosaic virus in tomato, *Physiological Plant Pathology*, 20(1), 109–117.

11- HAK, H., SPIEGELMAN, Z. (2021): The Tomato brown rugose fruit virus movement protein overcomes *Tm-2(2)* resistance in tomato while attenuating viral transport, *Molecular plant-microbe interactions*, 50

12- HANSSEN, I. M., LAPIDOT, M., THOMMA, B. P. H. J. (2010): Emerging viral diseases of tomato crops, *Molecular plant-microbe interactions*, 23(5), 539–548

13- JONES, J. B., ZITTER, T. A., MILLER, S. A. (2016): *Front Matter, Compendium of Tomato Diseases and Pests*, Second Edition, APS Press/The American Phytopathological Society, USA, i–vi.

14- KOLE, C. (2011): *Wild crop relatives: genomic and breeding resources: vegetables*. Springer, Berlin, Heidelberg

15- LEVITZKY, N., SMITH, E., LACHMAN, O., LURIA, N., MIZRAHI, Y., BAKELMAN, H., SELA, N., LASKAR, O., MILROT, E., DOMBROVSKY, A. (2019): The bumblebee *Bombus terrestris* carries a primary inoculum of Tomato

brown rugose fruit virus contributing to disease spread in tomatoes, *PLoS ONE*, 14(1), 1–13.

16- LI, R., GAO, S., FEI, Z., LING, K.-S. (2013): Complete genome sequence of a new tobamovirus naturally infecting tomatoes in Mexico, *Genome announcements*, 1(5), e00794-13

17- LURIA, N., SMITH, E., REINGOLD, V., BEKELMAN, I., LAPIDOT, M., LEVIN, I., ELAD, N., TAM, Y., SELA, N., ABU-RAS, A., EZRA, N., HABERMAN, A., YITZHAK, L., LACHMAN, O., DOMBROVSKY, A. (2017): A new israeli tobamovirus isolate infects tomato plants harboring *Tm-2*^{2} resistance genes, *PLoS ONE*, 12(1), 1–19.

18- MAAYAN, Y., PANDARANAYAKA, E. P. J., SRIVASTAVA, D. A., LAPIDOT, M., LEVIN, I., DOMBROVSKY, A., HAREL, A. (2018): Using genomic analysis to identify tomato *Tm-2* resistance-breaking mutations and their underlying evolutionary path in a new and emerging tobamovirus, *Archives of Virology*, 163(7), 1863–1875.

19- MESHI, T., MOTOYOSHI, F., MAEDA, T., YOSHIWOKA, S., WATANABE, H., OKADA, Y. (1989): Mutations in the tobacco mosaic virus 30-kD protein gene overcome *Tm-2* resistance in tomato, *The Plant cell*, 1(5), 515–522.

20- OKADA, K., KUSAKARI, S., KAWARATANI, M., NEGORO, J., SATOSHI, T. O., OSAKI, T. (2000): Tobacco mosaic virus is transmissible from tomato to tomato by pollinating bumblebees, *Journal of General Plant Pathology*, 66(1), 71–74

21- OLADOKUN, J. O., HALABI, M. H., BARUA, P., NATH, P. D. (2019): Tomato brown rugose fruit disease: current distribution, knowledge and future prospects, *Plant Pathology*, 68(9), 1579–1586.

22- PANNO, S., CARUSO, A. G., BLANCO, G., DAVINO, S. (2020): First report of Tomato brown rugose fruit virus infecting sweet pepper in Italy, *New Disease Report*, 41(20), 588–2044

23- PASSAM, H., KARAPANOS, I., BEBELI, P., SAVVAS, D. (2007): A review of recent research on tomato nutrition, breeding and post-harvest technology with reference to fruit quality, *The European Journal of Plant Science and Biotechnology*, 1(1), 1–21

24- RAZDAN, M. K. and MATTOO, A. K. (2006): *Genetic improvement of solanaceous crops volume 2: Tomato*, CRC Press, USA, pp666

25- SALEM, N. M., CAO, M. J., ODEH, S., TURINA, M., TAHZIMA, R. (2020): First report of tobacco mild green mosaic virus and tomato brown rugose fruit virus infecting *Capsicum annuum* in Jordan , *Plant Disease*, 104(2), 601.

26- SALEM, N., MANSOUR, A., CIUFFO, M., FALK, B. W., TURINA, M. (2015): A new tobamovirus infecting tomato crops in Jordan, *Archives of Virology*, 161(2), 503–506.

27- SAMARAH, N., SULAIMAN, A., SALEM, N. M., TURINA, M. (2021): Disinfection treatments eliminated tomato brown rugose fruit virus in tomato seeds, *European Journal of Plant Pathology*, 159(1), 153–162.

28- SOLER-ALEIXANDRE S, LÓPEZ C, CEBOLLA-CORNEJO J, NUEZ F (2007): Sources of resistance to pepino mosaic virus (PepMV) in tomato, *HortScience*, 42:40–45.

29- SPANÒ, R., FERRARA, M., GALLITELLI, D., MASCIA, T. (2020): The role of grafting in the resistance of tomato to viruses, *Plants*, 9(8), 1–20.

30- STRASSER, M. and PFITZNER, A. J. P. (2007): The double-resistance-breaking Tomato mosaic virus strain ToMV1-2 contains two independent single resistance-breaking domains, *Archives of Virology*, 152(5), 903–914.

31- VAN DE VOSSENBERG, BART T L H, VISSER, M., BRUINSMA, M., KOENRAADT, H. M. S., WESTENBERG, M., BOTERMANS, M. (2020): Real-time tracking of Tomato brown rugose fruit virus (ToBRFV) outbreaks in the Netherlands using Nextstrain, *Plos one,* 15(10), e0234671

32- WEBER, H., SCHULTZE, S., PFITZNER, A. J. (1993): Two amino acid substitutions in the tomato mosaic virus 30-kilodalton movement protein confer

the ability to overcome the *Tm-2(2)* resistance gene in the tomato., *Journal of Virology*, 67(11), 6432–6438.

33- YAN, Z., MA, H., WANG, L., TETTEY, C., ZHAO, M., GENG, C., TIAN, Y., LI, X. (2021): Identification of genetic determinants of tomato brown rugose fruit virus that enable infection of plants harbouring the *Tm-2* ^{2} resistance gene , *Molecular Plant Pathology*, 1–11.

34- YKEMA, M., VERWEIJ, C. W., DE LA FUENTE VAN BENTEM, S. (2020): tomato plant resistant to tomato brown rugose fruit virus, *Patentscope*, 143pp.https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020147921&tab=PCTBIBLIO

8. PUBLICATIONS

1- Jewehan, A., Salem, N., Tóth, Z., Salamon, P., & Szabó, Z. (2021). Screening of *Solanum* (sections *Lycopersicon* and *Juglandifolia*) germplasm for reactions to the tomato brown rugose fruit virus (ToBRFV). *Journal of Plant Diseases and Protection*, 1-7. https://doi.org/10.1007/s41348-021-00535-x. **(IF: 1.928)**

2- Kiemo, F. W., Salamon, P., Jewehan, A., Tóth, Z., & Szabó, Z. (2021). Detection and elimination of viruses infecting sweet potatoes in Hungary. *Plant Pathology*. https://doi.org/10.1111/ppa.13519. **(IF: 2.590)**

3- Jewehan, A., Salem, N., Tóth, Z., Salamon, P. and Szabó, Z., 2022. Evaluation of responses to tomato brown rugose fruit virus (ToBRFV) and selection of resistant lines in *Solanum habrochaites* and *Solanum peruvianum* germplasm. *Journal of General Plant Pathology*, pp.1-10. **(IF: 1.449)**

4- Jewehan, A., Kiemo, F. W., Salem, N., Tóth, Z., Salamon, P., & Szabó, Z. (2022). Isolation and molecular characterization of tomato brown rugose fruit virus mutant breaking the 1 tobamovirus resistance found in wild *Solanum* species. *Archives of Virology.* **(IF: 2.574)**

Conference

Ahmad Jewehan, Pál Salamon, Lilla Hajnik, Nida Salem, Zoltán Szabó: Identification of new resistance source in Solenaceae against ToBRFV**.** MBK Scientific Conference. https://abc.naik.hu/esemenyek/2020-jovoteremto-tudomany-mbk-napok-30