Tomato brown rugose fruit virus (ToBRFV)
Current status and perspectives

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ARO. The Volcani Center

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Adolph Mayer described “the mosaic disease of tobacco”
(published in 1886) tobacco mosaic disease

Plant virology emerged from the landmark *Tobacco mosaic virus* (TMV) studies by Mayer, Ivanowski, and Beijerinck. Beijerinck was the first to use the term virus in a modern context.

Cucumber mosaic diseases (1-4) reported in 1935, UK. “MOSAIC DISEASES OF THE CUCUMBER”, Ainsworth, 1935. Cucumber green mottle mosaic virus (CGMMV)
• Tobacco mosaic virus (TMV)
• Tomato mosaic virus (ToMV)
• **Tomato brown rugose fruit virus (ToBRFV)**
• Tomato mottle mosaic virus (ToMMV)
• Cucumber green mottle mosaic virus (CGMMV)
• Pepper mild mottle virus (PMMoV)
• Paprika mild mottle virus (PaMMV)

37 species:
Transmitted by contaminated seeds, mechanical contact, preserved infectious in soil
Tobamoviruses and resistance genes in vegetables

Cucumber green mottle mosaic virus (CGMMV)

Tomato brown rugose fruit virus (ToBRFV)

Pepper mild mottle virus (PMMoV)

Resistance genes in commercial vegetable varieties

Cucurbits
* Ineffective R genes
** Tolerant rootstocks

Tomato
Tm-2²

Pepper
L⁴
Breaking $Tm-2^2$ resistance in tomatoes
First report in October 2014
Disease symptoms of ToBRFV
Disease symptoms of ToBRFV
Disease symptoms of ToBRFV

(Luria et al., 2017)
ToBRFV detection in tomato plants

Morphological and serological characterization of viral particles and coat protein.

Monitoring the distribution of ToBRFV disease in tomatoes grown in greenhouses in Israel


http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0170429
In field or greenhouse/tunnel house

- Agro-technique work
- Contaminated tools
- Dispersion by pollinators and animals

In field or greenhouse/tunnel house

- Survival in soil

At the nursery

- Contaminated trays
- Grafting knives
- Irrigation by sprinklers

- Insufficient seed disinfection treatments

CGMMV disease cycle

- Primary inoculum
- Secondary spread
- Contaminated seeds
- Infected seedlings

Preservation in weeds
ToBRFV modes of spread

Secondary spread

1. Tools, hands...
2. Pollinator insects

Primary Inoculum sources

1. Seeds
2. Soil
3. Weeds
Transmission by hands 86%±0.5

Reingold *et al.*, 2015

IR = Infectivity rate
Mechanical transmission of ToBRFV and PepMV

<table>
<thead>
<tr>
<th>Plant no.</th>
<th>ToBRFV1</th>
<th>ToBRFV2</th>
<th>ToBRFV3</th>
<th>Mixed1</th>
<th>Mixed2</th>
<th>Mixed3</th>
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**IR ≥ 9/ mixed ≥9**

**ToBRFV**

**IR ≥ 1/ mixed ≥4**

**PepMV**

**IR = Infectivity rate**
The bumblebee *Bombus terrestris* contributes to *Tomato brown rugose fruit virus* spread in tomatoes

I, ToBRFV infected plant tissue parts, L- leaves, S- sepal P- petal, SC-stamen encompassing the carpel

The bumblebee *Bombus terrestris* contributes to ToBRFV spread in tomatoes

The bumblebee* *Bombus terrestris* contributes to ToBRFV spread

Levitzky et al. (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): e0210871. [https://doi.org/10.1371/journal.pone.0210871](https://doi.org/10.1371/journal.pone.0210871)
Primary Inoculum sources

1. Seeds
2. Soil
3. Weeds
ToBRFV detection in tomato seeds
(Serology-based methods)
Current study: ToBRFV detection in tomato seeds (molecular and high-throughput sequencing technologies)
Soil examination for CGMMV particles

Virion purifications followed by visualization of viral particles by TEM

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Soil disinfection of tobamoviruses

1. Using intermediate media

These viruses are able to enter the plant cells through the wounded roots.
Careful planting
Soil disinfection using stabilized chlorine

Planting pit preparation

Application before planting

Klor Bac

2,000 ppm. for soil disinfection

Application in large scale via the irrigation system one day prior to planting.

1,000 ppm. for structure disinfection
Studying the efficiency of disinfectants

South R&D center (2016-2019)

Virus inoculum

Application of the disinfectant

Planting
### Comparison between three formulations of stabilized chlorine on ToBRFV infection in tomatoes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rep. 1</th>
<th>Rep. 2</th>
<th>Rep. 3</th>
<th>Total</th>
<th>Infectivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klor Bac</td>
<td>2/50</td>
<td>6/50</td>
<td>8/50</td>
<td>16/150</td>
<td>AB 10.7</td>
</tr>
<tr>
<td>ChloRun</td>
<td>2/50</td>
<td>5/50</td>
<td>2/50</td>
<td>9/150</td>
<td>B 6</td>
</tr>
<tr>
<td>Taharn</td>
<td>4/50</td>
<td>3/50</td>
<td>1/50</td>
<td>8/150</td>
<td>B 5.3</td>
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<tr>
<td>Positive control</td>
<td>31/50</td>
<td>20/100</td>
<td>46/100</td>
<td>97/250</td>
<td>A 38.8</td>
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<tr>
<td>Negative control</td>
<td>1/50</td>
<td>------</td>
<td>------</td>
<td>1/50</td>
<td>2</td>
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## ToBRFV in tomatoes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rep. 1</th>
<th>Rep. 2</th>
<th>Rep. 3</th>
<th>Infectivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilized hydrogen peroxide</td>
<td>4/50</td>
<td>2/55</td>
<td>6/46</td>
<td>7.9</td>
</tr>
<tr>
<td>GreenUp-D</td>
<td>0/52</td>
<td>0/48</td>
<td>0/49</td>
<td>0</td>
</tr>
<tr>
<td>Taharan</td>
<td>0/53</td>
<td>0/52</td>
<td>0/51</td>
<td>0</td>
</tr>
<tr>
<td>Control wounded roots</td>
<td>6/52</td>
<td>5/57</td>
<td>17/51</td>
<td>17.5</td>
</tr>
<tr>
<td>Control not wounded</td>
<td>3/51</td>
<td>4/51</td>
<td>--------</td>
<td>6.9</td>
</tr>
<tr>
<td>Negative control</td>
<td>0/53</td>
<td>--------</td>
<td>--------</td>
<td>0</td>
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## CGMMV in cucumber

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rep. 1</th>
<th>Rep. 2</th>
<th>Rep. 3</th>
<th>Infectivity %</th>
</tr>
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<tr>
<td>Stabilized hydrogen peroxide</td>
<td>2/48</td>
<td>1/50</td>
<td>3/49</td>
<td>4.1</td>
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<tr>
<td>GreenUp-D</td>
<td>2/46</td>
<td>0/49</td>
<td>0/49</td>
<td>1.4</td>
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<tr>
<td>Taharan</td>
<td>0/52</td>
<td>0/50</td>
<td>0/47</td>
<td>0</td>
</tr>
<tr>
<td>Control wounded roots</td>
<td>13/52</td>
<td>9/50</td>
<td>1/37</td>
<td>15.2</td>
</tr>
<tr>
<td>Control not wounded</td>
<td>1/50</td>
<td>0/52</td>
<td>--------</td>
<td>1</td>
</tr>
<tr>
<td>Negative control</td>
<td>0/51</td>
<td>--------</td>
<td>--------</td>
<td>0</td>
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</tbody>
</table>
Ongoing studies on ToBRFV
Genetic resistance in tomato to ToBRFV

Moshe Lapidot and Ilan Levin

Institute of Plant Sciences
Volcani Center, ARO, ISRAEL

Plant Sciences
Avner Zinger
Dana Gelbart
Ben Avni
Zion Machbash

Hebrew University
Dani Zamir
To identify and characterize ToBRFV-resistant tomato genotypes, ~200 tomato genotypes were screened for viral resistance.

Results:
- A relatively large number of tolerant genotypes were identified.
- A single resistant genotype was identified.
The tolerance trait is recessive and segregated as a **single recessive gene**
Resistance is polygenic - it is controlled by at least two interacting loci: one is recessive and the other in semi-dominant.
Development of ToBRFV resistance in Solanum lycopersicum via genome editing

Michael Kravchik, Bekele Abebie, Yula Shnaider
Reenu Kumari, Diana Leibman
Amit Gal-On

Department of Plant Pathology
ARO Volcani Center, Israel
Schematic representation of *SITOM1* and *SITOM3* genomic map and the sgRNA and target sites

*Analysis of tomato lines for mutation within the target genes*
12 dpi

17kDa
# ToBRFV host range

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Early symptoms 4–7 dpi</th>
<th>Systemic symptoms description 7–14 dpi</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Capsicum annuum</em> (pepper) *Cv’s Maor, (L¹), Fiona (L³, Sw-5), Romans and Lyri (L⁴, Sw-5)</td>
<td>HR</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td><em>Chenopodium murale</em></td>
<td>YNL, BNL</td>
<td>MM, LL</td>
<td>+</td>
</tr>
<tr>
<td><em>C. amaranticolor</em></td>
<td>YNL</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td><em>C. quinoa</em></td>
<td>YNL</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>YNL</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td><em>Nicotiana benthamiana</em></td>
<td>NL</td>
<td>LL, PC</td>
<td>+</td>
</tr>
<tr>
<td><em>N. clevelandii</em></td>
<td>BNL</td>
<td>LY</td>
<td>+</td>
</tr>
<tr>
<td><em>N. glutinosa</em></td>
<td>NL</td>
<td>MM</td>
<td>+</td>
</tr>
<tr>
<td><em>N. tabacum cv. occidentalis</em></td>
<td>M</td>
<td>MM</td>
<td>+</td>
</tr>
<tr>
<td><em>N. tabacum cv. rustica</em></td>
<td>+</td>
<td>MM</td>
<td>+</td>
</tr>
<tr>
<td><em>N. tabacum cv. samsun</em></td>
<td>MM</td>
<td>M</td>
<td>+</td>
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<tr>
<td><em>N. tabacum cv. samsun N.N</em></td>
<td>NL</td>
<td>NS</td>
<td>-</td>
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<td><em>N. tabacum cv. sylvestris</em></td>
<td>NL</td>
<td>MM</td>
<td>+</td>
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<td><em>Petunia hybrida</em></td>
<td>-</td>
<td>NS</td>
<td>+</td>
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<tr>
<td><em>Solanum tuberosum</em> (potato) cv. Nicola*</td>
<td>-</td>
<td>NS</td>
<td>-</td>
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<tr>
<td><em>S. nigrum</em> (black nightshade)</td>
<td>-</td>
<td>MM/NS</td>
<td>+</td>
</tr>
<tr>
<td><em>S. melongena</em> (eggplant) cv’s. Classic, 206</td>
<td>-</td>
<td>NS</td>
<td>-</td>
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</tbody>
</table>

Early symptoms, local symptoms developed on the inoculated leaf 4–7 days post inoculation (dpi): hypersensitivity response (HR), necrotic lesions (NL) yellowing necrotic lesions (YNL), brown necrotic lesions (BNL), mottling (M). Systemic symptoms description 7–14 days post infection: no symptoms (NS); mild mottling (MM); Leaf lesion (LL). Mosaic (M); plant collapse (PC); Leaf yellowing (LY). Positive ELISA results, >3 of negative value are depicted in (+), while negative results marked (-).

doi:10.1371/journal.pone.0170429.t002
Grafted tomato plants on eggplant rootstock
Current experiment: ToBRFV root infection of grafted plants

S R S R

ToBRFV root infection
Conclusions

• Phytosanitation, disinfecting the greenhouse structure and trellising ropes

• Using ToBRFV free seed lots

• Pre-planting treatment with “stabilized chlorine”, using resistant rootstocks, intermediate medium addition to the planting pits

• Early identification and removal of primary infected plants

• Careful planting / Disinfection of working tools
Thank you for your attention

Funding:
Ministry of Agriculture,
Chief Scientist Israel
Pepper plants harboring L1,3,4 hypersensitive response (HR) to ToBRFV infection