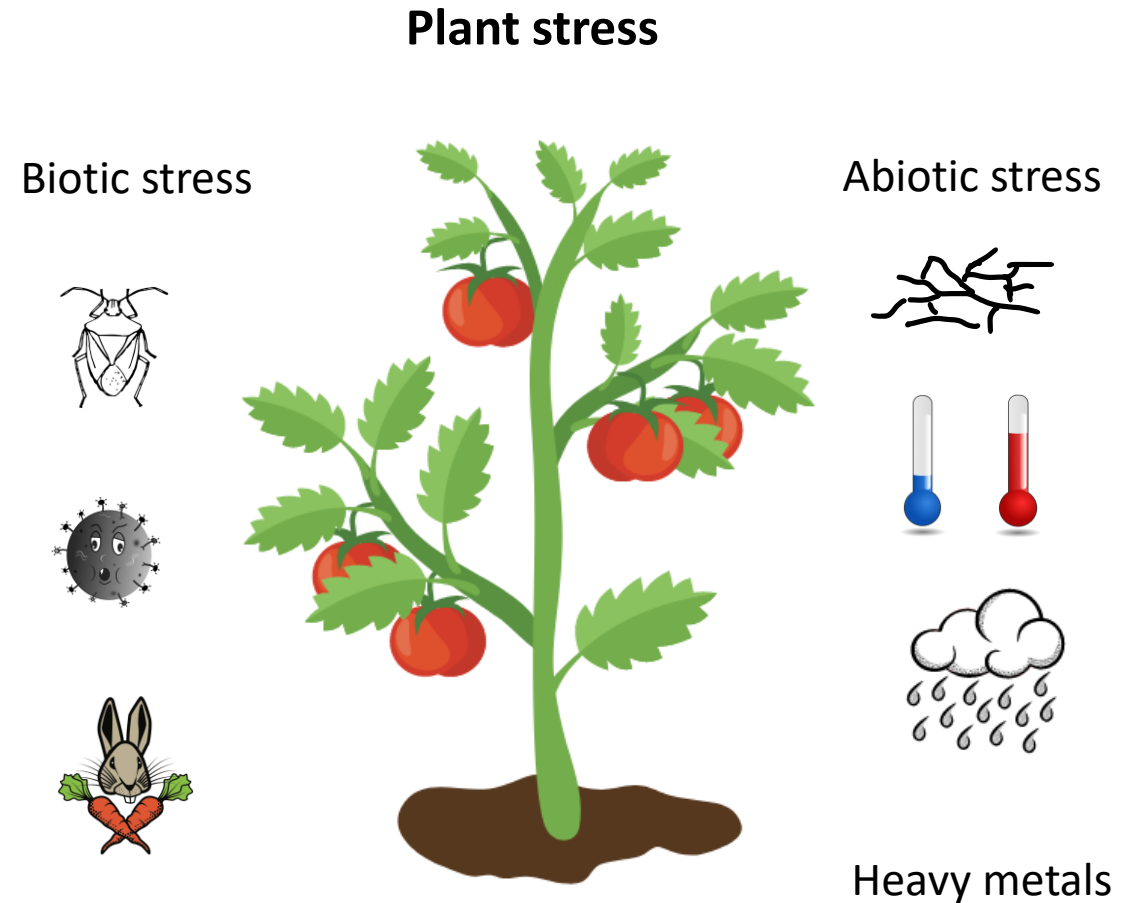


Plant virus interaction - a challenge

Plovdiv, 21 Apr 2022

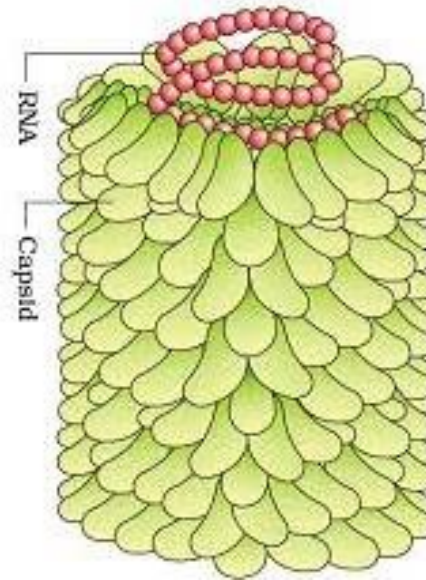
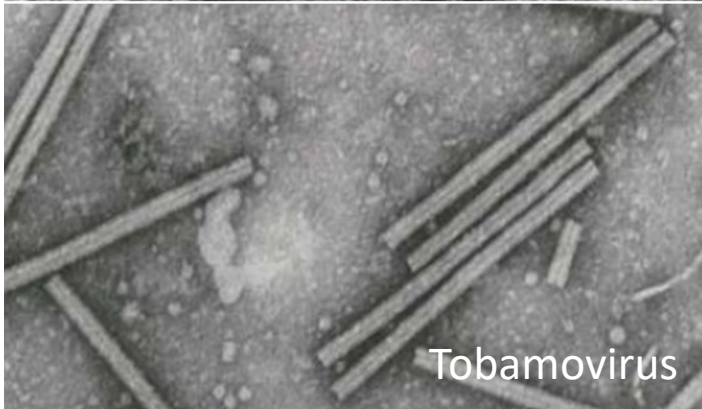
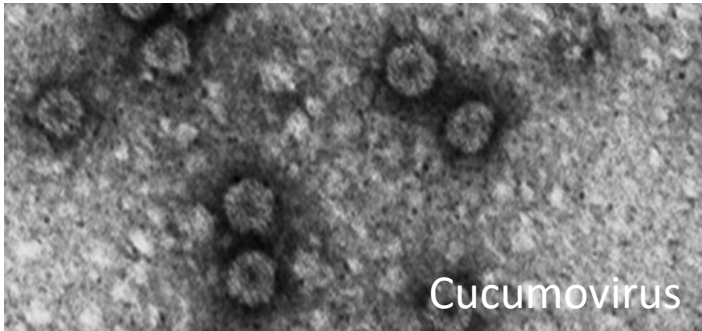
Biotic stress in plants:

This is a type of interaction between a plant and other living organisms, specifically **viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds**, that leads to deprivation of nutrients and consequently to reduced plant vigor as well as death of the host plant in extreme cases. In agriculture, biotic stress is a major cause of pre- and postharvest losses.



What is a plant virus? Live or death?

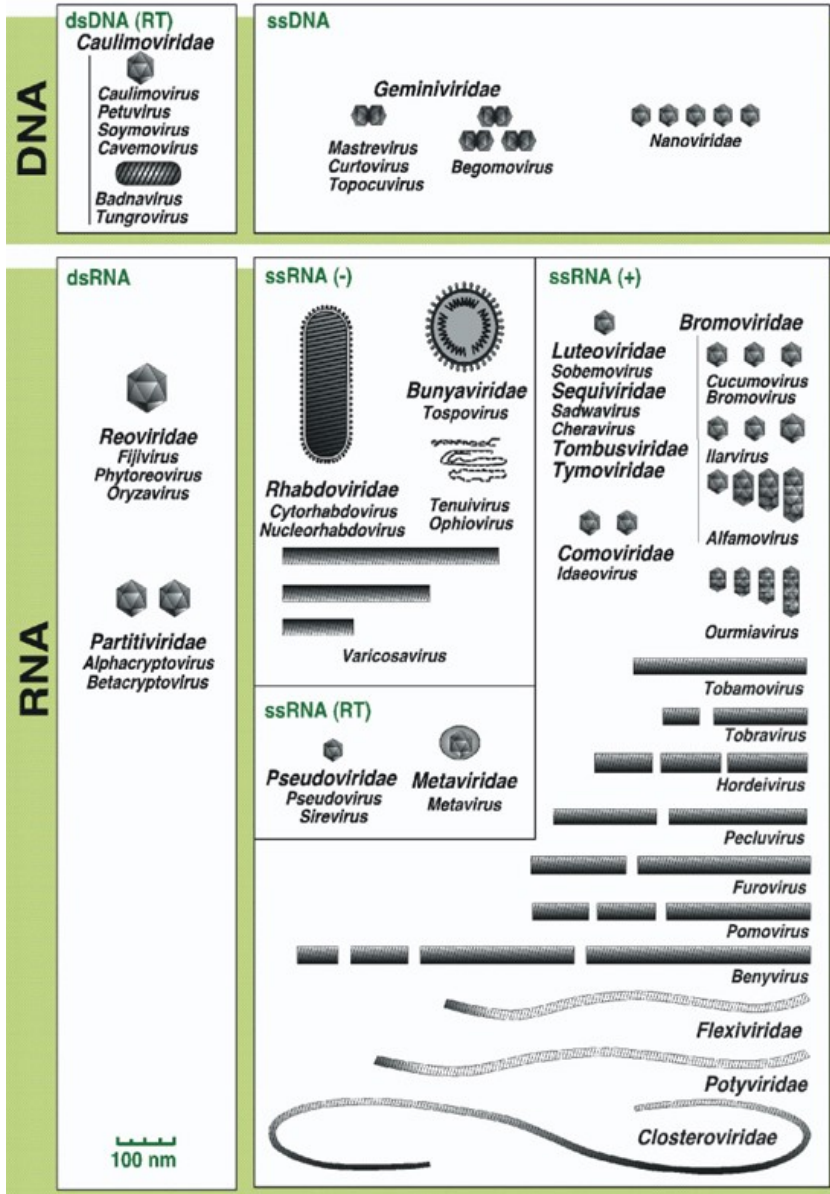
- **Very small organisms – 15-17nm 700-1000nm**
cell parasites.
- **Mandatory parasites**
propagation only in a living cell.



Structure of a viral pathogen

- Genome
- Protective coat

Families and Genera of Viruses Infecting Plants



Classification of plant viruses

DNA

dsDNA

ssDNA

RNA

dsRNA

ssRNA(-)

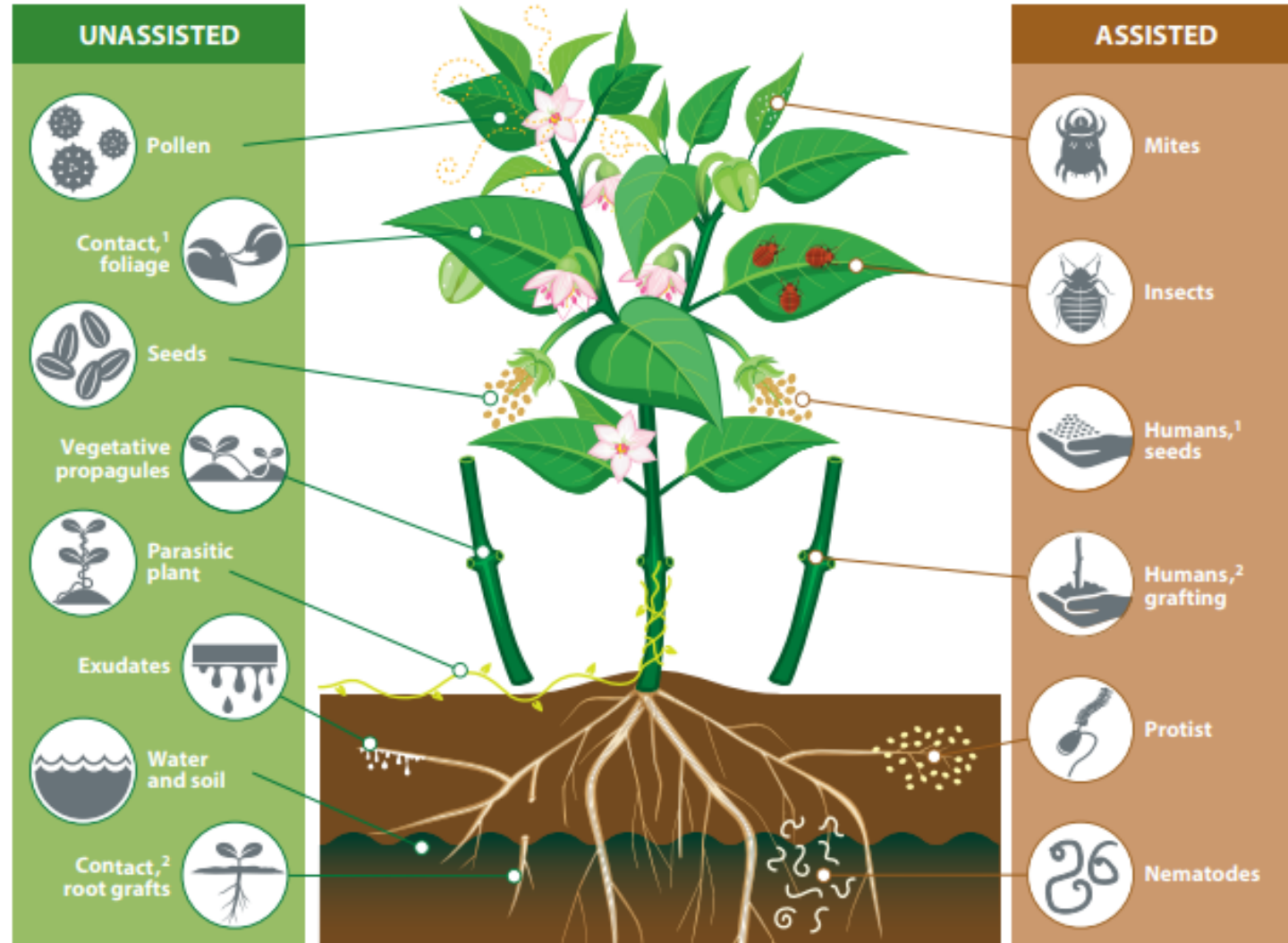
ssRNA(+)

ssRNA (RT)

What viruses can cause to plants



Virus transmission pathways



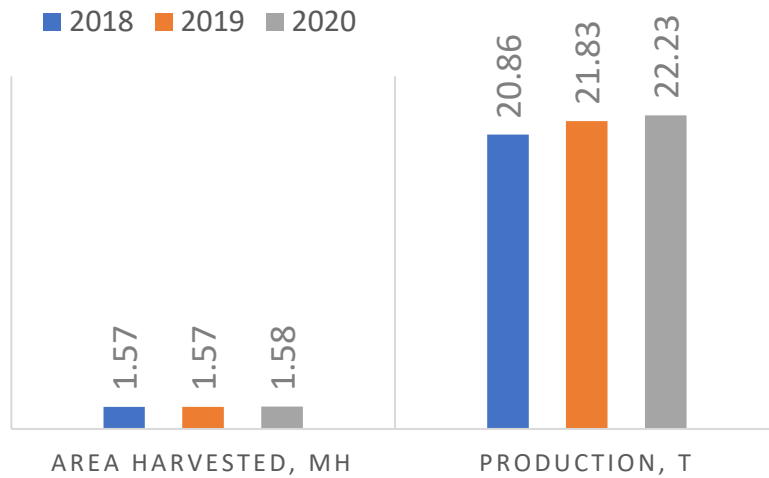


Tomato production worldwide

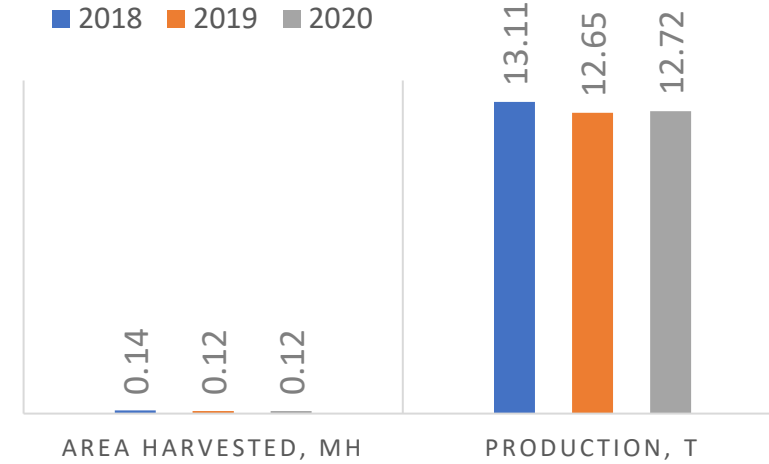
Year	Area, MH	Production, MT	Yield, kg/ha
2018	5,00	180,23	36,013
2019	4,99	183,01	36,609
2020	5,05	186,82	36,979

Tomato production worldwide

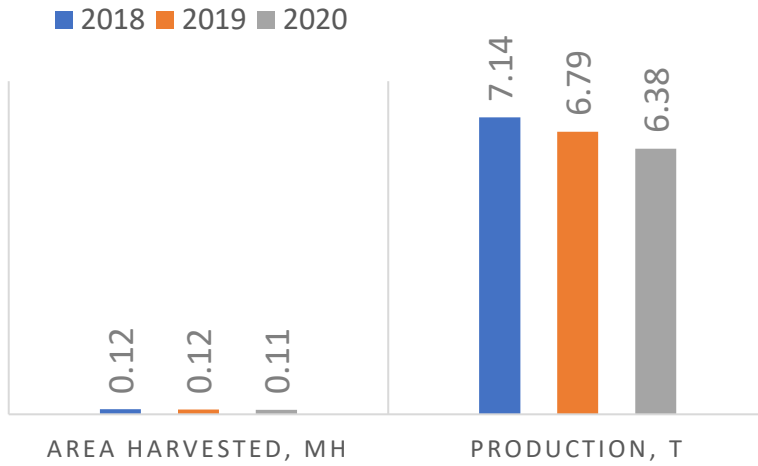
AFRICA



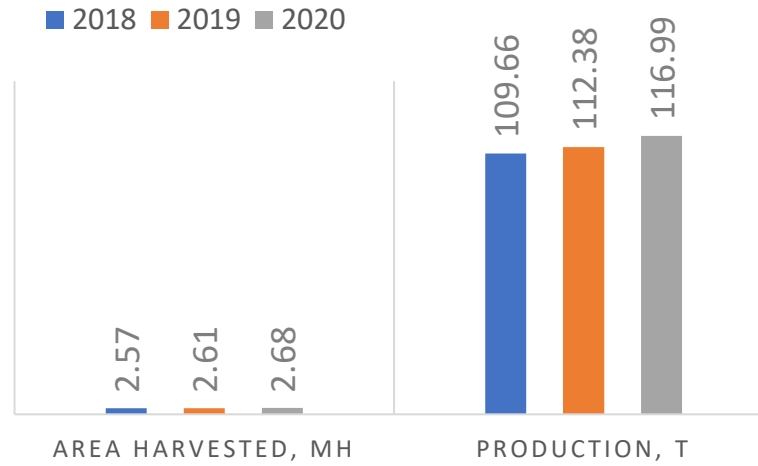
NORTHERN AMERICA



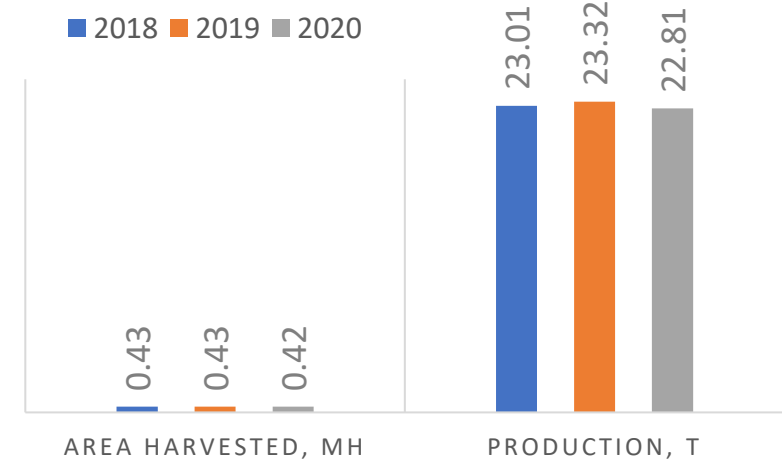
SOUTH AMERICA



ASIA



EUROPE



Tobamoviruses infecting Tomato

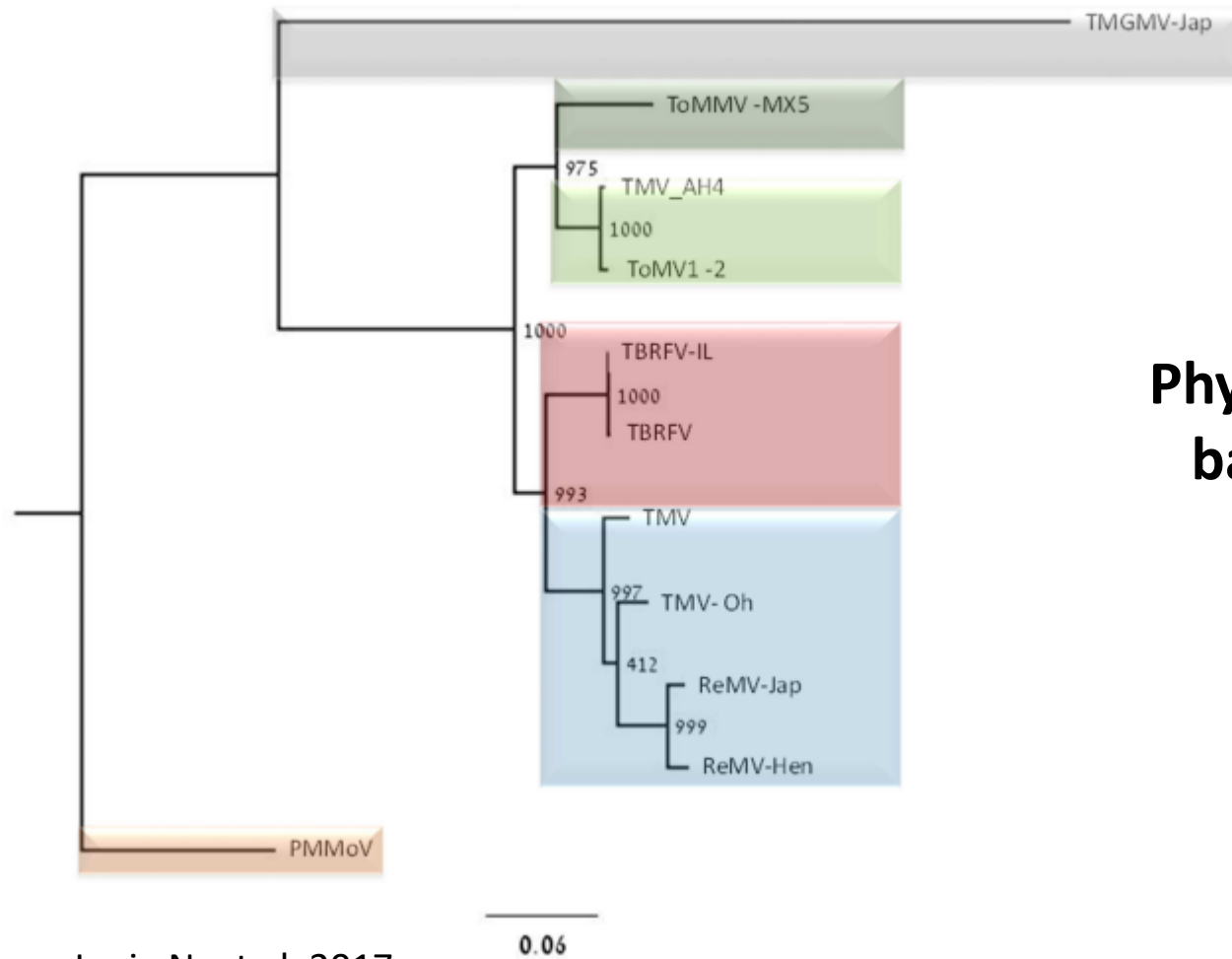
Tobacco mosaic virus (TMV)

Tomato mosaic virus (ToMV)

Tomato mild mottle virus (ToMMV) – 2013, Mexico

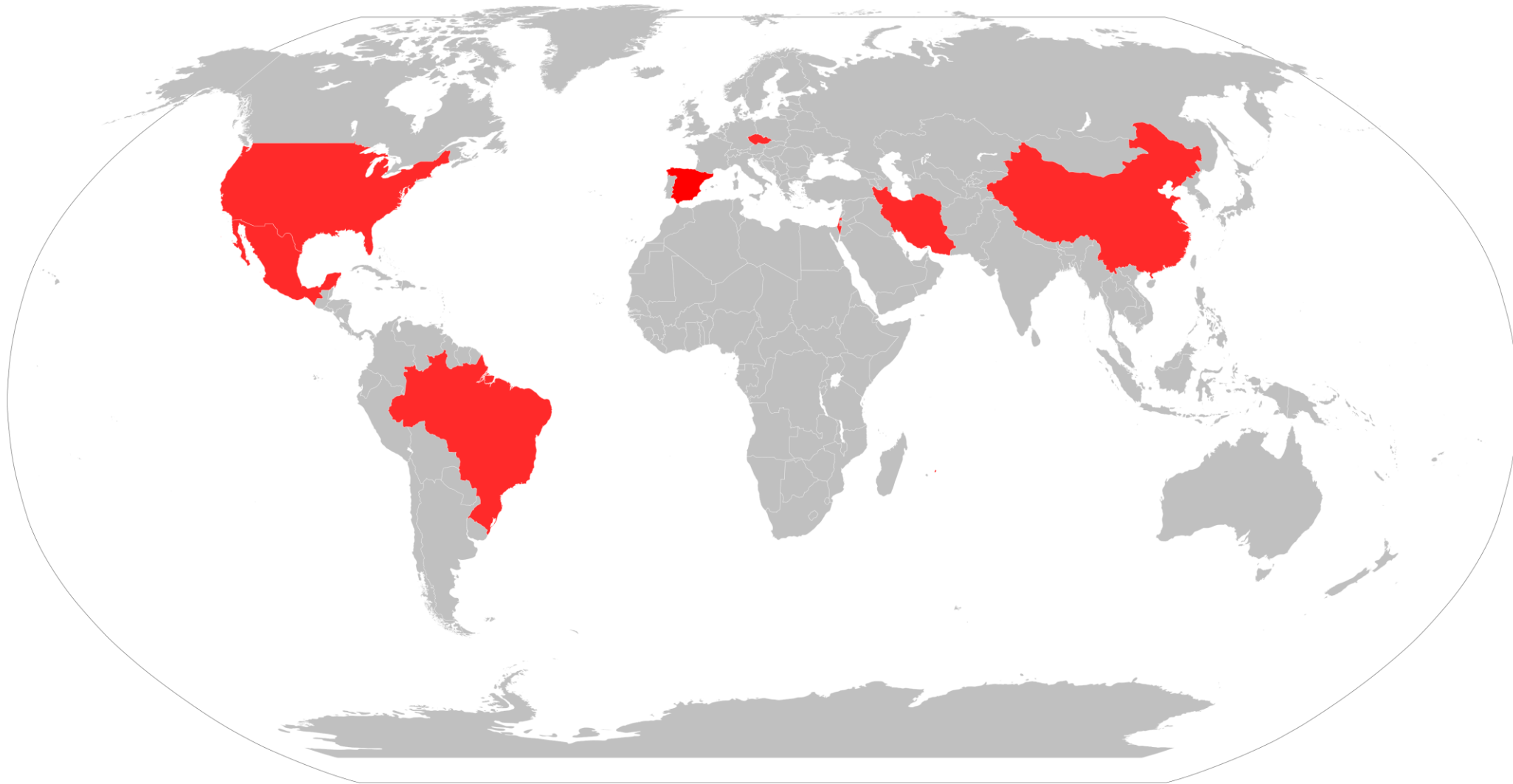
Tomato brown rugose fruit virus (ToBRFV) – 2015, Jordan





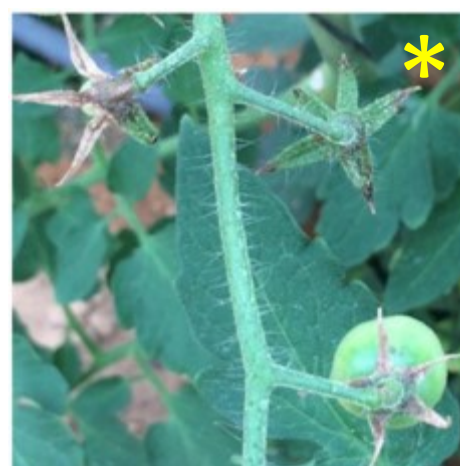
**Phylogenetic tree of Tobamovirus members
based on deduced amino acid sequences**

Distribution of ToMMV



A world map illustrating the global distribution of the genus *Euphorbia*. The map uses three colors to denote different species: red for *E. pulchra*, orange for *E. tiriacula*, and grey for *E. corollata*. *E. pulchra* is found in North America, Europe, North Africa, the Middle East, and parts of Asia. *E. tiriacula* is found in Central America, the Caribbean, South America, India, Southeast Asia, and Japan. *E. corollata* is found in South America, Africa, Australia, and parts of Asia and the Pacific.

Natural infection of ToBRFV in tomato



Luria et al. 2017

Reaction of indicator plants inoculated with ToBRFV



N. rustica



N. tabacum



Ch. murale



Ch. quinoa



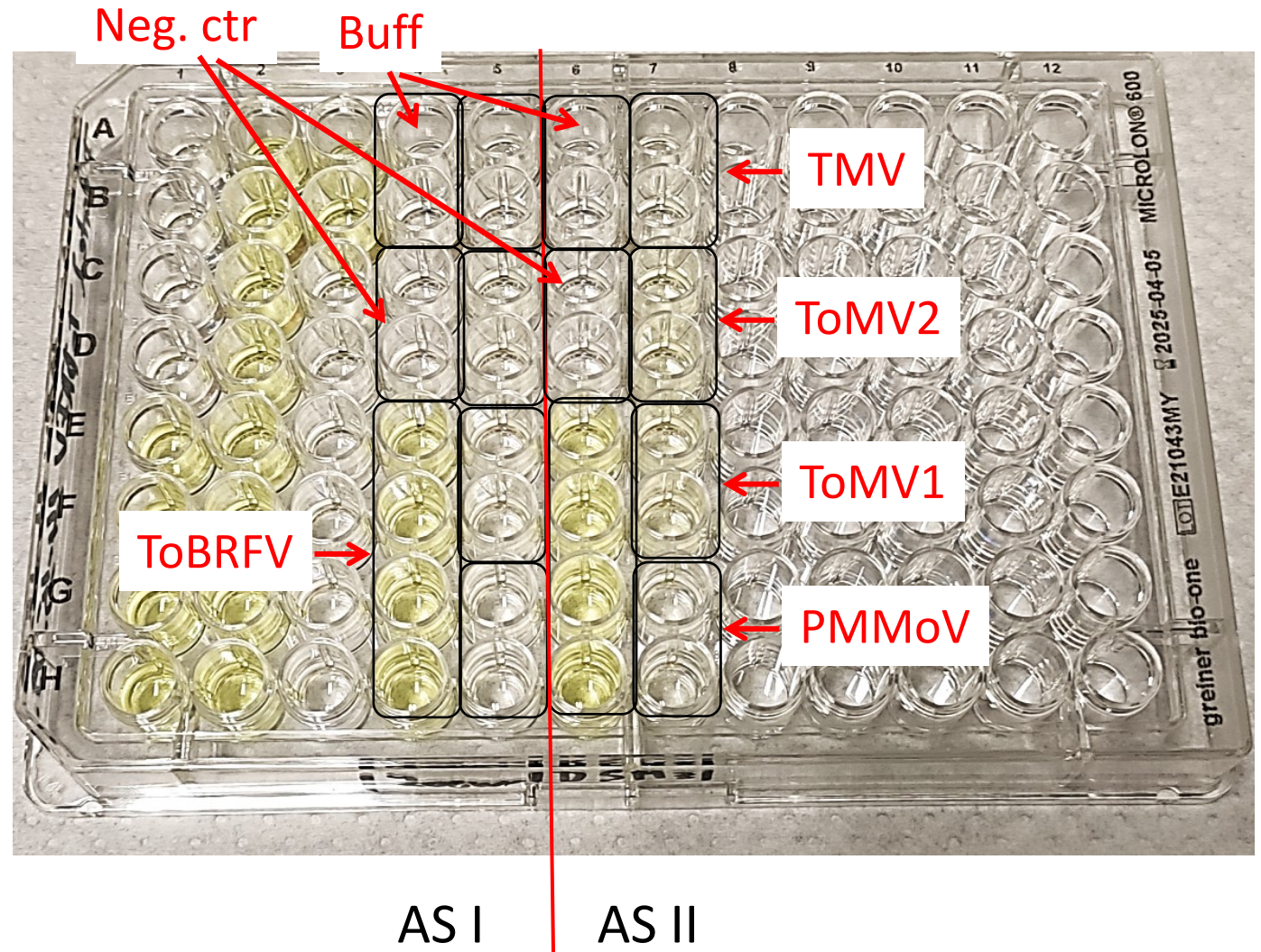
S. lycopersicum cv. Money maker

Detection of ToBRFV

ELISA test

Commercial test available:

- DAS-ELISA
- Lateral Flow Tests



ELISA test: cross-reaction of ToBRFV AS with ToMV

Molecular detection and sequencing of ToBRFV

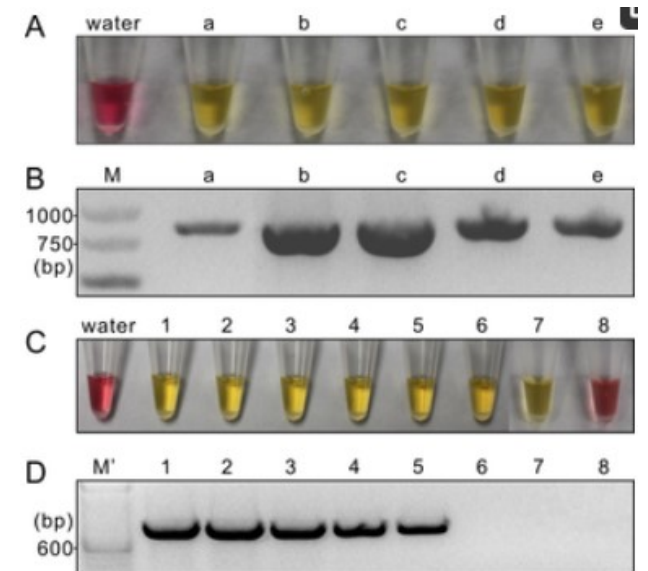
RT-PCR - Alkowni et al. 2019; Rodríguez-Mendoza et al. 2019; Beris et al. 2020;

qRT-PCR - Bernabé-Orts et al. 2021; Fidan et al. 2021

Loop-mediated isothermal amplification (LAMP) – Sarkers et al. 2020

CRISPR-Cas12a – Alon et al. 2021

NGS - Luria et al. 2017, Pasev et al. unpublished



Sarkers et al. 2020

Transmission

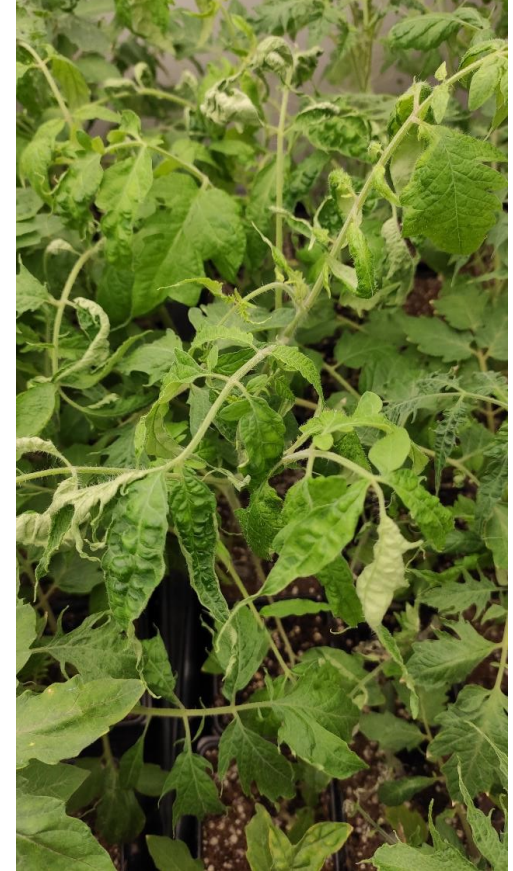
Mechanically



Bumble bees (*Bombus terrestris*)

Water

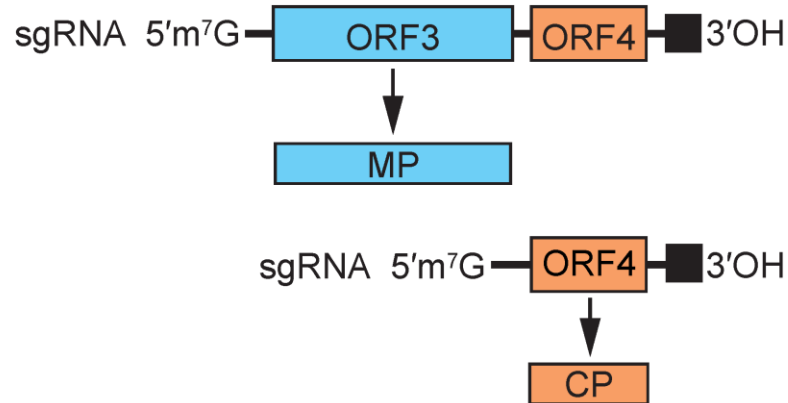
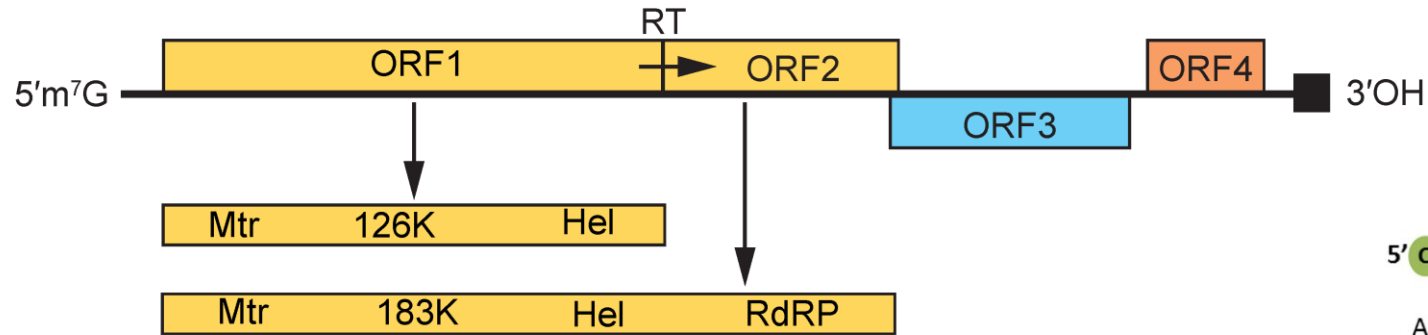
Seeds



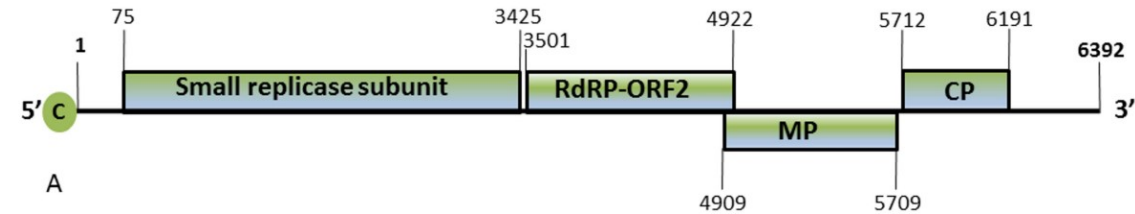
Infected plant debris in the soil

Genome organization of TMV and ToBRFV

tobacco mosaic virus, TMV (6,395 nts)

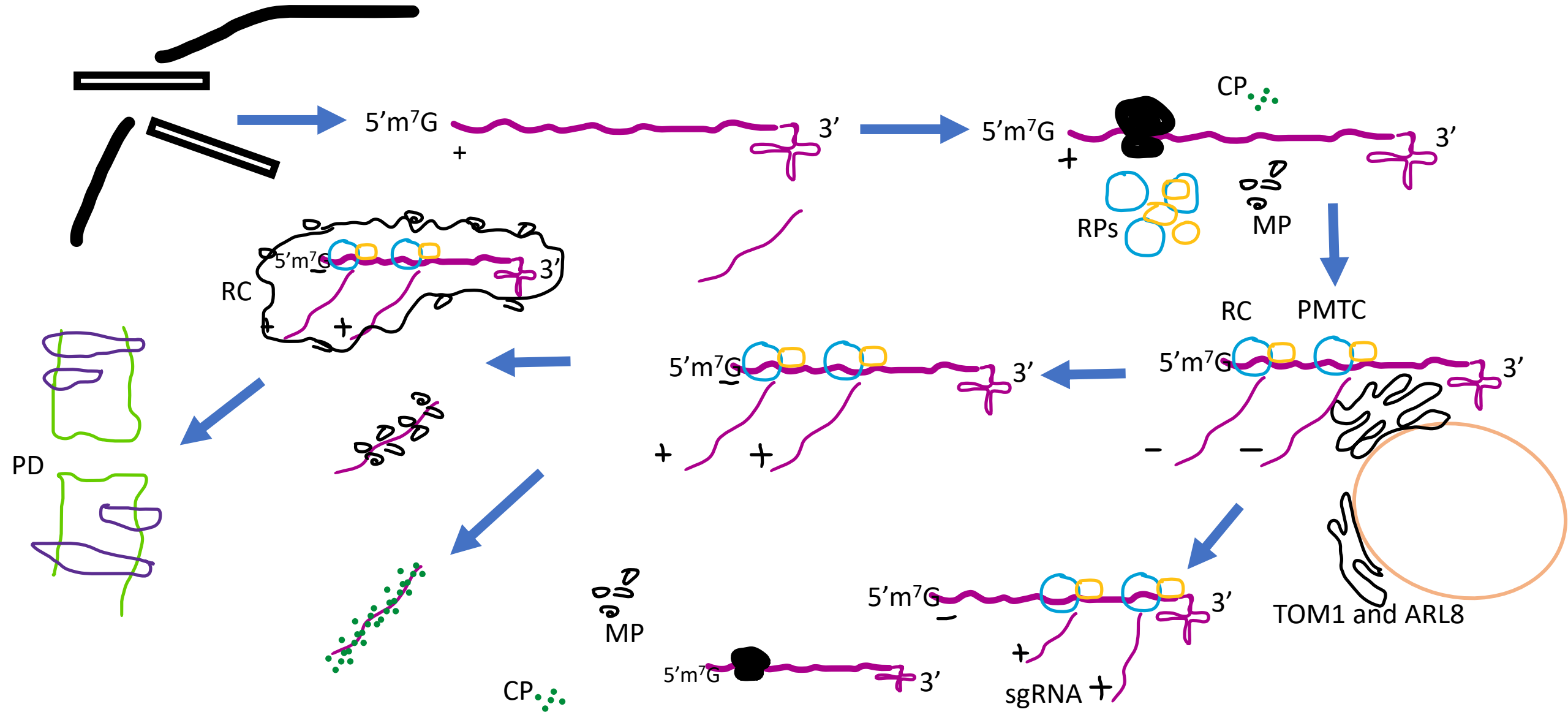


ToBRFV (6392 nts)



Luria et al. 2017

A scheme for viral replication of TMV



Durable resistance to a disease is resistance that remains effective during its prolonged and widespread use in an environment favorable to the disease .

Johnson, R. (1984). A Critical Analysis of Durable Resistance.
Annual Review of Phytopathology, 22(1), 309–330.

Interactions between Tm resistance genes in tomato and ToMV strains

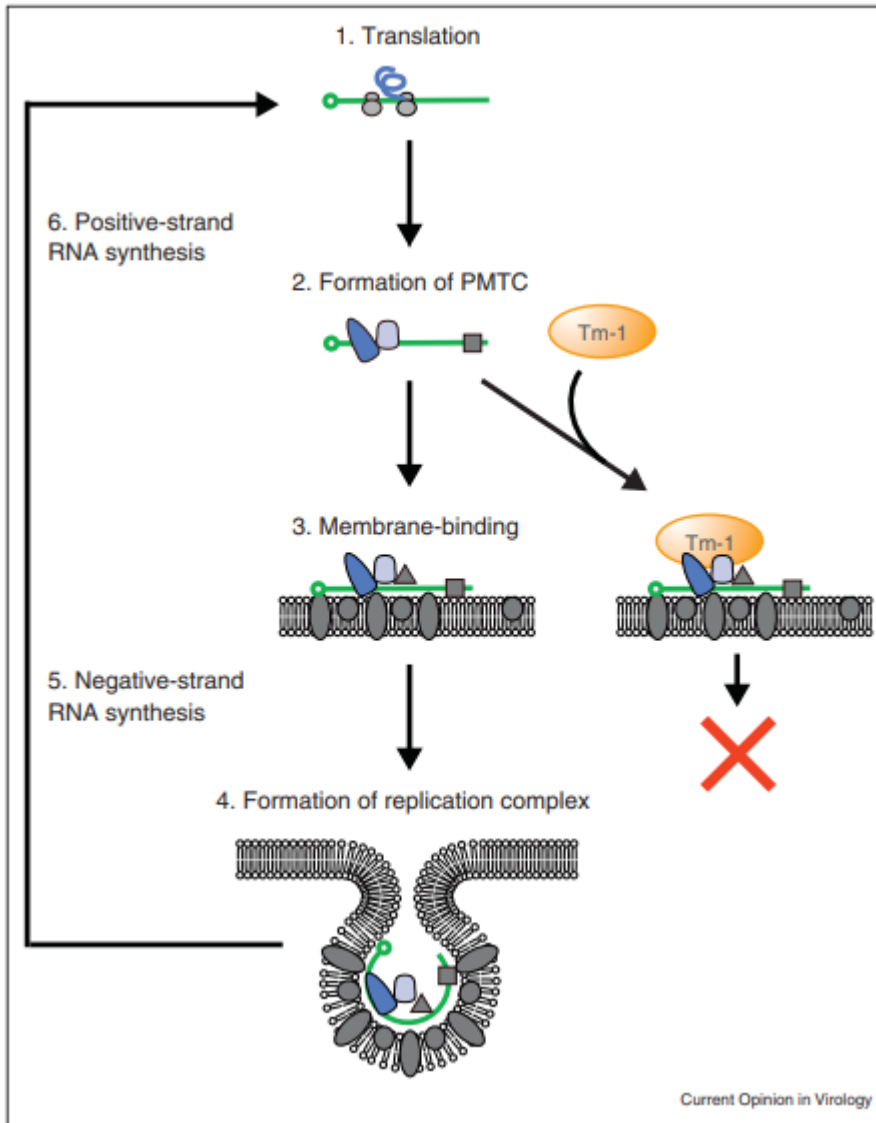
Genes	0	1	2
+	S	S	S
Tm1	R*	S	R*
Tm2	R	R	S
Tm2 ²	R	R	R

S – susceptible, R – resistant.



Recognition of viral pathogen in the host cell

Model of inhibition of ToMV by *Tm-1* gene

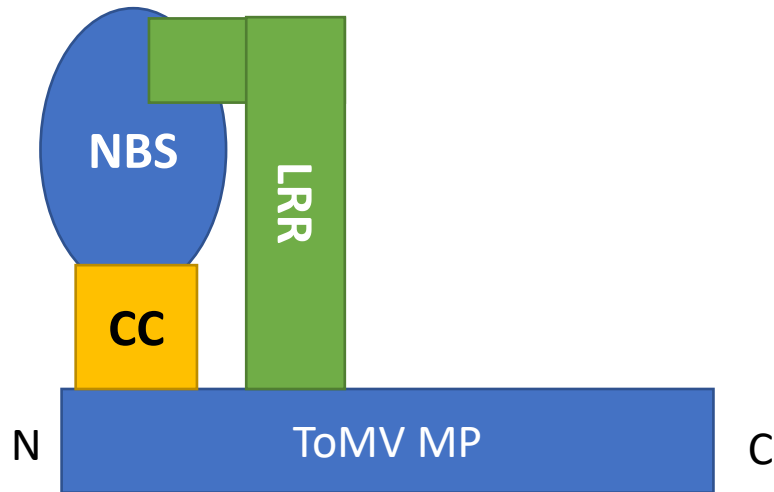


Tm-1 ~ 80 kDa soluble protein TIM barrel structure, encoded by *Tm-1* gene, located on Chr. 2 originally identified in *Solanum habrochites*

Tm-1 inhibits multiplication of the ToMV by binding Hel domain

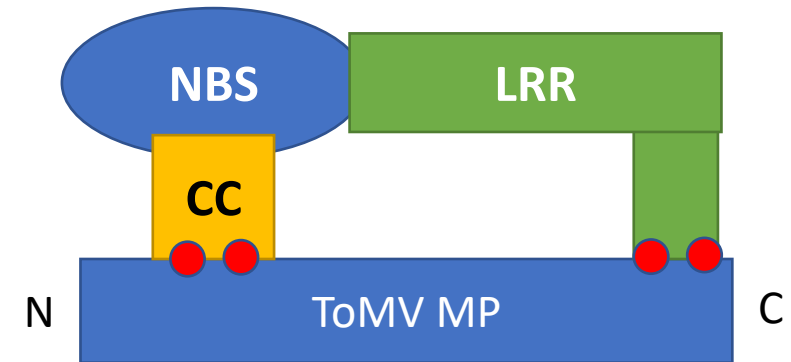
No defense response is induced

Model of action of *Tm2* and *Tm2²* resistance genes



Tm2

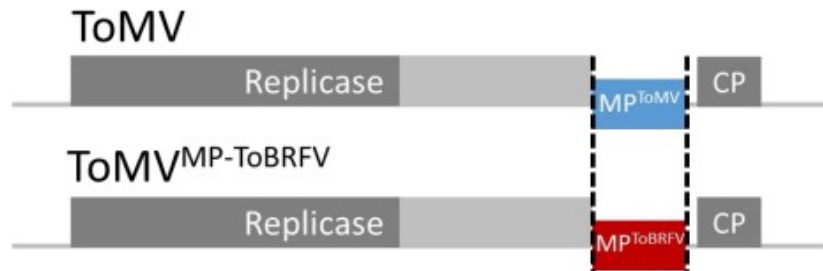
C- terminus required for recognition



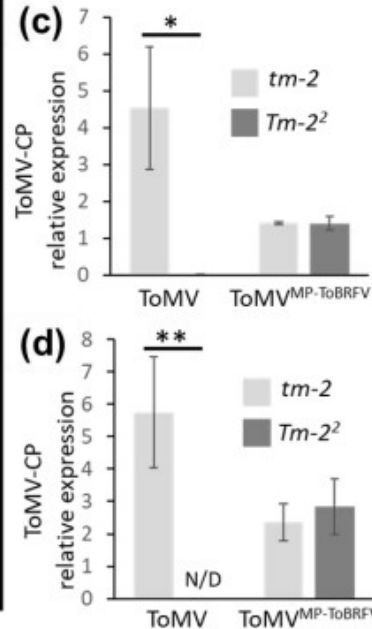
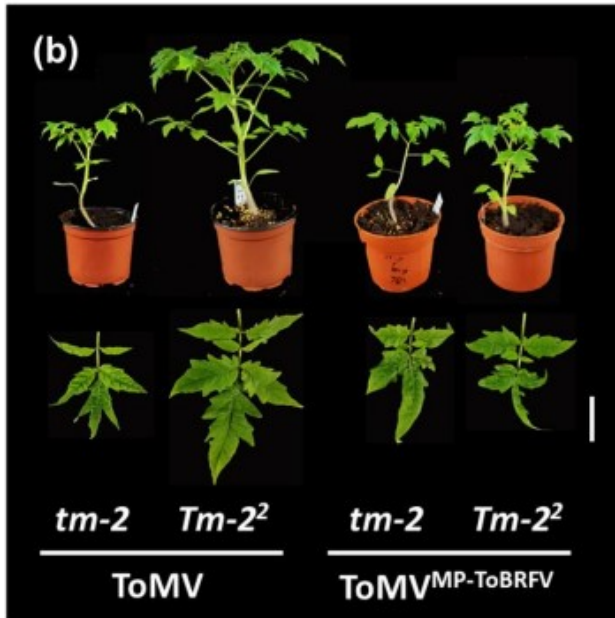
Tm2²

- Originally *Tm2* found in *Solanum peruvianum*
- Both located on Chr 9
- Behave as alleles
- Hypersensitive response if heterozygous

Why ToBRFV overcomes the Tm2² resistance?



- ToBRFV movement protein
- Resistant braking elements located between 1-216 aa of MPToBRFV
- MP ToBRFV moves less effectively compared to the TMV and ToMV MPs suggesting attenuated MP function



Virus	Genotype	Symptoms	Infected
ToMV	<i>tm-2/tm-2</i>	12/12	12/12
ToMV ^{MP-ToBRFV}	<i>tm-2/tm-2</i>	11/11	11/11
ToMV	<i>Tm-2²/Tm-2²</i>	0/10	0/10
ToMV ^{MP-ToBRFV}	<i>Tm-2²/Tm-2²</i>	11/11	11/11

Perspectives for resistance

Natural resistance



VC532 - tolerant X Money Maker (No Tm2²)

F1 susceptible → **Tolerance recessive**

F2 → **~ 25% No symptoms**

Recessive resistance,
Chromosome 11

VC554 - resistant X LA2706 (susceptible)

F1 tolerant → **Partially dominant**

F2 → **~ 40% No symptoms**

two factors >>> one dominant or semidominant

Chr 11 and Chr 2

Perspectives for resistance

CRISPR/Cas9-mediated targeted mutagenesis

Homologs of TOM1 in tomato

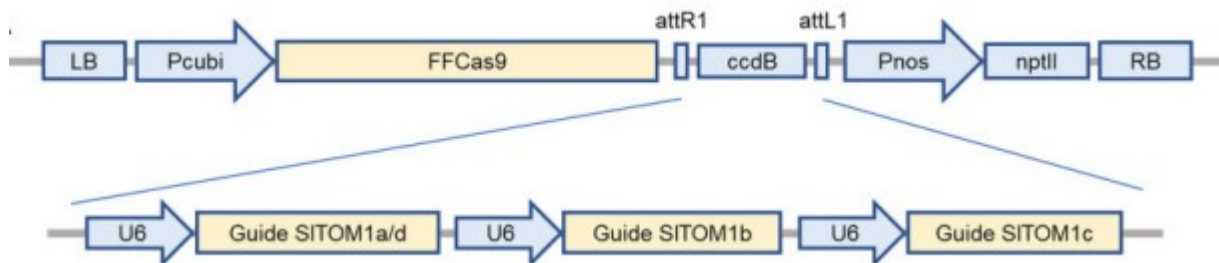
SITOM1a Solyc04g008540

SITOM1b Solyc01g105270

SITOM1c Solyc02g080370

SITOM1d Solyc01g007900

SITOM1a–d - genes homologs to TOM1 in *A. taliana*



Construct introduced in tomato Craigella GCR26

By crosses between mutants or with WT were produced single-, double-, triple-, and quadruple mutant lines

Guide RNA	(3')GGUUCAGAACGTAAGAAGUGUUG(5')
<i>SlTOM1a</i> (wt)	---GATATGCCAAGTCTTGCATTCTTCACAACCTTTTGC---
	D M P S L A F F T T F
	116
<i>Sltom1a-1</i>	---GATATGCCAAGTACTTGCATTCTTCACAACCTTTTGC---

Guide RNA	(3')GGUGCUCAAGCGGGUAGAAACU(5')
<i>SlTOM1b</i> (wt)	---TGGAACACCACGAGTTTCGCCCCATCTTTGATTGGTT---
	G T P R V R P I F D W
	21
<i>Sltom1b-1</i>	---TGGAACACCACGAG-----CCCATCTTTGATTGGTT---

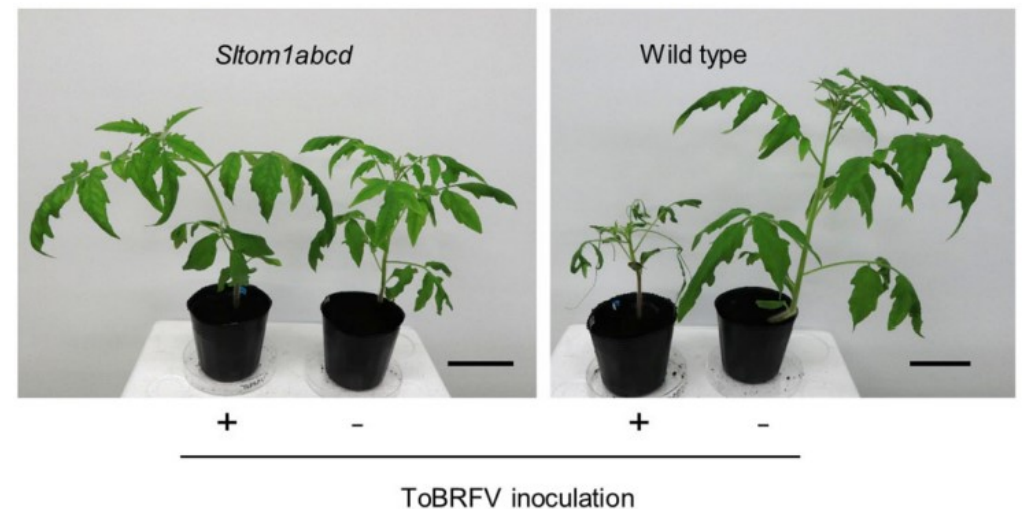
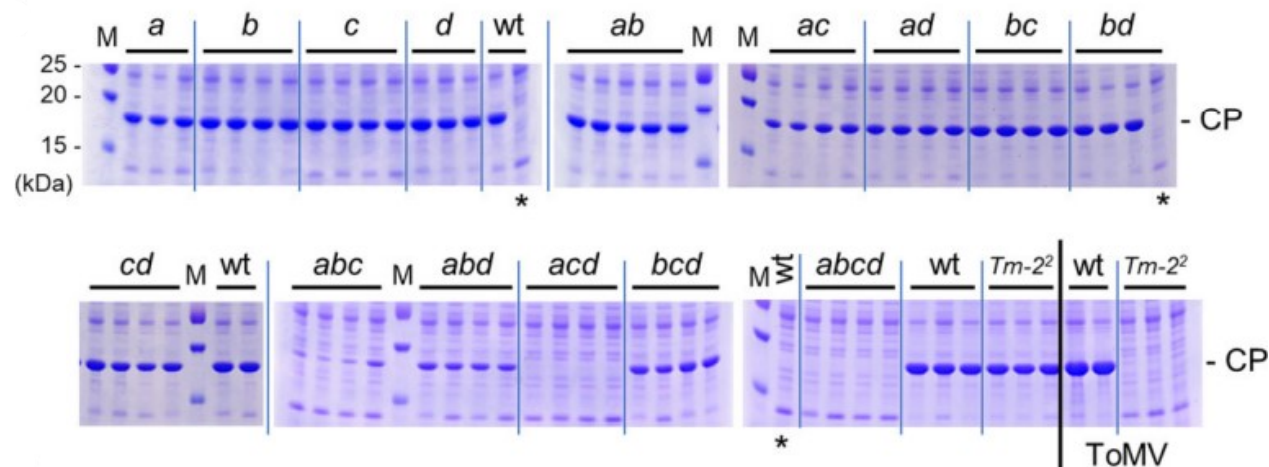
Guide RNA	(5')GCCGAUUGACAUCGCCGGUCCGG(3')
<i>SlTOM1c</i> (wt)	---GTCGTCGCCGATTGACATCGCCGGTCCGGTGACCA---
	S S P I D I A G P V T
	15
<i>Sltom1c-1</i>	---GTCGTCGCCGATTGACATCGCC-GTCCGGTGACCA---

Guide RNA	(3')GGUUCAGAACGTAAGAAGUGUUG(5')
<i>SlTOM1d</i> (wt)	---GATATGCCAAGTCTTGCATTCTTCACAACCTTATGC---
	D M P S L A F F T T Y
	116
<i>Sltom1d-1</i>	---GATATGCCAAGT--TGCATTCTTCACAACCTTATGC---

Perspectives for resistance

CRISPR/Cas9-mediated targeted mutagenesis

SITOM1a-d - genes homologs to TOM1 in *A. taliana*



Quadruple mutations lead to resistance to ToBRFV

Thank you