



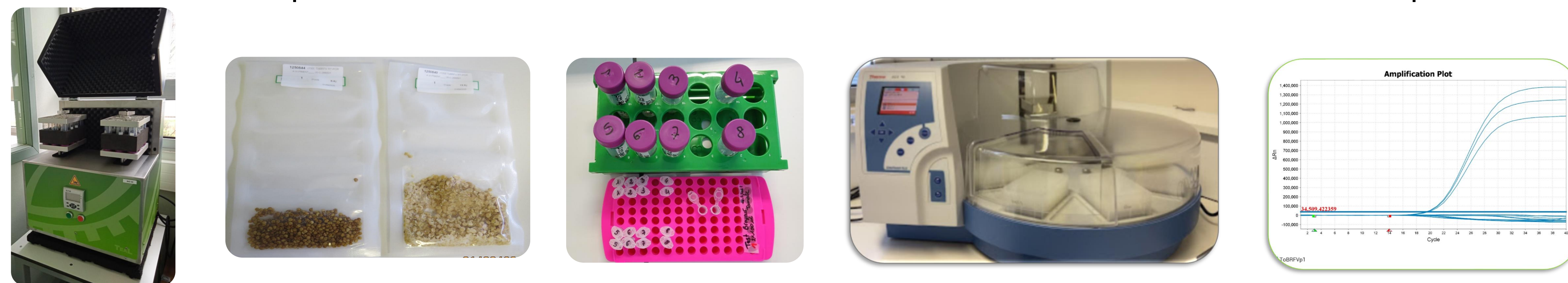
Tomato brown rugose fruit virus (ToBRFV) was first observed in 2014 and 2015 on tomatoes in Israel and Jordan, outbreaks have since occurred worldwide. ToBRFV causes a wide range of symptoms and is a major concern for growers of tomato and pepper and has been classified as « Emergency measures » in France since the end of 2019.

To improve management of this threat, two research areas were studied: detection and variety resistance.

Detection

Seed Extract (SE) RT-qPCR detection method

The performance criteria of the method developed by ISHI-Veg using CaTa28 and CSP1325 primers and probes were studied in 2019 and the method was used at Geves until 2021. Subsequently this method was replaced by the official French method ANSES/LSV/MA066 using the primers and probes developed by Menzel & Winter (in press). More than 8000 seed samples have since been tested for ToBRFV at GEVES and less than 0.1% were positive.



Biological relevance

The performance of the biotest was also investigated on a few samples. The results obtained raise questions for further investigation about sensitivity. The biotest on *Nicotiana tabacum* cv. Xanthi reveals the presence of infectious virions in a sample, whereas the SE-RT-qPCR method detects a relatively small fragment of RNA. The difference in analyte raises the question of biological relevance, in terms of risk of seed transmission of ToBRFV, in the case of positive SE-RT-qPCR results.

| Dilution | Tomato healthy seed extract (Rep I) + diluted ToBRFV infected tobacco leaf homogenate | | | Tomato healthy seed extract (Rep II) + diluted ToBRFV infected tobacco leaf homogenate | | |
|----------|---|------------|-------------------------|--|------------|-------------------------|
| | Ct CaTa28 | Ct CSP1325 | Number of local lesions | Ct CaTa28 | Ct CSP1325 | Number of local lesions |
| 1/100 | 13.24 | 14.38 | 27 | 12.87 | 14.13 | 12 |
| 1/1000 | 16.27 | 17.63 | 0 | 16.42 | 17.6 | 0 |
| 1/10 000 | 19.7 | 21.04 | 0 | 19.58 | 21 | 0 |

Disease resistance testing

ISF differential sets ToBRFV

GEVES conducted the characterization of 2 ToBRFV isolates on pepper and tomato Tobamovirus differentials to establish the differential table published by ISF.

| Pepper | | | | | | |
|----------------------------------|---|-----------------|-------------|----------------|--------|----|
| Pepper Tobamovirus Group | 0 | 1 | 2 | 3 | ToBRFV | |
| ISF code | TMV: 0*, 1*, 2 ToMV: 0*, 1*, 2 BPMoV ToBRFV | TMGMV PaMMV* | PMMoV: 1.2* | PMMoV: 1.2.3.* | | |
| Differential Set | Gene | | | | | |
| Lamu*, Early Calwonder* | | S | S | S | S | S |
| Tisana, Yolo Wonder* | L1 | HR | S | S | S | HR |
| Tabasco* | L2 | HR | HR | S | S | HR |
| Solario F1*, Novi 3*, PI 159236* | L3 | HR | HR | HR | S | HR |
| Tom4*, PI260429* | L4 | HR | HR | HR | HR | HR |

S = susceptible; HR = Highly Resistant
 * = differential hosts and isolates that are used by the vegetable seed industry.

| Differential hosts | Gene present | Race | | | |
|----------------------------------|------------------|----------|----------|----------|---------|
| | | ToMV: 0* | ToMV: 1* | ToMV: 2* | ToBRFV* |
| Monalbo*, Marmande* Early Pak 7* | - | S | S | S | S |
| Mobaci* | Tm-1 | HR | S | HR | NT |
| Moperou 161* | Tm2 | HR | HR | S | S |
| Momor*, Geneva 80, Gourmet | Tm2 ² | HR | HR | HR | S |

S = susceptible; HR = Highly Resistant
 * = differential hosts and isolates that are used by the vegetable seed industry.
 NT = not tested



Resistance test mastered on tomato

GEVES developed a ToBRFV bioassay adapted from the CPVO Tobamovirus protocol. Presence of virus in plant with few or without symptoms is confirmed by RT-PCR to distinguish intermediate resistance (IR) from high resistance (HR).

GEVES coordinates a CPVO project (ToBR-Ag) to set up an official protocol for evaluation of resistance of tomato and pepper to ToBRFV.

| Variety | Class 0 | Class 0 (necrosis) | Class 1 | Class 2 | Class 3 | Class 4 | Disease index | Proposal biotest + RT-qPCR |
|----------------------------|---------|--------------------|---------|---------|---------|---------|---------------|---|
| A | 1 | 4 | 4 | 7 | 16 | 0 | 51.56% | IR (with low symptoms and virus multiplication) |
| B | 0 | 0 | 0 | 32 | 0 | 0 | 50.00% | IR (with low symptoms and virus multiplication) |
| C | 21 | 10 | 2 | 0 | 0 | 0 | 1.52% | IR (with no symptoms but virus multiplication) |
| Susceptible control | 0 | 0 | 0 | 0 | 0 | 31 | 100.00% | Susceptible |
| Susceptible control (Tm-1) | 0 | 0 | 0 | 0 | 0 | 33 | 100.00% | Susceptible |

