

1. Content of the 'Topic Description' document

1.1. Topic area

Diagnostics, field detection, surveillance

1.2. Links to the Euphresco Strategic Research Agenda

The topic addresses the following objective(s) of the 2017-2022 Euphresco Strategic Research Agenda

- Objective 2017-R-2.2: to expand knowledge on transmission of disease and pathogens for healthy planting material
- Objective 2017-R-5.1: to understand the biological significance of a positive molecular diagnosis
- Objective 2017-R-7.1: to validate cost-effective and socially acceptable phytosanitary measures for consignments (pre-border and at border)

1.3. Topic title

Effect of disinfection treatments for elimination of ToBRFV in tomato seeds

1.4. Description of the problem the research should solve

Tomato brown rugose fruit virus (ToBRFV) currently causes problems in tomato and pepper cultivation worldwide (Luria *et al.*, 2017, Salem *et al.*, 2019). Tobamoviruses are very stable and will remain infectious for long periods of time when present in crop debris, soil and on surfaces. ToBRFV is easily transmitted mechanically (via contaminated tools, hands, clothing, direct plant-to-plant contact, and bumble bees) and seed-to-seedling transmission is expected to play a role in the spread of the virus (Levitzky *et al.*, 2019, Salem *et al.*, 2021). Infectivity on seeds is preserved for up to several years (Dombrovsky and Smith, 2017).

Since 2019, the EU has emergency measures in place against ToBRFV to prevent introduction and further spread of this virus. The measures include testing requirements for movement and introduction of seeds of *Solanum lycopersicum* and its hybrids and *Capsicum* spp. In addition, there is a requirement for testing of at least 20% of the consignments of seeds and plants for planting of *Solanum lycopersicum* and *Capsicum* spp. upon entry into the EU. Reliable and harmonised protocols for the detection of ToBRFV in tomato and pepper seeds are in place and recently been validated in the Euphresco project 2019-A-327 'Validation of molecular tests for the detection of tomato brown rugose fruit virus (ToBRFV) in seed of tomato and pepper'.

ToBRFV has repeatedly been detected on batches of tomato and pepper seeds. Seed treatments, including those with hydrochloric acid and trisodium phosphate, may reduce or eliminate infectious virus particles. In addition, the protocol used for the extraction (acid extraction is prescribed by the EU regulation for tomato seeds) may have an effect on the abundance of virus particles. Several seed treatments have already been tested on ToBRFV or on other Tobamoviruses, but these treatments were not always effective in 100% elimination of Tobamoviruses. Treatment effectiveness may vary depending upon the involved virus species and severity of the contamination.

Specifically for ToBRFV, Samarah *et al.* (2020) and Davino *et al.* (2020) have shown that several seed treatments have the ability to inactivate ToBRFV, but that ToBRFV was still detectable by real-time RT-PCR in most cases (Davino *et al.*, 2020). In particular, treatments with 10% trisodium phosphate and 2.5% sodium hypochlorite solution yielded the most promising results. No transmission was shown in bioassays on seedlings that were grown from these treated seeds. The experiments by Davino and collaborators were performed on relatively small numbers of seeds and require confirmation on a larger scale, particularly regarding the biological significance of any residual post-treatment ToBRFV. Considering the high plant-to-plant transmission rate of ToBRFV even a single infected plant may eventually infect an entire greenhouse during the cultivation cycle. On a more general note, the relation

between infection levels (or the ct value in a real-time RT-PCR test) and seed transmission is still elusive. Seeds from healthy mother plants may become infected post-harvest, which may result on positive RT-PCR test with ct-values of which the biological significance is currently unclear.

The aim of the project is to evaluate the efficacy of disinfection treatments on ToBRFV-infected seeds and understand the biological significance of positive RT-PCR tests in seeds. This information will contribute to ToBRFV management.

1.5. Description of the expected results

- Substantiation on the efficacy of disinfection treatments on ToBRFV-infected seeds to be able to evaluate their application as a risk-management measure
- Better understanding of the biological significance of a positive molecular diagnosis in which the low levels of viral RNA are detected.

1.6. Beneficiaries of this research product

The beneficiaries of this research project are national plant protection organizations, policy makers, seed industry and tomato, growers.

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Netherlands Food and Consumer Products Safety Authority, the Netherlands Martijn Schenk M.Schenk1@nvwa.nl	-Determining of the effects of several treatment on real-time RT-PCR-test values; -Grow outs of (un)treated seeds and subsequent testing on the level of seed transmission; Contact person: TBD
2. Austrian Agency for Health and Food Safety, Austria Sylvia Bluemel sylvia.bluemel@ages.at	- Determining of the effects of treatment on real-time RT-PCR-test values; Contact person: Sabine Grausgruber-Groeger E.mail address: sabine.grausgruber-groeger@ages.at
3. New Zealand Ministry for Primary Industries (MPI), New Zealand Aurelie Castinel Aurelie.Castinel@mpi.govt.nz	-Contribution to be detailed; Contact person: Craig Homer E.mail address: Craig.Homer@mpi.govt.nz
4. All Russian Plant Quarantine Center, Russian Federation Yuri Shneyder yury.shneyder@mail.ru	-Evaluation of different treatment methods on virus persistence in seeds; -Testing of different RT-real-time PCR tests, to improve the sensitivity and specificity of treatment evaluation methods; Contact person: Elena Karimova E.mail address: elenavkar@mail.ru
5. International Seed Federation, Switzerland Rose Souza Richards R.SouzaRichards@worldseed.org	- Review of documents (e.g. drafts project plans, protocols, publication paper etc., as needed). - Evaluation of different treatment methods on virus persistence in seeds.

	<p>- Provision of material for the grow outs of (un)treated seeds (by reaching out to the ISHI seed companies)</p> <p>Contact Person: Rose Souza Richards E.mail address: R.SouzaRichards@worldseed.org</p>
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1.8. Research project partnership outside Euphresco

Euphresco funding ensures a certain level of transnational collaboration among Euphresco member countries. It is possible, if the funding consortium is interested, to contact funding organisations or research groups outside the geographical area covered by Euphresco members. The Euphresco coordinator could advertise the research topic in order to have an enlarged collaboration. If funders are interested in this possibility, please check the case below:

The funding consortium of the topic mentioned in section 1.2 requires that the topic is advertised outside the Euphresco network

Information to define the profile of sought partners could be useful (but not mandatory): country/region (if there are preferences), skills/expertise required, etc.

1.9. Any other relevant information on content

None.

2. Euphresco management aspects of the project

2.1. Indication of the topic budget

Funding organisation	Mechanism	Total Budget
1. NVWA (NL)	NC	€
2. AGES (AT)	NC	€
3. MPI (NZ)	NC	€
4. VNIKR (RU)	NC	€
5. ISF (CH)	NC	€

2.2. Expected duration of the project (only for non-competitive topics)

24 months

2.3. Identification of project coordinator

Has the research project coordinator been identified?

- Yes
 No

2.4. Any other relevant information on topic organisation and management

None.