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-6.1: to test and validate methods for *in situ* detection and identification of pests

1.3. Topic title

Development and validation of rapid and sensitive techniques for cost-effective diagnostics of plant pests

1.4. Description of the problem the research should solve

Disease-causing pathogens, including viruses and phytoplasmas, are responsible for global economic losses in various horticultural commodities. While molecular testing has improved, there is a need to improve methods for extracting viruses and viroids from host tissues, and to validate these methods for use in regulatory diagnostic settings.

In recent years, new and emerging viruses and viroids have been reported in tomato worldwide, including *Southern Tomato Virus* (STV), *Tomato Brown Rugose Fruit Virus* (ToBRFV), *Pepino Mosaic Virus* (PePMV) and *Potato Spindle Tuber Viroid*. Seed transmission may be one of the major pathways for the spread of tomato viruses over a long distance (e.g., from country to country) and contributes greatly to a wider distribution of the emerging tomato viruses.

Both conventional and real-time PCR tests are highly sensitive and available for many of the known tomato viruses; however, the PCR-based technology can only be employed to reveal the specific targets (strain, species, or group-specific) and is highly dependent on the known genetic sequence of the targeted organisms and may not be reliable for revealing new strains, isolates, recombinants or variants. In the situation of mixed infection, PCR-based diagnostic methods can only be employed to exclude one (or a few) pathogens at a time and will take a long time to reveal all viral pathogens. Fortunately, High-Throughput Sequencing (HTS) technology has led to a revolution in virus discovery and exciting new possibilities for the diagnosis of viruses, viroids and other organisms. The application of massively parallel sequencing approaches, and subsequent bioinformatics analysis for viral sequences, carries the promise using HTS for routine generic detection of viruses and other pathogens. Depending on the needs of regulatory testing, HTS may be employed for detecting multiple targets in host plants (including seedlings) and botanical seeds and for identifying pathogens without any prior knowledge.

1.5. Description of the expected results

- Development and validation of HTS and PCR-based protocols for total RNA extraction from various plant materials (i.e., seedlings and botanical seeds). Tests of interest will cover the detection and identification of viruses such as PePMV, STV, and ToBRFV as well as emerging begomoviruses such as *Tomato yellow leaf curl Sardinia virus* (TYLCSV) or *Tomato leaf curl New Delhi virus* (ToLCNDV);
- Cost benefit analysis comparing HTS with other conventional molecular diagnostic techniques;

- Development of a regulatory policy framework to support the application of protocols for surveillance and certification testing to restrict the spread of harmful tomato viruses and viroids and manage tomato diseases caused by these pathogens;
- In addition to improving testing efficiency and diagnostic accuracy through the development and validation of new, cost-effective diagnostic techniques, the outcomes of the project will help address broader problems: drive further development of diagnostic protocols to standardise testing of target pests, prevent the introduction and spread of new invasive alien species, and ensure continued access to export markets.

1.6. Beneficiaries of this research product

The project will benefit to National Plant Protection Organizations, diagnostic laboratories, horticulture industry and the seed industry.

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Canadian Food Inspection Agency, Canada Loren Matheson Loren.Matheson@canada.ca	-Project coordination; -Development/evaluation/validation of HTS and PCR-based methods for tomato viruses; Contact person: Huimin Xu E.mail address: Huimin.Xu@canada.ca
2. Department of Agriculture, Water and the Environment, Australia Con Goletsos ACPPO@agriculture.gov.au	-Development/evaluation/validation of PCR tests; -Optimisation of sampling and nucleic acid protocols for HTS analysis; Contact person: Brendon Reading E.mail address: Brendon.Reading@awe.gov.au
3. Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel Yael Meller Harel YaelM@moag.gov.il	-Contribution to be provided; Contact person: E.mail address: Ahmada@moag.gov.il
4. The State Plant Service under the Ministry of Agriculture, Lithuania Arunas Beniusis arunas.beniusis@vatzum.lt	-Contribution to be provided; Contact person: E.mail address:
5. All Russian Plant Quarantine Center, Russian Federation Yuri Shneyder yury.shneyder@mail.ru	-Contribution to be provided; Contact person: Yuri Shneyder E.mail address: yury.shneyder@mail.ru
6. Department for Environment Food and Rural Affairs, United Kingdom Iain Dummett Iain.Dummett@defra.gov.uk	-Validation of extraction methods for HTS focussed on tomato leaf/fruit/seed; -PCR tests for ToBRFV to support frontline delivery, PepMV and PSTVd and other viroids;

	-LAMP test for ToBRFV (looking to expand to other pathogens - could be a useful tool for use in outbreak management) Contact person: Adrian Fox E.mail address: Adrian.Fox@fera.co.uk Contact person: Ian Adams E.mail address: Ian.Adams@fera.co.uk Contact person: Edward Haynes E.mail address: Edward.Haynes@fera.co.uk
7. US Department of Agriculture, Animal and Plant Health Inspection Service, United States of America David Schimmelpfennig david.schimmelpfennig@usda.gov	-Validation of methods for extraction of nucleic acids from plant tissues with focus on seeds and medium- to high-throughput extraction methods; -Validation of PCR-based diagnostic tests; -Provide expertise on antibody production for virus diagnostics and immunoassays; -Validation of HTS protocols and analysis; Contact person: Bright Agindotan E.mail address: bright.agindotan@usda.gov Contact person: Yazmín Rivera E.mail address: Yazmin.Rivera@usda.gov
8. Oklahoma State University, Unites States of America Cardwell, Kitty kitty.cardwell@okstate.edu	-Electronic Diagnostic Nucleic acid Analysis (EDNA); Contact person: Cardwell, Kitty E.mail address: kitty.cardwell@okstate.edu Contact person: Andres Espindola Camacho E.mail address: andres.espindola@okstate.edu Contact person: Francisco Ochoa Corona E.mail address: ochoaco@okstate.edu

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 [a]	Mechanism [b]	Total Budget [c]
1. CFIA (CA)	NC	€
2. DAWE (AU)	NC	€
3. MOAG (IL)	NC	€
4. WATZUM (LT)	NC	€
5. VNIIKR (RU)	NC	€
6. Defra (GB)	NC	€
7. APHIS (US)	NC	€
8. OKState (US)	NC	€
total		€

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.

[a] First member is project coordinator. A minimum of two partners are necessary for each proposal. Add lines as needed.
[b] Please indicate the preferred mechanism (e.g. real pot RP; virtual pot VP; non-competitive NC), or several mechanisms if there is flexibility.
[c] Optional, as this amount can still change in the next phase. In-kind contribution should also be indicated in this column.