## 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

Rapid and efficient detection and identification of severe strains of citrus tristeza virus

### 1.4. Description of the problem the research should solve

Citrus tristeza virus (CTV) (Genus Closterovirus) is responsible for one of the most devastating virus diseases of citrus worldwide (Roistacher, 1991). The virus is present in all continents and the most common symptom is the quick decline observed in different citrus species such as sweet orange (*Citrus sinensis*), mandarins (*C. reticulata*), grapefruits (*C.paradisi*), kumquats (*Fortunella* spp.) and limes (*C. aurantiifolia)* when grafted on sour orange (*C. aurantium*) or lemon (*C. limon*) rootstocks; other severe expressions of CTV infection are the seedling yellows, observed primarily in the nursery, and stem pitting that causes irregular growth of phloem tissues producing pits in the wood of sweet orange and grapefruit, low fruit-bearing and significantly reduced quality.

To date, six major CTV reference strains have been described: T36 (Karasev *et al*., 1995), T3 (Hilf *et al*., unpublished), VT (Mawassi *et al*., 1996), T68 (Harper, 2013), T30 (Albiach-Marti *et al*., 2000) and RB (Harper *et al*., 2010) mainly based on their genomic features. However, the relationship between genotype and phenotype is still unclear and a classification based on phenotype is not advisable (Harper, 2013).
Until recently, strain identification has been possible using a lengthy protocol based on the combination of serological, molecular and biological indexing tests. In particular, monoclonal antibodies (MCA13) (Permar *et al*., 1990) and the multiple molecular markers (MMM) (Hilf *et al*., 2005) have been found useful to diagnose severe CTV strains. The MCA13 antibody is no longer commercially available, which negatively impacts the diagnostic protocol currently in use. The project will aim to develop fast and efficient molecular tests for the detection and identification of CTV strains and in particular, the severe strains.

### 1.5. Description of the expected results

Project activities will focus on: a) gathering information on available diagnostic tools or tests that are currently used or being developed, b) collecting reference material to cover the diversity of CTV isolates c) performing *in silico* analysis of viral cDNA sequences and design primers or probes for the molecular detection and identification of CTV strains and d) organizing a test performance study to validate the test(s) identified/developed. The project's expected result is the development and validation of molecular test(s) for the detection and identification of severe strains of CTV.
Work will be organized as follows:
**January 2023-March 2023**: To collect information on the molecular tests available or in development; to agree on a common methodology for monitoring and sampling.
**April 2023- June 2023**: To monitor and sample plant material from the field; to identify relevant material in reference collections. The plant material from the field or from collections will be used for the molecular characterization and for the test performance study.

**July 2023-October 2023**: To agree on the protocols for sequence analysis and design the primers and probes for the molecular test
**November 2023- February 2024**: To develop the protocols and the material for the test performance study
**March 2024-June 2024**: To perform the test performance study: to send samples and protocols, to perform the tests, to send the results to the TPS organizers, to analyse the results.

### 1.6. Beneficiaries of this research product

The project will benefit official laboratories responsible for the diagnosis of CTV, growers and nurseries worldwide.

### 1.7. Research funders and research contribution/ distribution

In the framework of the CIHEAM/Euphresco initiative on the Plant Health research priorities for the Mediterranean region[1], the following organizations have preliminarily expressed an interest to be involved in a research project on citrus tristeza virus.

| Funding organisation | Research activity and researchers involved |
|---|---|
| 1. International Center for Advanced Mediterranean Agronomic Studies-Bari, Italy<br><br>Anna Maria D'Onghia<br>donghia@iamb.it | -Project coordination;<br>-To gather information on available diagnostic tests currently under development;<br>-To design primers or probes for the molecular detection and identification of CTV strains;<br>-To participate in the test performance study to validate the test(s) identified/developed;<br><br>Contact person: Khaled Djelouah<br>E.mail address: djelouah@iamb.it |
| 2. Department of Agriculture, Water and the Environment, Australia<br><br>Con Goletsos<br>PHSgovernancegroups@agriculture.gov.au | -To share information and experience on available diagnostic tests;<br>-To collect reference material to cover the diversity of CTV isolates;<br>-To participate in the test performance study to validate the test(s) identified/ developed;<br><br>Contact person: Nerida Donovan<br>E.mail address:<br>nerida.donovan@dpi.nsw.gov.au<br><br>Contact person: Andrew Geering<br>Email address: a.geering@uq.edu.au |
| 3. Federal Ministry for Agriculture, Regions and Tourism, Austria<br><br>Sylvia Blümel<br>sylvia.bluemel@ages.at | -Participation in the test performance study to validate the test(s) identified/ developed;<br><br>Contact person: Juliane Reiterer<br>E.mail address: Juliane.reiterer@ages.at |

[1] https://www.youtube.com/watch?v=WJ04QW4ImYA

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| 4. Croatian Agency for Agriculture and Food, Croatia<br><br>Ivan Poje<br>ivan.poje@hapih.hr | -To participate in the test performance study to validate the test(s) identified/ developed:<br><br>Contact person: Jasna Milanovic<br>E.mail address: jasna.milanovic@hapih.hr |
| 5. Plant Pathology Research Institute, Agricultural Research Centre, Egypt<br><br>Ahmed Kamal El-Attar<br>ippc.egypt@gmail.com<br>ippc@capq.gov.eg | -To survey in the main *Citrus* (*Citrus sinensis*, *C. reticulata*, *C. grandis*, *C.aurantifolia*, *C. lemon*) producing nurseries and orchards in Egypt;<br>-To participate in the test performance study to validate the test(s) identified/ developed:<br><br>Contact person: Ahmed El-Attar<br>E.mail address: ippc.egypt@gmail.com; ippc@capq.gov.eg<br><br>Contact person: Ahmed Soliman<br>E.mail address: amsma33@hotmail.com<br><br>Contact person: Hala A. Amin<br>E.mail address: hala-amin@arc.sci.eg; halaaminaly@gmail.com |
| 6. French Agency for Food, Environmental and Occupational Health & Safety, France<br><br>Geraldine Anthoine<br>geraldine.anthoine@anses.fr | ANSES Plant Health Laboratory - Unit for Tropical Pests and Diseases:<br>-To collect reference material to cover the diversity of CTV isolates (CTV isolates from French overseas regions);<br>-To characterize in-house reference materials used for validation by digital PCR;<br>-To co-organize (organization and methodological aspects) and participate in the TPS to validate the test(s) identified/developed;<br><br>Contact person: Aude Chabirand<br>E.mail address: aude.chabirand@anses.fr<br><br>ANSES Plant Health Laboratory - Quarantine Unit:<br>-To collect reference material to cover the diversity of CTV isolates (in house reference material);<br>-To participate in the TPS to validate the test(s) identified/developed;<br><br>Contact person: Jean-Philippe Renvoisé<br>jean-philippe.renvoise@anses.fr |
| 7. Benaki Phytopathological Institute, Greece<br><br>Panos Milonas<br>p.milonas@bpi.gr | -To collect information on available diagnostic tools or tests that are currently being developed;<br>-To collect reference material to cover the diversity of CTV isolates; |

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| Irene Vloutoglou<br>i.vloutoglou@bpi.gr | -To perform *in silico* analysis of viral DNA sequences and design primers or probes for the molecular detection and identification of CTV strains;<br>-To participate in the test performance study to validate the test(s) identified/ developed;<br><br>Contact person: Christina Varveri<br>E.mail address: c.varveri@bpi.gr<br><br>Contact person: Despoina Beris<br>E.mail address: d.mperi@bpi.gr<br><br>Contact person: Ioanna Malandraki<br>E.mail address: i.malandraki@bpi.gr |
| 8. Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel<br><br>Yael Meller Harel<br>YaelM@moag.gov.il | -To provide viral sequences material that could be used for the project;<br>-To participate in the TPS to validate the test(s) identified/developed;<br><br>Contact person: Ahmed Abu-Ras<br>E.mail address: Ahmada@moag.gov.il |
| 9. Ministry of Agriculture, Food and Forestry Policy, Italy<br><br>Masci Alberto<br>a.masci@politicheagricole.it | -To gather information on available diagnostic tests currently under development;<br>-To perform *in silico* analysis of viral DNA sequences and design primers or probes for the molecular detection and identification of CTV strains;<br>-To participate in the test performance study to validate the test(s) identified/developed;<br><br>Contact person: Luca Ferretti<br>E.mail address: luca.ferretti@crea.gov.it<br><br>Contact person: Andrea Gentili<br>E.mail address: andrea.gentili@crea.gov.it<br><br>Contact person: Marta Luigi<br>E.mail address: marta.luigi@crea.gov.it |
| 10. National Research Center, Italy<br><br>Angelantonio Minafra<br>angelantonio.minafra@ipsp.cnr.it | -To participate in the test performance study to validate the test(s) identified/developed;<br><br>Contact person: Giuliana Loconsole<br>E.mail address:<br>giuliana.loconsole@ipsp.cnr.it<br><br>Contact person: Maria Saponari<br>E.mail address: maria.saponari@ipsp.cnr.it |
| 11. Institute for Agricultural Research, Morocco | -To collect leaf samples from symptomatic and asymptomatic citrus trees; |

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| Faouzi Bekkaoui faouzi.bekkaoui@inra.ma | -To analyse samples by serological and molecular tests;<br>-Molecular characterization of CTV isolates in the case of their detection in Moroccan conditions;<br>-To participate in the development and validation of molecular tests established as part of the project for detecting severe CTV strains circulating in the agroecosystems under investigation;<br><br>Contact person: Fouad Mokrini<br>E.mail address: fouad.mokrini@inra.ma |
| 12. Ministry of Agriculture, Palestine<br><br>Ahmad Fattum<br>ahmadfattum@yahoo.com | -To survey CTV in Palestinian orchards;<br>-To provide samples for the molecular characterization of strains present in Palestine;<br><br>Contact person: Samer Jarar<br>E.mail address: samer.jarrar@moa.pna.ps<br>samerjarrar72@gmail.com<br><br>Contact person: Rola Sameer<br>E.mail address:<br>rola.mahmoud@moa.pna.ps<br>rola_s_m@yahoo.com<br><br>Contact person: Salamah Shabib<br>E.mail address: salamshbib@gmail.com<br><br>Contact person: Mazen Salman<br>E.mail address: m.salman@ptuk.edu.ps |
| 13. National Institute for Agricultural and Veterinarian Research, Portugal<br><br>Leonor Cruz<br>leonor.cruz@iniav.pt | -Contribution to be detailed;<br><br>Contact person: Margarida Teixeira<br>E.mail address: margarida.teixeira@iniav.pt |
| 14. University of Algarve, Portugal<br><br>Natália Tomás Marques<br>nmarques@ualg.pt | -To gather information on diagnostic tests that are available or in development;<br>-To collect material (mild strains present in Portugal);<br>-To perform in silico analysiss of viral DNA sequences and design primers or prorbes for the diagnosis of CTV;<br>-To co-organize and participate in the TPS to validate the test(s) identified/ developed;<br><br>Contact person: Natália Marques<br>E.mail address: nmarques@ualg.pt |
| 15. Valencia Institute for Agricultural Research, Spain<br><br>Luis Rubio Miguelez<br>lrubio@ivia.es | -Bioinformatic analysis of CTV sequences;<br><br>Contact person: Luis Rubio Miguelez<br>E.mail address: lrubio@ivia.es |

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| 16. Bioreba AG, Switzerland<br><br>Marco Kaiser<br>kaiser@bioreba.ch | -Contribution to be detailed;<br><br>Contact person:<br>E.mail address: |
| 17. Ministry of Agriculture, Tunisia<br><br>Asma Najar<br>asmanajara@yahoo.fr | -To collect reference material to cover the diversity of CTV isolates;<br>-To perform *in silico* analysis of viral DNA sequences and design primers or probes for the molecular detection and identification of CTV strains;<br><br>Contact person: Asma Najar<br>E.mail address: asmanajara@yahoo.fr<br><br>Contact person: Imen Hamdi<br>E.mail address: imenhamdi@yahoo.fr |

### 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. CIHEAM (Int) | | € |
| 2. DAWE (AU) | | € |
| 3. AGES (AT) | | € |
| 4. HAPIH (HR) | | € |
| 5. ARC (EG) | | € |
| 6. ANSES (FR) | | € |
| 7. BPI (GR) | | € |
| 8. MOAG (IL) | | € |
| 9. CREA (IT) | | € |
| 10. CNR (IT) | | € |
| 11. INRA (MA) | | € |
| 12. MoA (PS) | | € |
| 13. INIAV (PT) | | € |
| 14. UALG (PT) | | € |
| 15. IVIA (ES) | | € |
| 16. Bioreba (CH) | | € |
| 17. MoA (TN) | | € |
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### 2.2. Expected duration of the project (only for non-competitive topics)

36 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