

Disease spreading and borderless scientific cooperation: the case of 'Candidatus Phytoplasma phoenicium'

Almond witches' broom (AlmWB) is one of the most threatening phytoplasma diseases of peach and almond trees. 'Candidatus Phytoplasma phoenicium' is the pathogen associated with the disease in Iran and Lebanon, two countries where outbreaks have been officially reported. The disease has rapidly spread and caused

severe damage in peach orchards in Lebanon and killed over a hundred thousand almond trees in Iran. All Mediterranean countries are very concerned about keeping AlmWB out of their territories in which peach and other stone fruits are widely cultivated. The EPPO Pest Risk Analysis (PRA) for AlmWB underlined the need for an efficient protocol for the reliable detection and identification of the associated pathogen.

The main goal of the Euphresco project 2017-F-234 'Set-up of reliable protocols for the detection and identification of 'Candidatus Phytoplasma phoenicium' (DIPCAPP)' was to provide knowledge to support surveillance activities in order to prevent the introduction of 'Candidatus Phytoplasma phoenicium' into new areas, such as in Europe, where the vector(s) of the phytoplasma may occur.

Seven research partners from France, Iran, Italy (2 laboratories), Lebanon, Russia and Slovenia joined forces to validate several diagnostic tests for the detection of genetically distinct 'Candidatus Phytoplasma phoenicium' strains through a test performance study.

Six different PCR-based tests were evaluated:

- PCR test targeting the 16S-23S rDNA genes, specific for AlmWB phytoplasma (IX-B) (Jawhari, *et al.*, 2015);
- Nested PCR test targeting the inmp gene, specific for AlmWB phytoplasma (IX-B) (Quaglino et al., 2015);
- Nested PCR test targeting the 16S rDNA gene using generic phytoplasma primers, followed by RFLP analysis of patterns to identify the AlmWB phytoplasma (IX-B) (Molino Lova *et al.*, 2011);





- PCR test using the barcoding primers, followed by sequencing to detect the AlmWB phytoplasma (EPPO 2016);
- Real-time PCR test for the detection of phytoplasma in general, not specific for AlmWB (Christensen *et al.*, 2004, EPPO 2018);
- Real-time PCR test for the detection of AlmWB phytoplasma (IX-B) (Jawhari, et al., 2015).

Two tests, a conventional and a real-time PCR, have shown reliable and comparable results for the diagnosis of 'Candidatus Phytoplasma phoenicium' on DNA samples extracted from young almond and peach leaf midribs and veins. One on these tests was used to confirm the first report of 'Candidatus Phytoplasma phoenicium' in Apulia, Italy (Nigro et al., 2019). The results of the project will be used to prepare an EPPO Diagnostic Protocol on the pest and to support harmonization of diagnostic approaches in the region. The DIPCAPP project is an excellent example of cooperation among scientists coming from EU and non-EU countries and of how Euphresco can facilitate international collaboration on shared priorities by joining efforts and sharing information and scientific knowledge.

Project ID: 'Set-up of reliable protocols for the detection and identification of 'Candidatus Phytoplasma phoenicium' (DIPCAPP)

References

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