Euphresco

Success story

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Development of LAMP based protocol for accurate, reliable, fast and affordable diagnostics of *Candidatus* Phytoplasma solani

Phytoplasmas are cell-wall-free plant pathogenic bacteria; they have a broad range of plant hosts and diseases of many important crops are associated with these pathogens. At least ten phytoplasma ribosomal subgroups have been associated with grapevine yellows diseases, which have great economic impact on viticulture. In Europe, the main phytoplasmas associated with grapevine yellows are the causal agent of flavescence dorée and '*Candidatus* Phytoplasma solani', which cause bois noir.

The detection of phytoplasmas is difficult due to their uneven distribution within the host and low titer, which can be affected by the season. Different PCR-based protocols for detection of grapevine yellows phytoplasmas have been developed. Although their sensitivity and specificity are sufficiently high when they are properly applied, the procedures are time-consuming, require expensive laboratory equipment and cannot be performed in the field because of the lack of convenient portable instruments.

More recently it has been proven that methods based on loop-mediated isothermal amplification (LAMP) circumvent the real-time PCR sensitivity to inhibitors present in plant extracts and their isothermal nature makes LAMP-based methods suitable to be deployed in the field. Because of its speed, robustness, simplicity and affordable cost, the use of LAMP is gaining popularity for diagnostics in plant health, including flavescence dorée phytoplasma detection, for which a LAMP assay has been developed in the course of the FP7 project VITISENS.

One of the objectives of the project GRAFDEPI2 is to develop a LAMP-based protocol for the detection of *C.* P. solani. Test performance studies were organized to obtain validation parameters for the *C.* P. solani LAMP protocol, as well as for the recently developed LAMP assays for flavescence dorée phytoplasma.

LAMP assay for *C.* P. solani has been shown to be specific and extremely sensitive. As for the LAMP protocol for flavescence dorée the whole detection procedure is shortened from one day (for the real-time PCR-based detection) to less than one hour; this time also includes sample preparation with homogenization without DNA extraction. The whole procedure has been tested and validated according to the EPPO standard PM 7/98 (2), including comparison with qPCR tests for flavescence dorée and *C.* P. solani.

A test performance study has been organized to validate LAMP protocols for the detection of *C.* P. solani and flavescence dorée phytoplasmas. Ten laboratories from the research and plant protection area from Europe and Australia participated in the trials. The results are currently being evaluated and will be made public with the final report of the project. The validation data will be stored in the EPPO database on Diagnostic Expertise (http://dc.eppo.int/validationlist.php).

Project ID: Grapevine flavescence doreé (FD) follow up Vitisens, GRAFDEPI and Qdetect (GRAFDEPI2)