SCIENTIFIC INNOVATION FACTSHEET

SEROLOGICAL TESTS TO DETECT GRAPEVINE YELLOWS PHYTOPLASMAS

Set up friendly detection methods for identification of selected phytoplasmas associated with grapevine yellows

■ THE PROBLEM ADDRESSED

Improved tools for monitoring grapevine yellows presence

Grapevine yellows (GY) are severe diseases associated with several phytoplasmas, insect vectors, and host plants, widespread in different grapevine cultivation regions. Collectively, the GY, "flavescence dorée" (FD), "bois noir" (BN) and aster yellows (AY), are considered as a complex disease model based on multiple variables (phytoplasma host plants and insect vectors), each behaving often differently in diverse ecosystems. So far, the detection of GY phytoplasmas has relied on a set of molecular techniques that target the genome of the pathogen. Even though these techniques are sound and reliable, the diagnostic scheme is relatively expensive and lacks suitability for large scale monitoring methods. Serological tests were therefore evaluated under the TROPICSAFE activities to overcome the scarcity of the antigenic protein, assess the test sensitivity when the pathogen is present at low titer and compare their specificity to the results obtained with phytoplasma molecular identification.

• Grapevine yellows infected plants.

■ THE PRACTICE/INNOVATION PROPOSED BY TROPICSAFE.

Raising phytoplasma-specific antibodies

To overcome the scarcity of the phytoplasma antigenic proteins, the TROPICSAFE activities were focused on culturing grapevine aster yellows phytoplasmas in artificial medium and developing the synthesis of "flavescence dorée" antigenic proteins using a bacterium expression system. Both technologies allowed the production of antigenic preparations suitable for producing specific antibodies against the two phytoplasmas. These antibodies are now available for designing new serological tests to complement the molecular phytoplasma detection.

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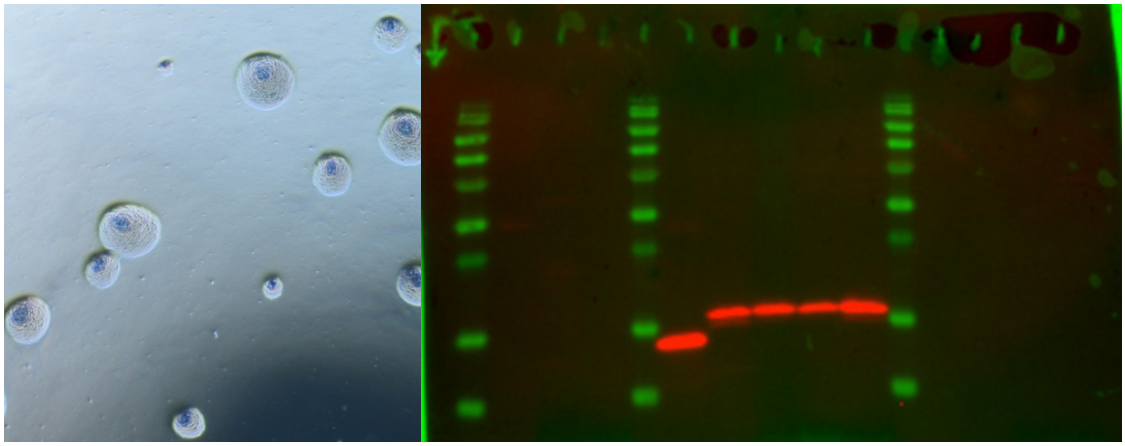
■ HOW IS TROPICSAFE IMPLEMENTING IT?

Development of serological tests

The antibodies were evaluated for serological applications, giving a priority to low-cost techniques routinely applied in the grapevine testing laboratories for other pathogens.

The grapevine aster yellows antibodies were applied to the immunofluorescence antibody assay (IFAS), a technique routinely applied to detect bacteria. A sound enzyme-linked immunosorbent assay (ELISA) could be developed for FD phytoplasmas: the protocol was challenged for its diagnostic performances by comparison with quantitative PCR test. Moreover, the FD-ELISA test was evaluated by testing a whole range of samples including grapevine leaves, insect vectors, and alternative host plant species. The assessment of the diagnostic performances (specificity and sensitivity) was addressed by a double check based on molecularly identified samples.



• On the left: colonies of grapevine aster yellows cells growing in solid medium photographed under bifocal microscopy at 40 X used as antigenic preparation. On the right: Western blot analysis of IgG anti "flavescence dorée" strain 16SrV-D recombinant protein (from Trivellone *et al.*, 2019).

■ HOW IS IT WORKING?

Testing grapevine yellows antibodies

The ELISA test was proven to be a reliable tool to specifically detect the FD presence in symptomatic grapevine plants. It works also for testing the main phytoplasma vector *Scaphoideus titanus*, and *Alnus glutinosa* and *Clematis vitalba* alternative host plants. Along three years of field sampling, two "flavescence dorée"-ELISA tests, targeting two diverse strains, were shown to be 100% strain specific in ELISA and were confirmed by qPCR. The exclusion of cross reactions among known strains makes the test suitable for one-step strain identification. The specificity values ranged from 80% to 100%, depending on the sample (plant or insect), while the preliminary assessment revealed a diagnostic sensitivity ranging from 58% to 64%. Even though the sensitivity values seem to be critical, this ELISA test might be used in the monitoring programs as a preliminary screening of a large number of samples; the samples resulting negative can then be tested by more sensitive molecular methods such as qPCR. The application of this protocol will produce about 50% reduction of costs for large screening programs.

Furthermore grapevine aster yellows antibodies were tested by ELISA and IFAS methods to evaluate their suitability for phytoplasma detection in plant samples. The two methods showed a good specificity to the aster yellows phytoplasma cultures, while the IFAS assay gave promising results also with infected plant tissues.

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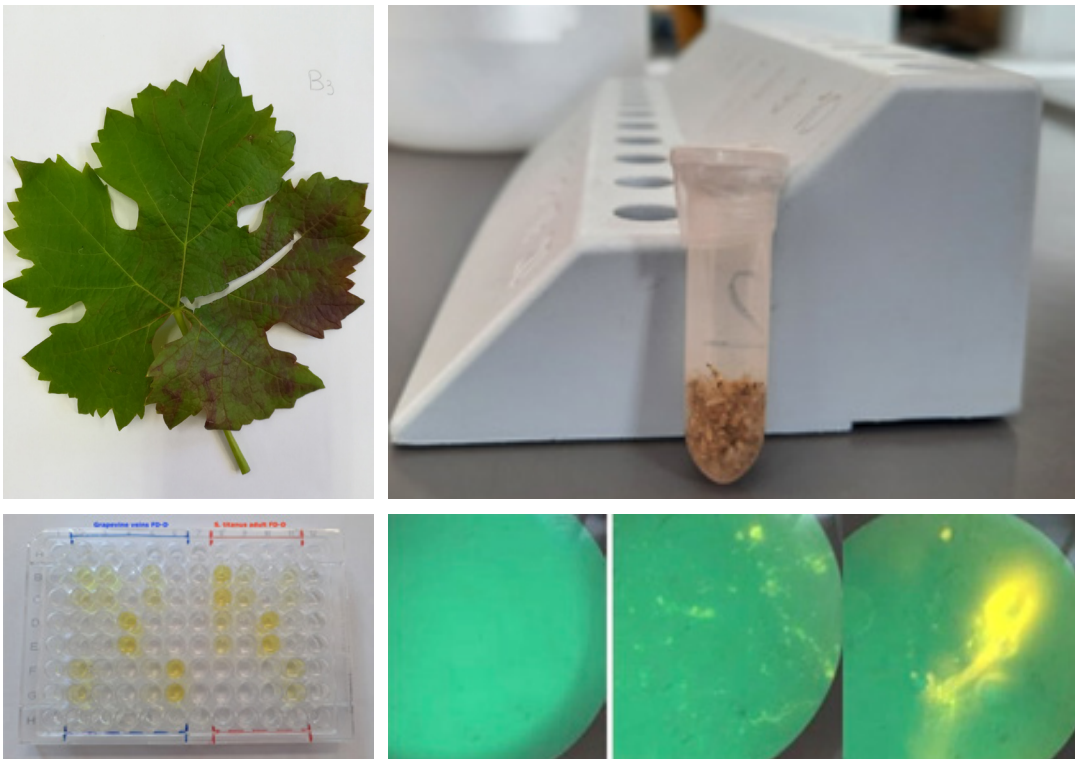

• Top from left: grapevine samples and *Scaphoideus titanus* adult sample for "flavescence dorée" ELISA test. Bottom from left: ELISA results for "flavescence dorée" and IFAS grapevine aster yellows detection of healthy periwinkle, cultivated aster yellows phytoplasma, aster yellows infected periwinkle tissue (from Contaldo *et al.*, 2019).

KEY WORDS

Antiserum production, "flavescence dorée", aster yellows, serological detection.

FURTHER INFORMATION

Regulation (EU) 2019/2072 of 28 November 2019. http://data.europa.eu/eli/reg_impl/2019/2072/oj

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