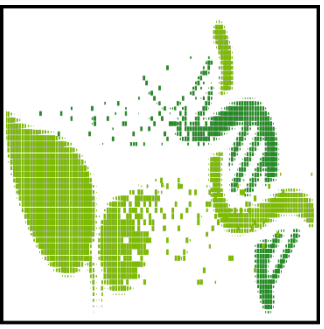
Euphresco

# Success story

October 2021

## Faster, cheaper identification of emerging viruses

Pest diagnosis is performed by official laboratories upon request of National Plant Protection Organizations, growers or traders, in samples that inspectors have collected *in situ* (a consignment, a place of production, an outbreak area, a buffer zone, *etc*.). Resources allocated to official laboratories have decreased over time, while trade in plants and plant products, and consequently the amount of material to be tested, has increased steadily. On-site detection and identification tests, that are both high-throughput, scalable and affordable have the potential to accelerate diagnosis (which is especially important in the case of perishable goods) and to relieve pressure on laboratories. Among the technologies under development and validation, the Oxford nanopore technology allows the generation of thousands up to millions of sequences on a portable high-throughput sequencer.



**The objective of the VIRFAST project was to evaluate the use of Oxford nanopore high-throughput sequencing technology for the diagnosis of plant viruses on different matrices (e.g. banana, citrus, pineapple) and on samples with increasing complexity (i.e. pure virus, plant infected with one virus, plant infected with several viruses).**

The project consortium analysed the performance of the Oxford nanopore sequencer when using crude extract material instead of RNA extracted from plant material. Early results from an inter-laboratory comparison indicated that the viruses can be detected when using crude extract, which allows for quicker and easy-to-use protocols that can be used on-site. Despite these promising results, the technology is not yet ready for routine diagnosis, as the sequencing threshold and, consequently, the analytical sensitivity of the test was greatly weakened when using crude extract material. However, it should be noted that validation was performed using dehydrated samples and moreover some participating laboratories used the Oxford nanopore sequencer on crude extract material for the first time. Better analytical sensitivity is expected when working with fresh tissue and if the users are familiar with the use of crude extract material.

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The library preparation used in the project was a time-consuming process, but new developments for rapid library preparation with the use of enzymes resistant to PCR-inhibitors have the potential to improve the performance of the technology. These enhancements should open to the possibility of using the Oxford nanopore sequencer to monitor an outbreak in the field, in a molecular biology laboratory or in remote locations with limited access to plant diagnostics facilities.

During the project, and in collaboration with the EU project Valitest, a survey was carried-out among project partners on the use of on-site testing by diagnostic laboratories. In this survey, the use of immunological tests, such as lateral flow device, immunoprinting or immunofluorescence, and molecular tests such as loop mediated isothermal amplification or recombinase polymerase amplification were considered. In total, 27 laboratories from 20 EU countries replied to the survey. Twelve laboratories are already using on-site testing with an estimated volume ranging from 25 to 2 500 tests per year. The main context for using on-site testing kits is field inspection (63%), and these tests are also used as screening tests in the laboratory (19%). On-site tests in the laboratory can save time and are easy to use. However, the ability to take immediate decision was considered an important criterion for choosing on-site tests for less than half of the responders. The three main limitations for the wider use of on-site tests are the lack of validation data, the limited analytical sensitivity and their cost. The absence of tests for some pests also limits use. Participants identified the pests for which they consider tests should be developed/validated: for viruses, plum pox virus (3 responses), for fungi and oomycetes *Phytophtora ramorum* (4 responses), and for bacteria *Erwinia amylovora*, *Ralstonia solanacearum* and *Xylella fastidiosa* (4 responses each).

Project ID: Faster, cheaper identification of emerging virus problems (VIRFAST)