Interlaboratory comparison of molecular methods for the detection of *Xylella fastidiosa* in plant and insects

Loconsole G, Zicca S, Ligorio A, Altamura G, Cavalieri, Saponari M

National Research Council (CNR), Institute for Sustainable Plant Protection (IPSP), 70126 Bari, Italy

In 2020, an Interlaboratory Comparison (IC) was organized for the identification of *Xylella fastidiosa* (Xf) by qPCR in plant and insect samples. In the framework of this IC a test performance study (TPS) and a proficiency test (PT) involving 16 and 14 laboratories, respectively, were organized. All participating laboratories processed the samples (crude sap containing inactivated bacterial cells) from the DNA extraction step by comparing different procedures.
The TPS aimed to compare the performance of different DNA extraction procedures, whereas, the PT assessed the proficiency of diagnostic laboratories carrying out Xf diagnosis.
The values of accuracy, repeatability and reproducibility recorded for the three DNA extraction procedures ranged from 98.52% to 99.30% when testing plant samples, and from 94.07% to 98.75% for insect samples, with a the detection limit of approx. 10^2 CFU/ml. Overall, the data gathered confirmed the robustness of the procedures previously validated (CTAB, DNeasy mericon Food kit - Qiagen), and allowed to extend the panel of DNA extraction procedures currently available for processing the wide range of plant matrices to be checked for Xf (i.e Maxwell® RSC PureFood GMO and Authentication Kit-Promega). Both commercial kits tested, combined with automatized platforms, yielded better performance than the manual CTAB-based procedure.
All laboratories participating to the PT proved to be highly proficient (values close to 100% for all performance criteria), regardless the procedure of extraction, the qPCR master mix and the amplification conditions used. However, some of the extraction methods used in few laboratories produced lower yield and quality of the total DNA recovered, thus producing higher values of quantitation cycle in qPCR.
In conclusion, the TPS provided useful information on additional extraction procedures that can be used to efficiently detect the pathogen in different plant and insect matrices. While the PT provided hints on the efficiency of the diagnostic laboratories operating in 10 different EU/non-EU Countries.

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