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DNA extraction methods for *in situ* LAMP testing of quarantine phytopathogens, long-term storage and safe movement of infected plant samples

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Rapid, sensitive, *in situ* detection methods are highly needed to monitor plant quarantine pathogens at borders, in nurseries, and as part of surveillance programs. In addition, it is important to safely move infected plant material from sampling sites to authorized diagnostic laboratories, especially when vector-borne pathogens are involved, to avoid the risk of passive movement of infected vectors into pathogen-free areas. Finally, it is necessary to use long-term storage methods to optimize the organization of laboratory analysis, especially during optimal periods for pathogen monitoring.

As concerns pathogen detection, molecular methods are the most reliable as specificity and sensitivity, being based on detection and amplification of target sequences from reference genes in the pathogens' nucleic acids. PCR-based methods, the most commonly used, amplify target regions using polymerase and two specific primers during a series of repeated thermal cycles. However, these methods are costly because of the need for expensive reagents, high-tech equipment such as thermal cyclers and skilled personnel.

A valid alternative to PCR-based methods is the molecular LAMP assay, described by Notomi *et al.* (2000), because it is a specific, rapid, inexpensive, and easy-to-use diagnostic method.amplifies DNA or RNA fragments using DNA strand displacing, thus allawing the production of high amounts of DNA in a short time. LAMP works under isothermal conditions and can be highly specific because it uses four to six primers capable of hybridizing six to eight regions of the target sequence. Usually, this method does not require post-reaction processing because the results can be rapidly observed by indicators. Consequently, it can speed up the diagnostic process compared with a PCR-based method. LAMP is also highly tolerant to sample inhibitors, allowing it to be used directly on raw DNA extracted from infected or infested products.

For the many advantages of the LAMP assay, CIHEAM in Bari has focused research on this method by developing efficient DNA extraction methods, instead of using leaf macerates, for the detection of two major quarantine pathogens which are also vector-transmitted: the *X. fastidiosa* bacterium that infects numerous host plant species worldwide and is devastating centuries-old olive trees in southern Italy; and the "Flavescence dorée" phytoplasma that is the most damaging agent of grapevine yellowing disease in many European grape-producing countries.

The membrane-based DNA extraction method combined with real-time LAMP assay has been developed for the detection of *Xylella fastidiosa*. It consists in printing stem sections of infected olive or oleander samples onto nitrocellulose membranes and use discs of printed membranes to rapid nucleic acid extraction. Compared to plant samples, printed membranes can be easily handled and delivered to the laboratory without moving the infected plant material and pathogen vectors. Moreover, printed mebranes can be easily stored at room temperature for subsequent LAMP analysis.

The leaf disc DNA extraction method combined with real-time LAMP assay has been developed for detecting the "grapevine flavescence dorée" phytoplasma. In this method three leaf discs with veins/sample are dissected using the inner part of 2 ml Eppendorf tube cap and the same tube is used for DNA extraction and LAMP assay. This method proved faster and easier than using leaf macerates, allowing the plant sample to be safely moved before analysis by dissecting the discs in Eppendorf tubes directly in situ. It also proved effective for long-term storage at -20°C with the advantage of keeping leaf disc samples in the same Eppendorf tube to be used for LAMP analysis.

All extraction methods combined with the real-time LAMP assay were as sensitive as real-time PCR. However, the choice of the best DNA extraction method depends from the pathogen and/or plant species.