Direct Tissue ImmunoBlot Assay (DTBIA), an efficient tool for the mass detection of Xylella fastidiosa in infected olive trees

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in
Xylella fastidiosa & the Olive Quick Decline Syndrome (OQDS). A serious worldwide challenge for the safeguard of olive trees

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 121

2017
pages 61-64

Article available online / Article disponible en ligne à l’adresse :
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Direct Tissue ImmunoBlot Assay (DTBIA), an efficient tool for the mass detection of Xylella fastidiosa in infected olive trees

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Following the first identification of Xylella fastidiosa (Xf) under field conditions from the province of Lecce (Puglia region, southeastern Italy), in association with a devastating disease of olive known as “olive quick decline syndrome” (OQDS) (Saponari et al., 2013), the pathogen was characterized as a strain of the subspecies Pauca (Cariddi et al., 2014) and found to be transmitted primarily by a xylem fluid-feeding insect vector Philaenus spumarius (L.) (Hemiptera: Aphrophoridae); moreover, apart from olive, other host species of the bacterium were identified, most of which are ornamentals or belong to the typical Mediterranean bush.

To face this threat, the local Plant Protection Services requested an immediate monitoring campaign in the region, in order to establish the exact distribution of the pathogen and to limit further spread of the bacterium. Before to conduct a large-scale monitoring program for Xf detection in Puglia, the validation of ELISA and PCR protocols was necessary. In this context the CIHEAM-Bari laboratory was part of the ring-test with other accredited laboratories and ELISA was recognized as the official diagnostic assay in large-scale monitoring; while PCR was identified as a method for the confirmation of ELISA assay.

Following these key studies, thousand samples of different plant species, primarily olive trees, were efficiently tested using ELISA for routine pathogen detection (Loconsole et al., 2014). However, despite all precautions that can be taken, the risks associated with the handling and safe movement of plant material to the laboratory which can often carry infected vectors, remained very high and were considered as critical issues for avoiding dissemination of this pathogen to “pathogen-free” areas, in which most of the laboratories are located.

In order to perform mass analyses of plant samples directly in the field, thus avoiding the potential pathogen spread through sample delivery, Direct Tissue Blot Immunoblot assay (DTBIA), an on-site rapid diagnostic assay that requires very little sample manipulation, was investigated in the CIHEAM Bari laboratories, as an alternative diagnostic tool to ELISA. This technique was already successfully applied in the mass detection of some citrus disease agents, mainly Citrus tristeza virus and Citrus psorosis virus (Garnsey et al., 1993; Cambra et al., 2000; D’Onghia et al., 2001; Djelouah and D’Onghia, 2001). Some attempts to use DTBIA applications were also reported for the detection of X. fastidiosa from citrus affected by variegated chlorosis (Garnier et al., 1993) and for other infectious agents (Lin et al., 1990).

The DTBIA technical protocol for the detection of Xf (Djelouah et al., 2014) was achieved by using plant tissues collected from different Xf infected olive and oleander trees from four orchards located in Gallipoli, Parabita and Taviano municipalities (Salento peninsula). In this framework, different explants, nitrocellulose membranes and reagents were analysed. The adopted DTBIA protocol by using Enbiotech kit was compared with ELISA and PCR tests through the use of specific antibodies to X. fastidiosa (Loewe Biochemica GmbH and Agritest) and the RST31/ RST33 set of primers targeting the 16S rDNA gene (Loconsole et al., 2014; Minsavage et al., 1994) respectively. In both procedures, the olive and oleander samples were correctly categorized as positive and negative.
The fresh mature twigs (2-5 mm in diameter), collected from the four quadrants of the tree, showed very distinct and homogeneous stained areas and proved to be the best explant for pathogen detection; moreover, the 0.45 µm nitrocellulose membranes evidenced better results in terms of blot colour reaction. Similarly, concerning the protein-binding sites, the use of 1% fat milk solution, coupled with a gentle stirring on a shaker for 1 h, performed better than BSA, which has been commonly used in previous research (Garnsey et al., 1993; Garnier et al., 1993; D’Onghia et al., 2001). Interestingly, no apparent difference was observed between fresh, 1 month stored blotted membranes as well as between membranes printed directly in the field, and those printed in the laboratory.

Results obtained using the DTBIA also highlighted the importance of adopting appropriate sampling for effective pathogen detection in large scale monitoring especially in the early stages of infection. Because the bacterium is unevenly distributed within the canopy, and because four twigs can be printed in each quadrant of the grid drawn on a membrane, two quadrants can be allocated to each tree to be tested, so that a number of representative twigs is therefore analysed. Whereas, young suckers should be avoided for sampling since they are probably infected at a later stage because of the xylem translocation of the bacterium.

The overall results of this protocol by using the Enbiotech commercial Kit proved that the technique is user-friendly, fast and does not require sophisticated equipment or highly skilled operators. DTBIA is an accurate and highly reliable serological test for processing a large number of samples. The efficiency comparable to ELISA and PCR, combined with the advantages of easier handling, speed and cost, makes DTBIA a valid alternative to ELISA in large-scale surveys for occurrence of Xf. Thus, it could be proposed as an effective alternative method for on-site detection of Xf. Moreover, the printing of membranes directly in the field prevents infections from spreading to Xf-free areas, through the movement of plant material with pathogen vectors for laboratory testing.

In table 1 is reported the DTBIA protocol for the detection of *Xylella fastidiosa*
Table 1. DTBIA PROTOCOL FOR THE DETECTION OF *XYLELLA FASTIDIOSA*

<table>
<thead>
<tr>
<th>MEMBRANE PREPARATION &amp; TISSUE BLOTS</th>
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<tbody>
<tr>
<td>Distinct squares are delimited on nitrocellulose membranes 0,45 µm pore size with high affinity for binding proteins.</td>
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<tr>
<td>Smooth cut sections are made from cross sections of mature twigs (2 mm in diameter), then gently pressed to the nitrocellulose membranes.</td>
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<tr>
<td>Each twig is printed twice in grid. Printed membranes are left to dry for 20 - 30 min at room temperature.</td>
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<tr>
<th>BLOCKING ASPECIFIC SITES</th>
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<tr>
<td>The membranes are incubated in 1% fat milk solution for 1h at RT on an orbital shaker, for the saturation of protein-binding sites.</td>
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<tr>
<td>The membranes are washed with PBS Buffer (0.05% Tween 20) on an orbital shaker 3 times x 3 min each.</td>
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<tr>
<th>ADDITION OF Xf SPECIFIC ANTIBODIES &amp; MEMBRANES STAINING</th>
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<tr>
<td>The membranes are immersed for 2h in a solution containing Xf specific alkaline phosphatase-conjugated antibodies.</td>
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<tr>
<td>The conjugated antibodies solution is discarded and the membranes are washed three times using the washing solution.</td>
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<tr>
<td>The membranes are stained by immersion in a solution obtained by dissolving one tablet of Sigma FastTM BCIP-NBT in 10ml of distilled water, until a purple-violet colour appears in the positive samples (around 2-3 min). The reaction is stopped by washing the membranes with tap water.</td>
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<th>MEMBRANES READING</th>
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<td>The membranes are observed under a low power magnification lens (X10-X20). The presence of purple-violet coloration on stem section reveals the presence of <em>X. fastidiosa</em> infection.</td>
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References


