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

Djelouah K., Frasheri D.

in

D'Onghia A.M. (ed.), Brunel S. (ed.), Valentini F. (ed.).
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

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

Khaled Djelouah, Dajana Frasheri

CIHEAM - Istituto Agronomico Mediterraneo di Bari, Italy

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 Immunoassay (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*

MEMBRANE PREPARATION & TISSUE BLOTS	
Distinct squares are delimited on nitrocellulose membranes 0,45 µm pore size with high affinity for binding proteins.	
Smooth cut sections are made from cross sections of mature twigs (2 mm in diameter), then gently pressed to the nitrocellulose membranes. Each twig is printed twice in grid. Printed membranes are left to dry for 20 - 30 min at room temperature.	
BLOCKING ASPECIFIC SITES	
The membranes are incubated in 1% fat milk solution for 1h at RT on an orbital shaker, for the saturation of protein-binding sites.	
The membranes are washed with PBS Buffer (0.05% Tween 20) on an orbital shaker 3 times x 3 min each.	
ADDITION OF *Xf* SPECIFIC ANTIBODIES & MEMBRANES STAINING	
The membranes are immersed for 2h in a solution containing *Xf* specific alkaline phosphatase-conjugated antibodies.	
The conjugated antibodies solution is discarded and the membranes are washed three times using the washing solution.	
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.	
MEMBRANES READING	
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 *X. fastidiosa* infection.	

References

Cambra M., Gorris M.T., Román M.P., Terrada E., Garnsey S.M., Camarasa E., Olmos A., Colomer M., 2000. Routine detection of citrus tristeza virus by direct immunoprinting-ELISA method using specific monoclonal and recombinant antibodies. Proceedings 14th Conference of International Organization of Citrus Virologists, Campinas, Brazil 1998, 34-41.

Cariddi C., Saponari M., Boscia D., De Stradis A., Loconsole G., Nigro F., Porcelli F., Potere O., Martelli G.P., 2014. Isolation of a *Xylella fastidiosa* strain infecting olive and oleander in Puglia, Italy. Journal of Plant Pathology 96(3), 1-5.

D'Onghia A.M., Djelouah K., Frasheri D., Potere O., 2001. Detection of citrus psorosis virus by direct tissue blot immunoassay. Journal of Plant Pathology 83, 139-142.

Djelouah K., D'Onghia A.M., 2001. Detection of citrus psorosis virus (CPsV) and citrus tristeza virus (CTV) by direct tissue blot immunoassay. In: D'Onghia A.M., Menini U., Martelli G.P. (eds.). Improvement of the citrus sector by the setting up of the common conservation strategies for the free exchange of healthy citrus genetic resources. Bari: CIHEAM, 2001. Options Méditerranéennes: Série B. Etudes et Recherches 33, 109 -114.

Djelouah K., Frasheri D., Valentini F., D'Onghia A.M., Digiaro M., 2014. Direct tissue Blot Immunoassay for detection of *Xylella fastidiosa* in olive trees. Phytopathologia Mediterranea, 53, 3, 559-564.

Garnier M., Chang C.J., Zreik L., Rossetti V., Bové J.M., 1993. Citrus variegated chlorosis: serological detection of *Xylella fastidiosa*, the bacterium associated with the disease. Proceedings 12th Conference of International Organization of Citrus Virologists, New Delhi, India 1992, 301-305.

Garnsey S.M., Permar T.A., Cambra M., Henderson C.T., 1993. Direct tissue blot immunoassay (DTBIA) for detection of Citrus tristeza virus (CTV). Proceedings 12th Conference of International Organization of Citrus Virologists, New Delhi, India 1992, 39-50.

Lin N.S., Hsu H.Y., Hsu H.T., 1990. Immunological detection of plant viruses and a mycoplasma-like organism by direct tissue blotting on nitrocellulose membranes. Phytopathology, 80, 824-828.

Loconsole G., Potere O., Boscia D., Altamura G., Djelouah K., Elbeaino T., Frasheri D., Lorusso D., Palmisano F., Pollastro P., Silletti M.R., Trisciuzzi N., Valentini F., Savino V., Saponari M., 2014. Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. Journal of Plant Pathology 96, 7-14.

Minsavage G.V., Thompson C.M., Hopkins D.L., Leite R.M.V.B.C., Stall R.E., 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84, 446-461.

Saponari M., Boscia D., Nigro F., Martelli G.P., 2013. Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Puglia (Southern Italy). Journal of Plant Pathology 95, 668.