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Detection of citrus psorosis virus (CPsV) and citrus tristeza virus (CTV) by direct tissue blot immunoassay

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Tristeza and psorosis are severe diseases of citrus with a worldwide distribution (Roistacher, 1991). The causal agents of these diseases, citrus tristeza virus (CTV) and citrus psorosis virus (CPsV), which belong to the genus closterovirus (Kitajiama *et al.,* 1964) and ophiovirus (Milne *et al.,* 2000) respectively, have a different distribution in the host plant. The need to provide alternative methods to biological indexing to meet certification and fulfil E.U. sanitary requirements for the production of propagating materials free from these citrus pathogens, and to detect their presence and incidence in citrus groves, prompted different laboratories to set up highly reliable, user friendly and low-cost protocols for serological diagnosis of most citrus.

Direct tissue blot immunoassay (DTBIA), a sensitive and simple technique that requires very little sample manipulation, has been applied for the rapid detection of CTV (Garnsey *et al.,* 1993; Cambra *et al.,* 2000; Djeouah *et al.,* 2001) and CPsV (Djelouah *et al.,* 2001; D'Onghia *et al.,* 2001, proving to be more sensitive and cheaper than ELISA.

Different types of tissues were used for diagnostic assays of these pathogens: stems and leaf petioles for CTV (Bar Joseph *et al.,* 1979; Cambra *et al.,* 2000); mature leaves for CPsV (Djelouah *et al.,* 2000); flower explants for the detection of both viruses (D'Onghia *et al.,* 2001; Djelouah *et al.,* 2001).

Among these explants, the best virus concentration and homogeneous distribution of CPsV and CTV were observed on flowers (Djelouah *et al,* 2001; D'Onghia *et al.;* 2001); in these last experiments, blots from a single ovary consistently detected CPsV and CTV, showing that ovaries are surely the best organs.

High sensitivity, short assay time and limited costs are the main advantages of DTBIA, which also represents a very convenient and safe system for shipping blotted membranes from one place to another. The short blossoming period of most citrus species may not represent a limit to the use of DTBIA for large-scale routine testing since storing flowers at 20°C for up to 1 year does not seem to affect test results.

The last investigations (Mrani *et al.,* 2001; Djelouah unpublished data) confirmed that DTBIA using ovaries is the most satisfactory procedure for the sensitive detection of CPsV and CTV. Sampling is simple and easy because few flowers/tree are sufficient for reliable testing; ovaries can be processed fresh or after up to 1 year's storage at 20°C.

Spotted membranes can be stained immediately or stored and processed after 1 year, using the same type of buffers without apparent loss of sensitivity. Commercial antibodies with high sensitivity and specificity, are also available for both viruses.

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