SUMMARY

Detection of *Citrus psorosis virus* (CPsV) by direct tissue blot immunoassay (DTBIA) was attempted using different tissues from CPsV-infected sources. Freshly cut surfaces of different plant organs were gently pressed on nitrocellulose membranes, exposed to a CPsV monoclonal antibody (MAb Ps29) conjugated with alkaline phosphatase, and stained with BCIP-NBT Sigma fast. For comparison, some explants were tested also by DAS-ELISA using the same MAb and a polyclonal antiserum for plate coating. Purple staining (positive reaction) was readily observed when flower explants were used, especially the ovary. DTBIA from ovaries correlated with DAS-ELISA, proving reliable and sensitive for the rapid detection of CPsV infections.

Key words: *Citrus psorosis virus*, diagnosis, immunoprinting, DTBIA.

Psorosis, a severe disease of citrus with a worldwide distribution (Roistacher, 1993), is caused by *Citrus psorosis virus* (CPsV), genus *Ophiuvirus* (Milne et al., 2000). For many years, laborious and costly indexing on citrus indicators was the only diagnostic method available (Roistacher, 1993) but laboratory procedures, such as ELISA (D’Onghia et al., 1998; Potere et al., 1999; Alioto et al., 1999, 2000; Djelouah et al., 2000) and RT-PCR (Garcia et al., 1997; Legarreta et al., 2000; D’Onghia et al., 2001), are now being utilised.

Direct tissue blot immunoassay (DTBIA), a technique that requires very little sample manipulation, has been used for the rapid detection of *Citrus tristeza virus* (CTV), proving to be more sensitive and cheaper than ELISA (Garnsey et al., 1993) and comparing well with the more recently described in situ immunoassay (ISIA) (Youjian et al., 2000). Thus, as reported in the present paper, DTBIA was tested for the rapid detection of CPsV.

Preliminary assays were done on roots, stems, leaves, fruit pedicels and flowers from five citrus genotypes (Table 1) from a field-grown collection of the Mediterranean Agronomic Institute (IAMB) and the University of Bari (UBA), all of which were known to be infected with CPsV, as determined by indexing and serology (Potere et al., 1999; Djelouah et al., 2000). Explants from a CPsV-free ‘Navelina’ orange were used as controls.

The high background in the blots from roots and the uncertain reactions given by stems and fruit pedicels, suggested to concentrate on leaves and flowers, which appeared to be more promising sources of antigen. Tests were done twice at blooming time using no less than 50 samples of different types of leaves (young expanding, fully expanded, and mature) and flowers, from each of the CPsV-infected sources. Open and closed flowers were collected, dissected into style, stigma, and ovary, which were blotted immediately or after storage at -20 and -70°C for 20, 60, and 120 days.

Table 1. *Citrus psorosis virus*-infected sources used in the tests.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Species/variety</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAMB-UBA -191X</td>
<td>Navelina orange</td>
<td>Italy</td>
</tr>
<tr>
<td>IAMB-UBA -269X</td>
<td>Bonanza orange</td>
<td>Italy</td>
</tr>
<tr>
<td>IAMB-UBA -654X</td>
<td>Grapefruit</td>
<td>Italy</td>
</tr>
<tr>
<td>IAMB-UBA -655X</td>
<td>Lemon</td>
<td>Italy</td>
</tr>
<tr>
<td>P216</td>
<td>Dweet tangor</td>
<td>USA</td>
</tr>
</tbody>
</table>

DTBIA was as described by Garnsey et al. (1993) with minor modifications. A fresh cut was made with a sharp razor blade across each sample and the cut surface was gently pressed on a Bio-Rad transblot nitrocellulose membrane. Leaf blades were rolled up before cutting and blotting. Gloves or tweezers were used when handling the membranes and in the blotting process.

Blotted membranes were allowed to dry for 20-30 min at room temperature and, before testing, were
marked with a pencil, so as to record the position of individual samples. Membranes were then placed in a 1% solution of BSA in distilled water and incubated for 2 h at room temperature, or overnight at 4°C in a plastic container on a shaker stirrer to block protein-binding sites. After washing with PBS containing 0.05% Tween 20, blotted membranes were exposed for 3 h to an alkaline phosphatase-conjugated monoclonal antibody (MAb Ps29) to CPsV at 1:250 dilution in conjugate buffer (Potere et al., 1999).

Membranes were stained by dissolving one tablet of BCIP-NBT Sigma fast in 10 ml distilled water and incubated until a purple-violet colour appeared in the positive control. The reaction was stopped by washing with tap water. After drying at room temperature, the membranes were observed with a low power (10x or 20x) magnification lens.

Leaves and whole flowers assayed by DTBIA were also tested by DAS-ELISA as described (Potere et al., 1999), using a polyclonal antiserum for plate coating and the alkaline phosphatase-conjugated Mab Ps29 at 1:500 dilution in conjugate buffer as second antibody. Explants from the tree infected with the CPsV isolate used for raising Mab Ps29 served as positive controls.

Colour development on blotted membranes usually appeared within 10 min from the addition of substrate and the reaction was stopped 5-10 min later.

Purple-stained areas were observed in imprints of all CPsV-infected flower explants (ovary, style and stigma) (Fig. 1). The localization and distribution of the stain was intense and homogeneous in blots from ovaries (Figs 1 and 2) and less clear-cut in those from stiles and stigmas (Fig. 1). Little or no reaction was observed in blots from leaves (Fig. 2). Control blots remained virtually unstained (Figs 1 and 2). The totality of ovaries from closed flowers were DTBIA-positive, whereas positive reactions from open flowers were fewer (ca 80%). Interestingly, no apparent differences were found between fresh and frozen ovary explants from closed flowers, regardless of the duration of the storage in the cold.

DAS-ELISA confirmed the results of immunoprinting. Strong positive reactions were obtained, especially from ovaries which showed OD_{405} readings from 35 to 65% higher than readings from leaves.

Based on the above, the conclusion can be drawn that DTBIA correlates with ELISA and is a sensitive and simple procedure for CPsV detection. Ovaries are better organs than leaves for DTBIA, in line with previous observations that reported lower concentration and irregular distribution of CPsV in infected leaves as compared with flowers (Djelouah et al., 2000; D’Onghia et al., 2001). Interestingly, in our experiments blots from a single ovary consistently detected CPsV, whereas with CTV, multiple prints from the same sample were needed (Garnsey et al., 1993).

High sensitivity, short assay time and limited cost 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 flowering 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 or -70°C for up to four months, apparently does not affect the results of the test.
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REFERENCES


Fig. 2. Evidence that only blots from CPsV-infected ovaries are consistently and intensely stained (positive reaction). A few blots from leaves show a light staining, but no staining is visible in those from healthy control tissues.


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