Further Serological Characterization of Two Tobravirus Isolates from Algeria and Libya

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Abstract
Antisera against purified particles of tobravirus isolates from Libya (LYV66-91) and Algeria (AlgRI0) were produced and used for their serological comparison with other isolates of pea early-browning tobravirus (PEBV), including one of the broad bean yellow band tobravirus (BBYBV) and with the PRN strain of tobacco rattle tobravirus (TRV:PRN). DASELISA, DAC-ELISA and dot-blot BLISA showed that the Algerian and the Libyan isolates represent two different serotypes. In DAS-BLISA, the Libyan isolate was similar to the Dutch isolate of PEBV (isolate El 16). In DAC-ELISA, the Algerian isolate reacted strongly with PEBV:BBYB and the TRV:PRN antibodies but not with those to the Libyan or the Dutch isolates of PBBV. Using dot-blot ELISA, the Libyan isolate was serologically closely related to the Dutch isolate of PBBV and distinct from the Algerian isolate of PEBV, PEBV:BBYB and TRV:PRN.

Key words: Pea early browning tobravirus, tobacco rattle tobravirus, diagnosis, ELISA

Introduction
Pea early-browning tobravirus (PEBV) was originally described from Western Europe (Bos and van der Want, 1962; Gibbs and Harrison, 1964) as a soil-(nematode-) and seed-borne virus able to cause severe disease in pea and often symptomlessly infecting other plant species, particularly legumes. Russo et al. (1984) isolated a virus from faba bean in Southern Italy, broad bean yellow band tobravirus (BBYBV), which they considered a distinct tobravirus, serologically unrelated to PEBV. Soon thereafter, Robinson and Harrison (1985) provided evidence that BBYBV is a serotype of PEBV, designated PEBV:BBYB, rather than a distinct tobravirus.

Within the framework of the ICARDA research on viruses of cool season food legumes in West Asia and North Africa (WANA) (Makkouk et al., 1988) and through a linkage project with the Research Institute for Plant Protection (IPPO-DLO) in Wageningen, two tobravirus isolates were recently recovered from faba bean (Vicia faba) in Libya and Algeria. They were studied at IPO-DLO and found to be different from each other. The Libyan isolate (LYV66-91) was closely related to the Dutch type strain of PEBV (El 16) (Bos et al., 1993), and the Algerian isolate (AlgR10) was related to, but biologically different from the Italian type strain of PEBV:BBYB serotype of PEBV (Mahir et al., 1992). LYV66-91 reacted in ISEM with antisera to El 16 but not with that to BBYB (Bos et al., 1993), whereas AlgR10 reacted with antisera to BBYB only (Mahir et al., 1992). This paper describes the production of antisera against the LYV66-91 and AlgR10 isolates as diagnostic reagents for fltrthersurveying in the region, for germplasm testing for seed infection and for resistance screening. It flther characterizes the serological relationship between these isolates and strains of PEBV and TRV, and evaluates the usefullness of the antisera produced for sensitive detection of the two isolates using different ELISA variants.

Materials and Methods

Virus isolates and maintenance: The Libyan isolate (LYV66-91) was originally obtained by mechanical inoculation from a faba bean leaf sample collected in 1991 near Tajura, Libya, and later identified as PEBV (Bos et al., 1993). The Algerian isolate (AlgR10) was derived from a sample of faba bean leaf provided by A. Oufiroukh, Institute National de Protection des Vegetaux (INPV), El-Harrach, Algiers, Algeria, identified earlier as a deviant isolate of PEBV:BBYB serotype of PEBV (Mahir et al., 1992). Both isolates were preserved in desiccated leaves and stored over calcium chloride (CaCl2) in the IPO collection and at ICARDA. The LYV66-91 isolate was propagated and maintained in Nicotiana clevelandii, and AlgR10 in faba bean or N. rustica by serial mechanical inoculation.

Virus isolates used for comparative serological tests were PEBV (E413) provided by Dr. L. Bos, The Netherlands; the PRN strain of tobacco rattle virus (TRV:PRN) and PBBV:BBYB supplied by Dr. D.J. Robinson, Dundee, 5CR, Scotland.

Virus purification: LYV66-91 and AlgR10 were purified from infected N. clevelandii and N. rustica, respectively, using a method slightly modified from those described by Cooper and Mayo (1972) and Lister and Bracker (1969). Both isolates were purified by using the same procedure. Systemically-infected leaves were harvested 15-20 days after plant inoculation and ground at room temperature in 0.1 M potassium phosphate, pH 7.4, containing 0.01 M citric acid (trisodium) and 0.1 per cent thioglycollic acid (1 g tissue 2 ml buffer). The homogenate was kept at -20°C for 2-5 days, thawed overnight at 4°C, and then heated at 50°C for 10 mm. The sap was expressed through cheesecloth, mixed with a quarter volume of a 1:1 mixture of chloroform:butanol, stirred at room temperature for 15 mm and then centrifuged at 10,000 rpm for 10 min.
procedure was that of Lommel
Direct Antigen Coating ELISA (DAC-ELISA).

Absorbance at 405 nm was measured 12 h after addition of
mixture was diluted 1:1000 in conjugate buffer.

protein A conjugated to alkaline phosphatase and the
substrate.

when the Fab fragment of TRV:PRN was used in
was measured 1 h after addition of the substrate.

was coated with 1 µg/ml of gammaglobulin and the
substrate.

were coated with I µg/ml of gammaglobulin and the
buffer.

were measured at 405 nm was measured 30 mm after addition of the substrate.

conjugate was diluted 1:2000. Absorbance at 405 nm was measured 30 mm after addition of the substrate.

Dot-blot ELISA. This test was conducted by using
chromogenic substrates nitro blue tetrazolium (NBT) and
bromo-chloro-indolyl phosphate (BCIP) (Makkouk et al.,
1993). All antisera were diluted 1:1000 and the goat anti-
rabbit alkaline phosphatase conjugate was diluted 1:2000.
Samples were applied on the nitrocellulose membrane
(NCM) using a Manifold I System (Schleicher & Schuell).
Reactions were evaluated visually 15 min after the addition of
the substrate.

Results and Discussion

Virus purification: Following the purification procedure
described above, particles of LYV66-91 and AlgR10 gave
two opalescent bands after centrifugation in sucrose density
gradients (Fig. 1). The presence of the virus in both bands
was confirmed by its reactivity with PEBV:BBYB antisera
(AlgR10) or to El 16 antisera (LYV66-91) using the dot-
blot test. The A260/280 ratio of the purified preparation of
AlgR10 was 1.16, which is close to the value for
PEBV:BBYB reported by Russo et al. (1984), The M601280
ratio for LYV66-91 was 1.13 which is close to the value for
PEBV reported by Harrison (1973). Assuming an extinction
coefficient for PEBV of 3, the yield of purified virus
was calculated to be 4.6 mg/kg of leaf for Al–10 and
7.5 mg kg⁻¹ for LYV66-91 (average of three
preparations). The UV absorption profiles (Fig. 1) for the two
isolates, also suggested that the virus bands are very well
separated from non-virus components. In addition, the ratio
of the long IL to short (S) particles in the Algerian isolate
was higher than that of the Libyan isolate. This result was
consistent in the three preparations made independently.
However, since the Algerian isolate was purified from
N. rustica and the Libyan isolate from N. clevelandii, it is not
clear whether the difference in LIS particle ratio is
dependent on host or virus strain.

Serology: Results obtained from DAS-ELISA (Table 1)
indicated that the Libyan and Algerian isolates of PEBV
hardly cross react, if at all. The Libyan isolate of PEBV
(LYV66-91) reacted strongly with antisera to the
homologous virus or to El 16 but not with that to AlgR10.
In contrast AlgR10 reacted strongly with its homologous
antisera but not with LYV66-91 or El 16 antisera. These
reactions suggest that the Libyan isolate is serologically
closely related to the Dutch PEBV isolate but the Algerian
isolate is not. Results obtained also suggested that the
antisera produced against the Algerian and Libyan isolates
are of good quality as indicated by the high specificity and
the low heterologous and healthy reactions observed.
When five tobavirus antisera were used in a comparative
study (Table 2), reactions obtained showed again that the
Libyan isolate is serologically closely related to the Dutch
isolate but not to the AlgR10, PEBV:BBYB or the type strain
of TRV (TRV:PRN). In contrast, the Algerian isolate reacted
strongly with the homologous antisera and with antisera
to PEBV:BBYB and TRV:PRN and no reaction was obtained
against antisera to the Libyan isolate or the Dutch strain
of PEBV (El 16). Dot-blot ELISA also clearly showed that the
Algerian and the Libyan isolates of PEBV strongly differ
serologically (Fig. 2).
Table 1: Detection of LYV66-91 and AlgR10 in leaf extracts and purified preparations when tested by DAS-ELISA

<table>
<thead>
<tr>
<th>Antigen extract</th>
<th>LYV66-91</th>
<th>AlgR10</th>
<th>E116</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYV66-91 N. benthamiana (leaf)</td>
<td>0.97*</td>
<td>0.11</td>
<td>0.65</td>
</tr>
<tr>
<td>LYV66-91 (purified: 1 µg/ml)</td>
<td>2.92</td>
<td>0.10</td>
<td>1.43</td>
</tr>
<tr>
<td>AlgR10 N. rustica (leaf)</td>
<td>0.14</td>
<td>0.74</td>
<td>0.10</td>
</tr>
<tr>
<td>AlgR10 (purified: 1 µg/ml)</td>
<td>0.12</td>
<td>1.99</td>
<td>0.09</td>
</tr>
<tr>
<td>Buffer (0.2 M KPO₄, pH 6)</td>
<td>0.12</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>Healthy (N. rustica)</td>
<td>0.12</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Healthy (N. benthamiana)</td>
<td>0.11</td>
<td>0.09</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*A405 nm values were recorded after 1 h of substrate incubation

Table 2: Differentiation among PEVB isolates by their reaction with six polyclonal antibodies when tested by DAC-ELISA, and DAS-ELISA using the Fab fragment in case of TRV:PRN

<table>
<thead>
<tr>
<th>Antisera used</th>
<th>DAC-ELISA</th>
<th>DAS-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>----------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Antigen extract</td>
<td>Host</td>
<td>AlgR10</td>
</tr>
<tr>
<td>E 413</td>
<td>N. rustica</td>
<td>0.00*</td>
</tr>
<tr>
<td>LYV66-91</td>
<td>N. benthamiana</td>
<td>0.02</td>
</tr>
<tr>
<td>AlgR10</td>
<td>N. rustica</td>
<td>2.75</td>
</tr>
<tr>
<td>TRV:PRN**</td>
<td>Nicotiana sp.</td>
<td>0.58</td>
</tr>
<tr>
<td>PEVB:BBYB**</td>
<td>Faba bean</td>
<td>2.75</td>
</tr>
</tbody>
</table>

*A405 values were recorded after 30 min. of substrate incubation for DAC-ELISA and 24 h for DAS-ELISA (TRV:PRN, Fab fragment), and after substrating healthy values, which ranged between 0.11-0.37 for Nicotiana spp. And 0.12-0.38 for faba bean.

**Extracted tissue for these viruses was sent to us by mail from SCRI, and possibly partially degraded before use.

Fig. 1: UV (254 nm) absorption profiles of purified virus preparations obtained from N. rustica infected with Algerian isolate (AlgR10) or N. cleyelandii infected with Libyan isolate (LYV66-91) after centrifugation on sucrose gradients at 30,000 rpm for 50 min. (Beckman SW41).

However, the Algerian isolate of PEVB was serological close to PEVB:BBYB but not identical. AlgR10 antiserum reacted strongly with the homologous antigen and with PEVB:BBYB and not as strongly with TRV:PRN. Likewise, PEVB:BBYB antiserum reacted with AlgR10 and LYV66-91 with five polyclonal antibodies when tested by Dot-blot ELISA.

Fig. 2: Reaction of purified preparations (1 µg/ml) of AlgR10 and LYV66-91 with five polyclonal antibodies when tested by Dot-blot ELISA.

antiserum reacted with AlgR10 and PEVB:BBYB antigens but not with TRV-PRN. These reactions suggest that AlgR10 has some serological relatedness to the type strain of TRV, but PEVB:BBYB does not. Some differences were also reported in their host reactions: PEVB:BBYB and AlgR10, both infect faba bean systemically but they differ appreciably in their reaction on N. rustica, N. tabacum, and Petunia hybriaa.
(Mahir et al., 1992) and the symptoms observed in faba bean were less severe than those described by Russo et al. (1984) for BBYBV. The Algerian isolate produced a very clear systemic line pattern on *N. rustica* and *N. tabacum* White Burley, whereas the Libyan isolate did not produce symptoms on these species (Bos et al., 1993; Mahir et al., 1992).

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References


