Comparison of three serological assays for the detection of potyviruses

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ABSTRACT: Sensitivity of three serological assays such as indirect F(ab')2 enzyme-linked immunosorbent assay (ELISA), dot immunobinding assay (DIBA) and electro-blot immunoassay (EBIA) in detecting cowpea aphid-borne mosaic, henbane mosaic and potato Y potyviruses was compared. DIBA was found to be most sensitive detecting up to 100 pg of virus followed by F(ab')2 ELISA which could detect up to 1 ng of virus in purified preparations diluted with sap extracts from respective non-infected tissues. EBIA could detect up to 100 ng of the virus.

Key words: Potyvirus, detection, sensitivity, serology, F(ab')2 ELISA, dot immunobinding assay (DIBA), electro-blot immunoassay (EBIA)

The genus Potyvirus in the family Potyviridae (Shukla et al., 1994) constitute the large number of plant viruses and collectively cause the greatest agricultural losses (Zettler, 1990). Members of the Potyvirus are characterized by long, flexuous, rod-shaped particles, 680-900 nm long and 11 nm wide, host cell-associated characteristic pinwheel type inclusion bodies, and aphid transmission (Hollings and Brunt, 1981; Brunt et al., 1996). In India, more than fifty potyviral diseases are reported (Mali, 1985; Varma and Ramachandran, 1996) and some of which are economically important (Varma, 1988).

Most potyviruses are transmitted through seeds or vegetative propagules collected from infected plants, necessitating sensitive detection of the virus in propagating materials as a part of sound management strategy. Sensitivity, accuracy, reproducibility, cost and adaptability to field conditions are some of the criteria to be considered while using an assay for routine detection.

Serology is the most widely used practice for detecting viruses in infected planting material and also for establishing the identity of the causal virus (Moghal and Francki, 1976; Hollings and Brunt, 1981; Dijkstra and deJager, 1998). Serological methods such as agglutination test, ELISA, dot immunobinding assay (DIBA), immunosorbent electron microscopy (ISEM), electro-blot immunoassay (EBIA) and radio immunosorbent assay (RIA) have been deployed by various workers for the detection of potyviruses (Shukla et al., 1994). However, comparative study of different serological methods in detecting potyviruses is limited. In this paper, the efficacy of three serological assays for the detection of three potyviruses has been compared.

MATERIALS AND METHODS

Virus Isolates

The virus isolates used were: cowpea aphid-borne mosaic (CABMV) (Bhat, 1993), henbane mosaic (HMV) (Bhat, 1993) and potato Y (PVY) (Bhat et al., 1997, 1999) potyviruses. PVY isolates from potato (PVY-P) and eggplant (PVY-E) were maintained and propagated through sap transmission on Nicotiana tabacum cv. White Burley. Similarly, HMV was maintained on henbane and CABMV on cowpea cv. V-513.

Virus Purification

Method of Moghal and Francki (1976) was employed for the purification of all the viruses except that the extraction buffer contained 0.2M urea and pelleting run was carried out using 5 ml of 30% (w/v) sucrose pad per centrifuge tube. Virus yields were calculated by taking spectral readings at 260 nm on the basis of E1% 0.1% = 2.5.

Antisera Production

Freshly purified preparations of CABMV, HMV,
PVY-P and PVY-E were used to make antisera in New Zealand white rabbits. Each antigen type containing 0.5 mg of virus was mixed with Freund's incomplete adjuvant (1:1) and injected into rabbits intramuscularly. A total of four injections were given at 10 days interval and bleeds were collected 15 days after the last injection.

**Serological assays**

*F*(ab\')\_2*-ELISA*

*F*(ab\')\_2*-ELISA was performed as per Barbara and Clark (1982) and Clark *et al.* (1986) except that a blocking step was included after antigen wash. Wells of microtitre plates (Corning, USA) were coated with *F*(ab\')\_2 fragments of specific antibodies at 1 μg/ml. Known amount of purified virus diluted with sap extract from non-infected tissues was used as source of antigen. Buffer and sap extracted from non-infected tissues were used as negative controls. Specific antisera and protein A alkaline phosphatase conjugate (Sigma Chemical Co.) were used at 1:1000 dilution. Readings of ELISA were read at 405 nm 1 h after substrate addition using a Dynatech ELISA reader.

**Electro-blot immunoassay (EBIA)**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970) using LKB 2001 apparatus. The stacking and separating gels contained 5% and 12% acrylamide respectively. Known amount of purified virus diluted in 10 μl of sap extracted from healthy plants was mixed with an equal volume of the Laemmli sample buffer (Laemmli, 1970) and heated for 3 min in boiling water before loading on the gel. EBIA was performed after SDS-PAGE as described by Rybicki and Von Wechmar (1982) using nitrocellulose membrane and alkaline phosphatase conjugated secondary antibodies. After electroblotting at 20 V for 2-4 h, the nitrocellulose membranes were developed as described in Bhat *et al.* (1997). Both specific antisera and alkaline phosphatase labeled antirabbit IgG (Sigma Chemical Co.) were used at 1:1000 dilutions.

**Dot immunobinding assay (DIBA)**

Dot immunobinding assay (DIBA) was performed as per Hammond and Jordon (1990). Known amount of purified virus diluted in 10μl sap extracted from the respective healthy plants was used for spotting on the nitrocellulose membrane. Similarly, 10μl sap extracted from healthy plants were also spotted on the nitrocellulose membrane to serve as negative control. After spotting, membranes were air-dried and developed as described in EBIA test (Bhat *et al.*, 1997). Specific antisera and alkaline phosphatase labeled antirabbit IgG (Sigma Chemical Co.) were used at 1:1000 dilutions.

**RESULTS AND DISCUSSION**

Our previous study (Bhat, 1993) has shown that both double antibody sandwich (DAS)-ELISA and indirect F(ab\')\_2-ELISA were equally sensitive while direct antigen coated (DAC)-ELISA was relatively less sensitive in detecting CABMV, HMV, PVY-E and PVY-P (data not shown). Since DAS-ELISA necessitates the production of IgG-enzyme conjugate for each virus, indirect F(ab\')\_2-ELISA was preferred in the present study. In F(ab\')\_2-ELISA, CABMV, HMV, PVY-E and PVY-P were detected up to 10 ng of virus in purified preparations diluted with sap extracts from respective non-infected tissues (Table 1). The detection limit for each virus was raised to 1 ng when purified virus preparations diluted in buffer alone were used as antigen (data not shown).

In DIBA, all the four viruses were detected up to 1 ng of virus in purified preparations diluted with sap extracts from respective non-infected tissues (Table 1). They were detected up to 100 pg of virus in purified virus preparations diluted in buffer alone (data not shown).

In EBIA, CABMV, HMV, PVY-E and PVY-P were detected up to 100 ng of virus in purified preparations diluted in healthy sap (Table 1).

As planting material serves as the main source of primary inoculum for many potyviruses, indexing is essential to check their further spread. Present study revealed that sensitivity of F(ab\')\_2-ELISA, EBIA and DIBA ranged from 1-100 ng for detecting potyviruses in purified preparations diluted with sap extracts from respective non-infected tissues. Sensitivity of detection was enhanced 10 times if purified virus preparations were diluted in buffer alone. The presence of plant extract may be interfering in the viral antigen binding, thus reducing the sensitivity of detection level. The presence of sap in extracts was also reported to inhibit viral antigen binding in the case of potato leaf roll and African cassava mosaic viruses (Mowat and Dawson, 1987). The DIBA was 10 times sensitive than F(ab\')\_2-ELISA in terms of total antigen detectable. Banttari and Goodwin (1985) also observed DIBA to be eight times sensitive than DAS-ELISA for the detection of potato viruses S, X and Y. Besides high sensitivity, DIBA was rapid and could be done in less than 8h. This would greatly enhance the output and thus facili-
Table 1. Detection limits of potyviruses in F(\(ab\))\(_2\)-ELISA*, DIBA* and EBIA*

<table>
<thead>
<tr>
<th>Antigen conc./dilution</th>
<th>CABMV</th>
<th>HMV</th>
<th>PVY-E</th>
<th>PVY-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>10(\mu)g</td>
<td>++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>1(\mu)g</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>100 ng</td>
<td>++</td>
<td>++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>10 ng</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>1ng</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
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<tr>
<td>100pg</td>
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<td>+</td>
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</tr>
<tr>
<td>10pg</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1pg</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

*A 405 values (after deducting the values obtained for healthy samples) were classed as > 0.91 (+++); 0.61-0.90 (+++); 0.31-0.60 (++); 0.11-0.30 (+); < 0.10 (-).

*+++*, strong; **++**, medium; +, weak; -, no reaction.

state the detection of potyvirus in large samples (Martin, 1998). In general, sensitivity of EBIA has been reported to be one-half nanogram of viral protein (Shukla et al., 1994). Low levels of detection in EBIA (100 ng) in the present study may be due to an incomplete transfer of viral proteins onto nitrocellulose membrane through electro-blotting. Though EBIA was initially reported to be useful for the detection of plant viruses (O'Donell et al., 1982; Rybicki and Von Wechmar, 1982), it appears more useful to check the purity of antigens and antibodies, detection of in situ degradation of antigens as well as for the detection of distant serological relationship (Koenig, 1988).

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REFERENCES


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