Indexing of leaf and seed samples of tomato and bell pepper for tobamoviruses

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Tomato (Lycopersicon esculentum Mill.) and bell pepper (Capsicum annuum L.) are the major commercial vegetable crops. Tomato mosaic tobamovirus (ToMV) and Tobacco mosaic tobamovirus (TMV) cause significant yield loss and reduce fruit quality in tomato and bell pepper (3,15,7). ToMV and TMV are extremely stable and easily transmitted mechanically from seeds to seedlings during transplantation (3,14).

In India, incidence of TMV in tomato (13) and pepper (12) has been reported based on indicator plant test. Cherian and Muniyappa (5) indexed tomato seed samples for TMV by indicator plant (Nicotiana glutinosa) test and ELISA. So far occurrence of ToMV and TMV in commercial seed samples of tomato and bell pepper has not been reported. Further, the bell pepper fields are not indexed for the presence of TMV. This paper distinguishes the tobamoviruses infection in leaf and seed samples. Seed samples collected from seed companies were also indexed for ToMV/TMV.

Tomato and bell pepper crops were surveyed during 1993-1996 in Karnataka. 153 locations covering an area of 156 ha of tomato and 40 locations representing 19 ha of bell pepper were surveyed. For each field of one hectare, five randomly selected rows were counted for healthy and diseased plants, and the per cent disease incidence was calculated based on the proportion of infected plants. Leaf and fruit samples were collected from infected plants. The seeds from tomato fruits were extracted after fermentation and dried under shade. Bell pepper fruits were cut longitudinally into two halves and the seeds were removed and air-dried in shade. Fifty-eight seed samples of tomato and four of bell pepper were also collected from seed companies (Karnataka State Seed Certification Agency, KSSCA) and other agencies.

Tomato seed/leaf samples (each 0.3 g) were extracted in 3 ml of 0.05 M phosphate buffer (pH 7.2) using pestle and mortar. The samples of bell pepper were extracted in 0.03 M disodium hydrogen phosphate containing sodium diethyl dithiocarbamate (3 ml) and activated charcoal (75 mg/ml). The sap was applied to indicator plants. A local lesion host, N. glutinosa, for both TMV and ToMV and N. sylvestris and N. tabacum cv. White burley, which react with systemic infection to TMV, but with necrotic local lesion (NLL) to ToMV were used.

Leaf and seed samples were also tested for ToMV/TMV by following the direct antigen coating ELISA (10). Seed/leaf samples (1/100, w/v) of tomato and bell pepper were extracted in antigen buffer. The extracts were allowed to react separately with polyclonal antisera to ToMV and TMV obtained from the Danish Government Institute of Seed Pathology for Developing Countries (DGISP).
Mosaic was recorded in tomato and bell pepper in a majority of fields surveyed. Symptoms were recorded in 114 of 153 tomato fields (75%) and in 28 out of 40 bell pepper fields (70%). Mosaic was the common symptom in different varieties of tomato. Some plants showed fern-leaf and shoe stringing, which was common in local varieties of tomato grown in and around Mysore. In a few tomato fruits blotchy ripening symptoms were observed. The percentage of diseased plants ranged from 3 to 95. Mosaic, mottle and vein banding were observed in bell pepper. Ringspots were observed on green fruits. Premature abscission of fruits was noticed in severely infected plants. Disease incidence ranged from 1 to 90%.

Of the 182 tomato leaf samples tested on *N. sylvestris* and *N. tabacum* cv. White burley, 177 samples induced NLL on both the hosts indicating infection with ToMV (Table 1). However in ELISA, the leaf samples reacted positively with both ToMV and TMV antiserum. Of 65 tomato seed samples, 11 caused NLL on *N. sylvestris* and *N. tabacum* cv. White burley indicating they had probably ToMV, but no TMV infection, while two samples produced systemic symptom indicating infection with TMV. Among the seed samples 46 (70%) reacted with both ToMV and TMV antiserum. Two samples reacted positively with only TMV antiserum.

Extracts from 47 of the 56 bell pepper leaf samples (84%) and all seed samples caused NLL on *N. glutinosa* and systemic symptoms on *N. sylvestris* and *N. tabacum* cv. White burley indicating that only TMV was present (Table 1). Similar results were obtained for ELISA, except for one seed sample which reacted for both TMV and ToMV antiserum.

This study confirmed the occurrence of ToMV in tomato and of TMV in bell pepper. The study also proved the occurrence of ToMV/TMV in commercial seed samples of tomato and bell pepper. ToMV has been reported previously in seeds collected from naturally and artificially infected tomato plants (5).

The cropping pattern in Karnataka, mainly in the southern part, allows continuous year round cultivation of both crops. Infected plant debris and seeds act as inoculum sources for ToMV/TMV. Therefore, clearing of plant debris or crop rotation with a non-host is necessary to control tobagoviruses. Tomato debris in the soil act as

<table>
<thead>
<tr>
<th>Crop source</th>
<th>Total No. of samples tested</th>
<th>No. of samples positive on</th>
<th>No. of samples positive in ELISA for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>N. glutinosa</em></td>
<td><em>N. sylvestris</em>, White burley</td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
<td>NLL</td>
<td>NLL, SI</td>
</tr>
<tr>
<td>Leaf</td>
<td>182</td>
<td>177 (97)</td>
<td>177 (97)</td>
</tr>
<tr>
<td>Seeds from infected fruits</td>
<td>7</td>
<td>7 (100)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Seed companies</td>
<td>KSSCA</td>
<td>16</td>
<td>1 (6)</td>
</tr>
<tr>
<td></td>
<td>Private Seed Companies</td>
<td>42</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Bell pepper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>56</td>
<td>47 (84)</td>
<td>0</td>
</tr>
<tr>
<td>Seeds from infected fruits</td>
<td>6</td>
<td>6 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Seed companies</td>
<td>Private Seed Companies</td>
<td>4</td>
<td>1 (25)</td>
</tr>
</tbody>
</table>

* One sample reacted positively with only TMV

Figures in parenthesis show the percentage of samples that reacted positively with ToMV or TMV

NLL: Necrotic local lesion; SI: Systemic infection; KSSCA: Karnataka State Seed Certification Agency
ToMV inoculum source for up to five months after tomato harvest in Taiwan (9). Use of healthy seeds or treatment of seeds with hydrochloric acid, trisodium phosphate or heat (4,1,9) should be considered to control tobamoviruses.

*N. glutinosa* is a sensitive indicator plant. However, as ToMV and TMV cannot be differentiated in this host the two other *Nicotiana* spp. were also used to differentiate the tomato virus isolates as ToMV or TMV, as tobacco mild green mosaic *tobamovirus* (TMGMV) and pepper mild mottle *tobamovirus* (PMMoV) do not infect tomato (8). Differentiation of ToMV and TMV based on their differential reaction has been reported (11). Bell pepper can be infected by all four tobamoviruses but TMGMV and PMMV have not been reported from India (8). Further, PMMV causes small white dot-like lesions on *N. glutinosa*, whereas ToMV produces large NLL (16), confirming that the virus occurring in bell pepper samples from Karnataka is TMV.

More seed samples were positive for ToMV or TMV when tested by ELISA than in biological tests, thus indicating high sensitivity of ELISA. Further, ELISA can detect even inactivated virus particles, as they are serologically active. As infectivity of ToMV or TMV in/on seeds often decreases or may be lost during storage, seed health testing based only on ELISA could be inappropriate and disadvantageous to the seed supplier. A biological test using sensitive test plants would give more reliable information on the presence of infective virus in the seed sample. Nevertheless, ELISA could be used initially as a quick test followed by biological tests on positive samples.

Our earlier studies revealed cross-reactivity of ToMV antiserum with TMV and TMV antiserum with ToMV (6). Monoclonal antibodies to the type strain of TMV can differentiate TMV from other tobamoviruses (2). The study has demonstrated the predominance of ToMV in tomato and TMV in bell pepper.

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**REFERENCES**


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