Local and Systemic Spread of Tobacco Mosaic Virus in Transgenic Tobacco

Biology Department, Washington University, St. Louis, Missouri 63130

Expression of a chimeric gene encoding the coat protein (CP) of tobacco mosaic virus (TMV) in transgenic tobacco plants confers resistance to infection by TMV. We investigated the spread of TMV within the inoculated leaf and throughout the plant following inoculation. Plants that expressed the CP gene [CP(+)] and those that did not [CP(-)] accumulated equivalent amounts of virus in the inoculated leaves after inoculation with TMV-RNA, but the CP(+) plants showed a delay in the development of systemic symptoms and reduced virus accumulation in the upper leaves. Tissue printing experiments demonstrated that if TMV infection became systemic, spread of virus occurred in the CP(+) plants essentially as it occurred in the CP(-) plants although at a reduced rate. Through a series of grafting experiments, we showed that stem tissue with a leaf attached taken from CP(+) plants prevented the systemic spread of virus. Stem tissue without a leaf had no effect on TMV spread. All of these findings indicate that protection against systemic spread in CP(+) plants is caused by one or more mechanisms that, in correlation with the protection against initial infection upon inoculation, result in a phenotype of resistance to TMV.

INTRODUCTION

We have previously shown that expression of the tobacco mosaic virus (TMV) coat protein (CP) gene in transgenic tobacco (Powell Abel et al., 1986; Nelson et al., 1987) and tomato plants (Nelson et al., 1988) inhibits TMV infection. Subsequent studies have shown protection of tobacco and tomato against alfalfa mosaic virus (AlMV) (Loesch Fries et al., 1987; Tumer et al., 1987; van Dun et al., 1987), potato virus X (PVX) (Hemmenway et al., 1988), and cucumber mosaic virus (Cuozzo et al., 1988) and tobacco rattle virus (van Dun and Bol, 1988) by expression of genes encoding the respective CPs in transgenic plants.

One manifestation of the CP-mediated protection by TMV CP against TMV infection occurs early in the infection cycle, and is overcome by inoculation with viral RNA (Nelson et al., 1987) and by TMV treated at pH 8.0 (Register and Beachy, 1988). These studies, carried out with a local lesion host and with isolated protoplasts, have led to the hypothesis that expression of the CP gene blocks an early step in TMV infection, perhaps the uncoating of the virus after inoculation. Although protoplasts and local lesion hosts may be used as assay systems for studies of early events in infection, such systems cannot be used to study subsequent events.

To detect effects of the CP on local and systemic spread of virus, it is necessary to study infection of a systemic host expressing the CP gene. Several groups reported a delay in development of systemic disease symptoms (Loesch Fries et al., 1987; Nelson et al., 1987; Tumer et al., 1987; van Dun et al., 1987) in transgenic plants that expressed the TMV and AlMV CP genes after inoculation with TMV or AlMV, respectively. However, in each case the finding was coincident with the observation that the inoculated leaves of the transgenic plants were less heavily infected than were the control plants. Thus, the low level of virus and the delay in systemic symptoms could be due in part to the lower level of virus in the inoculated leaves. Transgenic plants inoculated with viral RNA, which in some cases overcame protection, were not monitored for systemic virus accumulation (Loesch Fries et al., 1987; Nelson et al., 1987; van Dun et al., 1987). In classical cross-protection experiments in which a mild strain of cucumber mosaic virus was used to protect against a more severe strain, the challenge strain replicated to low levels in the inoculated leaves and failed to spread into the upper leaves (Dodds et al., 1985). Dodds et al. (1985) postulated that "the mechanism which prevents initial infection with virions of the challenge virus may also function to prevent movement of infection within the plant."

In this paper we compared the local and systemic spread of TMV in transgenic plants that express the TMV CP gene [CP(+)] with those that do not [CP(-)]. By using quantitative assays, tissue printing assays, and plant grafting experiments, we determined that plant leaves that
express the CP gene have a reduced rate of local spread within the inoculated leaves and a reduced rate of systemic spread of virus from inoculated leaves to upper parts of the plant. Furthermore, we showed that systemic spread apparently occurs primarily via the vascular tissues in CP(+) and CP(−) plant leaves.

RESULTS

Local and Systemic Spread of TMV in CP(+) and CP(−) Plant Lines

Plant lines that were CP(+) or CP(−) were inoculated with viral RNA to overcome the protection in CP(+) lines described earlier (Powell Abel et al., 1986; Nelson et al., 1987). Plants were monitored for virus replication by measuring CP accumulation in the inoculated leaf, the stem section above the inoculated leaf, and upper leaves (Figure 1). Figure 1 shows there was no TMV accumulation in any of the tissues sampled until 3 days post-inoculation (DPI) when TMV concentrations began to increase in the inoculated leaves of both the CP(+) and CP(−) plants. At 5 days DPI the accumulation of TMV in the inoculated leaves of both plant lines was the same, whereas virus accumulation in the stem and upper leaves of CP(−) plants was much higher than in the CP(+) plants. Interference in systemic spread of TMV was thus apparent in the CP(+) plants in spite of equivalent accumulation of virus in the inoculated leaves of the CP(+) and CP(−) plants. The background expression level of CP in the CP(+) plants was below the sensitivity of the enzyme-linked immunosorbent assay (ELISA) used in these experiments and did not affect assay results.

To determine whether the spread of virus within both inoculated CP(+) and CP(−) leaves was similar, TMV RNA was inoculated at specific sites using a drawn Pasteur pipette (Konte and Fritig, 1984). As shown in Table 1, virus accumulation was essentially the same in CP(+) and CP(−) leaves within the 2.5-mm radius of the inoculation site. Accumulation of virus outside of the 5-mm diameter disc area was significantly lower in the leaves of CP(+) plants compared with the CP(−) plants. This suggests that local movement of infection was reduced but not fully inhibited as a result of expression of the CP gene.

In a subsequent set of experiments, tissue print analysis was performed to identify in which tissue(s) TMV moves and to characterize further how virus spread is reduced in CP(+) plant lines. In these experiments plants were inoculated with 8 μg/mL TMV RNA, a very high level of inoculum. The CP(−) plants showed systemic disease symptoms by 3 DPI, whereas the CP(+) plants did not show symptoms until 5 DPI. Figure 2 shows that at 4 DPI the CP(−) plants showed the presence of TMV in all tissue examined, whereas the CP(+) plants showed no antigenic reaction except that due to expression of the CP gene. The midrib and lamina of the inoculated leaf and stem sections above and below the inoculated leaf of the CP(−) plants had large areas of strong antigenic reaction primarily in the vascular tissues, indicating substantial virus accumu-

![Figure 1](image-url) ELISA Assays of TMV Accumulation in CP(+) and CP(−) Plants Over Time.

(A) TMV accumulation in the inoculated leaves of CP(+) and CP(−) plants. CP(+) (□) and CP(−) (●) plants were inoculated with TMV RNA and accumulation was monitored by ELISA.

(B) TMV accumulation in the stem between the inoculated leaf and systemic leaves of CP(+) and CP(−) plants. CP(+) and CP(−) plants were inoculated with TMV RNA and accumulation was monitored by ELISA.

(C) TMV accumulation in the systemically infected leaves of CP(+) and CP(−) plants. CP(+) and CP(−) plants were inoculated with TMV RNA and accumulation was monitored by ELISA.
Accumulation and spread of tobacco mosaic virus in coat protein-expressing [CP(+)] and nonexpressing [CP(-)] plants after point inoculations with viral RNA. Inoculations were performed in four different areas on a fully expanded leaf using a flame-polished Pasteur pipette. Leaf discs and rings were taken with cork borers as described in Methods. The criterion for a successful infection was arbitrarily set at greater than 2.5 ng CP ± 0.4 ng. Variability was due to variation in ELISA plates. Harvests were taken at 5 DPI. Values for CP accumulation are means ± SD.

Table 1. Accumulation and Spread of TMV in Inoculated Leaves after Point Inoculations

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>Infections*</th>
<th>Protein</th>
<th>Infections*</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coat</td>
<td></td>
<td>Coat</td>
<td></td>
</tr>
<tr>
<td>3404 [CP(+)]</td>
<td>20/39$^b$</td>
<td>57 ± 20$^c$</td>
<td>15/39$^a$</td>
<td>6.7 ± 3.2$^a$</td>
</tr>
<tr>
<td>3404 [CP(-)]</td>
<td>20/40</td>
<td>43 ± 13</td>
<td>18/40</td>
<td>14.5 ± 8.0</td>
</tr>
<tr>
<td>316 [CP(-)]</td>
<td>24/39</td>
<td>47 ± 19</td>
<td>23/39</td>
<td>14.3 ± 9.2</td>
</tr>
</tbody>
</table>

Accumulation and spread of tobacco mosaic virus in coat protein-expressing [CP(+)] and nonexpressing [CP(-)] plants after point inoculations with viral RNA. Inoculations were performed in four different areas on a fully expanded leaf using a flame-polished Pasteur pipette. Leaf discs and rings were taken with cork borers as described in Methods. The criterion for a successful infection was arbitrarily set at greater than 2.5 ng CP ± 0.4 ng. Variability was due to variation in ELISA plates. Harvests were taken at 5 DPI. Values for CP accumulation are means ± SD.

Movement of TMV through CP(+) and CP(-) Stem Sections

Grafting experiments (see Methods) were initiated to determine whether long-distance transport of TMV was reduced in CP(+) tissue compared with CP(-) tissue as shown in the inset of Figure 3. These experiments included infection by TMV of the inoculated leaf on the CP(-) rootstock and subsequent movement through the plant. When the stem sections grafted between the CP(-) rootstock and apical section were derived from a CP(+) plant line and contained a leaf, fewer plants developed disease symptoms compared with grafted plants that contained a CP(-) stem section. Interestingly, when a CP(+) stem section lacking a leaf was used, the rate of disease development was identical to the case when a CP(-) section was used (Figure 3). Grafted plants with the CP(-) stem sections developed systemic symptoms within 8 DPI, indicating that the grafting procedures did not in and of themselves induce the resistance to systemic spread. ELISA experiments documented that TMV accumulation was much lower in grafted plants that did not develop symptoms than those that did, as shown in Table 2. TMV accumulated equally in the inoculated leaves of all the plants. By contrast, at 10 DPI there was little accumulation of TMV in upper leaves of grafted plants that contained leaf-bearing CP(+) sections. The result was the same when the CP(+) line was the local lesion host, Nicotiana tabacum cv Xanthi nc, (plant line 757; Nelson et al., 1987). There was no accumulation of TMV in the upper leaves as measured by ELISA at 15 DPI (data not shown). Stem sections (bearing leaves) from transgenic plants that expressed genes other than the CP gene provided no protection against systemic spread of the infection, as assayed immunologically by ELISA (data not shown). Grafted plants that had a stem section that expressed genes encoding the TMV movement protein (TMV-30K) (Deom et al., 1987) or chloramphenicol acetyltransferase (CAT) (Chen et al., 1988) genes accumulated an average of 7.66 µg and 13.10 µg of TMV per gram, fresh weight, respectively, after 15 DPI, whereas plants with CP(+) stem sections accumulated an average of 0.86 µg of TMV per gram, fresh weight, or less during the same time period.

When CP(+) stem sections lacked a leaf, there was no protection against systemic spread of TMV. To investigate whether the whole leaf was required to affect protection, an experiment was carried out in which one-fourth, one-half, three-fourths, or the full leaves on the CP(+) stem sections were excised. In each case the midrib remained intact. Figure 4 shows that the degree of resistance to systemic spread was proportional to the leaf area that remained. These observations were corroborated with ELISA data (Table 3), which showed that the accumulation of TMV was about the same (not more than 2 times different) in leaf tissue on the grafted stem section, regardless of the amount of tissue that remained. The amount of virus that accumulated in the upper leaves was much less in those plants that contained three-fourths or a full leaf on the grafted stem segment when compared with plants with smaller leaf areas. This result suggests that leaves on the grafted section can become infected, but larger...
leaves are less likely to transmit virus out of the tissue than are smaller leaf segments. Overall, TMV accumulation was greater in this experiment completed during the summer than in previous ones because the higher summer temperature caused a greater accumulation of the virus (Nejidat and Beachy, 1989).

To determine whether the effect in grafted plants was specific to the spread of TMV, leaves on the rootstock segment of the grafted plants were inoculated with potato virus X. Systemic spread of PVX in all grafted plants was independent of the genotype of the stem section used because there was no difference in the amount of PVX that accumulated in the systemic leaves of all plants. By 14 DPI, plants that contained a stem section that expressed the CP(+) TMV-30K, or CAT gene had accumulated no less than 2.30 µg, 2.06 µg, or 1.60 µg of PVX per gram, fresh weight, respectively, in the upper systemic leaves.

**DISCUSSION**

Plants that express the TMV-CP gene are protected against infection by inhibiting a step at or before uncoating of the TMV particle (Register and Beachy, 1988, 1989). This inhibition can be overcome by inoculating plants with TMV RNA (Nelson et al., 1987). We have determined that once infection has been established in the CP(+) plants there is a low but significant reduction in the rate of cell-to-cell spread of the infection near the site of infection (Table 1). The molecular basis for the decrease remains to be determined. In contrast to the inoculated leaves, virus accumulation in leaves above those that were inoculated was dramatically lower in CP(+) plants compared with CP(-) plants (Figure 1). This result has been corroborated through several similar experiments that differed in the number of plants tested and the duration of the experiment (L.A. Wisniewski, P.A. Powell, R.S. Nelson, and R.N. Beachy, unpublished data). The reduction in virus accumulation in the systemic leaves is independent of the resistance that blocks initial infection because it is not overcome by inoculation with TMV RNA.

**Table 2. ELISA Assays of TMV Accumulation in Systemic Leaves of Plants with Grafted Intersections**

<table>
<thead>
<tr>
<th>Genotype of Grafted Stem Section</th>
<th>Leaf Position</th>
<th>Leaf Position</th>
<th>Leaf Position</th>
<th>Leaf Position</th>
<th>Leaf Position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>[TMV]/g, fresh weight (µg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP(+) with leaf</td>
<td>0</td>
<td>0.00</td>
<td>0.07</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.05</td>
<td>0.06</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>CP(+) without leaf</td>
<td>0</td>
<td>0.00</td>
<td>0.12</td>
<td>0.14</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.70</td>
<td>8.26</td>
<td>6.14</td>
<td></td>
</tr>
<tr>
<td>CP(-) with leaf</td>
<td>0</td>
<td>0.00</td>
<td>0.06</td>
<td>0.02</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3.81</td>
<td>5.73</td>
<td>1.73</td>
<td>7.50</td>
</tr>
<tr>
<td>CP(-) without leaf</td>
<td>0</td>
<td>0.00</td>
<td>0.06</td>
<td>0.67</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.05</td>
<td>7.59</td>
<td>12.87</td>
<td></td>
</tr>
</tbody>
</table>

TMV accumulation in grafted plants in which the grafted stem section either expressed [CP(+) or did not express [CP(-)] the TMV CP gene and either contained or did not contain a leaf as measured by ELISA.

*1, inoculated leaf; 2, leaf on grafted stem section; 3, lower leaf on upper section; 4, middle leaf on upper section; 5, upper leaf on upper section.

*CP(+)*, plant line 3404.

*CP(-)*, Xanthi tobacco plants.
Figure 4. Percent TMV Infection in Plants Containing CP(+) Intersections with Different Leaf Areas.

Percentage of grafted plants with CP(+) stem sections that contained no leaf, one-fourth, one-half, three-fourths, or a whole leaf that developed systemic symptom development over time after inoculation with 0.35 μg/mL TMV.

Tissue print analysis of virus accumulation in stem sections of CP(+) and CP(−) plants infected with TMV documented the reduced rate of spread of TMV from the inoculated leaves in CP(+) plants (Figure 2). In control plants, virus accumulated in stem tissue above and below the inoculated leaf, consistent with the suggestion of phloem-associated virus movement (Esau, 1956). Furthermore, there was a large accumulation of TMV in vascular tissues near the apex of the plant. The parenchyma tissue surrounding the vascular tissue in the stem was subsequently invaded, followed by infection of the apex and more extensive spread into the parenchyma tissue. The same pattern of virus spread was observed in CP(+) and CP(−) plant lines but the invasion was delayed by approximately 2 days in CP(+) plant lines. On the basis of these analyses, it appears that TMV is not spreading by an alternate pathway in CP(+) compared with CP(−) plants. Rather, there appears to be reduced capacity to complete a normal function of infection such as loading into the vascular tissue. This may be a result of the high level of expression of the chimeric CP gene in these tissues because the cauliflower mosaic virus 35S promoter is known to be highly expressed in phloem and phloem-associated tissues (Jefferson et al., 1987).

Although the pathway of virus spread can be traced via the tissue prints, it is unknown in what form TMV is transported. The antibody reactions indicated that the CP is, in some way, involved in the process. Oxfelt (1975) found that TMV-CP, but not necessarily virion assembly, is required for efficient long-distance spread of TMV. Similar conclusions were drawn by Takamatsu et al. (1987), who used infectious TMV transcripts derived from a mutant cDNA clone. This is consistent with the studies of Dorokhov et al. (1983, 1984), who concluded that long-distance spread of TMV is via an RNA-associated virus particle composed of several proteins including CP. However, this particle was found only in infected leaves but not in the phloem of infected plants. Esau and Cronshaw (1967) reported finding TMV particles in phloem cells; however, these studies were carried out with plants that were chronically infected, and it was not determined whether the virus was synthesized in the phloem elements before they reached maturity or moved into these cells from surrounding tissue. Whatever the transport form, it is likely that the presence of CP in the phloem and associated cells interferes with its long-distance spread and systemic disease development.

The grafting experiments described here further documented that CP expression reduces the amount of virus accumulation in systemic leaves. Figure 3 shows that only those plants containing a stem section from a CP(+) expressing plant delayed movement of the virus from the inoculated CP(−) rootstock to the CP(−) section above the graft union. Furthermore, the amount of leaf tissue present on the stem section affected the amount of protection (Figure 4, Table 3). This observation is difficult to explain at present and will require further study to understand.

<table>
<thead>
<tr>
<th>Amount of Leaf on Grafted Stem Section</th>
<th>Leaf Position</th>
<th>DPI 1</th>
<th>DPI 2</th>
<th>DPI 3</th>
<th>DPI 4</th>
<th>DPI 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Full</td>
<td>45.65</td>
<td>0.16</td>
<td>0.28</td>
<td>0.21</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Full Leal</td>
<td>0.98</td>
<td>0.14</td>
<td>0.35</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/2 Leaf</td>
<td>0.29</td>
<td>0.23</td>
<td>0.41</td>
<td>2.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/4 Leaf</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No Leaf</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. ELISA Assays of TMV Accumulation in the Systemic Leaves of Grafted Plants That Contain Intersections with Different Leaf Areas: Effect of Leaf Tissue on the Grafted Stem Section on Systemic Spread of TMV as Measured by ELISA

a Grafted stem sections included various parts of a leaf from no leaf to a full leaf.

Leaf positions are as described in Table 2.
better the role of the leaf tissue in limiting virus spread. The leaf may simply act as a sink for the virus, which, after entering the leaf, cannot readily establish an infection, thereby reducing the likelihood of replication and achieving sufficient titer to enable virus spread. Alternatively, the leaf may translocate a component, perhaps CP, into the stem tissue, which itself inhibits the transport of the virus.

In conclusion, we provide evidence that expression of a TMV-CP gene in transgenic plants reduces the rate of virus accumulation in systemic leaves when the initial block to infection is overcome. Taken together with the earlier reports that TMV-CP interfered with an early stage in infection (Register and Beachy, 1988, 1989), these results lend support to a hypothesis that the CP-mediated resistance in CP(+) plants results from several sites of this interference or that several different but additive mechanisms are present.

METHODS

Transgenic Plant Lines and Virus Inoculum

TMV strains U1 and PV230 were isolated and purified as described by Nelson et al. (1987). Both strains produce chlorotic lesions on the inoculated leaves of Nicotiana tabacum cv Xanthi, with those from PV230 being more easily observed. U1 forms a light-green/dark-green mosaic pattern, whereas PV230 forms a white or yellow/green mosaic on systemically infected leaves. RNA was purified from the U1 strain as described by Bruening et al. (1976). Transgenic tobacco (N. tabacum cv Xanthi, a systemic host) lines expressing a chimeric gene encoding the U1 CP were lines 3646 and 3404 (Powell Abel et al., 1986). The 3646 line was the R2 generation and homozygous for CP expression, whereas 3404 was the R1 generation and heterozygous for CP expression. Nopaline accumulation was assayed as described by Rogers et al. (1986). Control plants were either line 316, which contained the vector used for transformation but without the CP gene (Powell Abel et al., 1986; Nelson et al., 1987), or the nontransformed systemic host.

Systemic Spread of Virus in CP(+) and CP(−) Plants Infected with TMV

Seedlings were germinated and grown in the greenhouse (Nelson et al., 1987) until four leaves were fully expanded (average height, 20 cm). Plants were then moved to a growth chamber with a day length of 12 hr and average day and night temperatures of 25°C and 18°C, respectively. The third fully expanded leaf was mechanically inoculated with 8 µg/mL TMV RNA. Inoculation was accomplished as described by Powell Abel et al. (1986) using TMV RNA in distilled, diethylpyrocarbonate-treated, autoclaved water. The leaves were rinsed with water 30 min after inoculation. Two plants from each treatment were destructively sampled daily beginning at 2 DPI. Leaf and stem tissues were frozen in liquid N₂ and stored at −70°C. The tissue was later homogenized in PBS (10 mM NaPO₄, 150 mM NaCl, pH 7.2) containing 0.05% Tween 20 and 0.2% ovalbumin and analyzed for CP accumulation by ELISA (double-sandwich procedure, Clark and Barr-Joseph, 1984). Duplicates of each sample, standards, and blanks were included on each microtiter plate. Readings of absorbance were taken at 30 min, 1 hr, and 2.5 hr on an ELISA reader (Biotek, Inc.). Data for each treatment/day combination were averaged and compared with other treatment/day combinations.

Point Inoculation with TMV RNA

In some experiments plants were inoculated at four sites on a leaf using a drawn-out, flame-polished Pasteur pipette (Hoeker and Bensen, 1960; Konte and Fritig, 1984). The leaf was first dusted with carborundum, then inoculated by dipping the end of the pipette into 10 µg/mL TMV RNA, touching it to the leaf surface, and turning the pipette slightly. Plants were then placed in a growth chamber with a day length of 14 hr and average day and night temperatures of 23°C and 19°C, respectively. Light intensity was approximately 250 µE m⁻² sec⁻¹. An inner disc (6-mm diameter) and outer ring (20-mm diameter) of leaf tissue were sampled at 5 DPI, frozen in liquid N₂, and later analyzed for CP accumulation by ELISA.

Tissue Print Experiments

Tobacco plants expressing the CP gene (line 3646) and nontransgenic plants (cv Xanthi) were grown in the greenhouse until the youngest primary leaf was fully expanded (approximately 10 cm). Ten plants of each line were mechanically inoculated (as described above) with 8 µg/mL TMV RNA. Plants were kept in the greenhouse throughout the experiment. Systemic movement of TMV was determined by a modified tissue print method developed by Cassab and Varner (1987). Nitrocellulose sheets were boiled in distilled water for 3 min, soaked in 0.2 M CaCl₂ for 30 min, and dried on paper towels. Fresh tissue was hand-cut with a razor blade into 1-mm sections. Each section was washed for 3 sec in distilled water and blotted on absorbent tissue. The section was placed on the pretreated nitrocellulose and blotted with hand pressure for 30 sec. All blots were stored at 4°C until the experiment was completed and then treated for antigen detection using protocols developed for immunoblots (Towbin et al., 1979). Antigen was detected with anti-TMV primary antibody and secondary antibody conjugated with alkaline phosphatase (Promega Biotec).

Plant Grafting Experiments

Seeds from the CP(+) line 3404 and control plants were grown in the greenhouse as described above; 5-week-old plants were used in grafting experiments. Grafted plants were produced by joining a stem section from either CP(+) or CP(−) plants to a rootstock and an apical section of a CP(−) plant (inset, Figure 3). Half of the grafted plant sections contained a leaf and half did not. Ten plants were produced of each treatment type for a total of 40 plants. Grafted plants were placed in a growth chamber with low light intensity and high humidity for 2 weeks and then moved to the greenhouse. Plants were allowed to acclimate in the greenhouse for 4 days before inoculation. A fully expanded leaf on the root-
stock was inoculated with 0.35 μg/mL TMV as described above. Symptoms of disease development were scored on the leaves above the graft union until 15 DPI. ELISAs were also performed on tissue by taking two leaf punches (1-cm diameter) from each of the following leaves at 6 and 10 DPI: inoculated, grafted section, lower systemic, middle systemic, and upper systemic leaves.

Grafting experiments were also done using stem sections of plants that expressed different genes. The tobacco lines used expressed genes encoding either the TMV-CP (lines 3404 and 3402, Powell Abel et al., 1986; Xanthi nc tobacco line 757, Nelson et al., 1987), the TMV movement protein (30-kD protein, line 277, Deom et al., 1987), chloramphenicol acetyltransferase (CAT, line 6-37A, Chen et al., 1988), or no foreign genes. All heterologous genes were under the control of the 35S promoter from cauliflower mosaic virus. Because not all lines were not homozygous for the gene of interest, nopaline assays and protein gel blots were used to determine which plants expressed or did not express the gene in question. The nonexpressing plants from transgenic lines and nontransgenic tobacco plants were used as controls. All grafted stem sections contained a leaf in these experiments. Symptoms of disease development were scored on the leaves above the grafted stem section at 6 DPI through 18 DPI. ELISAs were also performed on extracts from 6 DPI, 10 DPI, and 15 DPI.

In other experiments parts of the leaf on the grafted stem section were excised so that one-fourth, one-half, three-fourths, or the full leaf remained, or the whole leaf was removed. Ten plants were produced of each treatment type for a total of 50 plants. Plants were inoculated as described above, and symptom development in the leaves above the grafted stem section was observed until 10 DPI. TMV accumulation was measured by ELISA at 6 DPI, 8 DPI, and 10 DPI.

Grafted plants were also inoculated with 0.35 μg/mL PVX, and PVX accumulation was measured on extracts at 6 DPI, 8 DPI, 10 DPI, 12 DPI, 15 DPI, and 18 DPI by ELISAs. Antibody to PVX was obtained from Dr. W. Kaniewski (Monsanto Company, St. Louis, MO).

ACKNOWLEDGMENTS

We thank Mike Dyer and Sally Leitner for maintaining the plants for these experiments and Dr. James Register for helping to perform ELISA and point inoculation experiments. This research was supported by a grant from the Monsanto Company.

Received January 18, 1990; revised April 2, 1990.

REFERENCES


genic tomato plants expressing coat protein from tobacco mosaic virus. Biotechnology 6, 403–409.


Local and systemic spread of tobacco mosaic virus in transgenic tobacco.
L A Wisniewski, P A Powell, R S Nelson and R N Beachy
Plant Cell 1990;2:559-567
DOI 10.1105/tpc.2.6.559

This information is current as of October 28, 2017