Initial infection of roots and leaves reveals different resistance phenotypes associated with coat protein gene-mediated resistance to Potato mop-top virus

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Resistance to the pomovirus Potato mop-top virus (PMTV) was studied in potato (Solanum tuberosum cv. Saturna) and Nicotiana benthamiana transformed with the coat protein (CP) gene of PMTV. The incidence of PMTV infections was reduced in tubers of the CP-transgenic potatoes grown in the field in soil infested with the viruliferous vector, Spongospora subterranea. However, in those tubers that were infected, all three virus RNAs were detected and virus titres were high. The CP-transgenic N. benthamiana plants were inoculated with PMTV using two methods. Following mechanical inoculation of leaves, no RNA 3 (the CP-encoding RNA homologous to the transgene) was detected in leaves, but in some plants low amounts of RNA 3 were detected in roots; RNA 2 was readily detected in leaves and roots of several plants. Inoculation of roots using viruliferous S. subterranea resulted in infection of roots in all plants and the three PMTV RNAs were detected. However, no systemic movement of PMTV from roots to the above-ground parts was observed, indicating a novel expression of resistance. These data indicate that the CP gene-mediated resistance to PMTV specifically restricts accumulation of PMTV RNA 3, and is more effective in leaves than roots. Furthermore, expression of resistance is different depending on whether leaves or roots are inoculated. Data do not exclude the possibility that both a protein-mediated and an RNA-mediated resistance mechanism are involved.

Introduction

Potato mop-top virus (PMTV, genus Pomovirus) causes symptoms known as ‘spraing’ consisting of brown arcs and circles in the flesh of potato tubers, sometimes visible also on the tuber surface (Harrison & Jones, 1971; Sandgren, 1995; Mølgaard & Nielsen, 1996; Jeffries, 1998). Depending on the potato cultivar, other symptoms such as stunting of shoots and yellowing of leaves may develop (Harrison, 1974). Infection with the virus causes some quantitative yield loss, but the qualitative losses are more important. There are differences in susceptibility among cultivars. For example, cv. Saturna, widely used in the Scandinavian potato-processing industry, is very sensitive (Sandgren, 1995; Nielsen & Mølgaard, 1997). Cultivars Appell and Desireé, on the other hand, are more resistant to PMTV infection. Cultivar Bintje is tolerant and infected tubers are symptomless (Kurppa, 1990). PMTV is known to occur in Northern Europe, Canada, China, Japan and the Andean region of South America (Jeffries, 1998).

PMTV is transmitted by Spongospora subterranea f. sp. subterranea (Jones & Harrison, 1969), an obligate parasite belonging to the family Plasmodiophoraceae, kingdom Protista (Margulis & Schwartz, 2000). S. subterranea itself is also a pathogen and causes powdery scab on potato tubers (Harrison et al., 1997). The symptoms on mature tubers are tiny, hollow lesions filled with brown powder consisting of resting spores. Germinating resting spores release zoospores that attach to and penetrate the roots of the host plant. After penetration, the zoospore becomes a multinucleate plasmodium divided into segments to form zoosporangia. Each of these contains four to eight uninucleate (secondary) zoospores that can re-infect roots (Hims & Preece, 1975). Infection with S. subterranea is efficient under cool (14–20 °C) weather conditions and high humidity (Teakle, 1988) which, consequently, are also the conditions most favourable for PMTV infections. The zoospores can acquire virions of PMTV when S. subterranea develops in virus-infected host cells. The details of the
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Fig. 1. Genetic organization of PMTV, including a tRNA-like structure and an anticodon for valine (Val) at the 3' end of the three RNAs (for details, see Savenkov et al., 1999; Savenkov, 2002). The clones (probes) used for detection of the viral RNAs in plants in this study are indicated.

mechanism of acquisition are still uncertain. Inoculation occurs soon after the vector has penetrated the host cell. PMTV is located inside the zoospores emerging from vegetative sporangia. It also resides in resting spores capable of surviving in soil for more than 15 years (Jones & Harrison, 1969, 1972; Campbell, 1996), which makes it possible for PMTV to remain infective in field soil for a long period.

The particles of PMTV are tubular and rigid, 18–20 nm in diameter and 100–150 nm or 250–300 nm in length (Harrison, 1974). The genome of PMTV consists of three single-stranded positive-sense RNA molecules (Fig. 1). RNA 1 (6–1 kb) encodes the viral replicase (Savenkov et al., 1999). RNA 2 contains four open reading frames (ORFs) (Scott et al., 1994), of which three encode putative proteins similar to the triple gene-block (TGB) proteins involved in cell-to-cell movement of other viruses (Lauber et al., 1998). Expression and functions of the fourth ORF in RNA 2 are not known (Scott et al., 1994). RNA 3 encodes two proteins, a 20 kDa coat protein (CP) and a 67 kDa read-through (RT) protein (Kashiwazaki et al., 1995; Sandgren et al., 2001). The RT domain may be involved in vector transmission (Tamada et al., 1996; Reavy et al., 1998).

Since sufficiently effective natural resistance to PMTV is not yet available in potato cultivars, pathogen-derived resistance (PDR) has been tested as an alternative. In some cases PDR can be RNA-mediated, which does not require expression of the protein. Instead, the transgene mRNA may induce the host RNA silencing system to specifically target and degrade the mRNA and the homologous viral RNA (reviewed in Waterhouse et al., 2001). PDR can also be protein-mediated, which is best described for the CP-mediated PDR to Tobacco mosaic virus (TMV, genus Tobamovirus). It may operate via prevention of disassembly of virus particles at entry to the cells (Beachy, 1999). In Nicotiana benthamiana transformed with the CP gene from a Scottish PMTV, isolate T, the expression level of PMTV CP varied in different transgenic lines, but all lines were immune or highly resistant to PMTV (Reavy et al., 1995). In some transgenic lines, virus replication could be detected in a small proportion of plants but no symptoms developed (Barker et al., 1998a). Strong resistance was only expressed if the CP transgene was translatable, and all lines were resistant irrespective of the steady-state levels of transgene RNA transcript or protein (Barker et al., 1998a). When these transgenic N. benthamiana plants were inoculated with PMTV, RNA 1 and RNA 2 but not RNA 3 could be detected in both inoculated and systemically infected leaves, while all three RNAs could be detected in non-transgenic plants (McGeachy & Barker, 2000). This indicates that RNA 1 and 2 are capable of replication and long-distance movement in the absence of RNA 3 and, while transgene CP or RNA inhibits accumulation of RNA 3, it does not inhibit the replication of RNA 1 and 2.

The same transgene construct as described for N. benthamiana above has also been used to transform potato cv. Saturna (Barker et al., 1998b). Six transgenic lines (designated as AM lines) were tested for resistance to PMTV in a greenhouse by growing them in pots of soil from a Scottish field known to be infested with viruliferous S. subterranea. The steady-state
levels of the transgene mRNA varied between lines but all lines were resistant to PMTV (Barker et al., 1998b).

The aim of this study was to test the CP-transgenic lines of Saturna for resistance to PMTV under field conditions. In natural conditions PMTV infects roots and tubers, and infection rarely spreads from them to the above-ground parts of potato plants (Kurppa, 1989). Therefore, expression of resistance to PMTV was compared between roots and leaves (a comparison not made previously) using the CP-transgenic resistance to PMTV was compared between roots and leaves of potato plants (Kurppa, 1989). Therefore, expression of infection rarely spreads from them to the above-ground parts

Field experiments with potatoes. The field trial with the transgenic Saturna lines was carried out in a field known to contain viruliferous S. subterranea in Brunskog, Halland, Southern Sweden (permit 22-1387/99, National Board of Agriculture, Sweden). The seed tubers were pre-sprouted for 2 weeks in the glasshouse and planted on 5 May 1999. The test field was divided into four blocks. All lines were randomly planted within each block amongst many other potato cultivars not described further in this report. The first weeks of the summer (June) were colder and wetter than normal, conditions known to promote S. subterranea infections, while later the growing season was warm and dry and the crop was irrigated. Weeds were controlled mechanically before planting. Hilling was done during the cultivation. Fertilizing was done with NPK (8:7:16) (1000 kg/ha) and calcium nitrate (250 kg/ha), and twice on foliage with boron (60 g/ha) and manganese (500 g/ha). Late blight (causal agent Phytophthora infestans) was controlled with the fungicide fluzinam (‘Shirlan’, Zeneca Agro Scandinavia; seven times 0.21/ha and four times 0.41/ha). Insect infestations were prevented by treatment with the pyrethroid ester insecticide esfenvalerate (‘Sumialpha’, Du Pont de Nemours; twice 0.21/ha). Potatoes were manually harvested on 27 and 28 September. All tubers of a transgenic line in the same block were stored in the same net bag at 4°C for 10 weeks. They were then maintained at room temperature (ca. 18°C) for 2 weeks prior to visual inspection for symptoms on the surface and in the tuber flesh, determination of virus titres by ELISA, and analysis for the presence of viral RNAs by Northern and dot-blot hybridization (see below).

Experiments with N. benthamiana. N. benthamiana plants were inoculated with PMTV by two different methods: by mechanical inoculation to leaves or by growing the plants in soil infested with viruliferous S. subterranea. In both types of experiment, roots and leaves were tested for virus infection.

Mechanical inoculation was carried out with sap extracted from PMTV-infected leaves of 5-week-old N. benthamiana. The leaves were ground in distilled water and the sap rubbed onto two full-grown leaves dusted with Carborundum. Plants were grown in a greenhouse at 9°C/15°C (night/day) with supplementary lighting to provide a 16 h photoperiod. Plants were fertilized weekly (N:P:K:S:Ca:Mg = 5:1:4:0:4:0:3:0:4).

Inoculation of N. benthamiana with S. subterranea was done using soil obtained from the surface of tubers harvested from the field experiment, because soil collected in this manner was anticipated to be enriched with resting spores of S. subterranea. The soil was air-dried at 7°C, which is known to enhance release of zoospores from the resting spores after moistening. A pot (2.5 litre) was filled with peat-based compost, a hole (5 cm deep) made in the soil was filled with the S. subterranea-infested soil, and a small seedling of N. benthamiana planted into the infested soil. Three seedlings were planted in each pot. Plants were grown as above.

Virus detection by ELISA. The eight largest tubers from each line and block (32 tubers per line in total) were selected for ELISA to detect PMTV. A sample from the centre of the tuber was excised with a knife, placed in a small polyvinyl bag, and 2 ml of extraction buffer (Rowhani et al., 1998) was added. A homogenate was obtained by crushing the potato piece in the buffer with a hammer. The uppermost fully-expanded leaves of N. benthamiana plants (if not stated otherwise) were collected in polyvinyl bags, weighed, extraction buffer added at 3 ml/g leaf material, and the leaves ground in the bags.

From the homogenate, 100 µl was transferred to one well of a microtitre plate, pre-coated with PMTV IgG (raised in rabbit), followed by incubation at +4°C overnight. The rest of the procedure was as described elsewhere (Copeland, 1998). The substrate, p-nitrophenyl phosphate (Sigma), was added and absorbance measured at a wavelength of 405 nm using a microplate reader (Benchmark, Bio-Rad) after 2 h of incubation at room temperature. Samples with an A_{405} value greater than twice the negative control were deemed to be PMTV-infected.

Tissue print-immunoblot. A few infected (ELISA-positive) tubers with symptoms were studied for the distribution of PMTV by tissue-printing. The tuber was transected and the cut surface pressed onto a nitrocellulose membrane (0.45 µm; Trans-Blot, Bio-Rad). The membrane was incubated for 1 h at room temperature in TBS (20 mM Tris–HCl, 500 mM NaCl, pH 7.5) containing 2% nonfat milk powder and 2% Triton X-100. Then, virus detection was carried out using the PMTV IgG (diluted 1:1000), anti-rabbit IgG conjugated with alkaline phosphatase (Sigma) (diluted 1:2000) and substrate (NBT + BCIP) as described elsewhere (Abad & Moyer, 1992). The membrane was rinsed in distilled water to stop the reaction.

Extraction of viral RNA. Two infected (if available) and two non-infected tubers from each line were selected, based on ELISA data, to be tested for PMTV RNAs by dot-blot analysis. The sample for RNA extraction was taken at the same time and from the same part of the tuber as the sample for ELISA. The sample (100 mg) was crushed with a hammer in 1.5 ml of CTAB-buffer (Chang et al., 1993) to obtain a homogenate which was incubated at 65°C for 10 min. The same volume of chloroform–isoamyl alcohol (24:1) was added, followed by centrifugation at 8400 g for 10 min. The water phase was transferred to a new tube, 1/4 vol. of LiCl (10 M) was added, followed by incubation at 4°C overnight. The sample was centrifuged for 12 min at 13200 g, the pellet resuspended in 150 µl of SSE (Sambrook et al., 1989) and extracted with 1 vol. of chloroform–isoamyl alcohol, vortexed, and centrifuged for 5 min at 3000 g. The aqueous phase was transferred to a new tube and RNA precipitated by addition of 2 vols of 95% ethanol and incubation at −20°C for 2 h. RNA was collected by centrifugation at 13200 g for 10 min at 15°C and the pellet was washed with 70% ethanol. The pellet was finally resuspended in 50 µl of RNase-free water. The integrity and concentration of the RNA was analysed on a 1% agarose gel. Total RNA
hybridization with probe labelled with digoxigenin. Overnight. RNA was cross-linked to membrane by UV-light before cross-linking was carried out according to the manufacturer’s instructions. Total RNA extracted from potato tubers, or roots or leaves of \textit{N. benthamiana}, was dotted onto a nitrocellulose membrane (Bio-Rad) and cross-linked with UV-light. Hybridization with the probes specific to PMTV RNAs and detection of signals using anti-digoxigenin-AP, CSPD and Lumi-Film (Boehringer Mannheim) were carried out according to the manufacturer’s instructions and as described elsewhere (Pallás et al., 1998).

The membrane used for dot-blot analysis of the PMTV RNAs was hybridized with radioactively labelled probes for \textit{Tobacco rattle virus} (TRV) and \textit{ribosomal} RNA. A plasmid containing the c\textit{DNA} of RNA 1 of TRV and another plasmid containing a \textit{ribosomal} RNA gene isolated from \textit{tobacco} (\textit{N. tabacum}) were labelled with \textit{[^32P]}d\textit{CTP} using the \textit{Radprime II} random prime labelling system (Amersham Pharmacia) and hybridization was carried out according to the manufacturer’s instructions. After hybridization the membrane was washed at 65 °C, 2 × 15 min in 5 × \textit{SSC}+0.5 % SDS and 2 × 15 min in 1 × \textit{SSC}+0.5 % SDS, and then exposed to a PhosphorImager screen overnight.

**Northern blot hybridization.** Probe p419 was used for detection of PMTV RNA2. Probe p66 was used for detection of the transgene transcript in tubers of \textit{cv. Saturna}, which has not been tested previously. The RNA extracts were re-precipitated and resuspended in the appropriate buffer for Northern analysis, as described by Sambrook et al. (1989). The RNAs were separated by electrophoresis on an agarose gel (1:5 %) and blotted to a Hybond-\textit{N”} membrane (Amersham Pharmacia) overnight. RNA was cross-linked to membrane by UV-light before hybridization with probe labelled with digoxigenin.

### Table 1. Detection of PMTV by ELISA in tubers of CP-transgenic (AM) Saturna lines and two non-transformed Saturna lines (AMC and Saturna) obtained from different tissue culture collections and grown from seed tubers in the field

<table>
<thead>
<tr>
<th>Line no.</th>
<th>No. of PMTV-positive tubers</th>
<th>ELISA reading ($A_{405}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PTMV-negative tubers</td>
<td>PTMV-positive tubers</td>
</tr>
<tr>
<td>AM4</td>
<td>3/32</td>
<td>0.07–0.21</td>
</tr>
<tr>
<td>AM5</td>
<td>2/32</td>
<td>0.06–0.15</td>
</tr>
<tr>
<td>AM6</td>
<td>1/32</td>
<td>0.07–0.12</td>
</tr>
<tr>
<td>AM9</td>
<td>2/32</td>
<td>0.07–0.10</td>
</tr>
<tr>
<td>AM10</td>
<td>1/32</td>
<td>0.07–0.17</td>
</tr>
<tr>
<td>AM12</td>
<td>0/32</td>
<td>0.06–0.11</td>
</tr>
<tr>
<td>AMC</td>
<td>6/32</td>
<td>0.07–0.09</td>
</tr>
<tr>
<td>Saturna</td>
<td>7/32</td>
<td>0.06–0.13</td>
</tr>
</tbody>
</table>

Tubers with $A_{405}$ values two times higher than the negative controls were deemed to be virus-positive. A total of 32 tubers per line was tested.
Resistance to *Potato mop-top virus*

not detected, which was similar for transgenic and non-transgenic tubers (data not shown). To compare the amounts of RNA 2 and RNA 3 more precisely, dot-blot analysis was carried out using dilution series of the total RNA extracted from the PMTV-infected tubers. The results showed a positive correlation between the concentrations of RNA 2 and RNA 3. The results also showed that infected transgenic tubers contained no less viral RNA 2 and RNA 3 than the infected non-transgenic tubers (data not shown).

Expression of the transgene mRNA in tubers was tested by Northern blot hybridization and a transcript with the expected electrophoretic mobility was detected in some infected and non-infected tubers of the same transgenic line (data not shown). There was no correlation between detectable amounts of transgene transcript and PMTV infection. No signal for an RNA corresponding to the electrophoretic mobility of RNA 3 was detected in tubers that were negative in ELISA and RNA dot-blot (data not shown).

The test field was known to contain nematode vectors (*Trichodorus* spp.) viruliferous with TRV. The symptoms of TRV can sometimes be difficult to distinguish from the symptoms caused by PMTV. Although cv. Saturna is known to express good field resistance to TRV (Engsbro, 1973), an RNA dot-blot analysis using a probe for TRV was carried out to examine if any tuber with symptoms was infected with TRV. The results were negative (data not shown). Therefore, TRV was an unlikely cause of the spraing symptoms in our experiments, which supports the other lines of experimental evidence mentioned above indicating that the observed spraing symptoms were caused by PMTV.

Forty-five tubers were taken at random from the different transgenic and non-transgenic Saturna lines and tested for *Potato virus Y* (PVY), *Potato virus A* (PVA) and *Potato virus S* (PVS) by ELISA (for antibodies, see Oruetxebarria et al., 2000). These viruses are transmitted efficiently by aphids in a non-
persistent manner and occur in potato crops in Southern Sweden (J. Valkonen, unpublished data). In greenhouse experiments, Saturna has shown resistance to PVA and the ordinary strain group isolates of PVY (Valkonen & Palohuhta, 1996). Our ELISA results were negative, except for one tuber that was infected with PVS, but not with PMTV. The important implication of these data was that infections with other viruses were not common and, therefore, could not have suppressed the CP gene-mediated resistance to PMTV in the tubers that were PMTV-infected. This scenario had to be tested in light of the recent data indicating that infection with heterologous viruses can suppress virus-specific resistance in transgenic plants (Mittler et al., 2001; Savenkov & Valkonen, 2001).

**PMTV infection in leaves and roots of transgenic**

*N. benthamiana*

A total of 25 CP-transgenic plants and two wild-type plants was mechanically inoculated with PMTV. The non-transgenic plants were systemically infected with PMTV at 35 days post-infection (p.i.) ($A_{105}$ values of virus-positive leaves 0.38–0.62), as expected, but no transgenic plant was ELISA-positive ($A_{105}$ values for transgenic *N. benthamiana* 0.08 ± 0.02; non-inoculated control plants 0.08 ± 0.01). Ten transgenic plants were tested at random for RNA 2 and RNA 3 using RNA dot-blot hybridization. In five of them (Fig. 4, plants A4, A5, B4, B5 and E4), RNA 2 but not RNA 3 was detected in non-inoculated leaves. The roots in four of these plants contained RNA 2 but not RNA 3, but the roots of one of them contained also RNA 3 (Fig. 4, A9). In two additional plants (Fig. 4, C9 and D9), roots contained small amounts of both RNA 2 and RNA 3, but no viral RNA was detectable in leaves. The probe p448 detected the RT domain of the RNA 3 (Fig. 1) and not the CP transgene mRNA homologous to the 5' part of RNA 3.

Fig. 4. Detection of PMTV RNA 2 and RNA 3 in roots and leaves of *N. benthamiana* by dot-blot hybridization. The leaves and roots of 20 transgenic plants and a few non-transgenic control plants were tested. Ten transgenic plants (F) were inoculated by growing them in soil infested with viruliferous *S. subterranea* (columns 2, 3, 6 and 7). Ten additional plants (M) were grown in non-infested soil and the leaves of these plants were mechanically inoculated with PMTV (columns 4, 5, 8 and 9). Leaves and roots were tested from all plants at 63 days p.i. Each RNA sample dot (1 µg total RNA) represents a different plant. The same blot was hybridized with the probe for RNA 2 (p419) and RNA 3 (p448). Staining with methylene blue was done to verify possible loading and concentration differences between RNA samples. Controls: A1, leaf of a non-transgenic, healthy plant; B1, root of a non-transgenic, PMTV-infected plant; C1, leaf of a non-transgenic, PMTV-infected plant; D1, the roots of the plant of C1; E1, no RNA spotted. Northern blot analysis (to the right at the bottom) on the total RNA extracted from leaves was carried out to verify the specificity of the probes to viral RNA and hybridized with the same probes for RNA 2 (3–0 kb) and RNA 3 (3–1 kb) as used for the dot-blot. Lane a, healthy non-transgenic plant; lane b, PMTV-infected non-transgenic plant; lane c, healthy CP-transgenic plant. Note that the probe p448 detects the RT domain of the RNA 3 (Fig. 1) and not the CP transgene mRNA homologous to the 5' part of RNA 3.
of signals would have resulted from PMTV-containing zoospores and negative was clear-cut (Fig. 4). It is unlikely that these signals were quite strong, and the difference between positive PMTV CP (data not shown). In all these tests, the positive in transgenic and non-transgenic plants using the antibodies to (Fig. 4). Tissue immunoblots on roots revealed strong signals that were tested amongst the 43 plants grown in infested soil (Fig. 4). Ten of these transgenic plants were tested also with RNA dot-grown in the same soil was systemically infected. The leaves of strains of \\textit{S. subterranea} .

However, in contrast to leaves, RNA 2 and RNA 3 were readily detected in roots in seven of the ten transgenic plants that were tested amongst the 43 plants grown in infested soil (Fig. 4). Tissue immunoblots on roots revealed strong signals in transgenic and non-transgenic plants using the antibodies to PMTV CP (data not shown). In all these tests, the positive signals were quite strong, and the difference between positive and negative was clear-cut (Fig. 4). It is unlikely that these signals would have resulted from PMTV-containing zoospores of \textit{S. subterranea} infecting the roots and not from PMTV replication.

Discussion

In this study, virus resistance in transgenic potato and \textit{N. benthamiana} expressing the CP gene derived from the PMTV RNA 3 was investigated, with special attention to resistance expression in tubers and roots as compared to leaves. The data show that phenotypic expression of the CP gene-mediated resistance to PMTV is different depending on whether leaves or roots are inoculated.

In transgenic \textit{N. benthamiana} plants, inoculation of leaves with PMTV resulted in strong suppression of RNA 3 accumulation in the foliage, whereas readily detectable amounts of RNA 2 accumulated in the leaves of a few inoculated plants, as in a previous study (McGeachy \& Barker, 2000). Assays for RNA 1 were not carried out for all plants, but it was expected to occur in plants containing RNA 2 because it encodes the RdRp required for viral RNA replication. These data indicate that resistance expression in leaves was targeted to RNA 3, the viral RNA that is homologous to the transgene. RNA sequence-specific resistance to viruses is often explained by RNA silencing, a cytoplasmic RNA surveillance mechanism that can be induced by an mRNA and be targeted to any homologous RNAs, including viruses (reviewed in Baulcombe, 1999; Waterhouse \textit{et al.}, 2001). Thus, the CP gene-mediated resistance to PMTV RNA 3 may be associated with RNA silencing.

Unlike previous studies, roots of the mechanically inoculated transgenic plants were also tested for viral RNAs. Detectable amounts of RNA 2 and RNA 3 were found in several of them. However, RNA 2 was more commonly detected than RNA 3. The low but detectable levels of RNA 3 in roots indicated that some initial and/or low levels of RNA 3 replication occurred in the inoculated leaves, followed by systemic movement to roots. In conclusion, the accumulation of RNA 3 was suppressed in roots in transgenic plants compared with non-transgenic control plants, but the suppression of RNA 3 accumulation was even greater in leaves of transgenic plants.

A different picture of resistance expression appeared following inoculation of roots with viruliferous zoospores of \textit{S. subterranea} , because all infected plants had high titres of both RNA 2 and RNA 3 in roots, but no plant seemed to be systemically infected because there was an apparent absence of viral RNAs in leaves. It was intriguing that transport of virus did not occur from roots to leaves, although virus RNAs were transported from leaves to roots following inoculation of leaves. It is possible that infection of roots could have induced systemic resistance, which inhibited accumulation of viral RNAs in leaves. A systemic signal generated in the cells undergoing RNA silencing can precede virus infection and precondition uninfected cells and tissues for RNA silencing (Waterhouse \textit{et al.}, 2001). Taken together, although we have no direct evidence, our results do not exclude the possibility that the CP gene-mediated resistance to PMTV may be associated with RNA silencing.

In general there is little information as to whether virus resistance functions similarly in roots and leaves, and usually only leaves have been examined in the previous studies describing transgenic resistance to viruses. Our data indicate that the CP gene-mediated resistance to PMTV is expressed differently in roots and leaves. This may be because resistance mechanisms, such as RNA silencing, are poorly induced in roots, or because infection of roots with the zoospores and plasmodia of \textit{S. subterranea} alters the ability of root cells to be induced for and/or express resistance.

Previous studies have indicated that strong resistance is only expressed if the PMTV CP transgene is translatable, but that, on the other hand, all transgenic lines were resistant irrespective of the steady-state levels of transgene RNA transcript or protein (Barker \textit{et al.}, 1998a). Examples of transgenic resistance are frequently categorized as either RNA-based or protein-based, but the resistance expressed in the transgenic plants of this study is difficult to explain with only one model. Protein-mediated resistance is not known to have a specific RNA target, such as the suppression of RNA 3 accumulation in this study. On the other hand, translatability of the viral CP transgene is not known to be required for RNA silencing. Thus, it is possible that the CP gene-mediated resistance to PMTV operates via two mechanisms, one of which is RNA-based (associated with RNA silencing), while the other is protein-based, interfering with viral genome amplification and/or movement.

The data presented here on resistance to PMTV at the whole plant level are generally in agreement with those obtained in previous studies (Reavy \textit{et al.}, 1995; Barker \textit{et al.}, 1998a, b) using the same transgenic lines. The differences in results probably reflect the more challenging conditions for the durability of resistance (differences in the isolates of PMTV, strains of \textit{S. subterranea} , temperature and/or growth conditions) in the experiments reported here, differences in the assay.
methods, or any combination of these. In the greenhouse experiments in Scotland using local field soil infested with viruliferous *S. subterranea*, no infection with PMTV was detected in the tubers of the CP-transgenic lines of Saturna, whereas 10% of the controls were infected (Barker et al., 1998b). In our study carried out in a field in Southern Sweden, all transgenic lines of Saturna showed reduced incidence of PMTV infections in tubers (7% as compared to 20% in controls), although it is notable that line AM12 was not infected. Similarly, only two CP-transgenic *N. benthamiana* plants of 99 (2%) grown in the Scottish soil contained detectable infection of PMTV in roots (Reavy et al., 1995), whereas the roots of seven plants of ten (70%) grown in the Swedish soil were infected with PMTV. Despite these differences in results, comparison of the two experimental species, potato and *N. benthamiana*, indicated similar resistance phenotypes that were similarly expressed in both species. Our data showed that the incidence of infection was reduced in the tubers and roots, but once they were infected, resistance did not significantly reduce virus accumulation in either species.

Our results extend the understanding of the CP gene-mediated transgenic resistance to PMTV by providing novel data and describing comparisons of resistance expression in leaves and roots and in relation to different methods for virus inoculation. To our knowledge this is the first study in which the expression of resistance has been shown to differ in the vector–virus interaction rather than the vector–root cell interaction. Our results with PMTV suggest that in situations where transgenic resistance in the crop plant is required to be effective in roots, resistance tests on leaves alone may not be a reliable indicator of what may happen in the roots. Our work also emphasizes the importance of testing the resistance of transgenic plants in several locations because of the influences a particular environment may have on the expression of resistance.

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