Transformation of tobacco and potato with cDNA encoding the full-length genome of *Potato leafroll virus*: evidence for a novel virus distribution and host effects on virus multiplication

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A full-length cDNA copy of the genome of *Potato leafroll virus* (PLRV) was introduced into the genome of tobacco and potato plants by *Agrobacterium tumefaciens*-mediated transformation. Transgenic lines were obtained in which the transgene was readily detected by PCR with DNA extracted from T1 tobacco seedlings and clonally multiplied potato plants. PLRV-specific genomic and sub-genomic RNAs, coat protein antigen and virus particles were detected in transgenic plants. Aphids fed on the transgenic tobacco plants readily transmitted PLRV to test plants. Infected transgenic tobacco plants, like non-transgenic (WT) PLRV-infected plants, displayed no symptoms of the infection but transgenic plants of potato were severely stunted. In parallel tests, the mean PLRV titres in WT tobacco plants and transgenic tobacco plants were 600 and 630 ng virus/g leaf, respectively, although differences in PLRV titres among transgenic plants were much greater than those among infected WT plants. In similar tests with potato, the mean PLRV titre of WT plants was 50 ng virus/g leaf whereas higher concentrations (up to 3400 ng virus/g leaf) accumulated in transgenic potato plants. In tissue prints of stems, PLRV was detected in similar proportions of phloem cells in transgenic and infected WT plants. In transgenic tobacco and potato plants, but not in infected WT plants, a few stem epidermal cells also contained virus. From tissue prints of transgenic tobacco leaves, it was estimated that about one in 40000 mesophyll cells contained virus, but in transgenic potato, a greater proportion of mesophyll cells was infected.

Introduction

*Potato leafroll virus* (PLRV; genus Polerovirus; family Luteoviridae) has a single-stranded RNA genome and is aphid-transmitted in a persistent non-propagative manner (Waterhouse et al., 1988; Mayo & Ziegler-Graff, 1996). PLRV is introduced into the vascular tissue of plants by aphid vectors and, except in plants co-infected with certain other viruses (e.g. see Atabekov et al., 1984; Barker, 1987), remains largely restricted to the phloem tissues of its hosts.

The study of PLRV gene function by reverse genetics is made unusually difficult because the virus is not mechanically transmissible. One approach to this problem has been to use agroinfection, in which *Agrobacterium* cultures carrying a DNA copy of the virus genome in a Ti plasmid are injected directly into plant tissues (Leiser et al., 1992; Mutterer et al., 1999). As with aphid-inoculation, the resulting infections are limited to phloem tissue. An alternative way of inducing virus replication in a plant is by transformation with a full-length (biologically active) cDNA copy of a virus genome, but the methods are time consuming. For example, plants have been transformed with cDNA corresponding to full-length RNA genomes of *Tobacco mosaic virus* (Yamaya et al., 1988), *Brome mosaic virus* (Kaido et al., 1995), *Potato virus X* (Angell & Baulcombe, 1997) and PLRV (Prüfer et al., 1997). For PLRV, this has presented an
opportunity to study the behaviour of PLRV and its putative 17 kDa movement protein in cells outside the phloem tissue (Schnitz et al., 1997). In some of the above examples, virus accumulated in transgenic plants in amounts similar to, or greater than that accumulated in conventionally inoculated plants (Yamaya et al., 1988; Prüfer et al., 1997). In other instances, much less virus accumulated (Kaido et al., 1995; Angell & Baulcombe, 1997). This effect has been attributed to gene silencing induced by the transgene (Angell & Baulcombe, 1997).

We report here the results of experiments with transgenic tobacco and potato plants obtained by transformation with full-length infectious cDNA to PLRV RNA. Expression of the PLRV genome did not result in the accumulation of large amounts of PLRV in tobacco, although in potato, much larger amounts of PLRV accumulated than in infected WT plants.

Methods

DNA and RNA manipulations. For preparation of cDNA and plasmid DNA, digestion with restriction enzymes, filling-in reactions with the Klenow fragment of DNA polymerase I of *E. coli*, ligations and transformations of *E. coli*, procedures were essentially as in Sambrook et al. (1989). The *E. coli* strain used was Nova-Blue (Novagen), the Agrobacterium tumefaciens strain used was LBA4404 (Clontech). Sequencing of double-stranded DNA was as described by Murphy & Kavanaugh (1988).

Plasmid constructions. Previously described cDNA clones of the Canadian isolate of PLRV (Keese et al., 1990; Kawchuk et al., 1989) were taken as a source for the construction of full-length cDNA in plasmid Donavan (Angell & Baulcombe, 1997), the Agrobacterium tumefaciens strain used was LBA4404 (Clontech). Sequencing of double-stranded DNA was as described by Murphy & Kavanaugh (1988).

In vitro mutagenesis (Kunkel et al., 1987) was used to remove non-viral sequences at the 5′-end of the putative 35S promoter transcript. For this purpose, the plasmid p35SUP100 was shortened by digestion with Sall, removing all but the 5′ and 3′ termini of the viral cDNA from the vector, to give p35SUPAS. The mutagenic oligonucleotide, 5′ CATTTCATTTCGGAGAGGACAAAAAGAATAACCCAGG 3′ contained 35S promoter sequence up to the transcript start site (italicized) and virus sequence (bold) between positions 1 and 16. The resulting plasmid was named p35SUPASM10. The Apal/Saal fragment from p35SUP100 was replaced by the same fragment from p35SUPASM10 to give p35SUP110.

The *Nhe* fragment of p35SUP110 was finally cloned into XbaI-linearized pBIN19 (Bevan, 1984) to give pBNUP110. The plasmid was mobilized into *A. tumefaciens* by direct DNA transformation according to An et al. (1988).

Plant transformation and culture of transgenic plants

Transformation of *Nicotiana tabacum* cv. ‘Samsun’ and potato was according to Barker et al. (1993) and Barker et al. (1992), respectively, using *A. tumefaciens* containing plasmid pBNUP110 described above.

Tobacco. Transformed plantlets, designated AW1 to AW35, were transferred to a glasshouse and grown to maturity. Mature seeds were collected from self-fertilized flowers and retained for further use. The resistance of seedlings (T0 progeny) to kanamycin was tested as described by Barker et al. (1993). Transgenic plants for other tests were obtained from unselected seedlings germinated in compost, and grown to maturity in peat-based compost that was treated regularly with liquid fertilizer.

Potato. Stem tissue pieces of potato genotypes cv. Maris Piper and the SCR breeding line G8107(1) were transformed, but it was not possible to obtain transgenic lines of Maris Piper because callus grew poorly and died without regenerating transgenic shoots. Transformation of G8107(1), which has strong host-mediated resistance to PLRV accumulation (Barker & Woodford, 1992), produced vigorously growing callus from which shoots were regenerated. Following thermotherapy treatment (see below), in vitro plantlets of four transgenic lines were obtained that were transferred to a glasshouse. Transgenic plants grew poorly, but a few tubers were produced. After storage for approximately 6 months, they were planted to produce the progeny plants with which most tests were made.

Detection of transgenic plants by PCR. Total leaf DNA extracts were prepared essentially as described by Reavy et al. (1997). DNA was tested for the presence of PLRV cDNA by PCR amplification using oligonucleotide primers designed to amplify sequences from the 5′-most 537 nt, the 3′-most 1686 nt or the 3′-most 538 nt of ORF 5 of the PLRV genome (Mayo et al., 1989).

Assay and quantification of RNA transcript. A PCR product amplified from a plasmid containing the sequence of the coat protein (CP) gene of PLRV (Mayo et al., 1989), was labelled with digoxigenin (Boehringer Mannheim) to make a probe as described by Webster & Barker (1998). The oligonucleotide primers used were 5′ TCAATGCTCCATTGTCATGTCCC 3′, which is complementary to the virus sequence between positions 490 and 512, and the second strand primer, 5′ TACGTATACAAAAAGAATACCAGGAGAAAT TACGGAGAGG 3′, which corresponds to the virus sequence (bold) from position 1 to position 24, with added sites for *Bst* II (underlined) and *SnaB*I (italicized). The PCR product was cloned into pT7-Blue (Novagen) to produce pT75P. *p* T75P was cut with *Apa*I and *SnaB*I and ligated into *Apa*I/*Not*I (filled-in)-linearized pUP99a to give pUP100. The KpnI site of pGem3Zf (+) was removed by cutting with EcoRI and *Sal*I and, after the fill-in reaction, the vector was religated to give pG3DE/S. The HindIII (filled-in) fragment of pRT103 (Töpfer et al., 1987) containing the 35S promoter of *CaMV* mosaic virus and the corresponding transcription termination sequence was cloned into the HindIII (filled-in) site of pG3DE/S, to give pG35S1. This vector produces unique *Nhe*I restriction sites flanking the expression cassette that are not present in the PLRV cDNA sequence. The *Bst* II/KpnI fragment of pUP100 was cloned into KpnI and *Xho*I (filled-in) linearized plasmid pG35S1 to give p35SUP100.

In vitro mutagenesis (Kunkel et al., 1987) was used to remove non-viral sequences at the 5′-end of the putative 35S promoter transcript. For this purpose, the plasmid p35SUP100 was shortened by digestion with Sall, removing all but the 5′ and 3′ termini of the viral cDNA from the vector, to give p35SUPAS. The mutagenic oligonucleotide, 5′ CATTTCATTTCGGAGAGGACAAAAAGAATAACCCAGG 3′ contained 35S promoter sequence up to the transcript start site (italicized) and virus sequence (bold) between positions 1 and 16. The resulting plasmid was named p35SUPASM10. The Apal/Saal fragment from p35SUP100 was replaced by the same fragment from p35SUPASM10 to give p35SUP110.

The *Nhe*I fragment of p35SUP110 was finally cloned into XbaI-linearized pBIN19 (Bevan, 1984) to give pBNUP110. The plasmid was mobilized into *A. tumefaciens* by direct DNA transformation according to An et al. (1988).
Transmission with PLRV full-length genome

Characterization of transgenic lines of potato

In attempts to transform cv. Maris Piper, only a few non-transgenic shoots regenerated from the callus which eventually died. Samples of the callus were tested by ELISA using an anti-PLRV CP antibody and a positive reaction was obtained. It is possible that the growing callus was transgenic and the stress caused by accumulating PLRV inhibited callus growth and shoot regeneration. By using the methods described in this paper, it has been possible to transform cv. Maris Piper using other transformation vectors (H. Barker, unpublished results).

Transformation of the PLRV-resistant *Solanum tuberosum* clone G8107(1) produced vigorously growing callus from which sixteen independent regenerating shoots were removed and micropropagated *in vitro*. Five lines (BF10, BF16, BF17, BF19 and BF21) were found to contain PLRV (determined by ELISA of the initially transformed plantlets), and DNA extracts from these lines yielded a specific product when assayed by PCR using primers that annealed to the readthrough region of the CP gene. PLRV could not be detected in other plantlets and PCR tests on DNA extracted from these plants did not yield a product.

Tests for agroinfection as the source of PLRV in transgenic lines

Some of the transformed tobacco plants (T₀ generation) contained substantial amounts of PLRV, although they were not transgenic and their subsequent seed generations (T₁) did not accumulate PLRV. It was assumed that such plants had become infected by ‘agroinfection’ during tissue culture but that infection had not been passed to the seed generation, in contrast to the situation with transgenic lines AW3 and AW14. Because the transformed potato plants were propagated clonally and not through seed, it was possible that PLRV accumulating in transformed potato plants was, at least in part, the result of agroinfection during the process of tissue culture. To test this, *in vitro* plantlets were ‘heat-treated’ by incubation for periods of 4 h at 39 °C alternating with 4 h at 20 °C. Such thermotherapy treatment of PLRV-infected non-transgenic plantlets for 25 days, freed them of PLRV infection. However, plants of lines BF10, BF16, BF17, BF19 and BF21 still contained high concentrations of PLRV following thermotherapy treatment for 54 days. These lines were transferred to the glasshouse for further propagation. BF10, BF16, BF19 and BF21 produced a few tubers that were stored for approximately 6 months at 4 °C before planting to produce the progeny plants on which most tests were made.

Symptom development in transgenic tobacco and potato

Transgenic plants of AW3 and AW14 tobacco were indistinguishable from either virus-free or PLRV-infected WT plants (symptomless in our glasshouse conditions). In the...
glasshouse, plants of BF lines grown from tubers initially did not look much different from PLRV-infected WT plants of G8107(1), which do not show characteristic ‘leafroll’ symptoms of PLRV infection. However, as plants aged, most leaves of BF plants, except those at the top of the stem, became necrotic and died prematurely and plants became severely stunted in comparison to infected WT plants of G8107(1), which continued to grow without showing symptoms. BF plants remained severely stunted throughout their lives and produced a few small tubers. In vitro transgenic BF plantlets grew more slowly than the non-transgenic WT controls, but otherwise were indistinguishable and showed no visible symptoms.

Expression of the PLRV transgene

Tobacco. T1 plants of lines AW3 and AW14 were assayed by ELISA to determine the amounts of PLRV antigen in young, fully expanded leaves. Plants used for these tests were tested by PCR prior to ELISA and no non-transgenic segregants were found. For ELISA, plants were sampled approximately 10 weeks after sowing. Of 20 plants of each line, 15 of AW3 and 16 of AW14 contained PLRV antigen. Virus was not detected in further tests on the virus-free transgenic plants after an additional 2 weeks. The concentration of PLRV in plants that contained detectable antigen varied greatly among plants of the same line. Thus, plants of AW3 with the highest concentration of antigen contained 12-fold more than the plant with the lowest concentration and, in line AW14, the difference was 18-fold (Fig. 1). The test on 12-week-old plants gave very similar results (data not shown).

When the amounts of PLRV antigen in young, fully expanded leaves of 8-week-old AW3 transgenic plants and infected WT plants were compared, the mean titres of eight infected WT plants and ten AW3 plants were 597 (± 146) ng and 627 (± 231) ng virus/g leaf, respectively (SEM in parentheses). The differences in PLRV titre among AW3 plants were much greater than those among aphid-inoculated WT plants. Indeed, PLRV could not be detected in several leaf samples from the AW3 plants. In further tests, PLRV contents were measured in leaves of different ages. Similar PLRV contents were found in AW3 transgenic and infected WT plants, but a clear difference was observed in that whereas a few leaves of AW3 plants were apparently virus-free, virus was detected in all leaves of infected WT plants (Table 1). In other experiments, some AW3 plants that were found to be virus-free in several ELISAs of sequentially produced leaves were also tested by immunoblotting, but no PL RV antigen was detected (data not shown). Thus, a proportion of transgenic plants (approximately 25%) are virus-free throughout their lives, whereas other transgenic plants contain PLRV and, although a few leaves may be virus-free, a variable amount of PLRV accumulates in different leaves. Seeds collected from self-fertilized flowers of the virus-free transgenic plants were sown; the T2 seedlings were also found to be virus-free by ELISA. However, the PLRV-infected T1 plants produced T2 seedling progenies that were PLRV-infected.

Potato. Plants grown from transgenic tubers and progeny tubers from WT PLRV-infected G8107(1) and cv. Maris Piper

Table 1. PLRV titre in leaves from different regions of AW3 transgenic and infected wild-type tobacco plants

<table>
<thead>
<tr>
<th>Plant no.</th>
<th>Infected wild-type plant</th>
<th>Transgenic AW3 plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper</td>
<td>Middle</td>
</tr>
<tr>
<td>1</td>
<td>220</td>
<td>240</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>210</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>210</td>
</tr>
<tr>
<td>5</td>
<td>190</td>
<td>110</td>
</tr>
<tr>
<td>6</td>
<td>63</td>
<td>190</td>
</tr>
</tbody>
</table>
plants were tested by ELISA (four or five plants per clone). Very little virus could be detected in leaf tissue of G8107(1) plants (mean of 50 ng/g tissue) whereas high concentrations (mean of 3400 ng/g leaf tissue) accumulated in the BF transgenic plants. In comparison, a mean of 2700 ng/g leaf tissue accumulated in secondarily infected plants of the PLRV-susceptible cv. Maris Piper. The resistance of WT G8107(1) to PLRV accumulation in comparison to the susceptible cv. Maris Piper was established by Barker & Woodford (1992). Tubers were collected from these plants and a second set of plants was grown for further tests in which leaf and stem tissues were tested (Table 2). The amount of PLRV in leaves of G8107(1) plants was below the limit of detection by ELISA, but was readily detectable in leaves of transgenic BF lines and cv. Maris Piper (Table 2). PLRV was detected in stem tissue of G8107(1) plants but was at a higher concentration in the stems of cv. Maris Piper and the older stem tissues of BF plants (Table 2). Symptoms in the BF plants were more severe than in the first test and possibly the lower concentration of PLRV detected in leaves was a reflection of environmental influences because the two tests were done at different times of the year.

Properties of PLRV in AW3 plants

Virus-like particles with the characteristic morphology of PLRV were readily detected by immunosorbent electron microscopy (ISEM) of leaf extracts from AW3 plants that had given positive results by ELISA. In tests in which scions from five AW3 plants were grafted to virus-free WT tobacco plants, all five WT receptor plants became infected. When *M. persicae* that had been raised on two AW3 plants were transferred (five aphids per plant) to virus-free receptor plants of *P. floridana*, *N. clevelandii* or *N. tabacum*, 22 of 31 receptor plants became infected.

Table 2. PLRV titre in leaf and stem tissue of transgenic and infected wild-type potato plants

<table>
<thead>
<tr>
<th>Potato clone</th>
<th>Area of stem sampled</th>
<th>PLRV concentration (ng virus/g tissue)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>G8107(1) (non-transgenic control)</td>
<td>Top</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>0</td>
</tr>
<tr>
<td>BF16 (transgenic)</td>
<td>Top</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>NT</td>
</tr>
<tr>
<td>Maris Piper (non-transgenic)</td>
<td>Top</td>
<td>2300</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>750</td>
</tr>
</tbody>
</table>

* Data are means of measurements made on four plants of each clone. NT, Not tested.

Analysis of PLRV-specific RNA

Northern blots of RNA extracted from PLRV-infected plants of AW3 were similar to blots of RNA from PLRV-infected WT plants; two bands were detected, one of 6 kb (genomic RNA) and one of 2.5 kb (sub-genomic RNA). In general, relatively low amounts of PLRV-specific RNA could be extracted from tobacco compared with infected WT potato. Transgenic AW3 plants in which PLRV-CP could not be detected by ELISA or immunoblotting did not contain detectable PLRV RNA. RNA extracted from an AW3 transgenic plant in which PLRV-CP was detected, and from a WT PLRV-infected tobacco plant, was separated into poly(A) + and poly(A)— fractions and tested in RNA dot blots by hybridization with the CP probe. Only the poly(A)— RNA from infected WT plants hybridized with the probe, whereas both fractions of RNA from the AW3 plant hybridized strongly with the probe (data not presented). Presumably the AW3 RNA extracts contained polyadenylated RNA transcripts of the PLRV cDNA in the AW3 genome.

PLRV in isolated tobacco protoplasts

Mesophyll protoplasts were isolated from leaves of PLRV-infected AW3 tobacco plants and from infected WT plants, and analysed for their PLRV antigen and RNA contents by ELISA or Northern blotting. In eight tests, no PLRV was detected in protoplasts extracted from infected WT plants; in one test the protoplast sample contained 81 ng virus/10⁶ protoplasts. In contrast, virus was detected in all nine preparations of protoplasts from leaves of transgenic tobacco plants at levels in the range 36–220 ng virus/10⁶ protoplasts. Genomic and sub-genomic PLRV RNAs were readily detected by Northern blotting in RNA extracted from transgenic mesophyll protoplasts or in RNA from leaves similar to those from which the
protoplasts were prepared. No PLRV-specific RNA was found in mesophyll protoplasts prepared from infected WT tobacco plants.

**Location of PLRV by tissue printing**

Tissue prints of leaves and stems were made to assess the number and distribution of cells containing PLRV. Stained foci of indoxyl precipitate were identified in prints from transgenic and infected WT plants, but were not seen in any of the prints made from virus-free plants. It was assumed that the stained foci indicated the location of infected cells, although we cannot exclude the possibility that some infected cells contained insufficient PLRV to be detected by staining.

**Tobacco.** Tissue prints of stem sections showed that the majority of phloem cells in AW3 transgenic and infected WT plants were unstained, and thus that relatively few cells were infected (estimated to be less than 5% of the phloem companion cells) (Fig. 2b, c, d). In approximately 25 sections from each of AW3 and infected WT plants, means of 16 and 26 infected cells per section, respectively, were observed. A few infected cells were also observed in epidermal tissue of stem sections from some AW3 transgenic plants (a mean of about one cell per section), but PLRV was never detected in epidermal cells from infected WT tobacco plants (Fig. 2d vs b).

Tissue prints were also made of leaf lamina from which the lower epidermis had been removed by peeling. Tissue pieces were pressed onto nitrocellulose membranes to leave an imprint of the exposed mesophyll tissue. Within the region of vascular bundles of AW3 transgenic and infected WT tobacco plants (identified as an obvious track of a vein in which relatively few cell contents were deposited on the tissue print), some infected cells were observed which were assumed to correspond to phloem cells (Fig. 2f). Infected ‘mesophyll’ cells were also observed in leaves of AW3 transgenic plants (Fig. 2g, h) but not in infected WT plants. Because such cells were not seen alongside a track left by a vein, they could be
Table 3. Distribution of PLRV-infected cells in stem sections of transgenic and WT potato plants

<table>
<thead>
<tr>
<th>Potato clone</th>
<th>Area of stem sampled</th>
<th>Mean number* of PLRV-infected cells in stem</th>
<th>Phloem bundle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Epidermis</td>
<td>External</td>
</tr>
<tr>
<td>G8107(1) (non-transgenic control)</td>
<td>Top</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BF lines (transgenic)</td>
<td>Top</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Maris Piper (non-transgenic)</td>
<td>Top</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>0</td>
<td>28</td>
</tr>
</tbody>
</table>

* Data are means of counts from stem sections made from four plants of each clone; on average five sections per area of stem tissue sampled were made from each plant.

distinguished from infected cells in the region of vascular tissue. In some samples of AW3 leaves, mesophyll cells were aggregated to form small clusters (Fig. 2h). Infected mesophyll cells were found in only a few tissue prints; a total of 36 infected cells were found in 37 cm² of leaf prints made from AW3 leaves. On the basis of the entire mesophyll of a leaf yielding about 8 × 10⁶ protoplasts, we estimate that about one in 40000 mesophyll cells of these leaves had accumulated detectable amounts of virus.

Potato. Tissue prints were made of stem and leaf tissue from plants of the BF lines, and PLRV-infected WT Maris Piper and clone G8107(1). Far fewer infected phloem cells were found in stem tissue of infected plants of clone G8107(1) and infected cells were largely restricted to the internal phloem bundles in comparison with Maris Piper plants in which many infected cells were found in the internal and external phloem (Table 3; Fig. 3a vs c). Derrick & Barker (1997) found a similar distribution of infected phloem cells in potato clones that differed in susceptibility to PLRV accumulation, and suggested that a characteristic of PLRV-resistant clones [such as G8107(1)] was that infection was restricted in cells of the external phloem bundles. When the numbers of stained foci in plants of BF lines and G8107(1) plants were compared, there were more infected cells in the external phloem of the BF clones than in G8107(1) (Table 3). However, a more notable difference was that infected cells were detected in stem epidermis of transgenic BF lines, but not in the infected WT Maris Piper and G8107(1) plants (Table 3; Fig. 3a, c). Many infected cells were observed in mesophyll of transgenic BF plants, although these were often distributed in clusters (Fig. 3d). Thus, in many of the prints examined, the number of stained cells was low, but occasionally leaf areas with large amounts of stained foci were observed near areas with few stained cells (Fig. 3d).

Discussion

Only two transgenic lines of tobacco were obtained by transformation with a full-length cDNA copy of the PLRV genome; less than 14% of the primary transformants. The small number of transgenic plants obtained from this transformation is not characteristic of other tobacco transformation experiments in our hands; usually rates of ca 70% being obtained (e.g. Barker et al., 1993). T1 seedlings of the two transgenic lines contained PLRV antigen from an early stage in their growth. This is the first report of luteovirus transmission through seeds, albeit in a highly artificial situation. The results of several tests (ISEM, infectivity assay, detection of genomic and sub-genomic RNAs by Northern blotting) suggested that the PLRV accumulating in transgenic tobacco and potato plants was fully functional, and did not differ from the PLRV that accumulated in infected WT plants.

The PLRV-infected transgenic T1 tobacco plants were symptomless, as were naturally infected WT tobacco plants. Likewise, symptoms did not develop on infected WT G8107(1) potato plants, but plants of the transgenic BF lines were very severely stunted, died prematurely, and produced very few small tubers. Relatively little PLRV accumulated in infected WT tobacco and G8107(1) potato plants, both being poor hosts for PLRV when inoculated by using viruliferous aphids. A major difference between the transgenic tobacco and potato lines was that although PLRV contents of transgenic AW3 tobacco plants were similar to, or less than, those in infected WT tobacco, considerably more PLRV accumulated in leaf
tissue of transgenic BF potato plants than in infected WT G8107(1) plants. Thus, plants of our BF lines resemble those of Prüfer et al. (1997) whose potato plants transformed with a full-length PLRV genome contained much higher levels of viral RNAs and protein, and had a reduced lifespan. We were not able to transform the susceptible potato cv. Maris Piper, and it seems possible that we were able to obtain the BF lines only because the parental clone G8107(1) has a high level of resistance to PLRV accumulation (Barker & Woodford, 1992).

Stem tissue prints of transgenic and infected WT tobacco and G8107(1) potato plants, showed that less than 5% of the available phloem companion cells in stem tissue were infected. The major difference between transgenic plants and infected WT plants was that in transgenic tissue, PLRV-infected cells were in tissues other than the phloem. Thus, many infected cells were found in the stem epidermis of transgenic potato plants although there were rather fewer in tobacco plants. Moreover, in transgenic tobacco plants, PLRV was also found in a few mesophyll cells, although such cells were infrequent (approximately 1 in 40,000). In transgenic potato, a greater proportion of mesophyll cells was infected, although these had an irregular distribution with some areas containing many and other areas containing relatively few.

On average, more PLRV accumulated in AW3 plants than in AW14 plants, although PLRV could not be detected in about 25% of transgenic AW3 or AW14 plants. Further studies are required to reveal why the accumulation of PLRV differs so much between lines and individual plants. The 25% of transgenic plants that were consistently virus-free contained no PLRV genomic and sub-genomic RNA. It is possible that
such plants do contain PLRV but in such low amounts that it cannot be detected, or that the amount of transcribed RNA from the PLRV transgene is insufficient to initiate infection. The reason such transgenic plants do not become infected is being investigated.

Although all cells of plants transformed with full-length PLRV cDNA should produce infective PLRV RNA, only a very small proportion of cells accumulated detectable amounts of virus. Transgenic tobacco and potato differed in that much more PLRV accumulated in transgenic potato than in equivalent infected non-transgenic plants, but in transgenic and infected tobacco plants the amounts of PLRV were similar. Nevertheless, the majority of cells in transgenic plants did not accumulate PLRV, which suggests that they were expressing some form of resistance to the establishment of infection. The difference between hosts may reflect a differential resistance effect, and further study of this may be of value in work on transgene or host gene-mediated resistance.

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