Evidence for RNA-mediated defence effects on the accumulation of Potato leafroll virus

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In plants infected with Potato leafroll virus (PLRV), or other luteoviruses, infection is very largely confined to cells in the vascular system. Even in tobacco plants transformed with PLRV full-length cDNA, in which all mesophyll cells should synthesize infectious PLRV RNA transcripts, only a minority of the mesophyll cells accumulate detectable amounts of virus. We have explored this phenomenon further by transforming a better PLRV host, Nicotiana benthamiana, with the same transgene, by superinfecting transformed plants with Potato virus Y and by producing tobacco plants in which cells contained both PLRV cDNA and DNA encoding the P1/HC-Pro genes of the potyvirus Tobacco etch virus. A greater proportion of cells in superinfected plants or in doubly transgenic plants accumulated PLRV than did in singly transgenic tobacco plants. However, most cells in these plants did not accumulate virus. To investigate restriction of the multiplication of viruses containing PLRV sequences, transgenic plants were infected with a chimeric virus that consisted of Tobacco mosaic virus (TMV) containing genes for either the coat protein (CP) of PLRV or jellyfish green fluorescent protein (GFP) in place of the TMV coat protein. The virus that encoded PLRV CP spread more slowly and accumulated less extensively than did the virus that expressed GFP. The results support the suggestion that an RNA-mediated form of resistance that resembles post-transcriptional gene silencing operates in non-vascular cells and may be part of the mechanism that restricts PLRV to vascular tissue in conventionally infected plants.

Introduction

Like other luteoviruses, Potato leafroll virus (PLRV) cannot be transmitted to virus-free plants by mechanical inoculation. In the natural situation, PLRV is transmitted by aphid vectors that introduce particles into the vascular tissue of plants where PLRV multiplies and remains largely restricted within phloem tissue. Barker (1987) and van den Heuvel et al. (1995) have shown that a very small proportion of non-phloem cells of PLRV-infected plants of Nicotiana clevelandii and potato can contain virus particles. This spread into non-phloem tissue can be enhanced if plants are co-infected with certain other viruses (Atabekov et al., 1984; Barker, 1987, 1989). These findings suggest that the PLRV is normally restricted to phloem tissue because the virus lacks movement functions that can operate in epidermal and mesophyll cells (see for review, Taliansky & Barker, 1999), and/or because PLRV cannot suppress putative host defence responses in non-vascular tissues (Voinnet et al., 1999; Waterhouse et al., 1999). Complementation and enhancement of PLRV movement by co-infecting viruses is thought to result from the supply of either of these functions. PLRV does multiply in tobacco and potato mesophyll protoplasts that have been inoculated in vitro (Barker & Harrison, 1982; Miller & Mayo, 1991), and it has been proposed (Harrison & Mayo, 1983) that isolated protoplasts lack host defence responses that operate in intact tissues to prevent PLRV movement and multiplication.

Tobacco and potato plants have been transformed with a full-length (biologically active) cDNA copy of the PLRV genome by Franco-Lara et al. (1999). Although all cells of these transformed plants should have produced infective RNA by
transcription, only a very small proportion accumulated detectable amounts of the virus. Transgenic tobacco and potato differed in that much more PLRV accumulated in transgenic potato than in equivalent infected non-transgenic plants, whereas in transgenic and infected non-transgenic tobacco plants the amounts of PLRV were similar. It was suggested that the majority of cells in transgenic plants did not accumulate PLRV because they were expressing some form of resistance to the establishment of infection (Franco-Lara et al., 1999).

Recently, it was shown that resistance to PLRV multiplication in non-vascular tissues can be overcome when plants are also infected with umbraviruses. Thus, PLRV was mechanically transmissible when plants were inoculated with PLRV particles mixed with extracts of plants infected with Pea enation mosaic virus-2 (PEMV-2) or another umbravirus, Groundnut rosette virus (GRV) (Mayo et al., 2000). This effect involves limited movement in mesophyll tissues and loading into phloem in inoculated leaves, followed by unloading from phloem to infect mesophyll tissues in systemically infected leaves (Ryabov et al., 2001). The same effect was obtained when PLRV particles were mixed with extracts of plants infected with a recombinant Cucumber mosaic virus (CMV) that expressed the ORF4-encoded movement protein (MP) of GRV, but not if the CMV 2b gene, which functions as a suppressor of gene silencing (Brigneti et al., 1998), had been made non-translatible. It was suggested by Ryabov et al. (2001) that PLRV can accumulate and spread in mesophyll tissues when (i) some form of RNA-mediated defence (RMD), possibly involving post-transcriptional gene silencing (PTGS), is blocked and (ii) cell-to-cell movement function is complemented. This suggestion is supported by results reported recently by Savenkov & Valkonen (2001). They showed that another gene silencing suppressor, helper-component protease (HC-Pro) of the potyvirus Potato virus A, can enhance accumulation of PLRV in phloem tissues, although it cannot mediate unloading of PLRV from the phloem.

In this paper we report results of experiments to examine in more detail the restriction on virus accumulation in transgenic plants that express a full-length cDNA copy of the PLRV genome. We show that expression of HC-Pro can dramatically enhance the accumulation of PLRV, a result that reinforces the suggestion that PTGS-like RMD is involved in limiting the accumulation of PLRV in both phloem and mesophyll tissues.

Methods

Transgenic tobacco plants and hybridization. Transgenic tobacco plants were as follows: line AW3 contained the full-length genome of PLRV as described by Franco-Lara et al. (1999); lines UoB and X-27-8 contained Tobacco etch virus (TEV) P1/HC-Pro in cvs Havana 425 and Xanthi, respectively, as described by Carrington et al. (1990) and Mallory et al. (2001), respectively. Crosses were made in Dundee, Scotland, UK, using AW3 plants as female parents and pollen samples from plants of UoB and X-27-8 grown in South Carolina, USA; pollen was used within a week of arrival. Hybridizations were made using standard procedures after emasculation of the AW3 flowers. Control crosses were made by using pollen from plants not containing TEV P1/HC-Pro, namely Havana 425 plants transformed with ‘empty’ vector for UoB or non-transformed cv. Xanthi plants for line X-27-8.

Transformation of Nicotiana benthamiana. Pieces of N. benthamiana stem tissue were transformed as described by Benvenuto et al. (1991) using Agrobacterium tumefaciens containing plasmid pBNUP110 as described by Franco-Lara et al. (1999). Plasmid pBNUP110 contains a full-length cDNA copy of the genome of PLRV under the transcriptional regulation of the 35S promoter from Cauliflower mosaic virus. Seven rooted plantlets (T₀ generation) were transferred to an aphid-proof glasshouse kept at about 20 °C, their flowers were allowed to self-fertilize, and seed was collected. T₁ seedlings from six of the seven plantlets were grown and tested to determine if they were transgenic by PCR or by ELISA (see below). For PCR, total leaf DNA was prepared essentially as described by Reavy et al. (1997), and tested for the presence of PLRV cDNA by PCR amplification using oligonucleotide primers designed to amplify sequences within the PLRV coat protein (CP) gene (Franco-Lara et al., 1999).

Inoculation of plants with viruses. A culture of PTVV{+} (an isolate kindly provided by the Scottish Agricultural Science Agency, East Craigs, Edinburgh, Scotland, UK) was maintained in Nicotiana tabacum by manual transmission. Test plants of N. tabacum and N. benthamiana were infected by manual inoculation. Non-transgenic WT plants were inoculated with PLRV by exposure for 3 days to five viruliferous Myzus persicae that had been reared on PLRV-infected potato, cv. Maris Piper, as described by Franco-Lara et al. (1999).

Plasmids, generation of chimeric cDNA constructs and in vitro transcription. The plasmid pTMV(∆C)-GFP, in which the CP gene of Tobacco mosaic virus (TMV) was deleted and replaced by the DNA encoding jellyfish green fluorescent protein (GFP), was generated from a full-length cDNA clone of TMV (TMV30B) and has been described earlier (Ryabov et al., 1999). Plasmid TMV(∆C)PLRV-CP was designed to produce infectious RNA transcripts of TMV with the TMV CP gene replaced by the CP gene from PLRV. A fragment containing the PLRV CP gene was amplified by PCR by using the PLRV cDNA clone pBNUC110 as a template (Franco-Lara et al., 1999) and oligonucleotides 5'-GGCCCTT-AATTAAATGATACGGGTCTTGC 3' and 5'-GGCATCTGAGTTACTATTTGGGGTTTTGCAAAG 3'. RNA templates were synthesized with T7 RNA polymerase using the mMESSAGE mMACHINE T7 Kit (Ambion). Transcripts were inoculated directly to leaves of N. benthamiana plants by rubbing carborundum-dusted leaves with the transcription products derived from 0.2 μg of plasmid template.

Assay of PLRV by ELISA and tissue printing. The accumulation of PLRV antigen was assessed and quantified by ELISA as described by Franco-Lara & Barker (1999). Immunoprints of leaf lamina, from which the lower epidermis had been removed by peeling with forceps, were made as described by Franco-Lara et al. (1999). In developed prints, the presence of PLRV was apparent as discrete spots (stained cells) of purple indoxyl precipitate. These were counted and photographed at low magnification in a Leica MZ FLIII microscope. The number of cells per unit area of leaf was estimated by counting the number of green spots of cell contents imprinted on the membrane.
Results

N. benthamiana expressing PLRV-FL cDNA

Previous work with plants transformed with full-length PLRV cDNA used tobacco and potato plants (Franco-Lara et al., 1999). In current work, N. benthamiana was transformed in order to investigate a Nicotiana species known to support greater accumulation of PLRV than tobacco and, unlike tobacco, to be susceptible to a chimeric virus used in our experiments.

PLRV (detected by ELISA using anti-CP antibodies) accumulated in leaf tissue of T1 seedlings from the four lines of N. benthamiana tested; line CW1 was selected for all further tests. Although leaf extracts from the majority of individual CW1 T1 seedlings gave a strong signal in ELISA for PLRV CP, some seedlings gave signals that did not differ from those given by non-transgenic N. benthamiana. In one experiment, 48 of 67 CW1 T1 seedlings were found to be PLRV-infected and PCR tests on DNA extracts showed that these PLRV-infected seedlings contained DNA encoding the CP gene of PLRV, whereas PLRV-free seedlings did not. In this and other tests the ratio between PLRV-infected and PLRV-free (transgenic:non-transgenic) plants was always close to this ratio of 3:1, and we conclude that line CW1 contains a single locus of integrated T-DNA in which the PLRV-FL transgene is active.

PLRV accumulation in transgenic and PLRV-infected WT N. benthamiana

The PLRV-infected T1 plants of line CW1 grew more slowly and were smaller than uninfected WT plants, but there were no other symptoms of infection. Similar stunting effects were observed in WT N. benthamiana plants that had been inoculated by viruliferous aphids, but there were no other symptoms of infection.

In all subsequent experiments, ELISA of CW1 seedlings was done prior to further experimentation and non-infected plants (assumed to be non-transgenic segregants) were discarded. PLRV accumulation in leaf tissues of CW1 plants was assessed by ELISA and immunoprinting and, in some experiments, was compared with that in infected WT N. benthamiana plants. In contrast to earlier results with potato plants (Franco-Lara et al., 1999), there were no distinguishing trends among samples taken from different leaf positions or from plants of different ages. ELISA values were little different between CW1 and infected WT plants [mean A405 of 0.71 (n = 33) and 0.68 (n = 28) respectively at a dilution of 1 g in 10 ml extraction buffer]. However, ELISA values for samples from transgenic N. benthamiana CW1 plants (n = 12) were about 5-fold greater than those for samples from transgenic tobacco AW3 plants (n = 6).

Comparison of PLRV accumulation in mesophyll cells of N. benthamiana and tobacco

Immunoprinting of leaf tissue from CW1 and PLRV-infected WT N. benthamiana plants detected antigen in many cells of tissue that appeared to be mesophyll. There were means of 2.46, 12.7 and 10.8 cells that contained detectable PLRV per cm2 in prints of leaf tissue from AW3 (tobacco), CW1 (N. benthamiana) and PLRV-infected WT N. benthamiana respectively (approx. 70 cm2 of leaf immunoprints were examined from each plant type). Such cells were not obviously associated with a vein. Some tracks left by veins could be seen and stained cells were associated with some of these (for example, see Fig. 2(f) in Franco-Lara et al. (1999)]. In CW1 and PLRV-infected WT N. benthamiana, about 25% of the stained cells occurred singly and were irregularly distributed but most were in clusters of between two and six adjacent cells (Fig. 1a, b, c) and a few clusters contained up to 20 cells (Fig. 1d). In AW3 leaves, there were fewer infected cells and clusters of infected cells were less numerous and smaller, but in a few areas there were many. In one area of an AW3 leaf approximately 15 mm across, about 1 in 20 of the cells were infected. In other areas only about 1 in 5000 of the cells were infected.

Effects of inoculation with PVY on PLRV accumulation

CW1 plants were inoculated with PVY and, 12 days after inoculation, systemically infected leaves were tested by ELISA and immunoprinting. The PLRV titre in these leaves was about 5-fold greater and the number of infected mesophyll cells was about 4-fold greater than in unoinoculated control CW1 plants (approximately 1 in 24 mesophyll cells were infected in PVY-infected CW1 plants, and in some areas of the tissue up to 1 in 10 mesophyll cells were stained). However, infected cells in these areas were interspersed with unstained cells, and the appearance was different from that of clusters in immunoprints of leaves inoculated with TMV\(\text{ACP}\)PLRV-CP in which there were few unstained cells in infected areas (see below).

After inoculation of AW3 plants with PVY, the amounts of PLRV in leaf tissue samples were compared with those in leaves of non-inoculated plants. PLRV titre changed little between 13 and 38 days post-inoculation (p.i.) (Fig. 2); the mean titres were 240 ng/g leaf in non-inoculated plants, and 1600 ng/g leaf in PVY-infected plants. From immunoprints of the non-inoculated AW3 plants, 1 in 3000 mesophyll cells were found to be stained, whereas in PVY-infected AW3 plants, 1 in 625 mesophyll cells contained PLRV and clusters of up to four infected cells were found. Thus potyvirus infection resulted in a 5- to 6-fold enhancement in the accumulation of PLRV in the transgenic plants. This raised the possibility that PLRV in the transgenic line was limited by PTGS.

Effects of TEV P1/HC-Pro on PLRV accumulation

The experiment above used virus infection to elicit the production in transgenic cells of HC-Pro. In more direct tests,
Fig. 1. Immunoprints of leaf mesophyll tissue developed for PLRV after removing the lower epidermis. Magnification bar in (a) is for (a)–(g) and represents 0.5 mm. Prints of half leaves (approx. 40 x 20 mm) are shown in (h)–(k). (a) PLRV-infected WT N. benthamiana showing infected cells, some dispersed and some in pairs; (b) PLRV-infected WT N. benthamiana showing a typical cluster of six cells; (c) CW1 N. benthamiana leaf showing a typical cluster of five cells; (d) CW1 N. benthamiana leaf showing a typical cluster of approx. 20 cells; (e) WT non-inoculated N. benthamiana; (f) PLRV-infected WT N. benthamiana 4 days p.i. with TMV(ΔCP)PLRV-CP; (g) WT N. benthamiana 8 days p.i. with TMV(ΔCP)PLRV-CP; (h) WT non-inoculated N. benthamiana; (i) WT N. benthamiana 8 days p.i. with TMV(ΔCP)PLRV-CP; (j) PLRV-infected WT N. benthamiana 8 days p.i. with TMV(ΔCP)PLRV-CP; (k) CW1 N. benthamiana 8 days p.i. with TMV(ΔCP)PLRV-CP.
TEV P1 was produced as a result of potyvirus infection and suggests that expression of HC-Pro diminishes, at least in part, the resistance to PLRV accumulation in the mesophyll.

### Inoculation of N. benthamiana with TMV(ACP)PLRV-CP

As an alternative system for examining PTGS-like effects on the accumulation of PLRV RNA sequences, we used the chimeric virus TMV(ACP)PLRV-CP in which the CP gene of TMV RNA had been replaced by the CP gene of PLRV. This virus accumulates in N. benthamiana but not in N. clevelandii or N. tabacum. It did not move systemically, probably because the CP is essential for long-distance movement of TMV (Carrington et al., 1996) and because PLRV CP is unable to provide this function.

When TMV(ACP)PLRV-CP was inoculated to WT N. benthamiana plants, large areas (up to 15 mm across) containing many stained cells were visible by eye at 4 days p.i., and at 8 days p.i. approximately 80% of the leaf area (approx. 70 cm² of immunoprints examined) was strongly stained (Fig. 1i). By microscopy, it was possible to see that the majority of individual cells in these visibly stained areas were strongly stained with indoxyl precipitate (Fig. 1g). These results suggested that virus had moved from cell to cell in these leaves. The result was different when TMV(ACP)PLRV-CP was inoculated to PLRV-infected WT N. benthamiana or CW1 plants. In these plants, by 4 days p.i. antigen-containing cells were in clusters of up to approximately 200 cells (Fig. 1f). At 8 days p.i. the areas of staining were much larger and clusters of up to several hundred cells could be seen in the microscope, and by eye approximately 35% of the leaf area was stained (Fig. 1j, k; 160 cm² of immunoprints examined). As a control, we inoculated plants with the chimera TMV(ACP)-GFP in which the TMV CP gene was replaced by sequences encoding GFP. In these plants, and in contrast to results with TMV(ACP)PLRV-CP, there was no difference in virus dis-

### Table 1. PLRV titre in leaf samples from progeny plants from crosses between AW3 and P1/HC-Pro lines U-6B and X-27-8

<table>
<thead>
<tr>
<th>Cross between AW3 plants as female parents and pollen from other sources</th>
<th>PLRV titre* (ng/g leaf)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW3 × WT Havana 425</td>
<td>168</td>
<td>±12</td>
</tr>
<tr>
<td>AW3 × U6B</td>
<td>1467</td>
<td>±333</td>
</tr>
<tr>
<td>AW3 × WT Xanthi</td>
<td>292</td>
<td>±49</td>
</tr>
<tr>
<td>AW3 × X-27-8</td>
<td>4129</td>
<td>±830</td>
</tr>
</tbody>
</table>

* PLRV titre in leaf samples was measured in six plants from each progeny at the 6/7 leaf stage.
tribution (measured as that of GFP fluorescence) between WT N. benthamiana and CW1 N. benthamiana. These results suggest that the delay in spread of TMV(∆CP)PLRV-CP in inoculated leaves of PLRV-infected WT N. benthamiana and CW1 plants occurred because of specific resistance against the virus containing PLRV sequences.

Discussion

A small proportion of mesophyll cells (about 2%) in transgenic CW1 N. benthamiana contained detectable amounts of PLRV. Surprisingly, we also found that approximately 2% of mesophyll cells in PLRV-infected WT N. benthamiana that had been inoculated by aphids also contained virus. The titres of PLRV, estimated by ELISA, and the extent of mesophyll cell infection were about the same in CW1 plants and in PLRV-infected WT N. benthamiana. Franco-Lara et al. (1999) found very few stained mesophyll cells (less than 1 in 40 000) in tobacco plants transformed with the same PLRV-FL transgene, but no stained mesophyll cells were found in PLRV-infected WT tobacco plants. Because stained mesophyll cells occurred in WT N. benthamiana plants inoculated with PLRV, we assume that such cells were infected following cell-to-cell movement of PLRV from cells within vascular tissue, as was shown by Barker (1987) and van den Heuvel et al. (1995) in N. clevelandii and potato respectively. In CW1 plants, mesophyll cells could be infected via a similar route, or as a result of transcript of the full-length PLRV transgene initiating infection in particular cells, as was found in tobacco by Franco-Lara et al. (1999). However, because similar numbers of infected mesophyll cells were detected in transgenic and WT plants, it seems likely that the majority of stained mesophyll cells in CW1 plants became infected via the same route, as do the cells in WT plants inoculated with PLRV.

Immunoprints of leaves from CW1 and PLRV-infected WT N. benthamiana showed that a large proportion of stained mesophyll cells were located in clusters (groups of between 2 and 20 cells). This finding prompted a re-examination of AW3 tobacco plants because such clusters were not detected by Franco-Lara et al. (1999). In immunoprints of leaves from AW3 plants, we found that a proportion of stained mesophyll cells were also located in small clusters, although such clusters were generally not as large as those in N. benthamiana leaves. Furthermore, after examining a large area of immunoprints from AW3 leaves (250 cm²), it was apparent that stained cells were not evenly distributed, and that in some areas of some leaves stained cells were much more numerous than in other areas. For example, in a small area (approx. 5 mm across) in one leaf print, about 1 in 20 of the cells contained detectable PLRV. However, stained cells were interspersed with apparently non-infected cells. Similar clusters may have been present in the tobacco plants assessed by Franco-Lara et al. (1999) but not identified because the sampled tissues contained few cell clusters. In contrast, in N. benthamiana leaves stained cells were distributed relatively evenly.

A similar effect has been demonstrated by Angell & Baulcombe (1997), who transformed plants with amplicons of the non-phloem-limited virus Potato virus X (PVX). The amplicons comprised the intact PVX genome modified to carry the β-glucuronidase (GUS) reporter gene. Angell & Baulcombe (1997) suggested that the transgenic plants carrying these constructs displayed several phenotypes attributable to PTGS, one of which was that expression of GUS activity (and apparently of PVX) was restricted to single cells, or small groups of cells. These plants thus resemble those of CW1 or AW3 that, although presumably synthesizing full-length PLRV transcript RNA in all cells, accumulated virus in only a few. As discussed by Franco-Lara et al. (1999), this may indicate that a resistance mechanism is induced in these transgenic plants that restricts the establishment of infection. Our findings indicate some characteristics of this resistance and suggest that it results, at least in part, from PTGS.

This idea was further supported by experiments with the chimeric virus TMV(∆CP)PLRV-CP. When this virus was inoculated to WT plants, virus multiplication was extensive in inoculated leaves; infected areas were visible by 4 days p.i., and about 80% of the leaf area was infected by 8 days p.i. By contrast, when CW1 and PLRV-infected WT N. benthamiana plants were inoculated with TMV(∆CP)PLRV-CP, virus spread and accumulation was slower, and, although by 8 days p.i. many cells were infected, only 35% of the leaf area was infected. This delay (partial resistance) seems to be PLRV sequence-specific, because TMV(∆CP)-GFP (which lacked PLRV CP sequences) replicated and spread rapidly in infected and PLRV-free tissues.

Although in many instances transgenes are designed to maximize expression, transgene expression can be downregulated by PTGS. PTGS is manifested as the reduction in steady-state levels of specific RNAs after expression of exogenously introduced homologous nucleotide sequences (Marathe et al., 2000; Ding, 2000; Vance & Vaucheret, 2001). PTGS involves the systemic spread of a silencing signal that directs sequence-specific RNA degradation. Recently, it has been shown that PTGS and virus resistance are related phenomena. Plant viruses can both induce and be the targets of PTGS (Ratcliff et al., 1997; Covey et al., 1997). Indeed, Angell & Baulcombe (1997) have shown that replicating viral RNA can induce PTGS. Several aspects of our observations suggest that the phenomena we report here resemble those of PTGS. Thus, in plants transformed with the PLRV-FL transgene, only a few cells accumulated detectable amounts of PLRV, even though infectious PLRV transcript was presumably produced in all cells. Possibly the PLRV transgene was transcribed and PLRV replicated first in a few cells and after that a silencing signal induced in these cells spread to other cells in the plant, where it prevented further accumulation of PLRV. It is also possible that such a process could contribute to the restriction
of PLRV accumulation to very few cells after movement out of phloem tissue in infected WT plants.

In either WT N. benthamiana infected by aphids or CW1 N. benthamiana, most of the cells that contained PLRV were in small groups. Co-infection of CW1 and AW3 plants with PVY, or crossing AW3 plants with TEV P1/HC-Pro transgenic plants, increased the number of PLRV-infected cells. Although in these circumstances the clusters of infected cells remained fairly small, these clusters were more numerous than those in plants not infected with PVY or not containing TEV P1/HC-Pro. These clusters of cells may represent areas of tissue in which the putative silencing effect has been downregulated by a signal emanating from some of the first cells to support virus replication. Such clusters of cells were present in AW3 tobacco, but with the rare exception of a couple of relatively large areas of staining, clusters were smaller than those in N. benthamiana. It is possible that a small increase in anti-PTGS activity has a greater effect in N. benthamiana than in tobacco plants.

It has been generally accepted that some plant viruses, such as potyviruses, can suppress PTGS (Anandalakshmi et al., 1998; Brigneti et al., 1998; Kasschau & Carrington, 1998), and potyviral HC-Pro protein has been identified as a specific suppressor of PTGS (Anandalakshmi et al., 1998; Brigneti et al., 1998). Recently Savenkov & Valkonen (2001) showed that HC-Pro protein expressed in PLRV-infected transgenic plants enhanced titres of PLRV in the phloem. Similarly, the levels of PLRV accumulation and the numbers of cells that contained PLRV in AW3 and CW1 plants were significantly increased when plants were co-infected with the potyvirus PVY. Furthermore, in the progeny of crosses between AW3 plants and tobacco plants that contained sequence encoding TEV P1/HC-Pro, the accumulation of PLRV and the number of infected cells were increased between 10- and 20-fold. These results show that in AW3 and CW1 plants there is resistance to PLRV replication that is also partially alleviated in mesophyll tissues by the presence of potyviral P1/HC-Pro. Recently we have demonstrated that another silencing suppressor, 2b protein encoded by CMV, is able to facilitate multiplication of PLRV in mesophyll tissues (Ryabov et al., 2001). Taken together, these results (Savenkov & Valkonen, 2001; Ryabov et al., 2001; and the present work) strongly suggest that the observed increases in accumulation of PLRV in certain mesophyll cells are the result of suppression of PTGS.

However, even though the PTGS-like silencing was suppressed in mesophyll cells, most remained apparently virus-free. The CMV silencing suppressor protein 2b can only assist PLRV to spread in mesophyll tissue when supplemented by an umbravirus movement protein (Ryabov et al., 2001), indicating that cell-to-cell movement of PLRV is also defective in these tissues. Thus, either suppression of silencing was incomplete because of the absence of an HC-Pro effect on systemic silencing, as suggested by Mallory et al. (2001), or an additional resistance mechanism also operated, or both. It seems unlikely that PLRV moves extensively from cell to cell in mesophyll/epidermis tissues forming areas (groups) of infected cells because PLRV, like other luteoviruses, lacks a cell-to-cell movement function in mesophyll/epidermis. Our results are consistent with PLRV being principally confined to the phloem of conventionally infected plants because of a combination of a failure to suppress gene silencing (Voinnet et al., 1999; Waterhouse et al., 1999) and a lack of a cell-to-cell movement function protein, as suggested by Atabekov et al. (1984) and Barker (1989).

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References


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