

Plants transformed to express the entire genome of *Potato leafroll virus*

H. Barker, M.A. Mayo, K. McGeachy, F. Franco-Lara¹, U. Commandeur² & R.R. Martin³

Introduction *Potato leafroll virus* (PLRV) is transmitted in a persistent non-propagative manner by aphids that put virus into the vascular tissue of plants, where it remains largely restricted to the phloem cells. PLRV is not mechanically transmissible but plants can be agroinoculated by direct injection of *Agrobacterium tumefaciens* cells that carry a DNA copy of the virus genome in a Ti plasmid. Transcripts of the plasmid then initiate infection. As when aphids inoculate plants, the resulting infections are limited to phloem tissue. An alternative way of initiating virus replication in a plant is by stable transformation with a full-length (biologically active) cDNA copy of a virus genome, and this has been done for a number of viruses with RNA genomes. In recent work, we have transformed tobacco and potato plants with full-length infectious cDNA to the PLRV genome. These plants are providing novel and unexpected insights into the replication of PLRV and its interaction with the host genome.

Transformation of plants and characterisation of transgenic lines A full-length cDNA copy of the PLRV genome was cloned into a plasmid used for *Agrobacterium tumefaciens*-mediated transformation. The construct was used to transform tissues of *Nicotiana tabacum* cv. 'Samsun' and the potato genotypes cv. Maris Piper and the highly PLRV-resistant SCRI breeding line G8107(1). The transgene contains the PLRV sequence under the transcriptional control of the 35S constitutive promoter from *Cauliflower mosaic virus*, and the selectable marker gene, NPTII, that confers resistance to kanamycin.

Two transgenic lines of tobacco were obtained and seeds were collected from

self-fertilised flowers. The transgene was detected in DNA extracted from T₁ seedlings by PCR. The observed segregation data of kanamycin-resistant:sensitive seedlings from lines AW3 and AW14 indicate that T-DNA was inserted at two loci in the genome of the T₀ parental plants.

It was not possible to obtain transgenic lines of Maris Piper expressing PLRV, because transformed callus grew poorly, then turned brown and died without regenerating shoots. ELISA showed that PLRV coat protein (CP) was made in the callus cells; it is possible that PLRV replication in the callus caused stress that inhibited growth and shoot regeneration.

Transformation of *S. tuberosum* clone G8107(1), which has strong host-mediated resistance to PLRV accumulation, produced vigorously-growing callus from which shoots regenerated. Plantlets of four transgenic lines (BF#lines) were transferred to the glasshouse and grown to maturity to produce a few tubers.

PLRV expression in transgenic plants

Tobacco: Many T₁ seedlings of the two transgenic tobacco lines were found to contain PLRV antigen (detected by ELISA of leaf tissue) from an early stage in their growth. Transgenic plants were visually indistinguishable from infected wild-type (wt) plants and bore no symptoms. No PLRV could be detected in approximately 25% of T₁ transgenic plants by serological tests, and PLRV RNA was not detected by hybridization tests in such plants. The concentration of PLRV detected by ELISA varied greatly among plants of the same line. Thus, plants of line AW3 with the highest concentration of antigen, contained 12-fold more than the plant with the lowest concentration, and in line



¹ Present address: Facultad de Biología Aplicada, Universidad Militar Nueva Granada, Carrera II, NO-101-80, Bogotá, Colombia

² University of Aachen (RWTH), Institute for Molecular Genetics and Botany (Biologie I), Worringerweg 1, D-52074 Aachen, Germany

³ USDA-ARS HCRL, Northwest Center for Small Fruit Research, 3420 NW Orchard Ave. Corvallis, OR 97330, USA

Host pathogen interactions & crop protection

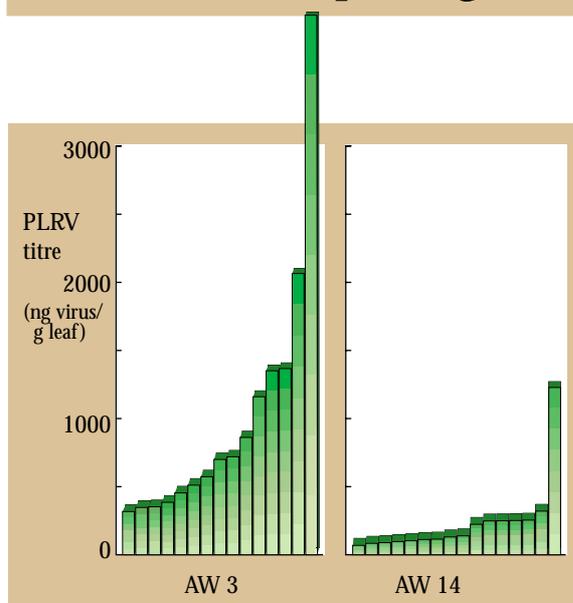


Figure 1 Detection of PLRV antigen in transgenic plants of lines AW3 and AW14, 10 weeks after sowing. Data from five AW3 and four AW14 plants that were transgenic but in which no virus could be detected, are not shown. The bars represent virus titre (ng virus/g leaf) in young fully-expanded leaves from individual plants.

AW14 the difference was 18-fold (Fig. 1). However, occasionally virus could not be detected in leaf samples of plants that produced other infected leaves, presumably because the sensitivity of the ELISA was insufficient to detect the low concentration present. The amounts of PLRV antigen in young fully expanded leaves of AW3 transgenic plants and of infected wt plants were similar; in 8 week-old plants, the mean titres were about 600 ng virus/g leaf.

Potato: Glasshouse-grown plants of BF lines initially did not look substantially different from infected wt plants of G8107(1); they did not show the 'leafrolling' symptoms characteristic of PLRV infection. However, as the plants aged, most leaves of BF plants, except those at the top of the stem, became necrotic and died prematurely and plants were severely stunted in comparison to PLRV-infected wt

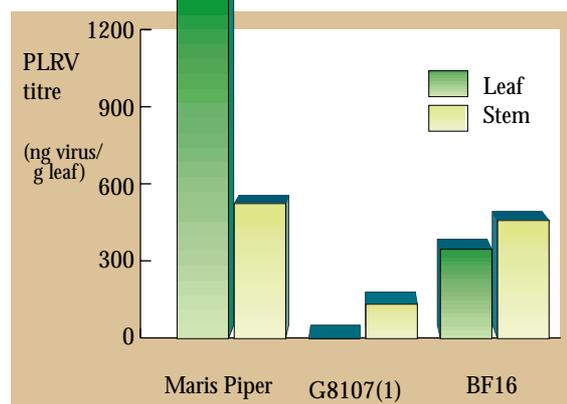


Figure 2 Detection of PLRV in leaf and stem tissue of wt infected plants of Maris Piper, line G8107(1) and transgenic line BF16.

plants of G8107(1), which continued to grow without symptoms. BF plants remained severely stunted throughout their lives and only produced a few small tubers. Plants grown from these tubers, and tested by ELISA, contained high concentrations of PLRV, whereas plants grown from tubers of infected non-transgenic G8107(1) contained very little virus (Fig. 2). More PLRV could be detected in stem tissue than in leaves of G8107(1) and BF plants, but the reverse was true for Maris Piper plants (Fig. 2). Both leaf and stem tissue of infected wt plants of the susceptible cv. Maris Piper contained high concentrations of PLRV (Fig. 2).

Properties of PLRV in transgenic tobacco plants

The virus-like particles that accumulated in the transgenic plants were indistinguishable from those of PLRV from conventionally infected plants, in immunosorbent electron microscopy (ISEM) tests. The PLRV that accumulated in the transgenic plants infected all receptor plants, when scions from transgenic plants were grafted to virus-free, non-transgenic tobacco plants, and was transmissible by *Myzus persicae* to virus-free receptor plants of *Physalis floridana*, *N. clevelandii* or *N. tabacum*.

Location of PLRV by tissue printing The number and distribution of cells

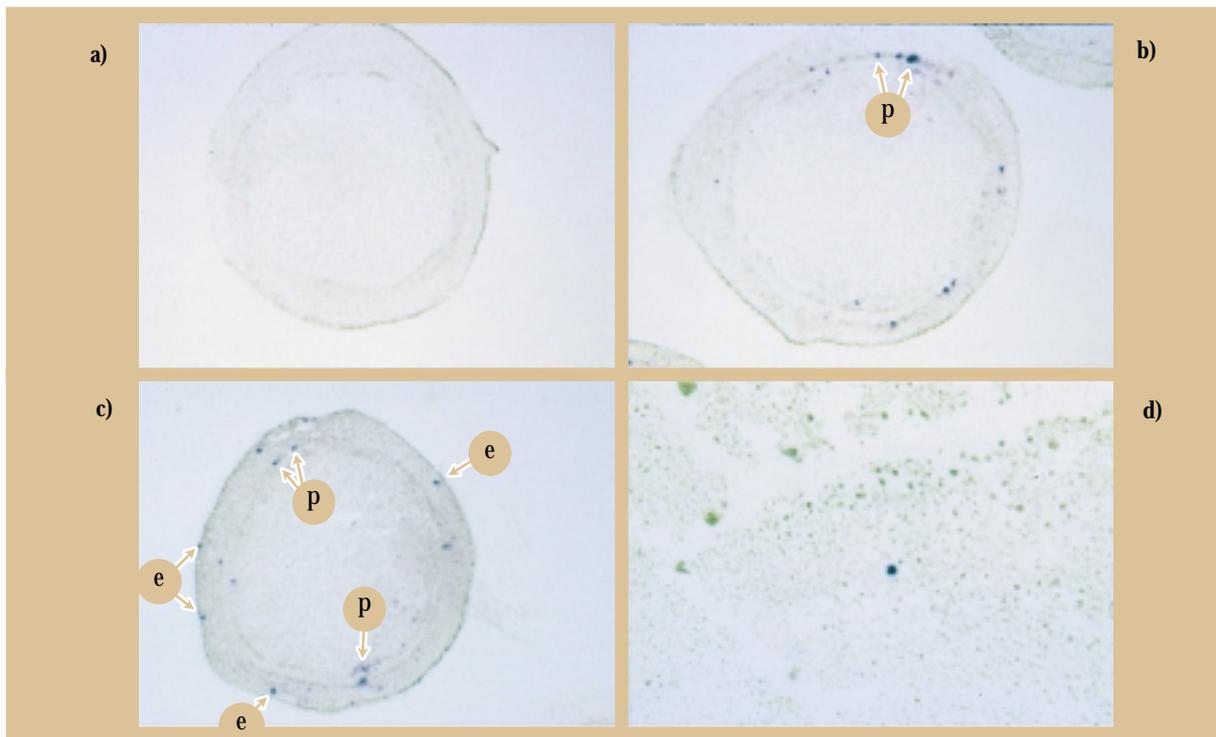


Figure 3 Detection of PLRV-infected cells in tissue prints. (a) Stem print of a virus-free non-transgenic tobacco. (b) Stem print of a PLRV-infected non-transgenic tobacco. Stained phloem cells are labelled 'p'. (c) Stem print of transgenic AW3 tobacco. Stained phloem cells are labelled 'p' and stained epidermal cells are labelled 'e'. (d) Leaf print of transgenic AW3 tobacco showing one stained cell.

containing PLRV was assessed in leaves and stems of transgenic plants by making tissue prints (immunologically stained imprints of tissues on a nitrocellulose membrane). Stained spots, indicating the location of infected cells, were readily identified in transgenic and infected wt plants, but were not seen in any of the prints made from virus-free plants.

Tobacco: Tissue prints of tobacco stem sections showed that the majority of phloem cells in transgenic and infected wt plants were unstained, with less than 5% of the phloem companion cells estimated to be infected (Fig. 3b and 3c). However, a few infected cells were also observed in epidermal tissue of stem sections from some transgenic tobacco plants (a mean of about one cell per section), but PLRV was never detected in epidermal cells from infected wt plants (Fig. 3c vs. 3b).

Tissue prints were also made of leaf lamina of transgenic tobacco from which the lower

epidermis had been removed by peeling. Tissue pieces were pressed onto nitrocellulose membranes to leave an imprint of the exposed tissue. Infected 'mesophyll' cells were observed in leaves of transgenic tobacco plants (Fig. 3d) but not in infected wt plants. In some transgenic tobacco leaves, mesophyll cells were aggregated to form small clusters. 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 transgenic leaves. We estimate that about one in 40000 mesophyll cells of these leaves had accumulated detectable amounts of virus.



Potato: Tissue prints of stem sections of Maris Piper showed that many phloem cells in infected wt plants were stained, although far fewer cells were infected in PLRV-infected G8107(1) (Fig. 4b) (less than a third in comparison to Maris Piper). Many phloem cells were infected in stems of transgenic plants of BF lines.

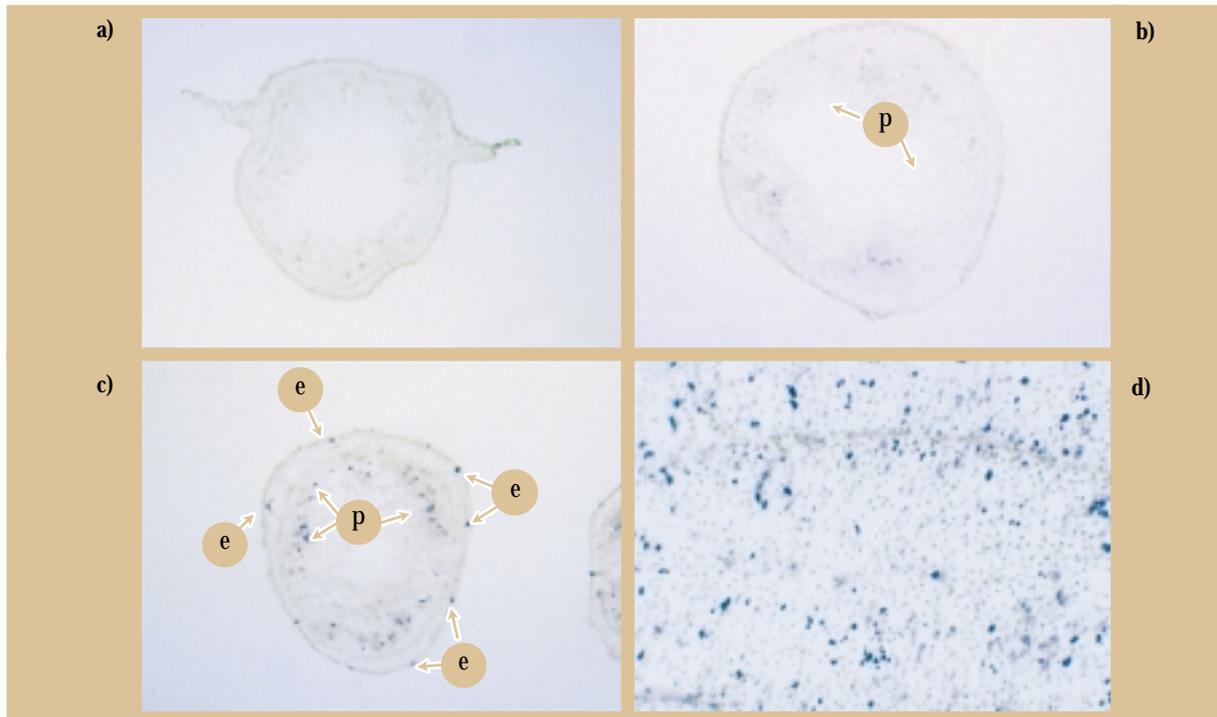


Figure 4 Detection of PLRV-infected cells in tissue prints. (a) Stem print of a virus-free non-transgenic potato. (b) Stem print of a PLRV-infected non-transgenic G8107(1) potato. Stained phloem cells are labelled 'p'. (c) Stem print of transgenic BF16 potato. Stained phloem cells are labelled 'p' and stained epidermal cells are labelled 'e'. (d) Leaf print of transgenic BF16 potato showing many stained cells.

More interestingly, a substantial proportion of infected cells also were observed in stem epidermal tissue, although PLRV was never detected in epidermal cells of infected wt G8107(1) plants (Fig. 4c vs. 4b). Tissue prints of leaf lamina of transgenic plants of BF lines showed that many cells were infected in some samples (Fig. 4d). However, the distribution of infected mesophyll cells was very erratic and some areas of leaf contained no infected cells, while neighbouring areas of the same leaf were very heavily infected.

Discussion The simplest expectation for plants transformed with cDNA copies of the PLRV genome is that all cells will produce transcript RNA and virus will thus multiply in all cells. But this did not happen with tobacco or with the highly PLRV-resistant potato clone. Thus, some mechanism was restricting PLRV multiplication in most of the cells of these plants. It is possible that the failure of the susceptible potato cultivar to survive the transformation process

was because too many cells accumulated virus and the effects were too debilitating. If this simple model is correct, then the approach of transforming plants with entire genomes has the potential for exploring the mechanism(s) by which cells can resist PLRV multiplication after the virus RNA genome arrives in the cell. This contrasts with the normal, and more complex, situation in which virus inocula must travel within the inoculated plant in order to reach the tissues in which resistance might be expressed.

This approach thus separates the infection process into two phases. The extra-cellular phase includes biological effects on the vector and on the host. In other words, it is a compound of the components of the delivery process. The intra-cellular phase concerns the establishment of the infection and the accumulation of the progeny virus. This novel dissection of the infection process could lead us to new insights into how plants naturally resist infection by PLRV.