Fluorescent tagging of *Potato leafroll virus*

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Potato leafroll virus (PLRV) is an important pathogen of potato crops. The disease it elicits can cause significant crop losses and is a constraint in the production of high quality virus-free seed potato crops. PLRV is one of several viruses in the family *Luteoviridae* that cause significant diseases in a variety of crops world-wide. One of the defining characteristics of viruses in this family is that they are transmitted by aphids in a circulative, persistent fashion. In recent years, research at SCRI has focused on critical events in the transmission cycle of PLRV in its aphid vector(s), both for their intrinsic scientific interest and in a search for an "Achilles heel" in virus biology at which control measures could be aimed.

Figure 1

Transmission occurs when virus particles are taken up by aphids feeding on infected plants, absorbed from the gut, transported from the aphid haemolymph (= body fluid) into the aphid accessory salivary glands and then excreted into new plants when the aphid vector feeds. Another characteristic of viruses in the family *Luteoviridae* is that they are relatively specific for certain aphid species, presumably because of molecular interactions at cell boundaries during the elaborate transmission process. Electron microscopy has revealed some details of these processes (*SCRI Ann. Rep.* 1996/1997, 164-167) but what happens between the arrival of an aphid at a new plant and the plant becoming infected by PLRV is something of a 'black hole'. As with many such unexplored areas, new methods of molecular biology are now starting to shine light into this part of the PLRV infection cycle.

In previous Annual Reports, it has been shown how DNA encoding the jellyfish green fluorescent protein (GFP) can be added to virus genomes so that virus multiplication causes host cells to become fluorescent. This has revealed where viruses multiply and details of how they move between infected and healthy cells (*SCRI Ann. Rep.* 1997/1998, 67-70). This fluorescent tagging has now been achieved with PLRV by modifying the virus genome – the first time that this has been done with the genome of a luteovirus.

illustrates the modification principle. DNA that encodes GFP was inserted near the 3' end of open reading frame (ORF) 5 of PLRV. This ORF codes for P5, a minor protein that is thought to lie on the surface of PLRV particles. The effect of the insertion of the GFP gene was to replace the C-terminal 100 amino

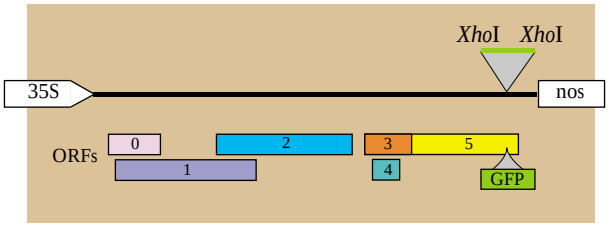

Figure 1 Construction of pPLRV-GFP. cDNA that corresponded in sequence to PLRV RNA was inserted either between the 35S promoter and the nos terminator in a Ti plasmid or downstream of a sequence with T7 RNA polymerase promoter activity. The encoded open reading frames (ORF; numbered 0 to 5) are shown in different colours. ORF 5 was cut with *Xho*I and cDNA that encoded GFP was inserted as shown.

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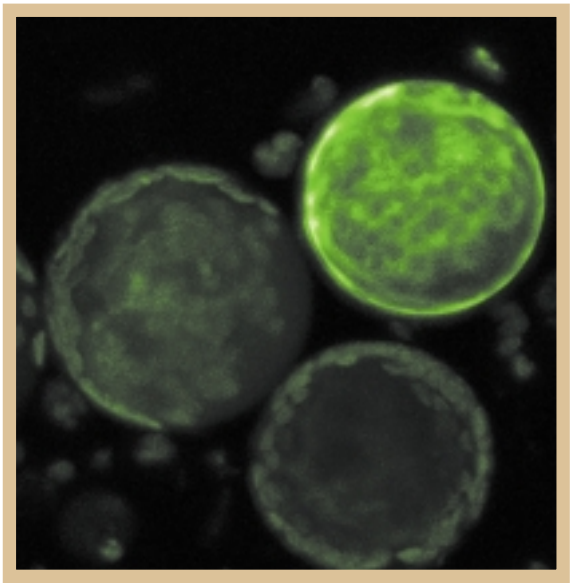

Figure 2 Protoplasts from a sample electroporated with transcript RNA corresponding in sequence to PLRV-GFP. The protoplasts were cultured at c. 20^{O}C for 3 days. The protoplast at the top right was fluorescent, demonstrating infection by PLRV-GFP, those in the bottom left were not infected.

acids of P5 with the amino acid sequence of GFP. It was known from work with mutant strains of PLRV that virus that lacks this part of P5 is able to multiply in infected plants. Plasmid DNA that contained the entire sequence of PLRV RNA, together with the added GFP gene was propagated in bacteria, purified from them and then used as a template for the synthesis of genomic RNA of PLRV-GFP. This was used to electroporate tobacco protoplasts. After culture for 3 days, a number of the electroporated protoplasts were shown by light microscopy to be fluorescent, that is they contained GFP (Fig. 2). When proteins in these protoplasts were examined by immunoblotting, the protein molecules containing P5 were larger than P5 molecules from control protoplasts infected with unmodified PLRV by an amount that corresponded in size to the added GFP. Thus most, or all, of the P5 molecules that were synthesized in protoplasts infected with PLRV-GFP contained extra amino acid sequence, presumably GFP. Examination of extracts by electron microscopy showed that the protoplasts contained PLRV-like particles. These bound to EM grids that had been coated either with antibodies to PLRV particles or with antibodies specific to GFP (Fig. 3a,b). When these trapped particles were incubated with gold-labelled antibodies to GFP, gold was bound to the particles (Fig. 3c). These results show that the PLRV particles carry GFP molecules on their

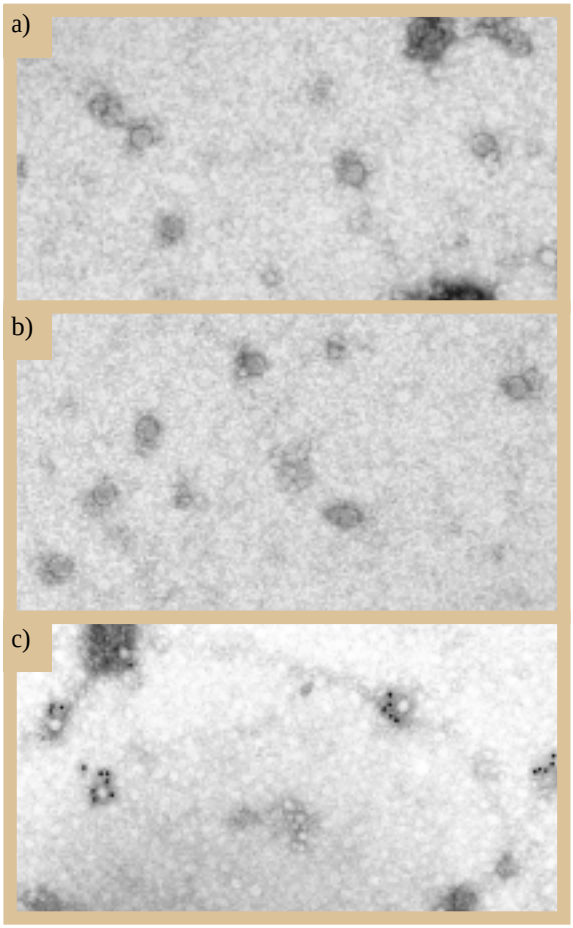

Figure 3 Immunosorbent electron microscopy of extracts of protoplasts infected with PLRV-GFP. (a) particles trapped by antibodies to PLRV particles; (b) particles trapped by antibodies to GFP; (c) particles trapped by antibodies to PLRV particles and then decorated with antibodies to GFP that had been conjugated to gold (black dots).

surfaces, presumably attached to the C-terminal part of the P5 protein carried by a proportion of the major coat protein molecules.

When aphids were fed through membranes on extracts of protoplasts infected by PLRV-GFP for 24 hours and then transferred to test plants, these became infected. Thus, the particles that were carrying GFP on their surfaces were able to pass through the bodies of vector aphids and enter new host plants to initiate infection. When the leaves on which the aphids had fed were examined by light microscopy about 2 weeks after aphid feeding, a small number of cells were fluorescent. These cells were of various types, including epidermal cells and trichomes, but very few such cells were adjacent to other fluorescent cells (Fig. 4). Although PLRV infected these plants systemically,

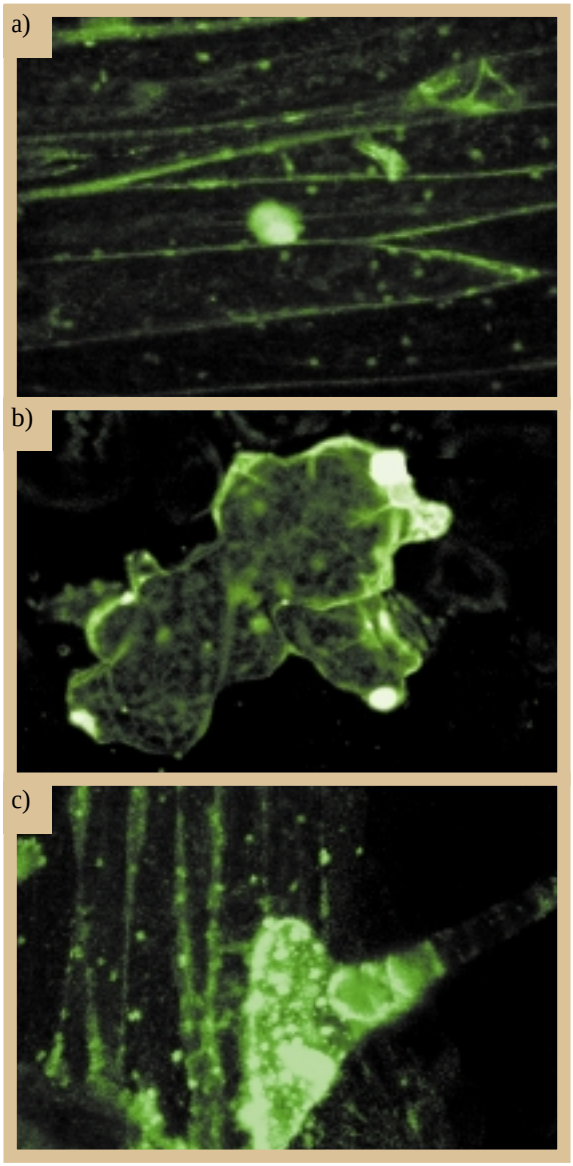

Figure 4 Cells in leaves that had been probed by aphids carrying PLRV-GFP. Some cells of the vascular tissue (a), the epidermis (b), and trichome cells (c) were shown to be infected.

there was no spread of fluorescence. Reverse transcription/PCR with PLRV RNA-specific primers showed that systemically infected tissues of these plants contained P5 genes that had lost some or all of the inserted GFP-coding sequences, presumably because of the deletion that allowed the PLRV to spread.

An alternative method of infecting plants with PLRV in a DNA form is by agroinoculation. In this method, DNA copies of the PLRV genome are inserted into the Ti plasmid and this is introduced into plant cells by injection (agroinoculation) into petioles or infiltration into leaf spaces. As with inoculation by aphids, agroinoculation resulted in infected plants, but none of the infected plants contained PLRV identical to the inoculum PLRV-GFP. All the progeny virus had deleted genomes. Agroinfiltration resulted in cells that were fluorescent because PLRV-GFP had multiplied, but none of the plants became infected.

These results show that the modification of adding the GFP gene to the genome of PLRV results in a genome that cannot move from cell to cell. The oversize RNA was encapsidated in particles that were then transmissible by aphids, and thus were intact, but cell-to-cell movement was not possible. The plants infected by aphids transmitting PLRV-GFP, or by agroinoculation, contained PLRV particles that contained RNA with all or most of the added GFP gene removed by deletion. Thus, some feature of the spread of PLRV infection is prevented if the genome of the virus is oversize.

Nevertheless, what were revealed for the first time by the experiments in which aphids carrying PLRV-GFP fed on plants, were the cells into which viruliferous aphids had introduced PLRV. These cells were of various types, which shows the extent to which aphids probe different leaf cells sufficiently long to transmit PLRV. The system therefore has promise as a means of assessing how the behaviour of aphids relates to the introduction of PLRV into leaf cells.