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Characterisation of a tobamovirus from trailing petunias

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Abstract

A tobamovirus has been identified as being involved in a devastating disease of trailing petunia. Results from indicator plants and ELISA suggested that the tobamovirus was a strain of *Tobacco mosaic virus* (TMV). This was confirmed from the full sequence of the coat protein gene and a partial sequence of the replicase gene. Sequence analysis revealed that TMV isolated from diseased petunia had high identity (ca. 98–99) with TMV vulgare type sequences reported from Korea and Japan. Mechanical inoculation of 23 varieties, representing 21 species of pot and bedding plants with the petunia isolate of TMV confirmed that 11 were infected by the petunia isolate of TMV, although several species remained symptomless after three weeks. This highlights a clear risk to a number of commercially important pot and bedding plant species from TMV infected trailing petunias.

Introduction

In 1995, there was a well-publicised disease problem in trailing petunias in the UK (Campos, 1995) and elsewhere in Europe (Lesemann, 1996; Bellardi et al., 1996). In the UK, a crop with an estimated UK retail value of £15 million was suddenly withdrawn from sale due to widespread infection with plant viruses. Individual growers' losses in the UK were as high as £100,000 due to the necessary destruction of affected crops (Spence et al., 1996). A number of viruses have been reported to infect trailing petunias. Preliminary investigations (Spence et al., 1996; Wright and Spence, 1997) revealed that two viruses with virus particle lengths of 300 nm (rod-shaped) and 750 nm (flexous rod-shaped) were often found together suggesting a tobamovirus and a potyvirus, which has recently shown by Boonham et al. (1999) to be an isolate of *Potato virus Y* (PVY^{NTN}). Also reported, but incidental to the problem were *Alfalfa mosaic virus*, *Tomato spotted wilt virus* and PVY$^{\circ}$ (Spence et al., 1996). It is likely that the virus problem started by infected propagation material being used, but the extent to which viruses have been spread by other means is not clear. Tobamoviruses have no known insect vector but are readily spread in plant sap by plant to plant contact, handling by workers, on glasshouse structures and utensils and by plant propagation. The virus also persists in plant debris and on glasshouse benching so that strict hygiene is necessary to control its spread. Tobamoviruses have a very wide host range so there is a risk of spread of virus to other crops in the glasshouse. The objective of this study was to characterise the tobamovirus involved in causing disease in infected trailing petunias.

Materials and methods

Virus symptoms were observed in three samples of diseased Surfinia™ trailing petunias obtained from three independent batches of nursery stocks within the UK. Virus isolates were maintained by sap inoculation to petunia (Surfinia™) and *Nicotiana tabacum* White Burley. Leaf samples of trailing petunias thought to be infected by virus were examined in the electron microscope using the 'quick dip' method (Brandes, 1957).

Direct antibody sandwich (DAS) ELISA was used for serological characterisation (Clark and Adams, 1977). Coating globulin prepared from purified antiserum against the following tobamoviruses: *Tobacco mosaic virus* (TMV Type strain), *Tomato mosaic virus* (ToMV) and *Pepper mild mottle virus* (PMMV) was applied at 1 μg/ml and antibody conjugated to alkaline phosphatase was used at 1/1000. Absorbence values ($A_{405\,nm}$) were measured 60 min after addition of the substrate and a result was considered to be positive if the absorbence was a least twice that of mean uninfected control sap. A universal potyvirus-detecting monoclonal antibody (Agdia, USA) was used in plate trapped antigen (PTA) ELISA for determining mixed infections by potyviruses. Several *Nicotiana* indicator species were sap inoculated with petunia tobamovirus and symptoms were observed after three weeks. Three six-week-old plug plants of twenty-three varieties of twenty-one of the most popular species of bedding and basket plants were sap-inoculated with petunia tobamovirus. Symptoms were recorded weekly and after three weeks, plants were tested for systemic infection by ELISA using antibodies to TMV Type strain.

For molecular analysis of the tobamovirus, the three isolates were sap inoculated to *N. tabacum*. RNA was extracted from infected tobacco, according to the method described by Napoli et al. (1990). RT-PCR was carried out using a commercial kit, with PCR products being cloned into pCR2.1 (Invitrogen) for subsequent analysis and sequencing. Primers designed upstream from the start of the coat protein gene (TMV-CP-F 5′CGCCGAATCGGATTCGTT3′) and downstream of the stop codon (TMV-CP-R 5′TTATGCATCTTGACTACC3′) based on the TMV vulgare genome sequence (Goelet et al., 1982) were used to clone the coat protein gene. Two degenerate primers designed against the amino acid sequences 'CPADVTH' and 'MYKVDA' which are highly conserved amongst tobamoviruses, were used to generate clones of the replicase gene, equivalent to nucleotide positions 2992–3389 on the TMV vulgare genome (Goelet et al., 1982).

Results

Virus symptoms in trailing petunia were distinct and included leaf mosaic, chlorotic mottling, vein clearing and distortion of leaves and stems (Figure 1) and growth of the whole plant was sometimes stunted. Some flowers also developed petal-break symptoms (Figure 2). Rigid rod-shaped virus particles approximately 300 nm long and 18 nm wide were observed when trailing petunia leaf samples were examined in the electron microscope, which indicated infection by a tobamovirus. In some samples, there was a mixed infection with flexous rod-shaped particles of approximately 750 nm long, which indicated the presence of a potyvirus (Figure 3). Additional infections of potyvirus were confirmed in several samples using the universal potyvirus-detecting monoclonal antibody (Agdia, USA) in ELISA. Petunia tobamovirus reacted positively in ELISA to antibodies against the Type strain of TMV and did not react with antibodies against ToMV and PMMV (data not shown).

Petunia tobamovirus induced systemic mosaic symptoms in *N. tabacum* White Burley, *N. occidentals*, *N. rustica*, *N. glutinosa* and *N. clevelandii*. In comparison, a ToMV isolate from tomato induced only local lesions in *N. tabacum* White Burley, *N. occidentalis*, *N. rustica*, *N. glutinosa* and *N. clevelandii*. Of the 23 varieties of pot and bedding plants inoculated, 11 were infected by TMV as shown by ELISA, although several species remained symptomless after three weeks (Table 1).

Two individual clones containing the coat protein gene from each of the three tobamovirus isolates were sequenced and shown to be identical from the three samples. To confirm that the tobamovirus sequences isolated from tobacco were the same as the tobamovirus present within Surfinia™, two additional clones were produced directly from a Surfinia™ sample and sequenced. These sequences were found to be identical to the sequences of the three isolates generated by RT-PCR from the virus in tobacco. A BLAST search using the coat protein gene sequence showed petunia tobamovirus was very similar to nucleotide sequences of the vulgare type strains of TMV reported from Korea and Japan. Amino acid similarities were initially analysed using BLAST, then analysed and aligned in detail using CLUSTAL V.

The identity between TMV petunia and TMV vulgare was 99.4% at the amino acid level within the coat protein. This represents only a single amino acid difference at the fourth amino acid from the C-terminus (TMV-vulgare G, TMV-petunia S). The identity of TMV-petunia with other tobamoviruses were significantly lower, for example 84% with ToMV (Accession number X02144), 57% with TMV strain Ob (Accession number D13438) and 44% with a TMV crucifer strain (Accession number Z29370), at the amino acid level.

Figure 1. Leaf mosaic and distortion of leaves and stems in Surfinia™ trailing petunia caused by TMV infection.

Figure 2. Petal-break symptoms in Surfinia™ trailing petunia caused by TMV infection.

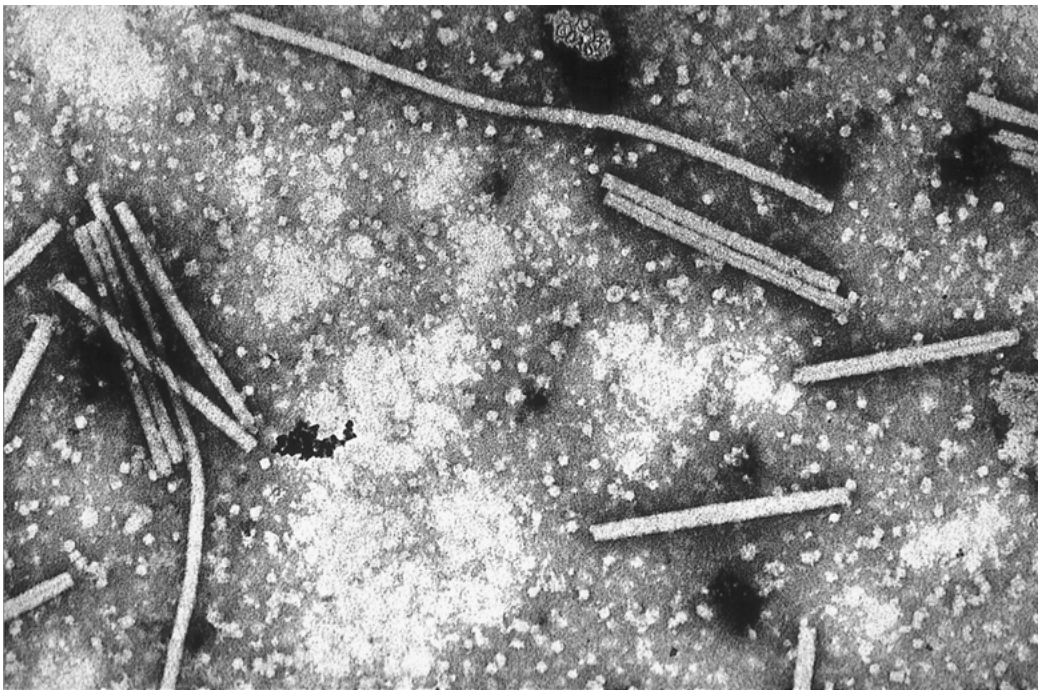

Figure 3. Electron micrograph of a mixed infection of virus particles approximately 300 nm long and 18 nm wide (tobamovirus) and flexous rod-shaped particles of approximately 750 nm long (potyvirus) observed in a Surfinia™ trailing petunia leaf sample.

An identical analysis was carried out with clones generated to a 397-nucleotide region within the replicase gene. All clones sequenced from the three samples were identical. Initial BLAST searches, followed by detailed CLUSTAL V alignment, revealed amino acid identities of 99.4% with TMV strain vulgare (Korean) (Accession number J02415) and the TMV strain ssp.NC82 (Korean) (Accession number X68110); 98.7% with TMV strain OM (Japanese) (Accession number D78608) and 98.1% with TMV Kokubu (Japanese) (Accession number P03572). There were lower similarities with other tobamoviruses such as 88% with ToMV (Accession number X02144) and 73% with pepper mild mottle virus (Accession number M81413).

Discussion

Tobamovirus-type particles were observed in infected trailing petunia samples using electron microscopy. ELISA confirmed that the tobamovirus was TMV and not ToMV or PMMV. There were several petunia samples which were also infected by a potyvirus shown to be PVY^{NTN} (Boonham et al., 1999). The results of the inoculation of *Nicotiana* species supports the ELISA tests showing that the tobamovirus isolates were TMV and not ToMV, as ToMV does not induce systemic symptoms in the *Nicotiana* species inoculated. This was confirmed by sequence analysis of the coat protein gene and a partial sequence within the replicase genes, which clearly revealed that the TMV isolated from diseased petunia had high amino acid identity (ca. 98–99%) with TMV isolates from Korea and Japan. The 397-nucleotide region of the replicase gene has been shown to be responsible for some tobamoviruses and strains being able to overcome the Tm-1 gene present in tomato (Rast, 1975; Meshi et al., 1988) and the N gene within tobacco (Padgett et al., 1997). The nucleotide sequence analysis of the TMV-petunia suggested it was a vulgare type strain, with no significant changes within the replicase or coat protein genes. This was confirmed by inoculation onto tobacco plants containing the N and N′ genes and tomato plants containing the Tm-1 gene. In all inoculations the expected results were obtained, with no resistance breaking observed (results not presented).

Table 1. Reaction of 23 varieties of pot and bedding plants inoculated with the petunia isolate of TMV. Infection determined by ELISA

Species	Variety	Symptoms	$OD_{405\,nm}$	ELISA
Antirrhinum	Chandelier Rose Pink	—	0.068	−
Bacopa	Pink Domino	—	0.093	+
Bidens	Goldie	—	0.034	−
Brachycome	Blue	—	0.042	−
Brachycome	Strawberry Mousse	—	0.058	−
Convolvulus	—	—	0.093	+
Diascia	Coral Belle	Necrotic local lesions	0.187	++
Felicia	Amelloides Variegata	—	0.448	++
Fuchsia	Trailing Eva Boerg	—	0.256	++
Geranium	Decora Lavender	—	0.043	−
Helichrysum	Gold	—	0.040	−
Impatiens	Double Peach Ice	—	0.037	−
Lamium	White Nancy	—	0.039	−
Lobelia	Richardii Blue	—	0.071	+?
Lysimachia	Lyssi	—	0.049	−
Marguerite	Jamaica Primrose	—	0.040	−
Mentha	Suaveolens	—	0.040	−
Nepeta	Variegata	—	0.038	−
Osteospermum	Springstar Castor	—	0.073	+?
Petunia	Million Bells Blue	Systemic mosaic	0.106	+
Scaevola	New Wonder	—	0.075	+?
Verbena	Tapien Lilac	Systemic mosaic	0.092	+
Verbena	Temari Pink	Systemic mosaic	0.115	+
Petunia infected with TMV control		Systemic mosaic	0.240	++

The mean $OD_{405\,nm}$ for four samples of healthy petunia was 0.035; + = positive ELISA result where OD > 2× the mean healthy petunia control; ++ = strong positive; +? = questionable positive result, − = negative result.

The results of the inoculation of pot and bedding plants with TMV confirmed that several of these species are susceptible to infection by TMV and that TMV infections in trailing petunias may therefore spread to a number of commercially important pot and bedding plant species. All of the species listed in Table 1 are vegetatively propagated in commercial pot and bedding plant production systems and the crops are subject to considerable handling at various stages of production. As many of the species infected by TMV were symptomless after three weeks, the virus could be transmitted to more susceptible plants unknowingly. The sudden and widespread occurrence of TMV in petunias was caused largely by the distribution of infected propagation material and further spread during crop handling. TMV is readily transmitted by sap and on cutting tools so it is desirable that growers start with virus-tested plants, if available and observe strict hygiene practices to reduce the risk of spreading virus to other crops.

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