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Short communication

Potato virus Y from petunia can cause symptoms of potato tuber necrotic ringspot disease (PTNRD)

Neil Boonham[1,*], Matthew Hims[1], Ian Barker[1] and Nicola Spence[2]
[1]*Central Science Laboratory, Sand Hutton, York, YO41 1LZ, ENGLAND;* [2]*Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, England;* [*]*Author for correspondence (Phone: 904 462000; Fax: 904 462111; E-mail: n.boonham@csl.gov.uk)*

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Abstract

A potyvirus known to be an important agent involved in causing a disease of trailing petunias, was identified as being a member of the necrotic strain of potato virus Y (PVY) using a number of monoclonal antibodies. The sequence of the coat protein gene for the PVY isolate was determined and when compared with sequences for other PVY strains it was shown to cluster closely with isolates of PVY^{NTN} and to have a recombination point present within the coat protein common with other isolates of PVY^{NTN}. When inoculated onto potato tuber necrotic ringspot disease (PTNRD) susceptible potato cultivars the petunia isolate was found to be capable of causing necrotic tuber symptoms, consistent with those caused by other isolates of PVY^{NTN}. Due to the number of similarities it is thought the petunia isolate belongs to the PVY^{NTN} group of isolates. Out of 24 species of bedding and pot plant crops tested, 19 were shown by mechanical inoculation to be susceptible to PVY, highlighting not only a clear risk to a number of commercially important plant species from PVY^{NTN} infected trailing petunias, but also other susceptible crops grown in these areas.

In 1994/95 a viral problem in trailing petunias resulted in crop losses in the UK (Campos, 1995) and elsewhere in Europe (Lesemann, 1996; Bellardi et al., 1996) estimated to be worth millions of pounds. The symptoms in petunia are distinct and include leaf mosaic, yellow mottling, vein clearing and distortion of leaves and stems. Colour breaking in flowers was often observed and the overall growth of the whole plant may be stunted (Spence et al., 1996; Bellardi et al., 1996). The disease was found to be associated with tobacco mosaic virus (TMV), (Sharp, 1994; Spence et al., 1996) and a strain of potato virus Y (PVY), which reacted with antiserum to the necrotic strain of PVY (PVY^{N}), either singly or in mixed infections. The role of TMV in the disease and its involvement in symptomology is still being characterised. It is thought the virus problem was widespread as a result of the use of infected propagation material, whilst subsequent spread probably occurred with handling and insect transmission (Spence et al., 1996; Lesemann, 1996; Bellardi et al., 1996). The potyvirus was confirmed in stocks of petunia imported from The Netherlands and Germany, often with infection levels of 100% (Sharp, 1994). Most growers of trailing petunias also produce a wide range of other bedding and ornamental species and it is not known if the viruses in trailing petunias represent a real risk to these other species.

PVY is the type member of the genus *Potyvirus*, and is the most economically important virus infecting potato. In conjunction with potato leaf roll virus and potato virus X it is responsible for crop losses in potato amounting to £30–50 million per year in the UK (Hull, 1984). PVY is widespread, known naturally or experimentally to infect up to 342 species of 69 genera

in 27 families (Edwardson and Christie, 1991). The virus is transmitted by up to 40 aphid species belonging to 20 different genera (Sigvald, 1984). There are three main PVY strains, described on the basis of systemic and local symptoms in selected cultivars of *Nicotiana tabacum* and *Solanum tuberosum*. PVY – necrotic (PVY^N) causes veinal necrosis on *N. tabacum*, PVY – common or ordinary strain (PVY^O) causes mild mottling on *N. tabacum* (Shukla et al., 1994; de Bokx and Huttinga, 1981). Members of the PVY^C strain induce a hypersensitive response in potato cultivars bearing the *Nc* resistance gene (Cockerham, 1970). A fourth group of isolates resembling members of the PVY^N strain but which are involved in potato tuber necrotic ringspot disease (PTNRD) (Le Romancer and Nedellec, 1997), are referred to as PVY^{NTN}.

The objective of this study was to further characterise (using serological and molecular techniques) the isolate of PVY involved in the disease in trailing petunias, and assess the risk, if any, of its spread into other ornamental crops.

The PVY isolate from petunia was obtained by the UK Plant Health and Seeds Inspectorate and sent to Central Science Laboratory (CSL), from a stock of petunia Surfinia which originated in The Netherlands. The presence of virus in plant samples was determined by a standard direct double antibody sandwich ELISA, using buffers described by Clark and Adams (1977), and a range of polyclonal and monoclonal antibodies (Tables 1 and 2). In order to assess whether other pot and bedding plants are at risk from PVY from infected trailing petunias, 3 six-week-old plants of 24 varieties belonging to 21 of the most popular species of pot and bedding plants were sap inoculated on a single leaf with the petunia isolate of PVY. Symptoms were recorded weekly and after 3 weeks, a bulk of 3 samples was taken (top, middle and bottom of each plant) and tested for infection by ELISA. The PVY isolate was inoculated onto susceptible cultivars of potato (Nadine, Pentland Crown and Pentland Ivory). The plants were mechanically inoculated 2 months post planting and tubers harvested 2 months post inoculation. Tubers were stored at 18 °C and monitored for the development of necrotic rings. PCR products representing 1201 bp of the 3′ end of the PVY genome were amplified using a proof reading RT-PCR system (Expand, Boehringer Manheim), and primers specific for the poly-A tail (5′-AAC TGG AAG AAT TGG CGG CCG CAG GAA TTT TTT TTT TTT TTT TTT-3′; linker added to increase Tm) and the NIB gene (5′-RGC YTT CAC TGA AAT GAT GG-3′). PCR products were cloned into pGEM-T (Promega). Sequencing of PCR products was carried out on minipreps (Wizard preps, Promega) using an ABI automated sequencer (Sequiserve, Germany). Phylogenetic analysis was carried out using the Dnapars program from the PHYLIP package (Felsenstein, 1989) (Figure 1). The statistical significance of the branching was estimated by performing

Table 1. Serological testing of petunia isolate of PVY, and control isolates of PVY^N, PVY^{NTN} and PVY^O, against a range of monoclonal antibodies (Bioreba – cat: 112921 and cat: 112722; Adgen – cat: 1051-05 and cat: 1052-52) and a recombinant antibody (Boonham and Barker, 1998). Results as a mean A_{405} (of duplicate wells) following 1 h of incubation with substrate and blanking on air. Positive ELISA results are $>2\times$ the uninfected control and are highlighted in bold

Antibody	Mean $A_{405\,nm}$–1 h				
	Uninfected	Petunia PVY	PVY^N	PVY^{NTN}	PVY^O
Bioreba monoclonal cocktail	0.097	**1.288**	**1.222**	**0.973**	**1.886**
Bioreba N specific	0.095	**1.436**	**1.264**	**1.271**	0.147
Adgen N specific	0.089	**2.843**	**2.156**	**2.048**	0.089
Adgen O/C specific	0.090	0.094	0.091	0.092	**1.906**
Recombinant O specific	0.110	0.108	0.092	0.101	**2.457**

Table 2. $OD_{405\,nm}$ readings following ELISA using Agdia Potyvirus Mab for inoculated plants (Agdia inc, USA), and systemic symptoms following inoculation of ornamental plant species with the petunia isolate of PVY. The mean $OD_{405\,nm}$ for 4 samples of healthy petunia was 0.041

Species	Variety	$OD_{405\,nm}$	Symptoms detected
Antirrhinum	Chandelier Rose Pink	0.157	–
Bacopa	Pink Domino	0.168	–
Bidens	Goldie	0.123	–
Brachycome	Blue	0.092	–
Brachycome	Strawberry Mousse	0.037	–
Convolvulus		0.045	–
Diascia	Coral Belle	0.089	–
Felicia	Amelloides Variegata	0.146	–
Fuchsia	Trailing Eva Boerg	1.155	–
Geranium	Decora Lavender	0.073	–
Helichrysum	Gold	0.948	–
Impatiens	Double Peach Ice	0.064	–
Lamium	White Nancy	0.337	–
Lobelia	Richardii Blue	0.140	–
Lysimachia	Lyssi	0.236	–
Marguerite	Jamaica Primrose	1.385	+
Mentha	Suaveolens	1.279	–
Nepeta	Variegata	0.517	–
Osteospermum	Springstar Castor	0.143	–
Petunia	Trailing Million Bells Blue	0.136	–
Scaevola	New Wonder	2.470	–
Verbena	Tapien Lilac	0.083	–
Verbena	Temari Pink	2.534	+

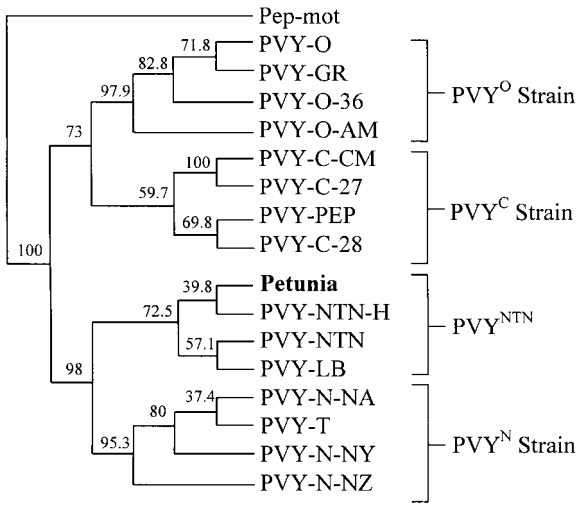

Figure 1. Phylogenetic tree generated using PHYLIP, based on the coat protein sequences of 4 isolates of each of the main strains (PVY^{N}, PVY^{O}, and PVY^{C}) and also PVY^{NTN}. The numbers above each branch indicate the percentages of bootstrap analysis which support the grouping at each node.

100 replications of bootstrap resampling from the original data using SEQBOOT (Felsenstein, 1989). Four sequences for each of the strains were included in the analysis (PVY strain, followed by name and database *accession number*) PVY^{NTN}: PVY-NTN-H, *M95491* (Thole et al., 1993); PVY-LB, *X92078* (Revers et al., 1996); PVY-NTN, X*79305* (VanDenHeuvel et al., 1994); PVY^{N}: PVY-T, *D12570* (Hidaka, unpublished); PVY-N-NZ, *M22470* (Hay et al., 1989); PVYN-NA, *U09508* (Dhar, 1997); PVYN-Ny, *Z70237* (Chachulska et al., 1997); PVY^{O}: PVYO, *U09509*, (Singh, unpublished), PVY-O-Am, *X14136* (Bravo-Almonacid and Mentaberry, 1989); PVY-O-36, *S74810* (Hataya et al., 1994); PVY-Gr, *M81435* (Griffin, unpublished); PVY^{C}: PVY-C-CM (CSL, unpublished); PVY-pep, *U10378*, (Daquino, unpublished); PVY-C-27, *AF012026*, PVY-C-28, *AF012027*, (Blanco-Urgoiti et al., 1998). Pepper mottle potyvirus was included as an outgroup in the analysis, PepMot, *M96425* (Vance et al., 1992).

The petunia isolate of PVY was tested using a range of commercially available monoclonal antibodies and

a recombinant antibody, following isolation onto *Nicotiana tabacum* (Table 1). The isolate reacted strongly with both monoclonal antibodies specific for PVY^N strain and showed no cross reaction with either monoclonal antibodies or recombinant antibodies specific for PVY^O. After 3 weeks, plants which had developed symptoms included Marguerite (Jamaica Primrose) and Verbena (Temari Pink), which both showed leaf mosaic and had high ELISA readings. A number of other test plants gave high ELISA values likely to be indicative of systemic infection by PVY but no systemic symptoms (Table 2). In contrast, an inoculated petunia plant used as a control developed veinal chlorosis in the leaves and a distinct flower colour break symptom. The petunia isolate of PVY was tested for its ability to cause necrotic ringspot symptoms on tubers of 3 susceptible varieties. Following storage of tubers, necrosis was observed in each variety inoculated with the petunia isolate of PVY (see Table 3). In phylogenetic analysis the sequence of the petunia isolate (Accession number AJ133454) clustered closely with 3 isolates of PVY^{NTN} from Austria, Hungary and Lebanon. This group was separated from the PVY^N cluster with a bootstrap value of 98%. Closer inspection of the sequence revealed that this isolate (in common with the other PVY^{NTN} isolates compared) had a recombination event present within its coat protein as shown previously for other isolates of PVY^{NTN} (Revers et al., 1996).

Using both serological and molecular methods, the PVY isolate from petunia has been identified initially as being within the PVY^N strain and more specifically as being an isolate of PVY^{NTN}, being very similar to isolates of PVY^{NTN} in many respects. In particular the isolate was shown to have a recombination event within its coat protein and was also able to induce necrosis on the tubers of susceptible potato cultivars.

When a number of bedding and pot plant crops were inoculated with the petunia isolate of PVY, 2 species showed symptoms, whilst 19 of 24 gave indication of systemic infection with PVY by ELISA, although they did not develop symptoms (Table 2). PVY infection in trailing petunias therefore represents a clear risk to a number of commercially important pot and bedding plant species. In addition this spread of a damaging isolate of PVY in ornamental hosts is a clear risk to potato, tomato and even tobacco crops in areas where these three are grown in close vicinity and ornamentals can clearly act as a source of inoculum for the virus.

Table 3. Pathogenicity of petunia isolate and control isolates tested on potato, as the percentage of tubers exhibiting necrotic ringspot symptoms following two months of storage – indicates no tubers harvested

Potato cultivar	Tubers showing symptoms (%)		
	PVY-petunia	PVY^O	PVY^{NTN}
Pentland Crown	7.4	0	28
Pentland Ivory	21.4	0	29
Nadine	100	0	—

PVY^{NTN} causes a damaging disease of potato, called PTNRD, characterised by superficial rings which protrude at first and then become sunken and necrotic, often becoming more pronounced during storage (Beczner et al., 1984). The disease seriously damages the tubers for table use, processing and seed and when susceptible cultivars are grown, tubers exhibiting symptoms can reach as much as 90% of the entire crop (Le Romancer and Nedellec, 1997). First described in Hungary in 1982 (Beczner et al., 1984) the disease subsequently spread widely across Europe, being reported in many countries Germany (1984), Czechoslovakia (1988), Austria (1990), Yugoslavia (1990), France (1991), Belgium (1992), Great Britain (1992) and Denmark (1992) (Weidemann and Maiss, 1996).

This outbreak of disease in trailing petunia is the first record of PVY^{NTN} being involved in causing disease in a host other than potato or tomato (which is thought to be most important as a reservoir host for PVY in potato, where potato and tomato are grown in neighbouring fields) (Le Romancer and Nedellec, 1997). The occurrence of the disease appears to be coincident with the infection of petunia with a tobamovirus and the spread across Europe of a new and damaging strain of PVY in potatoes.

The sudden and widespread occurrence of PVY^{NTN} in petunias was caused largely by the distribution of infected vegetatively propagated material. However, PVY is readily transmitted by aphids and in sap hence it is desirable that growers start with virus free plants, if available, and observe strict hygiene and aphid control practices to minimise the risk of virus spread to other crops, both in and outside of the glasshouse.

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