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A novel hypersensitive resistance response against potato virus A in cultivar 'Shepody'

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Abstract The potato cultivar 'Shepody' is susceptible to a number of potato viruses including potato virus Y (PVY, potyvirus) but was found to possess extreme resistance to another potyvirus, potato virus A (PVA). 'Shepody' plants were resistant to PVA infection in manual and graft inoculations. PVA replication was not detected in any of the inoculated plants by ELISA, an infectivity assay and RT-PCR. However, 'Shepody' plants grafted with shoots containing PVA developed a novel symptomology which resembled a virus infection in appearance and in rate of translocation to the entire plant. Efforts to transmit the symptom-inducing agent manually failed. Graft-inoculation to potato virus indicator plants and PVA-susceptible potato plants showed that the symptom inducer was PVA at an extremely low concentration, detected using RT-PCR followed by Southern blot assay. Tubers from grafted but resistant 'Shepody' plants had necrotic surfaces and internal spots. PVA was detected from necrotic areas but not from the non-necrotic ones. However, plants resulting from necrotic tubers were free from aerial leaf symptoms observed in grafted plants and produced non-necrotic normal tubers. A traceback of the parental lineage of 'Shepody' indicated that the resistance had been introgressed from the cultivar 'Bake King'. Analysis of progeny of a cross of resistant 'Shepody' to the susceptible 'Goldrus' indicated that this resistance is controlled by two independent dominant complementary genes in contrast to monogenic resistance reported for other potato viruses.

Key words Complementary genes · Extreme virus resistance · Genetics · Necrotic tubers · Restricted virus distribution · *Solanum tuberosum*

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Introduction

Potato virus A (PVA), a member of the family *Potyviridae*, along with potato viruses Y (PVY) and V (PVV), are all members of the genus *Potyvirus* that infect potato crops (Jones 1990, Barker 1997). PVA incites a mild mosaic in potato, or it may remain latent in infected plants. However, in combination with potato virus X (PVX), it incites a severe disease known as "potato crinkle" (Mac Lachlan et al. 1953). PVA is transmitted by various species of aphids (MacLachlan et al. 1953), and aphid transmission is the primary mode of the spread of the virus in the field. Although PVA occurrence in North America is sporadic, incidences of 9–11% are not uncommon in certain cultivars (Singh and Smith 1977; Singh and Boiteau 1987; Petrunak et al. 1991). In recent years, the prevalence of PVA in some potato production areas in Europe has also been noted (Rajamäki et al. 1998). At least four strains of the virus have been determined on the basis of symptoms in potato cultivar 'King Edward' (Valkonen et al. 1995; Rajamäki et al. 1998). Since the symptoms in infected potatoes are usually mild but vary with potato cultivars, PVA can reduce the potato crop yield from approximately 10% to as high as 40% (Bartels 1971). Control of aphid-borne potyviruses is difficult, and the most effective means of preventing their spread is by the use of durable conventional or transgenic resistance.

Resistance to PVA is present in some wild *Solanum* species (Cockerham 1970; Valkonen et al. 1996) and has been introgressed into the cultivated potato (*Solanum tuberosum*). On the basis of PVA-induced symptoms in potato plants, two main types of resistance have been reported. One is an extreme resistance (ER), in which very few or no visible symptoms are observed and the virus is very difficult to recover. This type of resistance is conferred by *R* genes (Cockerham 1970; Valkonen et al.1996). The R_y genes from *S. stoloniferum* (Cockerham 1970) and *S. tuberosum* subsp. *andigena* (Munoz et al. 1975) have been recently shown to provide comprehensive resistance to PVA, PVY and PVV (Barker 1996, 1997; Hämäläinen et al. 1998). The second type of resis-

tance, in which PVA inoculation produces necrotic symptoms in the top part of grafted plants (top-necrosis) and in tubers, is termed a hypersensitive resistance (HR). From such plants the virus can be easily recovered, and this resistance is conferred by *N* genes (Valkonen et al. 1996). An analysis of North American potato cultivars has shown that about 33% possess HR to PVA (Bagnall 1961) and that this type of resistance is so effective under natural conditions that such cultivars have been termed "field-immune". This characteristic is easily introgressed, and a large number of progeny have been shown to possess HR in crosses of parents having field immunity (Webb and Schultz 1959).

We report here a novel type of HR to PVA in cv 'Shepody' which does not possess the R_y gene. It reacts with PVA upon graft-inoculation by the development of necrotic pin-point lesions on the leaves followed by chlorotic mosaic-like symptoms and by the production of necrotic tubers. In contrast to the monogenic inheritance of ER and HR (Valkonen et al. 1996) the HR in 'Shepody' appears to be under the control of two independent dominant complementary genes. This type of resistance and the phenotypic response do not clearly fit the resistance categories elaborated above. A preliminary report on PVA resistance has appeared elsewhere (Singh 1997).

Materials and methods

Virus isolate and host plants

An isolate of PVA obtained from commercial potato fields in New Brunswick, Canada and used in previous studies (Singh 1982; Singh and Singh 1998) was maintained in plants of *Nicandra physaloides* and potato cultivar 'Russet Burbank'. Virus-free plantlets of potato cultivars 'Goldrus', 'Green Mountain', 'Russet Burbank', and 'Shepody' from the Plant Propagation Centre (New Brunswick Department of Agriculture and Rural Development, Fredericton, N.B.) were grown in a greenhouse at 18°–22°C, with a 14-h daylength supplemented with fluorescent illumination. Additional plants were raised from field-grown tubers of cv 'Bake King', and numbered seedlings F34011, F58050, and K113–1, the latter used in the pedigree of cv 'Shepody'.

Mechanical and graft inoculations and assay methods

For mechanical inoculation infected leaves were first ground in a buffer solution (0.01 *M* sodium phosphate containing 0.4% sodium sulphite, pH 7.5) to provide a 1:5 dilution of sap and then manually inoculated to young plants in the five- to six-leaf stage. To check for extreme resistance, we cleft-grafted potato plants with PVA-infected shoots from potato plants (scions). As many as six scions were cleft-grafted on some plants with multiple stems (rootstocks) to provide heavy inoculum pressure. Plants were monitored for symptom appearance at weekly intervals and assayed at 30, 60 and 90 days post-inoculation by enzyme-linked immunosorbent assay (ELISA) (Singh et al. 1996). In addition, the scions and the rootstocks of each grafted plant were tested 60 days post-grafting by reverse transcription polymerase chain reaction (RT-PCR) (Singh and Singh 1998) to confirm the presence of PVA in the scions and to determine the status of PVA in the rootstocks. Tubers harvested 90 days post-grafting were observed for necrotic symptoms and, after a cold storage of 5 months, were planted in the greenhouse. The resulting plant leaves were monitored for PVA by ELISA and RT-PCR.

Bioassay on virus indicator plant

All scions and rootstocks were also tested for PVA using the indicator plant *Physalis angulata* (Singh 1982). Additionally, the leaf extracts from axillary growths of grafted plants showing chlorotic mosaic symptoms were manually inoculated to various indicator plants of common potato viruses and a viroid. These were: *Datura metal* (virus M, PVM); *Gomphrena globosa* (PVX); *Lycopersicon esculentum* 'Sheyenne' (potato spindle tuber viroid, PSTVd and PVA); *Nicandra physaloides* (PVA); *Nicotiana debneyi* (virus S, PVS); *N. tabacum* 'Samsun' (PVA and PVY); *P. angulata* (PVA), *P. floridana* (PVY); *Scopolia sinensis* (PSTVd), *Solanum demissum* (PVY) and 'Russet Burbank' potato for any other potato virus(es). Plants were observed for symptoms of PVA and other viruses for 6–9 weeks. All inoculated plants were tested by ELISA for PVA and other viruses.

To determine if the chlorotic mosaic symptoms-inducing factor was graft-transmissible, scions with symptoms were cleft-grafted to 'Russet Burbank' plants and tested for the presence of PVA by ELISA. Additionally, they were grafted to various PVA indicator plants(see above) and assayed for virus by RT-PCR for 6–10 weeks. In another variation of the graft-transmission test of the symptom-inducing factor, *P. angulata* scions were grafted onto 'Shepody' shoots with chlorotic symptoms. PVA and other virus-es/viroid positive and negative controls were always included in each transmission and assay.

Enzyme-linked immunosorbent assay (ELISA)

PVA was assayed by a double antibody sandwich form of ELISA as described earlier (Singh et al. 1996). Polyclonal antibodies against PVA were obtained from Boehringer Mannheim Canada (Dorval, Quebec) and used for coating (1:67 dilution) ELISA plates (Immulon 1, Dynatech Laboratories, Chanatilly, Va.). The enzyme-conjugate dilution was 1:150. Leaf sap was prepared by a Pollähne roller press (4 drops/1.5 ml of extraction buffer). Samples of each extract were tested in duplicate wells, and the absorbance was measured at 405 nm (A_{405}).

Reverse transcription polymerase chain reaction (RT-PCR)

PVA was detected from leaves and dormant tubers by RT-PCR as described (Singh and Singh 1998). In brief, tuber sap was obtained using a tuber slicer (Electrowerk, Behcke and Co, Hannover, Germany), 150 µl of sap was mixed with 300 µl of the extraction buffer (0.1 *M* TRIS-HCl, pH 7.4, 2.5 m*M* $MgCl_2$) containing 6 units of RNase-free DNase I (Boehringer Mannheim). This mixture was incubated at 37°C for 10 min, then 200 µg of Proteinase K (Promega, Madison, Wis.) and 1% sodium dodecyl sulphate were added and incubated for an additional 10 min at 65°C, followed by a nucleic acid extraction with phenol-chloroform isoamyl alcohol and the precipitation of nucleic acid with isopropanol. One microliter (70 ng) of RNA was used for reverse transcription and 5 microliters of cDNA was used for the amplification reaction, essentially as described (Singh and Singh 1996). Amplification was carried out for 30 cycles in a Peltier thermal cycler (PTC-200, MJ Research, Watertown, Mass.). Each cycle consisted of a 1-min duration of denaturation at 94°C, primer annealing at 60°C, and primer extension at 72°C. Ten microliters of amplified product was electrophoresed on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. The 255 -bp product was identified using a size marker (DNA ladder, Gibco BRL, Md.).

Southern blot analysis

A digoxigenin (DIG)-labelled PVA probe was generated by RT-PCR using the purified PVA as template. RT-PCR conditions were the same as described above except that the dNTPs possessing Digoxigenin-dUTP were used. RT-PCR-amplified PVA fragment was fractionalized on an 1.5% agarose gel containing a trace

amount of ethidium bromide and observed on the UV box. The gel was blotted to MagnaCharge filter (Micron Separations) by capillary action with 20 × SSC for 16 h. After washing briefly with 2 × SSC, the filter was dried at 37°C and then was exposed to UV light for 1 min.

Prehybridizaiton was carried out at 42°C in the prehybridization solution (5 × SSC, 50% formamide, 0.1% sodium lauroylsarcosine, 0.02% SDS, and 2% blocking reagent (Boehringer Mannhem, Germany)] for 2 h. Thereafter, DIG-labelled PVA cDNA fragment was added to the prehybridization solution and hybridized for 16 h at 42°C. After washing twice with 2 × SSC + 0.5% SDS and then twice with 0.5 × SSC + 0.2% SDS at 65°C, the filter was exposed for the detection of DIG-labelled nucleic acid according to the manufacturer's instructions (Boehringer Mannhem, Germany), and disodium-3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-*yl*)phenyl phosphate(CSPD) was used as the substrate for the anti-DIG-alkaline phosphatase(AP) (Boehringer Mannhem, Germany). The filter was exposed to X-ray film (X-OMAT AR, Kodak) for 20 min at room temperature, and the film was developed.

Results

Evidence for PVA resistance of cv 'Shepody'

During an evaluation of the PVA susceptibility of PVY transgenic potato plants, it was discovered that transgenic 'Shepody' plants failed to become infected when inoculated with PVA by aphid, graft and manual inoculation. At the same time a high percentage of PVY transgenic 'Russet Burbank' plants became infected with PVA by the three methods. To determine whether the PVA resistance of 'Shepody' was due to a PVY transgene, we manually inoculated non-transgenic 'Shepody' plants with PVA. As a control, two cultivars highly susceptible to PVA were also included. None of the 30 'Shepody' plants became infected with PVA (Table 1), although 65% of inoculated 'Goldrus' and 53% of 'Russet Burbank' plants became infected with PVA (Table 1). Plants of both susceptible cultivars developed mild mosaic symptoms in the leaves. However, there were no symptoms of any kind in manually inoculated 'Shepody' plants. ELISA readings of individual plants confirmed the PVA infection in symptomatic plants of 'Goldrus' and 'Russet Burbank'. On average, in this test the PVA-infected plants had A_{405} values of 0.364–0.588, and non-infected plants had an A_{405} of less than 0.1 (Table 1).

Since manual inoculation of PVA failed to produce 100% infection of the highly susceptible cultivars, graft inoculation was performed. Healthy rootstocks of 'Shepody' were grafted with PVA infected scions from 'Goldrus' and 'Russet Burbank', and scions from healthy 'Shepody' were grafted on PVA-infected rootstock of 'Goldrus' and 'Russet Burbank' (Table 1). New growths of scions and rootstocks were observed for symptoms and tested for virus by ELISA at 30, 60 and 90 days post-grafting. ELISA tests of new axillary growth showed that 'Shepody' plants did not become infected with PVA during the 90-day post-graft period, although the virus was always recovered from infected scions or rootstocks of the other cultivars (Table 1). In a 90-day post-graft test, PVA concentrations in susceptible 'Goldrus' and 'Russet Burbank' ranged from an A_{405} of 0.828 to 1.211, while in resistant cv 'Shepody' the absorbance was limited to an A_{405} of less than 0.09 (Table 1).

In another test the inoculum pressure of PVA was increased by multiple grafting with PVA-infected scions of multi-stemmed 'Shepody' rootstocks. Five plants with five to six stems were grafted with infected scions and maintained for 90 days post-grafting. None of these became infected with PVA as determined by ELISA.

In addition to the ELISA test, all grafted plants were also tested by RT-PCR for the 60-day post-graft period. All the infected scions showed a strong PVA-specific, 255-bp DNA band, while none of the rootstock of 'Shepody' developed the PVA-specific band.

Infectivity assays of scions and rootstocks of grafted plants

All plants of 'Shepody' grafted with PVA infected scions or onto PVA-infected rootstocks were checked for PVA by inoculation to local lesion host *P. angulata* seedlings. Local lesions ranging in numbers from one to ten were detected in plant tissues of 'Goldrus' and 'Russet Burbank' but not in those of 'Shepody'.

Table 1 Resistance of 'Shepody' to manual and graft inoculation of PVA

Cultivars	Number of plants		ELISA (A_{405} nm)
	Inoculated	Infected	Means ± SD
Manual inoculation			
Goldus	34	22	0.588 ± 0.169
Russet Burbank	34	18	0.364 ± 0.108
Shepoldy	30	0	0.091 ± 0.025
	Number grafted	Scions	Rootstocks
Graft inoculation			
Goldrus[a]/Shepoldy[b]	25	0.929 ± 0.537	0.059 ± 0.019
Russet Burbank[a]/Shepody[b]	24	0.828 ± 0.277	0.059 ± 0.019
Shepody[b]/Goldrus[a]	25	0.086 ± 0.057	1.211 ± 0.464
Shepody[b]/Russet Burbank[a]	24	0.086 ± 0.057	1.011 ± 0.305

[a] Plants infected with PVA
[b] Plants not infected with PVA

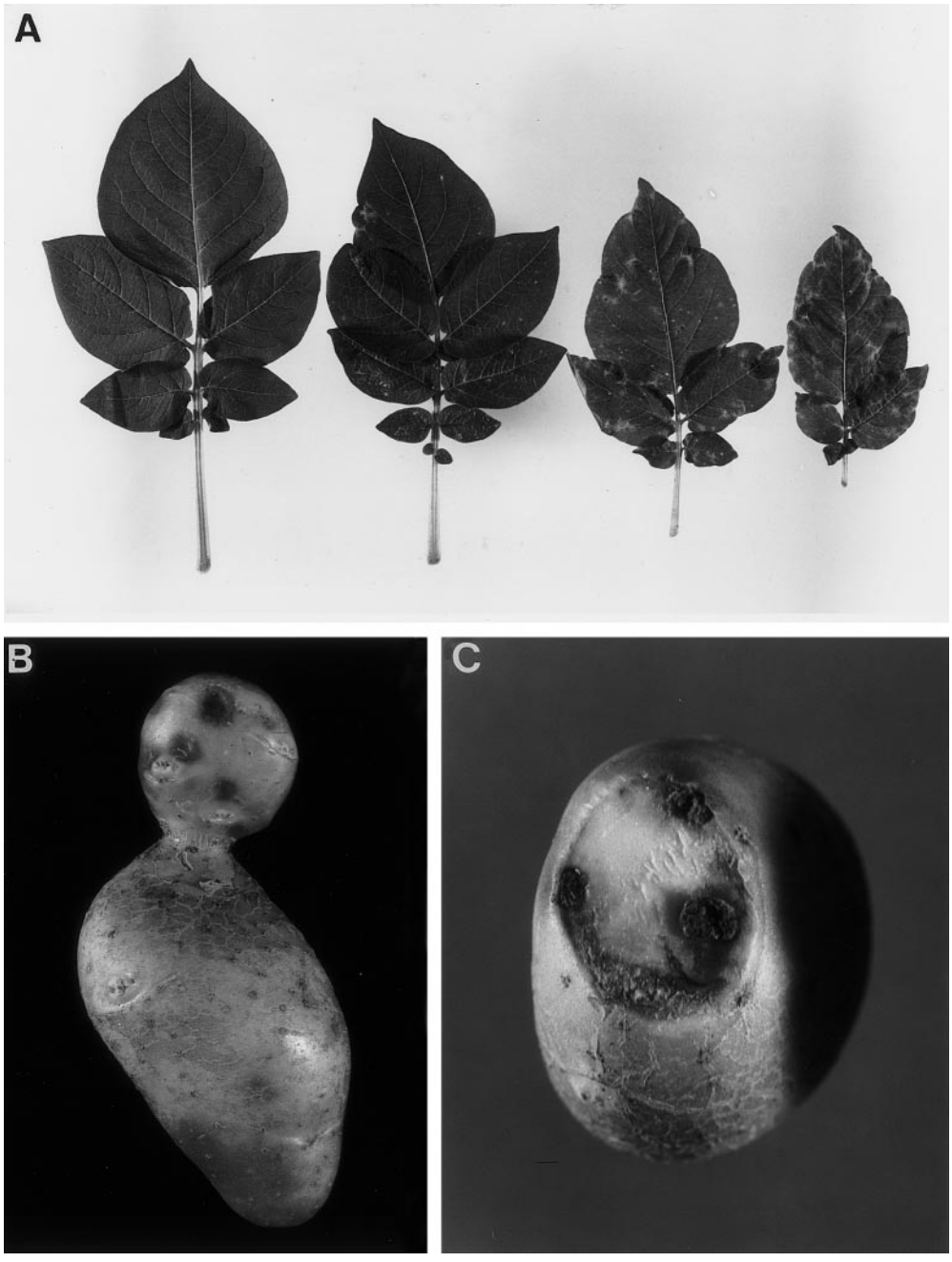

Fig. 1 A 'Shepody' leaves with chlorotic mosaic symptoms. *Far right* leaf with severe symptoms, *middle* two leaves with faded symptoms, *far left* is a healthy leaf. **B, C** Necrotic tuber symptoms in grafted 'Shepody' plants. **B** External necrotic symptoms, **C** internal necrotic and hardened tissues

Novel response in 'Shepody' plants grafted with PVA-infected material

'Shepody' plants grafted with PVA-infected scions occasionally developed necrosis, immediately below the graft-union (top-necrosis), which spread downward, and within 2–3 weeks the rootstock was killed. However, the majority of 'Shepody' plants (more than 95%) developed within 2–3 weeks a novel group of symptoms in which the axillary growths of the rootstock had chlorotic mosaic to etched leaf-like symptoms. Initially, a pin-point chlorotic dot appeared on the leaves, then the centres of the dots became necrotic, while chlorosis spread in each direction. Finally, both the green and chlorotic areas increased in intensity, and a chlorotic mosaic pattern became dominant. This symptom was most severe in the young leaves and faded away as the leaves became older (Fig. 1A). It appeared in all the new growths of 'Shepody' rootstocks and was virus-like in its rapid appearance throughout the plant. In scions of 'Shepody' on the infected rootstocks of the other cultivars, these symptoms were observed in vigorously growing 'Shepody' scions only. However, their appearance was delayed by 1–2 weeks compared to the symptoms in the 'Shepody' rootstocks.

Attempts to transmit the chlorotic mosaic factor

Since the symptoms observed in the axillary growth of 'Shepody' grafted with PVA-infected scions resembled those of virus infection, attempts were made to manually transmit the factor causing the symptoms to common potato virus indicator plants. No symptoms developed in plants of *D. metel*, *G. globosa*, *L. esculentum* 'Sheyenne', *N. physaloides*, *N. debneyi*, *N. tabacum*, *P. angulata*, *P. floridana*, *S. sinensis*, and *S. demissum*. Under the same environmental conditions, control plants inoculated with the respective viruses and viroid developed symptoms.

In another test, 26 axillary shoots from 'Shepody' with chlorotic mosaic symptomology were grafted onto virus-free plantlets of 'Russet Burbank'. No symptoms of any kind were observed on 'Russet Burbank' plants during 6 weeks of incubation. Under the same environmental conditions, 10 plants of 'Russet Burbank' grafted with PVA-containing shoots developed typical PVA symptoms. ELISA and RT-PCR tests also failed to detect any PVA in chlorotic mosaic 'Shepody' scions grafted onto the 'Russet Burbank' plants.

Similarly, 5 plants of 'Shepody' with chlorotic mosaic were grafted with healthy scions of *P. angulata* to determine if PVA-type lesions would appear in *P. angulata*. None developed symptoms, and no PVA was detected in any of the *P. angulata* scions by ELISA.

Finally, indicator plants, which become infected systemically with PVA (tomato, tobacco and *N. physaloides*), were graft-inoculated with PVA-containing scions and monitored by RT-PCR up to 8 weeks. Of the 4 grafted tomato plants 3 showed a faint band after 4 weeks and a stronger band 8 weeks after grafting; these were specific to PVA. This observation indicated that PVA can be recovered, by RT-PCR, from symptom-bearing 'Shepody' shoots grafted to highly susceptible tomato plants.

Association of PVA with chlorotic symptoms in 'Shepody' rootstocks

Recovery of PVA from tomato plants 4–8 weeks after grafting with 'Shepody' scions with chlorotic symptoms indicated that PVA is capable of multiplying and translocating in 'Shepody' plants. Therefore, 20 plants of 'Shepody' exhibiting very strong chlorosis symptoms in their axillary shoots were assayed by RT-PCR and RT-PCR followed by Southern blot, 30 days after grafting. As shown in Fig. 2A strong PVA-specific bands were detected by RT-PCR in 10 PVA-infected 'Goldrus' scions but only weak bands in 12 'Shepody' root-stock samples (the weak bands did not reproduce in the photograph). However, when RT-PCR products were probed with Digoxigenin labelled PCR probes in a Southern blot, all 12 rootstock samples showed strong bands of PVA (Fig. 2B).

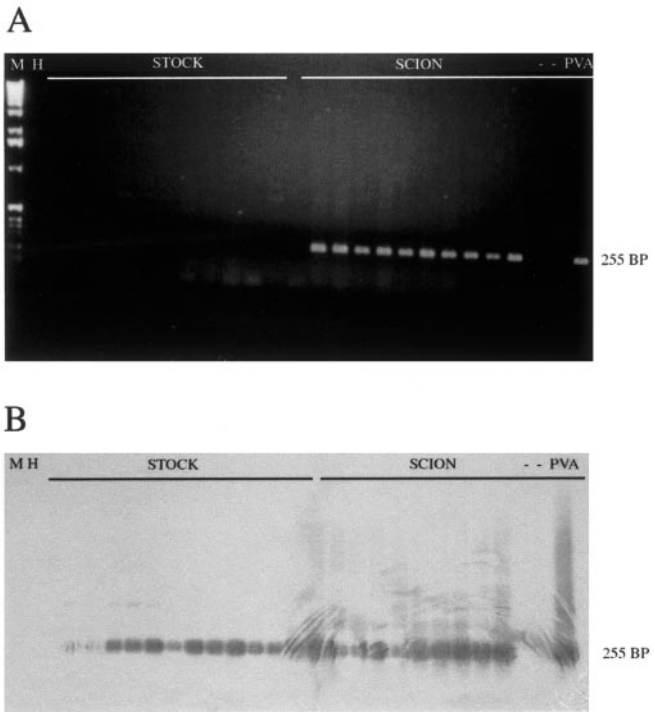

Fig. 2 A RT-PCR analysis of grafted 'Shepody' stocks and 'Goldrus' scions. *M* size markers, *H* healthy plant, *STOCK* 12 'Shepody' leaf samples; *SCION* 10 'Goldrus' leaf samples, –, cDNA and PCR controls without templates, *PVA* purified PVA. The PVA-specific fragment (255 bp) is indicated. Faint PVA bands in the stocks were visible on the gel but did not reproduce in the photograph. **B** Southern blot of the samples used in **Fig. 2A**, after transfer from gel to nylon membrane using a digoxigenin-labelled probe generated by RT-PCR (255 bp). *Lanes* for *M, H, STOCK, SCION*, –, and *PVA* are the same as in **2A**

Symptoms in 'Shepody' tubers

'Shepody' rootstock grafted with PVA-infected scions developed tubers with various degrees of necrosis (Fig. 1B). The surface necrotic symptoms continued internally, and the tissues were hard and dry (Fig. 1C). Not all plants produced tubers with necrotic symptoms. Of the 31 plants in one test, necrotic tubers were produced in 18. PVA was detected by RT-PCR in necrotic areas of the tubers but not from the non-necrotic areas. Plants developed from necrotic tubers were free from chlorotic mosaic symptoms, and PVA was not detected in these plants by ELISA or RT-PCR.

Trace-back of PVA resistance in parental lineage of 'Shepody'

Plants of cvs 'Bake King' and 'Green Mountain', and three numbered seedlings, F34011, F58050 and K34011, the latter belonging to the pedigree of cv 'Shepody' were grafted with PVA-containing scions. 'Green Mountain' and K113–1 developed mild mosaic symptoms, while others were symptomless for PVA (Table 2). ELISA tests

Table 2 Trace-back for sources of PVA resistance in parental lineage of 'Shepody'

Potato parental lines	Number of plants grafted (stocks)	Symptoms	ELISA (A_{405} nm)
Bake King	10	Symptomless	0.16 ± 0.017
F34011	12	Symptomless	0.719 ± 0.210
F58050	15	Mosaic	0.902 ± 0.242
Green Mountain	15	Mosaic	1.493 ± 0.454
K-113-1	12	Symptomless	1.550 ± 0.355
Shepody	30	Symptomless	0.092 ± 0.039

Table 3 The expected segregation ratio of duplex ($R_1R_1r_1r_1R_2R_2r_2r_2$) × nulliplex ($r_1r_1r_1r_1r_2r_2r_2r_2$) cross

Parental		Progeny	Segregation frequency	
Female	Male	Genotypes	Resistant	Susceptible
$R_1R_1R_2R_2$	$r_1r_1r_2r_2$	$R_1R_1r_1r_1R_2R_2r_2r_2$	1	0
$R_1R_1R_2r_2$		$R_1R_1r_1r_1R_2r_2r_2r_2$	4	0
$R_1r_1R_2R_2$		$R_1r_1r_1r_1R_2R_2r_2r_2$	4	0
$R_1r_1R_2r_2$		$R_1r_1r_1r_1R_2r_2r_2r_2$	16	0
$R_1R_1r_2r_2$		$R_1R_1r_1r_1r_2r_2r_2r_2$	0	1
$r_1r_1R_2R_2$		$r_1r_1r_1r_1r_2r_2r_2r_2$	0	1
$r_1r_1R_2R_2$		$r_1r_1r_1r_1R_2R_2r_2r_2$	0	1
$r_1r_1R_2r_2$		$r_1r_1r_1r_1R_2r_2r_2r_2$	0	4
$R_1r_1r_2r_2$		$R_1r_1r_1r_1r_2r_2r_2r_2$	0	4
$r_1r_1r_2r_2$		$r_1r_1r_1r_1r_2r_2r_2r_2$	0	1
Ratio (R_1-R_2-: $r_1r_1r_2r_2$)			25 (0.6944)	11 (0.3056)

showed that except for 'Bake King' and 'Shepody' other cv and numbered seedlings were susceptible to PVA (Table 2). The symptoms of chlorotic mosaic and tuber necrosis were observed in 'Bake King' althoug they were less extensive than in cv 'Shepody'.

Inheritance of the PVA resistance

Two hundred and forty-eight F_1 progenies from a cross of resistant ('Shepody') × susceptible ('Goldrus'), were tested for PVA reaction following graft-inoculation with PVA-infected scions. Grafted rootstocks of 'Shepody' were tested 60 days post-grafting by RT-PCR and one tuber each from 147 randomly selected plants was tested after harvest. In the plant tests 172 were resistant to PVA and 76 were susceptible; in the tuber test 103 were resistant to PVA and 44 were susceptible. These data provide a good fit for the existence of two independent dominant complementary genes controlling resistance in cv 'Shepody' (Table 3).

Discussion

Resistance to PVA in North American and European cultivars has shown different trends. North American studies have shown that many cultivars possess HR (Bagnall 1961). When cultivars are grafted with PVA-containing shoots, they develop top-necrosis and also produce severely necrotic tubers. The virus is readily detected from such plants. In contrast to the North American situation, European cultivars have been shown to respond to PVA infection by HR as well as by ER (Ross 1986; Barker 1996, 1997). The genes for HR are referred to as N_a and for ER as R_a (Ross 1986; Barker 1996, 1997; Hämäläinen 1998), and these are monogenically inherited (Valkonen et al. 1996). Many cultivars containing R_y genes show comprehensive resistance to PVA, PVY and PVV (Jones 1990; Barker 1997), and thus the *R* genes are preferred for introgression into new cultivars.

Our study showed that 'Shepody' plants grafted with PVA-infected shoots occasionally develop top-necrosis and that most of the grafted plants produce necrotic tubers (Fig. 1B,C); this aspect of the 'Shepody' reaction resembles an HR response. However, PVA is not recoverable from such plants and tubers by bioassay, ELISA or the highly sensitive method of RT-PCR (Table 1). Thus, the 'Shepody' reaction to PVA may consist of a combination of HR and ER. In addition, with the inclusion of the appearance of the virus-like, but manually non-transmissible, chlorotic mosaic symptoms throughout the plant (Fig. 1A), the response becomes truly a novel phenomenon of virus resistance. As far as we know, this type of resistance phenotype has not been reported before. It may represent a simultaneous expression of HR and ER and thus may involve either two different genes or else the ER response happened so rapidly that the amount of PVA accumulation was insufficient to induce HR (Benson and Hooker 1960; Valkomen 1994). This leads to the possibility that 'Shepody' may contain both N_a and R_a genes for PVA alone because 'Shepody' is not resistant to potyvirus PVY. Alternatively, both genes could be of the R_a type because some *R* genes have been shown to react after virus inoculation with a necrosis much less severe than is produced by genotypes expressing *N* genes (Benson and Hooker 1960; Barker

and Harrison 1984; Valkonen 1994). It appears that this alternative applies to the situation of 'Shepody' grafted with PVA-infected scions. In the majority of the grafted plants, necrosis is not the predominant symptom, and only occasional necrotic streaks were observed on the stems. In addition, virus multiplication was extremely low and only definitely detected in chlorotic leaves after Southern blot assay of the RT-PCR products (Fig. 2B).

The disease response data of the F_1 generation provided evidence for the inheritance of resistance. It was assumed that the resistance is due to a pair of independent dominant complementary genes, R_1 and R_2. A resistant plant should have the genotype R_1___ and R_2___. Thus, the resistant tetraploid parent, cv 'Shepody', is duplex for both resistant genes, i.e., $R_1R_1r_1r_1R_2R_2r_2r_2$, whereas the susceptible tetrapliod parent, cv 'Goldrus' is nulliplex for both genes, i.e., $r_1r_1r_1r_1r_2r_2r_2r_2$. Further, we assume that both loci are located close to the centromere and thus chromosomal segregation has taken place. Ten genotypes are expected from the gametes produced by 'Shepody' (Table 3). The overall expected segregation ratio of resistant versus susceptible progenies from the cross 'Shepody' × 'Goldrus' is 25 (resistant): 11(susceptible). The observed ratio obtained in the plant test of 172 resistant and 76 susceptible is 0.6935:0.3065 with a χ^2=0.0001. The observed ratio obtained with the tuber test of 103 resistant and 44 susceptible is 0.7007:0.2993 with a χ^2=0. Both indicate a perfect fit to the hypothesis of two independent complementary dominant genes. This evidence is in contrast to the monogenic-dominant inheritance observed in other investigations (Valkonen et al. 1996). It is possible that the resistant parent used in their studies was quadruplex (e.g., $R_1R_1R_1R_1$) or triplex (e.g., $R_1R_1R_1r_1$) for one of the pair of dominant resistant genes.

In an attempt to trace-back the sources of PVA resistance in 'Shepody', we were interested to note that 'Shepody' is susceptible to several potato viruses including the potyvirus PVY (Young et al. 1983). Among its immediate parents, 'Bake King' was found to have a similar resistance to PVA (Table 2) (Singh 1997). Among 'Bake King''s parents, 'Merrimack' and 'Merrimack''s immediate parent USDA seedling B96–56 are known to exhibit HR to PVA (Bagnall 1961). Therefore, the partial HR observed in 'Shepody' could have been introgressed through this HR lineage of 'Shepody'. Although leaf symptomology is novel and only a low concentration of PVA is recovered from grafted 'Shepody' plants, the presence of trace amounts of necrosis in plant stems and the extensive necrosis of the tubers suggest that the resistance observed in 'Shepody' could be of the HR type.

Use of PVY-transgenic potato plants to assess the PVA reaction in 'Shepody' shows that the natural resistance of 'Shepody' has not been affected by the incorporation of the PVY transgene. In comparative tests with several PVY-transgenic cultivars, 'Shepody' remained 100% resistant to PVA when tested with ELISA, while PVA-susceptible cultivars with the PVY transgene became 55–100% infected with PVA (data not shown).

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