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Extreme resistance to potato virus V in clones of *Solanum tuberosum* that are also resistant to potato viruses Y and A: evidence for a locus conferring broad-spectrum potyvirus resistance

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Abstract Extreme resistance to the potato V potyvirus (PVV) was found in four potato cultivars that contain *Ry* genes from *Solanum stoloniferum*. When plants of these cultivars, were inoculated by grafting in shoot tips from PVV-infected tomato plants, necrotic symptoms developed in some cultivars, although a full hypersensitive reaction was not elicited, while other cultivars were symptomless. PVV replication was not detected in any of the inoculated plants by ELISA, an infectivity assay of leaf extracts by manual inoculation to *Nicotiana benthamiana* indicator plants, or by 'return grafting' of shoot tips taken from newly developed shoots of the potato plants to virus-free indicator plants of tomato. These methods readily detected PVV infection in inoculated plants of cv 'Flourball', which does not contain an *Ry* gene and is susceptible, and in cvs 'Maris Piper' and 'Dr Macintosh', which contain gene *Nv* conditioning a hypersensitive reaction to inoculation. One of the *Ry*-containing cultivars, 'Barbara', has been previously shown to contain two genes that control extreme resistance, defined as no viral replication in intact plants, to the potyviruses potato viruses Y and A (PVY and PVA). These genes are: Ry_{sto}, which conditions resistance to PVY and PVA, and gene *Ra*, which conditions resistance to PVA only. It was found that in genotypes from a progeny of the cross 'Barbara' (Ry_{sto}/Ra) × 'Flourball' (*ry*/*ra*), extreme resistance to PVV segregated with gene Ry_{sto}. It is proposed that either gene Ry_{sto} conditions broad-spectrum extreme resistance to the distinct potyviruses PVY, PVA, and PVV or that Ry_{sto} represents a family of genetically closely linked genes each controlling resistance to a specific virus.

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Introduction

The phenotype of extreme virus resistance can be characterized as little or no response following inoculation and the failure to recover infectious virus from inoculated plants. Comprehensive extreme resistance to the potyviruses potato virus Y (PVY) and potato virus A (PVA) was one of seven phenotypic responses to be recognized by Cockerham (1970) in *Solanum tuberosum* containing *Ry* genes introgressed from *Solanum stoloniferum*. Ross (1986) listed approximately 20 European potato cultivars that contain *Ry* genes, but whether they are present in any non-European cultivars is unknown. Cockerham (1970) further designated *Ry* genes depending on the phenotype they conditioned; thus, Ry_{sto} conditions extreme resistance to PVY and PVA, and gene Ry^{na}_{sto} conditions extreme resistance to PVY and a hypersensitive reaction (HR) to PVA. In the context of this paper, HR is defined as a severe necrotic reaction to inoculation accompanied by the death of shoot apices but with the ready detection of virus replication in inoculated plants. Barker (1996) found that potato cultivars 'Barbara', which contains gene Ry_{sto}, also contains the previously unrecognized gene *Ra*, which confers extreme resistance to PVA only.

Mesophyll protoplasts isolated from two potato cultivars containing *Ry* genes introgressed from *S. stoloniferum* supported much lower levels of PVY replication than did protoplasts from HR clones following inoculation with RNA (Barker and Harrison 1984). Thus, while the *Ry* genes from *S. stoloniferum* do not confer immunity, they do condition a high level of extreme resistance in intact plants and protoplasts.

Potato virus V (PVV) was previously considered to be a strain of PVY, but it has now been recognized as a distinct potyvirus by Fribourg and Nakashima (1984), Jones and Fuller (1984), and Jones and Fribourg (1986). Calvert et al. (1980) found that most potato cultivars develop HR following graft inoculation with PVV, and Jones (1990) proposed that a single gene, *Nv*, which occurs in many potato cultivars, controls HR to this virus. In view of the fact that several cultivars containing *Ry* genes have extreme resistance to PVY and PVA, it would be prudent to also assess their resistance to PVV. This paper describes both tests that demonstrate that several cultivars containing gene *Ry* have extreme resistance to PVV and an investigation on the genetic control of resistance to PVV in cultivar 'Barbara'.

Materials and methods

Potato plant material

All plant material was grown in soil-less potting compost in an aphid-proof glasshouse at approximately 20°C. Stocks of virus-free potato cultivars were maintained in the glasshouse and propagated from tubers. Cultivars 'Barbara', 'Fanal', and 'Pirola' were kindly provided by Prof. Hans Ross, Germany; cultivars 'Corine' and 'Maris Piper' were obtained from commercial seed sources; 'Flourball' and Dr 'Macintosh' were from the Scottish Crop Research Institute potato germplasm collection. True seed progenies produced from a cross between 'Barbara' (Ry_{sto}, *Ra*) and 'Flourball' (*ry*, *ra*) were sown, and each seedling plant (genotype) was grown until tubers could be collected.

Virus resistance tests on cultivars and genotypes were made on tuber-grown plants.

Virus inoculation and resistance testing

An isolate of PVV obtained from the potato cultivars 'Arran Banner' was provided by the Scottish Agricultural Science Agency (SASA), East Craigs, Edinburgh, and was maintained in culture by manual transmission to plants of tomato (cv 'Moneymaker') or *Nicotiana tabacum* (cv 'Samsun'). Virus was transmitted to potato by graft inoculation using scions (shoot tips) from infected tomato plants which were 'cleft grafted' onto stems of potato test plants (approximately 0.3 m tall) from which the shoot apex had been removed. Two or three lateral meristems were allowed to grow, and tests for virus replication were made on tissue from the new shoots.

A standard test was used to assess resistance of graft-inoculated potato plants. Between 20 and 35 days after inoculation, at least two enzyme-linked immunosorbent assays (ELISA) were made on leaf extracts from each plant. Viral replication was also assessed in each plant by attempting to recover virus by 'return grafting', in which scions taken from newly developed shoots of the inoculated plants approximately 30 days after inoculation were grafted into virus-free tomato indicator plants. Occasionally, leaf extracts of test plants were manually inoculated to *Nicotiana benthamiana* indicator plants. Infection in the indicator plants was assessed by ELISA 3–4 weeks after inoculation.

Enzyme-linked immunosorbent assay

PVV was assayed by the antibody-trapped antigen form of indirect ELISA essentially as described by Barker et al. (1993). The following components were each incubated for 3 h at 37°C (unless stated otherwise) in polystyrene microtitre plates (NUNC): 1 μg/ml γ-globulin from an anti-PVY polyclonal antiserum (kindly provided by R. Burns, SASA), which traps particles of PVV; extracts of leaf samples (1 g of leaf/10 ml extraction buffer) for 16 h at 4°C; 1 μg/ml γ-globulin from a specific detecting monoclonal antibody to PVV (Scottish Crop Research Institute antibody collection); 1 μg/ml alkaline phosphatase-goat anti-rabbit IgG (Sigma); 0.6 mg/ml 4-nitrophenyl phosphate (Boehringer Mannheim) at room temperature for 2 h, then at 4°C for 16 h. Substrate absorbance was measured at 405 nm (A_{405}) using a Titertek Multiskan photometer (ICN Flow). A plant was considered to be infected if the leaf extract gave an A_{405} signal at least twice that given by an extract from a virus-free control plant.

Results

Reaction of potato cultivars to inoculation with PVV

Tests were made on several occasions to determine the reaction of the potato cultivars to inoculation with PVV; in each test two or three plants of each cultivar were graft-inoculated. Plants of cv 'Flourball' (susceptible control) occasionally developed systemic symptoms of mild mottle, although these could be difficult to identify, and virus was detected by ELISA, the infectivity assay, or 'return grafting' (Table 1). Plants of cultivars 'Dr Macintosh' (*Nv*) and 'Maris Piper' (*Nv*) developed necrotic lesions, necrotic streaks in the veins of the upper leaves, and eventually HR as characterized by the death of shoot tips and severe necrosis leading to the collapse of lower leaves; virus was detected in these plants by ELISA, infectivity assay, or 'return grafting'

Table 1 Reaction of a selection of potato cultivars to inoculation with PVV

Potato cultivars	Known potyvirus resistance genes[a]	Behaviour following inoculation of plants with PVV		
		Symptoms[b]	ELISA[c]	Virus recovery[d]
Barbara	Ry_{sto}, *Ra*	LN	−	−
Corine	Ry^{na}_{sto}	LN	−	−
Dr Macintosh	*Na*, *Nv*	HR	+	+
Fanal	Ry_{sto}	(LN)	−	−
Flourball	None	MM	+	+
Maris Piper	*Na*, *Nv*	HR	+	+
Pirola	Ry_{sto}	−	−	−

[a] Prior to this study, resistances known to be controlled by these genes were: Ry_{sto}, extreme resistance to PVY and PVA; Ry^{na}_{sto}, extreme resistance to PVY and hypersensitive reaction (HR) to PVA; *Ra*, extreme resistance to PVA; *Na*, HR to PVA; *Nv*, HR to PVV

[b] LN, Limited necrosis (data for 'Fanal' is in parentheses because necrosis appeared only infrequently); HR, hypersensitive response with generalised necrosis; MM, mild mottle

[c] In almost all tests, A_{405} values were 10- to 20-fold greater than those given by virus-free plants

[d] Virus recovery was determined by infectivity assay of foliage from inoculated plants, and by 'return grafting'. +, Virus recovered by both methods; −, no virus detected

(Table 1). Plants of cultivars containing *Ry* genes varied in their symptom response. Cultivars 'Barbara' (Ry_{sto}) and 'Corine' (Ry^{na}_{sto}) usually reacted with some necrosis in leaf veins, and also in stems, although this was often restricted to tissue immediately under the graft. However, this reaction was more limited than that which occurred on plants of 'Dr Macintosh' (*Nv*) and 'Maris Piper' (*Nv*). When necrosis did develop in 'Barbara' and 'Corine', it appeared later than that which developed in cultivars with HR, and it only occasionally caused shoot death. In general, cultivars 'Fanal' (Ry_{sto}) and 'Pirola' (Ry_{sto}) did not react to inoculation, apart from a very few leaves of 'Fanal' which became necrotic in some tests but not in others. However, despite the appearance of some necrosis in inoculated plants of cultivars containing *Ry* genes, PVV was never detected in leaf extracts by ELISA or by infectivity assay or 'return grafting' (Table 1).

Reaction of a progeny from a cross between 'Barbara' and 'Flourball' to inoculation with PVV

Plants of 38 genotypes from the progeny of a cross between 'Barbara' (Ry_{sto}, *Ra*) and 'Flourball' (*ry*, *ra*) were graft-inoculated with PVV. Plants of 2 genotypes developed mild mottle symptoms after inoculation, but no other plants developed symptoms. When the results from ELISA, 'return grafting' tests, and infectivity assays were examined, 19 genotypes were resistant (no virus multiplication detected), and 19 were susceptible (virus multiplication readily detected) (Table 2).

The resistance of individual genotypes to PVV was compared to their resistance to PVY and PVA identified in previous tests (Barker 1996). The 19 genotypes which were resistant to PVY (also resistant to PVA conditioned by the presence of gene Ry_{sto}) were resistant to PVV (Table 2). However, of the 29 genotypes that were resistant to PVA, 10 were susceptible to PVY (conditioned by the presence of gene *Ra*), and these were also susceptible to PVV, and 19 were resistant to both PVA and PVV (Table 3). The segregation ratios for resistance to these viruses was examined using a number of genetic models (Tables 2 and 3). The data in Table 2 best fit a model in which there is one locus controlling extreme resistance to PVY and PVV. However, the possibility cannot be discounted that there are two loci controlling extreme resistance which are closely linked in coupling phase. The data in Table 3 best fit a model in which there are two independent loci, one locus controlling extreme resistance to PVA and PVV and a second controlling extreme resistance to PVA only. Thus, in cultivar 'Barbara' extreme resistance to PVY, PVA, and PVV appears to be conditioned by gene Ry_{sto} (or a series of linked genes at one locus) and that to PVA only by gene *Ra* .

Discussion

On the basis that the necrotic reaction to PVV inoculation was limited and viral replication could not be detected (despite using very sensitive bioassays), cultivars 'Barbara', 'Corine', 'Fanal', and 'Pirola' appear to have extreme resistance to PVV. This type of extreme resistance (no detectable virus multiplication in intact plants) has not been reported previously for PVV. These results contrast somewhat with those of Jones (1990) who found that plants of cultivars 'Corine' and 'Pirola' reacted hypersensitively to inoculation with PVV. The results reported in this paper show that although there was a limited hypersensitive response

Table 2 Phenotypic ratios for resistance to PVY and PVV in progeny 93.VT.32 derived from cross 'Barbara' × 'Flourball'

	Genetic models tested[b]	Segregation following inoculation with[a]				Segregation on inoculation with PVY^N and PVV[a]			
		$PVY^{N\,c}$		PVV		Phenotype with Y/V reaction:			
Observed data		R 19	S 19	R 19	S 19	R/R 19	R/S 0	S/R 0	S/S 19
Expected ratios	1	1 : 1		1 : 1		1 : 1 : 1 : 1			
	2	1 : 1		1 : 1		1 : 0 : 0 : 1			
	3	1 : 1		1 : 1		1 : 2 : 2 : 1			
χ^2 (P)	1	0 (> 0.99)		0 (> 0.99)			N/A[d]		
	2	0 (> 0.99)		0 (> 0.99)			0 (> 0.99)		
	3	0 (> 0.99)		0 (> 0.99)			N/A		

[a] S, Susceptible; R, resistant and no virus multiplication detected
[b] Model 1: two independent loci, one of which controls extreme resistance to PVY, and the second which controls extreme resistance to PVV. Model 2: either one locus controlling resistance to PVY and PVV; or two loci controlling resistance as in model 1 but linked in coupling phase. Model 3: two loci controlling resistance as in model 1 but linked in repulsion phase
[c] Plants of genotypes that were resistant to PVY^N were also resistant to PVA.
[d] N/A, χ^2 test for a particular model is not appropriate because of missing phenotype class(es) in progeny

Table 3 Phenotypic ratios for resistance to PVA and PVV in progeny 93.VT.32 derived from cross 'Barbara' × 'Flourball'

	Genetic models tested[b]	Segregation following inoculation with[a]				Segregation on inoculation with PVA and PVV[a]			
		PVA[c]		PVV		Phenotype with A/V reaction:			
Observed data		R 29	S 9	R 19	S 19	R/R 19	R/S 10	S/R 0	S/S 9
Expected ratios	1	1 : 1		1 : 1		1 : 1 : 1 : 1			
	2	1 : 1		1 : 1		1 : 0 : 0 : 1			
	3	1 : 1		1 : 1		1 : 2 : 2 : 1			
	4	3 : 1		1 : 1		2 : 1 : 0 : 1			
χ^2 (P)	1	10.53 (> 0.01)		0 (> 0.99)			N/A[d]		
	2	10.53 (> 0.01)		0 (> 0.99)			N/A		
	3	10.53 (> 0.01)		0 (> 0.99)			N/A		
	4	0.035 (0.5–0.9)		0 (> 0.99)			0.053 (0.95–0.98)		

[a] S, Susceptible; R, resistant and no virus multiplication detected
[b] Model 1: two independent loci, one of which controls extreme resistance to PVA, and the second which controls extreme resistance to PVV. Model 2: either one locus controlling resistance to PVA and PVV; or two loci controlling resistance as in model 1 but linked in coupling phase. Model 3: two loci controlling resistance as in model 1 but linked in repulsion phase. Model 4: two independent loci, one controlling extreme resistance to PVA and PVV and the second locus controlling extreme resistance to PVA only
[c] Plants of some genotypes were resistant to PVA and also PVY^N; the rest were resistant to PVA only
[d] N/A, χ^2 test for a particular model is not appropriate because of missing or additional phenotype class(es) in progeny

with cultivar 'Corine', necrotic symptoms were never observed with cv 'Pirola'. It is possible that different virus isolates and environmental conditions used in my tests and in those of Jones (1990) may explain these differences. Indeed, the symptoms observed here were not identical in tests done at different times of the year. Jones (1990) did not report whether viral multiplication occurred in the plants he tested.

Cockerham (1970) identified a number of *Ry* genes from *S. stoloniferum* to which he gave specific designations and which corresponded to the various phenotypic responses he observed. It was recently shown in cv 'Barbara' that there are at least two separate genes controlling extreme resistance: gene Ry_{sto}, which confers resistance to PVY and PVA; and the previously undescribed gene, *Ra*, which confers resistance to PVA only (Barker 1996). Cultivar 'Pirola' contains Ry_{sto} but not *Ra*, but the precise genetic constitutions of 'Fanal' and 'Corine' are unknown, although it is assumed that they contain Ry_{sto} and Ry^{na}_{sto}, respectively (Barker 1996).

In progeny from a cross between 'Barbara' (Ry_{sto}, *Ra*) and 'Flourball' (*ry*, *ra*), resistance to PVV was found in genotypes carrying gene Ry_{sto} but not in those containing gene *Ra* only. Thus, these results suggest that Ry_{sto} confers broad-spectrum resistance to three potyviruses, namely potato viruses A, Y, and V. This is an intriguing result, although several questions remain. For example, is Ry_{sto} a single gene or a series of closely linked genes at one locus, each mediating resistance to a specific virus, and what is the nature of the virus-encoded elicitor of the resistance gene? Gene Ry_{sto} joins a short list of other genes which have been shown to confer broad-spectrum virus resistance. These include the *N* gene from *Nicotiana glutinosa* and the $TM\text{-}2^2$ gene from tomato, both of which confer broad spectrum resistance to tobamoviruses (reviewed by Culver et al. 1991 and Fraser 1990). Fisher and Kyle (1994) found evidence that a single gene from *Phaseolus vulgaris* may be responsible for conditioning resistance and/or lethal necrosis to nine potyviruses. They proposed that this simply inherited broad-spectrum resistance indicates that closely related viruses may have evolutionary conserved structures or processes necessary for pathogenesis that can be interrupted by the product(s) of a single host gene or a tightly linked series of genes (Fisher and Kyle 1996). Dogimont et al. (1996) reported that gene *Pr4* in *Capsicum annuum* confers resistance to PVY and pepper mottle virus (PeMV), which have 92% homology in the coat protein sequence. The three distinct potyviruses used in this study have relatively little sequence homology. For example, between PVY and PVA there is 48% identity in the amino acid sequence of the whole genome. When just the coat proteins are compared, there is 59% sequence identity between PVY and PVA, 58% between PVV and PVA, and 71% between PVY and PVV. The viral-encoded elicitors of some resistance genes have been identified. The resistance elicitors have been found to be the virus coat proteins in the potato virus X/gene *Rx* system (Bendahmane et al. 1995) and the tobacco mosaic virus/gene *N′* system (Culver and Dawon 1991). For some resistance genes, small specific amino acid changes in the viral-encoded proteins have been shown to alter the resistance phenotype. For example, Bendahame et al. (1995) showed that for the gene *Rx* in potato, which confers

extreme resistance to potato virus X (PVX), the substitution of a single residue in the PVX coat protein influences elicitor activity. Similarly, Knorr and Dawson (1988) demonstrated that for the *N′* gene of *Nicotiana sylvestris*, the alteration of a single amino acid in the coat protein of TMV was capable of changing the plant/virus interaction from a susceptible to a resistant response. Presumably, there is a conserved domain within the viral-encoded protein that binds to a receptor in the resistant host, and changes within this domain can influence elicitor activity. In the potyvirus/gene *Ry* system, the coat protein gene of PVY should be regarded as a potential resistance elicitor, although other viral-encoded proteins should also be considered. In future, the interaction between potyviruses and *Ry* and *Ra* genes should provide an immensely valuable tool by which to study the nature of host resistance genes and their viral elicitors.

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