

Resistance to potato virus A and potato virus Y in potato cultivars grown in Finland

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Phenotypic expression of resistance to potato Y potyvirus (PVY) and potato A potyvirus (PVA) was tested in 24 potato cultivars and an advanced breeding line using graft-inoculation under controlled conditions in the glasshouse. Resistance phenotypes were determined based on symptom expression and systemic infection detected with DAS-ELISA. Tubers were harvested from the PVA-inoculated plants and tested for PVA with ELISA. Sixteen potato cultivars expressed hypersensitive resistance (HR) to the strain group Y⁰ of PVY. Ute expressed extreme resistance (ER) to PVY (strain groups Y⁰ and Y^N) and PVA, and eight cultivars (Amazone, Bintje, Fambo, Posmo, Record, Rosamunda, Saturna and Van Gogh) expressed ER to PVA. These cultivars produced no PVA-infected tubers (tubers of Record were not tested). Matilda and Nicola expressed HR to PVA. The tubers of graft-inoculated Matilda produced no PVA-infected shoots, whereas shoots from Nicola tubers developed necrosis and severe mosaic symptoms and were PVA-infected based on results from ELISA. Comparison with purified PVA antigen (using ELISA) indicated that the secondarily infected shoots of the 14 PVA-susceptible cultivars contained 206–804 ng of PVA antigen per gram of leaf tissue.

Key words: hypersensitive response, extreme resistance, potyvirus, *Solanum tuberosum*

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Introduction

Potato A potyvirus (PVA) and potato Y potyvirus (PVY) belong to the large and agriculturally important family *Potyviridae* (Shukla et al. 1994). They can be transmitted mechanically, but are usually transmitted by aphids in the field in a non-persistent manner. PVA and PVY occur in

potato worldwide and can cause yield losses up to 40% and 80%, respectively (Hooker 1981).

Some potato cultivars (*Solanum tuberosum* L.) show necrotic lesions or top necrosis, i.e. a hypersensitive resistance response (HR), following infection with PVY and/or PVA (Cockerham 1943, Ross 1952, MacLachlan et al. 1953, Jones 1990). Expression of HR can reduce the spread of PVY and PVA in the potato crop in the field,

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but it seldom completely prevents it (Ross 1952). HR is usually virus strain-specific. Thus, virus isolates can be grouped according to their ability to elicit a particular hypersensitivity gene or genes in potato, and this forms the basis of the strain group concepts for PVY (Jones 1990) and PVA (Valkonen et al. 1995). Extreme resistance (ER) to PVY and/or PVA, defined as a very low incidence of infection in intact plants with extremely low virus titer in infected plants, is also known to exist in potato species. For reliable identification of ER in potato plants, graft-inoculation must be carried out (Benson & Hooker 1960, Valkonen et al. 1994). ER is functional against all strains of the potyvirus (Ross 1986, Valkonen 1994). Furthermore, ER to PVA and, in some potato genotypes, HR to PVA are also functional against PVY. HR and ER to PVY and/or PVA in potato are dominant and inherited monogenically (Cockerham 1943, 1970, Jones 1990, Ross 1986).

PVY is economically the most important virus infecting potatoes in Finland (Kurppa 1983). Strains of the ordinary strain group (Y^O) and the tobacco vein necrosis strain group (Y^N) of PVY exist in Finland, of which Y^N seems to be the more prevalent (Kurppa 1983). Many potato cultivars express specific HR to Y^O , whereas no HR to Y^N is known in cultivated potato. Thus, ER is required for the efficient control of all strains of PVY (Jones 1990). PVA is less prevalent than PVY in potatoes in Finland (Kurppa 1983). However, recent observations suggest that in certain cultivars, e.g., Sabina, Puikula and Pito, and also Hertha and Hankkijan Timo, PVA is prevalent and more important than previously recognized. The strains of PVA infecting potatoes in Finland have not been systematically grouped according to the strain group concept for PVA (Valkonen et al. 1995). Moreover, the resistance to PVA has not been systematically examined in most of the potato cultivars currently grown in Finland.

In this study, the phenotypic expression of resistance to PVA and Y^O was examined in potato cultivars and an advanced potato breeding line, all of which are commonly grown, or are bred to be grown, in Finland and are included in the

healthy seed potato production scheme at the Seed Potato Center, Tyrnävä. Experiments were carried out under controlled conditions in the glasshouse to permit reliable detection of the resistance phenotypes. Graft-inoculation was used to identify cultivars expressing ER, which provides efficient control of PVA and PVY in the field.

Material and methods

Pathogen-free minitubers of 25 potato cultivars (Table 1) were obtained from the Seed Potato Center, Tyrnävä. Tubers were planted in soil and plants were grown for virus inoculation under natural daylight in a glasshouse during March-May in 1995. The mean daily minimum and maximum temperatures were 18°C and 24°C.

An isolate of PVA (A-9506) was obtained from potato cv. Hankkijan Tanu infected in the field in Tyrnävä. Tobacco (*Nicotiana tabacum* L. cv. Samsun NN) was mechanically inoculated with the virus but developed no symptoms. The virus was back-inoculated mechanically to healthy plants of Hankkijan Tanu. It reacted serologically with polyclonal antibodies (Pab) to PVA (Boehringer Mannheim, Germany) and a monoclonal antibody to PVA (Mab no. 58/0; Browning et al. 1995) obtained from the Scottish Agricultural Science Agency (SASA), Scotland. It did not react with Pabs to PVY, potato X potyvirus, potato M and S carlaviruses (Boehringer Mannheim) and the Mab no. 53/8 to potato V potyvirus obtained from SASA. The isolate of PVY (PVY^O-UK) used in this study was described by Valkonen and Mäkäräinen (1993). A Finnish isolate of Y^N was kindly provided by A. Kuusela, Institute of Plant Protection, Agricultural Research Centre, Jokioinen, Finland. PVA, Y^O and Y^N were maintained in potato cvs. Hankkijan Tanu, Pito and Valtti, respectively, in the glasshouse.

Side-graft inoculation, using apical shoots of the virus-infected potato cultivars, was carried

Table 1. Phenotypic responses in potato cultivars following graft-inoculation with PVY^O and PVA. Symptoms were observed and the virus titres were determined in the uppermost fully-expanded leaves using DAS-ELISA four weeks after graft-inoculation (PVY^O and PVA) and four weeks after the emergence of the sprouts (tuber-borne infection with PVA).

Cultivar	Potato virus Y ^O		Potato virus A				
	Symptoms in graft-inoculated plants ^a	Resistance phenotype ^b	Symptoms in graft-inoculated plants ^a	Tuber-borne infection			Resistance phenotype ^b
				Symptoms ^a	Virus concentration (ng/g) ^c		
				Mean	s.d.		
Amazone	M	S	ns	ns	0		ER
Asterix	NL	H	ns	ns	475	130	S
Bintje	M	S	Npp	ns	0		ER
Fambo	TN	H	ChS	ns	0		ER
Gloria	M	S	ns	Ru	277	65	S
Hankkijan Tanu	NL, SM	H	ns	ns	689	171	S
Hankkijan Timo	NL, M	H	Mo	Ru, M	362	137	S
Hertha	M	S	Y	Y	259	130	S
Kardal	NL	H	M	ns	251	38	S
LH-152-80	NL	H	ns	ns	278	101	S
Matilda	TN	H	NL	ns	0		H
Nicola	NL	H	TN	NL, SM	78	31	H
Posmo	NL	H	Npp, ChS	ns	0		ER
Puikula	M	S	M	SM	300	59	S
Record	NL	H	Npp, ChS	*	*		ER(?)
Rosamunda	TN	H	ns	ns	0		ER
Sabina	M	S	M	ns	445	84	S
Satu	NL, LD	H	ns	ns	206	89	S
Saturna	NL	H	ns	ns	0		ER
Sieglinde (Siikli)	NL	H	ns	M	804	219	S
Sini (Jo-0912)	TN	H	ns	ns	329	123	S
Ute	nsi	ER	ns	ns	0		ER
Van Gogh	SSI	S	ChS	ns	0		ER
Vento	MM	S	MM	ns	439	224	S
Vital	NL	H	ns	Ru, M	262	57	S

^aChS, chlorotic spots; LD, leaf-drop; M, mosaic; MM, mild mosaic; Mo, mottle; NL, necrotic lesions and/or vein necrosis; Npp, necrotic pin-point lesions; Ru, rugosity; SM, severe mosaic; SSI, systemic symptomless infection; TN, top necrosis; Y, yellow blotches; ns, no symptoms; nsi, no symptoms and no infection based on ELISA; *, not determined.

^bER, extreme resistance, based on no virus detected with ELISA and no necrosis observed except limited pin-point lesions; H, hypersensitive resistance response, based on the development of necrosis and virus titers detected with ELISA in the graft-inoculated plants; S, susceptible, based on no necrotic response and high virus titers detected with ELISA.

^cMean concentration from six plants; s.d., standard deviation; *, not determined.

out according to Valkonen and Mäkäräinen (1993). Five plants of each potato cultivar were graft-inoculated with PVA and Y^O two weeks after shoot emergence. Additionally, five plants of cv. Ute were graft-inoculated with Y^N. One shoot per plant was graft-inoculated with one virus-infected potato scion and other shoots were

removed. One non-inoculated plant of each potato cultivar was grown as a control. Symptom development was observed until 28 days after inoculation. Virus titres were tested in the uppermost fully-expanded leaves using DAS-ELISA (Valkonen and Mäkäräinen 1993). PVA was tested using the Mab no. 58/0 and the alkaline

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phosphatase(AP)-conjugated Mab no. 58/0 obtained from SASA (Browning et al. 1995). PVA particles were purified from PVA-infected Tanu essentially as described by Browning et al. (1995). Virus concentration in the purified PVA preparation was determined spectrophotometrically and by comparison with known concentrations of a protein marker (Sigma) in gel electrophoresis (SDS-PAGE; Laemmli 1970). PVA concentrations in the leaf samples were determined by comparison with known concentrations of the purified PVA particles using DAS-ELISA. PVY was tested using the Pabs and AP-conjugated Pabs to PVY obtained from Boehringer.

Tubers of the PVA-inoculated potato plants were harvested eight weeks after inoculation and stored at 3°C for three months. Six tubers of each potato cultivar were planted in soil and grown under natural daylight in the glasshouse in September-October. Symptoms were observed and PVA titres were tested as described above four weeks after shoot emergence.

Results and discussion

Of the 25 cultivars tested, only Ute expressed ER to PVY and PVA (Table 1): no symptoms were observed, no Y⁰, Y^N and PVA were found following graft-inoculation, and no PVA was detected in the shoots grown from the tubers of the PVA-inoculated plants [ELISA readings (A₄₀₅) were similar (0.00 – 0.02) for the graft-inoculated and non-inoculated Ute regarding detection of PVY and PVA]. Ute carries genes from the wild potato species *S. stoloniferum* Schlecht. et Bché. (Ross 1986). Thus, it is likely that the comprehensive ER to PVY (and PVA) in Ute is controlled by the gene *Ry_{sto}* from *S. stoloniferum* (Ross 1986). Sixteen cultivars expressed HR to Y⁰. These were not tested with Y^N because it is known that the HR to Y⁰ in *S. tuberosum* is not elicited by Y^N and that *S. tuberosum* has no HR to Y^N (Ross 1986, Jones 1990). Seven cultivars were susceptible and ex-

hibited mosaic symptoms following infection with Y⁰. Van Gogh showed no symptoms (Table 1). This is consistent with the observations that Van Gogh can be severely infected with PVY in the field but remains symptomless (L. Pietilä, personal communication).

Nine cultivars (including Ute) were extremely resistant to PVA (Table 1). The necrotic pinpoint lesions observed in the plants of Bintje, Posmo and Record, following graft-inoculation with PVA, were similar to the lesions described in a few extremely resistant potato genotypes graft-inoculated with PVY (Ross 1986). As no tubers of the graft-inoculated Record were tested for PVA infection, ER to PVA in this cultivar could not be confirmed (Table 1). This caution in the determination of the resistance phenotype of Record is based on the observation that the graft-inoculated plants of many cultivars during this study had similar ELISA readings (i.e., negative) to the non-inoculated plants (data not shown). However, all six tubers of each of these cultivars produced shoots with high PVA titers, indicating that they were susceptible to PVA (Table 1). In Matilda and Nicola, necrotic lesions developed on the leaves and PVA was detected with ELISA following graft-inoculation, which indicated expression of HR. Fourteen potato cultivars were susceptible to PVA, but in many of them the shoots with secondary PVA infection (i.e., grown from the infected tubers) showed no symptoms (Table 1).

Stegemann and Schnick (1985) listed the susceptibility to PVY in many European potato varieties, 14 of which were included in this study. Susceptibilities were indicated by the numbers 1 (very low susceptibility) to 9 (very strong susceptibility) and by 0 (resistant). Additionally, HR and ER were specifically indicated for a few cultivars. However, the strains and strain groups of the PVY isolates used, and the method of testing, were not reported, but the evaluations were likely to have been based on observations in the field. Thus, the previously reported resistances (Stegemann and Schnick 1985) and the phenotypic resistance responses observed in this study cannot be readily compared. For example, no

cultivar which expressed characteristic HR to Y^o in this study was listed as hypersensitive to PVY by Stegemann and Schnick (1985). Susceptibility to PVA in Amazone, Bintje, Gloria, Nicola, Saturna, Sieglinde and Ute was low (2) or very low (1), and the susceptibility to PVA in Hertha was intermediate (5) according to Stegemann and Schnick (1985). Results from our study showed that Amazone, Bintje, Saturna and Ute express ER to PVA, Nicola expresses characteristic HR to PVA, and Gloria, Hertha and Sieglinde are susceptible to PVA. Record was hypersensitive to PVA according to Stegemann and Schnick (1985), whereas our results suggested that Record expresses ER to PVA. The differences between the results from this study and the list of Stegemann and Schnick (1985) are probably attributable to 1) the different virus strains used; 2) different methods used for testing the resistance expression; 3) the difficulty of observing HR reaction in the cultivars infected in the field

in the previous studies, and 4) some cultivars which are susceptible to PVY or PVA following graft-inoculation can have quantitative resistance to PVY and/or PVA which is expressed as a low incidence of infection in the field if the infection pressure, resulting from aphid infestation, is low (Davidson 1980).

In conclusion, this study reports the phenotypic expression of resistance to PVA and PVY in selected potato cultivars currently grown in Finland as determined under controlled conditions. The data are useful to agriculturalists and breeders as the utilization and deployment strategies for resistance to PVA and PVY will be partially determined by the locally prevalent virus strain groups and the comprehensive or strain-group-specific resistance of the potato cultivars and breeding lines.

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SELOSTUS

Suomessa viljeltävien perunalajikkeiden kestävyys A- ja Y-virusta vastaan

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Tutkimuksessa testattiin 24 perunalajikkeen ja yhden kotimaisen perunan jalostuslinjan kestävyyttä perunan A- ja Y-virusta vastaan. Testattavat perunat saastutettiin viruksilla varttamalla ne kasvihuoneessa. A-viruksella saastutetuista kasveista otettiin talteen mukulat, jotka idätettiin ja versot testattiin. Viruskestävyyden tyyppi määritettiin oireiden perusteella ja mittaamalla viruspitoisuudet ELISA:n avulla. Kuudessatoista lajikkeesta havaittiin hypersensitiivistä kestävyttä (yliherkkyttä) Y-viruksen roturyhmää Y⁰ vastaan, mikä ilmeni kuoliolaikkujen tai latvakuolion kehittymisenä. Ute havaittiin äärimmäisen kestäväksi Y- ja A-virusta vastaan. Amazone, Bintje, Fambo, Posmo, Record, Rosamunda, Saturna ja Van Gogh olivat äärimmäisen kestäviä vain A-virusta vastaan. Äärimmäinen kestävyys ilmeni siten, että kasveihin ei kertynyt ELISA:lla havaittavia viruspitoisuuksia. Lisäksi kasvit pysyivät oireettomina tai lehtiin ilmestyi pistemäisiä kuoliolaikkuja tai pieniä kloroottisia

laikkuja. A-viruksella saastutettujen äärimmäisen kestävien lajikkeiden mukuloista kasvaneissa versoissa ei ollut A-virusta. Matildassa ja Nicolassa ilmeni hypersensitiivistä kestävyttä A-virusta vastaan. Matildan mukuloista kasvaneissa versoissa ei ollut A-virusta, mutta Nicolan mukuloista kasvaneet versot olivat viroottisia ja niissä ilmeni kuoliota sekä selkeitä mosaiikkioireita. A-viruksen tartuttamat, viroottisista mukuloista kasvatetut versot olivat oireettomia useimmissa lajikkeissa. Tämän tutkimuksen perusteella ainoastaan Ute on luonnollisen kestävyytensä ansiosta suojattu sekä Y- että A-virukselta. Hypersensitiivisen kestävyuden tehokkuus vaihtelee lajikkeiden kesken ja eri ympäristöoloissa, ja kohdistuu vain tiettyyn tai muutamaan virusrotuun. Joissakin tässä tutkimuksessa alttiiksi todetuissa lajikkeissa Y- tai A-virussaastunta voi olla alhainen pellolla, mikä perustuu näiden lajikkeiden kykyyn vastustaa kirvojen leivittämää virusta.