A hypersensitive response-like mechanism is involved in resistance of potato plants bearing the \( R_{ysto} \) gene to the potyviruses potato virus Y and tobacco etch virus

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Potato plants carrying the \( R_{ysto} \) gene from \( Solanum stoloniferum \) are extremely resistant to a number of potyviruses, but it is not known at what stage of infection the resistance is expressed. The resistance may be due to \( R_{ysto} \) or to a closely linked gene. In this investigation, we used potato virus Y (PVY) and a tobacco etch virus construct that encodes \( \beta \)-glucuronidase (TEV-GUS) to monitor virus infections of potato plants. Systemic spread of either virus in resistant potato plants was not detectable by serology, RT–PCR, GUS assay or bioassay although each replicated in the initially infected cells of leaves from resistant potato cultivars and was transported into neighbouring cells. However, 3 days post-inoculation (p.i.) a necrotic reaction set in that stopped movement and accumulation of both viruses by 7 days p.i. The resistance reaction (probably a hypersensitive reaction) became visible as necrotic streaks on veins on the lower leaflet surfaces of some potato cultivars carrying the \( R_{ysto} \) gene and may be elicited by a common potyviral gene product.

Introduction

Potatoes can be infected by a large number of viruses [see Valkonen (1994) and references therein] and they are particularly vulnerable to virus infections because they are propagated mainly by tubers. This practice allows the transfer of viruses from one vegetative generation to the next. To overcome the devastating consequences of this type of virus transmission, many governments have implemented inspection and seed certification schemes that guarantee virtually virus-free seed potatoes. An even more effective strategy for avoiding virus diseases in potato crops is breeding for resistance to virus infections.

Natural genes for resistance to the main viral diseases of potato can be found in wild potato species (\( Solanum \) spp.) and can be integrated into the genome of cultivated potato (Valkonen, 1994). In this paper we deal with the dominant gene \( R_{ysto} \) from \( S. stoloniferum \) that was introduced in a number of potato cultivars more than 30 years ago (reviewed by Ross, 1986). This gene is effective against all potato virus Y (PVY) isolates (Fernandez-Northcote, 1983) including the tuber necrosis causing isolate PVY\(_{NTN}\) (Chrzanowska, 1995; Dolnicar, 1995; Le Romancer & Nedellec, 1997). To date, no breakdown of resistance has been observed. \( R_{ysto} \) or a closely linked gene may also confer extreme resistance to other potyviruses including potato virus A (PVA) (Cockerham, 1970), potato virus V (PVV) and tobacco etch virus (TEV) (Hinrichs \textit{et al.}, 1997) and we are not aware of any potyvirus that can overcome the resistance.

It is not known at what stage of virus infection the \( R_{ysto} \) resistance is expressed. No expression of the resistance gene is observed in protoplasts, because susceptible and extremely resistant potato cultivars do not differ significantly in the accumulation of PVY coat protein in inoculated protoplasts (Hinrichs \textit{et al.}, 1995). Barker (1996) has suggested the possibility that PVY and PVA may replicate in a few cells after inoculation of potato plants bearing the \( R_{ysto} \) gene. Here we report that TEV and PVY replicate in the initially infected cells of the leaves of extremely resistant potato cultivars and that these viruses move into neighbouring cells before the resistance reaction [probably a hypersensitive reaction (HR)] eventually stops movement and replication of the viruses.
Methods

Potato cultivars. The potato cultivars ‘Bettina’, ‘Pirola’ and ‘Ute’, which have been characterized as being extremely resistant to PVY, were obtained from the breeder Bavaria Saat, Brunneng, Germany. ‘Forelle’, another extremely resistant cultivar, was obtained from Nordsaat, Waterveredorf, Germany. The breeder of the PVY-susceptible cultivar ‘Quarta’ is Böhm, Lüneburg, Germany. Potato plants were grown in large pots in a growth chamber at temperatures of 21–23 °C with a light–dark cycle of 16/8 h. Fertilizer was applied once a week.

Viruses and virus strains. Particles of PVY*O, which belongs to the O strain group of PVY, and TEV-GUS were used as inoculum in these studies. TEV-GUS RNA was prepared by in vitro transcription of the plasmid pTEV?D-GUS.HC (Dolja et al., 1992; kindly provided by J. C. Carrington, Washington State University, USA). The RNA of TEV-GUS encodes the reporter protein β-glucuronidase (GUS; EC 3.2.1.31). Both viruses were maintained in Nicotiana tabacum L. ‘Ky14’; 8 or 10–14 days post-inoculation (p.i.), TEV-GUS particles or PVY particles, respectively, were purified as described by Hinrichs et al. (1995).

RNA was isolated from TEV-GUS and PVY particles according to Langeveld et al. (1991). Virus (0·5 mg) was resuspended in 0·5 ml extraction buffer (1 M Tris–HCl, pH 8·0, containing 100 mM LiCl, 10 mM EDTA and 1%, w/v, SDS), mixed with an equal volume of water-saturated phenol and incubated for 5 min at 65 °C. The phases were separated by centrifugation, and the aqueous phase was extracted twice more with phenol–chloroform (1:1, v/v). After extraction, RNA was precipitated in ethanol and dissolved in water at a concentration of 1 mg/ml.

Inoculation of plants. Celite-dusted leaves were inoculated mechanically with purified virus particles in 0·1 M phosphate buffer pH 7·2 or PVY RNA in 10 mM phosphate buffer, pH 7·2. Four weeks after PVY inoculation, one inoculated leaflet of each potato plant was homogenized and rubbed on a celite-dusted leaf of a tobacco ‘Ky14’ plant. Two weeks later the tobacco plants were checked for PVY symptoms.

GUS assays. In situ GUS assays were performed by vacuum infiltration of potato leaves with the colorimetric substrate X-gluc (5-bromo-4-chloro-3-indolyl β-glucuronic acid, cyclohexylammonium salt; from GBT, St Louis, Mo., USA) according to Jefferson (1987) as modified by Dolja et al. (1992). Infiltrated leaves were incubated overnight at 37 °C before they were cleared with 70% ethanol.

Fluorimetric assays for GUS activity in inoculated leaves were done essentially as described by Jefferson (1987) with the modifications described by Carrington & Freed (1990). The protein concentration in each leaf extract was measured according to Bradford (1976).

Serological assays. The presence of virus in inoculated potato plants was determined by Western blot analysis. Leaves from healthy and PVY- or TEV-infected potato plants were homogenized in sample buffer (Laemmli, 1970; 0·2 g tissue/ml) and applied to SDS–polyacrylamide gels (Laemmli, 1970). The separated proteins were transferred to Immobilon membranes (Millipore) according to Towbin et al. (1979). The membranes were probed with anti-PVY-IgG (Loewe Biochemica, Otterfingen, Germany) and anti-TEV-IgG (kindly provided by T. Pirone, Univ. Kentucky, Lexington, USA), respectively, and bound IgGs were detected by alkaline phosphatase-labelled goat anti-rabbit-IgG (Sigma) using standard techniques (Sambrook et al., 1989).

In situ immunostaining of mesophyll proplasts (prepared according to Hinrichs et al., 1995) and epidermal strips of PVY- or mock-inoculated potato leaves collected 0, 1, 2, 3, 4 and 10 days p.i. was done essentially as described by Luciano et al. (1989). The relative amount of virus in leaves collected on the same days was determined by direct double-antibody sandwich ELISA (Clark & Adams, 1977). A505 was measured with an ELISA reader. The anti-PVY antibody mentioned above was used for immunostaining and ELISA.

RT–PCR analyses. Total RNA was isolated from potato leaves that had been inoculated 4 weeks previously with PVY or buffer (mock-inoculation) by the method of Langeveld et al. (1991). First strand cDNA synthesis was performed using primer A {5’ ATGTCTTGTGACTTCAA-GTAG 3’}, complementary to nucleotide residues 9353–9327 in PVY RNA (Robaglia et al., 1989) according to the instructions of the supplier of the reverse transcriptase MMLV-RT (Gibco BRL). The reaction mixtures were incubated at 37 °C for 60 min, then heated to 95 °C for 6 min and finally cooled to 4 °C.

The cDNA was amplified by PCR in 50 µl reaction mixtures containing 0·5 µl of the RT reaction mixture, 50 pmol primer A and 50 pmol primer B {5’ TCACGTTCAATGAGAATG 3’}, nucleotide residues 8721–8740 in PVY RNA (Robaglia et al., 1989) according to the supplier of the Tag DNA polymerase (Promega). The samples were subjected to 30 cycles of amplification with periods of 30 s for melting at 94 °C, 1 min for primer annealing at 54 °C and 1 min for primer extension at 72 °C. In the last cycle, primer extension was extended to 8 min.

Results

PVY-susceptible (‘Quarta’) and extremely resistant potato plants (‘Bettina’, ‘Forelle’, ‘Pirola’ and ‘Ute’) were inoculated with PVY*O at the six to eight leaf stage (about 3 weeks after planting). Control plants were mock-inoculated with buffer. Four weeks after inoculation, the inoculated leaflet and a young, non-inoculated, fully developed leaflet of each plant were harvested and RNA was extracted from one half of the leaflet while the other half was homogenized in sample buffer for Western blot analysis. In all PVY-inoculated leaves, but not in the mock-inoculated leaves, PVY was detectable by RT–PCR (data not shown). However, only in ‘Quarta’ plants which were inoculated with PVY was virus detected in the systemic parts of the plant (Fig. 1). No virus could be detected by Western blot analysis (Fig. 1a) or by RT–PCR (Fig. 1b) in the younger leaves of mock-inoculated susceptible plants or in any of the extremely resistant potatoes. Inoculation of Nicotiana tabacum L. ‘Ky14’ plants with extracts of young leaves from PVY-inoculated potato plants homogenized in phosphate buffer confirmed these results (data not shown): ‘Ky14’ plants became infected only after inoculation with extracts from PVY-inoculated ‘Quarta’ plants. Thus, the virus is not able to spread systemically in potatoes carrying the Ry gene.

Potato leaves were inoculated with large concentrations of PVY (0·5 mg/ml) or PVY RNA (0·1 mg/ml) to maximize the numbers of primary infections. Samples of leaves that had been directly inoculated were subjected to ELISA for detection of the presence of virus. High absorbance values, presumably the result of residual inoculum, were obtained with 1–5 day samples of leaves of most of the cultivars that had been
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Fig. 1. Western blot (a) and RT–PCR (b) analyses of extracts of non-inoculated leaves of potato plants 4 weeks after inoculation with PVY. An anti-PVY IgG was used as primary antibody in Western blot analysis, while a 632 base sequence in the PVY coat protein coding region was detected by RT–PCR. Lanes in SDS–polyacrylamide gels (a) or 1.5% agarose gels (b) stained with ethidium bromide contained total protein extracts or RT–PCR products, respectively, from ‘Quarta’ [(Q) lanes 1, 2], ‘Bettina’ [(B) lanes 3, 4], ‘Forelle’ [(F) lanes 5, 6], ‘Pirola’ [(P) lanes 7, 8] and ‘Ute’ [(U) lanes 9, 10] after mock-inoculation [(m) lanes 1, 3, 5, 7, 9] or inoculation with PVY [(y) lanes 2, 4, 6, 8, 10].

Fig. 2. Analysis by ELISA (A_{405}) of accumulation of virus in potato leaves inoculated with PVY RNA (0.1 mg/ml) or mock-inoculated. Mean values from extracts of three inoculated leaves in two experiments are plotted.

Because earlier results had indicated that the extreme resistance is not expressed in protoplasts (Hinrichs et al., 1995), we did experiments to determine whether resistance is expressed in the initially inoculated cells and whether the virus is able to move into neighbouring cells. The lower surfaces of the leaves of potato plants were inoculated. Immediately after inoculation (10 min p.i.) and at daily intervals for 4 days the epidermis of inoculated leaves was removed and assayed for the presence of virus by immunostaining. In the 10 min p.i. samples, there was no staining of cells in the epidermal strips, but after 1 day p.i. single cells and groups of two neighbouring cells were stained in all cultivars tested. On the second day, clusters of two to five epidermal cells were stained. The number of cells in the stained clusters increased to six to ten until the fourth day, and again there was no difference between the cultivars (Fig. 3). In 10 day p.i. samples, there was a slight increase in the numbers of epidermal cells per cluster stained for PVY in the susceptible cultivar, but virtually no further increase in the extremely resistant cultivars.

To determine the extent to which virus had spread in mesophyll cells in the inoculated leaves, we isolated mesophyll protoplasts from leaves inoculated 4 days previously and subjected them to immunostaining analysis. While for ‘Quarta’, virus was detectable in 10–15% of protoplasts, fewer than 1% of the protoplasts isolated from the two extremely resistant potato cultivars, ‘Bettina’ and ‘Pirola’, were infected (data not shown).

Between 2 and 5 days after inoculation, necrotic streaks 2–4 mm in length appeared on the veins on the underside of inoculated with virus particles (data not shown). There was no evidence of the accumulation of progeny virus in the inoculated leaves of ‘Bettina’ or ‘Pirola’ plants and only a limited increase in ELISA values with leaves of ‘Quarta’ plants sampled 9 days p.i. Inoculation with viral RNA provided a clearer indication of the absence of accumulation of virus in the inoculated leaves of the resistant cultivars (Fig. 2). With both types of inoculum, the levels of virus produced in inoculated leaves of the susceptible ‘Quarta’ cultivar were moderate. These results demonstrate the limited spread of PVY in inoculated leaves, even of ‘Quarta’. 
inoculated leaves in cultivars ‘Forelle’, ‘Pirola’ and ‘Ute’ (Fig. 4). No streaks were visible on mock-inoculated plants or on leaves of the extremely resistant cultivar ‘Bettina’ or the susceptible cultivar ‘Quarta’ after PVY inoculation. The number, but not the length, of necrotic vein lesions seemed to be dependent on the inoculum concentration: 20 µg/ml PVY induced about 1-5 lesions per leaflet while a PVY inoculum of 1 mg/ml resulted in an average of 8 lesions per leaflet. These lesions were not observed or their number was significantly reduced after inoculation of the upper surface of leaves of ‘Forelle’, ‘Pirola’ and ‘Ute’. Cross sections through a lesion-containing vein revealed that collenchyma cells in particular were necrotic after PVY inoculation (Fig. 4d), although epidermal and mesophyll cells underlying the collenchyma cells sometimes also showed necrosis. No necrosis was observed in the vascular system. The period of time after inoculation before the necrotic reaction set in and the number of cells involved indicated that the virus moved from initially infected cells to neighbouring cells until the resistance response expressed as necrosis eventually stopped the virus.

Essentially the same type of lesion was detected in leaves of ‘Forelle’, ‘Pirola’ and ‘Ute’ after inoculation with TEV-GUS particles (Fig. 4c) and no lesions were produced in ‘Bettina’ and ‘Quarta’ leaves (data not shown). In previous experiments (Hinrichs et al., 1997) we were able to infect potato cultivars which were susceptible to PVY with TEV, but we were unable to generate systemic infections in cultivars carrying the Ry_{sto} gene. Because the same type of necrotic reaction was produced by PVY and TEV in the same cultivars and because resistance based on Ry_{sto} or a closely linked gene seemed to be effective against both viruses, we decided to study the resistance reaction in potato to TEV in detail with the aid of a TEV-GUS construct (Dolja et al., 1992). TEV-GUS has the advantage that it carries the Escherichia coli uidA gene (GUS) as a reporter gene to monitor the spread of virus in the plant. We used TEV-GUS particles purified from infected tobacco tissue instead of tissue extracts as the inoculum because initial experiments showed that the GUS enzyme in the leaf extracts interfered with the detection of GUS produced by translation of the viral RNA during the first 2 days p.i.

TEV-GUS- and mock-inoculated leaves of cultivars ‘Bettina’, ‘Pirola’ and ‘Quarta’ were vacuum infiltrated with the GUS substrate immediately after inoculation and at intervals of 24 h for 6 days (Fig. 5). No staining was observed in mock-inoculated leaves or in leaves that were infiltrated just after inoculation (data not shown). In leaves of all cultivars tested 1 day p.i., single cells, most of them in leaf hairs, became stained. This suggested that the virus usually entered the plant through leaf hairs after mechanical inoculation and that it replicated in the initially infected cells of susceptible and resistant cultivars (Fig. 6). By 2 days p.i., we observed blue areas three to six cells in diameter in ‘Quarta’ and smaller blue areas of up to four cells
Fig. 4. Necrotic streaks in veins 4 days after inoculation of lower surfaces of ‘Pirola’ leaves with PVY (b) or TEV-GUS (c) particles (250 µg virus/ml each) or after mock-inoculation (a). Cross-section through a PVY-induced necrotic area in a vein (d). Note that more collenchyma cells than epidermal cells are necrotic. Bar marker represents 100 µm.

in the resistant cultivars ‘Bettina’ and ‘Pirola’. The areas in ‘Quarta’ did not increase in size in a circular pattern but had spread along veins by the third day (Fig. 5). By 4 days p.i., parts of the main veins were blue after infiltration of the substrate and about 5 days p.i. the virus seemed to have spread from the inoculated leaflet into the stem of ‘Quarta’ (Fig. 5). This pattern was never observed in the leaves of the extremely resistant plants. With these, the infected areas grew in both cultivars until 4–5 days p.i. when they reached diameters of about ten cells. However, at 3 days p.i. these areas were not completely stained blue after infiltration of the GUS substrate but instead necrotic cells were always visible. Often only a few cells in the centre and at the border of the lesions were stained blue (Fig. 6c, d). In most cases the necrosis was complete after 6 days p.i.,
Fig. 5. TEV-GUS-inoculated leaves of ‘Bettina’, ‘Pirola’ and ‘Quarta’ plants collected at daily intervals from 1 to 6 days p.i. and stained for GUS activity. After incubation in the substrate solution the leaves were destained in 70% ethanol. Note small blue areas (arrows) in ‘Pirola’ 2 and 3 days p.i., necrotic vein lesions (arrowheads) in ‘Pirola’ 3 days p.i. and in ‘Bettina’ 6 days p.i. and necrotic lesions (arrowheads) in the intercostal region in ‘Pirola’ and ‘Bettina’ 4, 5 and 6 days p.i.
Resistance reaction to PVY and TEV in potato

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**Fig. 6.** GUS-stained leaves from potato plants inoculated with 250 µg TEV-GUS particles/ml (50 µl per leaflet). In ‘Pirola’ only single cells were heavily stained 2 days p.i. (a) and the virus appeared to initially infect a leaf hair (b). Necrotic lesions 3 days p.i. in a leaf vein from ‘Pirola’ (c) and in an intercostal region of ‘Bettina’ 5 days p.i. (d). Blue-stained cells were visible at the centre and border of the lesions. Cross-section through blue-stained areas 5 days p.i. revealed infection of most of the cells in a section from an inoculated ‘Quarta’ leaflet (e), while the spread of TEV-GUS indicated by the blue-stained cell surrounded by necrotic reacting cells stopped well before reaching the sieve elements in a ‘Pirola’ leaflet (f). Bar markers represent 100 µm.

i.e. no TEV-GUS-infected cells were detectable after infiltration of the substrate X-gluc. Apparently, both TEV-GUS and PVY trigger an HR in potato cultivars carrying the Ry_{sto} gene.

There was a small difference in the reaction between the two resistant cultivars after TEV-GUS inoculation. While ‘Pirola’ showed distinct necrotic streaks on the veins (Fig. 4c) in addition to the necrotic lesions in the intercostal region of the leaflet, the veinal necrosis was not so pronounced in...
‘Bettina’ although small necrotic streaks on the veins were visible especially after clearing the leaflet with 70% ethanol (Fig. 5).

The results of in situ staining of GUS were confirmed by the fluorimetric GUS assays of extracts of the inoculated leaves (data not shown). When leaves were inoculated with TEV-GUS particles (250 µg/ml), the GUS activity of the extremely resistant cultivars ‘Bettina’ and ‘Pirola’ remained stable over the entire observation period at about the level of mock-inoculated leaves of all cultivars tested. In contrast to the resistant cultivars, there was a steady increase in GUS activity in ‘Quarta’ from 1 days p.i. until the end of the experiment (7 days p.i.) when quantification of GUS activity showed a 1000-fold difference between ‘Quarta’ and the two resistant cultivars (data not shown).

Contrary to previous results (Hinrichs et al., 1997), we were only in very rare cases able to detect TEV-GUS in uninoculated parts of ‘Quarta’ plants. Only one out of 10 plants showed GUS activity 14 and 28 days p.i., but there was no activity in 7 and 21 days p.i. samples. No GUS activity was detected in the non-inoculated leaves of cultivars ‘Bettina’ and ‘Pirola’ 1, 2, 3 or 4 weeks after inoculation (data not shown). These results were confirmed by Western blot analysis (data not shown), where virus particles were also only detected in one out of 10 ‘Quarta’ plants tested.

**Discussion**

Extreme resistance of plants to viruses is defined by three criteria: localization of the virus to the primary infection site, restricted virus replication and little or no visible manifestation of the resistance effect (Valkonen, 1994; Barker, 1996). Our results lead to the conclusion that PVY did not spread systemically in the extremely resistant potato plants and that the resistance reaction inhibited replication and or short-distance movement in the inoculated leaves to an extent that PVY-susceptible tobacco plants did not become infected when inoculated with extracts of the leaves. Therefore, the criteria characterizing extreme resistance are fulfilled. This situation differs from the interaction between the potyvirus tobacco vein mottling virus (TVMV) and the resistant tobacco ‘Tennessee 86’, where the virus could be recovered from inoculated leaves as determined by infectivity assay (Gibb et al., 1989).

The extreme resistance also seems to be effective against infections with other potyviruses like TEV. Potato plants carrying the Ry gene never became systemically infected when inoculated with TEV even though the virus could spread in PVY-susceptible potato plants (Hinrichs et al., 1997). In experiments described here, the resistant plants did not become systemically infected with TEV-GUS as determined by serological and GUS analyses. However, since nine of ten plants of the susceptible cultivar ‘Quarta’ also failed to develop systemic infections when inoculated with TEV-GUS, the use of the modified TEV only provided significant information in experiments with inoculated leaves.

At what stage of infection is the resistance to potyviruses in the extremely resistant potato cultivars expressed? According to Barker & Harrison (1984), the genes for resistance to PVY are expressed in potato protoplasts. However, we have detected the accumulation of coat protein after inoculation with PVY of protoplasts from extremely resistant potato cultivars (Hinrichs et al., 1995). Thus, the Ry-gene is not effective in inhibiting PVY multiplication at the single cell level.

The immunostaining experiments demonstrated that the virus is replicated in the directly inoculated cells of leaflets of resistant plants and that the initial spread of virus to neighbouring epidermal cells proceeds in the same manner as it does in susceptible plants. Therefore, infection of mechanically inoculated cells and short-distance movement of PVY from such cells are not impeded by a resistance mechanism operating in epidermal cells during the first 4 days after inoculation.

To examine the extent to which PVY had spread from epidermal to mesophyll cells, we isolated protoplasts from potato leaves that had been inoculated 4 days p.i. and determined the proportion of them that were infected. Up to 15% of the protoplasts from PVY-inoculated ‘Quarta’ were positively stained. However, fewer than 1% of protoplasts from the inoculated leaves of ‘Bettina’ and ‘Pirola’ plants were infected and we could not exclude the possibility that these protoplasts became infected by the inoculation procedure itself. Since there were few if any epidermal protoplasts present in the mesophyll protoplast samples, it can be concluded that the movement of virus in the mesophyll tissue of the resistant cultivars was impeded.

Impaired cell-to-cell movement of PVY has also been suggested for pepper plants carrying the Yang (pr2) resistance gene (Arroyo et al., 1996) because the virus replicated in inoculated protoplasts but fewer than 1% of protoplasts isolated from leaves of resistant pepper cultivars 2 weeks after inoculation were infected. Gibb et al. (1989) found no infected mesophyll cells after inoculation of a resistant tobacco cultivar with TVMV, while in compatible virus–plant interactions more than 60% of the mesophyll cells became infected.

Analysis of leaves inoculated with TEV-GUS particles yielded results similar to those described for PVY. Nearly identical rates of cell-to-cell movement were detected in the epidermises of susceptible and resistant plants until 2 days p.i., when areas of three to six cells in diameter were stained blue (Fig. 5). In TEV-resistant N. tabacum ‘V20’ (Schaad & Carrington, 1996) and Solanum brevidens plants (Valkonen & Somersalo, 1996) inoculated with the same TEV construct, the short-distance movement of the virus in the epidermis was apparently also not impeded until 3 days p.i. While in S. brevidens, resistance is associated with restricted cell-to-cell movement in mesophyll tissue leading to a lack of systemic...
spread (Valkonen & Somersalo, 1996). Schaad & Carrington (1996) suggested that the block in long-distance movement of TEV-GUS in tobacco ‘V20’ is caused by inhibited entry into, or exit from, sieve elements. In contrast to the events in S. brevidens plants, the virus moved into the mesophyll tissue of the leaf after inoculation of potato plants bearing the Ry
sto gene, albeit to a limited extent. While the virus entered the phloem parenchyma and companion cells adjacent to the sieve elements in tobacco ‘V20’ (Schaad & Carrington, 1996), the movement of virus ceased in resistant potato plants well before it could enter these cells (Fig. 6f).

In the susceptible cultivar ‘Quarta’, only small regions of the epidermis of the inoculated leaflet had become infected by the end of the experiment (7 days p.i.), while Valkonen & Somersalo (1996) reported that nearly the entire epidermis of S. brevidens leaflets was infected at 2 days p.i. The infection foci in ‘Quarta’ leaflets expanded radially during the first 3 days p.i. similar to the behaviour of the virus in the tobacco line ‘V20’ (Schaad & Carrington, 1996), but after 3 days the blue infection foci developed mainly along the leaf vein, indicating that movement in the vascular system was more rapid than cell-to-cell movement (Fig. 5).

The restricted movement of TEV-GUS in extremely resistant potato plants was always associated with a necrotic response which resembled an HR (Fig. 5). However, we could not determine whether the HR was directly responsible for the limitation of virus replication and movement or whether the necrosis was only a by-product of the resistance reaction.

A striking form of necrotic streaking of veins was produced when the lower surfaces of leaves from ‘Forelle’, ‘Pirola’ and ‘Ute’ were inoculated with PVY or TEV-GUS (Fig. 4). The necrosis apparently started in the collenchyma cells (Fig. 4d) and spread from there into the surrounding tissue including the epidermis. It was completed by about 7 days p.i. Since the vascular system never developed necrosis, the phloem itself apparently did not directly participate in the resistance reaction by undergoing an HR and thereby destroying its capability to transport the virus. The resistance was probably effective before the virus had a chance to enter the phloem for long-distance movement. Our TEV-GUS experiments demonstrated that the virus did not spread concentrically in the leaflet of the susceptible cultivar ‘Quarta’ but in the direction of a leaf vein (Fig. 5). Therefore, PVY and TEV may also have moved mainly towards a vein in the resistant cultivars, thereby entering collenchyma cells. At that stage of infection, they set off a pronounced necrotic relaxation which became visible as veinal necrosis. Cells between veins also became necrotic (Figs 5 and 6) but their reaction was not as distinct as that of the veins, probably because the viruses were transported essentially to the veins, where the higher virus titre triggered a distinctive necrosis.

Signs of an HR in the form of necrotic lesions after PVY-inoculation of some potato cultivars carrying the Ry-gene from S. stoloniferum have been reported (Cockerham, 1970; Jones, 1990; Barker, 1996). In our work, no vein necrosis was observed after PVY- or TEV-inoculation of the extremely resistant cultivar ‘Bettina’ or the susceptible cultivar ‘Quarta’. The failure to detect veinal necrosis in ‘Bettina’ could be explained by the fact that the expression of the Ry-gene from S. stoloniferum is modified by other genes that are also involved in the resistance reaction (Flis, 1995).

Taken together, the results of our TEV and PVY experiments are very similar. Both viruses replicate in initially infected cells, move into neighbouring cells and trigger what is probably an HR in potatoes carrying the Ry
sto gene. These common features let us speculate that the product(s) of the Ry
sto gene or closely linked genes effectively prevent infections with TEV or PVY and that the resistance response is elicited by a common feature of potyviruses (Hinrichs et al., 1997). However, genetic analyses are necessary to determine whether resistance to potyviruses like PVA, TEV and PVV cosegregates in segregating potato populations. Transient expression of potyviral genes in extremely resistant cultivars will be used to determine which potyviral gene product actually triggers the resistance response.

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References

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