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Resistance to potato leaf roll virus and potato virus Y in somatic hybrids between dihaploid *Solanum tuberosum* and *S. brevidens*

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Summary. Many somatic fusion hybrids have been produced between a dihaploid potato *Solanum tuberosum* and the sexually-incompatible wild species *S. brevidens* using both chemical and electrical fusion techniques. *S. brevidens* was resistant to both potato leaf roll virus (PLRV) and potato virus Y (PVY), the viruses being either at low (PLRV) or undetectable (PVY) concentrations as determined by enzyme-linked immunosorbent assay (ELISA). The *S. tuberosum* parent was susceptible to both viruses. A wide range of resistance, expressed as a decrease in virus concentration to both viruses was found amongst fusion hybrids, four of which were especially resistant. The practicality of introducing virus resistance from *S. brevidens* into cultivated potatoes by somatic hybridisation is discussed.

Key words: Potato, *Solanum brevidens,* somatic hybrid, PLRV, PVY

Introduction

Potato leaf roll virus (PLRV) and potato virus Y (PVY) are the most important virus pathogens of potatoes. Both are transmitted by aphids, although in different ways (Kennedy et al. 1962). Insecticides can limit their spread (Marco 1980; Gibson et al. 1982) but the increasing incidence of insecticide-resistant *Myzus persicae* (Ffrench-Constant and Devonshire 1986), the principal virus vector, emphasises the need for alternative control methods. Single gene sources of immunity to PVY are available (Barker and Harrison 1984). However, although potato cultivars differ in resistance to PLRV (Barker and Harrison 1986), there appears to be neither immunity nor major gene resistance to this virus within either *S. tuberosum* or known sexually-compatible wild species (Ross 1986). Resistance to both PVY and PLRV has been reported in *S. brevidens,* a wild non-tuber-bearing species which is sexually incompatible with *S. tuberosum* (Jones 1979; Hermsen and Taylor 1979). Somatic hybridization techniques are now sufficiently well developed to allow the introduction of many characters from sexually incompatible species into the cultivated potato.

In recent work in our laboratory, protoplast culture and fusion techniques have been developed that are suitable for a range of agronomically advanced dihaploid *S. tuberosum* genotypes (Tempelaar and Jones 1985 a, b; Fish et al. 1987). Many somatic hybrids have been produced both by chemical and electrical fusion (Fish et al. 1987, 1988) between *S. brevidens* and PDH 40, a dihaploid *S. tuberosum* genotype derived from the PLRV and PVY resistant cv. Pentland Crown (De Maine 1982). In this paper, we report the results of experiments in which the resistance of some of these somatic hyprids to PLRV and to two common strains of PVY, PVY^O and PVY^N (Ross 1986), has been assessed.

Materials and methods

Plant material

The parental plants (dihaploid *S. tuberosum* PDH 40, *S. brevidens* CPC 2451), and somatic hybrid plants derived from chemical fusions (designated 65003, 65006, 65009, 65013, 65014, 70023, 70064, 70067) and electrofusion (designated 81011, 81012, 81025, 81078, 84042, 84111, 84118, 84140) were maintained as in vitro shoot cultures on Murashige and Skoog (1962) (MS) medium 20 mg l^{-1} sucrose 0.05 mg l^{-1} naphthalene acetic acid and 10 g l^{-1} agar at pH 5.8. For experiments with PLRV, plants were established from in vitro shoot cultures by planting them in EFF compost (EFF products, Guildford, Surrey) in

8 cm pots. The plants were grown in either a controlled environment room (250 µE m^{-2} s^{-1}, 18°–15°C, 12 h/day, 70/80% R.H) or in a glasshouse (natural daylight, ambient temperature, June–July 1987).

PLRV inoculation

The PLRV used was from naturally infected plants of the PLRV-susceptible potato cv. Maris Piper. In experiments, *Myzus persicae* reared on turnip cv. Snowball acquired the virus by feeding on detached leaves of PLRV-infected Maris Piper for 3–4 days. The aphids were then confined for 3–4 days on the test plants after which they were killed by spraying with the insecticide pirimicarb.

PVY inoculation

Isolates of PVY^{O} and PVY^{N}, obtained from naturally infected plants of potato cv. King Edward, were maintained separately in plants of tobacco *Nicotiana tabacum* cv. White Burley. In experiments, plants were kept in the dark ca 20 h and dusted with carborundum abrasive before being manually inoculated with sap from infected plants.

Enzyme-linked immunosorbent assay (ELISA)

The presence and/or titre of PLRV and of PVY in inoculated test plants was examined by ELISA using the method described by Clark and Adams (1977). Samples were prepared by macerating 0.5 g of tissue of young leaves in 2 ml extraction buffer followed by brief low-speed centrifugation to remove large debris. Antiserum and antiserum conjugated with alkaline phosphatase obtained from Bioreba AG were diluted 1:1000 as recommended. After incubation with substrate (nitrophenol phosphate), absorbance at 405 nm was read using a microplate reader (Titertek, Multiskan MC).

Immuno-specific electron microscopy (ISEM)

The number of virus particles present in selected PLRV and PVY inoculated plants was examined by ISEM according to Govier (1985), using the same PLRV and PVY antisera as used for ELISA.

Virus testing

1 Preliminary screen of chemical fusion somatic hybrids

S. brevidens (4 plants), PDH 40 (1 plant), Maris Piper (5 plants), and somatic hybrid plants (65003, 65006, 65009, 65013, 65014, 70023, 70064 and 70067, 2 plants each) were inoculated with 15 viruliferous *M. persicae* per plant, and virus antigen titre was assayed by ELISA 5 weeks later.

2 PLRV resistance of three somatic hybrids derived from chemical fusion

From the preliminary screen, the three somatic hybrids that contained least detectable PLRV (65009, 70064, 70067) were selected for further testing.

Single, 12 cm high plants of *S. brevidens*, Maris Piper, and hybrids (65009, 70064 and 70067) were inoculated twice, at a 7 day interval, with 50 viruliferous *M. persicae*. PLRV was assayed by ELISA and ISEM 8 weeks after the second inoculation.

3 In vivo comparison of PLRV titre in somatic hybrid 65009

2-fold serial dilutions in sap extract of healthy Maris Piper leaves were made with sap extract from Maris Piper (experiment 2) inoculated with PLRV and the virus titres of these extracts were compared with an undiluted extract of the PLRV-inoculated plant 65009 described in Sect. 2 above.

4 PLRV-resistance of electrofusion somatic hybrids

Single plants of *S. brevidens*, PDH 40, Maris Piper and hybrids (81011, 81012, 81025, 81078, 84042, 84111, 84118 and 84140) were inoculated three times at 2 week intervals with 50 viruliferous *M. persicae*. PLRV was assayed by ELISA 8 weeks after the first inoculation.

5 PVY resistance of chemical and electrofusion hybrids

Single plants of *S. brevidens*, PDH 40, Maris Piper, chemical fusion hybrids (65003, 65006, 65009, 65013, 65014, 70023, 70027, 70064 and 70067) and electrofusion hybrids (81011, 81012, 81025, 81045, 81068, 81078, 84042, 84087, 84111, 84126 and 84140) were inoculated with either PVY^{O} or PVY^{N} and assayed by ELISA 21 and 26 days later respectively.

Results

1 Preliminary screen of chemical fusion somatic hybrids

Samples from all plants except one (70064) inoculated with PLRV gave greater readings in the ELISA than uninoculated controls. This indicates that infection occurred in all except in this one plant (Table 1). However, values for inoculated *S. brevidens* were a tenth those of inoculated PDH 40 and Maris Piper and only slightly greater than those of uninoculated *S. brevidens*. The average absorbances for each of the inoculated somatic hybrids lay between that for the inoculated *S. brevidens* and that of inoculated PDH 40. This suggests that PLRV resistance from *S. brevidens* had been incorporated into the somatic hybrids.

2 PLRV resistance of three somatic hybrids derived from chemical fusion

Plants of 65009, 70064 and 70067 inoculated with PLRV and ELISA absorbance readings greater than uninoculated controls (Table 2) indicating that all plants had been infected. However, the absorbance readings of PLRV-inoculated 65009 and 70067 were considerably lower than that of PLRV-inoculated Maris Piper, indicating that there was much less virus antigen in these plants. Furthermore, no PLRV particles were seen on electron microscope grids treated with PLRV antiserum (ISEM) and incubated with sap from either 65009 or 70067 whereas they were readily observed on grids incubated with sap from PLRV-inoculated Maris Piper and 70064.

3 In vivo comparison of PLRV titre in somatic hybrid 65009

The absorbance reading of undiluted extract from PLRV-inoculated 65009 coincided with sap extract of

Table 1. ELISA absorbance readings (405 nm) of plants tested in the preliminary screen for PLRV resistance in somatic hybrids from chemical fusions

Treatment	Plant 1	Plant 2	ELISA absorbance reading (405 nm)[b]			Average	Chromosome no.
			Plant 3	Plant 4	Plant 5		
S. brevidens PLRV inoculated	0.107±0.130[a]	0.116±0.007	0.143±0.005	0.096±0.013		0.115	24
S. brevidens uninoculated	0.072±0.022	0.061±0.004				0.065	24
PDH40 PLRV inoculated	1.199±0.044					1.199	24
PDH40 uninoculated	0.072±0.022	0.104±0.018				0.094	24
Maris Piper PLRV inoculated	1.280±0.084	1.210±0.026	1.420±0.013	1.310±0.057	1.640±0.028	1.370	48
70067 PLRV inoculated	0.197±0.007	0.166±0.004				0.181	47
70064 PLRV inoculated	0.434±0.002	0.079±0.005				0.257	48
65009 PLRV inovulated	0.198±0.005	0.493±0.005				0.345	71
65003 PLRV inoculated	0.326±0.001	0.419±0.008				0.372	47
65013 PLRV inoculated	0.601±0.014	0.396±0.004				0.499	48
65014 PLRV inoculated	0.467±0.003	0.860±0.007				0.664	47
70023 PLRV inoculated	0.523±0.006	0.942±0.013				0.733	48
65006 PLRV inoculated	1.290±0.029	1.079±0.009				1.189	48

[a] Mean of three readings ± standard error of the mean
[b] ELISA reading after 75 min

Table 2. ELISA absorbance reading (405 nm) of somatic hybrids derived from chemical fusion, uninoculated or inoculated with PLRV

Genotype	ELISA absorbance reading (405 nm)[c]		Chromosome no.
	uninoculated	PLRV inoculated	
Solanum brevidens	0.059±0.005[a]	0.071±0.010	24
Maris Piper	0.076±0.005[a]	2.370±0.015	48
65009	0.079±0.011[b]	0.320±0.016	71
70067	0.057±0.006[b]	0.610±0.027	47
70064	0.067±0.008[b]	1.700±0.054	48

[a] Average of four readings per plant
[b] Average of five readings per plant
[c] ELISA reading after 5 h

PLRV-inoculated Maris Piper diluted between four and eight times (Fig. 1).

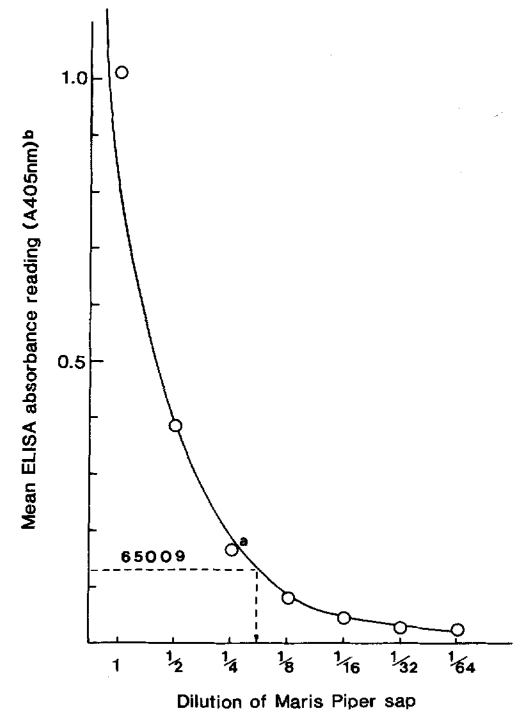

Fig. 1. A comparison of the ELISA absorbance values of PLRV-inoculated 65009 and sap from inoculated Maris Piper serially diluted in healthy sap

4 PLRV resistance of electrofusion somatic hybrids

All the electrofusion somatic hybrids inoculated with PLRV showed greater ELISA absorbance readings than uninoculated controls, indicating that they had all been infected with PLRV (Table 3). The absorbance reading for the inoculated *S. brevidens* did not exceed that of the uninoculated control. The values for most of the PLRV-inoculated hybrids lay between those of *S. brevidens* and PDH 40, suggesting that PLRV resistance had been transferred to some of the hybrids.

5 PVY resistance of chemical and electrofusion hybrids

PDH 40 was infected by both PVY^{O} and PVY^{N} whereas the ELISA absorbance readings for *S. brevidens* inoculated with PVY^{O} or PVY^{N} were not significantly ($P > 0.05$) greater than those of healthy plants and no

Table 3. ELISA absorbance readings (405 nm) of somatic hybrids derived from electrofusion

Genotype	Absorbance (405 nm) [b]		Chromosome no.
	uninoculated	PLRV inoculated	
Solanum brevidens	0.064 ± 0.016 [a]	0.036 ± 0.018	24
PDH40	0.12 ± 0.022	0.526 ± 0.013	24
Maris Piper	0.035 ± 0.010	2.24 ± 0.178	48
81025	0.059 ± 0.025	0.174 ± 0.020	45
84140	0.008 ± 0.001	0.114 ± 0.023	48
84118	0.041 ± 0.009	0.472 ± 0.026	47
81011	0.015 ± 0.007	0.117 ± 0.012	47
84111	0.022 ± 0.006	0.445 ± 0.013	71
81078	0.067 ± 0.028	0.511 ± 0.006	48
84042	0.015 ± 0.006	0.078 ± 0.005	48
81012	0.044 ± 0.014	0.613 ± 0.036	46

[a] Average of three readings per plant
[b] ELISA reading after overnight incubation

Table 4. ELISA absorbance readings (405 nm) of plants inoculated with PVY^O or PVY^N

Genotype	Chromosome no.	Isolate inoculated	
		PVY^O	PVY^N
Maris Piper	48	1.203 [a]	1.160 [a]
PDH 40	24	1.250	1.012
S. brevidens	24	0.039 (−ve)*	0.011 (−ve)*
S. brevidens	24	0.031	0.046
65003	47	0.448	1.373
65006	48	0.181	0.537
65009	71	0.053 (+ve)*	0.037 (+ve)*
65013	48	0.202 (+ve)	0.038 (+ve)
65014	47	0.561	0.021 (+ve)
70023	48	0.210	0.645
70027	46	0.719	0.629
70064	48	0.345	0.030 (+ve)
70067	67	0.164	0.104 (+ve)
81011	47	0.240	0.318
81012	46	0.834	0.946
81025	45	0.202	0.796
81045	78	0.113 (+ve)	0.213 (+ve)
81068	69	0.069	0.027 (−ve)
81078	48	0.174 (+ve)	0.026 (−ve)
84042	48	0.170	0.892
84087	nd**	0.183	0.395
84111	71	0.073	0.132
84126	nd	0.230	0.024 (−ve)
84140	48	0.250	0.262
Healthy controls			
S. brevidens		0.026 ± 0.0084 [b]	
PDH40		0.028 ± 0.0102 [b]	

[a] Mean of 2 readings
[b] Mean of 6 readings
* Results in parentheses are of ISEM tests; +ve = particles seen, −ve = no particles seen
** nd = not determined

virus particles were observed in ISEM tests of their sap (Table 4).

All fusion hybrids were infected with PVY^O and all but three (81068, 81078, 84126) were demonstrably infected with PVY^N. However, most absorbance values for hybrids were less than those of PDH 40, the susceptible parent, some by an order of magnitude. There was a significant correlation ($P < 0.05$) between absorbance values for hybrids inoculated with PVY^O and with PVY^N and generally those hybrids with less than 48 chromosomes gave larger ELISA readings than those with 48 or more.

Discussion

We have demonstrated that somatic hybrids between dihaploid *S. tuberosum* and *S. brevidens* (Fish et al. 1987, 1988), are resistant to PLRV and to PVY^O and PVY^N, ELISA readings indicating that only low virus antigen titres occurred in inoculated plants. This result confirms the earlier observations of PLRV resistance in *S. brevidens* and *S. tuberosum* somatic hybrids (Austin et al. 1985, Helgeson et al. 1986). However, in our results the dihaploid *S. tuberosum* genotype had been selected for its good agronomic character, and protoplast fusion has been achieved by chemical fusion (Fish et al. 1987) as well as the more efficient electrical fusion technique (Tempelaar and Jones 1985a; Fish et al. 1988).

The *S. tuberosum* parent, PDH 40, although derived from a resistant cultivar, appeared to be susceptible to both PLRV and PVY in our glasshouse experiments. The *S. brevidens* genotype used (CPC 2451) was very resistant to PLRV but, as has been reported for other genotypes of *S. brevidens* (Jones 1979), appeared not to be immune. *M. persicae* also infected all the somatic hybrids with PLRV. However, judging by their ELISA absorbance readings, several somatic hybrids (e.g. 65009, 84042, 84140) contained much less virus than the *S. tuberosum* parent, PDH 40, although none as low as *S. brevidens*. Although *S. brevidens* appeared to be immune to PVY, all the fusion hybrids were infected by at least one of the virus isolates but, as with PLRV, several of the plants had low virus titres. Therefore, for both viruses, aphids feeding on infected plants might be less likely to acquire virus and the spread of virus through crops should be decreased. A similar type of resistance to PLRV appears to be present in some UK potato cultivars (Barker and Harrison 1986). Several of the somatic hybrids showed no symptoms of either PLRV or PVY infection, but no comparisons were made between the growth of inoculated and healthy plants.

A spectrum of resistance against both PLRV and PVY was found in the somatic hybrids; there was also a wide range of phenotypes. Protoplast fusion hybrids ideally consist of two complete sets of chromosomes, one from each parent. Chromosome complements different from the expected number of 48 occurred amongst both the chemical and electrofusion hybrids (Fish et al. 1987, 1988), and part of the variation in virus resistance could derive from incomplete transfer of chromosomes from one or other parent. No correlation between chromosome number and PLRV resistance has been observed amongst the hybrids treated, but susceptibility to PVY did seem to be associated with low chromosome numbers. Variation could also be due to genetic changes induced during protoplast culture and regeneration. These could include increased DNA methylation (Brown and Lörz 1986), new cytoplasmic or nuclear genome combinations (Kemble et al. 1986) and aneuploidy as a result of multiple protoplast fusions (Fish et al. 1988). Regardless of its origin, this variation may be of considerable practical interest to plant breeders.

The majority of the highly resistant somatic hybrids have produced flowers and in initial crosses with one tetraploid *S. tuberosum* cultivar, one seed has been produced. However, since the parental *S. brevidens* is not immune to infection by PLRV and a broad spectrum of resistance to both PLRV and PVY was found amongst fusion progeny, it seems likely that, for both viruses, resistance is polygenic. If this is the case, it is interesting that such high levels of virus resistance and large numbers of resistance genes have been acquired in a species which does not overwinter vegetatively as tubers. Such resistance may be difficult for breeders to utilise since it may be diluted by backcrossing to *S. tuberosum* to remove undesirable "wild" characters. However, now that fusion between *S. brevidens* and *S. tuberosum* can be routinely accomplished, it may be worthwhile to screen for *S. brevidens* genotypes with greater levels of resistance than CPC 2451.

This work, in combination with our previous publications (Fish et al. 1987, 1988), and those of Austin et al. (1985, 1986) and Barsby et al. (1984) clearly demonstrate that protoplast fusion can be used to produce somatic hybrids between *S. tuberosum* and sexually incompatible *Solanum* species, and indicates the approach can be used to introduce potentially useful characters from such wild species into commercial potato cultivars.

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