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Resistance to potato virus Y in somatic hybrids between *Solanum etuberosum* and *S. tuberosum* × *S. berthaultii* hybrid

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Abstract Somatic hybrids between a potato virus Y (PVY) resistant *Solanum etuberosum* clone and a susceptible diploid potato clone derived from a cross between *S. tuberosum* Gp. Tuberosum haploid US-W 730 and *S. berthaultii* were evaluated for resistance to PVY. All but one of the tested somatic hybrids were significantly more resistant than cultivars 'Atlantic' and 'Katahdin'. However, none was as resistant as the *S. etuberosum* parent. One hexaploid somatic hybrid, possibly the product of a triple-cell fusion involving one *S. etuberosum* protoplast and two haploid×*S. berthaultii* protoplasts, was as susceptible to PVY infection as the cultivars. Tetraploid progeny of the somatic hybrids, obtained from crosses with Gp. Tuberosum cultivars, were neither as resistant as the maternal somatic hybrid parent, nor as susceptible as the paternal cultivar parent. It appears that the introgression of PVY resistance from (1EBN) *S. etuberosum* into (4EBN) *S. tuberosum* (EBN-endosperm balance number) will be successful through the use of somatic hybridization and subsequent crosses of the somatic hybrids back to *S. tuberosum*.

Key words Somatic hybrid · *Solanum etuberosum* *Solanum tuberosum* · Haploid×wild species hybrid Potato virus Y resistance

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Introduction

Yield reductions of up to 80% can occur in potato plants infected with potato virus Y (PVY), making it one of the most damaging of the potato viruses (Beemster and de Bokx 1987). The use of certified potato seed pieces is the most successful means for the farmer to avoid 15–30% reductions in yield from secondary PVY infection (van der Zaag 1987). In many areas of the world, however, seed certification programs do not exist, and farmers must either plant tubers that may be severely infected with virus, use minitubers or plantlets from in vitro culture, or use true potato seed (TPS). Even with seed certification programs, however, PVY can still decrease yields with current-season (primary) infection. Van der Zaag (1987) states that primary infection with PVY early in the growing season can cause considerable damage, almost comparable to that caused by secondary infection.

The breeding of resistant cultivars is one of the best means of combating PVY. Extensive work has been done in this area with resistances contributed by sexual crosses with several different potato species, most notably *S. stoloniferum* and *S. tuberosum* Gp. Andigena (Ross 1986; Beekman 1987). Other potential sources of extreme resistance/immunity to viruses such as potato leaf roll virus (PLRV) and PVY are the species in the series Etuberosa. Resistance to PLRV has been transferred from *S. brevidens* to its somatic hybrids with potato (Austin et al. 1985; Helgeson et al. 1986) . Similarly, PVY-resistant materials have been obtained by somatic hybridization of potato lines with *S. brevidens* (Gibson et al. 1988; Pehu et al. 1990).

Certain accessions of *S. etuberosum* have also been identified as possessing resistance/immunity to PVY (Hanneman and Bamberg 1986). A PVY resistant, diploid *S. etuberosum* clone has been used in somatic hybridizations to a Gp. tuberosum haploid×*S. berthaultii* hybrid (2n=2x=24). In the present article, we report the results obtained from the screening of the fusion parents, their somatic hybrids, and the sexual progeny of the somatic hybrids.

Materials and methods

Plant material and experimental design

Diploid *Solanum etuberosum* ('PI 245939') was obtained from the Inter-Regional Potato Introduction Project (IR-1) at Sturgeon Bay, Wis. This accession of *S. etuberosum* has immunity to potato virus X and Y and resistance to potato leaf roll virus (Hanneman and Bamberg 1986). Seeds of *S. etuberosum* were germinated *in vitro* on propagation medium (Haberlach et al. 1985), and one clone (designated 16-1) was selected for use in somatic fusions with a haploid×wild species hybrid (2n=2x=24) designated 463-4. Clone 463-4, a hybrid from a Gp. Tuberosum haploid US-W 730×*S. berthaultii* ('PI 265857') cross, was obtained from S. J. Peloquin and Lisa Darmo, University of Wisconsin, Madison, Wis. The somatic hybrids were given the designation E + BT, which represents the genomes present in the hybrids (E=*etuberosum* and BT=*berthaultii* and *tuberosum*). The generation and characterization of the somatic hybrids is described by Novy and Helgeson (1994).

The two parental clones (16-1 and 463-4), nine E + BT somatic hybrids, the haploid maternal parent (US-W 730) of 463-4, and the Gp. Tuberosum cultivars 'Katahdin' and 'Atlantic' were included in the study. 'Katahdin' has moderate resistance to PVY (Bawden and Kassanis 1946), while 'Atlantic' is susceptible. In mid-April, plants were established in 10 cm clay pots in the greenhouse from 2-week-old in vitro shoot cultures. A completely random design was utilized with five replicates (plants) of each clone. Plants were grown and inoculated under natural lighting supplemented with high-pressure sodium vapor lights. The temperature in the greenhouse was generally maintained at 21°C, although fluctuations of ±5°C were sometimes seen. For the follow-up study comparing somatic hybrids and their sexual progeny, the experimental design and cultural conditions were the same as above. However, plants were established in the greenhouse in mid-January rather than mid-April. Parental clones 16-1 and 463-4, five E+BT somatic hybrids, Gp. Tuberosum cvs '-Katahdin' and 'Atlantic', and five sexual progeny of somatic hybrid×Gp. Tuberosum cultivar crosses (designated EBT×T) were tested.

PVY inoculation and quantification

PVY_O isolate 2536, obtained from Phillip Berger (University of Idaho), was used to systemically infect *Nicotiana tabacum* cv 'Xanthi nc'. Infected tobacco leaves were ground in a mortar containing 1–2 ml of Sorenson's phosphate buffer (0.066 *M*, pH of 7.0) and a small amount of 'Celite'[1]. Sodium sulfite (0.02 *M*) was also included in the maceration buffer as a phenolic inhibitor (Hill 1984). Mechanical inoculation with the tobacco inoculum was conducted as described by Hill (1984, pp 27–28). The primary and the first two secondary leaflets of 2 different leaves were inoculated on each plant. The inoculated leaves were the youngest, fully-expanded leaves on the plant. Plants had been established in the greenhouse for approximately 4 weeks (initial study) and 3 weeks (follow-up study) prior to inoculation.

Three weeks (initial study) or 4 weeks (follow-up study) after PVY inoculation, 2 or 3 young, fully expanded leaves were collected from each test plant. The primary and the first 2 secondary leaflets were excised from the collected leaves until 1 g of fresh leaf material was obtained for each plant. The leaves were ground with a mechanized grinder until approximately 50 µl of sap was obtained. This volume of sap was diluted with 9 parts of PBS-tween extract buffer and assayed with a PVY-ELISA kit (Agdia, Granger, Ind.). Between each sample, the grinder was first rinsed with distilled water, followed by a solution consisting of 1% w/v of Alconox soap and 1% v/v commercial bleach, and a final rinse of distilled water. The grinder was then rinsed with PBS-Tween buffer solution (Agdia) and the next sample was added for grinding.

[1] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable

Results and discussion

In the initial study, somatic hybrids and their parental lines ranged from highly resistant to highly susceptible to PVY (Table 1). Clones 463-4, US-W 730, and 'Atlantic' were highly susceptible to PVY, with absorbance readings near or at 2.0 (the maximum absorbance value of our ELISA plate reader). 'Katahdin' showed an intermediate, but still high titer of 1.151. *Solanum etuberosum*, clone 16-1, was highly resistant with mean absorbance readings of 0.050.

All but one of the tested somatic hybrids were significantly more resistant to PVY than the tested cultivars or the potato fusion parent. Absorbance values of the one exception, somatic hybrid 2-2-11A, did not significantly differ from those of 'Katahdin'. Chromosome counts indicated that 2-2-11A was hexaploid, rather than tetraploid. Restriction fragment length polymorphism (RFLP) analysis (see Novy and Helgeson 1994) confirmed the presence of both parental genomes in the somatic hybrid. These data, together with the improved tuber characteristics, suggest that 2-2-11A is a triple-cell fusion product of two 463-4 protoplasts and one 16-1 protoplast. A similar increase in susceptibility to PVY observed by Pehu et al. (1990) in a hexaploid somatic hybrid of *S. brevidens* was also postulated to have an additional susceptible parental genome.

Although the somatic hybrids were significantly more resistant than the cultivars, with the exception of line 2-1-4c, none were as resistant as the *S. etuberosum* parent in the first test. These findings are comparable to results reported for PVY resistance of *S. brevidens* somatic hybrids (Gibson et al. 1988; Pehu et al. 1990). Thus, it would appear that the PVY resistances observed in species of the series Etuberosa are not simply dominant in their expression.

Somatic hybrids and their sexual progeny from crosses with 'Atlantic' and 'Katahdin' were compared in a second test (Table 2). Both 2-7-4d and 2-2-11A were about as susceptible as 'Katahdin' (Table 2). ELISA absorbance means of the sexual progeny (EBT×T clones) differed substantially. Three clones (EBT×T-2, 3 and 5) were as resistant as their somatic hybrid parents; the other two clones (EBT×T-1 and 4) had titer levels similar to those of 'Katahdin', the potato crossing parent. The fact that discrete and widely separated readings were obtained suggests that the resistance contributed by *S. etuberosum* might be controlled by one or a few major genes. However, it is difficult to draw conclusions from results obtained with only five individuals, which comprised the entire progeny population. The screening of a larger progeny population would be useful in this regard. Such a population has now been obtained by the cross of EBT×T-3× 'Katahdin'.

Three weeks after inoculation, the symptoms of PVY infection varied widely among the clones. *S. etuberosum*

Table 1 PVY resistance of somatic hybrids, fusion parents, and potato cultivars

Clone	Description	Absorbance
US-W730	Maternal parent of 463-4	2.0[a]
463-4	(*S. tuberosum*×S. *berthaultii*)	2.0[a]
Atlantic	Cultivar	1.806[a]
Katahdin	Cultivar	1.151a[b]
2-2-11A	Somatic hybrid	1.141a
2-7-4D	Somatic hybrid	0.580b
2-7-4A	Somatic hybrid	0.486bc
2-9-3B	Somatic hybrid	0.377bcd
2-9-3F	Somatic hybrid	0.324cd
2-9-1A	Somatic hybrid	0.322cd
2-1-3A	Somatic hybrid	0.317cd
2-1-4B	Somatic hybrid	0.308cd
2-1-4C	Somatic hybrid	0.197de
16-1	*S. etuberosum* fusion parent	0.050e
2-9-1A	Non-inoculated control	0.007

[a] Absorbance values above 2.0 were off scale for the instrument. These values and the values for 'Atlantic' were included in Fisher's LSD comparison

[b] Absorbance means followed by the same letters are not significantly different at P=0.5 (Fisher's LSD)

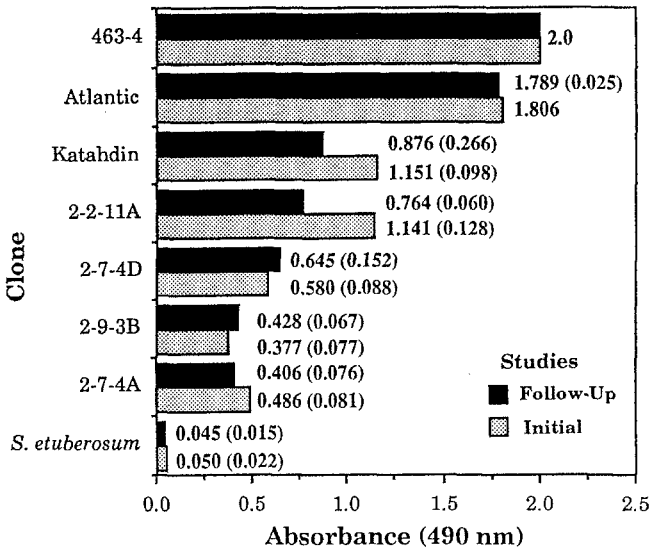

Fig. 1 PVY-ELISA absorbance means and standard errors (*in parentheses*) for eight somatic hybrids that were tested in two studies approximately 1 year apart

Table 2 PVY resistance of somatic hybrids and their progeny

Clone	Description	Absorbance
463-4	*S. tuberosum*×*S. berthaulti*	2.0[a]
Atlantic	Cultivar crossing parent	1.789a[b]
EBT×T-1	2-9-3B×Katahdin	1.358b
EBT×T-4	2-7-4D×Katahdin	1.068bc
Katahdin	Cultivar crossing parent	0.876cd
2-2-11A	Somatic hybrid	0.764d
2-7-4D	Somatic hybrid	0.645de
2-9-3B	Somatic hybrid	0.428ef
2-7-4A	Somatic hybrid	0.406ef
EBT×T-5	2-7-4A×Katahdin	0.329fg
2-3-10A	Somatic hybrid	0.288fg
EBT×T-3	2-9-3B×Atlantic	0.279fg
EBT×T-2	2-3-10A×Katahdin	0.265fg
16-1	*S. etuberosum* fusion parent	0.045g
EBT×T-1	Non-inoculated control	0.024

[a] Absorbance values above 2.0 were off scale for the instrument, thus these values were not included in Fisher's LSD comparison

[b] Absorbance means followed by the same letters are not significantly different at P=0.5 (Fisher's LSD)

clone 16-1 showed no symptoms of PVY infection, whereas clones US-W 730, 463-4, and 'Atlantic' were stunted with chlorotic and necrotic leaves and their lower leaves had usually fallen off by this time. 'Katahdin', clone 2-2-11A, and the susceptible EBT×T sexual progeny displayed most of the above-mentioned symptoms, but not to the severe degree observed in the highly susceptible lines. PVY infection in the somatic hybrids and their resistant EBT×T progeny was characterized by mottling and necrotic flecking of the lower leaves and stunting. Leaf drop did not occur.

Eight clones were included in both PVY screenings. Those clones and their respective absorbance values for each of the two studies are presented in Fig. 1. A combined ANOVA analysis of six of the eight clones was performed in order to evaluate the reproducibility of the results. The combined ANOVA analysis showed no differences at the 5% level of significance for the 'clone' and 'clone×study' components of variance, indicating the PVY screening procedure provided reproducible results. The statistical non-significance of the 'clone×study' component of variance allowed for a combined LSD test of the six clones. The combined LSD analysis confirmed that *S. etuberosum* had a higher level of resistance than the somatic hybrids which, in turn, were more resistant than 'Katahdin'. Clone 2-2-11A was again exceptional, with an increased susceptibility to PVY as compared to the other three somatic hybrids.

The evaluation of the E+BT somatic hybrids and their progeny indicates a level of PVY^O resistance that is significantly greater than that observed for the moderately resistant cultivar 'Katahdin'. These results indicate that a useful level of resistance may be incorporated by somatic hybridizations with *S. etuberosum*. However, this resistance has been tested with only one isolate of PVY^O, and further testing would be warranted with other isolates and strains. Also, high levels of inoculum and mechanical inoculation were used in these studies. Further evaluation of PVY resistance under field conditions would give a better idea as to the usefulness of the resistance.

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