Clones of *Solanum berthaultii* Resistant to Potato Spindle Tuber Viroid

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ABSTRACT

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Seventeen plant introductions (PI) of *Solanum berthaultii* were tested. Two clones (1726 and 1729) of *S. berthaultii* (PI 473340) were resistant to the mild and severe strains of potato spindle tuber viroid (PSTV). Both clones were resistant to PSTV by sap inoculation but were susceptible by graft inoculation. Even after graft inoculation, plants remained symptomless and PSTV was not detected by polyacrylamide gel electrophoresis. However, bioassay on *Scopolia sinensis* and dot-blot tests detected PSTV in such plants. Both clones were susceptible to potato viruses A, S, X, and Y by sap inoculation and to leafroll virus upon grafting. The importance of the PSTV resistance in these clones is discussed in connection with multiple insect resistance found in clones of *S. berthaultii*.

Potato spindle tuber viroid (PSTV) is a low-molecular-weight (5,25), circular RNA (19) with only 359 ribonucleotides (9). PSTV does not act as messenger RNA (4) and no protein products specific to the viroid infection have been found (2). However, the small size and apparent lack of a protein coat does not prevent PSTV from infecting a large number of plants belonging to several families (15,16,21,24). A number of tuber-bearing *Solanum* species, including widely grown potato cultivars (1,6,10,17,27) as well as species hybrids (30) are also susceptible to PSTV. Difficulty in symptom expression and potential yield losses on potatoes due to PSTV have been serious constraints for North American potatoes to overseas trade (23).

Seed certification programs were initiated in the early 1920s (13) to identify and eliminate PSTV from North American potato seed. Maintaining disease-free seed today requires sophisticated testing procedures and equipment (23). This approach to controlling PSTV is very costly. Clearly, eliminating PSTV is impractical in countries like China, where it is widespread (14). In China, 2 million ha or one-half of all potato production areas are used for spring-planted potatoes. One of China's breeding objectives, therefore, is to incorporate PSTV resistance into their potato cultivars (20). Valuable sources of PSTV resistance, however, are rare in *S. tuberosum* L. (10,17). Resistance was reported in *S. acaule* Bitt. (OCH 11603), but observations were preliminary with few details (11,12).

In an earlier study (27), it was observed that clones of *S. berthaultii* Hawkes plant introduction (PI) 265857 differed in reaction to PSTV. One clone appeared to be hypersensitive and could be used as an indicator plant (24), while others developed non-necrotic symptoms or were completely symptomless (27). Because of this range of reactions, I have tested additional PIs in a search for clones resistant to PSTV. Two such clones were found. These are the first suitable sources of resistance to PSTV and the presence of multiple insect resistance makes them even more valuable (3,7,28,29).

MATERIALS AND METHODS

One severe strain of the viroid (S-PSTV) from Fredericton and two mild strains (MA-PSTV and MB-PSTV) from S. A. Slack, University of Wisconsin, Madison, were used as inoculum. All were propagated in potato (*S. tuberosum* cultivars Jemseg or Russet Burbank). Test plants were inoculated either manually with a nucleic acid extract from infected plants (22,24) or by grafting small pieces of infected stem material. Inoculations were repeated unless test plants developed symptoms or PSTV was detected by other means.

Botanical seeds of 17 PIs of *S. berthaultii*, obtained from R. E. Hanneman, Jr., Potato Introduction Station, Sturgeon Bay, WI, were designated as 218215, 265857, 265858, 283069, 310925, 310926, 310927, 473330, 473331, 473332, 473333, 473334, 473335, 473336, 473337, 473338, and 473340. Seeds were treated with 1,500 ppm of gibberellic acid for 24 hr at room temperature, rinsed with distilled water, and planted in a soil:peat:sand mix (4:1:1). When seedlings were about 2–3 cm tall, they were transplanted in 12.5-cm-diameter clay pots containing soil mix. Each plant was further propagated clonally as three plants and, when plants were in the five- to six-leaf stage, they were inoculated with a nucleic acid extract containing PSTV (22,24). Inoculated plants were grown in a greenhouse at 27–32 C with a photoperiod of 14–16 hr. High humidity was maintained by covering greenhouse benches with 3–4 cm of moist peatmoss. Plants were fertilized with a 20-20-20 N-P-K solution every week to ensure vigorous growth.

Following inoculations, plants were observed for symptoms weekly for 9–10 wk. Plant symptoms consisted of reduced leaf size, veinal necrosis, and dwarfing of entire plants. PSTV infection was confirmed by using polyacrylamide gel electrophoresis (PAGE) (18,22), local lesion assay on *Scopolia sinensis* Hemsl. (21), and the dot-blot test (Agdia, Inc., Mishawaka, IN).

Reaction of other potato viruses was checked as previously described (26). Potato viruses A, S, X, and Y were mechanically inoculated while potato leafroll virus-infected tissue was grafted to stocks of *S. berthaultii*. Presence of viruses was monitored by ELISA (26).

RESULTS

Of the 1,700 seeds planted, 945 seedlings survived. Each seedling (clone) was tested in triplicate, and the PSTV reaction generally was uniform in all plants of a clone. All clones, except 28, developed PSTV symptoms after the first inoculation. Twenty-six of the 28 clones were found to be symptomless carriers of PSTV. PSTV was not detected in the remaining two clones 1726 and 1729 from PI 473340; further evaluations of these clones were done.

Table 1 summarizes the results of several inoculation tests. Vegetatively propagated plants of clones 1726 and 1729, mechanically inoculated three times within a 9-wk period, tested negative for PSTV. Plants of both clones simultaneously

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inoculated by grafting tested positive for PSTV. Graft-infected plants of neither clone showed any symptoms. Fewer plants became infected that were grafted with mild rather than severe PSTV strains (Table 1).

The viroid concentration in clones 1726 and 1729 infected by graft inoculation was very low and was not detected by the PAGE test, even 3 mo after inoculation. However, PSTV was detected by bioassay on *S. sinensis* as early as 3 wk after graft inoculation. Dot-blot hybridization also confirmed the presence of PSTV in graft-inoculated plants (Table 1).

Because PSTV was not detected by PAGE in sap- or graft-inoculated plants of either clone, an experiment was performed in which partially purified PSTV was mixed with sap from clones 1726 and 1729 incubated for 5 hr at 20 C and reextracted for PAGE. PSTV was recovered from the sap of both clones; thus, the absence of a 7S PSTV band in PAGE does not appear to be due to degradation in the sap of these clones.

Since clones of *S. berthaultii* have been reported to be resistant to potato virus X (8), and various insects including aphids (3,7,28), an attempt was made to determine the reaction of clones 1726 and 1729 to the other potato viruses. Both clones were susceptible to all the viruses. Potato virus X caused a necrotic spotting on clone 1729 but only a faint mottle on clone 1726. PVY also caused necrosis and leaf drop on clone 1729 but no obvious symptoms on clone 1726. PVA- and PVS- inoculated plants of both clones were symptomless, but viruses were detected by ELISA. PLRV-grafted plants showed slight rolling of leaves and virus was recovered from each plant.

TABLE 1. Effect of inoculation methods on the transmission of potato spindle tuber viroid to the resistant clones of *Solanum berthaultii*

Inoculation	Detection of viroid[a]		
	PAGE	Bioassay	Dot-blot
Mechanical			
Clone 1726 - S-PSTV	0/20	0/20	0/20
Clone 1726 - MA-PSTV	0/10	0/10	0/10
Clone 1726 - MB-PSTV	0/10	0/10	0/10
Clone 1729 - S-PSTV	0/20	0/20	0/20
Clone 1729 - MA-PSTV	0/10	0/10	0/10
Clone 1729 - MB-PSTV	0/10	0/10	0/10
S. sinensis - S-PSTV	10/10	10/10	10/10
Graft[b] with clone 1726			
ST-Tomato[c]/ Healthy 1726	0/3	3/3	3/3
ST-Potato[d]/ Healthy 1726	0/7	7/7	4/7
Healthy 1726/ ST-Tomato[c]	0/4	0/4	0/4
Healthy 1726/ ST-Potato[d]	0/8	4/8	4/8
Healthy 1726/ ST-Potato[e]	0/4	0/4	0/4
Healthy 1726/ ST-Potato[f]	0/6	0/6	0/6
Healthy 1726/ Healthy Tomato	0/4	0/4	0/4
Graft with clone 1729			
ST-Tomato[c]/ Healthy 1729	0/5	5/5	2/5
ST-Potato[d]/ Healthy 1729	0/8	7/8	6/8
Healthy 1729/ ST-Tomato[c]	0/4	4/4	4/4
Healthy 1729/ ST-Potato[d]	0/11	5/11	5/11
Healthy 1729/ ST-Potato[e]	0/3	0/3	0/3
Healthy 1729/ ST-Potato[f]	0/4	2/4	2/4
Healthy 1729/ Healthy Tomato	0/3	0/3	0/3
Viroid controls (MA, MB)	12/15	15/15	15/15

[a] Number of positive/number tested. Inoculum consisted of extracted nucleic acid solution and was used for all three tests. Bioassay done by inoculating *Scopolia sinensis*.
[b] Scion/stock.
[c] Severe strain of potato spindle tuber viroid in Shevenne tomato (S-PSTV).
[d] Severe strain of potato spindle tuber viroid in Jemseg potato (S-PSTV).
[e] Mild strain of potato spindle tuber viroid in Russet Burbank potato (MA-PSTV).
[f] Mild strain of potato spindle tuber viroid in Russet Burbank potato (MB-PSTV).

DISCUSSION

S. berthaultii clones 1726 and 1729 of PI 473340 apear to be highly resistant to PSTV because PSTV was not recovered after three inoculations with infectious nucleic acid extracts. One inoculation was sufficient to infect the other 943 clones. Although neither clone was immune to PSTV, it appears that the viroid concentration remained very low (Table 1) with no detectable increase even 3 mo after the plants were grafted.

Some accessions of *S. berthaultii* have been shown to possess resistance to potato viruses and potato insects, e.g., potato virus X (8); potato flea beetle, *Expitrix cucumeris* (Harris) (28); the green peach aphid, *Myzus persicae* (Sulzer) (7); the two-spotted spider mite, *Tetranychus urticae* (Koch) (7); thrips, *Thrips tabaci* (Lindemann) (7); and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (3). Therefore, *S. berthaultii* clones are being studied in various potato breeding programs (7,28) and genes for insect resistance have already been transferred to hybrids of *S. tuberosum* and *S. berthaultii* (7,29). In addition, some accessions of *S. berthaultii* have been shown to possess the desirable character of maintaining very low levels of glycoalkaloids (29). Thus, *S. berthaultii* is an ideal species in which to find resistance to PSTV, so that without too much additional effort, this resistance source could be used for cultivated potatoes. Clones 1726 and 1729 differ slightly in their leaf shape as well as in their growth habit, but both are readily multiplied by clonal propagation. Root development in excised cuttings takes place in 7–9 days and cuttings are ready to be inoculated in 21–28 days. Both clones flower profusely in a short-day environment and produce some tubers. Both clones have been shown to possess insect resistance (*unpublished*). Thus, they possess suitable qualities for cross-breeding purposes.

PSTV spreads mainly by contact of healthy foliage with diseased foliage or contaminated planting and cultivating machinery (23). The resistance to mechanical inoculation with PSTV observed in clones 1726 and 1729 should prove useful if it can be transferred to cultivated potatoes.

In recent years, the use of true potato seed for direct sowing of commercial crops has gained importance and it is the major objective of research at the International Potato Center, Lima, Peru, and at other institutes in developing countries (14). Fear of PSTV (which can be seed-transmitted in high percentages) is a major constraint in the international distribution of botanical seed. Therefore, for successful use of true potato seed in countries where PSTV is a problem, identification of suitable sources of resistance to PSTV can be of great value.

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