Reactions of Tuber-Bearing *Solanum* Species to Infection with Potato Spindle Tuber Viroid

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ABSTRACT

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A total of 555 plant introductions belonging to 81 tuber-bearing *Solanum* species were evaluated in greenhouse or field tests for their reactions to mild and severe strains of the potato spindle tuber viroid (PSTV). Plants tolerant to PSTV were found, but none were immune. Although most plants of all species displayed diagnostic symptoms, symptomless plants were observed for 38 species. None of the inoculated plants for 17 of the 38 introductions developed symptoms. Primary symptoms consisted of stunting of whole plants; reduced leaf size; and necrosis of petioles, leaf veins, and stems. Selection and clonal propagation before inoculation of plants that develop diagnostic symptoms when inoculated with either mild or severe strains of PSTV may be useful for bioassay purposes.

Potato spindle tuber viroid (PSTV) is carried through the pollen and seeds of potato (*Solanum tuberosum* L.) (3,10). It has been reported to occur in the tuber-bearing *Solanum* species of the major potato germ plasm collections, eg, the Commonwealth Potato Collection in Scotland (4), the International Potato Center in Peru (4), and the Inter-Regional (IR-1) Potato Project at Sturgeon Bay, WI (6). Harris et al (4) expressed the opinion that the increasing dispersal of *Solanum* species and the persistence of viroid infection in *Solanum* germ plasm constitutes a continued worldwide risk to potatoes. The primary effort to reduce this risk has been to screen germ plasm for freedom from PSTV. Information on the response of *Solanum* species to PSTV infection also would be useful to persons responsible for germ plasm maintenance.

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Although more than 200 plant species and varieties have been examined for their reaction to PSTV (11), only scattered reports of PSTV reactions in a few tuber-bearing *Solanum* species are known (1,2,4,6), and only the accession *S. acaule* (Och 11603) has been found resistant to infection (5). We report a systematic effort to determine the reaction of tuber-bearing *Solanum* species to PSTV. Because mild strains of PSTV exist that make reliable detection by tomato bioassay difficult (9), plant introductions (Plant Inventory numbers [PI]) were evaluated for utility as diagnostic hosts as well as for sources of resistance.

MATERIALS AND METHODS

One severe strain (PSTV-S) and two mild strains (PSTV-MA and PSTV-MB) of PSTV were used at Madison (7) and a mild strain of PSTV was used at Fredericton (14). Tuber-bearing *Solanum* species (PIs) were obtained from the IR-1 collection at Sturgeon Bay, WI, in the form of tubers or botanical seeds. Symptom readings recorded at both locations were similar and data were combined to simplify presentation. At Madison, plants were grown from botanical seeds that were treated with 1,500 ppm of gibberellic acid for 24 hr at room temperature, rinsed with distilled water, and planted in vermiculite. Before development of the first true leaf, eight to 11 plants of each PI were transplanted to steamed soil in Com-pak plant containers (A. H. Hummert Seed Co., St. Louis, MO). A tomato seedling (*Lycopersicon esculentum* Mill. 'Rutgers') was planted in each container as a PSTV inoculum control. Three or more plants of the same PI were planted in a separate Com-pak as healthy controls. After 5–7 days, plants were inoculated by a "cut-leaf" method that entailed rubbing the fresh-cut edge of a PSTV-infected Rutgers tomato leaf on PI leaves dusted previously with corundum. A droplet of buffer (0.1 M $NaPO_4$, pH 7.2) was sometimes placed on the cut tomato leaf to facilitate inoculation. Control plants were rubbed with a leaf from an uninfected tomato plant. About 1 min after inoculation, leaves were rinsed with distilled water. Plants were reinoculated 1 wk later and maintained in a greenhouse fitted with a sand bench to maintain high humidity at 28–30 C with a 16-hr photoperiod or were transplanted to the field at the University of Wisconsin Hancock Experimental Station in May or June.

Table 1. Reaction of selected tuber-bearing *Solanum* species to potato spindle tuber viroid

Species	Plant introductions (no.)	Total plants (no.)	Symptoms[a]			Test location[c]
			Range[b]	Mean	Predominant	
S. acaule	215	1,471	0.0–3.0	2.1	R,N	F,W
S. berthaultii	6	189	0.0–3.0	2.0	LD,N,LL	F,W
S. brachistotrichum	3	34	0.0–3.0	1.6	R	F,W
S. brachycarpum	6	59	0.0–3.0	2.3	R,N	F,W
S. brevidens[d]	2	13	0.0–3.0	0.5	LS	W
S. bulbocastanum	11	67	0.0–2.5	1.8	N,S	F,W
S. bukasovii	6	48	0.0–3.0	2.0	R,S,N	F
S. canasense	6	68	0.0–3.0	2.5	C,R,N	F,W
S. cardiophyllum	4	59	0.0–3.0	1.7	R,N	F,W
S. chacoense	8	88	0.0–3.0	1.5	N,R,S	F,W
S. chiquidenum	2	20	0.0–3.0	1.7	LS	F,W
S. circaeifolium	1	5	0.0–2.0	0.9	C,LS	F
S. commersonii	6	62	0.0–2.5	0.9	C,R	F,W
S. x *curtilobum*	6	51	0.0–3.0	2.7	LS	W
S. demissum	7	80	0.5–3.0	2.2	R,N	F,W
S. fendleri	6	83	0.5–3.0	2.0	LS	F,W
S. gourlayi	7	64	0.0–3.0	2.4	R,S	F,W
S. guerreroense	3	17	0.0–2.0	1.5	S,LS	F,W
S. hjertingii	7	40	0.0–2.0	1.2	R,LS	F,W
S. hougasii	4	39	0.0–3.0	2.7	R,S,N	F,W
S. immite	1	6	0.0–0.0	0.0	···	F
S. iopetalum	2	19	0.0–3.0	1.2	R,LS	F,W
S. jamesii	5	62	0.0–3.0	2.8	LL,N,S	F,W
S. kurtzianum	12	70	0.0–2.5	1.5	R,N,LS	F,W
S. lesteri	1	4	0.0–2.0	1.5	C,LS,N	F
S. lignicaule	1	12	0.0–2.5	1.5	N	F,W
S. limbaniense	1	7	0.0–2.0	1.6	R,N	F
S. lycopersicoides[d]	1	7	0.0–2.5	1.5	LS	W
S. multidissectum	4	17	0.0–2.0	0.6	LS	W
S. phureja	10	106	0.0–3.0	1.8	R,LS,S	F,W
S. pinnatisectum	5	57	0.0–3.0	1.4	N	F,W
S. sparsipilum	5	61	0.5–3.0	2.5	R,LS	F,W
S. stenophyllidium	2	17	0.0–3.0	2.0	R,LS	F,W
S. stenotomum	13	73	0.0–2.5	1.8	R,LS	F,W
S. stoloniferum	12	72	0.0–2.5	2.0	R,S,LS	F,W
S. sucrense	4	51	0.0–3.0	2.9	S,N,R	F,W
S. tarijense	7	101	0.0–3.0	2.8	N,R,S	F,W
S. weberbaueri	1	4	0.0–2.5	1.9	N,LS	F

[a] Symptoms were R = rosettelike growth; S = overall stunted; LS = leaves stunted (small leaf diameter); C = chlorosis of leaves; N = necrosis of stems, petioles or leaves; LL = necrotic lesions; and LD = leaf drop, after becoming necrotic or chlorotic.
[b] Plants were rated for symptoms biweekly for 8 wk after inoculation. Scale was 0–3 (0 = no symptoms to 3 = dead or dying).
[c] Work completed at Wisconsin (W) or Fredericton (F). Listing of data for individual PI lines is available on request from appropriate author.
[d] Non-tuber-bearing.

At Fredericton, tubers were planted after dormancy was broken. From each plant, four to six cuttings were rooted and transplanted to 12.5-cm clay pots containing a steam-sterilized soil mix (4:1:1 soil, peat, and sand). Plants were fertilized regularly with 20-20-20 fertilizer mix to ensure vigorous growth. When cuttings developed four to six leaves, they were mechanically inoculated with partially purified PSTV-RNA (12). As a control for PSTV infection, *Scopolia sinensis* (a local lesion host) was also inoculated and maintained in the same greenhouse. Two cuttings of each PI were inoculated with buffer to serve as healthy controls. After inoculation, plants were maintained at 27–32 C with a photoperiod of 14–16 hr. High humidity was maintained by partially submerging pots in 8-cm-thick, moist peat moss.

Plants were observed for PSTV symptoms biweekly for 8 wk after inoculation and were scored on a scale of 0–3, where 0 = no symptoms, 1 = stunting and reduced leaflets, 2 = stunting and tissue necrosis, and 3 = dead or dying plants.

Plants showing distinct symptoms were tested in composite form by electrophoresis on polyacrylamide gels (PAGE) (8,12) and those with weak or no symptoms were tested individually by PAGE and by bioassay on *S. sinensis* or Rutgers tomato.

RESULTS AND DISCUSSION

A total of 555 PI lines that included 81 *Solanum* species were screened for susceptibility to PSTV. No plants immune to PSTV were found. Susceptible reactions of individual plants in 38 species ranged from symptomless (tolerant) to very severe (Table 1). Also, symptom severity varied considerably among PIs belonging to the same *Solanum* species, as determined from

Table 2. Response of selected *Solanum* introductions to mild (MA and MB) or severe (S) strains of potato spindle tuber viroid 4 wk after inoculation[a]

Species	Plant introduction or collector number	MA		MB		S	
		$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*
S. acaule	Hof 1591 (PI 472640)	0.38[b]	1.06	1.25	1.49	0.75	1.39
	Hof 2090 (PI 472697)	1.75	0.71	1.25	1.04	0.38	1.06
	Oka 6085A (PI 472802)	0.12	0.35	1.00	1.41	2.38	0.52
	Hof 1571 (PI 472638)	2.25	1.16	1.25	1.49	2.12	1.25
	Oka 6082A (PI 472800)	1.12	1.55	0.50	1.07	1.50	1.60
	PI 3120275	2.18	1.25	2.00	1.07	1.22	1.55
	Och 9796 (PI 473435)	1.38	1.51	2.12	0.35	2.00	0.00
S. andreanum	WRF 1144	2.12	1.36	0.50	0.53	0.75	1.04
S. cardiophyllum	PI 184762	1.25	1.16	1.00	0.76	2.12	0.99
S. chomatophilum	PI 243341	2.75	0.71	2.50	0.53	2.50	0.53
S. × *curtilobum*	PI 186181	0.12	0.35	2.00	0.00	0.25	0.46
	PI 225650	1.50	1.41	1.00	1.31	1.00	1.31
S. hougasii	WRF 1736	2.50	0.92	1.12	1.55	0.88	0.99
S. megistacrolobum	PI 265879	1.75	0.89	1.00	0.76	1.62	0.92
	PI 210034	1.50	1.60	0.75	1.39	1.12	1.55
S. mochicense	PI 283114	1.50	0.92	1.12	1.55	2.25	0.46
S. multiinterruptum	PI 275272	2.25	1.04	2.00	0.92	1.12	0.64
S. sparsipilum	PI 230502	2.50	0.92	1.88	1.55	2.00	1.00
S. tarijense	PI 208881	0.75	1.39	0.57	0.93	0.62	0.92

[a] Greenhouse evaluations at Madison, WI, on eight inoculated and four healthy plants per introduction from botanical seed.
[b] Scale of 0–3 (0 = no symptoms to 3 = dead or dying). Values represent the mean ($\bar{X}$) and standard error (*SE*).

mean values computed for each PI. All inoculated plants of the following *Solanum* species expressed PSTV symptoms (mean symptom rating is given in parentheses after each species): *Abancayense* (1.5), *acroglossum* (3.0), *acroscopicum* (3.0), *agrimonifolium* (2.4), *amabile* (3.0), *ambosinum* (2.0), *andreanum* (3.0), *boliviense* (2.4), *brevicaule* (1.0), *capsicibaccatum* (2.8), *chancayense* (2.8), *chomatophilum* (2.9), *clarum* (2.8), *colombianum* (2.5), *etuberosum* (2.4), *gandarillasii* (2.5), *huancabambense* (3.0), *infundibuliforme* (2.7), *marinasense* (1.8), *medians* (1.6), *megistacrolobum* (2.8), *microdontum* (2.8), *mochicense* (2.9), *morelliforme* (3.0), *multiinterruptum* (2.8), *oplocense* (2.7), *oxycarpum* (2.2), *pampasense* (2.9), *papita* (2.4), *paucijugum* (3.0), *piurae* (3.0), *polyadenium* (2.4), *polytrichon* (2.2), *raphanifolium* (2.8), *sanctae-rosae* (2.7), *sogarandinum* (3.0), *spegazzinii* (1.9), *toralapanum* (2.9), *trifidum* (3.0), *tuberosum* spp. *andigena* (2.5), *venturii* (3.0), *vernei* (2.0), and *verrucosum* (2.4).

The most characteristic symptom of infected plants was stunting. Generally, new growth was typified by reduced leaflets and shortened internodes that produced a rosette plant. Tissue necrosis, especially veinal necrosis of leaflets, was associated with the more severe symptoms. Depending on the species, necrosis was observed on leaflet veins, petioles, and/or stems. In a few cases, necrosis was restricted to inoculated leaflets.

Symptoms were not observed on any inoculated plants of the following 17 PI lines: *S. berthaultii* (PI 310926 and PI 310927), *S. bulbocastanum* (PI 275199), *S. chacoense* (PI 133708), *S. guerreroense* (PI 161730), *S. hjertingii* (PI 275174), *S. immite* (PI 365330), *S. iopetalum* (PI 275182), *S. medians* (PI 310994), *S. stenotomum* (PI 234013), *S. stoloniferum* (PI 160226, PI 338617, and PI 275246), *S. vernei* (PI 230468 and PI 458370), *S. verrucosum* (PI 338624), and *S. multidissectum* (PI 210044). Bioassays and PAGE demonstrated that all inoculated plants were PSTV-infected. Similarly, in an earlier study, Singh (11) found that none of the inoculated plants in 51 non-tuber-bearing *Solanum* species developed PSTV symptoms but were infected.

Because several PI lines of the *Solanum* species developed diagnostic symptoms within 4 wk after inoculation, they were evaluated further as potential bioassay hosts. At Wisconsin, 50 PI lines were retested by inoculating greenhouse-grown seedlings with the MA, MB, and S strains of PSTV. Nineteen PIs developed marked symptoms after inoculation with all three PSTV strains (Table 2). *S. chomatophilum* (PI 243341) and *S. sparsipilum* (PI 230502) appeared to be exceptionally good prospects for use as bioassay hosts because mean scores of 1.96 and 1.64, respectively, for all three PSTV strains were recorded only 2 wk after inoculation. Symptom severity continued to increase (mean values increased and standard deviation decreased) until the test was concluded 8 wk after inoculation. Because detection of mild PSTV strains either by visual observation of potato varieties or by bioassay on tomato has not been reliable (7,9), the observation that many *Solanum* species are sensitive indicator hosts is important.

The potential usefulness of these *Solanum* species as indicator hosts for PSTV detection has been limited by the long period required to establish seedlings (frequently 4–6 wk) and by the variability among seedlings in response to PSTV inoculation. These limitations were circumvented at Fredericton by selection and clonal propagation before inoculation of plants that developed diagnostic symptoms when inoculated with either mild or severe strains of PSTV (Table 3). Among 16 species, clones 9, 10, and 26 of *S. berthaultii* (PI 265857) have been the most promising. Typical PSTV symptoms on clone 26 are illustrated in Figure 1.

It is clear from this study that individuals within and among the tuber-bearing *Solanum* species vary considerably in their response to PSTV infection. Symptomless plants are not uncommon in PSTV-infected seedling populations; however, immune plants were not found in this study. Therefore, continuous screening of *Solanum* germ plasm to maintain freedom from PSTV is necessary and justified. Clonal propagation of plants that are diagnostic for both mild and severe PSTV strains would be a valuable asset for rapid, accurate screening. Further evaluation of several selected clones is now in process (13).

Table 3. *Solanum* species from which individuals were selected for clonal propagation on the basis of diagnostic symptom development

Species[a]	Plant introduction or collector number	Symptoms
S. amabile	PI 365353	Profuse auxillary growth, stunted, bunchy plants
S. berthaultii	PI 265857	Necrosis, local lesions, death of plant
S. bukasovii	Hje 5902 (PI 473494)	Stunted growth, smaller leaves
S. canasense	Och 9795 (PI 473448)	Necrosis, death of plant
S. hougasii	PI 161726	Stunted growth, veinal necrosis
S. huancabambense	PI 365406	Smaller leaves, dwarf plant
S. jamesii	PI 458424	Local lesions, necrosis
S. kurtzianum	Oka 4961	Necrosis, death of plants
S. microdontum	PI 218226	Necrosis, death of plant
S. mochicense	PI 338616	Necrosis, death of plant
S. raphanifolium	PI 296126	Necrosis, death of plant
S. sparsipilum	PI 365343	Stunted leaves, veinal necrosis
S. stenotomum	PI 234007	Stunted leaves, veinal necrosis
S. stenotomum	PI 234009	Stunted leaves, veinal nerosis
S. trifidum	PI 285539	Necrosis, death of plant
S. venturii	PI 218220	Local lesions, stunting, stunted leaves

[a] Individual plants that developed symptoms within 3–4 wk were multiplied at Fredericton as clones by cuttings.

Fig. 1. Variability of potato spindle tuber viroid symptoms in *Solanum berthaultii* PI 265857. (Left) Clone 26, with severe leaf-drop symptoms; (middle) clone 80, rosetting of top leaves without necrosis; and (right) clone 15, symptomless (tolerant) plant.

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