Verticillium Wilt Resistance in Noncultivated Tuber-Bearing *Solanum* Species

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ABSTRACT

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Noncultivated tuber-bearing *Solanum* species were evaluated for resistance to Verticillium wilt (*Verticillium dahliae*). Four of the 66 species consistently showed high resistance when wilt symptom expression and stem colonization by *V. dahliae* were used as evaluation criteria. These were *S. berthaultii* (PI 265858), *S. chacoense* (PI 473402), *S. sparsipilum* (PI 311000), and *S. tarijense* (PI 473228). Five hundred sixty-eight accessions were obtained from the Inter-Regional Potato Introduction Project (IR-1) and tested under short-photoperiod greenhouse conditions and long-photoperiod field conditions. Some *S. tuberosum* accessions and other *Solanum* species, such as *S. microdontum*, showed resistance to wilt symptoms but not to pathogen colonization. None of the accessions was completely free of stem colonization by *V. dahliae* after repeated testing, indicating lack of immunity. The four most resistant species are diploids (2n = 24) with a common origin in Bolivia and northwestern Argentina. They can be hybridized with cultivated diploids, thereby serving as germ plasm resources.

A large portion of the U.S. potato germ plasm collection has recently been screened for resistance to Verticillium wilt, caused by *Verticillium dahliae* Kleb. This was accomplished at a number of locations, including Aberdeen, ID, with USDA, ARS, and CSRS special grant funds obtained through the Inter-Regional Potato Introduction Project (IR-1), which maintains the collection. Before this, the search for *Verticillium* resistance in wild *Solanum* species had been on a relatively small scale (4,7,8).

The development of Verticillium wilt-resistant cultivars suitable for the processing and fresh table-stock markets of the Pacific Northwest has been one of the major objectives of the USDA-University of Idaho potato breeding program at Aberdeen for many years (4). Certain tetraploid selections from this program have high levels of *Verticillium* resistance, but these have lacked the storage and culinary traits required by the specialized processing industries (3). It would be advantageous to this breeding effort if sources of *Verticillium* resistance were available that would produce effective field resistance in a high proportion of progeny. This would improve the chances of producing cultivars with high *Verticillium* resistance combined with the necessary market characteristics.

This work summarizes the screening of 568 accessions from the IR-1 collection for Verticillium wilt resistance at Aberdeen. The primary objective was the identification of superior parental germ plasm that could be used directly in breeding programs. This involved comparing the levels of resistance found in cultivars and advanced breeding selections with that present in wild *Solanum* species. A brief report of this work has been published (2).

MATERIALS AND METHODS

True seed (sexually propagated) or

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tuber families of more than 600 accessions were obtained from the IR-1 Project, Sturgeon Bay, WI. Seeds were germinated in flats and transplanted into a sand, vermiculite, peat (2:2:1) mixture in 8-cm plastic pots for greenhouse evaluations or in 8-cm peat pots for subsequent transplanting in the field. Seedling tubers were planted directly in the field.

1983 Field evaluation. Tuber families were evaluated in randomized complete blocks with three replicates (five plants per replicate). The field was a Declo silt loam at Aberdeen that had an average of 65 colony-forming units (cfu) of *V. dahliae* per gram of soil. Fertilizer (NPK) was applied as recommended for a cultivar Russet Burbank crop (6). Irrigation was by sprinkler as needed to maintain optimum soil water availability. Emergence was slow and erratic. Wilt severity was evaluated on 15 September (122 days after planting) based on plants dead or dying with typical Verticillium wilt symptoms. Stem sections were taken 5–10 cm above the soil surface on 23 September from accessions that showed little or no wilt and from *S. tuberosum* control clones to determine *V. dahliae* colonization levels. Colonization was determined on a plot basis by the method of Davis et al (3), using composite samples of one stem from each plant in the plot.

Many of the same accessions were also planted as seedlings in a field at Parma, ID, by Guy Bishop for the purpose of evaluating Colorado potato beetle resistance. Management practices were similar to those for the Aberdeen plots. Wilt readings were made on these on 30 August, and the data were combined with the data from Aberdeen for final determination of wilt resistance. Stem sections from the Parma plots were also taken and assayed as previously described for the Aberdeen plots.

1984 Greenhouse evaluation. True seed was germinated in perlite and transplanted in 8-cm plastic pots containing a potting mix (sand:vermiculite: peat; 2:2:1, v/v) amended with 472 cfu of *V. dahliae* inoculum per gram of potting mix. Slow-release fertilizer (6-10-4) was added only once, at mixing time. Fifteen individual plants per accession were arranged in three randomized complete blocks with five plants per replicate. The greenhouse was maintained between 12 C minimum night and 27 C maximum day temperature with an 11-hr photoperiod. An accession was not evaluated if fewer than eight individuals survived transplanting.

The criteria used to evaluate wilt resistance were: 1) time of appearance of first symptoms from 5 through 11 wk after transplanting, 2) the proportion of individuals showing symptoms and severity of symptoms, and 3) the proportion of individuals colonized by *V.*

Table 1. Evaluation of 66 *Solanum* species for Verticillium wilt reaction from 1983 to 1986

	Number of accessions evaluated[a]		Predominant
Solanum species	Greenhouse[b] (1984)	Field[c] (1983–1986)	Verticillium wilt reaction[d]
abancayense	1	2	S
acaule	11	5	S
acroglossum	1	1	S
acroscopicum	2	1	S
agrimonifolium	5	...	S
alandiae	2	...	S
amabile	2	2	S
ambosinum	...	3	S
andreanum	1	...	T
berthaultii	13	9	T-R
blanco-galdosii	1	...	S
boliviense	8	2	S
brachistotrichum	2	1	S
brachycarpum	4	2	S
brevidens	2	2	S
bukosovii	15	2	S
bulbocastanum	11	6	S
canasense	14	7	S
capsicibaccatum	3	2	S
cardiopyllum	11	4	S
chacoense	11	23	T-R
chancayense	2	2	S
chomatophilum	6	2	S
clarum	3	...	S
colombianum	5	1	S
commersonii	10	4	S
demissum	13	15	S
doddsii	1	2	M
etuberosum	3	1	S
fendleri	5	5	S
gourlayi	4	13	S
guerreroense	...	2	S
hjertingii	1	3	S
hougasii	2	4	S
huancabambense	1	2	M-R
immite	2	3	M
infundibuliforme	4	9	S
jamesii	2	7	S
kurtsianum	9	16	S
leptophyes	...	2	S
marinasense	3	5	M-S
medians	4	5	M
megistacrolobum	5	7	S
microdontum	7	15	S-T
multiinterruptum	3	...	S
oplocense	7	11	S-R
pampasense	2	3	S-M
phureja	9	17	S
pinnatisectum	1	6	S
polyadenium	3	4	S
polytrichon	1	2	S
raphanifolium	4	7	S-M
sanctae-rosae	2	...	S
sogarandinum	1	1	S
sparsipilum	5	15	T-R
spegazzinii	6	9	S-R
stenophyllidium	1	...	S
stenotomum	3	9	S
stoloniferum	4	9	S
sucrense	3	8	T-R
tarijense	10	20	T-R
toralapanum	2	...	S
tuberosum			
Andigena group	54	24	S-R
Tuberosum group	3	4	S
vernei	2	8	S-T
verrucosum	1	5	S

[a] Some evaluated more than once in field tests, some tested in both field and greenhouse.
[b] Up to five individuals in each of three replicates grown in 8-cm pots to which *Verticillium dahliae* inoculum had been added to produce 472 cfu/g of potting mix.
[c] Fields at Aberdeen, ID (silt loams, 5–65 cfu/g) and Parma, ID (unknown *V. dahliae* level). Tuber families in 1983 and 1984; true seed families in 1985 and 1986.
[d] S = susceptible, M = intermediate, T = tolerant, and R = resistant based on both wilt symptoms and stem colonization.

dahliae, which was determined by plating a thin cross section from a surface-disinfested stem of each plant onto 0.6% alcohol-0.01% streptomycin sulfate-0.8% agar medium. Classification of accessions into Verticillium wilt reaction categories was based on an index calculated as follows: [(fraction individuals with wilt) × (wilt severity 0–5 scale)] + (fraction stems colonized) ÷ [median time for symptoms to appear], multiplied by 10 to rank on a scale of 0–10. The lowest index recorded was 0.1; the highest was 8.2. Based on the index, the accessions were classified as follows: ≤0.9 = very resistant (VR), 1.0–1.9 = resistant (R), 2.0–2.9 = intermediate (M), 3.0–5.9 = susceptible (S), and ≥6.0 very susceptible (VS). A tolerant (T) designation was used for accessions that were in the VR or R category but had >20% stems colonized.

1984 Field evaluation. Conditions were the same as described for the 1983 evaluations, except the soil contained an average of 10 cfu of *V. dahliae* per gram. Wilt severity was determined on 6 September. Stem sections were collected and assayed as in 1983.

1985 and 1986 Field evaluations. Thirty accessions classed as resistant in prior tests were retested in 1985, and 19 accessions were retested in 1986. They were grown from true seed in the greenhouse in 8-cm peat pots in pathogen-free greenhouse mix. These were transplanted into the field in randomized complete blocks with 10 plants per replicate on 12 June 1985 and on 17 June 1986. The field used in 1985 was a sandy loam that contained 19 cfu of *V. dahliae* per gram of soil. Two fields were used in 1986, a sandy loam with 5 cfu of *V. dahliae* per gram and a silt loam with 26 cfu per gram. Cultural practices were the same as described previously. Foliar sprays of mancozeb were used to control early blight (*Alternaria solani*) both years. Wilt readings were made on 17 September in 1985 and on 15 September in 1986. Stem sections were sampled as composites of all individuals in each plot in 1985. Individual stems were collected separately in 1986 and assayed for stem colonization.

Cultivars, breeding selections, and tetraploid crosses of Aberdeen origin were included as controls in all tests. These were resistant and susceptible clones grown from small (about 25 g) seed pieces in the field tests or families grown from true seed in the greenhouse.

After all testing was completed, each accession was categorized into the following wilt reaction categories: R = resistant, T = tolerant, M = intermediate, and S = susceptible. Although procedures were not identical from year to year and between greenhouse and field, the most important criteria used to evaluate accessions in all cases were the percentage of individuals showing definite wilt symptoms and the degree of stem colonization by *V. dahliae*. The distinction between resistant and tolerant was arbitrary, being based on the amount of colonization relative to group Tuberosum controls. For either an R or T designation, wilt symptoms were slight or absent. An R rating indicated colonization was very low, whereas a T rating indicated colonization was similar to susceptible controls. An S rating indicated wilt symptoms were present in more than 10% of individuals and stem colonization was similar to susceptible controls. The M category was used for those accessions that did not fit clearly into the other categories.

RESULTS

The tuber-bearing *Solanum* species that were successfully tested for Verticillium wilt resistance between 1983 and 1986 are listed in Table 1 along with the number of accessions evaluated for each species and the predominant *Verticillium* reaction. In some cases, only one accession from a species was available for testing, and the individuals appeared to be resistant. Such species were not included in this report because the likelihood of escapes was high. On the other hand, a species was included if only one accession was tested and it was definitely susceptible.

Five hundred sixty-eight accessions representing 66 *Solanum* species were evaluated from 1983 through 1986. Although most were evaluated only once, some were tested repeatedly under both field and greenhouse conditions. There was a poor correlation ($r = 0.27$, $P = >0.99$) between the results of the 1983 field evaluations and the 1984 greenhouse test for 127 repeated accessions. This was due to the number of accessions that appeared resistant in the field but were susceptible in the greenhouse. The greenhouse test of 338 accessions in 1984 resulted in fewer accessions being classified as resistant or tolerant (19%) and more classified as susceptible (61%) compared with the field test of 220 accessions in 1983. In the 1984 greenhouse and field tests, however, the distribution of accessions into wilt categories was similar. More material was handled under better environmental control in the greenhouse. In addition, high inoculum levels were supplied uniformly and interference from other diseases was eliminated. Field testing did allow the individual plants to grow normally, although in both situations, most of the wild *Solanum* species failed to tuberize.

After the screening in the field and the greenhouse was completed, it became apparent that resistance was being consistently expressed in only a few species. These were tested further using the same accessions where possible. Accessions of *S. berthaultii*, *S. chacoense*, *S. sparsipilum* (and the closely related *S. sucrense* [1]), and *S. tarijense* consistently showed high levels of resistance (Table 2), surpassing highly resistant group Tuberosum families and clones. The breeding selection A66107-51 is a highly resistant cultivated type that has been tested extensively (3). The group Tuberosum family A8211 from a cross between Verticillium wilt-resistant parents (A66107-51 and Katahdin) was

Table 2. Verticillium wilt reactions and stem colonization by *Verticillium dahliae* in the most resistant *Solanum* accessions compared with *Solanum tuberosum* (group Tuberosum) families and clones

		Plants with wilt (%)			Stem colonization by *V. dahliae*			
					Log cfu/g of stem		Individuals colonized (%)	
Solanum species	Accession[a]	Two fields[b] (1986)	Field (1985)	Greenhouse wilt index (1984)	Field (1986)	Field (1985)	Two fields[b] (1986)	Greenhouse (1984)
berthaultii	PI 265858	0	0	1.6	1.9	1.6	24	0
chacoense	PI 473402	5	0	···	0.6	0.9	4	···
sparsipilum	PI 311000	7	0	0.6	1.3	0.6	7	10
tarijense	PI 473228	7	0	···	0.9	0.0	19	···
tuberosum	A8211	47	25	2.1	3.0	3.4	75	50
Russet Burbank	sus clone	38	100	···	3.5	3.8	95	···
A66107-51	res clone	0	0	···	2.0	3.2	33	···
LSD (5%)		14	18		1.4	1.6	18	

[a] Each accession was a group of closely related genotypes that could segregate as individuals for any character. These were grown as seedlings and transplanted into the field or greenhouse. Clones were grown from small (about 25 g) seed pieces.
[b] Mean values for both fields.

representative of a cross that would be expected to have a high incidence of resistant progeny with normal tuber maturity characteristics. Wilt reaction in the field and in the greenhouse, stem colonization level, and number of individuals colonized were considerably higher in this family than in resistant accessions of wild species. However, there were some individuals that segregated for susceptibility, even in the most resistant accessions, as shown by the percentage of individuals colonized (Table 2).

DISCUSSION

In general, resistant wild species were characterized by the following: Most accessions within the species were resistant or tolerant, most individuals within resistant accessions showed no wilt symptoms, and stem colonization by *V. dahliae* was low whether expressed as percentage of plants colonized or as colony-forming units per gram in composites of all plants.

The species with the highest levels of resistance of the 66 tested have the following relationships: *S. chacoense* and *S. tarijense* are diploids in the series *Commersonia* and are found in southern Bolivia and northwestern Argentina. *S. chacoense* extends into contiguous areas of northeastern Argentina, Paraguay, and Uruguay (5). *S. sparsipilum* is a diploid in the series *Tuberosa* found from central Peru through Bolivia (5). *S. berthaultii* is a diploid in the series *Tuberosa* that is thought to be a natural hybrid between *S. tarijense* and *S. sparsipilum* and is found in Bolivia and northwestern Argentina (5). These species, therefore, are closely related taxonomically and are derived from a relatively small geographical area in contrast to the entire range of the tuber-bearing *Solanum* species. The hot, dry regions of southern Bolivia and northwestern Argentina appear to be the common origin of these species with high *Verticillium* resistance.

Immunity was not present in the large population screened. Every accession that was tested repeatedly had some individuals that were colonized by *V. dahliae*. This is consistent with the findings of others (4).

Some *S. tuberosum* accessions and clones (cultivated forms), such as A66107-51, show high resistance to wilt symptom development and a degree of resistance to stem colonization. Such clones are comparable to accessions of the more resistant wild species, but there are probably individuals within these accessions with a much higher degree of resistance. These individuals would be valuable as parental germ plasm because they would potentially be an improvement over sources of resistance found in cultivated types. It is not known if improved *Verticillium* resistance can be transferred into cultivated group Tuberosum while maintaining normal tuberization and tuber bulking characteristics. Even though tuberization did not occur in most of the wild *Solanum* species tested, resistance did not seem to be conditioned by a juvenile physiological state, because 82% of the accessions were classed as intermediate or susceptible. At our latitude (43° N), it is difficult to maintain and use individuals from wild species as clones because of this lack of tuberization. Our approach has been to save individual plants that survive inoculation with *V. dahliae* in the greenhouse and maintain them until pollination can be achieved. Several successful crosses between resistant *S. chacoense* and *S. tarijense* plants (diploid 2n = 24) and cultivated diploids have been made. The progeny will be evaluated and used as parents with 2 N pollen-producing cultivated diploids where appropriate.

This study has identified specific accessions in the IR-1 collection from which individuals may be selected to serve as sources of superior Verticillium wilt resistance. These are PI 265858 (*S. berthaultii*), PI 473402 (*S. chacoense*), PI 311000 (*S. sparsipilum*), and PI 473228 (*S. tarijense*).

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