Identification of a Gene Conferring High Levels of Resistance to Verticillium Wilt in *Solanum chacoense*

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ABSTRACT

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Verticillium wilt (*Verticillium albo-atrum*) is an important disease affecting potato tuber yield and quality. In North America the major commercial cultivars are susceptible and management strategies for control of the pathogen rely mainly on soil fumigation and crop rotation. In this study 398 genotypes from accessions of *Solanum berthaultii*, *S. chacoense*, and *S. tarijense* were screened for resistance to *Verticillium albo-atrum*. Resistant genotypes were identified in all but two accessions; however, results indicate that tolerance is more common than resistance. We identified two genotypes in *S. chacoense* (PI 472819) that had low stem-colonization levels and also did not develop wilt symptoms when inoculated with *V. albo-atrum*. These genotypes and a susceptible genotype from PI 472810 (*S. chacoense*) were studied to determine genetic inheritance. Segregation ratios in F_1 , F_2 , and backcross populations indicated that resistance in one of the resistant genotypes (18-21R) was controlled by a single dominant gene. Transfer of the V_c gene to tetraploid germ plasm could provide effective and economical control of Verticillium wilt.

Verticillium wilt, caused by *Verticillium albo-atrum* Reinke & Berthier or *Verticillium dahliae* Kleb., is a common vascular disease causing severe yield and quality losses in many crops. In a recent survey in North America, Verticillium wilt was ranked as the most important disease of both seed and commercial potato crops, and the second greatest constraint on tuber yield (24). Pathogen-mediated reductions in net photosynthesis, transpiration, and increased leaf temperature (6) cause premature foliage senescence and yield loss. Disease symptoms in potato include wilting and leaf chlorosis and necrosis, while the tubers of infected plants develop necrosis in the vascular tissue that reduces tuber quality, in particular for the manufacture of french fries and chips (24). Abiotic factors such as moisture stress and high temperatures accelerate development of visual symptoms. Studies in Ohio (20) and Wisconsin (18,19) demonstrate a synergistic interaction between root-lesion nematodes (*Pratylenchus penetrans* (Cobb) Filipjev &

Schuur. Stekh.) and Verticillium wilt, further complicating disease control.

Management strategies for the control of the pathogen include soil fumigation, crop rotation (4), and development of resistant cultivars (8,9). While several recent cultivar releases have some resistance to the pathogen, the major potato varieties grown in North America are susceptible. The genetic mechanisms of resistance towards the soil-borne fungi of the taxon *Verticillium* spp. vary from polygenic in strawberry (27) and alfalfa (28) to a dominant single gene in cotton (3), sunflower (25), and tomato (26). Conclusions from studies with tetraploid *Solanum tuberosum* subsp. *tuberosum* L. suggest that inheritance is polygenic and complex (16,23). Screening for resistance in the non-cultivated diploid tuber-bearing wild *Solanum* species identified resistance but not immunity (8), and a recent study concluded that the resistance is polygenic and genetically complex (7). Thus, incorporation of this resistance into new cultivars is difficult and inefficient.

Research was undertaken to identify resistant clones in plant introductions of the diploid wild species *S. berthaultii* Hawkes, *S. chacoense* Bitter, and *S. tarijense* Hawkes known to contain resistant genotypes (11). Verticillium wilt-resistant clones were subsequently characterized to determine the level of resistance, host colonization, and genetic inheritance. This study describes identification of the first

single gene resistance towards Verticillium wilt in potato.

MATERIALS AND METHODS

Plant material. Botanical seed of accessions of *S. berthaultii* (PI 20881, 265857, 265858, 310971, 473331), *S. chacoense* (PI WRF267, 275141, 471819, 472810, 472819) and *S. tarijense* (PI 195206, 217458, 473228, 473236) reported to contain genotypes resistant to Verticillium wilt, were selected from the inventory maintained by the USDA Potato Introduction Station (Sturgeon Bay, WI; 11), treated with gibberellic acid (GA_3 ; 1.5 g liter⁻¹; Sigma-Aldrich Ltd. Ontario, Canada) and planted in seedling flats containing Cornell Mix (5). After 14 days (four-leaf stage), the seedlings were transplanted into Cornell Mix contained in 15-cm plastic pots and placed in a growth chamber (Convion Ltd., Winnipeg, Canada) with a 22/18°C day/night temperature regime and a 16-h photoperiod. Tubers were harvested from tuber-bearing genotypes 4 months after transplanting and stored for 4 months at 4°C. After natural dormancy release, sprouted tubers of a total of 398 genotypes (16 replications per genotype) of the accessions were planted in alternate sleeves in Cornell mix contained in 32-cell root trainers (Lamaire Industries, Ltd., Edmonton, Canada) and placed in a greenhouse. A root trainer consists of plastic sleeves which, when folded, form individual cells with the sleeves contained in a plastic tray. In the 32-cell root trainer, each sleeve forms 4 cells, and there are 8 sleeves per tray. In addition, 8 single 'eyes' from 5 control varieties with established disease reactions were included in the screening trial: susceptible, Russet Norkotah and Shepody; moderately susceptible, Russet Burbank; resistant, A66107-51 and A68113-4 (2,9).

Inoculum preparation and inoculation. An aggressive isolate of *V. albo-atrum*, obtained from infected potato plants from Alberta, Canada, was used in the study. Conidia were collected from 10- to 14-day-old cultures grown in potato dextrose broth at room temperature with shaking (100 rpm). Cultures were filtered through cheesecloth and conidia concentrated from the filtrate by centrifugation at $5,000 \times g$ for 25 min and then diluted with sterile deionized water to give a concentration of approximately 5×10^7 spores ml⁻¹.

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The plants were inoculated with the *V. albo-atrum* spore suspension 4 weeks after planting, using a modification of the procedure described by Huang and Hanna (15). The folding sleeve design of the roottrainer enabled removal of the intact plant from the container. Root injury was accomplished by removing the lower half of the root ball and inoculation by submerging the remaining portion of the root ball for 10 min in the spore suspension. Four control plants (a single sleeve) in each 32-cell roottrainer were root damaged but not inoculated.

Resistance evaluation. Disease reaction ratings were initiated 4 weeks after inoculation and conducted at weekly intervals for a further 4 weeks to confirm earlier ratings. Genotypes that showed no visual symptoms were rated as resistant (Table 1), while the reactions of susceptible genotypes included wilting, chlorosis, and necrosis of the lower leaves (Fig. 1). All resistant and a few of the susceptible genotypes from all accessions were retained for further study.

Vascular colonization by the pathogen was assessed for all resistant genotypes using the method described by Hoyos et al. (14). Five stems from several plants of each resistant genotype were removed after the final visual disease rating. Stem sections, approximately 15-cm long, were cut with a sterile scalpel at the soil line, thoroughly washed with water, immersed in 1% NaOCl for 30 s, and rinsed in sterile water. Plant sap was extracted using a roller press (Agdia Inc., Elkhart, IN) and collected in a sterile petri dish. A modified Nadakavukaren and Homer medium (22) was used to quantify pathogen invasion. The medium was prepared by suspending 10 g agar and 5 g potassium thiocyanate in 1 liter of distilled water. The medium was autoclaved for 20 min at 121°C and, prior to use, 10 ml of 95% ethanol, 1.0% streptomycin sulfate solution and 0.5% penicillin G (Sigma-Aldrich Ltd.) added. A 0.1-ml aliquot of the plant sap was pipetted into a 100- by 15-mm petri dish and 20 ml of the cooled medium added. Petri dishes were incubated at room temperature in

diffuse light for 10 days. The number of CFU were counted and used to estimate the total CFU in the 0.1-ml aliquot. The result was expressed as $\log_{10} (\text{CFU} + 1)/0.1 \text{ ml}$.

Genetic studies. Clones that were rated resistant and with zero or very low CFU as well as susceptible clones were included in genetic studies. Inoculation of these genotypes (Table 2) was repeated to confirm earlier results. Reciprocal crosses were made between resistant and susceptible *S. chacoense* clones and F_1 segregating populations derived from these hybridizations screened for resistance. An F_2 population was generated by hybridizing resistant F_1 siblings. Selfing was not successful, probably due to gametophytic self-incompatibility associated with S-alleles (13). To confirm our results, resistant F_1 hybrids were backcrossed to the susceptible parent. Hybridization, using emasculated plants, was carried out in a growth room (Convion Ltd.) with a 20/18°C day/night temperature regime and a 20-h photoperiod. Resistance screening (as previously described) of the segregating populations was carried out in a growth chamber (Convion Ltd.) with a 22/18°C day/night temperature regime and 16-h photoperiod. Each roottrainer contained 12 plants inoculated with the pathogen, and 4 control plants that were root-damaged but not inoculated and kept separate from the inoculated plants.

RESULTS

Resistant genotypes were identified by visual screening in all the accessions sampled, except for *S. chacoense* PI 275141 and *S. tarijense* PI 217458 (Table 1). Ratings for the varieties included in the experiment as controls were consistent with published disease reactions (2,9). No wilt symptoms were observed in resistant plants, whereas the disease reaction for susceptible plants included severe wilting, chlorosis and necrosis of the lower leaves, and eventual death of the plant (Fig. 1). Some variation was observed in the severity and occurrence of disease symptoms following inoculation in susceptible plants.

Extensive stem colonization by the pathogen occurred in most of the genotypes visually rated as resistant (Table 2). Two genotypes from *S. chacoense*, designated 18-21R and 18-7R from PI 472819, with low stem colonization levels, 0 and 0.30 $\log_{10}$ CFU/0.1 ml, respectively, were selected, and reciprocal crosses between these two resistant clones and a highly susceptible genotype designated 12-9S from PI 472810 were made (Table 3). Segregation ratios for the reciprocal hybridizations suggested that the 18-21R clone was heterozygous resistant and 12-9S homozygous susceptible for a single dominant gene controlling *Verticillium* wilt resistance, and that inheritance was nuclear (Table 3). Hybridizations with the 18-7R clone produced segregation ratios that

Table 1. Resistance to *Verticillium albo-atrum* in *Solanum chacoense*, *S. tarijense*, and *S. berthaultii* rated by visual foliage reaction^a

Accession no.	No. of genotypes screened	% resistant or tolerant
<i>S. chacoense</i>		
WRF 267	41	14.6
PI275141	15	0.0
PI471819	27	22.2
PI472810	35	25.7
PI472819	32	6.2
<i>S. tarijense</i>		
PI195206	33	6.1
PI217458	11	0.0
PI473228	20	20.0
PI473236	40	15.0
<i>S. berthaultii</i>		
PI208881	15	13.3
PI265857	45	14.0
PI265858	22	3.0
PI310971	46	18.0
PI473331	16	25.0

^a Roots damaged and inoculated with a spore suspension.

Fig. 1. Foliage reaction to inoculation with *Verticillium albo-atrum* of resistant (A) and susceptible (B) *Solanum chacoense* test cross progeny.

indicated complex inheritance and did not fit the expected ratio for a one- or two-gene model (data not shown).

The result for 18-21R $\times$ 12-9S was confirmed by crossing a resistant F₁ hybrid with a sibling resistant F₁ hybrid (selfing was not successful), and by backcrossing the resistant F₁ hybrid to the susceptible parent. Segregation ratios for both hybridizations were consistent with the expected ratios for a one-gene model and the dominance of resistance over susceptibility ($P > 0.99$). The proposed designation of the gene V_c reflects the *S. chacoense* source (Table 3). Our results suggest that inheritance of the resistance is simple, and nuclear.

DISCUSSION

To our knowledge this is the first study to report single gene resistance towards

Verticillium wilt in potato. Previous genetic studies of resistance towards Verticillium wilt in diploid and tetraploid *Solanum* species suggest complex polygenic inheritance (7,23). Furthermore, results indicate that tolerance is more common than resistance to Verticillium wilt in the *S. berthaultii*, *S. chacoense*, and *S. tarijense* accessions examined.

As reported by others (8,21), visual symptom expression and the magnitude of stem invasion by the pathogen were poorly correlated. This finding supports the distinction between tolerance (no visual symptoms with significant stem invasion) and resistance (no symptoms and very low stem invasion). Tolerance is undesirable because it facilitates proliferation of the pathogen in the soil. Results from this and other studies (7,23) suggest that genetic

mechanisms for the two disease reaction types are distinct in *S. chacoense*. Furthermore, inheritance is complex for tolerance and simple or complex for resistance.

In contrast to potato, in which the disease is widespread and expensive to control, the single gene resistance V_c in tomato has effectively controlled Verticillium wilt in North America for several decades. Within 10 years of the introduction of wilt resistant tomato cultivars, a new race of *V. dahliae* was reported that was not controlled by the V_c gene (1). However, only recently has *V. dahliae* race 2 become endemic in the tomato-producing areas of Ontario (10). Long-term durability of single gene resistance is frequently observed with soil-borne pathogens.

Identification of a dominant single gene conferring high levels of resistance to Verticillium wilt in *S. chacoense* provides an opportunity to transfer the gene directly to tetraploid progeny via 2N pollen production or by using *S. tuberosum* subsp. *tuberosum* dihaploids as an intermediate step (12). Development of tetraploid breeding clones, either duplex or simplex for the resistance, will ensure a high proportion of resistant progeny and thus accelerate development of commercial cultivars with high levels of resistance. Several successful crosses have been made between dihaploids of Atlantic, ND860-2 and F51013, and the resistant *S. chacoense* clone. Tuber traits and 2N pollen production of the progeny of these hybridizations are currently being examined. Search for a polymorphic DNA marker that will enable distinction between the homozygotes and heterozygotes and facilitate development of duplex parents is also currently underway. The recent identification of a co-dominant amplified DNA marker for Verticillium wilt resistance in tomato (17) may assist in the development of a potato marker, since the genomes of the two crop species are co-linear.

This study has identified a specific accession, PI 472819 (*S. chacoense*) in the

Table 2. Stem invasion by *Verticillium albo-atrum* in asymptomatic genotypes of *Solanum chacoense* accessions^w

Accession no.	Clone no.	CFU ^x log ₁₀ (CFU + 1)/0.1 ml
WRF 267	7	2.40 $\pm$ 0.90
	10	> 2.45
	25	0.60 $\pm$ 0.49
	30	2.06 $\pm$ 1.22
	33	> 2.45
	34	> 2.45
PI472810	3	1.99 $\pm$ 1.66
	12	0.76 $\pm$ 0.29
	14	1.49 $\pm$ 0.78
	20	> 2.45
	27	> 2.45
	31	1.54 $\pm$ 0.50
	33	1.54 $\pm$ 1.31
	36	1.85 $\pm$ 1.43
	52	2.20 $\pm$ 1.25
PI471819	2	2.34 $\pm$ 1.92
	5	1.00 $\pm$ 0.74
	9	1.52 $\pm$ 0.84
	10	1.98 $\pm$ 1.59
	12	1.86 $\pm$ 0.70
	23	1.83 $\pm$ 1.78
PI472819	7	0.30 $\pm$ 0.21 ^y
	21	0.00 ^z

^w Root-damaged plants inoculated with a spore suspension.

^x Mean of 15 stems.

^y Resistant parent (18-7R).

^z Resistant parent (18-21R).

Table 3. Segregation for Verticillium wilt resistance in crosses of *Solanum chacoense* clones from PI 472819 and 472810

Materials	No. of plants tested	Disease reaction				χ^2	P^z	Postulated genotypes	Phenotypic ratio resistant:susceptible
		Observed		Expected					
		R ^y	S ^y	R	S				
Parents									
18-21R (PI472819)	60	60	0	...	...	...	...	V _c V _c	
12-9S (PI472810)	54	0	54	...	...	...	...	v _c v _c	
F ₁ generation									
18-21 R × 12-9S	52	32	20	26	26	2.77	0.10	V _c v _c , v _c v _c	1:1
12-9S × 18-21R	176	86	90	88	88	0.09	0.75 – 0.90	V _c v _c , v _c v _c	1:1
F ₂ generation									
F ₁ -resistant hybrid × F ₁ -resistant hybrid	80	60	20	60	20	0.00	> 0.99	V _c V _c , V _c v _c , v _c v _c	3:1
Backcross generation									
12-9S × F ₁ -resistant hybrid	117	58	59	58	59	0.00	> 0.99	V _c v _c , v _c v _c	1:1

^y R = resistant, S = susceptible; assessed by inoculating the plants with a spore suspension of *Verticillium albo-atrum* and rating visual wilt symptoms.

^z Probability of $\chi^2 \leq$ the calculated value.

NRSP-6 collection (Sturgeon Bay, WI) with a dominant resistance gene for *Verticillium* wilt V_c in a diploid wild species of potato. Transfer of the gene to tetraploid germ plasm and the development of resistant commercial varieties should provide effective and economical control of *Verticillium* wilt.

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LITERATURE CITED

- Alexander, L. J. 1962. Susceptibility of certain *Verticillium* resistant tomato varieties to an Ohio isolate of the pathogen. *Phytopathology* 52:998-1000.
- Anderson, N., and Hoyos, G. 1987. Varietal responses of potato to *Verticillium* wilt. *Valley Potato Grower* 53(105):10-14.
- Barrow, J. R. 1970. Heterozygosity in inheritance of *Verticillium* wilt tolerance in cotton. *Phytopathology* 60:301-303.
- Ben-Yephet, Y., Frank, Z. R., MaleroVera, J. M., and DeVay, J. E. 1989. Effect of crop rotation and metham-sodium on *Verticillium dahliae*. Pages 543-556 in: *Vascular Wilt Diseases of Plants: Basic Studies and Control*, Vol. 28. Springer-Verlag, New York.
- Boodley, J. W., and Sheldrake, R. 1977. Cornell peat-lite mixes for commercial plant growing. *Cornell Univ. Coop. Ext. Div. Info. Bull.* 43.
- Bowden, R. L., and Rouse, D. I. 1991. Effects of *Verticillium dahliae* on gas exchange of potato. *Phytopathology* 81:293-301.
- Concibido, V. C., Secor, G. A., and Jansky, S. H. 1994. Evaluation of resistance to *Verticillium* wilt in diploid, wild potato interspecific hybrids. *Euphytica* 76:145-152.
- Corsini, D. L., Pavsek, J. J., and Davis, J. R. 1988. *Verticillium* wilt resistance in noncultivated tuber-bearing *Solanum* species. *Plant Dis.* 72:148-151.
- Corsini, D. L., Pavsek, J. J., and Davis, J. R. 1990. *Verticillium* wilt resistant potato germplasm A66107-51 and A68113-4. *Am. Potato J.* 67:517-525.
- Dobinson, K. F., Tenuta, G. K., and Lazarovits, G. 1996. Occurrence of race 2 of *Verticillium dahliae* in processing tomato fields in southwestern Ontario. *Can. J. Plant Pathol.* 18:55-58.
- Hanneman, R. E., and Bamberg, J. B. 1986. Inventory of tuber-bearing *Solanum* species. Bulletin 533. Research Division, College of Agriculture and Life Sciences, University of Wisconsin, Madison.
- Hermundstad, S. A., and Peloquin, S. J. 1987. Breeding at the 2X level and sexual polyploidization. Pages 197-210 in: *The Production of New Potato Varieties: Technological Advances*. Cambridge University Press, Cambridge.
- Howard, H. W. 1970. *Genetics of the Potato*. Logos Press Ltd., Wellingborough, England.
- Hoyos, G. P., Zambino, P. J., and Anderson, N. A. 1991. An assay to quantify vascular colonization of potato by *Verticillium dahliae*. *Am Potato J.* 68:727-742.
- Huang, H. C., and Hanna, M. R. 1991. An efficient method to evaluate alfalfa cultivars for resistance to *Verticillium* wilt. *Can. J. Plant Sci.* 71:871-875.
- Hunter, D. E., Darling, H. M., Stevenson, F. J., and Cunningham, C. E. 1968. Inheritance of resistance to *Verticillium* wilt in Wisconsin. *Am Potato J.* 45:72-78.
- Kawchuk, L. M., Lynch, D. R., Hachey, J., Bains, P. S., and Kulcsar, F. 1994. Identification of a codominant amplified polymorphic DNA marker linked to the *Verticillium* wilt resistance gene in tomato. *Theor. Appl. Genet.* 89:661-664.
- Kotcon, J. B., Rouse, D. I., and Mitchell, J. E. 1985. Interactions of *Verticillium dahliae*, *Colletotrichum coccodes*, *Rhizoctonia solani*, and *Pratylenchus penetrans* in the early dying syndrome of Russet Burbank potatoes. *Phytopathology* 75:68-74.
- MacGuidwin, A. E., and Rouse, D. I. 1990. Role of *Pratylenchus penetrans* in the potato early dying disease of Russet Burbank potato. *Phytopathology* 80:1077-1082.
- Martin, M. J., Riedel, R. M., and Rowe, R. C. 1982. *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. *Phytopathology* 72:640-44.
- Mohan, S. K., Davis, J. R., Corsini, D. L., Sorensen, L. H., and Pavsek, J. J. 1990. Reaction of potato clones and accessions of *Solanum* spp. to *Verticillium dahliae* Kleb. and its toxin. *Potato Res.* 33:449-458.
- Nadakavukaren, M. J., and Homer, C. E. 1959. An alcohol agar medium selective for determining *Verticillium* microsclerotia in soil. *Phytopathology* 49:527-528.
- Pavsek, J. J., and Corsini, D. L. 1994. Pages 403-409 in: *Inheritance of Resistance to Warm-growing-season Fungal Diseases*. J. E. Bradshaw and G. R. Mackay, eds. *Potato Gen.* CAB International, Wallingford, United Kingdom.
- Powelson, M. L., and Rowe, R. C. 1993. Biology and management of early dying of potatoes. *Annu. Rev. Phytopathol.* 31:111-126.
- Putt, E. D. 1964. Breeding behavior of resistance to leaf mottle or *Verticillium* in sunflowers. *Crop Sci.* 4:177-179.
- Schaible, L., Cannon, O. S., and Waddoups, V. 1951. Inheritance of resistance to *Verticillium* wilt in a tomato cross. *Phytopathology* 41:986-990.
- Wilhelm, S. 1955. *Verticillium* wilt of strawberry with special reference to resistance. *Phytopathology* 45:387-391.
- Zaleski, A. 1957. Reactions of lucerne strains to *Verticillium* wilt. *Plant Pathol.* 6:137-142.