Inheritance of Resistance to *Pythium myriotylum* Hypocotyl Rot in *Phaseolus vulgaris* L.

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ABSTRACT

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Resistance to *Pythium myriotylum* hypocotyl rot was found in several lines of *Phaseolus vulgaris*, including PI 203958 (N203), State Half Runner, Ore. 70-169-1M, and Wis. (RRR) 46. Broad sense heritability values, as determined from crosses between PI 203958 and the susceptible cultivars Cascade and Tenderwhite, were 0.643 and 0.651, respectively. The value of the cross Wis. (RRR) 46 × Tenderwhite was 0.290. Distribution of the F_2 populations appeared to be normal for all crosses.

Root and hypocotyl rot have been considered two of the major diseases of bean since they were first described in 1916 (5,6). Several different fungi have subsequently been reported to cause damage to roots and hypocotyls of bean plants (5–7,12,14,16). Researchers have found it difficult to introduce resistance into commercial cultivars without introducing other, unwanted traits (2,3,11). Dry bean cultivars with acceptable resistance to root rot have only recently been released (4).

In the Central Sands area of Wisconsin, *Pythium* spp. appear to be the major incitants of root and hypocotyl rot (12). Several breeding lines have been released through a program to develop snap bean lines resistant to these diseases (9,10). Resistant germ plasm has been evaluated in a root rot nursery at the University of Wisconsin Experimental Farm at Hancock. Experiments with isolates from diseased bean plants growing in the nursery (experimental area W-7) indicated that *Pythium myriotylum* Drechs. was the most frequent incitant of root and hypocotyl rot, although *P. ultimum* Trow and *P. irregulare* Buis. appear to be the major incitants of root rot in commercial fields (12,18). Lines that perform well in the nursery should, therefore, possess resistance to *P. myriotylum*. Although *P. myriotylum* is an incitant of root and hypocotyl rot in other bean production areas (7,8,14), resistance to it has not been previously reported.

We assessed *P. myriotylum* hypocotyl rot resistance in selected *Phaseolus vulgaris* germ plasm and measured the heritability of this resistance in crosses between resistant and susceptible lines. We developed a growth chamber test to eliminate possible effects of other pathogens.

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MATERIALS AND METHODS

Inoculum for all experiments was a modified Schmitthenner's liquid medium (19): 2.4 g sucrose, 0.27 g asparagine, 0.15 g KH_2PO_4, 0.15 g K_2HPO_4, 0.10 g $MgSO_4 \cdot 7H_2O$, 4.4 mg $ZnSO_4 \cdot 7H_2O$, 1.0 mg $FeSO_4 \cdot 7H_2O$, 0.07 mg $MnCl_2 \cdot 4H_2O$, 5.0 mg $CaCl_2 \cdot 2H_2O$, 2.0 mg thiamine HCl, 10.0 mg ascorbic acid, 50 mg β-sitosterol (dissolved by gently heating in 95% ethanol, 5 mg/ml), and 1 L of double-distilled H_2O.

After preparation, 100 ml of the medium was poured into each 500-ml Erlenmeyer flask. The medium was autoclaved for 15 min at 121 C, then allowed to cool to room temperature before each flask was seeded with an agar block from a 3-day-old culture of *P. myriotylum*. The flasks were incubated, without shaking, at 28 C in the dark for 10–14 days.

The resulting mycelial mats were removed, washed three times in double-distilled water, blended in a Sorvall Omni-mixer for 1 min at full power, and sonified for 45 sec at 75 W (Sonifier Cell Disrupter, model W185, Heat Systems, Ultrasonics, Inc., Plainview, NY). This mixture was centrifuged for 15 min at 2,590 *g*.

The pellet was again suspended in distilled water and centrifuged. The oospores and oogonia were counted in a hemacytometer. The remaining mycelium was not viable when plated on cornmeal agar. The oospores and oogonia were added to silica sand (natural grain, Ottawa Silica Co., Ottawa, IL) at a rate of 500 propagules per gram of sand.

In most experiments, the infested sand was added to sterilized aluminum pans (29 × 23 × 6 cm) to a depth of 4 cm. Six rows of captan-dusted *P. vulgaris* seeds (eight seeds per row) were placed on the surface of the sand and covered with an additional 1 cm of infested sand. The pans were placed in a 12-hr photoperiod growth chamber (light intensity: 2.7×10^4 W) at 28 C and watered daily to wet the soil surface. When the seedlings emerged (late crook-neck stage, usually 4 days after planting), pans were transferred to 32 C and watered daily to saturation; Hoagland's solution was added three times per week. Experiments were ended after 10 days of exposure to 32 C. Hypocotyl damage was evaluated for each plant on a 0–4 scale, where 0 = no symptoms; 1 = small, reddish lesions on the base of the hypocotyl; 3 = water-soaking of the hypocotyl base and an elongated, water-soaked streak extending up the hypocotyl; and 4 = collapse or death of the plant.

Resistant breeding materials that have been used in the development of the Wis. (RRR) 46 and Wis. (RRR) 83 breeding lines (9,10) were evaluated. Each breeding line was allotted one randomly selected row in each of three pans filled with infested sand. Each row was treated as a replicate when results were analyzed. The susceptible cultivar Tenderwhite was included in the test for comparison.

We evaluated the heritability of resistance in PI 203958 (N203) and Wis. (RRR) 46 by crossing them with susceptible cultivars and screening the F_2 populations of each cross for resistance. PI 203958 was crossed with the susceptible snap bean cultivars Tenderwhite and Cascade, and Wis. (RRR) 46 was crossed

Table 1. Resistance of selected *Phaseolus vulgaris* germ plasms to *Pythium myriotylum* hypocotyl rot

Line	DI[x]
Tenderwhite	4.0 a[y]
Ore. 71-1759	3.5 ab
Gloria[z]	2.7 bc
State Half Runner	2.5 cd
PI 203958	2.2 cd
Ore. 70-169-1M	1.8 d
Wis. (RRR) 46	1.5 d

[x] Disease index; evaluations of hypocotyl rot were made on a scale of 0 (healthy) to 4 (dead).
[y] Values are the mean of three replicates; values followed by the same letter are not significantly different ($P = 0.05$) as determined by Duncan's multiple range test.
[z] Used in the development of Wis. (RRR) 83 but not Wis. (RRR) 46.

with Tenderwhite. In all cases, reciprocal crosses were made. Back crosses of the F_1 of Wis. (RRR) 46 × Tenderwhite were made to the respective parents. The parents of each F_2 population were randomly allotted to three rows in each pan. The remaining three rows were allotted to the F_2 population.

Because space was limited, not all seeds of a given cross could be tested at once; two trials were thus run for each F_2 population. The individual plant disease index values from each trial were used to obtain a mean index value and a variance (s^2) for each population. Individual plant disease index values from both trials were also combined into a single population, and a population mean and variance were again determined. Broad sense heritability values were calculated where possible, using the methods of Warner (21) and Mahmoud and Kramer (15). The distribution of the F_2 population resulting from the combination of the two trials was compared with the expected normal distribution, as calculated on the basis of the population mean and standard deviation. A chi-square test was used to determine goodness-of-fit to the normal distribution. Adjusted chi-square (χ^2 Adj.) values were calculated by grouping class frequencies of less than five with the next disease class (13).

RESULTS

Table 1 presents the comparisons among sources of germ plasm for resistance to *P. myriotylum*. Ore. 70-169-1M, State Half Runner, and PI 203958 appeared to be the most resistant.

Table 2 presents the data about crosses between susceptible and resistant parents. No maternal effect on disease reaction was apparent, and the F_2 populations from the reciprocal crosses were thus not distinguished from each other in the analysis.

The broad sense heritability values in Table 2 were similar for all F_2 populations except Wis. (RRR) 46 × Tenderwhite (trial 2), which had a very low value (0.099). This exception may be caused by the high variance in the parental populations in the trial.

The χ^2Adj values for each cross indicated no significant difference between the population distributions and normal distributions. Figure 1 shows the parental and F_2 distributions.

DISCUSSION

Several lines that contributed to the development of Wis. (RRR) 46 possessed varying degrees of resistance to *P. myriotylum*. The resistance in Ore. 70-169-1M was apparently derived from PI 203958 (1), which has been used in several breeding programs because it is resistant to many pathogens (3,11,20); however, Pieczarka and Abawi (17) reported that PI 203958 is susceptible to *P. ultimum* root rot. State Half Runner, which has been reported to be resistant to *Fusarium solani* f. sp. *phaseoli* (Burk.) Snyd. & Hans. (3), performed well in field trials where *Pythium* spp. are believed to be the major incitants (2). The origins of State Half Runner are unclear, but it is probably not derived from PI 203958.

Although high levels of resistance were available (Table 1), the heritability of this resistance was only moderate (Table 2). When resistance has been difficult to transfer from lines with unacceptable plant type to commercially acceptable lines, Beebe (2) has suggested that traits conferring agronomic plant type be incorporated into the resistant lines. Wis. (RRR) 46 may be a good choice for this

Table 2. Resistance in the F_2 populations of selected *Phaseolus vulgaris* germ plasms to *Pythium myriotylum* hypocotyl rot

Line	Trial 1			Trial 2			Total		
	DI[a]	s^{2b}	N[c]	DI	s^2	N	DI	s^2	N
Tenderwhite	3.7	0.275	54	3.8	0.331	13	3.7	0.293	67
PI 203958	1.5	0.250	25	1.7	0.410	21	1.6	0.333	46
F_2	2.7	0.539	98	3.1	0.964	90	2.9	0.874	188
	BSH[d] =	0.514		BSH =	0.618		BSH =	0.643	
Cascade	3.7	0.380	11	3.8	0.446	12	3.7	0.278	23
PI 203958	1.5	0.250	25	2.0	0.188	22	1.7	0.387	47
F_2	2.8	0.731	118	2.6	1.116	95	2.7	0.940	213
	BSH =	0.557		BSH =	0.740		BSH =	0.651	
Tenderwhite	3.9	0.113	23	3.8	0.586	23	3.8	0.660	46
Wis. (RRR) 46	1.9	0.408	17	1.8	1.040	25	1.8	0.610	42
F_2	2.6	0.612	79	2.3	0.867	92	2.4	0.890	171
	BSH =	0.649		BSH =	0.099		BSH =	0.290	

[a] Disease index; mean disease rating (0–4) of N plants.
[b] Variance.
[c] Number of plants.
[d] BSH = broad sense heritability.

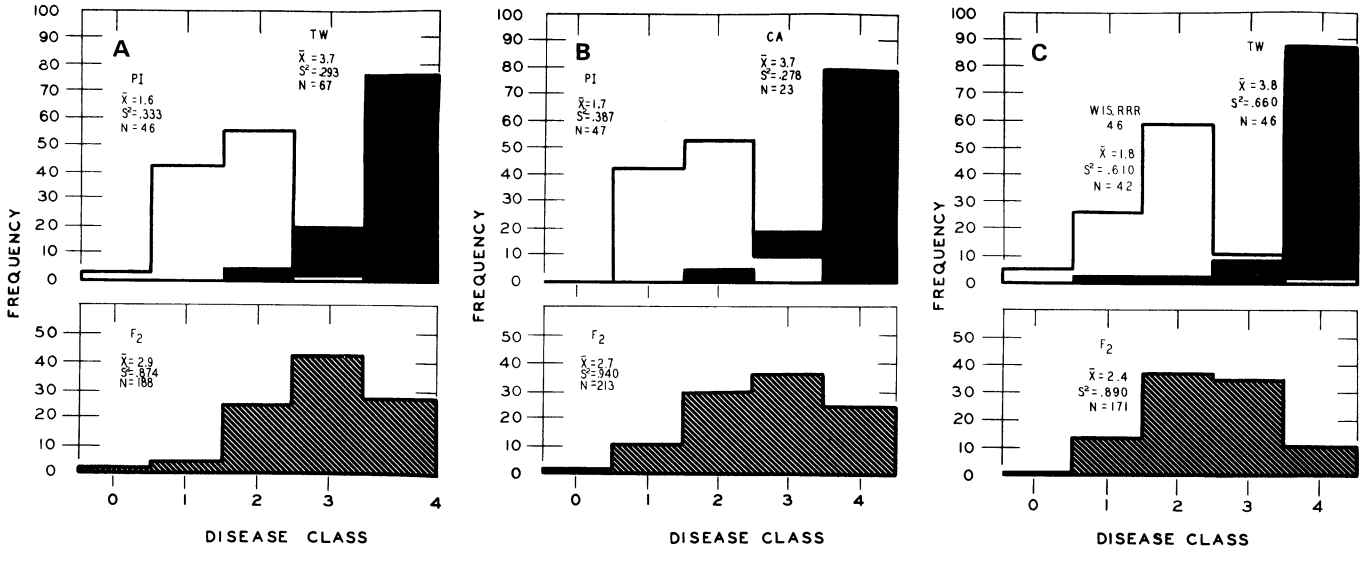

Fig. 1. Distribution of the F_2 and parental populations of selected *Phaseolus vulgaris* germ plasms. **(A)** PI 203958 (PI) × Tenderwhite (TW). $\chi^2 = 11.45$, 2 d.f., $P < 0.005$; χ^2Adj. = 0.373, 2 d.f., $0.75 < P < 90$. **(B)** PI 203958 (PI) × Cascade (CA). $\chi^2 = 2.53$, d.f. = 2, $0.250 < P < 0.500$; χ^2Adj. = 2.43, d.f. = 2, $0.250 < P < 0.500$. **(C)** Wis. (RRR) 46 × Tenderwhite (TW). $\chi^2 = 3.75$, d.f. = 2, $0.005 < P < 0.250$; χ^2 Adj. = 3.48, d.f. = 2, $0.005 < P < 0.250$.

procedure, because its plant type is already similar to that of commercial snap bean cultivars.

The normal distributions of the F_2 populations of all crosses indicated that dominance was not an important factor in the expression of resistance.

The resistance discussed herein refers only to hypocotyl rot and does not imply that resistance to root rot was also present. The root systems of all of the germ plasm sources listed in Table 1 were rated as susceptible to *P. myriotylum*. However, it cannot be concluded that useful root resistance was not present. The test may have been too severe to permit expression of root resistance. Further work is needed to determine the relationship between root and hypocotyl resistance and to determine whether resistance to *P. myriotylum* confers resistance to other *Pythium* spp.

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Seed of PI 203958 was obtained from F. A. Bliss, Department of Horticulture, University of Wisconsin, Madison, and multiplied in the greenhouse. State Half Runner, Cascade, and Tenderwhite seeds were obtained from commercial stocks produced in Idaho. Wis. (RRR) 46 was multiplied in root rot plots at the University of Wisconsin Agricultural Experiment Station, Hancock. Gloria was obtained from D. W. Burke, Washington Agricultural Experiment Station, Pullman, Ore. 71-1759 and Ore. 70-169-1M seeds were obtained from W. A. Frazier, Oregon State University.

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