

# Sources of Partial Resistance to Fusarium Root Rot in the *Pisum* Core Collection

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## ABSTRACT

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Fusarium root rot, caused by *Fusarium solani* f. sp. *pisi*, is one of the most important fungal diseases of pea and is found in most pea-growing areas around the world. Currently, no commercial cultivars are resistant to this pathogen. Availability of new sources of partial resistance could provide another tool for managing Fusarium root rot. In all, 387 accessions from the *Pisum* core collection were evaluated for resistance to Fusarium root rot in two independent experiments. Nonparametric analysis of variance conducted on ranks of disease severity for each accession indicated that the two experiments corresponded well. Forty-four plant introduction lines with a disease severity rating of 2.5 or less on a 0-to-5 scale (where 5 = completely rotted) were selected as being partially resistant to root rot. Immunity to Fusarium root rot was not found. Comparison of disease resistance data for Aphanomyces root rot and Fusarium root rot showed a weak, but significant and positive correlation. A complete listing of the data for the partial resistance of all accessions tested can be found at the National Plant Germplasm System website, United States Department of Agriculture-Agricultural Research Service.

Additional keywords: *Pisum sativum*

Fusarium root rot, caused by *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (F. R. Jones) W. C. Snyder & H. N. Hans, is an economically important fungal disease of pea (*Pisum sativum* L.) in most pea-growing areas around the world (6–8). Currently, no commercial cultivars are completely resistant to this pathogen. Fusarium root rot is managed by a combination of cultural practices, including tillage practices that avoid soil compaction and promote plant growth and use of high quality seed. Availability of new sources of genetic resistance could enhance the use of plant resistance as another tool for managing this disease.

Germ plasm collections are an invaluable resource for discovery of novel sources of resistance to plant pathogens (12). The *Pisum* germ plasm collection, encompassing 3,615 accessions as of March 2002, is maintained by the Western Regional Plant Introduction Station (WRPIS), located at Washington State University in Pullman. This germ plasm collection has been characterized for resistance to several soilborne plant pathogens, including Fusarium wilt races 1 and 2

(caused by *F. oxysporum* f. sp. *pisi*) and Aphanomyces root rot (caused by *Aphanomyces euteiches* Drechs.) (10,11). Since 1996, the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) research program has been characterizing accessions for resistance to Fusarium root rot. Characterization of the *Pisum* core collection for resistance to Fusarium root rot should provide data on the degree of partial resistance available and should identify novel sources of partial

resistance that may be useful in breeding programs.

Our objective was to identify sources of partial resistance against Fusarium root rot available in the *Pisum* core collection. The *Pisum* core collection is composed of 504 accessions from 57 countries, representing about 17% of pea accessions, and was designed to include accessions based upon documented concentrations of diversity and the entire range of adaptation (16). We evaluated 387 plant introduction (PI) accessions for resistance to Fusarium root rot in two independent greenhouse experiments. We also tested the hypothesis that accessions that are partially resistant to Fusarium root rot are also resistant to Aphanomyces root rot, using Aphanomyces root rot data available from the WRPIS website and the literature (10).

## MATERIALS AND METHODS

**Seed source.** Due to seed availability, a subsample of 387 PI accessions of the 504 accessions in the *Pisum* core collection were obtained from the USDA WRPIS, Pullman, WA. The accessions were of diverse origin and originated from 57 countries as well as unknown locations (Fig. 1) (16). In addition to *P. sativum*, which made up 93% of accessions, several subspecies of *P. sativum* were included (Table 1).

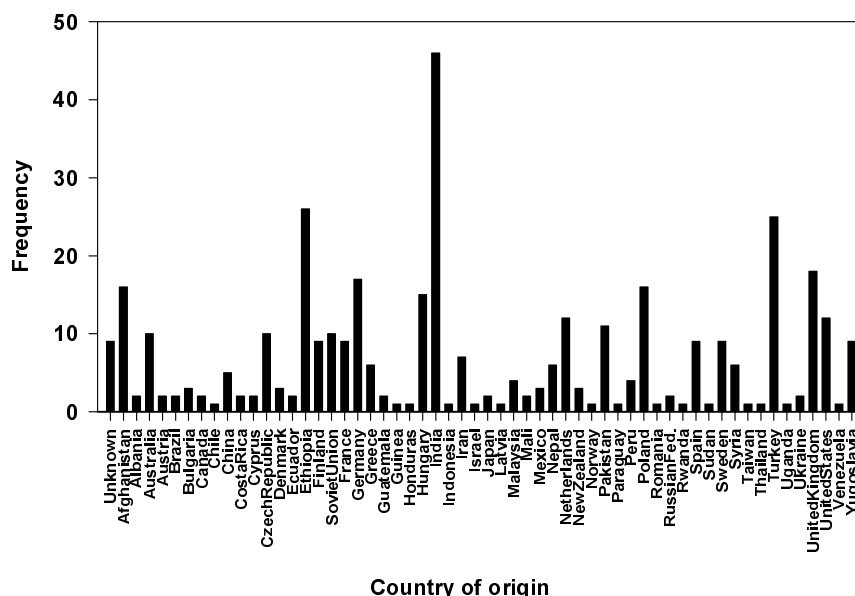


Fig. 1. Histogram of number of accessions from a subsample of the *Pisum* core collection evaluated by country of origin. Nine accessions were of unknown origin.

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**Inoculum.** Isolates of *F. solani* f. sp. *pisi* for these experiments were maintained in 10 g of a 1:1:1 soil:peat moss:perlite mixture that was crushed, mixed, and autoclaved in test tubes and stored at 5°C (17). A conidial suspension from a heavily sporulating, fresh culture in 2 ml of Kerr's medium (2) was delivered to soil mix and air dried to induce chlamydospore formation.

For each series of greenhouse experiments, the same five isolates were reiso-

lated on peptone-pentachloronitrobenzene (peptone-PCNB) agar by sprinkling a few soil grains on the agar (14). Individual 250-ml Erlenmeyer flasks containing 120 ml of Kerr's medium were inoculated with a 2-mm agar plug of the three best-growing isolates randomly selected from the five isolates grown on peptone-PCNB. Flasks were placed on an orbital shaker under 24-h light at room temperature for 6 days. Conidia were collected by straining cul-

tures through sterile cheesecloth. Strained suspensions of conidia for each of the three isolates of *F. solani* were combined and concentration of conidia was adjusted to  $10^6$  spores  $ml^{-1}$ . Inoculum was kept chilled (4°C) until use later the same morning.

**Greenhouse experiments.** Seed of a few accessions had to be scarified using a razor blade. Seed of each accession was inoculated by soaking seed in 50 to 60 ml of a conidial suspension in 100-ml beakers at room temperature overnight. Ten seed of each accession were planted after inoculation in single rows in plastic trays (27.3 × 51.1 × 6.4 cm [10 3/4 by 20 1/8 by 2 1/2 in.]; Landmarks, Akron, OH) with perlite in two replications per experiment. Three accessions were planted per tray. Two independent experiments were conducted in 1996 and 2001. Cv. Dark Skin Perfection was the susceptible control for each experiment. Plants were harvested after 20 days and roots were scored on a 0-to-5 scale, where 0 = no symptoms; 1 = slight hypocotyl lesions; 2 = lesions coalescing around epi- and hypocotyl; 3 = lesions starting to spread into the root system, with root tips starting to be infected; 4 = epicotyl, hypocotyl, and root system almost completely infected and only slight amount of white, uninfected tissue left; and 5 = completely infected root (5).

**Data analysis.** Greenhouse experiments followed a completely randomized design with two replications per accession. A mean disease severity score was given as an average over all 10 seedlings planted for each replication. Nonparametric statistics were used for data analyses because disease severity data were nonnormally distributed. To assess whether accessions evaluated in independent experiments resulted in the same ranking of accessions (i.e., no significant differences of median disease severity by accession among the two experiments), a nonparametric analysis of variance (ANOVA) based on ranks was conducted (1): first, ranks were assigned to all observations using PROC RANK (SAS User's Guide: Statistics; SAS Institute, Cary, NC) and, second, ranks were subjected to ANOVA using PROC ANOVA. Selections for the best accessions were arbitrarily made at a disease severity rating less than or equal to 2.5.

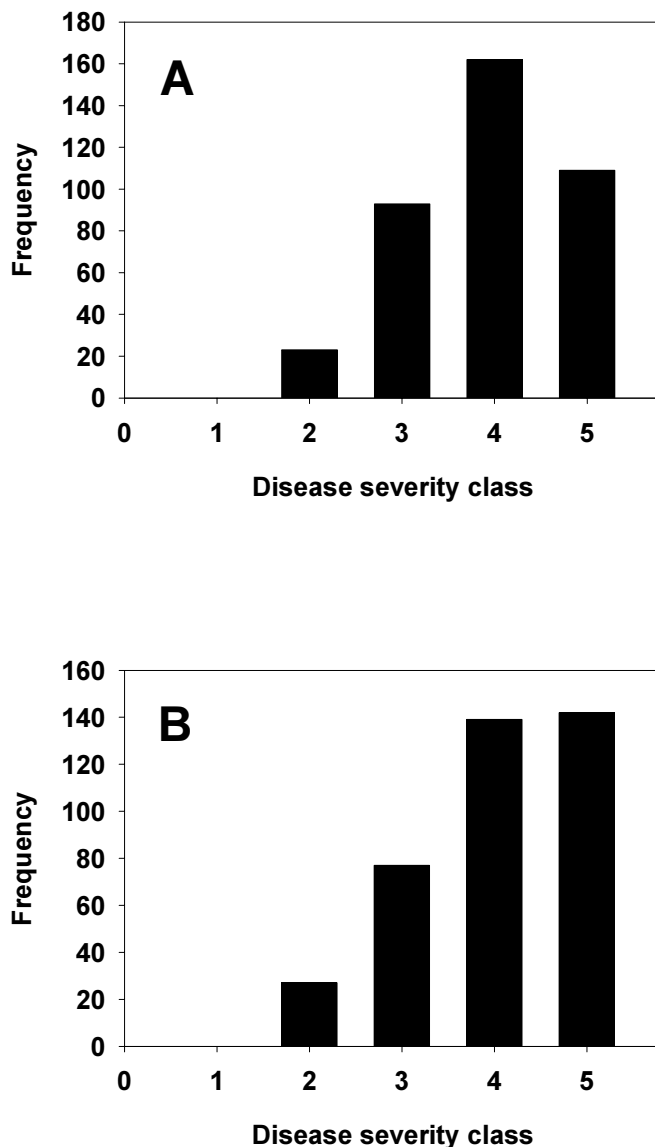
To assess whether resistance to *Aphanomyces* root rot and *Fusarium* root rot are related, data for *Aphanomyces* root rot submitted by D. K. Malvick to GRIN was obtained online (10). Downloaded data for *Aphanomyces* root rot is given in percent damage to plants. A Spearman rank correlation coefficient between *Aphanomyces* and *Fusarium* root rot scores was calculated using PROC FREQ.

## RESULTS

Two independent experiments were conducted to evaluate partial resistance and the results indicate that the two experi-

**Table 1.** Frequency of species in subsample of core collection evaluated for partial resistance to *Fusarium* root rot

Taxon	Frequency	Percent
<i>Pisum sativum</i>	361	93.3
<i>P. sativum</i> subsp. <i>abyssinicum</i>	4	1.0
<i>P. sativum</i> subsp. <i>elatius</i>	5	1.3
<i>P. sativum</i> subsp. <i>sativum</i>	15	3.9
<i>P. sativum</i> var. <i>arvense</i>	2	0.5
Total	387	100.0



**Fig. 2.** Histogram displaying the distribution of accessions in each disease severity class in **A**, first, and **B**, second experiment (n = 387). Disease severity was scored visually on a scale of 0 (= no disease) to 5 (= completely rotten) on uprooted and washed roots.

ments yielded qualitatively similar results. Nonparametric ANOVA conducted on ranks of disease severity for each accession rejected the hypothesis that there were significantly different rankings among accessions in the first and second experiment ( $P = 0.9447$ ).

Good levels of partial resistance to Fusarium root rot were identified, but disease severity scores for accessions ranged between 1.5 and 5, while most accessions had a disease severity score of 3 to 5 (Fig. 2). Forty-four accessions with very high partial resistance were identified in the *Pisum* core collection (Table 2). These accessions consistently scored below or equal to 2.5 on a 0-to-5 disease severity scale. Of the best accessions, 2, 7, 14, and 21 accessions scored a mean disease severity value of 1.75, 2, 2.25, and 2.5, respectively (Table 2). The standard deviation of severity on each of these accessions was

less than 1, except for three accessions (Table 2). Although most of the accessions presented in Table 2 were *P. sativum*, two were *P. sativum* subsp. *sativum* and one was *P. sativum* var. *arvense*. In general, accessions scoring below 3 were only found in *P. sativum*, *P. sativum* subsp. *sativum*, and *P. sativum* var. *arvense* (Fig. 3). The low number of accessions could confound this result for some of the taxa (Table 1).

Aphanomyces and Fusarium root rot data were significantly and positively correlated. The Spearman rank correlation was low at  $r_s = 0.25$  ( $Z = 4.9$ ;  $P < 0.001$ ), but a large dataset of 344 observations still resulted in a significant correlation.

## DISCUSSION

Several accessions of *P. sativum* with good levels of partial resistance to Fusarium root rot were identified. Forty-four accessions had a disease severity rating of

2.5 or less, with a standard deviation of less than or equal to 1.4. Only a few accessions from the PI collection previously evaluated were reevaluated in this project. Of the accessions shown in Table 2, PI164971 was previously scored as resistant, whereas PI166084, PI174921, and PI197990 were scored as 0.4, 1.5, and 3.0, respectively, on a 0-to-5 scale (3,4).

The methodology used for testing pea accessions for partial resistance to Fusarium root rot gave very repeatable results. This is supported by the good correspondence in rankings of accessions in the first and second experiment. A seed-soak inoculation method was used in this study and may have contributed to consistency of results. Inoculation procedures for other studies included growing test lines in infested soil (4) and spraying inoculum on seed at the time of planting (9). The methodology used in this study also was effi-

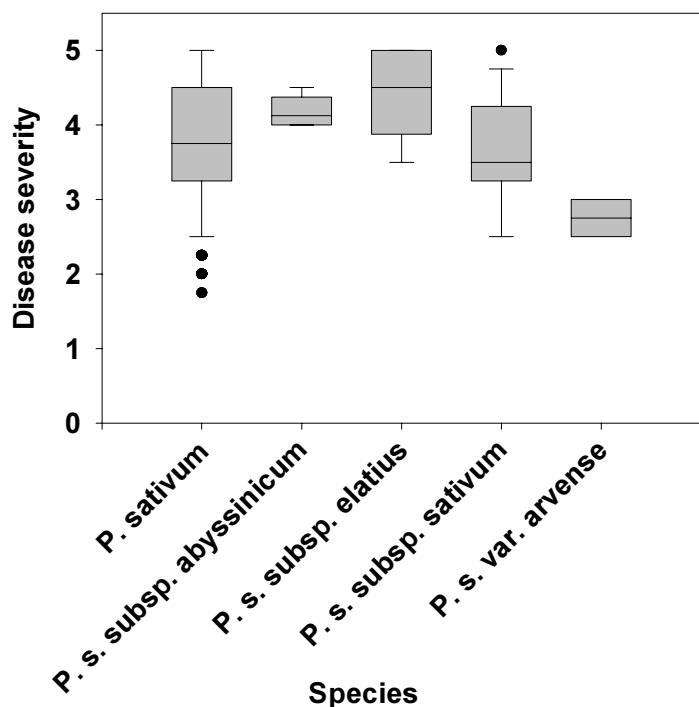
**Table 2.** Plant introduction (PI) accessions scoring less than or equal to 2.5 on a 0-to-5 disease severity scale<sup>a</sup>

Accession	Species	Origin	Flower color <sup>b</sup>	Seed pigmentation <sup>c</sup>	Mean disease severity	Standard deviation
PI203064	<i>P. sativum</i>	Finland	p	...	1.75	0.4
PI220174	<i>P. sativum</i>	Afghanistan	p	...	1.75	0.4
PI102888	<i>P. sativum</i>	...	p	m	2	0.0
PI125839	<i>P. sativum</i>	Afghanistan	p	m	2	0.7
PI195020	<i>P. sativum</i>	Ethiopia	p	m	2	0.0
PI198735	<i>P. sativum</i>	Afghanistan	m	m	2	0.0
PI207508	<i>P. sativum</i>	Afghanistan	p	m	2	0.0
PI220189	<i>P. sativum</i>	Afghanistan	p	...	2	0.0
PI222117	<i>P. sativum</i>	Afghanistan	p	...	2	0.0
PI121976	<i>P. sativum</i>	India	p	m	2.25	0.4
PI125840	<i>P. sativum</i>	Afghanistan	p	n	2.25	0.4
PI138945	<i>P. sativum</i>	Iran	p	m	2.25	0.4
PI175226	<i>P. sativum</i>	India	p	m	2.25	0.4
PI180693	<i>P. sativum</i>	Germany	p	m	2.25	0.4
PI180695	<i>P. sativum</i>	Germany	p	m	2.25	0.4
PI180702	<i>P. sativum</i>	Germany	p	n	2.25	1.1
PI184128	<i>P. sativum</i>	Yugoslavia	p	m	2.25	0.4
PI204306	<i>P. sativum</i>	Australia	p	...	2.25	0.4
PI219705	<i>P. sativum</i>	Pakistan	p	...	2.25	0.4
PI222071	<i>P. sativum</i>	Afghanistan	p	...	2.25	0.4
PI223526	<i>P. sativum</i>	Afghanistan	p	m	2.25	0.4
PI227457	<i>P. sativum</i>	Iran	p	m	2.25	0.4
PI413686	<i>P. sativum</i>	Hungary	p	m	2.25	0.4
PI116056	<i>P. sativum</i> subsp. <i>sativum</i>	India	p	m	2.5	0.0
PI116944	<i>P. sativum</i>	Afghanistan	p	m	2.5	0.0
PI163125	<i>P. sativum</i>	India	p	...	2.5	0.0
PI164612	<i>P. sativum</i>	India	m	...	2.5	0.7
PI166084	<i>P. sativum</i>	India	p	...	2.5	0.7
PI197450	<i>P. sativum</i>	Ethiopia	p	...	2.5	0.7
PI197990	<i>P. sativum</i>	Netherlands	p	...	2.5	0.7
PI215766	<i>P. sativum</i>	Peru	w	m	2.5	1.4
PI223527	<i>P. sativum</i>	Afghanistan	p	m	2.5	0.7
PI226561	<i>P. sativum</i>	Ethiopia	p	...	2.5	0.7
PI226564	<i>P. sativum</i>	Ethiopia	p/m	m	2.5	0.7
PI227258	<i>P. sativum</i>	Iran	p	m	2.5	0.0
PI244121	<i>P. sativum</i>	Netherlands	w	...	2.5	1.4
PI249645	<i>P. sativum</i>	India	p	...	2.5	0.7
PI251051	<i>P. sativum</i>	Yugoslavia	p/w	m	2.5	0.0
PI253968	<i>P. sativum</i>	Afghanistan	p	...	2.5	0.7
PI257592	<i>P. sativum</i>	Ethiopia	p	m	2.5	0.0
PI268480	<i>P. sativum</i> var. <i>arvense</i>	Afghanistan	p	...	2.5	0.0
PI271119	<i>P. sativum</i>	Mali	p	...	2.5	0.7
PI272194	<i>P. sativum</i>	Germany	p	...	2.5	0.0
PI505122	<i>P. sativum</i> subsp. <i>sativum</i>	Albania	p	m	2.5	0.7

<sup>a</sup> Data for origin, flower color, and seed pigmentation were obtained from GRIN.

<sup>b</sup> Abbreviations: m = mixed, p = pigmented, and w = white flower color; p/m = GRIN reports both pigmented and mixed score for flower color; p/w = GRIN reports both pigmented and white score for flower color.

<sup>c</sup> ... = Data not available in GRIN; m = mixed, n = nonpigmented green/white seed coat.



**Fig. 3.** Box plots of disease severity by subspecies of *Pisum sativum* evaluated for partial resistance to Fusarium root rot. The rectangles show the values below which 25% (lower side of box), 50% (the center line), and 75% (upper side of box) of the observations fall. The whiskers extending below and above the box represent the 5th and 95th percentiles, respectively. Dots represent outlying data points located beyond the 5th or 95th percentile.

cient in terms of cost and labor requirements and can be recommended for use in breeding programs.

Prior research indicated that PI accessions found to be partially resistant against Fusarium root rot were lavender-flowered with pigmented seed (4). In the current study, two accessions found to be resistant had white (PI215766 and PI244121) and two had mixed (PI198735 and PI164612) flower color (Table 2). Two additional accessions, PI226564 and PI251051, were reported in GRIN with a mixed score as either being pigmented/mixed or pigmented/white in flower color (Table 2). Two accessions found to be resistant had a non-pigmented white/green seed coat (PI125840 and PI180702). Thus, this study provides evidence that partial resistance to Fusarium root rot might not be linked to pigmented flower color and pigmented seed coats.

Comparison of disease data for Aphanomyces root rot (10) and Fusarium root rot showed a weak, positive correlation that was significant, primarily due to the large dataset (344 observations). Resistance to both diseases is likely based on several

genes or single genes (QTLs) conferring quantitative resistance (9). Several QTLs for resistance to Aphanomyces root rot recently have been identified (15). A previous report indicated that resistance to *Pythium ultimum* and *F. solani* may be conditioned by the same multiple, genetic factors (13). The Spearman rank correlation of  $r = 0.25$  between Aphanomyces and Fusarium root rots indicates that there is a relationship, but that it is not very strong.

This study identified 44 accessions in the *Pisum* core collection that show good partial resistance against Fusarium root rot. A complete listing of the data for the partial resistance of all accessions tested can be found at the National Plant Germplasm System website, USDA-ARS.

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