

Identification of *Pisum sativum* Germ Plasm with Resistance to Root Rot Caused by Multiple Strains of *Aphanomyces euteiches*

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ABSTRACT

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Aphanomyces root rot is a serious disease of pea (*Pisum sativum*), and additional sources of resistance are needed for development of disease-resistant cultivars. Accessions ($n = 123$) from the *P. sativum* Plant Introduction (PI) collection with the highest relative levels of resistance to one strain of *Aphanomyces euteiches* were previously identified from among approximately 2,500 accessions evaluated. The chosen 123 accessions were evaluated in this study for resistance to root rot caused by multiple strains of this pathogen. Five strains representing different US geographical locations and pathogenicity characteristics were used to evaluate pea seedlings in a greenhouse. Disease severity (DS) and percent loss of fresh biomass (inoculated vs. non-inoculated plants) were determined 15 days after inoculation. Significant differences ($P = 0.05$) in levels of DS and biomass loss (BL) occurred among the accessions after inoculation individually with the five strains. The relative rank of accessions based on DS and BL varied with the strain of *A. euteiches* used for inoculations. The 20 accessions with the lowest DS after inoculation with each strain were identified. Based on lowest DS, two accessions were among the 20 identified with all five individual strains, and four other accessions were among the 20 identified with four of the five strains. The results suggest that the *P. sativum* PI collection contains useful accessions for breeding programs aimed at developing pea varieties with resistance to *A. euteiches*.

Additional keywords: legume, Oomycota, pea

Pea (*Pisum sativum* L.) is widely grown throughout the world. In the United States, pea production for the canning and freezing industry is focused in the Midwest and Pacific Northwest. Disease can be a severe limiting factor to production in all areas where peas are cultivated. One serious disease of pea is *Aphanomyces* root rot caused by *Aphanomyces euteiches* Drechs. (6,8,16). *A. euteiches* is a soilborne organism classified in Kingdom Chromista, Division Oomycota. (15). *Aphanomyces* root rot occurs in many geographical regions, including Europe, Australia, Japan, Canada, and the United States (16). Persson et al. (17) reported that *A. euteiches* was the most yield-reducing pathogen of pea in Denmark and southern

Sweden. Average annual yield losses of pea due to *A. euteiches* are estimated at 10% in the Midwestern United States (6). Entire fields, however, are frequently destroyed during wet years.

Aphanomyces root rot has remained a destructive disease of pea for over 70 years (8) due to ineffective disease control methods. Crop rotation, host resistance, fungicides, and biological control agents have been investigated to manage this disease (6,16). These tactics have shown promise, but have not provided an adequate level of control for commercial production. Disease is reduced primarily through crop rotation and avoidance of fields highly infested with *A. euteiches*. Varieties with resistance to *Aphanomyces* root rot may be a key component in an integrated strategy for disease control. Several pea breeding lines have been developed that express resistance to *Aphanomyces* root rot (2,5,9); however, their resistance traits have not been incorporated into commercial cultivars.

Aphanomyces root rot resistance in pea has been difficult to achieve due to challenges in identifying and incorporating useful disease resistance traits, and because of pathogenic variability in populations of *A. euteiches* (1,4,11,13). Variability within populations of *A. euteiches* may cause inconsistent performance of disease-resistant pea lines in the field, and could influ-

ence the durability and effectiveness of resistance in different geographical areas (4,11,13,19). Breeding for resistance to *Aphanomyces* root rot may be enhanced if additional resistance traits that are effective against a variety of distinct strains of *A. euteiches* are identified. Few disease resistance genes in pea appear to be available to breeders. The United States Department of Agriculture (USDA) *P. sativum* Plant Introduction (PI) collection may be a source of resistance genes. The first extensive study of *Aphanomyces* root rot resistance in pea accessions was published in 1960 (10). In a recent study, 2,500 accessions, comprising most of the PI collection, were screened for resistance to *Aphanomyces* root rot using one isolate of *A. euteiches* (12).

The aim of this study was to identify the best sources of resistance to *Aphanomyces* root rot in the *P. sativum* PI collection. The 123 accessions evaluated in this study were previously identified to be those with the highest relative level of disease resistance in the PI collection (12). Our primary objective was to determine how the 123 accessions interact with five strains of *A. euteiches* that represent different pathogenicity characteristics and geographical areas.

MATERIALS AND METHODS

Strains of *A. euteiches*. Five strains of *A. euteiches* (Table 1) from different geographical areas with differing pathogenicity characteristics on pea, alfalfa, and bean were included in this study (11,13). Strains were characterized as *A. euteiches* according to the keys of Dick (3) and Scott (18). Strains of *A. euteiches* were isolated from soil by baiting with *P. sativum* seedlings (cv. Perfection 8221), and single zoospore cultures were prepared as described previously (11). All strains used for inoculations except Ae467 originated from single zoospore cysts. Cultures were maintained on corn meal agar (Difco Laboratories, Detroit) and stored at 4°C.

***P. sativum* accessions evaluated.** *P. sativum* accessions ($n = 123$) from the USDA Western Regional Plant Introduction Station in Pullman, Washington were evaluated (Table 2). These represent the best 5% of all available accessions based on percent loss of fresh biomass (inoculated vs. noninoculated plants) after inoculation with strain Ae467 of *A. euteiches* (12). Additional information on

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these and other *P. sativum* accessions is available in the USDA Germplasm Resources Information Network.

Pathogenicity studies. The 123 *P. sativum* accessions were inoculated separately with the five strains of *A. euteiches*. Pea cultivar Little Marvel was the susceptible control, and breeding lines MN313, MN314, and WI8904 were included as resistant controls (2,5). Waxed paper cups

(160 ml) containing vermiculite were sown with three or four seeds. Plants were grown in a greenhouse with a 12-h photoperiod and fertilized with 0.5× Hoagland's solution (7) 1 week after inoculation. Temperature was maintained at 23 ± 2°C. Zoospores were produced as described by Mitchell and Yang (14), counted with a hemacytometer, and adjusted to 500 zoospores/ml. The inoculum (5 ml) was in-

jected approximately 1 cm below the vermiculite surface next to seedlings 7 days after planting. Vermiculite in cups was saturated at time of inoculation and watered thereafter as needed to keep vermiculite moist. At 15 days after inoculation, the plants were washed and disease severity (DS) and fresh biomass were determined. DS was based on a 0 to 5 scale, where 0 = no macroscopic symptoms; 1 = a few small discolored lesions on roots; 2 = minor discoloration covering less than 90% of the root system; 3 = over 90% of root system brown or yellowed, but no symptoms on epicotyl or hypocotyl; 4 = all of root system brown or soft and epicotyl or hypocotyl shriveled or brown; and 5 = plant dead (2). Percent loss of biomass was based on fresh biomass of equal numbers of inoculated and noninoculated plants for each accession. Three replicate cups were used for noninoculated controls and for each combination of strain and plant host. The experiment was organized as a completely randomized design. The experiment was repeated once. DS and biomass loss (BL) values among all accessions inoculated with individual strains were subjected to analysis of variance and means were compared with Fisher's protected least significant difference test (SAS software, SAS Institute, Inc., Cary, NC).

RESULTS

Significant differences in resistance to *Aphanomyces* root rot were identified among the 123 accessions tested, although most were clearly susceptible. Over 88% of the 123 *P. sativum* accessions were susceptible (DS > 2.9) to each of the five strains of *A. euteiches* (Table 3). Fresh BL of most (75%) accessions was >30% after inoculation with each strain (*data not shown*). The DS and percent BL of accessions differed significantly ($P = 0.05$) after inoculation with the five strains. All 123 accessions were ranked from low to high DS and BL based on the five individual strains and the average across all strains. The 20 accessions with the lowest mean DS based on inoculation with each strain in two replicate experiments are listed in Table 4. The 20 accessions with the lowest DS and BL based on the mean DS and BL values from all strains combined in both experiments are shown in Table 5. Approximately 50% of the top 20 accessions were significantly better than the highly susceptible cultivar Little Marvel and the moderately resistant breeding line WI8904. The highly resistant breeding lines MN313 and MN314 expressed lower DS and BL than at least 50% of the top 20 accessions. The noninoculated control plants remained free of root rot symptoms in all replications and experiments.

The *P. sativum* accessions reacted differently with the five strains of *A. euteiches*. Individual strains caused significantly different ($P = 0.05$) DS and BL on all acces-

Table 1. Strains of *Aphanomyces euteiches* used for evaluation of resistance to *Aphanomyces* root rot in pea

Strain	Geographical origin	Pathogenicity to five pea germ plasm lines ^{a,b}				
		MN313	MN314	90-2079	WI8904	Little Marvel
Ae467	Wisconsin	-	-	-	+	+
AeMM183 (1)	Minnesota	-	-	-	-	+
AeMM62 (3)	Minnesota	-	-	+	+	+
AeOR5 (2)	Oregon	+	+	+	+	+
P134 (1)	Wisconsin	+	+	+	+	+

^a + denotes the strain incites disease severity >3 on a particular host based on severity of root infection, where 0 = a healthy plant with no symptoms and 5 = a dead plant.

^b MN313 and MN314 are resistant pea breeding lines (2); 90-2079 is a moderately resistant breeding line (9); WI8904 is a moderately resistant breeding line (5); and Little Marvel is a susceptible cultivar.

Table 2. *Pisum sativum* PI accessions evaluated for resistance against five strains of *Aphanomyces euteiches*

Accession	Accession	Accession	Accession	Accession
102887	196015	261613	347410	471190
116056	196023	269794	356982	471195
116944	206798	269802	357003	471314
143486	206811	272215	357006	471331
162909	206816	272216	357030	471336
164396	206824	272217	358612	471354
164669	210613	273681	358627	471357
165965	210641	275639	358630	471387
169600	210642	280608	358640	471388
170669	210678	286607	358667	471409
173058	210684	306592	358679	471410
174321	226562	312200	358693	471438
176721	244140	314798	365424	494077
179019	244141	320973	390795	494506
180471	244144	324702	390808	494508
181800	244151	324705	393487	494510
183910	244154	343267	411143	494511
185183	244158	343293	413686	494515
193579	244160	343958	413696	505080
193586	244162	343984	413703	505102
193589	244163	347284	429843	505133
193835	244184	347299	471114	512078
193846	244226	347301	471123	512087
193849	244259	347304	471128	
195629	257593	347327	471166	

Table 3. Percentage of 123 Plant Introduction accessions tested within various disease severity (DS) and percent biomass loss (BL) classes after inoculation individually with five strains of *Aphanomyces euteiches*^a

Strain	DS class ^b		BL class (%) ^c	
	0-2.9	3.0-5.0	0-50	50-100
Ae467	8	92	52	48
AeMM183(1)	3	97	19	81
AeMM62(3)	2	98	9	91
AeOR5(2)	11	89	65	35
P134(1)	6	94	63	37

^a Results shown are from one of two experiments conducted; results were similar for both experiments.

^b DS is based on severity of root infection, where 0 = a healthy plant with no symptoms and 5 = a dead plant. DS > 3.0 indicates a clear pathogenic interaction.

^c Percent loss of biomass is based on fresh biomass of inoculated vs. noninoculated plants.

sions except 411143, 413696, and 512078. Results were similar for both experiments. The top 20 accessions ranked by lowest DS and BL differed for the five strains (Table 4). Two accessions appeared on all five of the ranked lists based on DS (244162 and 393487), and four (210641, 269802, 411143, and 413696) appeared on four lists. One accession (393487) appeared on five ranked lists based on BL, and two accessions (244226 and 413696) appeared on four lists. Many of the best 20 accessions identified with individual strains differed from the best 20 identified with averages of DS and BL across all strains.

DISCUSSION

The main purpose of this study was to identify the *P. sativum* accessions from the PI collection that are most likely to be useful as sources of resistance to *A. euteiches*. Increasing the levels of resistance in pea to *A. euteiches* may be the most desirable approach to control of *Aphanomyces* root rot. *P. sativum* accessions were identified that may have good potential for use in pea breeding programs designed to improve resistance to *Aphanomyces* root rot.

Most accessions from the 123 evaluated in the present study were highly susceptible to *Aphanomyces* root rot. Some accessions had significantly lower levels of DS, BL, or both than the susceptible cultivar Little Marvel. Some of the best accessions were rated comparable to the *Aphanomyces*

root rot-resistant pea breeding lines WI8904, MN313, and MN314 using the seedling test conducted in vermiculite under conditions that may favor the pathogen.

Performance of accessions may differ if they were allowed to grow over a longer time period, or were evaluated in soil under different environmental conditions.

Table 5. Accessions of *Pisum sativum* ($n = 20$) with the lowest mean disease severity (DS) and percent fresh biomass loss (BL) based on evaluation individually with five strains of *Aphanomyces euteiches*^a

Accession	DS ^b	Accession	BL (%) ^c
210641	2.1	244162	17
411143	2.4	269802	22
210642	2.5	393487	23
393487	2.5	413696	24
244162	2.6	390808	26
269802	2.8	471354	26
413696	2.9	343984	28
343984	3.0	210642	29
505102	3.2	210641	29
494506	3.2	244226	29
512078	3.2	411143	29
286607	3.2	365424	30
471354	3.3	185183	30
324702	3.3	196015	30
471190	3.3	471409	31
358679	3.3	471166	33
165965	3.4	343958	34
272217	3.4	320973	34
494508	3.4	358630	34
206824	3.4	210684	34
MN313	2.2	MN313	21
MN314	1.7	MN314	13
WI8904	3.8	WI8904	46
Little Marvel	4.5	Little Marvel	58

^a Results are the combined mean from all inoculations with five individual strains in two experiments.

^b DS is based on severity of root infection, where 0 = a healthy plant with no symptoms and 5 = a dead plant. DS > 3.0 indicates a clear pathogenic interaction.

^c Percent loss of biomass is based on fresh biomass of inoculated vs. noninoculated plants

Table 4. *Pisum sativum* accessions ($n = 20$ per strain) with the lowest mean disease severity (DS) ratings based on individual evaluations with five strains of *Aphanomyces euteiches*^a

Strain Ae467		Strain AeMM183 (1)		Strain AeMM62 (3)		Strain AeOR5 (2)		Strain P134 (1)	
Accession	DS ^b	Accession	DS	Accession	DS	Accession	DS	Accession	DS
210641	0.9	210641	0.2	210642	1.1	411143	0.1	393487	0.2
210642	0.4	411143	2.3	210641	0.9	413696	0.5	324705	4.0
286607	1.4	210642	0.9	244162	2.4	272217	1.1	411143	1.8
269802	2.2	244162	2.3	393487	1.8	471190	0.1	471354	2.4
343984	2.3	275639	0.5	471438	1.1	505102	0.8	494077	3.9
210613	3.3	269802	1.8	324702	1.5	471195	0.1	505080	1.6
494508	2.9	393487	2.3	269794	2.1	393487	3.2	413696	3.8
393487	2.7	494506	2.8	165965	3.0	244151	1.3	244184	2.4
244162	2.9	413696	1.7	471409	2.3	358693	0.3	505102	3.4
471410	2.2	343984	2.1	494510	2.8	269802	1.8	226562	3.2
273681	2.2	206824	2.1	471357	2.5	358679	1.3	244162	2.4
358679	2.1	343958	2.0	347284	2.6	173058	0.1	206798	3.3
164669	2.3	471357	2.5	269802	3.0	244163	0.2	356982	3.6
343598	2.8	505133	2.5	41143	2.0	494506	1.0	494510	2.8
471331	2.8	471123	1.8	413696	3.0	512078	NT ^c	324702	4.1
358667	2.9	244144	1.6	512078	3.0	471354	2.3	244141	2.4
471387	1.9	505102	2.3	273681	2.2	244162	2.0	210641	2.4
471409	2.2	320973	2.7	365424	2.6	165965	1.1	358693	2.2
244151	4.1	244226	2.4	181800	2.8	471410	0.8	471123	4.3
494515	2.8	494515	2.8	179019	3.2	206824	2.4	512078	3.3
MN313	1.6	MN313	1.9	MN313	1.1	MN313	3.6	MN313	2.2
MN314	1.1	MN314	0.3	MN314	1.3	MN314	2.0	MN314	3.6
WI8904	4.6	WI8904	4.8	WI8904	3.9	WI8904	3.5	WI8904	1.7
Little Marvel	4.4	Little Marvel	4.5	Little Marvel	4.7	Little Marvel	4.0	Little Marvel	4.1
LSD ^d	1.4	LSD	1.6	LSD	1.3	LSD	2.3	LSD	1.3

^a Rankings for individual strains are based on the mean of DS values from two experiments. The DS values shown are for one of the two experiments; however, results were similar for both experiments.

^b DS is based on severity of root infection, where 0 = a healthy plant with no symptoms and 5 = a dead plant.

^c NT = not tested in this experiment, but was tested in replicate experiment.

^d LSD = least significant difference.

Regardless of these experimental considerations, the results suggest that some of the accessions identified may have relatively good potential for inclusion into breeding programs.

Five strains of the pathogen were shown to interact differently with the accessions. This demonstrates that pathogen isolate and strain selection can significantly affect the response of germ plasm to *Aphanomyces* root rot. The *P. sativum* accessions expressed different DS and BL after inoculation with the five strains of *A. euteiches*. These results suggest that minimal incorporation of pathogenic variability into breeding programs due to use of single isolates or single nurseries may have slowed progress in breeding for resistance. The overall best accessions may be those that have the relatively lowest DS and BL after inoculation with each of several different strains. Alternatively, the best accessions for breeding programs may be identified by combining the results from evaluation individually with a set of strains, and then ranking the pooled mean results. Another approach would be to use an inoculum "cocktail" consisting of a mixture of strains with different pathogenicity characteristics.

Inoculation with different strains may reveal various sources of resistance to *Aphanomyces* root rot. Accessions with dissimilar DS or BL after inoculation may demonstrate distinct interactions with unknown virulence factors in the different strains. Little is known of the traits controlling resistance to *Aphanomyces* root rot in *P. sativum*, and even less is known about the virulence and pathogenicity factors that are important in *A. euteiches*. The use of different pea accessions and strains of *A. euteiches* may be useful in studies delving into these questions. These results also illustrate the potential existence of strains with different pathogenicity and virulence

characteristics in different geographical areas and, furthermore, a corresponding need for different sources of resistance. Various sources of resistance in pea to *Aphanomyces* root rot may not be effective or stable against predominant strains of *A. euteiches* present in different parts of the United States.

This study reveals challenges and promise in understanding and controlling *Aphanomyces* root rot of pea. Some accessions in the *P. sativum* PI collection appear to have useful traits for resistance to *A. euteiches*; however, much work is required to determine how useful the traits may be. Significant challenges remain in developing commercially acceptable pea cultivars with resistance to *A. euteiches*. Continued study of disease resistance traits available in *P. sativum* germ plasm and interactions between germ plasm and distinct strains may lead to the development of pea cultivars with improved resistance to *Aphanomyces* root rot.

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