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Resistance to Fusarium Wilt Race 2 in the *Pisum* Core Collection

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Abstract. Plant breeders must be aware of sources of resistance to pathogens that affect their crops. Fusarium wilt caused by *Fusarium oxysporum* Schl. f. sp. *pisi* Snyd. & Hans. is a fungal disease that affects peas and is important worldwide. Resistance to the different races of the pathogen has been identified in adapted germplasm and from specific accessions in the United States World Collection of peas (*Pisum sativum* L.). The goal of this study was to evaluate the resistance to fusarium wilt race 2 in the *Pisum* core collection. Of the 452 accessions screened, 62 (14%) were resistant. The resistant accessions included accessions from *P.s.* ssp. *elatius* that were collected from 24 different countries. The wide distribution of resistance around the world precludes the identification of any single country or region as a source of resistance. Of the 62 accessions resistant to race 2, 39 are also resistant to race 1 based on data obtained from GRIN. One of the wild progenitors, PI 344012, possessed resistance to races 1 and 2.

Germplasm collections are valuable sources of resistance to numerous plant pests. The value and use of cool season food legume germplasm collections as sources of desirable traits has been reviewed by several authors (Ali et al., 1994; Muehlbauer, 1992, Muehlbauer et al., 1994). Core collections, as subsets of germplasm collections, have been developed for the grain legumes including chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), and pea (*Pisum sativum* L.) specifically to aid researchers in identifying regions of the world where genes of interest may be found (Simon and Hannan, 1995).

Fusarium wilt, caused *by Fusarium oxysporum* Schl. f. sp. *pisi* Snyd. & Hans., is an important fungal disease of peas in most pea growing regions around the world. Six races have been described. Race 1 was discovered in Wisconsin in 1924 (Linford, 1928) and was later found in Washington, Idaho and New York (Wade et al., 1938). Soon after resistance to race 1 was incorporated into cultivars, a second race which overcame resistance to race 1 was discovered (Snyder and Walker, 1935). This second race, designated race 2, was called near wilt since symptoms became noticeable later in the season than for race 1. Races 1 and 2 were the only economically important wilt races in the United States until race 5 appeared in northwestern Washington in 1963 (Haglund and Kraft, 1970). Races 1 and 2 are known to occur throughout the world, while races 5 and 6 are only important in western Washington State (Haglund and Kraft, 1979). Race 3 is present in Europe and England while race 4 is found in Canada (Hagedorn, 1984).

Near wilt is an increasing problem in southwestern Washington and northeastern Oregon. The disease is nearly always associated with short crop rotations, root rots and nematode damage. Secondary cortical decay often occurs with race 2 of fusarium wilt when other fungal diseases invade the root through existing wounds (Hagedorn, 1984).

Genetic resistance to races 1, 2, 5, and 6 is conferred by different single dominant genes (Hagedorn, 1984) and is available in numerous germplasm releases (Haglund and Anderson, 1987; Kraft and Giles, 1976; Kraft and Tuck, 1986). Resistance to race 2 was first discovered in an adapted breeding line in 1945 by researchers in Wisconsin (Hare et al., 1949). A selection from this line was later released as Delwiche Commando (Canner, 1945).

Genetic resistance is often concentrated in certain groups of germplasm that originate from a specific region in the world. It is valuable to understand where these regions are located such that the accessions from those areas can be fully exploited for their resistance. The goals of the current study were to 1) identify individual accessions in the *Pisum* core collection that possess resistance to race 2 of fusarium wilt and 2) determine if resistance was concentrated in a specific region of the world.

Materials and Methods

The *Pisum* core collection is composed of 504 accessions from 60 countries. However, due to insufficient quantities of seed, only 452 accessions were available for screening at the time of this study. Seed for each of the accessions were received from the USDA–ARS, Regional Plant Introduction Station (RPIS), Pullman, Wash. Two subspecies of *Pisum sativum* are represented in the core collection: *P.s.* ssp. *abyssinicum* A. Br. (8 accessions) and *P.s.* ssp. e*latius* Bieb. (13 accessions) (Table 1).

Ten to twelve seeds of each of the accessions were planted in trays containing sterile coarse perlite. Dark Skin Perfection, Little Marvel, and New Season, all having known reactions to race 2 (Hagedorn, 1984; Haglund and Anderson, 1987), were used as controls with each set of lines tested. Plants with three or four nodes were carefully removed from the perlite and submerged in a spore suspension for 5 min. While submerged in the spore suspension, about 1/4 to 1/5 of the basal roots on each seedling were removed with scissors. The plants were inoculated and replanted into the perlite and watered with Peter's solution (15–10–30) diluted to 1 $mg \cdot L^{-1}$ nitrogen (Scott's-Sierra Horticultural Products Co., Marysville, Ohio) plus micronutrients (Peter's Fertilizer Products, W.R. Grace and Co., Allentown, Pa.) daily. Air temperature in the greenhouse was 24 to 28 °C. All accessions were screened in two replications. The plants were scored for the disease reaction 3 and 6 weeks after inoculation.

The primary inoculum was derived from a single spore culture of *Fusarium oxysporum* f. sp. *pisi* race 2. Cultures were grown in a liquid media as described in Haglund (1989), Bhatti (1990), and

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Table 1. Distribution of resistance to fusarium wilt race 2 among accessions in the *Pisum* core collection.

Genus	Species	Subspecies	Accession (no.)	Reaction to race 2		
				Resistant	Mixed	Susceptible
Pisum	*sativum*	*abyssinicum*	8	0	7	1
Pisum	*sativum*	*elatius*	13	2	8	3
Pisum	*sativum*		431	60	231	140
Total			452	62	246	144

Tullu (1996). Conidial concentration was adjusted to 1×10^6 spores/mL as measured by a hemacytometer.

After disease development the number of healthy plants and the total number of plants inoculated were counted. Accessions with >90% of the plants showing no visible symptoms such as stunting, discoloration of the growing tip, or chlorosis and death of alternate stipules, were classified as resistant. Accessions with 20% to 89% asymptomatic plants were classified as mixtures. Accessions with <20% asymptomatic plants were classified as susceptible. The average of both replications were used to classify the accessions into resistant, mixed, and susceptible groups.

Data for resistance to fusarium wilt race 1 and tolerance to aphanomyces root rot for the accessions in the *Pisum* core collection was obtained from the Germplasm Resources Information Network (GRIN) (http:/www.ars-grin.gov/npgs/). This information was combined with the data for resistance to race 2 to evaluate if an association existed between resistance to races 1 and 2 as well as between resistance to race 2 and tolerance to aphanomyces root rot caused by *Aphanomyces euteiches* f. sp. *pisi* Drechs.

Results

The response of the three differential check lines—Dark Skin Perfection, Little Marvel, and New Season—to infection with fusarium wilt race 2 was as expected (Hagedorn, 1984). Dark Skin Perfection, which is resistant to race 1 but susceptible to race 2, had an average of 20% survival. Little Marvel, which is susceptible to both races, had an average survival rate of 23%; while New Season, which is resistant to both races, had an average survival rate of 80%. The lack of complete susceptibility or resistance in the differential lines may have come about via escaping infection in the case of the susceptible lines or, in the case of the resistant lines, death may have been due to infection by other fungi or from excessive loss of roots during inoculation. The classification of the accessions into the three categories was relatively conservative because only those accessions with fewer symptomatic plants than the resistant checks were classified as resistant and those with equal or fewer asymptomatic plants were classified as susceptible.

Of the 452 accessions, 62 (14%) were classified as resistant, 246 (54%) as mixtures of asymptomatic and symptomatic plants, and 144 (32%) as susceptible (Table 1). *Pisum sativum* ssp. *elatius* and *P.s.* ssp. *abyssinicum* are commonly regarded as the wild progenitors of common pea. Only 2 of the 21 wild subspecies accessions (PI 344010 and PI 344012) showed resistance to race 2 and both were from *P.s.* ssp. *elatius*. PI 344012, collected in Greece in 1969, was also resistant to race 1 (Table 2).

The large number of accessions (54%) which showed a mixed reaction to race 2 was not unexpected given the nature of how the plant introduction accessions are collected. Many of the accessions are landraces (natural mixtures) or mixtures of seed which are grown by farmers in many of the developing countries.

Data for resistance to race 1 of fusarium wilt was obtained from GRIN and compared to the data on resistance to race 2. Of the 62 accessions resistant to race 2, 39 were also resistant to race 1, 13 were resistant to race 2 but showed a mixed reaction to race 1, and 10 with resistance to race 2 were susceptible to race 1.

No association was detected between resistance to race 2 and tolerance to aphanomyces root rot. Only 387 accessions could be compared for an association between race 2 and aphanomyces root

Table 2. PI accessions of pea which show resistance to races 1 and 2 of fusarium wilt.

Accession	Country of origin	Wilt reaction	
		Race 1	Race 2[z]
PI 117264	Turkey	R	100
PI 121976	India	R	100
PI 125672	Austria	R	95
PI 163129	India	R	96
PI 164614	India	R	90
PI 166084	India	R	100
PI 179451	Syria	R	94
PI 179459	Turkey	R	90
PI 180693	Germany	R	100
PI 180699	Germany	R	90
PI 181958	Syria	R	100
PI 184130	Yugoslavia	R	90
PI 210561	Soviet Union	R	95
PI 244150	Netherlands	R	90
PI 261677	Netherlands	R	100
PI 269777	England	R	96
PI 269804	England	R	100
PI 269816	England	R	100
PI 269825	England	R	100
PI 271121	Germany	R	100
PI 272175	Germany	R	100
PI 274308	Pakistan	R	100
PI 280617	Estonia	R	100
PI 285718	Poland	R	95
PI 285719	Poland	R	96
PI 286430	Nepal	R	90
PI 314803	Australia	R	100
PI 324706	Romania	R	100
PI 343958	Turkey	R	91
PI 343968	Turkey	R	100
PI 343988	Turkey	R	93
PI 344012[y]	Greece	R	100
PI 347457	India	R	90
PI 347496	India	R	95
PI 356984	India	R	95
PI 356986	India	R	90
PI 390795	Peru	R	90
PI 409031	Germany	R	95
PI 505111	Syria	R	100

[z]Reaction to race 2 is the percent of plants with resistance.
[y]This accession is from the subspecies *Pisum sativum* ssp. *elatius*.

Table 3. Distribution of resistance (R = resistant, S = susceptible) to fusarium wilt race 2 in the *Pisum* core collection by country.

Country	Accession (no.)	R/mixed/S	Country	Accession (no.)	R/mixed/S
Afghanistan	16	5/2/9	Albania	2	0/2/0
Australia	10	2/3/5	Austria	2	1/1/0
Brazil	2	1/1/0	Bulgaria	3	0/2/1
Canada	4	0/1/3	Chile	1	0/1/0
China	9	0/8/1	Costa Rica	2	0/2/0
Cyprus	2	0/1/1	Czechoslovakia	13	1/6/6
Denmark	4	0/3/1	Ecuador	2	0/2/0
Estonia	1	1/0/0	Ethiopia	38	0/26/12
Finland	9	0/7/2	France	10	1/3/6
Germany	21	7/7/7	Greece	11	2/6/3
Guatemala	3	0/3/0	Guinea	1	0/0/1
Honduras	1	0/0/1	Hungary	19	0/6/13
India	50	8/33/9	Indonesia	1	0/1/0
Iran	9	0/7/2	Iraq	1	0/1/0
Israel	8	0/6/2	Japan	2	0/1/1
Latvia	1	0/0/1	Lebanon	1	0/1/0
Malaysia	4	0/3/1	Mali	2	0/2/0
Mexico	5	0/4/1	Nepal	7	1/5/1
Netherlands	14	2/6/6	New Zealand	5	0/1/4
Nigeria	1	0/1/0	Norway	1	0/1/0
Pakistan	11	3/7/1	Paraguay	1	0/1/0
Peru	5	1/2/2	Poland	17	4/5/8
Romania	1	1/0/0	Rwanda	1	0/0/1
Spain	10	0/3/7	Soviet Union	15	1/5/9
Sudan	1	0/0/1	Sweden	11	1/6/4
Syria	7	3/3/1	Taiwan	1	0/1/0
Thailand	1	0/1/0	Turkey	28	8/16/4
Uganda	1	1/0/0	Ukraine	2	0/0/2
United Kingdom	19	5/11/3	United States	12	1/5/6
Venezuela	1	0/1/0	Yugoslavia	9	1/5/3
Totals	231	40/124/68	Totals	221	22/122/76

rot due to incomplete data in GRIN. The average root damage caused by *Aphanomyces* for those lines resistant to race 2 was 69.2%, while those lines with either a mixed or susceptible reaction to race 2 had an average root damage of 67.9%.

Discussion

An understanding of the improvement status (cultivated type, cultivar, landrace, wild progenitor, etc.) of the resistant PI accessions would lend insight to the source and origin of resistance. Unfortunately, the improvement status of only half the resistant accessions could be determined with reasonable confidence because of insufficient reports left from the original collection. Of the 62 accessions resistant to race 2, 11 were cultivars; 15 were cultivated types; 2 were from the subspecies *P.s.* ssp. *elatius*, PI 344010, and PI 344012; 1 was a genetic line from Lamprect (#368); and 1 was a cross between *P.s.* ssp. *elatius* and 'Clamart', a breeding line in England. The improvement status of the remaining 32 accessions is unknown. Due to the large number of accessions with unknown improvement status and the fact that resistance was found in accessions which certainly differ in the degree to which they were developed, it is difficult to draw any conclusions regarding the origin of resistance to fusarium wilt race 2.

No specific region of the world could be identified as a source of resistance to race 2 of fusarium wilt since at least one accession from 24 of the 60 countries represented in the core collection showed resistance (Table 3). However, 33 of the 62 resistant accessions were obtained from five countries: Turkey (8), India (8), Germany (7), Afghanistan (5), and the United Kingdom (5) (Table 3). The large number of accessions classified as mixed in the collection is evident of the heterogeneous nature of germplasm accessions, particularly landraces and wild accessions. Each individual would be expected to be homozygous, however, mixtures of homozygous individuals result in heterogeneous populations. Many of the accessions show phenotypic variability and certainly contain genetic variability. That only 54% of the accessions were classified as mixtures is realistic and one could expect that the percentage would be higher.

The presence of all possible combinations of resistance and susceptibility to races 1 and 2 within the *Pisum* core collection was not unexpected since there is a loose association between the two loci (Grajal-Martin and Muehlbauer, 1992; Wade, 1929; Wells et al., 1949). Resistance to each of the four races of fusarium wilt found in the United States is conferred by different single dominant

Table 4. Summary of disease reactions for 407 PI accessions from the *Pisum* core collection to fusarium wilt races 1 and 2.

Race 2 reaction	Race 1 reaction			Total
	Resistant	Mixed	Susceptible	
Resistant	39	13	10	62
Mixed	127	53	37	217
Susceptible	77	16	35	128
Total	243	72	82	407

genes (Hagedorn, 1984; Muehlbauer, 1992). The gene for resistance to race 1 (*Fw*) is located on the *Pisum* linkage group four, 30 map units from the *Le* gene (Wade, 1929). More recent evidence indicates that placement of *Fw* on linkage group four may not be correct (N. Weeden, personal communication). Grajal-Martin and Muehlbauer (1992) studied the linkage between resistance genes for race 1 (*Fw*) and 2 (*Fnw*) and reported a recombination frequency of 46% indicating independent assortment. Wells et al. (1949) also reported a loose linkage between the two loci with a recombination frequency of 40%.

It has been hypothesized that the genetic resistance for common root rot, caused by *Aphanomyces euteiches* f.sp. *pisi*, and fusarium wilt may be linked (J. Kraft, personal communication). The data for resistance to common root rot was obtained from the Genetic Resources Information Network (GRIN) and combined with the data on resistance to fusarium wilt race 2. Based on the combined data, there does not appear to be any association for the lines included in the *Pisum* core collection. Overall resistance/tolerance to aphanomyces root rot is quite low in the core collection. Only one accession showing resistance to fusarium wilt race 2 can be considered tolerant to aphanomyces root rot with a root damage score <50% while two accessions showing a mixed or susceptible reaction to fusarium wilt race 2 could be considered tolerant to aphanomyces root rot. Therefore, with a nearly equal and low number of PI accessions showing resistance to both organisms and resistance to one and susceptibility to the other, it can not be concluded whether there is a direct linkage or association between the resistance genes.

This study identified 62 accessions in the *Pisum* core collection which were resistant to fusarium wilt race 2. Of these accessions, 39 were also resistant to race 1 and are listed in Table 2. A complete listing of the data for the resistant accessions and the remaining accessions tested can be found at the GRIN website (http:/www.ars-grin.gov/npgs/). The resistant germplasm identified in this study will be of direct use to breeders in the development of cultivars resistant to fusarium wilt race 2.

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