Resistance in *Pisum sativum* to Epicotyl Rot Caused by *Rhizoctonia solani*

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ABSTRACT

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Resistance to epicotyl rot caused by *Rhizoctonia solani* was correlated with epicotyl thickness among 20 pea lines tested when peas were exposed to 20 sclerotia per gram of soil ($r = -0.91$) or when epicotyls were inoculated directly with mycelial disks ($r = -0.88$). Severity of epicotyl rot could be predicted by measuring the epicotyl diameter of uninoculated 9-day-old seedlings, using the regression equation obtained from the original 20 test lines. Resistance to epicotyl rot was not related to anthocyanin pigmentation of the seed coat or rate of seedling emergence. The rate of tissue destruction caused by *R. solani* was also found to vary among pea lines. Of four fungicides applied separately as a slurry, only chloroneb adequately protected the cotyledons, epicotyl, and hypocotyl of Dark Skin Perfection from colonization by germinating sclerotia of *R. solani*.

Rhizoctonia solani Kühn (*Thanatephorus cucumeris* (Frank) Donk) is the cause of cotyledon rot, tip blight, and epicotyl rot of peas (*Pisum sativum* L.) (2). Various anatomical and biochemical factors have been associated with resistance to *R. solani* in beans (*Phaseolus vulgaris* L.), but comparable research has not been conducted with peas.

Fungistatic compounds and anatomical differences of seeds have been associated with resistance in beans to *R. solani* (1,8–10). In peas, only plant introduction accessions with anthocyanin-pigmented seed coats were reported resistant to both seed and epicotyl rot caused by *R. solani* (13), although a few pea lines lacking anthocyanin-pigmented seed coats have been reported to possess low levels of resistance to *R. solani* (12). Kraft (3) found that pea lines with the *A* gene for anthocyanin-pigmented seed coats, resistant or susceptible to *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyd. & Hans., contained the fungistatic anthocyanin pigment delphinidin in equal amounts. The fungistatic effect of the delphinidin was overcome if sufficient sugars were exuded during imbibition and germination.

Relatively large, robust bean seedlings with thick stems and fibrous or woody hypocotyls have been reported to be more resistant to *R. solani* (7–9). It appeared, however, that woodiness of the stem was related to speed of germination and seedling emergence (9), and this in turn was an aid in resistance to preemergence death (4,11).

In a previous study (6), two methods of inoculation and five types of *R. solani* inoculum were compared when testing 20 pea lines for epicotyl resistance. The results reported in this paper are an extension of that work, in which several host factors were evaluated to determine their role in the resistant response of peas to *R. solani* epicotyl rot. A portion of this work was reported previously (5).

MATERIALS AND METHODS

Twenty pea lines consisting of 4 breeding lines, 10 plant introduction accessions, and 6 cultivars (Table 1) were compared for anthocyanin pigmentation, rate of emergence, and epicotyl thickness, as they related to resistance. Eight of the 20 lines had anthocyanin-pigmented seed coats and 12 had seed coats without anthocyanin. These 20 lines varied in seed weight from 0.06 to 0.46 g/seed and were representative of some of the seed variability that exists in the genus *Pisum*. The average number of days to seedling emergence for each line was based on the number of plants emerged at 4, 5, and 6 days after planting (4).

Table 1. The relationship of seed coat pigmentation, emergence rate, and seedling epicotyl diameter with resistance in peas to *Rhizoctonia solani* stem rot

Pea lines	Mean disease rating[w] (inoculum source)		Seed coat pigmentation[x]	Mean days to emergence[y]	Epicotyl diam.[z] (mm)
	DAT	Sclerotia			
PI 223285	4.5 a	4.6 a	+	4.8	2.1
PI 166159	4.4 ab	4.7 a	+	4.7	2.1
PI 140165	4.6 a	4.1 b	+	4.7	2.0
PI 257593	4.3 abc	3.1 defg	+	4.6	2.7
Mini	4.1 bcd	3.7 c	−	5.3	2.4
Frimas	4.0 cde	3.4 cd	−	4.7	2.6
PI 194006	3.9 cde	2.9 efgh	+	4.6	2.6
PI 378159	3.8 def	3.2 def	−	5.0	2.7
Minn 108	3.7 efg	3.3 de	−	4.8	2.6
74SN5	3.7 efg	3.1 defg	−	5.2	2.8
M410	3.5 fgh	3.1 defg	−	4.9	2.5
Horal	3.5 fgh	2.9 efgh	−	5.1	2.6
74SN3	3.3 ghi	2.8 fgh	−	5.0	2.8
PI 244128	3.2 ghi	2.8 fgh	−	5.1	2.9
PI G19169	3.1 hij	3.3 cd	−	4.6	2.7
Finale	2.9 ij	3.3 cd	−	4.6	2.8
PI 189171	2.9 ij	2.5 hi	+	4.5	3.3
PI 197990	2.8 j	2.2 i	+	4.8	3.3
Dark Skin Perf.	2.8 j	2.3 i	−	4.9	3.0
B77-634-4	2.5 k	2.2 i	+	4.3	3.4

[w] Mean disease rating based on a scale increasing from 0 to 5, where 0 = no lesions and 5 = 75–100% of epicotyl girdled. Inoculum source: DAT = dextrose asparagine broth, 4-mm mycelial disks used as inoculum. Sclerotia = 20 sclerotia (250–425 μm) per gram of soil. Means within columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[x] + = Anthocyanin pigmentation, − = no anthocyanin pigmentation.

[y] Mean number of days until emergence calculated by the sum of each daily emergence times the days from sowing divided by the total emergence; calculated from two tests at 19 ± 1 C for 33 seedlings for each line.

[z] Average epicotyl diameter of 9-day-old uninoculated seedlings was determined midway between the cotyledons and the soil surface; calculated data represents an average measurement of 33 seedlings per line.

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Two methods of inoculation, consisting of either direct epicotyl inoculation with mycelial disks produced on dextrose asparagine (DAT) broth (14) or planting seeds into soil artificially infested with sclerotia, were used to compare host factors for resistance. Specific methods of inoculation and environmental conditions of screening were reported previously (6).

Epicotyl diameters of uninoculated seedlings were measured to the nearest 0.25 mm 9 days after emergence at the same site as the DAT inoculations. The epicotyl diameters of 22 additional pea lines were measured on 9-day-old healthy seedlings of each line while another set of these same pea lines was inoculated with DAT mycelial disks as reported previously (6).

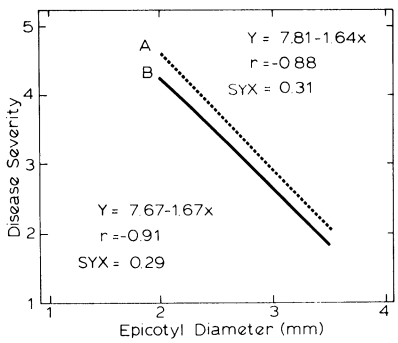

Fig. 1. Linear regression of mean seedling epicotyl diameter and mean stem rot severity rating for 20 pea lines: A = inoculated directly with DAT mycelial disks, or B = seeds sown in soil infested with 20 sclerotia (250–425 μm) per gram. Mean disease severity is based on a scale increasing from 0 to 5, where 0 = no lesions and 5 = 75–100% of epicotyl girdled.

Progression of disease development was determined over a 21-day period, using a resistant (Dark Skin Perfection [DSP]), intermediate resistant (PI 257593), and highly susceptible (PI 223285) pea lines. Ninety seeds of each test line were planted into 10 plastic pots (10 × 10 × 9 cm) containing field soil infested with 20 sclerotia per gram of soil (6). Disease severity was determined 6, 8, 12, 16, and 21 days after planting, on a scale of 0–5, where 0 = no lesions, 1 = one to few small discrete lesions, 2 = up to 25% of epicotyl girdled, 3 = 25–50% of epicotyl girdled, 4 = 50–75% of epicotyl girdled, and 5 = 75–100% of epicotyl girdled. This test was conducted twice.

To determine if cotyledon colonization affected the severity of epicotyl rot, four fungicides with known activity against *R. solani* were applied separately to seed of DSP. The fungicides were: captan, chloroneb, and pentachloronitrobenzene (all applied at the rate of 2.5 g a.i./kg of seed) and thiram (applied at 1.9 g a.i./kg of seed). All fungicides were applied as a slurry with 0.5 g methyl cellulose per kilogram of seed as a sticker. Control seeds were treated with water and sticker only. Seeds were planted in soil infested with 20 sclerotia per gram or in uninfested soil. The test was conducted twice, with 27 seeds planted in each test for each fungicide treatment.

RESULTS

Anthocyanin pigmentation of seed coats did not correlate with resistance to *R. solani* epicotyl rot (Table 1). The most susceptible lines (PI 140165, 166159, and 223285) as well as some of the most resistant lines (B77-634-4, PI 189171, and PI 197990) had pigmented seed coats. Also, the average rate of seedling emergence was not associated with resistance to epicotyl rot (Table 1). A significant negative correlation was found between pea epicotyl diameter and disease severity when seeds were exposed to sclerotia-infested soil ($r = -0.91$) or seedling epicotyls to DAT mycelial disks ($r = -0.88$) (Table 1, Fig. 1).

Using the linear regression equation obtained from the original 20 pea lines that had been screened with DAT inoculum ($Y = 7.81 - 1.64X$), a disease severity rating was predicted for 22 additional lines. Of the 22 lines tested, only one breeding line (244219-B) and one cultivar (Mexique) had disease severity ratings that deviated widely from the predicted disease rating (Table 2).

When stem rot severity was assessed for a resistant (DSP), intermediate (PI 257593), and highly susceptible (PI 223285) pea line over a 21-day period using sclerotia inoculum (Fig. 2), there was a rapid increase in disease severity during the first 12 days after planting for all three lines tested. However, the highly susceptible PI 223285 had significantly higher disease ratings in a shorter period of time than resistant DSP (Fig. 2).

When untreated seeds of the *Rhizoctonia*-resistant cultivar DSP (nonanthocyanin-pigmented seed coats) were planted in sclerotia-infested soil, cotyledons were usually heavily colonized 6 days after planting. In contrast, resistant or susceptible pea lines with anthocyanin-pigmented seed coats usually had intact, noncolonized cotyledons for up to 21 days. Of the four

Table 2. Actual and predicted seedling epicotyl rot severity of peas caused by *R. solani*, basing the prediction on average epicotyl diameter

Pea lines	Epicotyl diam.[y] (mm)	Mean disease severity		Deviation
		Predicted[z]	Observed	
244219-B	2.5	3.7	2.3	−1.4
Mexique	2.4	3.9	2.6	−1.3
81-952	2.7	3.4	2.7	−0.7
PI 174919	1.6	5.2	4.7	−0.5
PI 234263	2.8	3.2	2.7	−0.5
81-985	2.6	3.5	3.1	−0.4
Minn 494-A-9	2.2	4.2	3.8	−0.4
CM-034C	2.6	3.5	3.1	−0.4
Gentry	2.6	3.5	3.2	−0.3
Tiny	2.4	3.9	3.6	−0.3
81-1008	2.0	4.5	4.2	−0.3
81-942	3.0	2.9	3.1	−0.2
81-992	2.8	3.2	3.4	+0.2
81-1039	2.6	3.5	3.3	−0.2
B77-634-9	2.7	3.4	3.2	−0.2
Cobri	2.5	3.7	3.6	−0.1
Finette	2.7	3.4	3.3	−0.1
Amino	2.6	3.5	3.6	+0.1
Gen 059-81	2.7	3.4	3.3	−0.1
M64-333	2.5	3.2	3.2	0.0
Ph 91-3	2.8	3.2	3.2	0.0
81-939	2.5	3.7	3.7	0.0

[y] Epicotyl diameter measured on 9-day-old healthy seedlings midway between the cotyledons and the soil surface, calculated from 16 seedlings.

[z] Mean disease severity rating based on a scale increasing from 0 to 5 (0 = no lesions and 5 = 75–100% of epicotyl girdled) from seedlings inoculated with 4-mm mycelial disks produced on dextrose asparagine broth. Number of seedlings evaluated from each line varies from 15 to 29 seedlings due to poor germination of some lines. Predicted disease severity rating calculated from regression line $Y = 7.8 - 1.6X$.

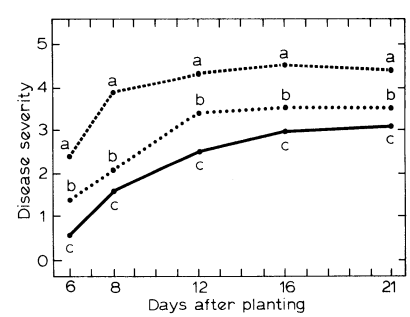

Fig. 2. Progression of epicotyl rot severity with time of exposure to 20 sclerotia (250–425 μm) of *R. solani* per gram of soil for Dark Skin Perfection (———); PI 257593 (·····); and PI 223285 (---------). Data points in vertical columns indicated by days after planting followed by the same letter are not significant at $P = 0.01$. Mean disease severity is based on a scale increasing from 0 to 5, where 0 = no lesions and 5 = 75–100% of epicotyl girdled. Data points represent a mean from two tests, with a total of 28 seedlings evaluated.

Table 3. Effect of fungicide treatments of Dark Skin Perfection seed on epicotyl and hypocotyl rot, and cotyledon colonization by *Rhizoctonia solani*

Treatment	Rate (g a.i./kg seed)	Disease severity ratings[x]		
		Epicotyl	Hypocotyl	Cotyledon
PCNB	2.5	2.7 a[y]	2.1 a	3.4 a
Thiram	1.9	2.4 a	2.0 a	3.7 a
Captan	2.5	2.4 a	1.9 a	2.8 b
Chloroneb	2.5	2.0 b	0.3 c	0.9 c
Infested soil control[z]	···	2.4 a	1.5 b	3.3 a
Uninfested soil control	···	0.0 c	0.1 c	1.0 c

[x]Disease severity rating based on a scale increasing from 0 to 5, where 0 = no lesions and 5 = 75–100% of epicotyl girdled. Means are for two tests with 54 seeds planted for each line within each treatment.

[y]Means within columns followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

[z]No fungicide treatment, seed treated with sticker and water only.

fungicidal seed treatments applied, only chloroneb adequately protected cotyledons of DSP from colonization by *R. solani*, and both epicotyl and hypocotyl rot were significantly reduced compared with untreated seed or seed treated with other fungicides (Table 3).

DISCUSSION

Pea epicotyl diameter was the only host factor studied that strongly correlated with resistance to epicotyl rot caused by *R. solani* (Table 1, Fig. 1). This correlation also occurred with the cornmeal-sand (cm-s) inoculation treatments reported previously (6); 3% cm-s ($r = -0.83$); 6% cm-s ($r = -0.87$). The relationship between epicotyl diameter and stem rot severity was a consistent feature regardless of inoculum treatment, even though there were significant differences in stem rot severity ratings between inoculum treatments (6). Epicotyl rot was also predicted by measuring the epicotyl diameter (Table 2).

Resistance to epicotyl rot differed with time of exposure to sclerotia inoculum (Fig. 2), and the disease developed in susceptible lines much faster than in a resistant line.

Seed coat pigmentation appears to have little relationship to resistance to epicotyl rot caused by *R. solani* in peas (Table 1). Seed coat pigmentation in PI accessions 223285, 166159, and 140165 possibly protected cotyledons from colonization by *R. solani* when grown in sclerotia-infested soil but failed to prevent severe epicotyl rot. In contrast to anthocyanin pigmentation, fungicide seed treatment with chloroneb reduced both epicotyl and hypocotyl rot of DSP, which possesses a measurable level of resistance to *R. solani*.

One of the most resistant pea lines in this study (B77-634-4) had the lowest average emergence rate of the 20 lines tested and possessed pigmented seed coats (Table 1). When this line was exposed to inoculum placed directly on the epicotyl, where such factors as average rate of seedling emergence and seed coat pigmentation should be less important, this line still displayed one of the highest levels of resistance. Of more importance, B77-634-4 had the largest epicotyl diameter of the 20 pea lines tested (Table 1).

The reason peas with large-diameter epicotyls are more resistant to *R. solani* than peas without thick epicotyls remains to be determined. However, selection for thick epicotyls could be a useful breeding and screening tool because many lines could be evaluated quickly and segregants saved without the pathogen's presence.

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