Brown Stem Rot Resistance and Its Modification by Soybean Mosaic Virus in Soybeans

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Joint contribution from Plant Science Research Division, ARS, USDA, and Iowa Agriculture and Home Economic Experiment Station. Contribution of the U.S. Regional Soybean Laboratory as Paper No. 720 and Journal Paper No. J-7147 of the Iowa Agriculture and Home Economic Experiment Station, Ames, Iowa; Project No. 1179.

The authors acknowledge assistance of Jean L. Hank, Federal Work-Study Program, and statistical analyses by Dan Niffenegger, Assistant Director, Biometrical Services, ARS, USDA, Beltsville, Maryland.

Accepted for publication 24 May 1972.

ABSTRACT

Resistance to brown stem rot (BSR) of soybeans was confirmed by artificial inoculations in the greenhouse. One line, P.I. 86.150, had 30% less BSR than P.I. 84.946-2, current principal source of BSR resistance for breeding purposes. Occurrence of soybean mosaic virus (SMV) in BSR-resistant lines was observed. Relationship between seed coat mottling, a symptom of SMV, and BSR resistance was inverse. When plants from nonmottled and mottled seed of BSR susceptible Clark cultivar and resistant P.I. 84.946-2 were inoculated with *Cephalosporium gregatum*, plants from mottled seed had half as much disease as did plants from nonmottled seed. Individual and combination inoculations of SMV and *C. gregatum* in BSR-susceptible Ontario cultivar further indicated half as much BSR with both virus and fungus as with *C. gregatum* alone.

Phytopathology 62:1314-1317.

Additional key words: Glycine max (L.) Merr.

Because brown stem rot (BSR), caused by *Cephalosporium gregatum* Allington & Chamberlain (1), of soybeans [*Glycine max* (L.) Merr.] is a major disease problem of the crop, resistance to the disease has been sought for many years by workers at the U.S. Regional Soybean Laboratory, Urbana, Ill. (2). The line P.I. 84.946-2, selected from among 2,060 soybean lines screened for BSR resistance, had a high proportion of disease-free plants during 12 seasons (2). Kunkel (6) evaluated 22 soybean lines and obtained evidence that resistance to BSR is horizontal.

Chamberlain & Bernard (2) observed that early maturing varieties of soybean had lower incidence of BSR. There is apparently also some relationship between varietal maturity and soybean mosaic virus (SMV) infection. Quiniones (7) obtained serological evidence that early-maturity cultivars of soybeans contain more SMV than do late-maturing cultivars. Symptoms of both, BSR and SMV, are uniquely identifiable. The pith-browning symptom of BSR not only makes possible identification of the disease, but also provides quantitative measure of severity (6). Kennedy & Cooper (4), Koshimizu & Iizuka (5), and Ross (9, 10) found infection by SMV caused soybean seed coat mottling in addition to the usual leaf symptom caused by mosaic viruses. Because no quantitative relationship has been shown between seed coat mottling and SMV infection, seed coat mottling only indicates presence or exposure to SMV.

This paper reports (i) evaluation of resistance of P.I. 84.946–2 and other resistant lines by artificial inoculation in the greenhouse; (ii) observations of SMV symptoms in BSR resistant lines; and (iii) investigation of a possible relationship between BSR resistance and SMV.

MATERIALS AND METHODS.—Selection of soybean lines and cultivars for these studies was based primarily on previous field reactions and limited artificial inoculation studies with *C. gregatum*. Clark 63, Lincoln, and Ontario were susceptible to *C. gregatum*, whereas Midwest and P.I. 84.946–2 were resistant (1, 2, 6, 11). Four plant introduction lines, P.I. 86.150, P.I. 88.820N, P.I. 90.138, and P.I. 95.769 were resistant to brown stem rot, and P.I. 69.507 and P.I. 171.434 were susceptible (R. L. Bernard, *personal communication*). Soybean seeds used in these studies were grown at Ames, Iowa, except as otherwise indicated.

Spore suspensions from 7-day-old potato-dextrose agar (PDA) cultures of *C. gregatum* grown at 23 C were used for inoculum. Spore concentration was standardized for each test using a Bausch & Lomb Spectronic 20 set at 540 nm. Inoculum was adjusted to 50-60% transmittance. Difco nutrient broth (12 g/liter) and NaCl (3.7 g/liter) were added to standard PDA for growing the fungus. Plants were grown in 10.1 cm clay pots, 2-4 plants/pot at 27 C. Soil was a 5:2:1 (Clarion sandy loam, peat, and sand) mixture, steam autoclaved at 15 psi for 4 hr. Natural daylight was supplemented with artificial VHO fluorescent light of 1,000 ft-c min for 16 hr/day. Twelve days after seeding, plants were inoculated using a 0.5-inch, 22-gauge hypodermic needle inserted into the stem between cotyledons. Penetration by the needle was ca. 0.24 inch down into the vertical axis of the stem. Pressure was applied to the plunger as the needle was removed from the stem. The volume of inoculum injected was less than 0.01 ml. Inoculated plants were incubated at 20 ± 4 C in the greenhouse. Disease severities, based on length of pith and vascular tissue discoloration, and plant height were evaluated 5 weeks after inoculation.

A soybean mosaic virus from cultivar Midwest (5) was used for SMV and *C. gregatum* inoculation studies with Ontario soybeans. Virus inoculum was crude sap from infected leaves diluted 1:7 (w/v) with 0.01 M phosphate buffer, pH 7. We inoculated primary leaves by rubbing inoculum mixed with 600-mesh Carborundum. Gauze pads saturated with the inoculum were used for rubbing. Primary leaves of Kentucky Wonder Wax Pole bean seedlings were similarly inoculated for virus assay by the local lesion method (8). Randomization of treatments within blocks, each block a replication, was the experimental design, except that complete randomization was used for SMV and *C. gregatum* inoculation studies with Ontario soybeans.

RESULTS.—*Resistance of plant introduction lines to BSR.*—Means of extent pith browning of susceptible plants ranged from 5.1 to 6.6 cm; whereas, the resistant P.I. lines were from 2.4 to 3.8 cm. One resistant line, P.I. 86.150, had 30% less pith browning than did P.I. 84.946–2 (Table 1). The other three P.I. lines, P.I. 90.138, 95.769, and 88.820N, were similar to P.I. 84.946–2 in resistance. Among susceptible controls of Clark 63, P.I. 69.507, and P.I. 171.434, the line P.I. 69.507 had the lowest mean value for pith browning, but not as low as those of the resistant lines.

TABLE 1. Seed coat mottling and pith browning of resistant and susceptible Plant Introduction soybeans tested in the greenhouse by artificial inoculation

Soybeans	Seed coat mottling of seeds planted	Extent of pith browning in stem[a]
	%	*cm*
P.I. 90.138	57.8	3.7
P.I. 86.150	37.0	2.4
P.I. 95.769	33.0	3.8
P.I. 88.82ON	2.0	3.6
P.I. 84.946-2 (resistant control)	1.8	3.5
Clark 63 (susceptible cultivar)	1.3	6.3
P.I. 69.507 (susceptible control)	0.3	5.1
P.I. 171.434 (susceptible control)	0.0	6.6

[a]Means of eight replications, two plants per replication.

During inoculation and incubation of BSR-resistant P.I. plants, we noticed viruslike symptoms in a large percentage of the plants, whether inoculated or noninoculated with *C. gregatum*. In contrast, all BSR-susceptible plants appeared healthy. Because seed coat mottling has been related to virus infection (4, 5, 9, 10), we obtained seed coat mottling data from seeds of the test P.I. lines rather than attempting to rate symptom severity of the plants. Seed coat mottling data (Table 1) were obtained by examining seed of bulk seed lots for these experiments.

There was an inverse correlation of seed coat mottling percentage with centimeters of BSR. Seed coat mottling percentages ranged from 0 to 57%. Lines equal to, or more resistant to, BSR than P.I. 84.946–2 had a higher percentage of seeds with seed coat mottling than BSR-susceptible lines. The susceptible controls, Clark 63, P.I. 69.507, and P.I. 171.434, had least seed coat mottling.

Brown stem rot reaction of nonmottled and mottled Clark and P.I. 84.946–2 soybeans.—The availability of nonmottled seeds of same cultivars or strains made possible further tests for SMV and BSR interaction. Seeds of Clark and P.I. 84.946–2, each with 25% mottled seeds, were obtained from R. L.

Bernard at Illinois. Although Iowa-grown seed lots had some mottled seeds (0.01 and 0.03 for Clark and P.I. 84.946–2, respectively), these were considered nonmottled and tested along with the heavily mottled seeds of these same cultivars for BSR reactions by artificial inoculation with *C. gregatum* (Table 2). Plants from mottled Clark and P.I. 84.946–2 had less pith browning than plants from nonmottled equivalents. The differences were twice as great between Clark plants, but only slightly greater in P.I. 84.946–2. For both lines, however, length of vascular tissue discoloration was approximately twice as great in plants from nonmottled seed as compared to plants from mottled seed.

TABLE 2. Pith browning, vascular tissue discoloration, and plant height of brown stem rot-susceptible and -resistant soybean plants grown from nonmottled and mottled seeds

Soybeans	Extent of pith browning in stem	Vascular tissue discoloration	Plant height
	cm	*cm*	*cm*
Susceptible			
Clark (nonmottled)			
Noninoculated	0[a]	0	24.0
Inoculated	3.0	9.5	20.4
Clark (mottled)			
Noninoculated	0	0	27.0
Inoculated	1.4	4.7	23.5
Resistant			
P.I. 84.946-2 (nonmottled)			
Noninoculated	0	0	45.5
Inoculated	2.1	6.9	38.9
P.I. 84.946-2 (mottled)			
Noninoculated	0	0	50.1
Inoculated	2.0	3.9	37.4

[a]Each value is the mean of 12 replications, two plants/replication.

Plant heights were consistently reduced in all plants inoculated with *C. gregatum* when compared with the noninoculated. Plants from nonmottled seeds were consistently shorter when compared with plants from mottled seed among the noninoculated plants, but not of inoculated. Among inoculated, plant height was lower in Clark plants from nonmottled seeds, but slightly higher in P.I. 84.946–2.

Virus assay by local lesion test.—When leaves of BSR-resistant Midwest, P.I. 84.946–2, and P.I. 81.650 and susceptible Clark 63 and Lincoln were assayed for virus, leaf extracts from the three soybeans resistant to *C. gregatum* produced local lesions on primary leaves of Kentucky Wonder Wax Pole bean, whereas no lesions were produced with extracts from BSR-susceptible Clark 63 and Lincoln (Table 3).

TABLE 3. Local lesions produced on primary leaf of Kentucky Wonder Wax Pole bean when inoculated with sap from brown stem rot (BSR)-resistant and susceptible soybeans in greenhouse

Soybeans	Local lesions[a]
BSR-resistant soybeans	
Midwest	
Leaf	3.5
Cotyledon	0.5
P.I. 84.946-2	
Leaf	1.5
Cotyledon	0
P.I. 81.650	
Leaf	1.35
BSR-susceptible soybeans	
Clark	
Leaf	0
Lincoln	
Leaf	0

[a]Means of four replications, two plants/replication and two leaves/plant.

Local lesions were produced with a cotyledon extract from Midwest, but not P.I. 84.946–2.

Interaction of C. gregatum and soybean mosaic virus.—The effect of SMV upon BSR development was further investigated by a combination of SMV and *C. gregatum* inoculations of BSR-susceptible Ontario soybeans. When both SMV and *C. gregatum* were inoculated into the same plant, pith browning and vascular discoloration were 46 and 41% less, respectively, of that inoculated with *C. gregatum* alone (Table 4). Plant height was reduced significantly (33%) where SMV was inoculated, whether alone or with *C. gregatum*, and only slight (8%) plant height reduction occurred with *C. gregatum* alone.

TABLE 4. Pith browning, vascular tissue discoloration, and plant height of Ontario soybeans when *Cephalosporium gregatum* and soybean mosaic virus (SMV) were inoculated separately and in combination

Treatments	Extent of pith browning in stem	Vascular tissue discoloration	Plant height
	cm	*cm*	*cm*
C. gregatum	2.83[a] x[b]	6.87 x	41.5 y
SMV	0 z	0 z	30.3 x
C. gregatum + SMV	1.53 y	4.10 y	30.8 x
Noninoculated	0 z	0 z	45.0 y

[a]Means of 30 plants/treatment.

[b]x, y, z indicate statistical difference at 1% level of significance if letters are different (Duncan's multiple range test).

DISCUSSION.—Resistance to brown stem rot was observed in four additional resistant P.I. lines when tested by artificial inoculation in the greenhouse. The results generally confirm previous field test results at Urbana, Ill. by R. L. Bernard (*personal communication*). Among the resistant lines, P.I. 86.150 had 30% greater resistance than the standard P.I. 84.946–2, making it a potentially valuable source of resistance for breeding programs. The other three lines had levels of resistance similar to P.I. 84.946–2. These results also indicate that it is possible to screen for BSR resistance by artificial inoculations in the greenhouse.

Differences in extent of symptom between BSR-resistant and BSR-susceptible lines were consistent from test to test. Extent of pith browning in either naturally or artificially SMV-infected plants was generally about half that in plants noninfected with SMV. Clark plants grown from naturally mottled seeds had approximately half the extent of pith browning observed in plants from nonmottled seed. When both *C. gregatum* and SMV were inoculated into BSR-susceptible Ontario, pith browning was approximately half as compared to pith browning in plants inoculated with *C. gregatum* alone. In all cases, the presence of SMV, whether naturally present or artificially inoculated, reduced pith browning by at least some significant amount. The relationship observed in these studies between *C. gregatum* and SMV in soybeans strongly indicates a possible interaction between the two pathogens or diseases.

The observed effect of SMV on BSR may possibly explain some of the problems encountered in genetic studies and BSR breeding programs. Chamberlain & Bernard (2) found large plant-to-plant and seasonal variability of reactions in both BSR-resistant and -susceptible soybeans. They found it difficult to study the inheritance of resistance to BSR because of this variability; their data did show selections from hybrid populations of P.I. 84.946–2 crosses to average more plants with less BSR than susceptible cultivars. A combination of backcrossing and bulk and pedigree selection has been used to develop better agronomic lines with resistance approaching that of P.I. 84.946–2. After 15 years of breeding, screening, and selection for BSR-resistant lines, no commercial variety has been released with the resistance of P.I. 84.946–2. The interaction of SMV with BSR may have contributed to the problem.

Crop rotation (3) is no longer feasible for BSR control because of the very large acreage of soybeans now being grown. Consequently, BSR continues to increase and becomes more serious each year. As previously indicated, variability of results is a major problem in breeding for BSR resistance. Our observations and results indicate that the resistance is associated with the presence of SMV, which may be a factor responsible for the variability encountered in studying and developing BSR-resistant soybeans. Therefore, new sources of BSR resistance and other methods of control are needed. Also, further studies should be made to more critically evaluate relative significance of SMV in currently available BSR-resistant P.I. lines, and to determine feasibility of their continued use and ultimate utilization.

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