Reaction of Soybean Cultivars to 14 Races of *Phytophthora megasperma* f. sp. *glycinea*

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ABSTRACT

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Soybean cultivars (*Glycine max*) were screened for reaction to 14 races of *Phytophthora megasperma* f. sp. *glycinea*. Eighty-five cultivars were found susceptible to all 14 races, whereas 37 were resistant to one or more races. Many plant-pathogen interactions resulted in reactions that were intermediate and could not be classified as either susceptible or resistant to *P. megasperma* f. sp. *glycinea*.

Phytophthora megasperma Drechs. f. sp. *glycinea* Kuan & Erwin (*Pmg*), which incites phytophthora root rot of soybean (*Glycine max* (L.) Merr.), occurs in most soybean-producing areas of the United States and Canada. Currently, several physiologic races of *Pmg* have been identified based on pathogenicity of the isolates to differential cultivars. Phytophthora root rot is one of the most serious diseases of soybeans when conditions are favorable. Host-plant resistance to the pathogen is controlled by dominant genes occurring at several loci (2,6,7,8). To date, the most effective means of controlling the disease has been the incorporation of resistance genes Rps_1^a, Rps_1^b, Rps_1^c, Rps_1^k, Rps_2, and Rps_3 into adapted cultivars. Once the genes are incorporated into a cultivar, however, there is a threat that new virulent races of the pathogen will increase in frequency enough to overcome host-plant resistance. For this reason, additional sources of resistance must be identified. The purpose of this study was to determine reactions of soybean cultivars to 14 races of *Pmg* and to identify new sources of resistance.

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MATERIALS AND METHODS

Seed of 122 cultivars of maturity groups 00–IV were obtained from R. L. Bernard, USDA. Soybean Germplasm collection, Urbana, IL. These cultivars, along with known differentials (Table 1), were inoculated with *Pmg* races 1–10, 12–14, and 16. Races 1–6 were obtained from F. W. Schwenk, Manhattan, KS, races 7–9 from A. F. Schmitthenner, Wooster, OH; and races 10 and 12–16 from B. L. Keeling, Stoneville, MS. Cultivars were not screened with race 15 because of low zoospore production by the isolate.

Zoospores used for inoculation were produced using a technique developed by F. W. Schwenk (*personal communication*). About 1 wk before zoospores were needed for inoculation, four small pieces

of lima bean agar (LBA) with mycelium were transferred into standard petri plates (100 × 15 mm) each containing 8 ml of LBA. Plates were incubated in the dark at 21 C for 3 or 4 days, at which time mycelium covered most of the agar. Fifteen milliliters of sterile distilled water was added to each plate under aseptic conditions and the plate returned to the incubator for 30 min. Water from each plate was discarded, another 15-ml portion of water was added, and the plates were incubated again for 30 min. This was repeated four times. After a fifth portion of water was added 2 hr after the first flooding, the plates were incubated for 6–12 hr. Zoospores in the rinse water were used to inoculate LBA in petri plates by adding a 1-ml portion of zoospore suspension to each plate and rotating to cover the agar with liquid. Zoospore concentrations were highest when LBA plates were inoculated with zoospores rather than with mycelium. These plates were incubated for 24–48 hr and flooded as described previously. The resulting zoospore concentration was above 100,000/ml. This zoospore suspension could be used either to inoculate other LBA plates to continue the zoospore producing cycle or to inoculate plants. A concentration of 10^5 zoospores per milliliter was used for inoculating plants.

Ten seeds of each cultivar were planted 2.5 cm deep in sand in 10-cm-diameter plastic pots. Plants were grown for 10 days in the greenhouse before they were moved to the laboratory for inoculation with zoospores. The hypodermic inoculation technique described by Schwenk et al (10) was used. Plants were injected three times below the cotyledonary node in each hypocotyl. Injections were made about 1 cm apart with a 25-gauge needle. Plants that had difficulty emerging, with enlarged, hard hypocotyls, were removed before inoculation. Five days after inoculation, a cultivar's reaction to a particular race of *Pmg* was classified as either susceptible (S = hypocotyl collapse and death), moderately susceptible (S* = majority of plants with killing lesions at five days), partially resistant (R* = majority of plants with nonkilling lesions), or resistant (R = no infection).

RESULTS AND DISCUSSION

Of the 122 cultivars screened, the following 85 were found susceptible to all 14 races of *Pmg*: A-100, Agate, Aksarben, Aoda, Bansei, Bavender Special A, Bethel, Blackeye, Burwell, Capital, Carlin, Chestnut, Chief, Cloud, Cypress No. 1, Delmar, Dunfield, Earlyana, Ebony, Elton, Emperor, Ennis I, Etun, Fabulin, Funk Delicious, Funman, Green Giant, Goku, Granger, Harbinosoy, Harmon, Hidatsa, Hokkaido, Hongkong, Hoosier, Ilsoy, Imperial, Jefferson, Jogun, Kabott, Kahala, Kaikoo, Kailua, Kanro, Kanum, Kura, Lincoln, Linman 533, Little Wonder, Macoupin, Manchu, Manchuria, Mandell, Mansoy, Mendota, Midwest, Mingo, Minsoy, Morse, Ontario, Pagoda, Pando, Patoka, Patterson, Pennsoy, Perry, Polysoy, Roe, Sac, Scioto, Shingto, Shiro, Sioux, Sooty, Sousei, Tastee, Waseda, Wayne, Wea, Williams, Willomi, Wilson, Wing Jet, Wisconsin Black, and Yellow Marvel.

Cultivars resistant to one or more of races 1–10, 12–14, and 16 of *Pmg* are listed in Table 2. The following cultivars were found resistant only to races 12 and 16 of *Pmg*: AK (FC 30.761 [FC = USDA Forage Crops Accession]), Corsoy, Early White Eyebrow, Flambeau, Goldsoy, Hark, Harosoy, Kagon, Mandarin, Mandarin (Ottawa), Peking, Poland Yellow, Richland, and Soysota. Pedigrees of Harosoy, Corsoy, and Hark (Fig. 1) include common parents resistant to *Pmg* races 12 and 16. Resistance to these two races was transferred from one cultivar to the next without selection.

Table 2 reports reactions of other cultivars resistant or partially resistant to at least one race of *Pmg*. The cultivars Chusei, Portugal, Toku, and Wolverine reacted similarly (resistant R or R* or susceptible S or S*) to 14 races of *Pmg*. With the exception of Portugal, all were plant introductions from Japan. Cultivars Norsoy and Ogemaw were both found partially resistant to race 16. No other cultivars reacted similarly to the 14 races of *Pmg*.

Many plant-pathogen interactions resulted in reactions that were intermediate and could not be fitted to the classification of susceptible or resistant (Tables 1 and

Table 1. Differential responses of soybean cultivars to inoculation with races 1–10, 12–14, and 16 of *Phytophthora megasperma* f. sp. *glycinea*

Cultivar	Genes for resistance	Reaction[y] (race) 1	2	3	4	5	6	7	8	9	10	12	13	14	16
Williams		S	S	S	S	S	S	S	S	S	S	S	S	S	S
Harosoy		S	S	S	S	S	S	S	S	S	S	R	S	S	R
Mukden	*Rps*$_1$[a]	R	R	S	S	S	S	S	S	S	R	S	R	S	R
Harosoy 63	*Rps*$_1$[a]	R	R	S	S	S	S	S	S	S	R	R	R	S*	R
Union	*Rps* $_1$[a]	R	R	S	S	S	S	S	S	S	R	S	R	S*	R
Voris 295		R	R	S	S	S	S	S	S	S	R	R	R	S*	R
Sanga	*Rps*$_1$[b]	R	S	R	R	R	R	R	R	R	S	S	R	R	S
Williams 79	*Rps*$_1$[c]	R	R	R	S	S	R	R	R	R	R	S	R	S	S
Williams 82	*Rps*$_1$[k]	R	R	R	R	R	R	R	R	R	R	S	R	R	S
PI 86972-1[z]	*Rps*$_3$	R	R	R	R	R	S	S	R	R	S	S	R	R	R
PI 86050	*Rps*$_1$,[c] *Rps*$_4$	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Altona		R	R	R	R	S	S	S	S	R	R	S	S	R	R
PI 103091		R	R	R	R	R*	R*	R	S	R	R	S	R	R	R

[y] S = Plants killed, S* = majority of plants with killing lesions, R* = majority of plants with nonkilling lesions, and R = no infection.
[z] PI = Plant Introduction.

Table 2. Soybean cultivars resistant to one or more of races 1–10, 12–14, and 16 of *Phytophthora megasperma* f. sp. *glycinea*

Cultivar	Reaction[a] (race) 1	2	3	4	5	6	7	8	9	10	12	13	14	16
Acme	R*	R*	R	R*	S	S	S	S	S	S	R*	S	S	R*
Black Eyebrow	S	S*	R	R*	S	R	S*	S*	S	R	S	S	S	R
Bombay	R	R	R*	R	R*	S	S	R	R*	S	S	S*	R	R*
Boone	R	R	S	S	S	S	S	S	S	R	S	R	S*	R
Cayuga	S	S*	R*	R*	S*	S	S	R	S*	S	S	S	S	R*
Chusei	R*	R*	R*	R*	S	S	S	R	S*	R*	R*	S	R*	R*
Columbia	S*	S*	S*	S*	S*	R*	S	R*	S	R*	S	S	S	R*
Fuji	R*	S	R*	R*	S	S	S	S*	S	S	S	S	R*	R*
Guelph	S	S*	S	S	S	S	S*	S	S	R*	S	S	S	S*
Habaro	R*	R	R*	R*	S*	S	S	R*	R	S	R	R	R*	R
Korean	S*	S*	R*	R*	R*	R*	S*	S	S	S*	S	S	S	S*
Manitoba Brown	S*	S*	R*	S*	S*	S	S	S	S	S	S	S	S*	R*
Miller 67	S*	S*	R*	R	S*	S	S	R*	S*	S	R*	S*	R*	R*
Norredo	R	R	R	S	S	R	R	R	R	R	S	R	S	S
Norsoy	S	S	S	S	S	S	S	S	S	S	S	S	S	R
OAC 211	R*	R*	R*	R	S*	S	S	R	R*	S	R*	S*	R*	R*
Ogemaw	S*	S*	S*	S*	S*	S	S	S*	S	S	S*	S	S	R*
Osaya	S	S	S	R*	S	S	S	R*	S	R*	S	S	S	R*
Portugal	R	R	R*	R	S	S	S	R*	S*	R	R	S*	R	R
Toku	R*	R	R	R	S*	S	S	R*	S	R	R*	S	R	R*
Virginia	R	R*	R*	R*	S	R*	R*	R*	S	R	S	R	R	R
Wolverine	R	R	R	R	S	S	S	R*	S	R	R	S	R	R*

[a] S = Plants killed, S* = majority of plants with killing lesions, R* = majority of plants with nonkilling lesions, and R = no infection.

2). In the past, most reports of *Pmg* screening have divided cultivar reactions into discrete classes, either resistant or susceptible. Reactions that were intermediate were excluded from discussion (1). In our study, we attempted to identify reactions that were intermediate by classifying them as either moderately susceptible (S*) or partially resistant (R*). Although these descriptions may not be complete, they give a basis for discussion.

Several hypotheses would seem plausible for explaining intermediate reactions. Explanations include 1) cultivar impurity, 2) variation in virulence within races, 3) cultivars with the CNS reaction type (6) of resistance that may give intermediate reactions depending upon the inoculation technique used, 4) morphological barriers present in the hypocotyl resisting pathogen growth, 5) differences in glyceollin production when challenged by *Pmg*, or 6) factors such as temperature, light intensity, seed quality, or seedling vigor.

Cultivar impurity would seem the simplest explanation for intermediate reactions. To test this hypothesis, single plants were selected randomly from several cultivars giving intermediate reactions to *Pmg*. The progeny of these plants, when inoculated, gave intermediate reactions similar to those described in the original screening. Apparently, cultivar impurity does not adequately explain intermediate reactions in our screening.

Avere and Athow (3) found isolates of *Pmg* varying in virulence when soybean cultivars were stem-inoculated with mycelium. They reported that weakly virulent isolates gave either nonkilling lesions or no infection when susceptible seedlings were inoculated. Their results are similar to the plant-pathogen combinations classified as partially resistant in our screening. In theory, if a cultivar is susceptible to all races of *Pmg*, a weakly virulent race could result in a moderately susceptible or partially resistant classification. Also, an extremely virulent race might result in the classifications of moderately susceptible or partially resistant even when the cultivar is resistant to that *Pmg* race. Cultivars with known reaction to a particular race must be used to measure relative virulence of an isolate and to determine reaction of cultivars whose reaction is untested. We found that zoospore concentrations of the inoculum also affect the pathogenicity of a race. The concentration of 10^5 zoospores per milliliter was used for all screenings, but this may have been high enough to overwhelm some otherwise *Pmg*-resistant cultivars if the isolate was extremely virulent. Variation in virulence within races may in part explain moderately susceptible or partially resistant reactions, although in most instances, differential cultivars reacted as expected, with other cultivars giving intermediate reactions.

When the cultivar CNS, which carries the gene Rps_2, is inoculated by inserting mycelium of *Pmg* into the hypocotyls of seedlings, some plants are killed, others develop lesions, and still others appear healthy (6). These results are also similar to cultivar classifications of moderately susceptible (S*) or partially resistant (R*) to *Pmg* in our screening (Tables 1 and 2). Cultivars classified as such in our screening should be screened again using the hydroponic inoculation technique used to identify the CNS reaction type of resistance (6).

Paxton and Chamberlain (9) have reported two types of resistance to *Pmg* in soybeans: resistance in young plant tissue (0–2 wk) where the production of a phytoalexin, glyceollin, appears to play a role; and *Pmg* resistance in older tissue that becomes woody because of sclerification of cells. Several cultivars in our screening, including Fuji, Osaya, and Ogemaw, had hypocotyls that were hardened and woody when inoculated 10 days after planting. A morphological barrier may be responsible for the pathogen being partially excluded and could explain the intermediate reactions. Another cultivar, Sanga, in our screening, however, developed hardened and woody hypocotyls early in the seedling stage but did not give intermediate reactions. Although a morphological barrier excluding the pathogen may explain intermediate reactions in some cases, it does not explain such reactions in the majority of cases found in our study.

Several researchers have shown the consistent production of glyceollin to be associated with incompatible soybean-*Pmg* interactions and have considered monogenic resistance the result of this production (5,12). Relative amounts of glyceollin accumulation have also been used as a marker in testing soybean cultivars for resistance to *Pmg* (11). In a preliminary experiment, J. D. Paxton (*personal communication*) found that Kanum, which is moderately susceptible to race 3, accumulated greater amounts of glyceollin when inoculated with this race than did the *Pmg*-susceptible cultivar Williams (Table 1). Apparently, intermediate, moderately susceptible, or partially resistant reactions to *Pmg* could be explained on the basis of a cultivar's ability to produce glyceollin when challenged with a particular race of *Pmg*. Intermediate classifications could result from intermediate accumulation or relative rates of accumulation of glyceollin that are greater than those in susceptible cultivars but less than those in cultivars with resistance to a particular race. More extensive studies are needed to determine if intermediate reactions to *Pmg* are an expression of intermediate glyceollin production.

Environmental factors such as light intensity and temperature may also affect the plant-pathogen interactions. In our screening, plants were grown in the greenhouse and then taken to the laboratory for inoculation. Temperatures were generally higher in the greenhouse than in the laboratory and could have affected the plant's capability to produce glyceollin (4). Temperature and light intensity in the laboratory were lower and varied less than in the greenhouse. These differences probably were not enough to put either plant or pathogen under stress, thus allowing an accurate measure of disease reaction.

Seed quality and seedling vigor varied somewhat among cultivars screened. Cultivars giving intermediate reactions to *Pmg* races were increased in 1980, which should have improved seed quality and vigor over older seed obtained from the germ plasm collection. When these cultivars were inoculated with *Pmg* races again, however, reactions were similar to those of the original screening.

Our study identified soybean cultivars with new sources of resistance to *Pmg*. Many soybean-*Pmg* interactions were also identified that could be classified only as intermediate but neither susceptible nor resistant. These intermediate interactions are best explained by the assumption that these cultivars carry the CNS reaction type of resistance (6).

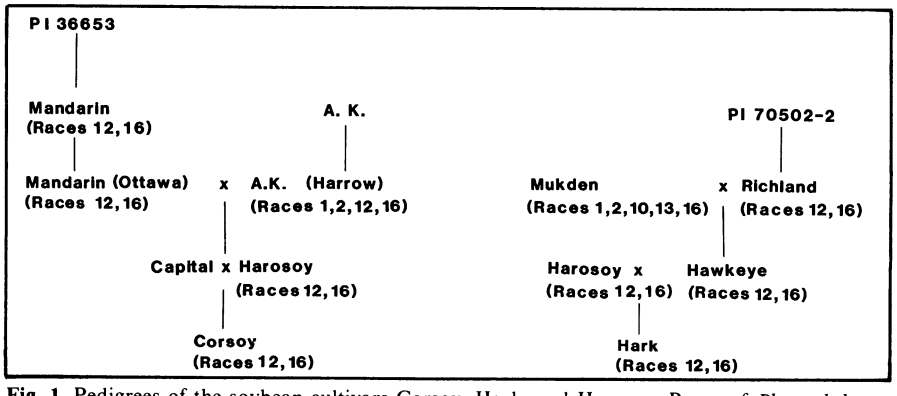

Fig. 1. Pedigrees of the soybean cultivars Corsoy, Hark, and Harosoy. Races of *Phytophthora megasperma* f. sp. *glycinea* to which cultivars are resistant are given in parentheses. PI = Plant Introduction.

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