A Greenhouse Technique for Assessing Phytophthora Root Rot Resistance in *Glycine max* and *G. soja*

D. L. Pazdernik, Department of Crop Sciences, **G. L. Hartman,** USDA, Agricultural Research Service and Department of Crop Sciences, and **Y. H. Huang** and **T. Hymowitz,** Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana 61801-4723

ABSTRACT

Pazdernik, D. L., Hartman, G. L., Huang, Y. H., and Hymowitz, T. 1997. A greenhouse technique for assessing Phytophthora root rot resistance in *Glycine max* and *G. soja*. Plant Dis. 81:1112-1114.

New sources of soybean (*Glycine max*) resistance to *Phytophthora sojae* are needed to provide effective resistance because of the rapidly changing race patterns of *P. sojae* in fields. The objectives of our study were to develop a method to screen *Glycine soja* for resistance to *P. sojae* and then use this methodology to screen *G. soja* lines for resistance to *P. sojae* races 1, 3, and 20. An agar plug-inoculation method, in which a 3-mm-diameter mycelial plug of the fungus was placed mycelium side down on cotyledons of 10-day-old soybean seedlings, was directly compared with the traditional hypocotyl inoculation method. There was no significant difference between the hypocotyl- and plug-inoculation methods when tested on four soybean differential lines using three *P. sojae* races. The plug-inoculation method then was used to screen 430 *G. soja* accessions for resistance to *P. sojae* race 3. Nine *G. soja* accessions were retested with races 1, 3, and 20. Of the 430 *G. soja* accessions tested, 22 accessions had survival rates higher than 75% and nine had rates higher than 90% against race 3. Additionally, five of the nine accessions that were tested again had greater than 60% survival against races 1, 3, and 20. These results suggest that the plug-inoculation method can be used as an alternative to the hypocotyl-inoculation method. Potential sources of new *P. sojae* resistance and/or tolerance may be present in *G. soja,* but additional genetic research is needed to determine if these sources are different from sources currently found in *G. max*.

Additional keywords: soybean disease resistance, soybean germplasm

Phytophthora root rot, caused by *Phytophthora sojae* H.J. Kaufmann & J.W. Gerdemann, is an important soybean (*Glycine max* (L.) Merr.) pathogen, which caused an estimated 560,300-t reduction in soybean yields during 1994 (20). Control of *P. sojae* has been accomplished mainly through the incorporation of race-specific genes. Thirteen single dominant resistance genes (*Rps* genes) have been identified at seven loci, and 45 *P. sojae* races with different reactions against these resistance genes have been described (1,15,18). Compatible races of *P. sojae* possibly have emerged as a result of selection pressure due to the presence of race-specific resistant cultivars (16), leading to the need for alternative sources of *P. sojae* resistance in the soybean germplasm pool. In addition to race-specific resistance, the development of tolerant cultivars, characterized as race-nonspecific, may prevent the buildup of any one specific *P. sojae* race (19). The development of tolerant or partially resistant cultivars with multiple race-specific resistance genes would be optimal for the control of *P. sojae* in soybean.

A fast, accurate, and precise screening method that can be used to identify sources of tolerant, partially resistant, and race-specific resistant soybean germplasm would aid in the development of cultivars for optimal control of *P. sojae*. Many *P. sojae* screening methods have been developed for tolerance (6,7,10,12,17,19) and race-specific resistance screening (4,5,8,9,13). One common tolerance screening method is the mycelium-inoculum-layer method (19), and a common, traditional method for screening large populations and new sources of germplasm for race-specific resistance is the hypocotyl-inoculation method (5).

New sources of soybean germplasm are continually being screened for *P. sojae* resistance by soybean researchers with the hypocotyl-inoculation method and other methods that damage the plant stem. The progenitor of cultivated soybean, a wild annual species (*Glycine soja* Sieb. & Zucc.), can readily hybridize with soybean and may contain novel race-specific and/or tolerance genes to *P. sojae*. However, an initial problem with screening *G. soja* for *P. sojae* resistance is that the stems are thin, and therefore the hypocotyl-inoculation and related methodologies cannot be used.

The objectives of our research were to develop a *P. sojae* screening method for *G. soja* and then screen *G. soja* lines for resistance to *P. sojae* races 1, 3, and 20.

MATERIALS AND METHODS

Alternative *P. sojae* screening method. Four soybean differential lines (Altona, Mack, Harosoy 63, and Harosoy) and three *P. sojae* races (1, 3, and 20) were used in the development of a *P. sojae* screening method that could be used on thin-stemmed seedlings. Two sets of 12 seeds per line were planted in 10-cm-deep flats containing a 1:1 mix of sterilized sand and soil. Following germination, the number of plants was thinned to 10 seedlings per line per set, with all four lines planted in each flat. Each flat contained eight total sets, four lines with two sets per line, of 10 seedlings each. The seedlings were allowed to grow for 10 days in a greenhouse with a 16-h photoperiod, a 26/21°C day/night temperature, and daily watering. One set of each line per flat was inoculated with *P. sojae* race 1, 3, or 20 using the traditional hypocotyl-injection method (5). The other set was inoculated using a new plug-inoculation method as described below with the same races of *P. sojae*.

P. sojae isolates were grown for 14 days on V8 juice agar as previously described (19). A 3-mm cork-borer then was used to cut 3-mm plugs out of the colonies. A single plug, mycelial-side down, was placed on a cotyledon immediately adjacent to the stem of each seedling. One *P. sojae* isolate was used to inoculate seedlings in an entire flat. Following inoculation with the hypocotyl- and plug-methods, all seedlings were lightly misted with water using a hand-atomizer to increase humidity and

Corresponding author: G. L. Hartman
E-mail: ghartman@uiuc.edu

Accepted for publication 17 June 1997.

Publication no. D-1997-0728-04R

then covered with a plastic dome that fit over individual flats. The dome-covered flats were placed about 1 m under black mesh shade cloth (80% light reduction) to prevent heat buildup inside the domes. After 5 days, the domes were removed. Two days later the shade cloth was removed and the number of seedlings that survived or died was counted for each inoculation method. If a plug fell from the cotyledon before infection, the seedling was counted as an escape. Five seedlings per line were injected with V8 juice agar without *P. sojae*, and V8 juice agar plugs without *P. sojae* were placed on an additional five seedlings per line to serve as controls.

The experimental design was a randomized complete block in a split-split plot arrangement with two replicates and 10 plants per replicate. The entire experiment was repeated four separate times. The whole plots were the three *P. sojae* races, the subplots were the four soybean lines, and the sub-sub-plots were the two inoculation methods. Because the percent survival data were binomially and not normally distributed, the data were transformed with the arcsine $(Y)^{1/2}$ transformation. The analysis was performed using Proc Anova within the SAS System, version 6.03 (14).

Screening *G. soja* germplasm. A set of 430 *G. soja* accessions was randomly chosen and tested with *P. sojae* isolate race 3 using the previously described plug-inoculation method. Twelve scarified seeds of each accession were planted in flats and grown under the greenhouse conditions previously described. The soybean differentials Mack and Harosoy 63 were used as controls. Nine *G. soja* accessions were chosen from the 430 accessions based on percent survival and tested in a replicated trial with *P. sojae* races 1, 3, and 20. The soybean differentials Altona, Mack, Harosoy 63, and Harosoy were used as controls. The same plug-inoculation method previously described was used for this experiment.

The experimental design was a randomized complete block in a split-plot arrangement with two replicates and 10 plants per replicate. The entire experiment was conducted three times. The whole plots were the three *P. sojae* races, and the subplots were the nine *G. soja* accessions and four soybean lines. The percent survival data were again binomially and not normally distributed, so the data were transformed with the arcsine $(Y)^{1/2}$ transformation. The analysis was performed using Proc Anova within the SAS System, version 6.03 (14).

RESULTS AND DISCUSSION

Alternative *P. sojae* screening method. Little difference was observed between the hypocotyl- and plug-inoculation methods based on four soybean differential lines and three *P. sojae* races (Table 1). These results suggest that the plug-inoculation method described here could be used as an alternative to hypocotyl injection. Advantages of the plug method include: (i) no seedling injury is inflicted, which prevents false susceptibles from being recorded; and (ii) more plants can be inoculated per unit time compared with the injection method. Disadvantages of the plug method include: (i) the seedlings must be kept moist longer, which requires more intensive care; and (ii) extra care must be taken to prevent the plugs from falling from the cotyledons before infection occurs. If the flats are moved or bumped, plugs could fall from cotyledons, resulting in no infection. This occurred in approximately 2% of our inoculations. Our plug-inoculation method is similar to the method using detached cotyledons (11); however, our method is nonwounding and does not require the removal of cotyledons. In addition, the plug inoculation produces similar results to the hypocotyl inoculation in terms of plant response and subsequent disease ratings, which differs from the detached cotyledon method.

Screening *G. soja* germplasm. Identification of new sources of resistance to *P. sojae* is critical to providing resistant germplasm because of the rapid development of *P. sojae* races (1,2). The initial screening of 430 *G. soja* accessions with *P. sojae* race 3 resulted in 22 accessions (5%) having a survival rate greater than 75% and an additional 28 accessions (7%) having a survival rate between 51 and 75%. Most of the accessions (325 lines or 75%) had a survival rate less than 25%. These results indicate that there may be *P. sojae* resistance, partial resistance, and/or tolerance in a small percentage of the *G. soja* collection to race 3. Further genetic study is required to determine if the *G. soja* lines are either resistant, partially resistant, or tolerant to race 3.

Table 1. Comparison of the hypocotyl- and plug-inoculation methods using four soybean differential lines and three races of *Phytophthora sojae*

Line	Race	Expected reaction	Plant survival (%)[w]		Significance[x]
			Hypocotyl	Plug	
Altona	1	R[y]	100	100	NS[z]
Altona	3	R	94	100	NS
Altona	20	R	88	100	$P > 0.05$
Mack	1	R	97	98	NS
Mack	3	R	100	100	NS
Mack	20	S	11	6	NS
Harosoy 63	1	R	95	93	NS
Harosoy 63	3	S	19	19	NS
Harosoy 63	20	S	2	17	$P > 0.05$
Harosoy	1	S	16	16	NS
Harosoy	3	S	9	14	NS
Harosoy	20	S	5	5	NS

[w] Plant survival is the number of plants that survived 7 days after inoculation divided by the total number of plants tested and then multiplied by 100.
[x] Statistical significance level between the hypocotyl- and plug-inoculation methods for each line by race combination.
[y] R = resistant and S = susceptible to *Phytophthora sojae* based on previous publications (3).
[z] NS = not significant.

Table 2. Percent survival of four *Glycine max* and nine selected *Glycine soja* lines to three races of *Phytophthora sojae*

Soybean line (MG[x])	Races		
	1	3	20
Altona (OO)	85 bc[y,z]	95 a	88 ab
Mack (V)	90 ab	95 a	9 ef
Harosoy 63 (II)	96 ab	9 b	5 f
Harosoy (II)	0 f	8 b	4 f
PI 101404B (II)	92 ab	100 a	4 f
PI 326581 (II)	77 cd	96 a	73 c
PI 339732 (IV)	68 d	91 a	80 bc
PI 366124 (V)	100 a	96 a	19 e
PI 378696B (VI)	21 e	96 a	84 ab
PI 407018 (V)	94 ab	88 a	8 f
PI 407063 (V)	66 d	91 a	94 a
PI 407085 (VI)	96 ab	90 a	62 d
PI 407086 (VI)	74 cd	95 a	80 bc

[x] MG = maturity group.
[y] Percent survival is the number of plants that survived 7 days after inoculation divided by the total number of plants tested and then multiplied by 100.
[z] Means followed by the same letter are not significantly different at $P = 0.05$.

To further characterize the accessions with the highest survival rate, *P. sojae* races 1, 3, and 20 were tested on nine *G. soja* accessions. These nine accessions had a survival rate greater than 90% during the initial screening with *P. sojae* race 3. All nine accessions had greater than 87% survival and no significant difference among accessions for survival rate when retested in a replicated trial with *P. sojae* race 3 (Table 2). Four of the nine accessions had survival rates greater than 90% when tested with *P. sojae* race 1, and accession PI 407063 had a 94% survival when tested with race 20.

Two accessions, PI 378696B and PI 407063, appear to be resistant to *P. sojae* races 3 and 20 but not to race 1 (Table 2). This result has not been documented in *G. max*, at least to our knowledge, suggesting that the resistance genes contained in these two accessions may be different from previously reported genes in *G. max*. Additional research is needed to determine if this source(s) of resistance is different from previously reported *G. max* sources.

Five of the nine accessions had greater than 60% survival when tested against all three *P. sojae* races (Table 2). This result suggests at least three possibilities. The first is that single resistance genes exist in *G. soja* that convey control over a large number of *P. sojae* races. The second is that a certain level of tolerance exists within these accessions. The third is that there is a combination of resistance and tolerance among these accessions. Additional *P. sojae* races need to be tested against these nine accessions to determine the broadness of this resistance and/or tolerance. Also, the resistance–tolerance genetic mechanisms of *G. soja* should be studied and compared with those of *G. max*.

ACKNOWLEDGMENTS

This research was supported in part by the Illinois Agricultural Experiment Station and a C-FAR grant (IDA CFAR 97 I-52CS). We thank Cecil Nickell for providing isolates of the fungus, Randy Nelson for providing germplasm, and Meredith Gardner for scarification of seeds.

LITERATURE CITED

1. Abney, T. S., Melgar, J. C., Richards, T. L., Scott, D. H., Grogan, J., and Young, J. 1997. New races of *Phytophthora sojae* with *Rps*1-d virulence. Plant Dis. 81:653-655.
2. Anderson, T. R., and Buzzell, R. I. 1992. Diversity and frequency of races of *Phytophthora megasperma* f. sp. *glycinea* in soybean fields in Essex County, Ontario, 1980-1989. Plant Dis. 76:587-589.
3. Athow, K. L. 1987. Fungal diseases. Pages 687-727 in: Soybeans; Improvement, Production and Uses, 2nd ed. J. R. Wilcox, ed. American Society of Agronomy Monograph 16, Madison, WI.
4. Buzzell, R. I., and Anderson, T. R. 1992. Inheritance and race reaction of a new soybean *Rps1* allele. Plant Dis. 76:600-601.
5. Haas, J. H., and Buzzell, R. I. 1976. New races 5 and 6 of *Phytophthora megasperma* var. *sojae* and differential reactions of soybean cultivars for races 1 and 6. Phytopathology 66:1361-1362.
6. Irwin, J. A. G., and Langdon, P. W. 1982. A laboratory procedure for determining relative levels of field resistance to *Phytophthora megasperma* f. sp. *glycinea*. Aust. J. Agric. Res. 33:33-39.
7. Jimenez, B., and Lockwood, J. L. 1980. Laboratory method for assessing field tolerance of soybean seedlings to *Phytophthora megasperma* var. *sojae*. Plant Dis. 64:775-778.
8. Kaufmann, M. J., and Gerdemann, J. W. 1958. Root and stem rot of soybean caused by *Phytophthora sojae* n. sp. Phytopathology 48:201-206.
9. Layton, A. C., Athow, K. L., and Laviolette, F. A. 1986. New physiologic race of *Phytophthora megasperma* f. sp. *glycinea*. Plant Dis. 70:500-501.
10. McBlain, B. A., Hacker, J. K., Zimmerly, M. M., and Schmitthenner, A. F. 1991. Tolerance to Phytophthora rot in soybean: II. Evaluation of three tolerance screening methods. Crop Sci. 31:1412-1417.
11. Morrison, R. H., and Thorne, J. C. 1978. Inoculation of detached cotyledons for screening soybeans against two races of *Phytophthora megasperma* var. *sojae*. Crop Sci. 18:1089-1091.
12. Olah, A. F., and Schmitthenner, A. F. 1985. A growth chamber test for measuring Phytophthora root rot tolerance in soybean seedlings. Phytopathology 75:546-548.
13. Rutherford, F. S., Ward, E. W. B., and Buzzell, R. I. 1985. Variation in virulence in successive single-zoospore propagations of *Phytophthora megasperma* f. sp. *glycinea*. Phytopathology 75:371-374.
14. SAS Institute. 1992. SAS/STAT Guide for Personal Computers. 6th ed. SAS Institute, Cary, NC.
15. Schmitthenner, A. F., Hobe, M., and Bhat, R. G. 1994. *Phytophthora sojae* races in Ohio over a 10-year interval. Plant Dis. 78:269-276.
16. Schmitthenner, A. F., and Walker, A. K. 1979. Tolerance versus resistance for control of Phytophthora root rot of soybeans. Pages 35-44 in: Proc. Soybean Seed Res. Conf., 9th. H. D. Loden and K. Wilkenson, eds. American Seed Trade Association, Washington, DC.
17. Tooley, P. W., and Grau, C. R. 1982. Identification and quantitative characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean seedlings. Phytopathology 72:727-733.
18. Tyler, B. M., Forster, H., and Coffey, M. D. 1995. Inheritance of avirulence factors and restriction fragment length polymorphism markers in outcrosses of the oomycete *Phytophthora sojae*. Molecular Plant-Microbe Interact. 8:515-523.
19. Walker, A. K., and Schmitthenner, A. F. 1984. Comparison of field and greenhouse evaluations for tolerance to Phytophthora rot in soybean. Crop Sci. 24:487-489.
20. Wrather, J. A., Anderson, T. R., Arsyad, D. M, Gai, J., Ploper, L. D., Porta-Puglia, A., Ram, H. H., and Yorinori, J. T. 1997. Soybean disease loss estimates for the top 10 soybean producing countries in 1994. Plant Dis. 81:107-110.