Screening Soybean for Resistance to *Heterodera glycines* Ichinohe Using Monoxenic Cultures[1]

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Abstract: A simple, rapid, and inexpensive method for evaluation of host-parasite interactions, based on monoxenic cultures, is described. Axenic root explants of *Glycine max* (L.) Merr., cultured on a holidic agar medium, were inoculated with axenic second-stage larvae of *Heterodera glycines* Ichinohe, Race 3. A clear separation of susceptible and resistant cultivars, based on numbers of mature female nematodes present after 3 wk at 25 C, was observed. The method described should aid researchers in the evaluation of the host response to infection by *H. glycines*.

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Evaluations of soybean (*Glycine max* [L.] Merr.) breeding lines for resistance to *Heterodera glycines* Ichinohe, the soybean cyst nematode (SCN), have been derived primarily from field and greenhouse studies. Basically, the methods involve growing soybean populations in SCN-infested soils using relatively high population densities of cysts and counting the number of adult females on the roots as the criterion for resistance. However, there are inherent problems associated with screening for resistance under these conditions, including lack of a homogeneous nematode population, nonuniform distribution of nematodes in the soil, interference by secondary pathogens, seasonal variation, and high cost.

An early report of soybean grafting experiments indicated that resistance to SCN was "genetically inherent in the entire resistant plant" (5). Therefore, it seemed plausible to utilize axenic root explants to observe soybean-SCN interactions under controlled environmental conditions. This study reports the results of the interactions of 15 soybean cultivars with SCN, Race 3, (SCN) as determined under gnotobiotic conditions.

MATERIALS AND METHODS

Fifteen soybean cultivars were selected from seed stocks at ARS, USDA, Beltsville, Maryland. Ten of the cultivars are generally accepted as susceptible to SCN 3: Dare (3), Delmar (3), Essex (4), Hawkeye (11), Hill (11), Hood (11), Lee (11), Perry (11), Kent, and Williams. The remaining five cultivars were previously reported as resistant to SCN 3: Centennial (3), Dyer (6), Forrest (7), Mack (2), and Pickett (1). Second-stage larvae (L2) were obtained from previously established monoxenic cultures of SCN 3 on Kent (9,10).

Axenic excised root cultures of the soybean cultivars were propagated as previously described (10). Three replicates of each cultivar with three root explants per petri dish were inoculated with 50 ± 2 axenic L2 suspended in 0.1 ml of sterile tap water. Petri dishes were sealed with Parafilm and incubated in the dark at 25 C in an environmental control chamber. Root cultures were examined with a stereomicroscope over a 3-wk period, and the number of adult females and males was determined. The number of adults developing on each cultivar was statistically compared using Duncan's multiple-range test.

RESULTS AND DISCUSSION

The average number of adult females per cultivar varied considerably (Table 1). Of the 10 susceptible cultivars, Essex had significantly ($P = 0.01$) more adult females than Hood, Hill, or Williams. Resistant cultivars Mack, Dyer, Forrest, Pickett, and Centennial had significantly fewer adult females than the susceptible cultivars. Although the average number of adult males ranged from 0.3 to 5.0, there were no significant differences among the means. These results generally agree with those of previ-

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Table 1. Average number of adult females and males of *Heterodera glycines*, Race 3, which developed on axenic root explants from 15 soybean cultivars grown in the dark at 25 C for 3 weeks.

Cultivars	Number of nematodes Females	Males
Essex	36 a*	2.3 a
Dare	28 a b	1.0 a
Kent	27 a b	4.3 a
Lee	27 a b	2.0 a
Perry	25 a b	0.3 a
Delmar	25 a b	3.0 a
Hawkeye	23 a b	2.7 a
Hood	21 b	5.0 a
Hill	19 b	3.7 a
Williams	18 b	2.0 a
Mack	6 c	2.3 a
Dyer	3 c	2.3 a
Forrest	3 c	2.0 a
Pickett	3 c	2.7 a
Centennial	2 c	3.3 a

Numbers followed by a letter in common are not significantly different ($P = 0.01$) according to Duncan's multiple-range test. Fisher's LSD = 11.58 ($P = 0.01$).

ous evaluations of SCN 3 to resistant and susceptible cultivars.

Monoxenic cultures should prove useful in screening and comparing resistance of soybean cultivars to SCN races. The screening method described is rapid, reliable, and relatively inexpensive. Analyses are derived from host-parasite interactions of a homogeneous nematode population. The ability to observe directly SCN development and host-parasite interaction should enhance studies concerning the mechanisms and physiology of resistance. A disadvantage of this method to soybean breeders is the inability to regenerate whole plants from experimental root explants. A modification of the procedure, whereby a plant could be regenerated, would be necessary if the breeder wished to screen F_2 seed. A more likely application of the technique, however, would involve testing later generation plant progeny from which remnant seed of resistant genotypes would be available. Therefore, the described method should provide a valuable aid to plant geneticists, breeders, and nematologists in evaluating resistance to the SCN.

LITERATURE CITED

1. Brim, C. A., and J. P. Ross. 1966. Registration of Pickett soybeans. Crop. Sci. 6:305.

2. Caviness, C. E., R. D. Riggs, and J. H. Walters. 1972. Registration of Mack soybeans. Crop Sci. 12: 396.

3. Caviness, C. E., and R. D. Riggs. 1976. Breeding for nematode resistance-soybeans. Pages 594-601 *in* L. D. Hill, ed. Proceedings of the world soybean research conference. Danville, Illinois: Interstate Printers and Publishers, Inc.

4. Caviness, C. E., H. J. Walters, R. D. Riggs, and D. Boquet. 1976. Soybean varietal performance, 1975. Arkansas Farm Research 25:2.

5. Chambers, A. Y., and J. M. Epps. 1967. Nature of soybean resistance to Heterodera glycines. Plant Dis. Rep. 51:771-774.

6. Hartwig, E. E., and J. M. Epps. 1968. Dyer soybeans. Crop Sci. 8:402.

7. Hartwig, E. E., and J. M. Epps. 1973. Registration of Forrest soybeans. Crop Sci. 13: 287.

8. Hartwig, E. E., and J. M. Epps. 1977. Registration of Centennial soybeans. Crop Sci. 17:979.

9. Lauritis, J. A., R. V. Rebois, and B. Y. Endo. 1981. Monoxenic culture of Heterodera glycines propagated on root cultures of susceptible soybean. J. Nematol. 13:447 (Abstr.).

10. Lauritis, J. A., R. V. Rebois, and L. S. Graney. 1982. Technique for gnotobiotic cultivation of Heterodera glycines Ichinohe on Glycine max (L.) Merr. J. Nematol. 14: 422-424.

11. Riggs, R. D., and M. L. Hamblen. 1962. Soybean-cyst nematode host studies in the family Leguminosae. Arkansas Agric. Exp. Stn. Report Series 110.