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# Resistance Genes in a 'Williams 82' × 'Hartwig' Soybean Cross to an Inbred Line of *Heterodera glycines*[1]

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*Abstract:* The number of resistance genes in soybean to soybean cyst nematode (SCN) *Heterodera glycines* was estimated using progeny from a cross of 'Williams 82' × 'Hartwig' (derived from 'Forrest'[3] × PI 437.654) screened with a fourth-generation inbred nematode line derived from a race 3 field population of SCN. Numbers of females developing on roots of inoculated seedlings were assigned to phenotype cells (resistant, susceptible, or segregating) using Ward's minimum variance cluster analysis. The ratio obtained from screening 220 $F_3$ soybean families was not significantly different from a 1:8:7 (resistant:segregating:susceptible) ratio, suggesting a two-gene system for resistance. The ratio obtained from screening 183 $F_2$ plants was not significantly different from a 3:13 (resistant:susceptible) ratio, indicating both a dominant (Rhg) and a recessive (rhg) resistance gene.

*Key words: Glycine max,* Hartwig, *Heterodera glycines,* PI 437.654, resistance soybean, soybean cyst nematode, soybean resistance gene.

Soybean cyst nematode (SCN) *Heterodera glycines* is the most serious disease of soybean in the north-central region of the United States (4). The increasing cost of nematicides and regulatory loss of the most effective chemicals have made planting of SCN-resistant soybean cultivars the primary and most economical management option for most growers. The number of genes involved and the nature of resistance have been postulated from a number of studies based on assessment of the phenotypic (resistant or susceptible) behavior with different nematode populations and plant cross combinations (2,3,11,13–15). Most studies have been carried out with field populations of SCN that are naturally heterogeneous, and none have used true SCN inbreds. The variability of SCN field populations is well documented (5,6,12,16). The goals of this study were to determine whether reduction of SCN variability by using homogeneous nematode inbreds can lead to a better understanding of soybean resistance to SCN, and to document the number and nature of resistance genes in the soybean cv. Hartwig (1).

## Materials and Methods

*Nematode inbred:* A true SCN inbred, obtained by sib-mating (9), was developed on the susceptible soybean cv. Essex by one of us (JMH) from a race 3 wild-type population from a South Carolina soybean field. Subsequently, the inbred was advanced to the $F_4$ generation (fourth sib-mating cross) at Purdue University. Each generation of inbreeding was carried out using 128 cavity seedling trays (cavity size 3 × 3 × 4.5 cm) filled with a 3 sand:1 soil mix. Two randomly chosen second-stage juveniles (J2) hatched from cysts were placed near the root of a 4-day-old seedling in a new cavity. The soybean plants were pruned periodically to minimize growth and reduce water use (8). After 3 months, plants were examined for presence of females on the roots, and a success rate of 1–3% was obtained. Each infested plant was transplanted into a 10-cm-d clay pot (3 sand:1 soil mix) along with new plants to increase the population. The inbreeding step was repeated three more times. At the $F_4$ generation, cysts were increased to provide inoculum for the phenotypic screening. A

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race test was performed (7,17) with 'Williams 82' as the reference susceptible.

*Plant crosses:* 'Williams 82' × 'Hartwig' (derived from 'Forrest'[3] × PI 437.654) crosses were made during the summer of 1992 to produce hybrids from which about 100 $F_1$ seeds were harvested. One third of these $F_1$ seeds were planted in the greenhouse to produce $F_2$ seeds. These seeds were bulked, and about 220 seeds were chosen randomly to produce $F_3$ seed.

*Phenotype evaluations:* Screening began with $F_3$ families, with each test replicated four times. Replicates were started about 2 weeks apart. Inoculum was prepared freshly by dissolving cyst wall and gelatinous matrix with a 50% solution of commercial bleach (5.25% sodium hypochlorite) to release eggs (5). Inoculum density for each replication was adjusted to 3,000 eggs and J2/ml. For each replication, two seeds from each family were germinated in sterilized sand in a 5-cm-d pot. The most vigorous 5-day-old seedling, one for each of the 220 $F_3$ families, was placed in a 2.5-cm-d × 7.5-cm-long glass tube containing 10 ml of water mixed with 1 ml of nematode inoculum. A sand-soil mix was added to cover the roots. Tubes were placed in a water bath designed to maintain a root zone temperature of 24 C, and the plants were allowed to grow for approximately 30 days. Plants were fertilized every 2 weeks with a 0.04% solution of 20-20-20 (NPK) fertilizer. At the end of this incubation period, each plant was washed out of its tube and developing females dislodged with a jet of water. The number of females developing on the root system was recorded. Each seedling was then replanted in a 15-cm-d pot and grown to produce $F_4$ seeds. A total of 183 $F_2$ and 17 $F_1$ seedlings were screened in the same fashion. Resistant and susceptible parents were included in each test.

*Analysis:* Numbers of females developing on roots were standardized using a $\log_{10}(x + 1)$ transformation and then subjected to SAS Ward's minimum variance cluster analysis (18). Data for the $F_3$ families were separated into cells of resistant, segregating, and susceptible phenotypes. Data for $F_2$ plants were assigned to either resistant or susceptible cells. Goodness of fit with appropriate genetic ratios was tested using chi-square.

## Results

In the race screening test, the nematode inbred used for this study behaved as a race 3 population. Indices of parasitism ([number of cysts developed on test differential/number of cysts on 'Williams 82'] × 100) for PI 88.788, PI 90.763, 'Peking,' and 'Pickett 71' were 3.9%, 0.4%, 2.5%, and 6.1%, respectively.

Numbers of developing females on individual $F_3$ plants ranged from 0 to 544. Average counts from four replications from each $F_3$ family ranged from 0 to 345. When Ward's minimum variance cluster analysis was used to assign means of transformed $F_3$ family data to one of three classes, the algorithm placed 12 families in the resistant cell, 99 in the segregating cell, and 109 in the susceptible cell. This 12:99:109 observation was tested for deviation from the two- and three-gene expected ratios. Chi-square analysis indicated that the observed ratio was not significantly different from the expected ratio for a two-gene system (Table 1). All 12 families within the resistance cell averaged 0 cyst. Within the segregating and susceptible cells, the average number of cysts per family ranged from 0.3 to 110 and from 111 to 345, respectively.

All 17 $F_1$ plants produced a susceptible response (Table 1). Ward's minimum variance cluster analysis divided the 183 $F_2$ plants into resistant and susceptible cells containing 34 and 149 plants, respectively (Table 1). The 34:149 observed ratio was tested for deviation from the ratios of several two-gene systems and was not significantly different from a single dominant and single recessive genetic model (Table 1).

## Discussion

The cv. Hartwig ('Forrest'[3] × PI 437.654), which was released as a Maturity

TABLE 1. Reaction of $F_1$, $F_2$, and $F_3$ soybean progeny from the cross 'Williams 82' × 'Hartwig' to an inbred of *Heterodera glycines* race 3.

| | Number of plants or families | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Observed | | | Expected | | | | | |
| Generation | R | Seg | S | R | Seg | S | Hypothesized genes | Ratio | $\chi^2$ |
| $F_1$ | 0 | — | 17 | 0 | — | 17 | | 0:1 | — |
| $F_2$ | 34 | — | 149 | 11 | — | 172 | rhg,rhg | 1:15 | 51.2 |
| | | | | 41 | — | 142 | Rhg,rhg | 3:13 | 1.5* |
| $F_3$ | 12 | 99 | 109 | 14 | 110 | 96 | 2 genes | 1:8:7 | 3.2* |
| | | | | 3 | 89 | 128 | 3 genes | 1:26:37 | 30.9 |

R = Resistant, Seg = Segregating, S = Susceptible.
* = Not significantly different from the expected ratio ($\alpha = 0.05$).

Group V SCN-resistant cultivar, was reported to be resistant to all known races of soybean cyst nematode (1). It appears that Hartwig will be incorporated as a source of SCN resistance in many new soybean cultivars. Thus, a clear understanding of its resistance gene(s) is desirable.

Genetic diversity and information about the number of genes involved in soybean resistance to SCN have been reported by several authors (2,3,11,14,15). Myers and Anand (13) discussed the phenotypes obtained with the progeny of a cross between 'Essex' and PI 437.654. In response to a race 3 field population, they reported results that fit a genetic model with one dominant and two recessive resistance genes. However, this study's data indicated a two-gene system in the 'Williams 82' × 'Hartwig' cross. This difference might be explained in two different ways. One explanation might be the presence of a common resistance locus shared by 'Williams 82' and 'Hartwig' based on an earlier suggestion (10) that 'Williams' might possess a resistance gene. Another possible explanation is that 'Hartwig' did not inherit all of the resistance genes present in PI 437.654. Comparative data from other 'Hartwig' crosses will be necessary to resolve the issue.

The genetic complexity and heterogeneity of SCN field populations have been obstacles in understanding the nature of soybean resistance to SCN. In addition, considerable variability can be introduced via different methods of inoculating plants, assessing phenotypes and the unbiased division of entries into resistant, segregating, and susceptible phenotypes. We believe that the ability to reduce a significant portion of this variability will allow a more accurate estimation of the genetic basis of resistance. The use of advanced true inbreds as inoculum, rather than heterogeneous field populations of SCN, is an important step toward reducing variability and producing more reliable and accurate data (9). In this study, a fourth-generation true inbred SCN line produced little variability among replications of parental screens included with every test.

The utilization of minimum variance cluster analysis to produce an unbiased separation among cells eliminates arbitrary designation of phenotypes. Unambiguous assignment of phenotypes is important in both the accurate estimation and the molecular mapping of resistance genes. The unbiased division is especially difficult in $F_3$ analyses, since $F_3$ families are not genetically fixed and segregation may still be occurring in many families. In most studies, the separation of samples into resistant, segregating, and susceptible cells is by nonstatistical methods. These nonstatistical divisions may be arbitrary or biased. Ward's minimum variance analysis, an unbiased statistical method for separating phenotypes, is applicable to all mapping populations because of the flexibility of designating the number of phenotypic cells.

Our analysis started with $F_3$ families because, unlike $F_2$ individuals, they may be

replicated. Replication plus use of SCN inbreds reduces the chance of misclassifying a family's phenotype. Comparison of the observed $F_3$ ratio to the appropriate two- and three-gene expected ratios showed that, in this cross, resistance was conferred by a two-gene system. Since all $F_1$ plants were susceptible, we knew resistance was not conferred by dominant gene(s) only. Analysis of an $F_2$ population clearly showed a dominant and a recessive gene system. Further analyses are needed to molecularly map these genes for their efficient use in developing new SCN resistant cultivars and to determine the uniqueness of these resistance genes.

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