Soybean Aphid Resistance in Soybean Jackson Is Controlled by a Single Dominant Gene

Curtis B. Hill,* Yan Li, and Glen L. Hartman

ABSTRACT

The soybean aphid, *Aphis glycines* Matsumura, has become established as a serious pest of soybean, *Glycine max* (L.) Merr., since it was first found in North America in 2000 and has caused millions of dollars in economic losses. While the application of chemical insecticides is the only means to control the soybean aphid at present, genetic resistance to the aphid was recently discovered in soybean. A single dominant gene named *Rag1* that controls resistance to the soybean aphid was found in the cultivar Dowling. Another cultivar found to have strong antibiosis-type resistance to the soybean aphid was Jackson. The primary objective of this study was to determine the inheritance of resistance to the soybean aphid in Jackson. Segregation of resistance was analyzed in F2 and among F2–derived F3 families produced from crosses between Jackson and the susceptible soybean cultivar Loda. Segregation of F2 plants was 247 resistant to 97 susceptible and fit a 3:1 genetic ratio (*P* = 0.17). Segregation among F2:3 families was not clear because a number of susceptible F2 plants did not produce a sufficient amount of seed for progeny testing. Ignoring the susceptible class, the segregation of F2:3 families fit a 1:2 (all resistant/segregating) ratio. These results indicated that a single dominant gene controlled resistance in Jackson. There is no known genetic relationship between Jackson and Dowling. The genetic relationship between *Rag1* in Dowling and the gene in Jackson is unknown.

The soybean aphid was found in North America in 2000 (Hartman et al., 2001) and has spread throughout the main soybean production areas (Ragsdale et al., 2004). The pest caused extensive economic losses in soybean in several midwestern states in 2003. Nearly 1.6 million ha of soybean were damaged in Minnesota with an estimated loss of US$80 million (Associated Press, 2003). In Illinois, about 0.5 million ha were damaged with an estimated loss of US$45 million (Steffey, 2004). Nearly 3 million ha of soybeans were sprayed to control the soybean aphid in the USA in 2003 (Landis et al., 2003), with US$9 to 12 million spent in Illinois alone (Steffey, 2004). Until the recent discovery of plant resistance to the aphid (Hill et al., 2004), chemical insecticide application was the only available means to control the pest.

Plant insect resistance is an important component of an integrated pest management program to control insects (Auclair, 1989; Harrewijn and Minks, 1989). It is a cost effective and environmentally safe control method (Luginbill, 1969), and it is a plant trait governed by the same genetic mechanisms that condition other plant traits (Auclair, 1989).

Strong antibiosis-type resistance to the soybean aphid was found in soybean germplasm, including the cultivars Dowling and Jackson (Hill et al., 2004; Li et al., 2004). There is no known genetic relationship between Dowling and Jackson (Hill et al., 2004). Resistance in the ancestral soybean cultivar Dowling was controlled by a single dominant gene named *Rag1* (Hill et al., 2006). Inheritance of resistance in Jackson has not been determined.

Knowledge on the inheritance of insect resistance is useful in the design of appropriate breeding procedures to develop resistant cultivars and for the identification of biotypes that may already exist or develop over time (Smith, 1989). Because qualitative, or simply inherited traits, require different breeding methods than quantitative traits controlled by many genes, the objective of this study was to determine the inheritance of soybean aphid resistance in Jackson.

MATERIALS AND METHODS

Cosses were made between the soybean aphid resistant cultivar Jackson and the susceptible soybean cultivar Loda as previously described (Hill et al., 2006). All crosses were made in one direction; pollen from Loda was transferred onto stigmata of Jackson flowers. Seed produced from individual crosses was harvested and planted separately for F1 seed production in a greenhouse maintained at 28°C with supplemental lighting provided by a mixture of 1000-W high intensity discharge and high pressure sodium vapor lamps set to give a 14-h photoperiod. F1 hybrid plants were distinguished from selves by the expression of flower color that was polymorphic among the parents. Loda had purple flowers whereas Jackson had white flowers. Purple flower is dominant over white (Takahashi and Fukuyama, 1919).

The parents and F1 plants were tested for soybean aphid resistance in a choice test in the greenhouse. Methods for plant culture, aphid infestation, and experimental design were described previously (Hill et al., 2004, 2006). Seeds were planted at a rate of one seed per pot in soilless media (Sunshine Mix, LC1, Sun Gro Horticulture Inc., Bellevue, WA) in 48-pot plastic inserts, with 12 rows of four pots (Hummert International, Earth City, MO, no. 1204) contained in flats without drainage holes (Hummert International, Earth City, MO, no. F1020). Genotypes were planted in four-pot rows. Ninety rows of the F2 population, six rows of Jackson, and 13 rows of Loda were randomized and interspersed throughout the test. Three weeks after aphid infestation, the level of aphid colonization on each individual plant was estimated by visually examining aphid density, aphid mortality, and plant damage on leaves and stems using the following 0-to-4 scale, where 0 = no aphids
present; 1 = few solitary live or dead aphids (dead aphid bodies present); 2 = several transient aphids (aphids possibly probing for a suitable feeding site present) with some viviparous aptera surrounded by a few nymphs; 3 = dense colonies; and 4 = dense colonies accompanied by plant damage, including leaf distortion and stunting (Hill et al., 2006). Plants in the F2 population were considered resistant when they had the phenotype of the resistant parent Jackson (rating 0, 1, or 2) and susceptible when they had the susceptible Loda phenotype (rating 3 or 4).

All F2 plants were transplanted to produce F2:3 seed (F2-derived F3 lines) for progeny testing and genotyping using previously described methods (Hill et al., 2006). Progeny from all F2 plants that produced at least 12 F3 seeds, regardless of F2 soybean aphid resistance phenotype, were planted with the parents and tested for aphid resistance in randomized four-pot rows as described above. Of the 344 F2 plants transplanted, 172 plants produced the minimum required 12 seeds. A minimum of 10 F2:3 plants was required to determine the F3 genotypes. There were 149 total F2:3 families out of the 172 planted that had at least 10 viable plants to rate for aphid resistance.

χ² tests were performed to test the goodness of fit of observed segregations among F2 plants and F2:3 families with different genetic ratios. Segregation among F2 families with a minimum of 10 plants was analyzed after classifying each family as homozygous resistant (all plants had a rating of 0 to 2), homozygous susceptible (all plants had a rating 3 to 4), and heterozygous (both resistant and susceptible plants were identified).

RESULTS

Expression of resistance in the Jackson × Loda F2 and F2:3 populations was qualitative. The frequency distribution of aphid colonization ratings was non-normal and skewed toward rating 1 (data not shown). Aphid colonization ratings for Jackson plants were 0, 1, or 2 and ratings for Loda were 3 or 4. Only the parental phenotypes were observed in the F2. Segregation of the F2 population was 247 resistant plants and 97 susceptible plants and fit a 3:1 ratio (P = 0.17).

From the randomly selected 149 Jackson × Loda F2:3 families, there were only 26 F2:3 families from F2 plants scored as susceptible that were included in the progeny tests. The ratio of 123:26 did not fit a 3:1 ratio and did not fully represent the F2 population. Progeny from susceptible F2 plants were under-represented in the F3 generation, probably because many susceptible F2 plants did not produce the minimum 12 seeds required for the progeny test. The plants may have been weakened by the aphid feeding damage and did not recover sufficiently after killing the aphids with insecticide after transplanting. The under-representation of susceptible F2 plants in the F3 generation prevented testing the fit to a complete 1:2:1 ratio with F2:3 families from susceptible F2 plants. Therefore, the fit of the segregation of just the F2:3 families from resistant F2 plants to a 1:2 resistant segregating ratio was tested. In the F2 generation, the following were found: 42 families with all resistant plants, 86 families with both resistant and susceptible plants, and 21 families with all susceptible plants (Table 1). The ratio of 42:86 resistant segregating or heterozygous F2:3 families significantly fit the 1:2 resistant segregating (heterozygote) ratio expected for a monogenic dominant gene (P < 0.001). Out of 26 F2 plants that were scored as susceptible, four F2 plants were actually heterozygous and one was homozygous for resistance and not susceptible, as originally scored in the F2 generation. Therefore, there was about a 19% rate of error in scoring susceptible F2 plants. Applying the 19% error rate for susceptible plants to the original F2 data gives an adjusted ratio of 265:79 resistant/susceptible F2 plants. This ratio also fit a 3:1 resistant/susceptible ratio (P = 0.38).

DISCUSSION

Segregation of Jackson × Loda F2 plants (247:97 resistant/susceptible) for resistance to the soybean aphid fit a 3:1 monogenic dominant inheritance pattern in the initial analysis before F2 progeny testing (P = 0.17) as well as after correcting the observed ratio for the error rate in scoring susceptible F2 plants that was found after progeny testing (P = 0.38). Analysis of the genotypes of the F2 plants identified after progeny testing was weakened by the under-representation of progeny from susceptible plants in the analysis. However, the ratio of 41 F2:3 families found with all resistant plants (homozygous resistant F2 plants) with 82 segregating families (heterozygous resistant F2 plants) from the total 123 progenies from resistant F2 plants that were tested, significantly fit a 1:2 homozygous resistant/heterozygous resistant ratio (P < 0.001). The results of this analysis together with the F2 phenotype analysis indicated that a single dominant gene controls resistance in Jackson.

According to information provided in GRIN, the Germplasm Resources Information Network, Jackson and Dowling have no known genetic relationship (Hill et al., 2004). It is possible that the soybean aphid resistance gene found in Jackson and Rag1 found in Dowling are unique and nonallelic; however, genetic allelism tests that involve analysis of segregation for aphid resistance in F2 plants from crosses between the two cultivars or lines homozygous for each gene are required to determine the genetic relationship between the genes. No known biotypes of A. glycines exist that

<table>
<thead>
<tr>
<th>F2 plant phenotype†</th>
<th>No. of F2 plants</th>
<th>F2 plant genotype‡</th>
<th>No. of F2:3 families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant (0–2 rating)</td>
<td>247 RR (all F2:3 plants resistant)</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Susceptible (3–4 rating)</td>
<td>97 RR (all F2:3 plants resistant)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

† Resistant = 0, 1, or 2 aphid colonization rating, where 0 = no aphids present, 1 = few solitary live or dead aphids present, 2 = several transient aphids present with some viviparous aptera surrounded by a few nymphs. Susceptible = 3 or 4 aphid colonization rating, where 3 = dense colonies and 4 = dense colonies with plant damage, including leaf distortion and stunting.
‡ Twelve seeds from each F2 plant were sown for testing and a minimum of 10 F2:3 plants were required to determine F2 genotypes.

<table>
<thead>
<tr>
<th>Total</th>
<th>344</th>
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<tr>
<td>Number</td>
<td>149</td>
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Table 1. Segregation of F2:3 progeny from Jackson × Loda F2 plants for resistance to the soybean aphid 21 d after infestation.
are distinguished by the reactions of plants possessing these genes (Hill et al., 2004). Due to the simple inheritance of the gene found in Jackson and the ease of distinguishing resistant from susceptible plants in aphid resistance bioassays, soybean breeders will be able to rapidly convert existing soybean cultivars into aphid resistant cultivars using efficient back cross breeding procedures. Breeders now have two sources of resistance with dominant soybean aphid resistance genes to use to combat the soybean aphid.

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REFERENCES


