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

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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 F_2 and among F_2–derived F_3 ($F_{2:3}$) families produced from crosses between Jackson and the susceptible soybean cultivar Loda. Segregation of F_2 plants was 247 resistant to 97 susceptible and fit a 3:1 genetic ratio ($P = 0.17$). Segregation among $F_{2:3}$ families was not clear because a number of susceptible F_2 plants did not produce a sufficient amount of seed for progeny testing. Ignoring the susceptible class, the segregation of $F_{2:3}$ 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.

T HE 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 soybean aphid (Hill et al., 2004), chemical insecticide application was the only available means to control the pest.

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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

Crosses 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 F_2 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. F_1 hybrid plants were distinguished from selfs 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 F_2 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 F_2 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 F_2 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 F_2 plants were transplanted to produce $F_{2:3}$ seed (F_2–derived F_3 lines) for progeny testing and genotyping using previously described methods (Hill et al., 2006). Progeny from all F_2 plants that produced at least 12 F_3 seeds, regardless of F_2 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 F_2 plants transplanted, 172 plants produced the minimum required 12 seeds. A minimum of 10 $F_{2:3}$ plants was required to determine the F_2 genotypes. There were 149 total $F_{2:3}$ families out of the 172 planted that had at least 10 viable plants to rate for aphid resistance.

χ^2 tests were performed to test the goodness of fit of observed segregations among F_2 plants and $F_{2:3}$ families with different genetic ratios. Segregation among $F_{2:3}$ 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 F_2 and $F_{2:3}$ 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 F_2. Segregation of the F_2 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 $F_{2:3}$ families, there were only 26 $F_{2:3}$ families from F_2 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 F_2 population. Progeny from susceptible F_2 plants were under-represented in the F_3 generation, probably because many susceptible F_2 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 F_2 plants in the F_3 generation prevented testing the fit to a complete 1:2:1 ratio with $F_{2:3}$ families from susceptible F_2 plants. Therefore, the fit of the segregation of just the $F_{2:3}$ families from resistant F_2 plants to a 1:2 resistant/segregating ratio was tested. In the F_3 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 $F_{2:3}$ families significantly fit the 1:2 resistant/segregating (heterozygote) ratio expected for a monogenic dominant gene ($P < 0.001$). Out of 26 F_2

Table 1. Segregation of $F_{2:3}$ progeny from Jackson × Loda F_2 plants for resistance to the soybean aphid 21 d after infestation.

F_2 plant phenotype†	No. of F_2 plants	F_2 plant genotype‡	No. of $F_{2:3}$ families
Resistant (0–2 rating)	**247**	**RR (all $F_{2:3}$ plants resistant)**	**41**
		Rr (resistant and susceptible $F_{2:3}$ plants)	**82**
		rr (all $F_{2:3}$ plants susceptible)	**0**
Susceptible (3–4 rating)	**97**	**RR (all $F_{2:3}$ plants resistant)**	**1**
		Rr (resistant and susceptible $F_{2:3}$ plants)	**4**
		rr (all $F_{2:3}$ plants susceptible)	**21**
Total	**344**		**149**

† 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 F_2 plant were sown for testing and a minimum of 10 $F_{2:3}$ plants were required to determine F_2 genotypes.

plants that were scored as susceptible, four F_2 plants were actually heterozygous and one was homozygous for resistance and not susceptible, as originally scored in the F_2 generation. Therefore, there was about a 19% rate of error in scoring susceptible F_2 plants. Applying the 19% error rate for susceptible plants to the original F_2 data gives an adjusted ratio of 265:79 resistant/susceptible F_2 plants. This ratio also fit a 3:1 resistant/susceptible ratio ($P = 0.38$).

DISCUSSION

Segregation of Jackson × Loda F_2 plants (247:97 resistant/susceptible) for resistance to the soybean aphid fit a 3:1 monogenic dominant inheritance pattern in the initial analysis before F_2 progeny testing ($P = 0.17$) as well as after correcting the observed ratio for the error rate in scoring susceptible plants that was found after progeny testing ($P = 0.38$). Analysis of the genotypes of the F_2 plants identified after progeny testing was weakened by the under-representation of progeny from susceptible plants in the analysis. However, the ratio of 41 $F_{2:3}$ families found with all resistant plants (homozygous resistant F_2 plants) with 82 segregating families (heterozygous resistant F_2 plants) from the total 123 progenies from resistant F_2 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 F_2 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 F_2 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

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

Associated Press. 2003. Aphids whittling soybean farmers' profits [Online]. Available at www.cnn.com/2003/US/Midwest/11/25/soybean.aphids.ap (posted 25 November 2003; verified 24 Mar. 2006).

Auclair, J.L. 1989. Host plant resistance. p. 225–265. *In* A.K. Minks and P. Harrewijn (ed.) Aphids: Their biology, natural enemies, and control. Vol. C. Elsevier, New York.

Harrewijn, P., and A.K. Minks. 1989. Integrated aphid management: General aspects. p. 267–272. *In* A.K. Minks and P. Harrewijn (ed.) Aphids: Their biology, natural enemies, and control. Vol. C. Elsevier, New York.

Hartman, G.L., L.L. Domier, L.M. Wax, C.G. Helm, D.W. Onstad, J.T. Shaw, L.F. Solter, D.J. Voegtlin, C.J. D'Arcy, M.E. Gray, K.L. Steffey, S.A. Isard, and P.L. Orwick. 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control [Online]. Available at www.plantmanagementnetwork.org/pub/php/. Plant Health Progress DOI 10.1094/PHP-2001-0205-01-HN.

Hill, C.B., Y. Li, and G.L. Hartman. 2004. Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44:98–106.

Hill, C.B., Y. Li, and G.L. Hartman. 2006. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci. 46:1601–1605.

Landis, D., M. Brewer, and G. Heimpel. 2003. Soybean aphid parasitoid questionnaire 2003 [Online]. Available at www.ncera125.ent.msu.edu/StateRpts2003MI.htm (verified 23 Mar. 2006) Michigan State Univ., East Lansing.

Li, Y., C.B. Hill, and G.L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera: Aphididae). J. Econ. Entomol. 97: 1106–1111.

Luginbill, J.P. 1969. Developing resistant plants—The ideal method of controlling insects. USDA, ARS. Prod. Res. Rep. 111. U.S. Gov. Print. Office, Washington, DC.

Ragsdale, D.W., D.J. Voegtlin, and R.J. O'Neil. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97:204–208.

Smith, C.M. 1989. Plant resistance to insects: A fundamental approach. John Wiley & Sons, New York.

Steffey, K.L. 2004. Soybean aphids in Illinois [Online]. www.ipm.uiuc.edu/fieldcrops/insects/soybean_aphids/workshop/SoybeanAphidsInIllinois_Steffey.ppt (verified 18 Mar. 2006). Univ. of Illinois at Urbana-Champaign.

Takahashi, H., and J. Fukuyama. 1919. Morphological and genetic studies on the soybean. 10. Hokkaido Agric. Exp. Stn., Hokkaido, Japan.