Influence of the *Rag1* and *Rag2* genes on aphid resistance and agronomic performance of soybean lines

Shaylyn L. Wiarda, graduate student, Agronomy, Iowa State University; Walter R. Fehr, distinguished professor, Agronomy, Iowa State University; Matthew E. O'Neal, associate professor, Entomology, Iowa State University

Introduction

Aphids have been causing economic damage to soybean in the United States since 2000 (Hartman et al., 2001). High aphid density on soybeans causes the yield to decrease due to plant damage including leaf puckering, plant stunting, reduced pod and/or seed counts, and smaller seeds (Rice et al., 2008). In addition, the black sooty mold fungus that grows on aphid honeydew has a negative influence on soybean performance by reducing photosynthesis (Beckendorf et al., 2008).

Insecticides have been used to manage aphid populations in commercial soybean production (Ragsdale et al., 2007). An alternative method of control would be to develop aphid resistant cultivars. Use of soybean cultivars with aphid resistance could reduce the amount of insecticide used and its damage to beneficial insects (Hill et al., 2006). In addition, organic farmers who cannot use broad-spectrum insecticides available to conventional soybean growers could use resistant non-GMO cultivars. Genes that confer resistance to soybean aphids through antibiosis have been identified in the soybean germplasm. Currently, the *Rag1* gene is commercially available in both GMO and non-GMO cultivars. However, populations of soybean aphids that can survive on *Rag1* have been found on a limited basis (Kim et al., 2008; Hill et al., 2010). To what extent this single gene can provide protection in light of these biotypes is not clear. Furthermore, it is not yet known if additional genes will be needed to maintain or even improve upon the resistance provided by *Rag1*. We investigated whether the performance of *Rag1* against soybean aphids under field conditions is improved with additional sources of aphid resistance.

The two genes for aphid resistance used in our study were *Rag1* and *Rag2*. The *Rag1* gene was found in the cultivar Dowling and the *Rag2* was identified in PI 200538 by scientists of the USDA-ARS and the University of Illinois at Urbana Champaign (UIUC) (Hill et al., 2006; Hill et al., 2009). The *Rag1* gene has been found to reduce aphid development in soybean lines without negatively influencing their agronomic and seed traits (Li et al., 2004; Kim and Diers, 2009; Mardorf et al., 2010). No data have been reported on the influence of the *Rag2* gene alone or in combination with the *Rag1* gene on aphid development or agronomic performance of soybean. One objective of our research was to compare aphid development on soybean lines with the *Rag1* and *Rag2* genes together, the *Rag1* gene alone, the *Rag2* gene alone, or neither resistance gene. A second objective was to assess the agronomic performance of the lines in the four combinations of the resistance genes.

Materials and methods

The parent lines used to develop the backcross population for our study were A08-123074 and LD08-89051a. A08-124074 was a BC2F2-derived line with the *Rag1* gene developed at Iowa State University. The donor of the *Rag1* gene was LD05-16521 developed at UIUC. The recurrent parent in the backcross was IA3027, a cultivar developed at Iowa State University. The line LD08-89051a with the *Rag2* gene was developed at UIUC.

The cross of A08-123074 with LD08-89051a was made at Santa Isabel, PR, during March 2009. The F1 seeds and seeds of A08-123074 were planted at the Agronomy and Agricultural Engineering Research Center near Ames, IA, during the summer of 2009. Three F1 plants were confirmed as hybrids through molecular analysis conducted by the laboratory of Brian Diers at UIUC with the SSR marker Sct_033. The F1 hybrid plants were used as males for backcrossing to A08-123074 to obtain 35 BC1F1 seeds.

The BC1F1 seeds were planted at Santa Isabel, PR, in October 2009. Nine BC1F1 plants were found to be heterozygous for both *Rag1* and *Rag2* through molecular analysis conducted by the laboratory of Brian Diers at UIUC. The SSR marker Satt 540 was used to select for *Rag1* and Sct_033 was used to select for *Rag2*. The double heterozygous plants were harvested individually to obtain BC1F2 seed.

The BC1F2 seeds from the nine BC1F1 individuals were planted as families at Santa Isabel, PR, in January 2010 (Table 1). IA3027RA1, an Iowa State cultivar with the *Rag1* gene, and LD08-89051a were planted as checks. The plants were genotyped at Iowa State Univ. for *Rag1* with the TaqMan assay for ss107913360 developed at UIUC (Kim et al., 2009). The TaqMan assay for *Rag2* was KS9-3 also developed at UIUC (Kim et al., 2010). The genotypic combinations selected were *Rag1Rag1/Rag2Rag2*, *Rag1Rag1/Rag2Rag2*, *Rag1Rag1/Rag2Rag2*, and *Rag1Rag1/Rag2Rag2* (Table 1). The selected plants were harvested individually. The BC1F2:3 seeds were used for two experiments.

Table 1. Number of lines of each genotype from nine BC1F1 families.

	Genotype*			
AX # (Family)	**R1/R2**	**R1/S2**	**S1/R2**	**S1/S2**
AX22055-D1-5	5	6	11	5
AX22055-D1-11	9	8	11	12
AX22055-D2-18	6	4	5	10
AX22055-D2-19	7	7	2	9
AX22055-D1-19	13	10	5	5
AX22055-D2-1	11	6	16	3
AX22055-D2-2	8	9	4	9
AX22055-D2-12	5	3	5	3
AX22055-D2-16	14	7	3	4
Total	78	60	62	60

*Genotype notation is as follows: R1=*Rag1* present; R2=*Rag2* present; S1=absence of *Rag1*; S2= absence of *Rag2*.

Experiment 1

The purpose of experiment 1 was to compare the agronomic performance of lines from the four genotypic classes. The experiment consisted of the 260 BC1F2:3 lines of the four classes obtained in Puerto Rico (Table 1) and the parents A08-123074 and LD08-89051a.

The experiment was planted as a randomized complete-block design with one replication at each of three locations in fields of the Agronomy and Agricultural Engineering Research Center near Ames. The entries were planted in a one-row plot 0.76 m long with a 1.02 m spacing between rows and a 1.07 m alley. The seeding rate was 20 seeds for each plot.

The plots at the three locations were rated for aphid infestation three times during the summer on 26 July, 5 August, and 13 August. The rating was based on the aphid scores ranging from 1 to 10 as defined by Mardorf et al. (2010).

The maturity and height of each plot was recorded before harvest. The plots were harvested individually with a stationary plot thresher. Each plot is being evaluated for seed yield, protein concentration, oil concentration, and seed size.

Table 2. Aphid scoring system used to evaluate phenotypic resistance. (Mardorf et al., 2010)

Score	Aphid population and plant description
1	No aphids, plants were normal and healthy.
2	Less than 10 aphids per plant, no colony formation.
3	11-100 aphids per plant, plants appeared normal and healthy.
4	101-249 aphids per plant, plants appeared normal and healthy.
5	250-300 aphids per plant, plants appeared normal and healthy. *
6	301-500 aphids per plant, plant appeared healthy.
7	501-800 aphids per plant, leaves slightly curly and shiny, young leaves and stems covered with aphids.
8	More than 800 aphids per plant, plant stunted, leaves curled, slightly yellow, light sooty mold and a few exoskeletons.
9	More than 800 aphids per plant, plant stunted, leaves severely curled, yellow, covered with sooty mold and exoskeletons.
10	More than 800 aphids per plant, plant severely stunted, leaves severely curled, yellow-brownish color, covered with sooty mold and exoskeletons, plant dying.

*The economic threshold is 273 ± 38 aphids per plant, often abbreviated to 250 aphids per plant, for susceptible cultivars (Ragsdale et al., 2007).

Experiment 2

One line for each of the four genotypic classes was prepared by bulking seed from the same BC1F2:3 lines of seven families planted on 19 May in experiment 1.

The experiment was designed to have five aphid treatment levels for each of the four genotypic classes: aphid-free, 675 aphids per plant; 25,000 cumulative aphid days (CAD); 50,000 CAD; 75,000 CAD. We summarized soybean exposure to aphids for large populations by calculating CAD as follows:

$$\text{CAD} = \sum_{i=1}^{n}\left(\frac{x_{i'} + x_{i-1}}{2}\right) * t_{i'} \quad \text{(Hanafi et al., 1989)} \quad \text{Ex: } 56.1 + \left(\frac{215 \ \ + 12.4}{2}\right) * 5 \ \ = 210$$

Where,

n = the number of sample dates,

x_i = the number of aphids per plant on sample date i,

x_{i-1} = the number of aphids per plant on sample date i-1 or the previous sample date, and

t = the number of days since previous sample.

The treatment of 675 aphids per plant was chosen because it represented the economic injury level of 674 ± 95 aphids per plant defined by Ragsdale et al. (2007). The treatments that resulted in aphid populations that exceed the EIL were abbreviated to 25K, 50K, and 75K throughout this manuscript. The 25K CAD treatment represented the highest infestation found in experiments that involved naturally occurring soybean aphid infestations, as described by Ragsdale et al. (2007). The 50K CAD and 75K CAD treatments represented the lowest infestations obtained in infested caged experiments, as described by Cantangui et al. (2009). To determine when treatment levels had been obtained, an average of the six replications was calculated for each entry.

The four genotypes and five aphid treatments were randomized as a factorial arrangement in a randomized complete-block design with six replications. The experiment was planted on 7 June, 2010 at the Agronomy and Agricultural Research Center near Ames. The rows were 0.61 m long with a 1.02 m row spacing and a 1.15 m alley.

There were 24 seeds planted in each plot.

Each plot was enclosed in a cage covered by netting. The cage frames were set up on 16 June, the nets were anchored in the soil on 25 June, and the nets were put over the frames to enclose the plants on 12 July. The plots were thinned to 10 plants on 18 June at stage V1 when the emergence was complete (Fehr et al., 1971).

Soybean aphids for this experiment were obtained from a laboratory colony at Iowa State University. The Iowa State colony was established from field-collected aphids found in soybean production fields in Jasper and Story counties in 2008. Additional field-collected aphids were added in 2009 from Story County. Aphids were maintained in a growth chamber under a 14/10 day-night cycle on Prairie Brand 2636NRR, an aphid susceptible cultivar. For aphid rearing in the field for our experiment, six 6.9 m rows of the susceptible cultivar IA3027 were planted on 26 May. Lab colony aphids were transferred to an outdoor aphid rearing enclosures in early June. Rearing enclosures measured 4.5 m x 2.4 m x 2.4 m. A fine mesh fabric covered each enclosure. The fabric was buried under the soil line to exclude predators from entering the aphid rearing enclosures.

All treatments, except the aphid-free, were initially infested on 12 July. A leaf with approximately 50 aphids was paper clipped to the top of the youngest fully expanded leaf on five plants in each plot.

Aphids were first counted 3 days after infestation. During the following weeks, aphids were counted twice a week to determine when the genotypes had 675 aphids per plant. Once that treatment level was reached for a genotype, the plots were sprayed with Warrior II (Lambda-cyhalothrin: Dow AgroSciences, Midland, MI). Whenever an insecticide was applied, we also sprayed the aphid-free controls and all previously sprayed treatments. On 29 July when 675 aphids per plant were found on the plots of the susceptible genotype without either of the two Rag genes, the plots for the 25K, 50K, and 75K CAD treatments of the three genotypes with one or both of the Rag genes were re-infested. A leaf with approximately 100 aphids was paper clipped to the top of the youngest fully expanded leaf on five plants in each plot. Aphids were counted on the plots twice a week through the rest of the summer. By 18 August, the aphid populations had reached a plateau and started to decline on all of the plots. Therefore, all the plots were sprayed and no additional aphid counts were made.

The maturity and height of each plot was recorded before harvest. The plots were harvested individually with a stationary plot thresher. Seed from each plot is being evaluated for seed yield, protein concentration, oil concentration, and seed size.

Results and discussion

Experiment 1

The maximum natural infestation on any line in the experiment was less than a rating of 3, which is less than the economic threshold. Therefore, no results on aphid development among the four genotypes were obtained. The data from the experiment will be used to determine if the *Rag1* and *Rag2* genes alone or in combination with each other influence agronomic performance under aphid-free conditions.

Experiment 2

The susceptible line without either the *Rag1* or *Rag2* genes reached 675 aphids/plant 16 days after the initial infestation. The line with *Rag1* alone and the line with *Rag2* alone reached 675 aphids per plant 24 days after the initial infestation. The line with both *Rag1* and *Rag2* never reached the 675 aphids per plant treatment level after the initial infestation; therefore, it was necessary to make a second infestation for this treatment on 29 July. It took 18 days after the second infestation to reach 675 aphids per plant for this treatment.

The susceptible line reached 25K CAD 24 days after the initial infestation. The lines with *Rag1* alone or *Rag2* alone required a second infestation to reach 25K. It took 18 days after the second infestation to reach the 25K treatment for these lines. The line with both *Rag1* and *Rag2* never reached 25K, even with the second infestation.

The susceptible line reached 50K 35 days after the initial infestation, but never reached the 75K treatment. We were unable to reach the 50K or 75K treatments on any of the aphid-resistant lines.

The results of the experiment indicated that aphid populations did persist on the three resistant lines; however, the rate of population growth was limited on all the resistant lines compared with the susceptible line. The *Rag1* gene alone or *Rag2* gene alone provided similar protection against development of the biotype of aphid used in our study.

The combination of the two genes provided additional protection that should be useful to soybean farmers.

Acknowledgements

This research was supported by the Hatch Act, State of Iowa; Iowa Soybean Association; and Raymond F. Baker Center for Plant Breeding. The authors gratefully acknowledge the technical support of Ryan Brace, Brian De Vries, John Gill, Susan Johnson, Justin Mardorf, and Grace Welke for germplasm development, planting and harvesting the research; Mike McCarville for cage construction; and Adam Varenhorst and Greg Vannostrand for applying insecticide.

References

Beckendorf, I., A., Cantangui, and W.E. Riedell. 2008. Soybean aphid feeding injury and soybean yield, yield components, and seed composition. Agron. J. 100: 237-246.

Catangui, M.A., E.A. Beckendorf, and W.E. Riedell. 2009. Soybean aphid population dynamics, soybean yield loss, and development of stage-specific economic injury levels. Agron. J.101: 1180-1092.

Fehr, W.R., C.E. Caviness, D.T. Burmood, and J.S. Pennington. 1971. Stage of development descriptions for soybeans, Glycine max (L.) Merrill. Crop. Sci. 11: 929-931.

Hanafi, A., E.B. Radcliffe, and D.W. Ragsdale. 1986. Spread of control of potato leafroll virus in Minnesota. J. Econ. Entomol. 82: 1201-1206.

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 glycine* on soybean in Illinois in 2000 and its potential control. Available at http://www.plantmanagementnetwork.org/pub/php/brief/aphisglycines/ (accessed 19 August 2010).

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.

Hill, C.B., K. Kim, L. Crull, B.W. Diers, and G.L. Hartman. 2009. Inheritance of resistance to the soybean aphid in soybean PI 200538. Crop Sci. 49: 1193-1200.

Hill, C.B., L. Crull, T.K. Herman, D.J. Voegtlin, and G.L. Hartman. 2010. A new soybean aphid (Hemiptera: Aphidiae) biotype identified. J. Econ. Entomol. 103: 509-515.

Kim, K., C.B. Hill, G.L. Hartman, M.A. Rouf Mian, and B.W. Diers. 2008. Discovery of soybean aphid biotypes. Crop Sci. 48: 923-928.

Kim, K., and B.W. Diers. 2009. The associated effects of the soybean aphid resistance locus *Rag1* on soybean yield and other agronomic traits. Crop Sci. 49: 1726-1732.

Kim, K., C.B. Hill, G.L. Hartman, D.L. Hyten, M.E. Hudson, and B.W. Diers. 2010. Fine mapping of the soybean aphid-resistance gene *Rag2* in soybean PI 200538. Theor. Appl. Genet. 121: 599-610.

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.

Mardorf, J., W.R. Fehr, and M.E. O'Neal. 2010. Agronomic and seed traits of soybean lines with the *Rag1* gene for aphid resistance. Crop Sci. 50: 1891-1895.

Ragsdale, D.W., B.P., McCornack, R.C. Venette, B.D. Potter, I.V. Macrae, E.W. Hodgson, M.E. O'Neal, K.D. Johnson, R.J. O'Neil, C.D. Difonzo, T.E. Hunt, P.A. Glogoza, and E.M. Cullen. 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 100: 1258-1267.

Rice, M.E., M. O'Neal, B. Lang, P. Pedersen, C. Pilcher, A. Robertson, D. Ragsdale, K. Ostile, E. Hodgson, R. Venette, and D. Voegtlin. 2008. Soybean aphid management: field guide 2008. Research Report CSI 0011. Iowa State University, Ames.