Identification of Sources of Soybean Aphid Resistance and Mapping of a Dominant Gene

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Abstract

Soybean, *Glycine max* (L.) Merr, is the world's most important source of vegetable oil and protein meal. Soybean ranks third only after corn and wheat in total acres grown for a seed crop in the USA. The Soybean aphid (SA) (*Aphis glycines* Matsumura) was first reported in the northern soybean growing region of the USA in 2000. By 2004, 80% of the U.S. soybean field was infested by SA. The SA is native to southeastern and eastern Asia and it is long been a pest of soybean in many Asian countries. Three plant introductions (PIs) (PI 243540, PI 567301B, and PI 567324) were identified as resistant to the SA biotype from Ohio. PI 243540 displayed strong antibiosis resistance such that SA was unable to survive on this PI in a non-choice test. The other two resistant PIs possessed mainly antixenosis type resistance. PI 243540 and PI 567301B were also resistant to the SA isolate from Illinois. A single dominant gene controlling SA resistance in PI 243540 was mapped to the soybean molecular linkage group (LG) F. Thus, the SA resistance in PI243540 is controlled by a gene that is independent from the *Rag*1 gene in cultivar Dowling. This new gene has been named as *Rag*2. The *Rag*2 gene has been transferred to elite U.S. soybean cultivars by backcross breeding and marker assisted selection.

Media Summary

The *Rag*2 gene conferring resistance to soybean aphids will be useful for developing soybean cultivars with soybean aphid resistance in USA and elsewhere.

Key Words

Soybean, aphid, *Rag*2, marker assisted selection

Introduction

The SA is native to southeastern and eastern Asia and it has long been a pest of soybean in many Asian countries and has recently become a pest in the USA and Canada (Wu et al. 2004; Hartman et al. 2001). The yield losses of soybean attributed to the aphid in Minnesota have been reported to be greater than 50% (Ostlie 2002) and up to 52% in China (Wang et al. 1994). Besides the yield loss of soybean from direct feeding damage, aphids can transmit certain plant viruses such as *alfalfa mosaic virus*, *soybean dwarf virus*, and *soybean mosaic virus* to soybean (Iwaki et al. 1980; Hartman et al. 2001). Currently available U.S. soybean cultivars are all susceptible to SA and producers depend on insecticide for controlling the aphid. Host plant resistance is most desirable for controlling the aphid. The first step in breeding a resistant cultivar is to identify the genetic sources of resistance to SA. Several research groups in the USA have recently identified SA resistant soybean germplasm (Mian et al. 2008).

The SA resistance in each of the two soybean cultivars - Dowling and Jackson – is controlled by single dominant genes (Hill et al. 2006a, b). The gene in Dowling was designated as *Rag1* and the gene in Jackson remained unnamed. Li et al. (2007) have mapped *Rag*1 and the unnamed gene from Jackson to the same genomic region on soybean LG M indicating that these two resistance genes may be allelic. Until recently it was believed that there was no biotype diversity of SA in North America (Hill et al. 2006a). However, Kim et al. (2008) have confirmed the existence of two biotypes of SA in North America and that the Ohio biotype of SA was able to break the resistance provided by *Rag*1 and *Rag*(Jackson) genes. The aphid resistance in PI 243540 is controlled by a single dominant gene (Kang et al. 2008). This report summarizes the recent and on-going research on SA resistance by the USDA-ARS in Wooster, Ohio, USA and collaborators.

Methods

The aphid screenings were conducted in greenhouses and in fields in Wooster, Ohio, USA as described by Mian et al. (2008). Nearly three hundred soybean lines were screened with SA collected from Wooster for identifying the source of aphid resistance. The SSR genotyping was performed using standard protocols. A mapping population from a cross between Ohio adapted cultivar 'Wyandot' and PI 243540 was used for mapping the gene. The phenotypic data were analyzed using SAS (SAS Institute 2002) and the marker data analysis and linkage mapping was accomplished by using the JoinMap 4.0 (Van Ooijen 2006) using the Kosambi mapping function.

Results

Three soybean accessions - PI 243540, PI 567301B, and PI 567324 - were identified as highly resistant against the Ohio biotype of SA (Table 1). Three other soybean accessions were identified to have partial resistance or tolerance to the aphid.

The study on inheritance of the aphid resistance gene in PI 243540 indicated that a single dominant gene controlled the aphid resistance in this soybean accession (Table 2).

The dominant gene was mapped on soybean LG F (Fig. 1) and this independent gene was named *Rag*2.

Conclusion

The aphid resistance in soybean PI 243540 is controlled by a simply inherited single dominant gene called *Rag*2. Due to simple inheritance of this gene it can be easily and quickly transferred to desired background via backcross breeding.

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Table 1: The aphid scores, plant damage scores, and aphid susceptibility indexes (ASI) from greenhouse confirmation choice test with the Ohio biotype of the aphid (adapted from Mian et al. 2008).

Line name	Score 14 DAI[+]	Score 28 DAI[±]	ASI[ψ]	Comment
PI243540	1.0	1.0	1.0	Resistant
PI567301B	2.0	1.0	2.0	Resistant
PI567324	2.0	1.0	2.0	Resistant
PI567318	3.2	1.8	5.8	Moderately Resistant
PI567321A	2.5	2.0	5.0	Moderately Resistant
PI567336A	3.7	2.2	8.1	Moderately Resistant
PI567543C	1.4	1.0	1.4	Res. check (Mensah et al., 2005)
PI567597C	1.5	1.0	1.5	Res. check (Mensah et al., 2005)
PI567598B	2.0	1.5	3.0	Res. check (Mensah et al., 2005)
Jackson	4.0	4.1	16.4	Res. check (Hill et al., 2004)

Dowling	4.7	4.6	21.7	Res. check (Hill et al., 2004)
Williams 82	4.7	4.0	18.8	Sus. check cultivar (IL)
Ohio FG5	4.7	4.7	21.7	Sus. check cultivar (OH)
Wyandot	4.8	4.8	23.0	Sus. check cultivar (OH)
LSD 0.05	0.7	0.7	2.9	

[+] Score 1=<25 aphids per plant, 2=25-100 aphids per plant, 3=101-200 aphids per plant, 4=201-400 aphids per plant, and 5=>400 aphids per plant.

[±] Score 1=<25 aphids and plant appears normal and healthy, 2=25-100 aphids per plant and plant appears normal and healthy, 3=101-300 aphids per plant and plant appears slightly stunted with slight yellowing of older leaves, 4=301-600 aphids per plant and plant appears moderately stunted with yellowing of older leaves and slight curling of young leaves, and 5=>600 aphids per plant and plant severely stunted with severely curled and yellow leaves and most of the stem and leaf surfaces covered with sooty mold and cast skins.

[Ψ] The aphid susceptibility index (ASI) was calculated by multiplying the aphid score at 14 DAI by the plant damage score at 28 DAI with a possible ASI between 1 and 25.

Table 2. Segregation of 341 F_2 plants in 11 $F_{1:2}$ families for resistance to the Ohio biotype of soybean aphid in a population of Wyandot × PI 243540 cross (adapted from Kang et al. 2008).

$F_{1:2}$ Family	No of plants	Observed[†]		Expected (3:1)			
		Res.	Sus.	Res.	Sus.	$\chi2$ value	P
1	37	24	13	27.8	9.3	2.03	0.15
2	40	29	11	30.0	10.0	0.13	0.72
3	19	15	4	14.3	4.8	0.16	0.69
4	36	28	8	27.0	9.0	0.15	0.70
5	36	27	9	27.0	9.0	0.00	1.00
6	28	23	5	21.0	7.0	0.76	0.38
7	24	20	4	18.0	6.0	0.89	0.35
8	37	27	10	27.8	9.3	0.08	0.78
9	36	31	5	27.0	9.0	2.37	0.12
10	13	10	3	9.8	3.3	0.03	0.87
11	35	27	8	26.3	8.8	0.09	0.77
Pooled	341	261	80	255.8	85.3	0.43	0.51

†Res (resistant) aphid scores 1 and 2 and Sus (susceptible) aphid scores 3, 4 and 5 where 1=no aphid present, 2=few (<25) solitary live or dead aphid bodies present, 3=some aphids (25 to 100) with some viviparous aptera surrounded by few nymphs present, 4=dense colonies on the upper half of the stem, underside of most leaves, and near the growing point of the plant with more than 300 aphids, and 5=similar or more severe aphid infestation as in score 4 accompanied by visible plant damage.

The single dominant gene in PI 243540 was mapped to soybean LG J using SSR markers (Figure 1). This gene was confirmed as independent from the *Rag*1 gene in cultivar Dowling that was mapped to LG M by Li et al. (2007) and named as *Rag*2. Rag2 gene was transferred to cultivar Wyandot by marker assisted backcross breeding.

Figure 1. Linkage map showing the position of the soybean aphid resistance gene (*Rag*2) on soybean genetic linkage group (LG) F. The map distances between adjacent markers are shown in centimorgan (cM).