Resistance to Soybean Aphid in Early Maturing Soybean Germplasm

Clarice Mensah, Christina DiFonzo, Randall L. Nelson, and Dechun Wang*

ABSTRACT

Since 2000, the soybean aphid (Aphis glycines Matsumura) has become a major pest of soybean [Glycine max (L.) Merr.] in North America. In the USA, there are currently no commercial soybean cultivars with aphid resistance and there are no reported resistance sources in early maturing soybean germplasm. The objectives of this study were to identify sources and types of resistance to soybean aphid from early maturing soybean germplasm. Over a 2-yr period, 2147 soybean accessions from maturity group (MG) 0 to III, originally from northern China, were evaluated for aphid resistance in a greenhouse and in field cages. The plants were hand-inoculated and aphid populations were evaluated 10 d after inoculation. A damage index (0–100%) was calculated for each accession. After 2 yr of evaluation and confirmation in choice tests, four MG III accessions from Shandong province, PI 567543C, PI 567597C, PI 567541B, and PI 567598B, were found to be resistant to the soybean aphid. Two of these accessions, PI 567541B and PI 567598B, possessed antibiosis resistance, preventing the aphids from reproducing on the plants in a no-choice study. PI 567543C and PI 567597C possessed antixenosis resistance. These resistant sources can be used to develop commercial cultivars with aphid resistance for the North Central states.

Soybean is the leading oilseed crop produced and consumed worldwide (Wilcox, 2004). In the past half century, the USA has been the world’s leading producer. In 2003, the USA produced 35% (65.8 million Mg) of the world’s total soybean (FAOSTAT, 2004). Soybean has many insect pests limiting its production, including the soybean aphid. A native to eastern Asia, the soybean aphid was not reported in the USA before July 2000. Since then, the insect has rapidly spread to the major soybean production areas in the USA and Canada (Plant Health Initiative, 2004). Outbreaks have been severe in the northern part of the midwestern USA and in Ontario, particularly in 2001 and 2003.

Several factors affect soybean aphid outbreaks, including environmental conditions, over-wintering success, cultural practices, natural enemies, and the synchronization of soybean and aphid development (Wu et al., 1999). The soybean aphid is the only aphid in North America that develops large colonies on soybean. Plant damage occurs when large numbers of aphids remove significant amounts of water and nutrients as they feed on leaves and stems, causing leaves to wilt, curl, yellow, and even drop. Other symptoms of direct feeding damage include plant stunting, poor pod fill, reduced pod and seed counts, smaller seed size, and nutrient deficiencies resulting in overall yield and quality reduction (DiFonzo and Hines, 2002). Significant yield loss (8–25%) occurs when the soybean plants are heavily infested by the aphid during the early reproductive stage (DiFonzo and Hines, 2002). Honeydew, a sticky substance excreted by soybean aphids onto the leaves, leads to the development of sooty mold, which affects photosynthesis and results in yield loss (Baute, 2004). During the feeding process, soybean aphids are capable of transmitting viruses including Alfalfa mosaic virus, Soybean mosaic virus, and Bean yellow mosaic virus. These viruses commonly occur together and form a disease complex that leads to plant stunting, leaf distortion and mottling, reduced pod numbers, and seed discoloration (Glogoza, 2002).

In the USA, soybean aphid research is still in its early stages. The aphid was first found in 2000 and annual infestations have been unpredictable (Steffey and Gray, 2004). Insecticides are the only available method of controlling soybean aphids in the USA. Although the use of insecticides can be a quick and easy way to control aphids, the ideal time to spray is not well defined. Insecticides also have many adverse effects such as killing beneficial insects, environmental contamination, and increased production costs (Sun et al., 2000). Aphid populations may surge when applications of insecticides are poorly timed or applied. Developing soybean varieties that are resistant to the aphid is a long-term solution to the aphid problem.

To develop aphid resistant varieties, sources of resistance must be identified. Sources of resistance to the soybean aphid are reported in China. In the late 1980s, two highly resistant varieties were found among 181 varieties evaluated (Fan, 1988). In 1991, resistance was also reported in soybean germplasm in China (Sun et al., 1991). The type of resistance, antixenosis or antibiosis, was not indicated in these studies. Antixenosis is nonpreference of insects for a host plant (Kogan and Orman, 1978). Antibiosis includes all adverse effects on an insect’s life history after a resistant host plant has been used for food (Painter, 1951). Knowing the type of resistance is important to fully understand and utilize resistant accessions in a breeding program. Hill et al. (2004) recently reported three lines with resistance to soybean aphid. PI 71506 (MG IV) has antixenosis and the cultivars Dowling (MG VIII) and Jackson (MG VII) are reported to have antibiosis resistance.

In 2002, when this research was initiated, there were no known sources of host plant resistance to soybean aphid in the USA. The objectives of this study were to:

Abbreviations: DI, Damage Index; MG, Maturity Group; PI, Plant Introduction.
(i) evaluate soybean germplasm from northern China in the USDA Soybean Germplasm Collection to identify sources of resistance to soybean aphids in early maturity groups and (ii) determine the resistance type of the identified sources.

**MATERIALS AND METHODS**

Soybean plant introductions (PI) from MG 0 to III were obtained from the USDA Soybean Germplasm Collection in Urbana, IL. A total of 2147 PIs were evaluated in 2002 and 2003, including five MG 0 accessions (PI 468920 to PI 597467), 530 MG I accessions (FC 03609 to PI 612761E), 979 MG II accessions (PI 253650A to PI 612758E), and 633 MG III accessions (FC 02108 to PI 612759D). Accessions originally from northern China were selected, as the climatic conditions are similar to those in the northern USA, where the soybean aphid over-winters, and because soybeans in that region have been exposed to aphids over the years. Williams 82 was included as a susceptible check in all experiments and one or more of the three resistant genotypes, Dowling, Jackson, and PI 71506 (Hill et al., 2004), were included as resistant checks. Both the susceptible and resistant checks were obtained from Dr. Glen Hartman, USDA-ARS at Urbana, IL. All PI's and checks were first evaluated in choice tests (Davis 1985), in which the aphids colonized genotypes they preferred, to identify PIs with either antixenosis or antibiosis. The resistant PIs were then re-evaluated in no-choice tests (Davis 1985), in which aphids were confined on plants of one genotype, to identify PIs with antibiosis resistance. The insects have no choice but to feed on the genotype on which they are confined. The no-choice test is also conducted to overcome the uneven distribution of insects, which normally occurs in choice tests, resulting in escapes (Saxena and Khan, 1984).

All experiments were performed on the campus of Michigan State University (MSU), East Lansing, MI. Soybean aphids were obtained from nearby naturally infested soybean fields for summer fieldwork, and from a colony maintained in growth chambers at the Field Crops Entomology Laboratory at MSU for winter greenhouse work.

**Summer Field Evaluation—Choice Test**

Two experiments were performed in the summers of 2002 and 2003 to evaluate soybean germplasm for aphid resistance. Summer plantings were done at the Agronomy Farm, MSU, in 12.2- × 18.3-m polypropylene cages with a 0.49-mm mesh size (Redwood Empire Wicking Co., Santa Rosa, CA) that are aphid- and predator-proof.

In 2002, 1043 PIs, the susceptible check (Williams 82), and a resistant check (Jackson) were evaluated in the field cage. The PIs and checks were planted on 26 June and each check was treated as an accession in the test. Five seeds per accession were planted in a plot 0.3 m long and with a row spacing of 0.3 m. Each accession was planted in a single plot without replication. At the V1 stage (Fehr and Caviness, 1977), two plants per accession were inoculated with two wingless aphids each on the partially expanded trifoliate, with a camel-hair brush. Aphids were obtained from naturally infested fields on the Agronomy Farm, MSU. The aphids were left to multiply and move among plants.

In 2003, a new set of 1103 PIs, the resistant checks (Dowling, Jackson, and PI 71506), and the susceptible check (Williams 82), were evaluated in two field cages. In each cage, a complete set of the PIs plus the checks were planted as a randomized complete block. Each check was treated as an accession in the test. The lines were sown on 30 May in one cage (Planting 1) and on 6 June in the second cage (Planting 2). The methods of inoculation, plot sizes, and evaluation procedures were the same as for the 2002 field evaluation.

**Winter Greenhouse Evaluation—Choice and No-Choice Tests**

A winter evaluation was performed in a large greenhouse with temperatures between 22 and 25°C to verify the results obtained in the field in 2002. The PIs planted in the field in 2002 were evaluated. Seeds were planted on 21 Nov. 2002 in the greenhouses at the Horticulture Research Farm at MSU. Three seeds of each genotype were planted in a plastic pot 22 cm in diameter and 23 cm deep. Each genotype was planted in a single pot without replication and the pots of all genotypes were randomly laid out on the benches in the greenhouse. The soil used in all greenhouse tests was Bacto High Porosity Professional Planting mix (Michigan Peat Company, Houston, TX). Two of the three plants were inoculated at the V1 stage (Fehr and Caviness, 1977) with two wingless aphids each on the partially expanded trifoliate.

A no-choice test was performed in the greenhouse from December 2003 to February 2004 to determine the type of resistance of each resistant source. Each pot was set up as described for the 2002 greenhouse plantings with two replicates and in a randomized complete block design. Each pot was isolated by the use of a no-see-um mesh cage (Venture Textiles, Inc., Braintree, MA). The entries in the no-choice test were the resistant PIs identified in the 2002 and 2003 evaluation in choice tests, the resistant check (Jackson), the susceptible check (Williams 82), and two soybean cultivars, Titan and Loda.

**Confirmation of Resistance**

In the summer of 2004, the PIs identified as potentially aphid resistant after 2 yr of evaluation, and Williams 82, were evaluated in the field to confirm the resistance found in previous tests. The experiment was set up as a randomized complete block design with three replications. Ten seeds were planted in each 0.6-m plot. All 10 plants were inoculated at the V1 stage (Fehr and Caviness, 1977) with wingless aphids as described earlier.

**Data Collection**

In all studies, except the confirmation of resistance test, aphid populations on inoculated trifoliate were counted 10 d after inoculation when the plants were at the V3 stage (Fehr and Caviness, 1977). Four weeks after inoculation, the plants in each accession were visually rated for susceptibility to soybean aphid by the rating scale shown in Fig. 1 (Zhuang, 1999). A damage index (DI) for each accession was calculated by the following formula (Zhuang, 1999): DI = ∑ (Scale value × No. of plants in the category)/(4 × Total no. of plants evaluated) × 100. The DI ranges between 0% for no infestation and 100% for the most severe damage. A DI of 30% or less was classified as resistant, whereas a DI of 30% or more was classified as susceptible. The 30% break point was chosen on the basis of our observation that a soybean genotype with a DI value less than 30% never showed symptoms of damage under high aphid pressure until the end of the season. In the second year of field evaluation, the plants were visually rated weekly from the second week through the fifth week after inoculation to determine and confirm the best time to carry out the visual rating.
Fig. 1. Illustration of the visual rating scale used to establish the Damage Index (DI). 0 = No aphids, plant appears normal and healthy; 1 = Less than 100 aphids per plant, plant appears normal and healthy; 2 = 101–300 aphids per plant, mostly on the young leaves and the tender stem at top of plant, plant appears normal and healthy; 3 = 301–800 aphids per plant, leaves slightly curly and shiny, young leaves and stems covered with aphids; 4 = More than 800 aphids per plant, plants stunted, leaves severely curled, yellow, covered with sooty mold and cast skins.

Statistical Analysis

The data for each year were analyzed by the PROC GLM procedure in the SAS statistical package V8 (SAS Institute, 1999). Means were separated by least significant difference (LSD) at the 5% probability level. Linear correlations between the average number of aphids per leaflet 10 d after inoculation and the DI were calculated by PROC CORR.

RESULTS

Choice Tests in 2002

In the first year of evaluation in the field cage, the average number of aphids per leaflet ranged from 0 to 500. In the greenhouse, the average number of aphids per leaflet ranged from 0 to 170. Results from the visual rating and calculation of the DI showed that 1008 and 973 of the accessions evaluated in the field and greenhouse, respectively, were susceptible to the soybean aphid (DI > 30%). Twenty-eight and 62 accessions did not germinate in the field and the greenhouse, respectively. The correlation between the average number of aphids per leaflet 10 d after inoculation and the DI of an accession was low ($r = 0.16, n = 1043, p < 0.0001$). Seven of the 1043 accessions appeared to be resistant (DI < 30%) to the aphid in the field cage, while eight accessions showed resistance in the greenhouse. Only three of these accessions were resistant in both the field and greenhouse evaluations. The accessions that showed resistance in only one test, field or greenhouse, were replanted in the greenhouse in the spring of 2003 and found to be susceptible. The resistant check, Jackson, had a DI of 55% in the field and 25% in the greenhouse. After the first year of evaluation, PI 567543C, PI 567597C, PI 567541B, and PI 567598B appeared to be resistant to the soybean aphid.

Choice Tests in 2003

In the second year of evaluation, the number of aphids per leaflet ranged from 0 to 326 for the first planting
and 0 to 244 for the second planting. On the basis of DI, 931 and 995 of the plants were found to be susceptible (DI > 30%) in Plantings 1 and 2, respectively. As in the previous year, the DI value 4 wk after inoculation did not reflect the aphid population 10 d after inoculation. The correlation between the average number of aphids per leaflet 10 d after inoculation and the DI ered with black sooty mold, while PI 603392 and PI 603418C 4 wk after inoculation. The susceptible check susceptible checks, and varieties in 2003. and 0 to 244 for the second planting. On the basis of DI, 931 and 995 of the plants were found to be susceptible (DI > 30%) in Plantings 1 and 2, respectively. As in the previous year, the DI value 4 wk after inoculation did not reflect the aphid population 10 d after inoculation. The correlation between the average number of aphids per leaflet 10 d after inoculation and the DI value 4 wk after inoculation was low (r = 0.20, n = 1103, p < 0.0001) in 2003.

Eight accessions were rated as resistant in Planting 1 and 10 accessions were rated as resistant in Planting 2. The difference in numbers of accessions rated as resistant was due to some accessions failing to germinate in both plantings. However there were only two accessions, PI 603392 and PI 603418C, which had a DI of <30% in both plantings. All the resistant checks had a DI of 25% in both plantings. In cases where germination did not occur in both plantings, the accessions were re-planted in the greenhouse in the winter of 2003 and found to be susceptible.

No-Choice Test
The six PIs rated as resistant in 2002 and 2003 choice tests (PI 567543C, PI 567597C, PI 567541B, PI 567598B, PI 603392, and PI 603418C) were evaluated for aphid resistance in a no-choice test. The no-choice study revealed that two of the six resistant PIs, PI 567541B and PI 567598B, had significantly lower DIs than the other PIs in the test. These two PIs had aphid damage similar to Jackson, the resistant check (Table 1). The other resistant PI and Titan had significantly (p ≤ 0.05) higher damage indices than PI 567541B and PI 603418C, but there were also significantly lower than Loda and Williams 82 (the susceptible check). There was a high correlation (r = 0.63, p = 0.048) between the average number of aphids per leaflet 10 d after inoculation and the DI of an entry.

Confirmation of Resistance
Resistance in the four accessions (PI 567543C, PI 567597C, PI 567541B, and PI 567598B) identified in the choice tests in 2002 was confirmed in 2004 (Table 2). At 3 and 4 wk after inoculation, highly significant differences (p < 0.0001) were found between the DIs for these four accessions and the DIs for PI 603392 and PI 603418C, identified in choice tests in 2003. The amount of damage to the plant as a result of aphid feeding was greater on the susceptible check than on PI 603392 or PI 603418C 4 wk after inoculation. The susceptible check appeared stunted, and its leaves were curled and covered with black sooty mold, while PI 603392 and PI 603418C showed none of these symptoms.

Discussion
In the USA, evaluation of soybean germplasm for resistance to the soybean aphid began as soon as the pest was discovered. Hill et al. (2004) evaluated 1542 soybean genotypes, mostly current North American soybean cultivars, and found resistance in three North American soybean ancestral lines: Dowling, Jackson, and PI 71506. These resistant genotypes belong to MG IV to VII that are not well adapted to the northern USA, where soybean aphids are most prevalent. In our study, we identified four resistant accessions (PI 567543C, PI 567597C, PI 567541B, and PI 567598B) belonging to MG III after evaluating 2147 soybean accessions in MG 0 to III. All of these primitive Chinese cultivars originated from Shandong province, but their resistance to the soybean aphid has never been reported in China. The resistance in these accessions can be readily incorporated into the elite soybean germplasm in the north central states.

During vegetative growth of soybean, aphid colonies were usually found at the growing points e.g., partially expanded young trifoliate, petiolo, and stems. At the reproductive stage the aphids became more widely dispersed on the plant and could be found on the underside of mature leaves, on lower stems, lateral branches, petiolo, and pods (Ragsdale et al., 2004). On the basis of our observations, most aphid colonies stayed on inoculated trifoliates for more than 10 d after inoculation, with the inoculated leaves still not overcrowded. Therefore, an estimate of the increase of the aphid population in the first 10 d can be obtained by counting aphids on the inoculated trifoliate 10 d after inoculation.

Weekly visual ratings using the method of Zhuang

Table 1. Results of the greenhouse no-choice test for the six accessions classified as resistant in evaluation trials, resistant and susceptible checks, and varieties in 2003.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Maturity Group</th>
<th>Average no. of aphids per leaflet</th>
<th>Damage Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI 567543C</td>
<td>III</td>
<td>8ab§</td>
<td>56b</td>
</tr>
<tr>
<td>PI 567597C</td>
<td>III</td>
<td>1a</td>
<td>62b</td>
</tr>
<tr>
<td>PI 567541B</td>
<td>III</td>
<td>1a</td>
<td>25a</td>
</tr>
<tr>
<td>PI 567598B</td>
<td>III</td>
<td>11bc</td>
<td>25a</td>
</tr>
<tr>
<td>PI 603392</td>
<td>III</td>
<td>5a</td>
<td>81c</td>
</tr>
<tr>
<td>PI 603418C</td>
<td>III</td>
<td>12c</td>
<td>77c</td>
</tr>
<tr>
<td>Jackson</td>
<td>VII</td>
<td>2a</td>
<td>25a</td>
</tr>
<tr>
<td>Titan</td>
<td>I</td>
<td>17c</td>
<td>71c</td>
</tr>
<tr>
<td>Loda</td>
<td>II</td>
<td>19c</td>
<td>83c</td>
</tr>
<tr>
<td>Williams 82</td>
<td>III</td>
<td>19c</td>
<td>100d</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9.4</td>
<td>61</td>
</tr>
</tbody>
</table>

§ Means followed by the same letters are not significantly different by the least significant difference test (p = 0.05).

Table 2. Damage Index (DI) based on three replications in 2004 for six putatively resistant accessions identified after 2 yr of evaluation, and a susceptible check, 3 and 4 wk after inoculation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Three weeks after inoculation</th>
<th>Four weeks after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI 567543C</td>
<td>25 a‡</td>
<td>25 a</td>
</tr>
<tr>
<td>PI 567597C</td>
<td>26 a</td>
<td>26 a</td>
</tr>
<tr>
<td>PI 567541B</td>
<td>25 a</td>
<td>25 a</td>
</tr>
<tr>
<td>PI 567598B</td>
<td>26 a</td>
<td>26 a</td>
</tr>
<tr>
<td>PI 603392</td>
<td>75 b</td>
<td>79 b</td>
</tr>
<tr>
<td>PI 603418C</td>
<td>75 b</td>
<td>79 b</td>
</tr>
<tr>
<td>Williams 82</td>
<td>83 c</td>
<td>100 c</td>
</tr>
<tr>
<td>Mean</td>
<td>47</td>
<td>51</td>
</tr>
</tbody>
</table>

‡ Averages of two replications.
(1999) showed that there was a clear difference in susceptibility or resistance among accessions 4 wk after inoculation when aphid densities reached their peak (data not shown). Thus DI values 4 wk after inoculation were used to determine susceptibility of the PI. Visual rating data 2 wk after inoculation were not used because of low aphid populations. Two weeks after inoculation, the method of Zhuang (1999) categorizes all the plants as either a 1 or 2 and the results are similar to counting aphids 10 d after inoculation. On the other hand, 5 wk after inoculation, the aphid populations started to decline because of overcrowding and development of winged aphids, which left the plants. Therefore, visual rating data 5 wk after inoculation were not used in the analysis.

Lin et al. (1992) showed that the soybean aphid colonizes soybeans in China at the early vegetative stage. Aphid populations increase gradually and reach a 10 to 15 d exponential growth phase coinciding with late vegetative to early reproductive stage of the plants. It is not surprising that 10 d after inoculation, at the early vegetative stage, a high percentage of our test plants had very few aphids per leaflet. Correlations were low between the number of aphids per leaflet 10 d after inoculation and the DI 4 wk after inoculation in the first and the second years of evaluation ($r = 0.16$ and $r = 0.20$, respectively). These low correlation values indicate that counting aphids on the inoculated trifoliate 10 d after infestation in the early vegetative stage is not an optimal method for determining the resistance or susceptibility of an accession. Counting the total number of aphids on the whole plant 10 d after inoculation would also not have helped to separate resistant from susceptible accessions because most aphid colonies did not move away from the inoculated trifoliate during the first 10 d after inoculation. It is advisable to count aphids on the whole plants in the late vegetative or early reproductive stage to identify truly resistant accessions. However, counting aphids is very tedious and time consuming. For large-scale evaluation of aphid resistance such as progeny evaluation in a breeding program, the method of Zhuang (1999) is more appropriate.

The six MG III accessions classified as resistant in evaluation trials, PI 567543C, PI 567597C, PI 567541B, PI 567598B, PI 603392 and PI 603418C, were identified in field and greenhouse choice tests. The no-choice test showed that PI 567541B and PI 567598B had adverse effects on the aphid and thus possessed antibiosis as defined by Painter (1951). The high DIs obtained in no-choice test for PI 567543C and PI 567597C (which were classified resistant in choice tests) is likely due to the change in feeding response of the aphid in choice and no-choice tests as found by Smith et al. (1994). Also, it is possible for a genotype classified as resistant in a choice test to be declared susceptible in a no-choice test (Tingey, 1986). PI 567543C and PI 567597C, while having lower DI values than Williams 82, are not resistant (Table 1). The high ($r = 0.63, p = 0.048$) correlation between the average number of aphids per leaflet 10 d after inoculation and the DI of an entry in the no-choice test is attributed to the fact that the entries chosen for this test were truly susceptible or resistant as found in previous evaluations. The inconsistent average numbers of aphids per leaflet for PI 567598B and PI 603392 (Table 1) strengthens the fact that counting of aphids 10 d after inoculation is not optimal for selecting aphid resistant plants. The method of Zhuang (1999) would still be the best to use in experiments with few entries.

The test conducted to confirm the resistance after 2 yr of evaluation revealed that PI 603392 and PI 603418C, both from Liaoning province, were not resistant to the soybean aphid. These plants, when evaluated in 2003 in the field cages, did not show symptoms of severe aphid infestation. According to Painter (1951), the type of resistance that enables a host plant to withstand infestation by insects without suffering severe damage is tolerance. PI 603392 and PI 603418C might be tolerant, but tolerance can only be confirmed with further yield and dry matter studies. These two accessions were not considered resistant after their poor performance in the confirmation test. Smith (1989) also observed that pseudo-resistance or false resistance may occur in normally susceptible plants. Resistance may have been induced temporarily by variations in temperature, daylength, soil chemistry, plant or soil water content, or internal plant metabolism. Also susceptible plants may simply escape damage because of incomplete infestation.

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**REFERENCES**


MENSAH ET AL.: APHID RESISTANCE IN EARLY MATURING SOYBEAN


