



# Resistance to soybean aphid among wild soybean lines under controlled conditions



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## ABSTRACT

The soybean aphid, *Aphis glycines* Matsumura, is frequently a serious pest of soybean in the U.S. and Canada, and an occasional pest of soybean throughout its native range in Asia. Large infestations of this aphid cause economic loss to soybean by reductions in seed yield and oil concentration. Host-plant resistance is a potential alternative to insecticides for managing soybean aphid, and various sources may be evaluated, including adapted cultivars, landraces, and wild ancestors of the crop. To date, few studies have evaluated wild soybean for resistance to soybean aphid. In this study, initial screening assays indicated 20 wild soybean lines with resistance to soybean aphid, and three of the 20 lines had notable resistance in subsequent choice and no-choice assays. Significantly fewer soybean aphids settled on lines PI 468397 A and PI 479749 than on susceptible lines in choice assays, and aphid populations were low and moderately low on these two respective lines in no-choice assays. Populations of soybean aphid on a third line, PI 549046, were equal to or less than those on a resistant check over 3 wks in a no-choice assay. Based on results of the respective choice and no-choice assays, resistance to soybean aphid was manifested as both antixenosis and antibiosis in PI 468397 A and PI 479749, and as antibiosis in PI 549046. This is apparently the first report of resistance to soybean aphid in these three lines, and they provide soybean breeders and pest management practitioners new sources for developing aphid-resistant soybean lines.

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## 1. Introduction

The soybean aphid, *Aphis glycines* Matsumura, is native to eastern Asia (Blackman and Eastop, 2000), and was discovered infesting soybean [*Glycine max* (L.) Merr.] in North America in 2000 (Ragsdale et al., 2011). It is an occasional insect pest of soybean in Asia (Wu et al., 2004), but has become a serious pest of soybean in the U.S. and Canada (Ragsdale et al., 2011). Soybean aphids colonize soybean fields in early summer, and their exponential growth results in several hundred to many thousand aphids per plant (Beckendorf et al., 2008; McCornack et al., 2008). Large infestations reduce plant biomass and cause economic loss by decreasing seed yield and oil concentration (Wang et al., 1996; Ragsdale et al., 2007; Beckendorf et al., 2008; O'Neal and Johnson, 2010). Several plant-disease viruses, such as *Soybean mosaic virus*, can be transmitted by soybean aphid (Clark and Perry, 2002; Burrows et al., 2005;

Wang et al., 2006), and these viruses may cause additional yield loss (Wang et al., 2006).

Repeated outbreaks of soybean aphids in North America are challenging pest management practitioners to develop environmentally responsible means to protect soybeans (Rutledge et al., 2004). However, the primary means of managing soybean aphid is by one to three mid-summer applications of insecticides (Ragsdale et al., 2011). Host-plant resistance is a potential alternative method for managing soybean aphid that may reduce economic and environmental costs associated with insecticide use in soybean-production systems (Hill et al., 2012; Hesler et al., 2013).

Various sources of germplasm may be evaluated for genetic variation in resistance to biotic stresses including aphids, and sources include elite cultivars grown in areas where resistance is needed, adapted landraces, non-adapted landraces, and wild ancestors of the crop (Harlan and de Wet, 1971; Smith, 2005). In the case of soybean, elite cultivars adapted to the northern production region of North America are classified into photoperiod-sensitive maturity groups from 00 to III (Pedersen, 2004). These elite cultivars characteristically lack resistance to soybean aphid, as evident by the rapid and extensive spread of this pest following its

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introduction and the subsequent economic yield loss in soybean it has caused (Ragsdale et al., 2011; Hill et al., 2012). Thus, additional sources of soybean germplasm need to be screened for aphid resistance, including other North American germplasm, non-adapted plant introductions (PIs) from Asia, and germplasm of the wild progenitor of cultivated soybean, *Glycine soja* Siebold and Zucc. (Lu, 2004; Hyten et al., 2006). However, genetic diversity is not equal among these sources, as the process of domesticating soybean, founder effects of introducing germplasm into North America, and intensive selection following its introduction have all greatly reduced this diversity (Hyten et al., 2006; Kim et al., 2012). Of these events, domestication had the most severe impact by halving overall genetic diversity and reducing the frequency of rare alleles by 81 percent (Hyten et al., 2006). Thus, wild soybean represents a rich source of genetic diversity compared to cultivated soybean, and it has been used extensively in several breeding programs for improving various traits in cultivated soybean such as disease resistance and tolerance to various stresses (Panthee, 2010).

Accordingly, given higher genetic diversity in wild soybean, one may argue that screening germplasm of wild soybean will also be worthwhile for identifying resistance to soybean aphid (Wu et al., 2004; Smith, 2005). To date, however, relatively few studies have evaluated wild soybean for resistance to soybean aphid. Sun et al. (1990) screened about 1000 Chinese accessions of wild soybeans and identified two of them as aphid resistant. Hill et al. (2004) found six plant introductions of wild soybean that were at least moderately resistant to soybean aphid. Given the limited number of studies that have tested for aphid resistance in wild soybean and its rich genetic potential, as well as the severity of soybean aphid outbreaks, especially in North America, further testing of wild soybean is warranted. This paper summarizes results of additional tests to identify and characterize resistance of wild soybean lines against soybean aphid.

## 2. Materials and methods

### 2.1. Overview of experiments

Altogether, four sets of experiments were conducted that included two sets of screening assays, and sets of no-choice assays and choice assays. The first screening assay determined a suitable resistant check for use in the other assays. This was followed by 10 screening assays of 135 wild soybean lines from early U.S. maturity groups 0 to III to identify lines putatively resistant to soybean aphid. Lines with the highest levels of resistance were advanced for further evaluation in two no-choice assays and three choice assays. The no-choice assays tested aphid population growth among lines as a measure of antibiosis, and three choice assays tested for antixenosis (non-preference) as a mode of resistance (Kishaba and Manglitz, 1965; Smith, 2005; Pierson et al., 2010). All assays were performed in environmental chambers (CMP 3245, Conviron, Winnipeg, MB, Canada) set with a 16:8 (L:D) photoregime, 22:18 °C (L:D) temperature range, and approximately 30% RH at the North Central Agricultural Research Laboratory, USDA-ARS, Brookings, SD, USA.

### 2.2. Soybean aphids

Soybean aphids used in assays were collected from cultivated soybean fields near Brookings County, SD, in August and October 2009, and maintained as a multiclinal stock colony for multiple generations on plants of soybean cultivar '91B91' (Pioneer Seed, DuPont Corp., Johnston, IA, USA) in growth chambers with settings identical to those used in assays. Newly collected aphids were caged and checked every few hours, and neonate offspring

deposited within the first 30 h were transferred to fresh, 2-wk-old soybean plants to ensure that they were free of circulative aphid-transmitted plant virus. Infested colony plants were maintained 3–4 weeks, and then infested shoots were cut and transferred to non-infested, 2-week-old soybean plants to perpetuate the colony (Hesler and Dashiell, 2008). When tested on soybean lines having one of the aphid-resistant *Rag* genes (Hill et al., 2012), soybean aphids from the colony had reduced populations analogous to the response of biotype 1 soybean aphids (data not shown).

### 2.3. Wild soybean germplasm

Lines of wild soybean were obtained as plant introductions (PIs) from the USDA-ARS Soybean Germplasm Collection (SGC), National Soybean Research Center, Urbana-Champaign, IL, USA. Seed of wild soybean in the SGC was originally collected from Asia and periodically increased in fields near the Center.

### 2.4. Initial assay to select resistant check

Five plant introductions (PI 468400 B, PI 424006 B, PI 468399 B, PI 468399 C and PI 518281) of wild soybean were screened initially to find a suitable resistant check. Resistance to *Soybean mosaic virus* was reported in the first four lines (NGRP, 2013), and they were being assayed as part of a larger screening project to identify aphid resistance (Hesler et al., 2012), as resistance to aphid-transmitted viruses may be due at least partially to vector resistance (Maule et al., 2007). The five wild soybean lines were prepared by placing two seeds of a line into an 8.5-cm square plastic pot filled with a 2:1:1 mixture of Vienna soil (fine-loamy, mixed Calcic Hapludolls), peat moss, and vermiculite (Hesler and Dashiell, 2008). The soil mix was saturated with water, and 12 d later pots were thinned to one seedling each based on uniform seedling growth among lines. The soil surface of each test pot was then covered with a thin (ca. 2 cm) layer of white sand to aid in seeing aphids that might drop during initial infestation. Two weeks after planting, one potted plant of each wild soybean line was placed into a 26.5 × 51-cm plastic tray, with each tray used as an experimental block. Test lines were arranged according to a randomized complete block (RCB) design with eight replications. Experiments commenced as 10 apterous, adult soybean aphids were transferred by artist's brush onto the abaxial surface of unifoliate leaves (5 per leaf) of each test plant (intermediate VC stage; Pedersen, 2004), and plants were then placed into environmental chambers. Two wks after infesting, assay lines were rated as resistant (<150 aphids per plant) or susceptible (>>150 aphids per plant) to soybean aphid, and stark differentiation was typical between susceptible and resistant lines (Hesler and Dashiell, 2008). Ratings were converted to proportions (number resistant per eight replicate plants), and Fisher's exact test was used to determine whether the proportions of resistant plants were independent of soybean line ( $\alpha = 0.05$ ; Zar, 2010). As ratings were not independent, the proportions of resistant plants per line were compared among lines using a Tukey-type mean separation (Zar, 2010).

### 2.5. Screening assays

Assay plants were prepared by placing two seeds of a line into a cylindrical peat pellet (36 mm diam; Ferry-Morse Seed Co., Fulton, KY, USA), and the seeded pellet was saturated with water. Ten to 12 d later, pellets were thinned to one seedling and transferred to individual 8.5-cm square plastic pots containing soil mix and a 2-cm layer of sand. Two wks after planting, one potted plant of each soybean line was placed into a plastic tray, with each tray used as a replicate block. Each tray held 18 pots, with individual pots

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