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Resistance to *Aphis glycines* among wild soybean accessions in laboratory experiments

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ABSTRACT

The soybean aphid (*Aphis glycines* Matsumura) is a major pest of soybean (*Glycine max* (L.) Merr.) in eastern Asia and in North America. Aphid-resistant soybean cultivars have been developed as a non-chemical management tactic, but viability of this tactic may depend on the availability of diverse resistance sources, including wild soybean (*Glycine soja* Siebold and Zucc.), in order to counter various resistance-breaking soybean aphid biotypes. In this study, 10 wild soybean accessions were identified as resistant in free-choice screening assays against avirulent soybean aphid biotype 1, and eight of the accessions were advanced for follow-up in two, 20-day-long no-choice assays. Two accessions, PI 65549 and PI 135624, did not differ from resistant check PI 549046 in number of aphids per plant after 10 and 20 days of infestation; accessions 99PI101404B, PI 549035 B, and PI 342618 A had more aphids than PI 549046 by day 20, but fewer aphids than known susceptible accessions. In a second no-choice assay, accessions 99PI81762 and PI 101404 A had fewer aphids than other test accessions but more aphids than PI 549046 by day 20. Because of particularly strong resistance in PI 135624 and PI 65549, these two accessions should be genetically characterized and tested for resistance against virulent soybean aphid biotypes.

1. Introduction

The soybean aphid (*Aphis glycines* Matsumura) is a pest of soybean (*Glycine max* (L.) Merr.), in eastern Asia and in North America (Ragsdale et al., 2011). It is native to eastern Asia, and has historically been a sporadic pest of soybean there; outbreaks can cause up to 52% yield reduction (Wang et al., 1994). Soybean aphid has been an invasive pest in North America since 2000 (Ragsdale et al., 2011). Although it has been found in nearly all soybean-producing states west of the Rocky Mountains, the pest status of soybean aphid in North America is largely confined to north-central production regions (Ragsdale et al., 2011). Soybean aphid infestations in North America were projected to cost $3.6 to $4.9 billion due to yield loss and control expenditures (Kim et al., 2008a).

Insecticides are the primary method of controlling soybean aphid (Hodgson et al., 2012), but host-plant resistance offers a promising alternative management option (Hesler et al., 2013). In general, a wide diversity of plant resistance sources will optimize the efficacy and durability of resistant crop lines (Smith, 2005; Mundt, 2014). This may be especially important for management of soybean aphid because various soybean aphid biotypes are able to overcome some of the major resistance (*Rag*) genes that have been identified (Kim et al., 2008b; Hill et al., 2010; Alt and Ryan-Mahmutagic, 2013; Fox et al., 2014). Biotype 2 is virulent to *Rag1* plants, biotype 3 surmounts resistance from *Rag2*, and biotype 4 overcomes resistance in plants with *Rag1*, *Rag2* or both genes. Avirulent soybean aphids are designated as biotype 1.

The domestication of crops has undoubtedly led to the loss of important pest resistance genes (Berlinger, 2008; van Doorn and de Vos, 2013; Zhang et al., 2017), and such loss through de-selection bottlenecks seems especially acute in the domestication of soybean (Hyten et al., 2006; Guo et al., 2010). Thus, the probability of finding aphid-resistant sources could be increased by including the screening of wild relatives of soybean (Hill et al., 2004; Yang et al., 2004; Hesler, 2013; Zhang et al., 2017). This can include wild soybean, *Glycine soja* Siebold and Zucc., which readily crosses with domesticated soybean and for which a large accession pool is available and not widely screened (Hyten et al., 2006; Zhang et al., 2017).

Various high throughput techniques have been developed to rapidly screen soybean germplasm in the laboratory and field (Hill et al., 2004; Mensah et al., 2005; Michel et al., 2010; Hesler et al., 2012; Bhusal

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et al., 2014), and many of these methods are adaptable for screening wild soybean as well (Hill et al., 2004; Hesler, 2013; Hesler and Tilmon, 2017). Hesler and Tilmon (2017) recently screened for soybean aphid resistance among 337 early-maturing wild soybean accessions in a series of assays in which soybean aphid were free to colonize various test accessions within environmental chambers. However, tests for resistance should extend beyond free-choice assays, as differences among accessions may be unduly influenced by relatively high attractiveness of some susceptible accessions rather than high unsuitability of other accessions (Berlinger, 2008). Such an effect could inflate the false discovery rate of resistant accessions (Broadhurst and Kell, 2006; Pusztai et al., 2013; Ganna et al., 2013). Accordingly, the identification of putatively resistant sources from screening trials may be followed up with no-choice cage assays that confine soybean aphid to individual accessions and better reflect limited host choice that soybean aphid faces in the field (Berlinger, 2008; Hesler et al., 2017b). The objective of this study was to report the results of screening assays that identified putative soybean aphid resistance among wild soybean accessions, as well as results from further evaluation of these accessions in no-choice assays.

2. Materials and methods

2.1. Overview of experiments

Experiments were conducted at the North Central Agricultural Research Laboratory, USDA-ARS, Brookings, SD, USA, and included eight screening assays and two no-choice assays of wild soybean accessions. Ninety-six unique, wild soybean accessions were screened, with 16 accessions tested in two assays, including 12 accessions that showed resistance in their first assay (Appendix). Accessions identified as aphid resistant in screening assays (see below) were advanced for further evaluation in the no-choice assays. Assays were performed in environmental chambers (PGR15 and PGW36, Conviron, Winnipeg, MB, Canada) set to a 16-h light per day, 22:18 °C (day:night) temperature range, and approximately 30% relative humidity.

2.2. Soybean aphids

Soybean aphids used in the assays were collected from cultivated soybean fields in Brookings County, SD, USA, in October 2009, and maintained as a multiclonal stock colony for multiple generations on 2- to 4-week-old soybean plants (soybean aphid-susceptible cultivar 'Brookings' [Jiang et al., 2014]; South Dakota State University, Brookings, SD, USA). Colonies were maintained in growth chambers using the same conditions as for assays (Hesler, 2013). Soybean aphids from the colony had responses to known resistance sources that were largely consistent with avirulent biotype 1 (Hill et al., 2012; Hesler, 2013).

2.3. Wild soybean

Plant introduction (PI) accessions of wild soybean were obtained from the USDA-ARS Soybean Germplasm Collection (SGC), National Soybean Research Center, Urbana, IL, USA. These germplasm accessions were originally collected from Asia, and seed was periodically produced and stored at the SGC. Individual accessions of wild soybean were identified with their respective PI number or with a number beginning with the digits '99' to denote a 1999 seed lot for which the original PI numbers could not be verified (Hesler and Tilmon, 2017).

2.4. Screening assays

Wild soybean accessions were screened in eight assays using slight modification of a free-choice assay in Hesler (2013). Assay plants were grown by placing two seeds of a wild soybean accession into a cylindrical peat pellet (36 mm diam; Ferry-Morse Seed Co., Fulton, KY, USA) saturated with water. Individual pellets were thinned to one seedling 12 days later and transferred into separate 8.5-cm square plastic pots containing a 2:1:1 mixture of soil (fine-loamy, mixed Calcic Hapludolls), peat moss, and vermiculite plus a 2-cm top layer of sand to provide a level surface for aphids to move across and to prevent fungus gnat infestation (Hanson et al., 2016). Two weeks after planting, one potted plant of each wild soybean accession in the VC developmental stage (fully expanded unifoliolate leaves; Pedersen and Licht, 2014) was set into a plastic tray, and each tray was used as one of eight replicate blocks. Each tray held 18 individually potted plants. The 18 plants comprised 14 different assay accessions, a resistant check (PI 549046; Hesler, 2013), a susceptible check (PI 522212 B; Hesler, 2013), and two aphid-source plants. The two source plants were situated at foci roughly equidistant from surrounding test accessions (Hesler, 2013). Source plants consisted of 4-week-old Brookings soybean plants that each had about 250 soybean aphids after infesting with 10 adult aphids 2 weeks earlier (Hesler and Tilmon, 2017). The source plants were clipped at soil level to induce wilting and subsequent dispersal of aphids onto assay plants, and the stem of each clipped source plant was attached by paper clip to a 10-cm long wooden skewer that was inserted 3 cm into the potted soil.

Test accessions were arranged in each replicate tray according to a randomized complete block design. Trays were placed in an environmental chamber, and 2 weeks later when plants had the second trifoliolate leaf newly unfurled (V2 stage), infestations of individual assay plants were rated in 50-aphid increments using an ordinal scale that ranged from a rating of 1 (50 or fewer aphids) to 6 (≥250 aphids).

A nonparametric, marginal effect analysis described by Shah and Madden (2004) was used for each assay to test whether soybean aphid-infestation ratings differed by plant accession. This "ANOVA-type" analysis (Shah and Madden, 2004; Khan et al., 2004) uses standard procedures and specialized macros in SAS (2012). In each screening assay, infestation ratings were ranked among the individual 128 assay plants (16 accessions × 8 replicates), with ties assigned a midpoint value based on the number of plants of the same rating (PROC RANK). Rankings were tested for variance by plant accession using PROC MIXED, utilizing mid-ranks, determined as the default in the rank procedure, to calculate the nonparametric test statistics and significance levels (Shah and Madden, 2004). The eight free-choice assays were analyzed separately. Sixteen accessions that were putatively resistant in one assay were repeated in a second assay.

When rankings varied significantly ($\alpha = 0.05$) by accession, the LSMEANS option was used to separate mean rankings among accessions (Shah and Madden, 2004). Accessions with a ranking that did not differ significantly from that of the resistant check in two screening assays were considered resistant, and eight such accessions were included in no-choice assays to confirm resistance (Berlinger, 2008). Medians of infestation ratings and mean rankings for each accession are reported (Shah and Madden, 2004).

2.5. No-choice assays

Because of the relatively high false discovery rate in high-throughput tests such as screening assays, only test accessions identified as resistant in two respective screening assays were advanced to confirmatory no-choice assays. A subset of eight test accessions identified in the free-choice tests were advanced for testing in a series of two no-choice assays in this study; the number of putatively-resistant accessions was limited to those eight based on funding constraints and project deadlines.

Putatively-resistant test accessions were compared within their respective no-choice assay by measuring soybean aphid populations at 10 and 20 days after initial infestation, when plants were in the V2 (two-trifoliolate) and late V3 (three-trfoliolate) stages, respectively. The assays had nine (assay 1) and seven (assay 2) wild soybean accessions,