Accessions of Perennial *Glycine* Species With Resistance to Multiple Types of Soybean Cyst Nematode (*Heterodera glycines*)

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Abstract

Soybean cyst nematode (SCN; *Heterodera glycines*; HG) is a widely occurring and damaging pathogen that limits soybean production. Developing resistant cultivars is the most cost-effective method for managing this disease. Genes conferring SCN resistance in soybean have been identified; however, there are SCN populations that overcome known resistance genes. In order to identify additional sources of resistance and potentially new resistance genes, 223 plant introductions (PIs) of *G. tomentella* and 59 PIs of 12 other perennial *Glycine* species were inoculated with HG Types 0, HG 2, and HG 1.2.3, and then 36 PIs out of this set were further evaluated with HG Type 1.2.3.4.5.6.7, a

population that overcomes all the resistance genes in soybean. Of 223 *G. tomentella* PIs evaluated, 86 were classified as resistant to three HG types, 69 as resistant to two HG types, and 22 as resistant to one HG type. Of the other 12 perennial *Glycine* species, all PIs of *G. argyrea* and *G. pescadrensis* were resistant to all three HG types. Of the 36 PIs challenged with HG Type 1.2.3.4.5.6.7, 35 were resistant with 16 showing no cyst reproduction. Our study confirms that there are high levels of resistance to SCN among the perennial *Glycine* species. This represents an untapped resource for use in genetic studies and for improving resistance to SCN in soybean.

Soybean cyst nematode (*Heterodera glycines*) (SCN) is the most damaging pathogen to soybean (*Glycine max* [L.] Merr.) production, incurring about 2.2% losses in production averaged over Argentina, Brazil, and the U.S.A., the three top soybean-producing countries in the world (Hartman 2015; Niblack and Riggs 2015). Soybean plants are damaged when juvenile nematodes feed on the vascular system to complete their life cycle, producing eggs inside cysts that protrude from the roots (Niblack and Riggs 2015).

Developing soybean cultivars with resistance to SCN is one effective approach for managing SCN. Although a number of sources of resistance have been discovered in plant introductions (PIs) in the USDA Soybean Germplasm Collection, mainly one source, PI 88788, has been employed in breeding programs (Joos et al. 2013). Shifts in SCN populations have led to a decrease in resistance in soybean cultivars derived from PI 88788 (Colgrove et al. 2002; Niblack et al. 2003, 2008), increasing the need to utilize other sources of resistance. Other sources found in soybean, like PI 437654 and PI 89772 (Anand 1992; Arelli et al. 2015; Nickell et al. 1999), have been not fully effective due to the vast genetic diversity of SCN populations.

The genetic variation in SCN populations was initially described by a race scheme in which the separation of different SCN populations was based on comparing female reproduction on four resistant differential lines (Golden et al. 1970). This system also was used to classify soybean genotypes based on responses to the various races ranging from immunity, or no reproduction, to highly susceptible (Schmitt and Shannon 1992). However, with the extensive genetic diversity of *H. glycines* populations, the race scheme was shown to be inadequate for defining

SCN populations. Therefore, the race scheme was replaced by a new scheme, which uses seven soybean indicator lines to classify nematode populations to HG types (Niblack et al. 2002).

There are only a few studies that have evaluated plant species other than *G. max* for resistance to SCN, although the pathogen is known to reproduce on other legume plants (Riggs 1992). The genus *Glycine* is composed of two subgenera, *Glycine* and *Soja*. *Soja* includes *G. max* and *G. soja*, an annual plant closely related and cross-compatible with soybean. The subgenus *Glycine* includes 24 perennial *Glycine* species, which mostly originate from diverse geographical areas in Australia, and are not cross-compatible with soybean (Hymowitz and Hartman 2015). Riggs et al. (1998) evaluated eight perennial *Glycine* species with one accession each of *G. argyrea*, *G. arenaria*, *G. canescens*, *G. curvata*, *G. cyrtoloba*, *G. latifolia*, and *G. microphylla*, and four accessions of *G. tabacina* and *G. tomentella* to three isolates of *H. glycines*, and determined that all were resistant with almost all having no cyst production. The most extensive study evaluated 491 accessions of 12 perennial *Glycine* species for resistance to *H. glycines* HG Type 0 and showed that all species, except *G. curvata* and *G. pindanica*, had at least one accession with an immune response (Bauer et al. 2007). In addition to SCN resistance, some perennial *Glycine* species have been reported with resistance to other soybean diseases including brown spot (Lim and Hymowitz 1987), Phytophthora root rot (Kenworthy 1989), powdery mildew (Mignucci and Chamberlain 1978), soybean rust (Hartman et al. 1992; Hymowitz 1995), Sclerotinia stem rot, and sudden death syndrome (Hartman et al. 2000). Thus, there is a reservoir of potentially new resistance genes in the perennial *Glycine* species that needs additional research and exploitation. These sources of resistance may be especially useful in cases where the pathogen population, like that of SCN, is complex. To our knowledge, published reports of perennial *Glycine* species evaluated for SCN resistance have used either only one HG type to characterize accessions (Bauer et al. 2007) or multiple HG types with a very limited number of accessions (Riggs et al. 1998). The objective of our study was to evaluate 13 perennial *Glycine* species against SCN HG Type 0, HG 2, HG 1.2.3, and HG 1.2.3.4.5.6.7. This information would provide a more comprehensive understanding of the resistance structure in perennial *Glycine* species to several populations of *H. glycines*.

Materials and Methods

SCN source and HG type confirmation on a soybean differential set. SCN HG Types 0, 2, and 1.2.3 were obtained from G. R. Noel, USDA-ARS, Urbana, IL, and multiplied once on the roots of the

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Trade and manufacturers' names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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susceptible soybean cultivar Lee 74 before beginning this study. The SCN HG Type 1.2.3.4.5.6.7 (TN23) was collected from a field in Missouri and maintained in the greenhouse by mass selection on PI 437654. The isolate was obtained from Dr. Colgrove at the University of Illinois, and had been multiplied on the resistant soybean accession PI 437654 just before use in our study.

The SCN HG Types 0, 2, 1.2.3, and HG 1.2.3.4.5.6.7 were confirmed on the standard susceptible cultivar Lee 74 and seven soybean indicator lines, Peking, PI 88788, and PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316 (Table 1; Niblack et al. 2002).

Table 1. *Heterodera glycines* (HG) types confirmed on seven soybean indicator lines based on positive or negative host compatibility

Indicator lines	Female index of each HG type ^z			
	HG 0	HG 2	HG 1.2.3	TN 23
PI 54840 (Peking)	-	-	+	+
PI 88788	-	+	+	+
PI 90763	-	-	+	+
PI 437654	-	-	-	+
PI 209332	-	-	-	+
PI 89772	-	-	-	+
PI 548316 (Cloud)	-	-	-	+
HG type determination	HG 0	HG 2	HG 1.2.3	HG 1.2.3.4.5.6.7

^z “+” indicates positive host compatibility as the number of females produced was equal to or greater than 10% of the number produced on the standard susceptible cultivar Lee; “-” indicates negative host compatibility as the number of females is less than 10% of cultivar Lee. Ratings are based on five replications for each soybean line and each HG type.

For each HG type test, five seeds of each indicator line were planted in five polyvinyl tubes with steam-pasteurized Torpedo sand (100 cm³), one seed per tube. The tubes were randomly arranged in plastic buckets (19 cm in diameter and 20 cm in height) filled with 5 liters of pasteurized Torpedo sand. A shallow hole was dug next to each 1-week-old seedling to inoculate by pipetting 1 ml of 2,000/ml egg solution of the assigned SCN HG type. Cysts were extracted and counted on each indicator line 35 days after inoculation and the female index (FI) was calculated based on the formula: FI = (mean number of females on an indicator soybean line / mean number of females on standard susceptible) × 100. The HG type of each SCN population was determined according to the FI of each indicator line with FI <10% indicating negative host compatibility and FI >10% indicating positive host compatibility. The resistance level was classified into four categories based on FI value: resistant with FI ≤9%, moderately resistant with FI 11 to 30%, moderately susceptible with FI = 31 to 60%, and susceptible with FI >60% (Schmitt and Shannon 1992). The plastic buckets with plants were kept in a water bath at 28°C in the greenhouse at 16 h of fluorescent light per day during the experiment.

Seed source and seed preparation of perennial *Glycine* species.

There were 223 *G. tomentella* PIs and 59 PIs of 12 other *Glycine* species obtained from the USDA-ARS Soybean Germplasm Collection (www.ars-grin.gov) that were tested in three experiments. The first experiment consisted of 223 *G. tomentella* PIs. The second experiment consisted of 59 PIs of 12 *Glycine* species. The third experiment was based on a selection from the first two experiments. For all experiments, seeds of the perennial *Glycine* species were scarified (required for germination) by removing a tangential portion of the seed coat on the side opposite the hilum with a sharp razor blade. Each

Table 2. *Glycine tomentella* accessions evaluated for their response to three *Heterodera glycines* (HG) types

Plant introductions	Response ^w		
	HG 0	HG 2	HG 1.2.3
320548, 339655, 339657, 339663, 373987, 373988, 440999, 441001, 441004, 441006-441010, 446950, 446980, 446989, 446996, 483218, 483223, 483225, 483227, 483228, 499919, 499920, 499927, 499928, 499933, 499934, 499939, 499941, 505202, 505212, 505224, 505225, 505228, 505230, 505231, 505233, 505235, 505239, 505240, 505243, 505245, 505247, 505248, 505250, 505252, 505254, 505255, 505257, 505261, 505262, 505266-505268, 505275, 505276, 505281, 505286, 505292, 505297, 509491, 509501, 509502, 537294, 546957, 546958, 563881, 563892, 563893, 563896, 563898, 563899, 573056, 573058, 573064, 573068, 573070, 573071, 583969, 583971, 583975, 591606, 599422, 604474, 604487, 604488, 604491, 604494	R	R	R
393556, 393557, 446963, 446984, 446985, 483220, 483224, 483226, 499938, 499951, 505209, 505210, 505218, 505219, 505234, 505277, 505279, 505285, 509499, 563878	R	R	MR
499930, 505258, 505260, 583973	R	MR	R
373980, 399479, 440998, 446946, 446951, 446954, 446956, 446957, 446958, 446959, 446981, 446988, 446998, 483219, 499916, 499935, 499936, 505203, 505229, 505238, 505244, 505249, 505269, 505269, 563897, 573062, 573066, 573067, 591609, 604476-604479, 604484, 604489, 604490, 604492, 604493	MR	R	R
441011, 505213, 505206	R	MR	MR
441005, 446955, 446994, 499937, 499945, 499947, 505215, 505220, 505226, 505280, 505294, 583974, 604481	MR	R	MR
446991, 505264, 505298, 505304, 563895, 563976, 595823	MR	MR	R
441012, 446995, 505217, 505232, 505236, 505237, 505256, 505263, 505272, 505282, 505303, 509500	MR	MR	MR
233051, 320547, 37399, 505246, 505271	R	R	S
505221, 505242, 591605, and 595824	R	S	R
499990	R	MR	S
446960 ^x , 446992, 505205	MR	R	S
446962 ^y , 505216 ^y	MR	S	R
505201 ^x	S	R	MR
505222 ^y	R	S	S
441000 ^z , 446982 ^z , 446993 ^{x,y,z}	S	R	S
441003 ^{y,z} , 446952 ^{y,z} , 505214 ^{y,z} , 563900 ^z	S	S	R
446953 ^z , 505211 ^z , 505241 ^z , 505278 ^z	S	MR	MR
446961, 446949 ^y , 563877	MR	S	MR
441002 ^{y,z} , 505300, 505301 ^{y,z}	S	S	MR
446947 ^y , 573063 ^y	MR	S	S
446983, 446948 ^{x,y,z} , 505274 ^{x,y,z} , 573059, 591607 ^x , 595822	S	S	S

^w Resistant (R) = adjusted female index (AFI) 0–10; moderately resistant (MR) = AFI 11–30; moderately susceptible (MS) = AFI 31–60; and susceptible (S) = AFI 61 or greater. Rating based on five replications over time.

^x Variable result in repeat for HG 1.2.3 from resistant to susceptible; classified as susceptible.

^y Variable result in repeat for HG 2.5.7 from resistant to susceptible; classified as susceptible.

^z Variable result in repeat for HG 0 from resistant to susceptible; classified as susceptible.

seed was placed in a rolled 6.5 mm diameter filter paper disk (No. 3 Whatman, Fisher Scientific) inside a single well of a 96-well ELISA plate (12 columns and 8 rows). Twenty microliters of distilled water were added to each well, and the plates were covered and incubated at 25°C under high humidity with 16 h of fluorescent light per day for 3 to 5 days until the seeds germinated.

Experimental set up and design. In the first and second experiment, PIs were inoculated with SCN HG Type 0, HG 2, and HG 1.2.3 (Tables 2 and 3). In the third experiment, 36 of the 282 PIs tested were further evaluated by inoculating with HG Type 1.2.3.4.5.6.7, a population that overcomes all the resistance genes in soybean

(Table 4). The soybean cultivar Lee 74 was included in these experiments as a susceptible check.

For the first and second experiment, each PI was inoculated with SCN HG Type 0, HG 2, and HG 1.2.3 separately in a completely randomized design with five replications for each treatment (PI entry and HG group). Seedlings germinated on filter paper were transplanted into clay pots (10 cm in diameter) filled with pasteurized Torpedo sand. A small divot about 2.5 cm deep was dug between the edge of each pot and the transplanted seedling, and 15 cysts of similar size of each SCN HG type were placed into the divot and covered with sand at the time of sowing. Each pot was an experimental unit. The inoculated seedlings were grown in a greenhouse room for 35 days at 27 ± 3°C. Supplemental illumination was provided by a mixture of high-pressure sodium vapor and metal halide lamps for a 16 h photoperiod. The five replications were completed individually over time.

For the third experiment, PIs were tested in water baths with controlled temperature using a randomized complete block design (RCBD) with 10 blocks (replications). Each block consisted of 37 polyvinyl chloride tubes (100 cm³) placed in two plastic buckets (19 cm diam. × 20 cm height) filled with 5 liters of pasteurized Torpedo sand (18 tubes in one bucket and 19 in the other). Seedlings of 36 perennial *Glycine* accessions and the standard susceptible check Lee 74 were randomly transplanted per block into pasteurized Torpedo sand, one plant per tube. Each tube was an experimental unit

Table 3. *Glycine* species accessions evaluated for their response to three *Heterodera glycines* (HG) types

<i>Glycine</i> spp.	Plant introduction	Response ^z		
		HG 0	HG 2	HG 1.2.3
<i>G. arenaria</i>	505204	R	R	R
<i>G. argyrea</i>	509451, 505151	R	R	R
<i>G. canescens</i>	440942	MR	R	R
<i>G. canescens</i>	446934	MS	MS	R
<i>G. canescens</i>	509458	MS	S	MR
<i>G. canescens</i>	573046	MS	S	R
<i>G. canescens</i>	509457	R	R	R
<i>G. canescens</i>	440932	R	MS	R
<i>G. canescens</i>	509455	R	R	MS
<i>G. canescens</i>	505154	R	R	S
<i>G. canescens</i>	509456	R	R	MR
<i>G. canescens</i>	573044	R	MS	MS
<i>G. canescens</i>	509454	R	MR	MR
<i>G. canescens</i>	573045	S	S	MR
<i>G. clandestina</i>	440960	MR	S	R
<i>G. clandestina</i>	509461, 505163	MS	R	R
<i>G. clandestina</i>	509468	R	R	R
<i>G. clandestina</i>	509462, 509466	R	MS	R
<i>G. curvata</i>	505167	MS	R	R
<i>G. cyrtoloba</i>	440962	MS	R	R
<i>G. cyrtoloba</i>	373993, 509472	R	R	R
<i>G. falcata</i>	509473	MS	R	R
<i>G. falcata</i>	509475	R	MS	R
<i>G. falcata</i>	440975	S	S	R
<i>G. latifolia</i>	253238	MR	MS	R
<i>G. latifolia</i>	505181	MS	MR	R
<i>G. latifolia</i>	440978	R	R	R
<i>G. latifolia</i>	509478, 509476	R	MS	R
<i>G. latifolia</i>	509479	S	R	R
<i>G. latifolia</i>	509480	S	MS	R
<i>G. microphylla</i>	505188, 509486	R	R	R
<i>G. microphylla</i>	446939	R	MS	R
<i>G. microphylla</i>	505196	R	MR	R
<i>G. microphylla</i>	509485	R	S	R
<i>G. microphylla</i>	509489	S	S	MS
<i>G. microphylla</i>	509483	S	R	R
<i>G. microphylla</i>	509487	S	MS	MS
<i>G. pescadrensis</i>	505195, 505197, 505199	R	R	R
<i>G. rubiginosa</i>	440954	R	R	R
<i>G. tabacina</i>	509494	MR	MS	MR
<i>G. tabacina</i>	339661, 509493	MS	MS	R
<i>G. tabacina</i>	373986	MS	S	MR
<i>G. tabacina</i>	373990, 509496	R	R	R
<i>G. tabacina</i>	509497	R	R	MS
<i>G. tabacina</i>	440990	R	MS	MS
<i>G. tabacina</i>	509492	R	MS	MR
<i>G. tabacina</i>	509490	R	S	MR
<i>G. tabacina</i>	446974, 509495	S	MS	MS
<i>G. tabacina</i>	509498	S	MR	S

^z Resistant (R) = Adjusted female index (AFI) 0–10; moderately resistant (MR) = AFI 11–30; moderately susceptible (MS) = AFI 31–60; and susceptible (S) = AFI 61 or greater. Rating based on five replications over time.

Table 4. Number of cysts recovered from selected *Glycine* species plant introductions after inoculation with soybean cyst nematode HG Type 1.2.3.4.5.6.7

<i>Glycine</i> spp.	Plant introduction	Chromosome (2n)	Cyst count ^y
<i>G. argyrea</i>	509451	40	0
<i>G. canescens</i>	440932	40	0
<i>G. clandestina</i>	509462	I ^z	0
<i>G. clandestina</i>	509468	40	0
<i>G. latifolia</i>	505181	I	1
<i>G. microphylla</i>	505188	40	1
<i>G. falcata</i>	509473	I	1
<i>G. cyrtoloba</i>	509472	40	2
<i>G. latifolia</i>	509479	40	2
<i>G. microphylla</i>	509486	I	3
<i>G. canescens</i>	573047	I	5
<i>G. pescadrensis</i>	505197	80	6
<i>G. tabacina</i>	446974	80	17
<i>G. tabacina</i>	373990	40	148
<i>G. tomentella</i>	339655	80	0
<i>G. tomentella</i>	339657	78	0
<i>G. tomentella</i>	441010	78,80	0
<i>G. tomentella</i>	446996	78	0
<i>G. tomentella</i>	483227	80	0
<i>G. tomentella</i>	505235	78	0
<i>G. tomentella</i>	505286	78	0
<i>G. tomentella</i>	509502	78 7	0
<i>G. tomentella</i>	573064	38	0
<i>G. tomentella</i>	604474	I	0
<i>G. tomentella</i>	320548	78,80	0
<i>G. tomentella</i>	505240	80	0
<i>G. tomentella</i>	583969	40	1
<i>G. tomentella</i>	604494	I	1
<i>G. tomentella</i>	499920	I	2
<i>G. tomentella</i>	573059	I	3
<i>G. tomentella</i>	446983	40	4
<i>G. tomentella</i>	563899	40	8
<i>G. tomentella</i>	546957	80	9
<i>G. tomentella</i>	446950	80	14
<i>G. tomentella</i>	373987	38	16
<i>G. tomentella</i>	505292	40	35
<i>G. max</i>	Lee 74	40	676
<i>G. max</i>	Hartwig	40	254
<i>G. max</i>	437654	40	224

^y Numbers averaged over two runs with three replications per run.

^z Chromosome number not known.

with 10 replications for each accession. Seedlings were inoculated with 2,000 eggs 3 weeks after seedlings were transplanted. Two seedlings per entry were sampled at 5 days post inoculation (DPI), 10 DPI, and 15 DPI to evaluate nematode development on each perennial *Glycine* plant compared with the susceptible check Lee at each time point. Roots were washed, stained with acid fuchsin (Byrd et al. 1983), and a dissecting microscope at 64× magnification was used to quantify juvenile nematodes of all stages (J2, J3, and J4) in the entire root system. The remaining four plants of each PI were kept in the plastic buckets and harvested at 35 DPI for cyst counting. Plastic buckets with plants were set in a 28°C water bath in the greenhouse. Supplemental illumination was provided by a mixture of high-pressure sodium vapor and metal halide lamps for a 16 h photoperiod. The experiment was completed twice with a different randomization for the second run.

Cyst extraction and counts. Thirty-five days after inoculation, plants were cut at the soil line and the content of each pot or tube was poured into a plastic bucket, and about 2 liters of water was added to make a soil suspension. The soil suspension was mixed well with pressurized water by hand for about 10 s, and then sediments were allowed to settle for 5 s leaving cysts floating. The suspension was poured through an 850 µm sieve to remove larger debris, and then over a 250 µm sieve. Finally, retained cysts and accompanying sediments were washed into a clean 50 ml plastic tube.

Cysts collected in each 50-ml tube were decanted on to a glass Petri plate, marked with a 1 mm² grid, and individually counted under a dissecting microscope (Olympus SZX16, Tokyo, Japan) at 25×. The mean cyst count was recorded over four plants of each PI. Significant variation in root size among the tested 282 perennial PI accessions was observed in experiment 1. To adjust for this, root size at washing was recorded based on a visual scale from 1 to 10 with 1 = smallest to 10 = largest. The FI was adjusted to accommodate size differences in the entire root system using an adjusted female index (AFI) calculated with the formula: AFI = (mean number of females on tested accession / root size) / (mean number of females on standard susceptible / root size) × 100. Classification of resistance or susceptibility was as follows: resistant (R) = AFI 0 to 10; moderately resistant (MR) = AFI 11 to 30; moderately susceptible

(MS) = AFI 31 to 60; and susceptible (S) = AFI 61 or greater. For the third experiment, FIs were calculated and the data from the two runs were tested for homogeneity of error variances before combining data.

Data analysis. For the first and second experiment, the AFI was used to separate the PIs into response categories. In the third experiment, nematode (J2-J4) counts were log transformed after adding 0.5 to the raw count data. Analysis of variance was done to compare nematode (J2-J4) counts among different PIs and at different time points (5 DPI, 10 DPI, 15 DPI) with the aov function in R 3.2.1 (R Core Team, Vienna, Austria), and multiple comparisons were done between each PI pairs and time point pairs using Tukey's HSD function in R 3.2.1. For cyst number comparison at 35 DPI in the third experiment, FI was used to separate the PIs into response categories.

Results

SCN genotype confirmation. All SCN populations used in this study were confirmed using seven indicator lines (HG type test) (Table 1). The first population had FI >10% on only PI 88788 and was classified as HG 2. The second SCN population had FI <10% on all seven indicator lines and was classified as HG 0. The third population had FI >10% on Peking, PI 88788, and PI 90763 and was classified as HG 1.2.3. The fourth population had FI >10% on all seven indicator lines and was classified as HG 1.2.3.4.5.6.7.

Evaluation of perennial *Glycine* species for resistance to HG 0, HG 2, and HG 1.2.3. Among the 223 *G. tomentella* PIs evaluated, resistance to the three SCN types was identified in 86 PIs (Table 2), while only six PIs were susceptible to all three SCN types. One hundred and thirty-one PIs showed a differential reaction among the three SCN types from resistant to susceptible.

Of the 12 other perennial *Glycine* species evaluated, SCN reproduction was detected on accessions in 10 species, including first time reports for *G. arenaria* and *G. rubiginosa* as hosts to SCN. All PIs of *G. pescadrensis* and *G. argyrea* had no cyst reproduction regardless of the HG type. Of the 59 PIs evaluated, only four PIs (*G. microphylla*: PI 509487 and PI 509489; *G. tabacina*: PI 446974 and PI 509495) were susceptible to all three SCN HG types; all others were resistant to at least one SCN HG type (Table 3). Each of the 12 species had individual accessions that were resistant to all the three HG types of SCN, and 16 PIs in total that were resistant to all three SCN HG types.

Evaluation of selected *Glycine* species for resistance to HG 1.2.3.4.5.6.7. Based on germination rate, only 16 perennial *Glycine* lines were sampled and stained. Two seedlings of each PI were sampled at each time point, and juveniles of all stages (J2-J4) were counted in the entire root system for each PI. Experiment three was conducted twice, and the root staining data were combined for the two experiments. Nematode (J2-J4) counts in perennial species were compared among different PIs at different time points. Root staining of the 16 perennial *Glycine* lines and susceptible check Lee showed that at juvenile counts were significantly different among different PIs ($P < 0.00001$). Lee 74 had significantly more cysts than all the perennial *Glycine* species, and among the perennial species, PI 505286, PI 509451, and PI 505235 had significantly more cysts than PI 509462, PI 573064, PI 339657, and PI 339655 (Table 5). At 5 DPI, all accessions harbored J2 (Fig. 1A), and the development status of nematode was not different between the susceptible check Lee 74 and perennial species. At 10 DPI, both J2 and J3 stages were observed in the roots of Lee 74 with nearly 50% being J3s (Fig. 1B); however, only J2s were observed in the roots of perennial *Glycine* accessions, indicating slower development of SCN in perennial *Glycine* species than in Lee 74. At 15 DPI, when most of the nematodes in the roots of Lee 74 had developed to J4s, most of the nematodes in the perennial *Glycine* accessions were at the J2 stage (Fig. 1C). Nematode number in the perennial species differed ($P < 0.01$) at different time points as counts (indiscriminative J2 to J4 stages) changed from 2.3 to 7.0 to 1.8 at 5, 10, and 15 DPI, respectively, with the 10 DPI count being different from 5 and 15 DPI. At 35 DPI, FI calculations on all 36 PIs except one were classified as resistant (Table 4). The one susceptible entry was *G. tabacina* PI

Table 5. Counts of *Heterodera glycines* juveniles in stained roots 5, 10, and 15 days after inoculation (DAI) and cysts counts 30 DAI on soybean cultivar Lee74 and on perennial *Glycine* species

Entry ^y	Juvenile count (DAI)			Cyst counts ^z
	5	10	15	
Lee74	61.0	88.0	134.0	89.1 a
PI 505286	0.5	13.0	2.0	4.9 b
PI 509451	1.5	8.5	2.0	4.2 b
PI 505235	5.5	22.5	3.0	3.6 b
PI 441010	4.5	8.5	1.0	2.5 bc
PI 604474	1.5	6.0	3.0	2.5 bc
PI 505188	4.0	10.5	3.0	2.1 b-d
PI 505181	1.5	21.0	1.5	1.8 b-e
PI 440932	7.5	4.0	0.0	1.8 b-e
PI 509502	0.5	7.0	2.5	1.7 b-f
PI 446996	3.5	10.5	0.0	1.6 b-g
PI 509468	1.0	8.0	0.0	1.6 b-g
PI 509473	1.3	6.8	0.8	1.4 b-g
PI 483227	0.5	8.5	0.0	0.8 c-g
PI 509462	4.5	6.5	0.0	0.4 d-g
PI 573064	0.5	4.0	1.0	0.3 e-g
PI 339657	0.5	2.5	0.0	0.2 fg
PI 339655	0.5	0.5	0.0	0.2 g

^y PI = plant introductions (PI); most are *G. tomentella* except for 509451 (*G. argyrea*), 509462 and 509468 (*G. clandestina*), 505181 (*G. latifolia*), and 505188 (*G. microphylla*).

^z Mean nematode counts were back transformed for presentation; means with different letters are significantly different at $P = 0.05$ based on Tukey's HSD test. Each mean is based on 20 experimental units (two samples per 10 replications).

373990. Of the resistant PIs, 14 had no cyst production while 21 had low cyst counts (Table 4).

Discussion

We evaluated 223 PIs of *G. tomentella* and 102 PIs of 12 other perennial species for resistance to SCN. In general, many of the PIs were classified as resistant to SCN and only a few as susceptible. These results are consistent with previous studies, in which immunity and high levels of resistance to SCN were identified in many PIs of the perennial *Glycine* species (Bauer et al. 2007; Riggs et al. 1998). In one of the earliest studies, Riggs et al. (1998) inoculated one PI from each of eight perennial *Glycine* species with four SCN isolates (races 1, 3, 5, and 14) and found all PIs as immune or resistant except for PI 440956, which was moderately resistant to one HG type. In our study, 282 accessions from 13 perennial *Glycine* species were tested for resistance to three different SCN populations with PIs in each of the eight species used by Riggs et al. (1998), identified as resistant. Bauer et al. (2007) reported that 56% of the PI entries representing 12 perennial *Glycine* species were immune or resistant to one HG type. In our study, 10 of the 12 perennial *Glycine* species were classified as immune or highly resistance to three HG types, indicating a much broader resistance in these PIs.

It is clear that the germplasm of the perennial *Glycine* species contains many PIs that are highly resistant to SCN whereas the majority of the soybean germplasm is susceptible to SCN (Wang et al. 2003; Young 1990). In soybean, only a few PIs have been reported to be resistant to a specific or several SCN populations by several genes including *Rhg1-3*, *Rhg4*, and *Rhg5* (Concibido et al. 2004; Jiao et al. 2015). SCN populations are known to overcome the resistance

sources (Mitchum et al. 2007; Niblack et al. 2008). Although an immune response was identified in PI 437654 to all HG types tested (Anand et al. 1988), field populations isolated from Brazil were able to reproduce on Hartwig (derived from PI 437654) (Dias et al. 1998), and the isolate TN23 (later classified as HG 1.2.3.4.5.6.7) isolated from a field isolate in Missouri in 2005 reproduced on PI 437654 (Bekal et al. 2003). The high level and broad resistance to SCN in the perennial *Glycine* species suggests that they may be very valuable sources to broaden the genetic base of soybean and develop new cultivars with more durable resistance to SCN.

The differential reaction of PIs within some of the perennial *Glycine* species is interesting because these perennial *Glycine* species evolved primarily in Australia and presumably without SCN in many different niches (Doyle et al. 2004; Sherman-Broyles et al. 2014). Because of their susceptibility to SCN, the species are hosts with some remnant susceptible genes from the common ancestor or ancestors of soybean and the perennial *Glycine* species. Since nonhost resistance to a pathogen has been defined as an entire species being resistant to all genetic variation of the pathogen (Niks and Marcel 2009), the only species that fit this criterion in our study was *G. argyrea*. However, no conclusions can be made until more accessions of the species are evaluated. *G. arenaria* and *G. rubiginosa* were identified as hosts of SCN, which were not been reported before. The extent of SCN hosts are not known, but other hosts outside the legume family have been reported (Riggs 1992; Venkatesh et al. 2000).

One of the resistance mechanisms used by nonhost plants is to prevent penetration of the J2 stage worms into roots (Colgrove and Niblack 2008). In our study, we did not see evidence of this in the perennial *Glycine* species, but root staining showed that the number of nematode

Fig. 1. Comparison of *Heterodera glycines* type 1.2.3.4.5.6.7 SCN development in the roots of perennial *Glycine* species and standard susceptible check Lee 74 stained with acid fuchsin. Fewer nematodes progressed from J2 to J3 or J4 in the perennial *Glycine* accessions. **A**, representative perennial *Glycine* accession, *G. tabacina* PI 505235 (left) and susceptible check Lee 74 (right) at 5 days post infection (DPI). **B**, SCN development at 10 DPI in *G. tabacina* PI 446974 (left) and Lee 74 (right). **C**, SCN development at 15 DPI in PI 505235 (left) and Lee 74 (right). Bar (—) represents 500 microns in each image.

entering roots of the perennial *Glycine* species were fewer than that of Lee 74, and that the development of further life stages past J2 was delayed or in the case of immunity did not occur.

Information on the genomics and the molecular characterization of SCN resistance in the perennial *Glycine* species is limited. A comparative genomics study showed that the linkage group (LG) 18 of *G. latifolia* had a high degree of collinearity with the chromosome 18 in soybean with no interchromosomal rearrangements (Chang et al. 2014). A genome wide association mapping study identified one locus containing four SNPs located in the linked regions (5.8–12.0 Mb) of known *Rhgl* on chromosome 18 in *G. soja* (Zhang et al. 2016). These studies suggest that the resistance genes to SCN in soybean and its relatives may be conserved. It is well known that the genetic base of soybean is very narrow with an estimation of 50% genetic diversity and 81% less minor alleles lost in the process of domestication (Hyten et al. 2006; Zhou et al. 2015). Since a novel locus on chromosome 19 of *G. soja* was found associated with SCN resistance (Zhang et al. 2016), the perennial *Glycine* species may harbor novel SCN resistance genes not found in soybean, and thus would be important in developing novel SCN resistance in soybean. Despite the challenges in utilizing the genetic resources in perennial *Glycine* species due to genetic barriers, additional work on hybridizing the perennial *Glycine* species and soybean along with techniques of gene cloning and gene transfer may provide an avenue to incorporate genes from the perennial *Glycine* species into soybean. In an earlier study, based on an interspecific cross between soybean and *G. tomentella*, hybrid backcrossed lines were shown to be resistant to *H. glycines* (Riggs and Schmitt 1988). With so much resistance in the perennial *Glycine* species, over 100 highly resistant PIs identified in our study, there is a good chance that some of the SCN resistant genes from these sources will be used in future breeding programs.

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