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RESEARCH

Pyramiding of Alleles from Multiple Sources Increases the Resistance of Soybean to Highly Virulent Soybean Cyst Nematode Isolates

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ABSTRACT

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is estimated to be the pathogen that causes the greatest economic loss to soybean [*Glycine max* (L.) Merr.] in the United States. Genetic resistance is an effective way to manage SCN. Resistance sources have been identified, and quantitative trait loci (QTLs) conferring resistance from these sources have been mapped. However, there is a need to diversify SCN resistance genes in cultivars, as most grown in the northern United States have resistance tracing only to the source PI 88788. The objective of this study was to determine the effectiveness of combinations of SCN resistance alleles from different sources in two populations formed via backcrossing. Population 1 segregates for a resistance QTL from both PI 567516C and PI 88788, whereas Population 2 segregates for the same QTL as Population 1 and two QTLs from PI 468916. Lines from both populations were evaluated with two virulent nematode isolates. Furthermore, a subset of lines from Population 2 (Population 2 Subset) was evaluated with two additional nematode isolates. The SCN resistance alleles from each source significantly increased SCN resistance compared with the alternative alleles. The effect of resistance alleles varied depending on SCN isolate and population, and there was generally an increase in resistance as more resistance alleles were stacked together. These results indicate that stacking multiple sources of resistance can be an effective means to increase broad-spectrum SCN resistance.

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Abbreviations: chr, chromosome**;** FI, female index; HG, *Heterodera glycines* Ichinohe; LG, linkage group; MAS, marker-assisted selection; PCR, polymerase chain reaction; QTL, quantitative trait locus; SCN, soybean cyst nematode; SNP, single-nucleotide polymorphism; SSR, simple sequence repeat.

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe [HG]) is the pathogen estimated to cause the largest economic loss to soybean [*Glycine max* (L.) Merr.] in the United States. From 2006 to 2009, the estimated average annual yield loss to SCN was 3.5 Tg, which represents a loss of >US$1 billion yr^{-1} for producers (Koenning and Wrather, 2010). The first identification of SCN in the United States was in North Carolina in 1953, and since this time, the pathogen has spread to most major soybean-producing areas (Tylka and Marett, 2014; Niblack and Riggs, 2015).

Rotation to nonhost crops such as corn (*Zea mays* L.) can be a means of SCN control; however, this method does not completely eradicate the pathogen from the soil due to SCN's ability to overwinter in soil for several years (Porter et al., 2001; Jackson et al., 2005; Miller et al., 2006). Nematicide seed treatments have recently been made available to producers, but they need to be combined with genetic resistance to protect yields (Tylka et al., 2015; Tenuta and Tenuta, 2015). Genetic resistance is the most effective method to control losses due to SCN, and over the past several decades, SCN resistance has been a primary focus of soybean breeders (Kopisch-Obuch et al., 2005). The mechanism of SCN resistance is not entirely understood. In resistant cultivars, the nematode penetrates the root and forms a syncytium; however, the syncytium either forms slowly or becomes necrotic soon

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after formation, causing the nematode to starve to death (Williamson and Hussey, 1996).

Genetic resistance to SCN in soybean was identified shortly after the pathogen was discovered in the United States. Caldwell et al. (1960) observed that resistance from PI 548402 ('Peking') was controlled by three genes named *rhg1*, *rhg2*, and *rhg3*. In a later study, Matson and Williams (1965) reported a fourth resistance gene from Peking named *Rhg4*. Rao-Arelli et al. (1992) identified another gene in PI 88788, which was given the designation *Rhg5* (Rao-Arelli et al., 1992). Soybean cyst nematode resistance was shown to be inherited as a quantitative trait in genetic mapping studies, and resistance genes were mapped as quantitative trait loci (QTLs) (Concibido et al., 2004). Two major mapped SCN resistance QTLs from Peking were given the gene designations *rhg1* and *Rhg4*, and these designations will be used in this article. The terms gene and QTL will be used interchangeably to simplify explanations.

Over 118 soybean accessions have been identified as resistant to SCN and therefore are considered potential resistance sources (Arelli et al., 2000). Despite the number of resistance sources available, resistance in cultivars has a narrow base, with only seven resistance sources commonly used by breeders. Several of these sources were found to share one or more resistance genes, further limiting diversity for resistance. In the northern United States, PI 88788 is the most widely used SCN resistance source in commercial cultivars. It has resistance to a broad range of HG types (Niblack et al., 2002), and cultivars with good agronomic traits have been developed from this source (Diers and Arelli, 1999; Cary and Diers, 2015). In the 2016 University of Illinois Soybean Variety Test, 254 SCN-resistant cultivars entered in the test had their source of SCN resistance listed. Of these cultivars, 249 (98%) had their resistance from PI 88788 (University of Illinois, 2016). The resistance allele at *rhg1* from PI 88788 was shown to be different from the allele at this locus from Peking, and the PI 88788 allele was given the designation *rhg1-b* (Kim et al., 2010). *rhg1* has been cloned, and the resistance has been determined to be conferred by copy number variation of three genes: an amino acid transporter, an α-synaptosomal-associated (α-SNAP) protein, and a WI12 (wound-inducible domain) protein (Cook et al., 2012).

With the heavy reliance on just a few sources of resistance, it is crucial to continue to search for and evaluate novel resistance sources to protect yields. This need is even more important when the ability of field nematodes to adapt to and overcome host plant resistance is taken into account. In a survey of soil samples taken in Illinois, 70% of the SCN populations studied could overcome resistance conferred by PI 88788 (Niblack et al., 2008).

Young (1999) identified PI 567516C as the only source of resistance to LY1, a highly virulent synthetic population of nematodes formed by mass mating HG type 1.2.3 females to HG type 1.2 males. Follow-up research confirmed this resistance in PI 567516C and its genetic distinction from 'Hartwig' (Anand, 1992), a highly resistant cultivar with resistance from PI 437654 (Chen et al., 2006; Arelli et al., 2009). In a population derived from a cross between PI 567516C and Hartwig, a genetic region on chromosome (chr) 10 (formally linkage group [LG] O) defined by the markers Satt592, Satt331, and Sat_274, was associated with resistance to LY1 (Arelli et al., 2010). A QTL was mapped to the same region on chr 10 in a population developed from a cross between PI 567516C and the susceptible cultivar 'Magellan' (Schapaugh et al., 1998; Vuong et al., 2010). The QTL conferred resistance to HG types 2.5.7, 0, 2.7, 1.3.5.6.7, and LY1, and it was confirmed in a recombinant inbred line population derived from the original PI 567516C × Magellan cross.

Breeders have also sought to increase the genetic diversity of SCN resistance by identifying resistant soybean relatives and using them as parents in mapping studies. Wang et al. (2001) mapped QTL alleles that significantly increased SCN resistance from the *Glycine soja* Siebold & Zucc. accession PI 468916 on both chr 15 (LG E) and chr 18 (LG G). The QTLs on chr 15 and chr 18 were later confirmed (Kabelka et al., 2005). The chr 15 QTL was designated cqSCN-006, and the chr 18 QTL was designated cqSCN-007. Further testing of these QTLs provided no evidence of linkage drag that would reduce yield (Kabelka et al., 2006). Kim and Diers (2013) fine mapped cqSCN-006 to a 803.4-kb region and cqSCN-007 to a 146.5-kb region. The region containing cqSCN-006 was recently reduced to a 212.1-kb interval and cqSCN-007 to a 103.2-kb interval (Yu and Diers, 2016). The abundance of genetic and phenotypic information for these QTLs make them good candidates for incorporation into other genetic backgrounds to determine whether they are appropriate for widespread use in breeding programs.

Stacking multiple QTLs from different resistance sources may provide more durable protection against SCN isolates that can overcome the resistance conferred by the genes commonly used by breeders. The objectives of this study are (i) to test the effect of SCN on a population segregating for *rhg1-b* from PI 88788 and the resistance allele from PI 567516C for the chr 10 QTL (herein referred to as CHR10) and (ii) to test the effect of SCN on a population segregating for *rhg1-b* from PI 88788 and the resistance alleles at cqSCN-006, cqSCN-007, and CHR10.

MATERIALS AND METHODS

Population Development

Population 1

Population 1 segregated for *rhg1-b* from PI 88788 and the SCN resistance QTL CHR10 from PI 567516C. Population development was initiated by crossing the recurrent parent LD00-3309 (Diers et al., 2006), a maturity group IV cultivar with SCN resistance tracing back to PI 88788, with PI 567516C, an

accession that does not have a resistance allele at *rhg1* but does have a resistance allele at CHR10 (Supplemental Fig. S1a). After each generation of crossing, plants were genotyped with markers linked to the QTL. Two generations of backcrossing were conducted to reach the BC_2F_1 generation. BC_2F_1 plants fixed for the *rhg1-b* allele and heterozygous for CHR10 were crossed to LD09-15628, a line developed by backcrossing the susceptible allele at *rhg1* into the background of LD00-3309. The cultivar IA3023 was the donor parent of the susceptible allele, and the line had been developed through four backcrosses (BC_4). The F_1 plant from the cross between LD09-15628 and the BC_2F_1 plant previously described was heterozygous for *rhg1-b* and CHR10. The population was advanced through single-seed descent in a greenhouse and field until the F_4 generation. After testing each F_4 plant with simple sequence repeat (SSR) markers linked to the QTL, plants heterozygous for either *rhg1-b* or CHR10 were eliminated and each remaining plant was hand harvested to produce 143 $F_{4:5}$ lines (Supplemental Table S1).

Population 2

Population 2 segregated for *rhg1-b* from PI 88788, the resistance QTLs cqSCN-006 and cqSCN-007 from PI 468916, and the resistance QTL CHR10 from PI 567516C. This population was developed through backcrossing by first crossing the recurrent parent 09SCNPOP11-9 to PI 567516C (Supplemental Fig. S1b). 09SCNPOP11-9 is a $BC_4F_{2:3}$ line fixed for the resistance alleles at cqSCN-006, cqSCN-007, and *rhg1-b* in the LD00-3309 background. After each generation of backcrossing, marker-assisted selection (MAS) was performed with markers linked to the QTLs. Two generations of backcrossing were conducted, and BC_2F_1 plants fixed for the *rhg1-b* allele, cqSCN-006, cqSCN-007, and heterozygous for CHR10 were crossed to LD09-15628. An F_1 plant from this cross that was predicted to be heterozygous for *rhg1-b*, cqSCN-006, cqSCN-007, and CHR10 was selected with genetic markers. Population 2 was advanced by single-seed descent in the greenhouse and field until the F_4 generation. Each F_4 plant was genotyped with markers linked to the QTLs, and plants heterozygous for any of the four QTLs were eliminated. The F_4 plants were grown to maturity and individually harvested to produce 106 $F_{4:5}$ lines (Supplemental Table S2).

Genetic Analysis

The F_4 plants in Populations 1 and 2 were genotyped to predict the resistance allele in each plant and to identify those plants homozygous for genetic markers linked to the QTLs. Genomic DNA was isolated from young trifoliolate leaves via a modified cetyltrimethyl ammonium bromide (CTAB) method (Keim et al., 1988). The DNA samples were tested with SSR markers linked to the QTL of interest by conducting polymerase chain reactions (PCR) according to the method described by Cregan and Quigley (1997). The plants were tested with Satt309 to detect *rhg1* (Cregan et al., 1999), Satt592 for CHR10 (Vuong et al., 2010), Satt491 for cqSCN-006, and Satt472 for cqSCN-007 (Kim and Diers, 2013). Products from PCR were separated on 6% (w/v) nondenaturing polyacrylamide gels by electrophoresis (Wang et al., 2003).

SCN Bioassay

The SCN bioassays for Populations 1 and 2 were conducted using two different SCN field isolates; the first was a HG type 1.2.3.4.5.6.7 (TN21) and the second a HG type 1.2.3.5.6.7. The bioassays were conducted separately for each isolate by infesting a single F_6 plant from each F_4–derived line in a nonreplicated test. The SCN isolates had been maintained for several generations and were obtained from Alison Colgrove at the University of Illinois, Urbana, IL. TN21 was originally collected from a field in Missouri, where it was selected on PI 437654 and then Hartwig, whereas HG 1.2.3.5.6.7 was collected from a field in Dekalb, IL. TN21 had been maintained in a greenhouse for ~140 generations, and HG 1.2.3.5.6.7 had been maintained for ~10 generations.

A subset of 13 F_4–derived lines from Population 2 (Population 2 Subset) was evaluated with two additional isolates. Lines were replicated three times by infesting F_6 plants with SCN isolates HG 2.5.7 and HG 1.2.3.4.5.6.7 (TN23). HG 2.5.7 had been previously maintained in the greenhouse for ~85 generations and TN23 for ~90 generations. Tests were conducted separately according to isolate. HG 2.5.7 was collected in a field in southwest White County, IL, and TN23 was collected in a field at the former Dixon Springs Agricultural Center in Simpson, IL, and it was originally selected on PI 437654.

In all tests, the following check cultivars and indicator lines were replicated four times: Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89722, 'Cloud', 'Lee 74', IA3023, LD00-3309, PI 567516C, and LD00-2817 (Diers et al., 2010). Lines, checks, and indicator lines were planted in a randomized design.

The bioassays were conducted in a greenhouse in a thermoregulated water bath system using modified methods described by Arelli et al. (2000) and Niblack et al. (2002). Briefly, polyvinyl chloride (PVC) tubes filled with a 1:1 sand to soil mix were placed in a plastic crock. Each tube was inoculated with ~2000 nematode eggs suspended in water. A single germinated seedling was planted in each tube, and an experimental unit was a tube with a plant. The crocks were suspended in a water bath maintained at 27 ± 1°C. The plants were watered as needed and grown under a 16-h daylength. After the test was maintained for 28 d, the tubes are immersed in water to remove the plants from the soil. Cysts were dislodged from each root on a nested 850-μm aperture over 250-μm aperture sieve using gentle water pressure, and the cysts were counted using a stereo microscope. A female index (FI) was calculated on a per-plant basis using the following equation (Golden et al., 1970): FI = 100(number of cysts per plant/average number of cysts on susceptible host 'Lee 74'). Lines were then classified according to Schmitt and Shannon (1992): resistant (FI < 10), moderately resistant (FI = 10–30), moderately susceptible (FI = 31–60), and susceptible (FI > 60).

Statistical Analysis

Marker-trait associations were tested individually using ANOVA by the PROC GLM function in SAS 9.4 (SAS Institute, 2013). Markers individually significant at $\alpha = 0.05$ were then placed in multivariate models, and all two-way and three-way interactions between markers were evaluated. Those not significant were removed from the model, and analysis was repeated. All factors were considered fixed. If the residuals

were not normal with homogeneous variances, FIs were $\log_{10}$ transformed. Reported *p*-values are based on transformed data; however, all other data have been back transformed to original units. The R^2 values from the multivariate ANOVA measure the percentage of total genotypic variance for resistance explained by the QTLs and their interactions. Means of genotypic classes were separated according to Duncan's multiple range test ($\alpha = 0.05$).

RESULTS

HG Type Tests

The SCN isolates HG 1.2.3.5.6.7, TN21, HG 2.5.7, and TN23 are thought to be near-homogenous because they have been maintained in the greenhouse for many generations. Female reproduction of all isolates was robust, with the number of females on the susceptible check Lee 74 ranging from 223 for TN23 to 402 for HG 1.2.3.5.6.7 (Table 1). All four isolates reproduced as expected on the indicator lines.

Population 1

Population 1 segregated for resistance alleles from PI 88788 and PI 567516C in a LD00-3309 background. The FI of lines in Population 1 had a continuous distribution for both SCN isolates (Supplemental Fig. S2). The range of FI for lines was 30 to 203 for HG 1.2.3.5.6.7 and 12 to 118 for TN21. LD00-3309, the recurrent parent and donor of the *rhg1-b* allele, had a FI of 65 for HG 1.2.3.5.6.7 and 42 for TN21. Phenotypic data were collected on 140 lines in the HG 1.2.3.5.6.7 test and 141 lines in the TN21 test.

Across all lines within the HG 1.2.3.5.6.7 and TN21 tests, significant ($P < 0.0001$) QTL effects were observed for *rhg1* and CHR10 (Table 2), resulting in lines containing the resistance alleles being more resistant than those with the alternative alleles. The magnitude of the effects was dependent on the QTL and SCN isolate. For HG 1.2.3.5.6.7, FI average across all lines in the population was 90. The FI difference between the homozygous classes for *rhg1* was −38, and this difference for CHR10 was −26. These negative values represent a decrease in reproduction due to the resistance alleles. There was no significant interaction between *rhg1* and CHR10, and the two QTLs together explained 40% of the total variation for resistance to HG 1.2.3.5.6.7. For TN21, the average FI across all lines was 52, and the difference between the homozygous classes was −19 for *rhg1* and −15 for CHR10. There was a significant interaction between the two loci for TN21, and *rhg1*, CHR10, and the interaction explained 50% of the total variation for resistance.

When genotypic classes were compared, similar trends were observed in the HG 1.2.3.5.6.7 and TN21 tests (Fig. 1). Lines that were homozygous resistant at one or two loci had a reduced FI compared with lines containing no resistance QTLs. Lines with the resistance allele at either CHR10 or *rhg1* were not significantly different from each other. Additionally, lines with the resistance alleles at both QTLs had a reduced average FI compared with lines with only one resistance allele or lines that were homozygous susceptible. Although adding resistance alleles resulted in improved resistance, the significant statistical interaction between *rhg1* and CHR10 observed in the TN21 test was the result of the combined effect of the resistance QTLs not being additive. This means that the increase in resistance was smaller than expected when the two resistance QTLs were combined according to the effect of the individual resistance alleles. For both isolates, the combined effect of the resistance alleles for both QTLs reduced the FI by ~50%. For TN21, the average FI of lines with resistance alleles for both QTLs was 38, whereas lines with no resistance alleles averaged 72. For HG 1.2.3.5.6.7, the mean FI for those lines with both resistance alleles was 62, whereas the average FI for lines with neither resistance allele was 125.

Population 2

Population 2 segregated for resistance alleles from PI 88788, PI 468916, and PI 567516C in an LD00-3309 background. There was a continuous distribution in the FI of lines in Population 2 for both SCN isolates (Supplemental Fig. S2). The range of FI for lines was 1 to 167 for HG 1.2.3.5.6.7 and 11 to 116 for TN21. Phenotypic data were collected on 105 lines in the HG 1.2.3.5.6.7 test and 104 lines in the TN21 test.

Across all lines, each of the four resistance alleles significantly ($P < 0.05$) increased resistance to both

Table 1. The reaction of four soybean cyst nematode (SCN) isolates to Lee 74, the *Heterodera glycines* (HG)-type indicator lines, and elite checks with known genetic resistance to SCN.

Isolate	No. females on 'Lee 74'	HG type§	Female index on indicator lines and elite checks†								
			1‡ Peking	2 PI 88788	3 PI 90763	4 PI 437654	5 PI 209332	6 PI 89772	7 Cloud	LD00-3309	LD00-2817
HG 1.2.3.5.6.7	402	1.2.3.5.6.7	45	13	11	1	15	15	53	65	58
TN21	371	1.2.3.4.5.6.7	66	23	45	44	26	34	43	42	44
HG 2.5.7	303	2.5.7	2	31	0	1	22	0	39	59	6
TN23	223	1.2.3.4.5.6.7	72	23	70	80	21	58	78	37	83

† Female index = 100(average number of females per line/average number of females on 'Lee 74') (Golden et al., 1970).

‡ Numerical designation of indicator line in HG-type test (Niblack et al., 2002).

§ HG type of isolate according to female indices.

Table 2. Effects of soybean cyst nematode (SCN) resistance quantitative trait loci (QTLs) on female reproduction in Populations 1 and 2 for *Heterodera glycines* (HG) 1.2.3.5.6.7 and TN21 nematode isolates.

		HG 1.2.3.5.6.7 female indices					HG TN21 female indices				
Population	QTL	Resistant allele†	Susceptible allele‡	Effect§	P value	R^2¶	Resistant allele	Susceptible allele	Effect	P value	R^2
Population 1	rhg1	70	108	–38	<0.0001	0.27	42	61	–19	<0.0001	0.31
	CHR10	76	102	–26	<0.0001	0.12	44	59	–15	<0.0001	0.18
Population 2	rhg1	22	43	–21	<0.0001	0.09	38	56	–18	<0.0001	0.16
	cqSCN-006	21	43	–22	<0.0001	0.06	39	56	–17	<0.0001	0.12
	cqSCN-007	17	47	–30	<0.0001	0.24	43	51	–8	0.0120	0.01
	CHR10	25	40	–15	<0.0001	0.07	44	51	–7	0.0315	0.04

† Mean of lines homozygous for the SCN resistance allele at the locus.

‡ Mean of lines homozygous for the SCN susceptible allele at the locus.

§ Difference between homozygous classes.

¶ Proportion of total genetic variation for resistance explained by the QTL.

HG 1.2.3.5.6.7 and TN21, and the magnitude of the QTL effect was dependent on the QTL and SCN isolate (Table 2). For HG 1.2.3.5.6.7, the average FI of the population was 33. cqSCN-007 had a numerically greater effect on reproduction than the other QTLs, and the FI difference between the homozygous classes ranged from −15 to −30 for the four QTLs. A statistically significant interaction ($P < 0.01$) was observed between *rhg1* and cqSCN-007. The combined effect of the QTLs and interaction explained 57% of the total variance for resistance to HG 1.2.3.5.6.7. For TN21, the average FI of the population was 48, and *rhg1-b* had a numerically greater effect on reproduction than the other resistance alleles. The differences in FI between the homozygous QTL classes ranged from −7 to −18. There were no significant interactions between QTLs, and together the QTLs explained 38% of total genetic variation associated with resistance to TN21.

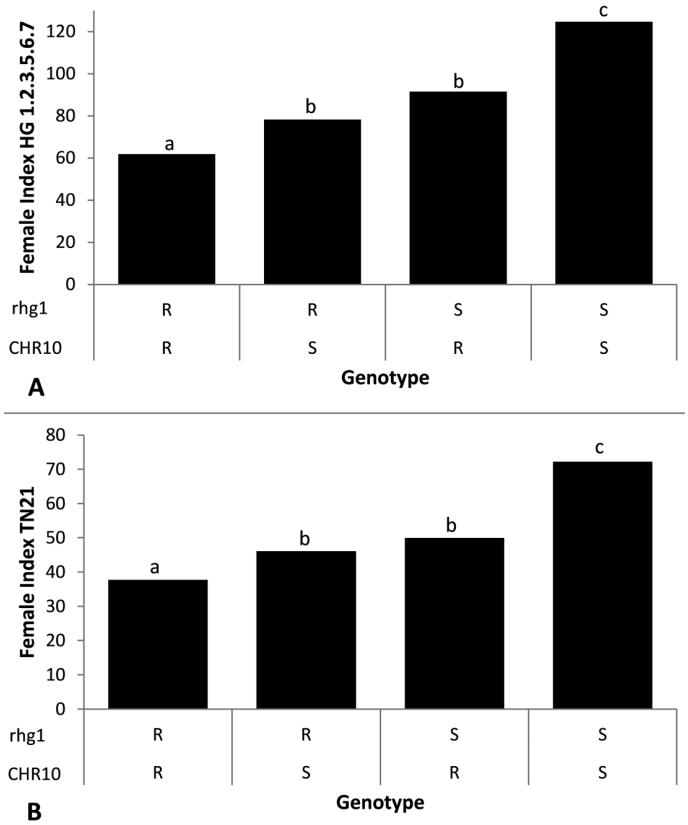

Fig. 1. Average female index values for Population 1 genotypes when evaluated with (A) *Heterodera glycines* (HG) 1.2.3.5.6.7 (n = 140) and (B) TN21 (n = 141) nematode isolates. "R" indicates homozygous for the resistance allele of the quantitative trait loci, whereas "S" indicates homozygous susceptible. Means with the same letter are not significantly different (α = 0.05). Mean separations are based on $\log_{10}$–transformed data.

For HG 1.2.3.5.6.7 and TN21, the mean FI of lines containing the resistance alleles at three or more QTLs were significantly ($P < 0.05$) lower than the mean of lines with no resistance alleles (Fig. 2a–2b). Generally, lines homozygous for all four resistance QTL alleles had reduced SCN reproduction compared with lines that only had a single QTL or lines that were homozygous susceptible. Lines carrying all four resistance QTLs and lines carrying resistance alleles at *rhg1*, cqSCN-007, and CHR 10 were classified as resistant to HG 1.2.3.5.6.7 and had average FIs of 4 and 7, respectively (Fig. 2a). Lines with the four resistance alleles and lines with the resistance alleles *rhg1-b,* cqSCN-006, and CHR10 were classified as moderately resistant to TN21 with average FIs of 29 and 28, respectively (Fig. 2b).

Population 2 Subset

Population 2 Subset consisted of the following genotypes: three lines containing no resistance alleles, three lines containing only the *rhg1-b* resistance allele, three lines containing the *rhg1-b* and CHR10 resistance alleles, and four lines containing the *rhg1-b*, cqSCN-006, cqSCN-007, and CHR10 resistance alleles. With the resistance QTLs confirmed in Population 1 and Population 2, the Population 2 Subset allowed for more extensive testing of resistance QTL stacks with two additional SCN isolates, HG 2.5.7 and TN23. There was a continuous distribution for the average FI of individual lines in the Population 2 Subset for both SCN isolates, and the FI of lines ranged from 10 to 88 for HG 2.5.7 and 23 to 100 for TN23 (Supplemental

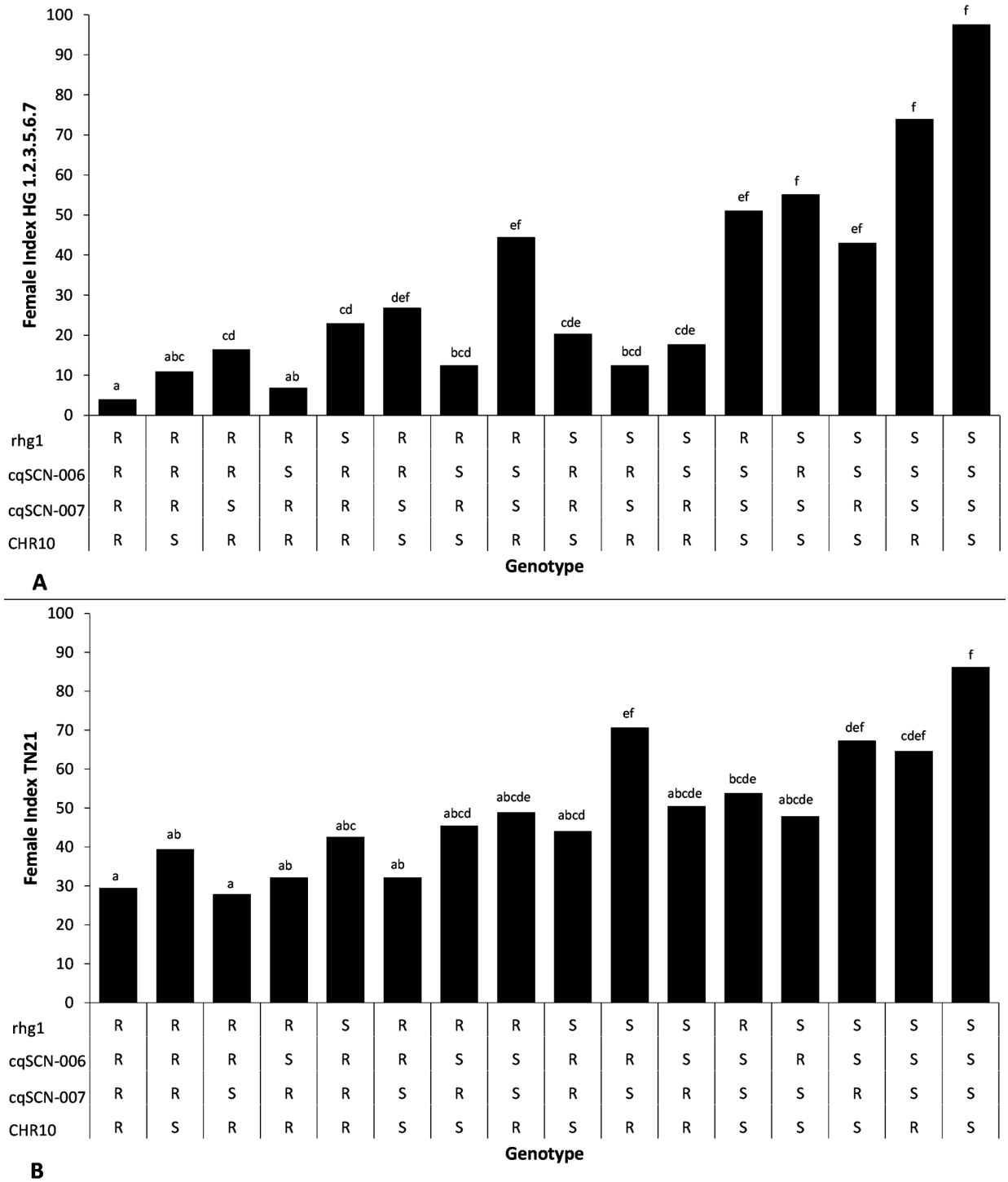

Fig. 2. Average female index values for Population 2 genotypes when evaluated with (A) *Heterodera glycines* (HG) 1.2.3.5.6.7 (n = 140) and (B) TN21 (n = 141) nematode isolates. "R" indicates homozygous for the resistance allele of the quantitative trait loci, whereas "S" indicates homozygous susceptible. Means with the same letter are not significantly different (α = 0.05). Mean separations are based on $\log_{10}$–transformed data.

Fig. S2). The average FI on LD00-3309 for HG 2.5.7 was 59 and was 37 for TN23.

For HG 2.5.7, lines that were homozygous for the resistance allele at all four QTLs had a reduced average FI compared with the other genotypic classes (Fig. 3a). The average FI of these lines was 15, which gives them a moderately resistant classification. Lines homozygous for the four susceptibility alleles had an average FI of 71, and this FI was decreased to 49 through the addition of *rhg1-b*. The FI of lines homozygous for both CHR10 and *rhg1-b* was 59, which was not significantly different from lines carrying *rhg1-b* alone. An improvement in resistance occurred when the resistance alleles at cqSCN-006 and cqSCN-007 were stacked with the *rhg1-b* and CHR10 resistance alleles.

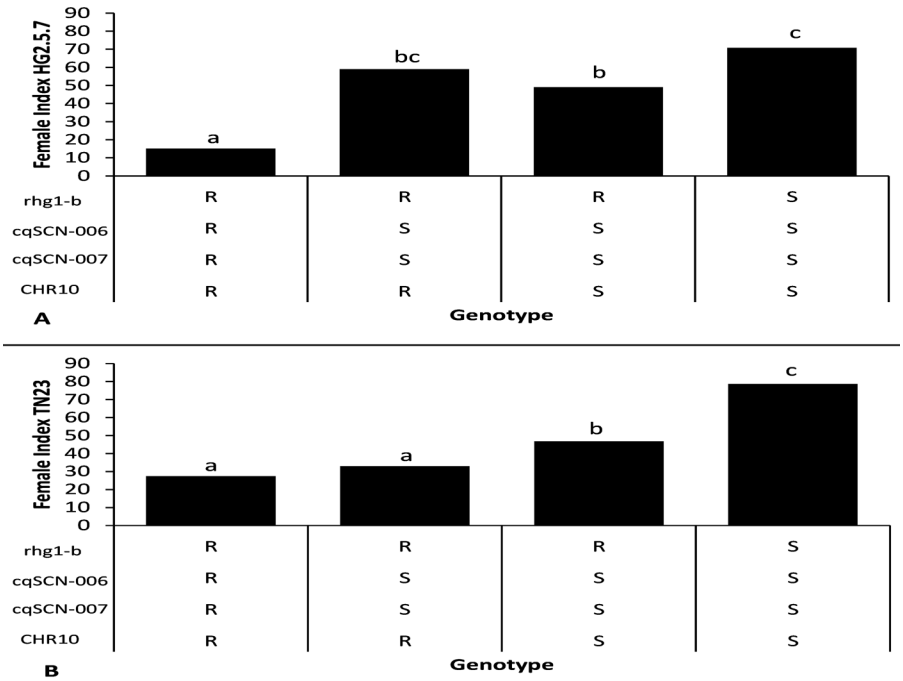

Fig. 3. Average female index values for Population 2 Subset (n = 13) genotypes when evaluated with (A) *Heterodera glycines* (HG) 2.5.7 (n = 140) and (B) TN23 (n = 141) nematode isolates. "R" indicates homozygous for the resistance allele of the quantitative trait loci, whereas "S" indicates homozygous susceptible. Means with the same letter are not significantly different (α = 0.05). Mean separations are based on $\log_{10}$–transformed data.

For TN23, lines with the two or four resistance QTL stacks had a significantly reduced FI compared with the other genotypic classes, and they were not significantly different from each other (Fig. 3b). The average FI of lines with all four resistance QTL alleles was 28, whereas the average of lines with the *rhg1-b* and CHR10 resistance allele stack was 33. Lines carrying the *rhg1-b* allele had a reduced FI versus lines with no resistance QTL alleles but had an increased FI versus lines with resistance stacks. Lines with no resistance QTL alleles were susceptible, with an average FI of 79.

DISCUSSION

The soybean populations used in this study were developed to test the effect of combinations of the resistance alleles *rhg1-b* from PI 88788, cqSCN-006, and cqSCN-007 from PI 468916 and CHR10 from PI 567516C on SCN reproduction. These QTLs were incorporated into an LD00-3309 background to form segregating populations of backcross lines. The QTLs are known to have large effects; therefore, population sizes were relatively small. The LD00-3309 background was selected because it is representative of high-yielding cultivars with PI 88788 resistance that are adapted to the Midwestern United States (Diers et al., 2006). All four resistance QTLs were detected across multiple nematode isolates, with each conferring partial resistance, and the magnitude of their effect on FI varied according to QTL and isolate. When lines contained multiple resistance alleles, lines classified as resistant and moderately resistant to multiple nematode isolates were observed. CHR10 from PI567516C has been noted as a promising novel SCN resistance QTLs because it has been shown to confer resistance to several SCN isolates (Young, 1999; Vuong et al., 2010). In our study, CHR10 generally had a smaller effect on nematode reproduction compared with the other QTLs tested; however, the addition of the CHR10 resistance allele did increase resistance in most combinations in which it was evaluated. Overall, our data suggest that broad-spectrum resistance to SCN can be significantly improved by stacking resistance alleles from multiple sources.

The accession PI 437654 confers resistance to a broad range of SCN isolates and has been used to develop highly resistant cultivars and germplasm lines (Anand et al., 1988; Diers et al., 1997; Wu et al., 2009; Diers et al., 2010). Despite its promise, few cultivars with resistance from PI 437654 have been released because it has been difficult to develop agronomically acceptable high-yielding cultivars with this source. Additionally, resistance conferred by PI 437654 is mediated by numerous QTL, which makes it challenging to achieve resistance similar to PI 437654 in elite cultivars (Concibido et al., 2004; Wu et al., 2009). For example, we observed that when inoculated with HG 1.2.3.5.6.7, LD00-2817, a high-yielding cultivar with SCN resistance from PI 437654 and/or Peking that was bred from Hartwig had a FI of 58, but PI 437654 only had a FI of 1 (Table 1). Despite the high level of resistance provided by PI 437654, two of the isolates used in our study had a FI >10 on PI 437654; TN21 had an average FI of 44, whereas TN23 had an average FI of 80. A reason for the high reproduction on PI 437654 is that both isolates were selected on PI 437654 or Hartwig, which has its resistance tracing to PI 437654. Our lines with the four gene stacks had reduced FI compared with PI 437654 with a FI of 29 for TN21 and 28 for TN23 (Fig. 1–3).

Pyramiding resistance in a single genetic background has been demonstrated as an effective way to improve the durability of host resistance to soybean pathogens and pests, including SCN. Kim et al. (2011) stacked SCN resistance alleles from exotic and domestic sources and observed increased resistance to the SCN isolates PA1, PA3, and PA5. Qualitative or quantitative gene pyramids in soybean have also been shown to enhance resistance to *Soybean mosaic virus* (qualitative), soybean aphid (*Aphis glycines* Matsumura) (qualitative), Phytopthora root rot (*Phytopthora sojae* Kaufm.

& Gerd.) (quantitative), and leaf-chewing insects (quantitative) (Dorrance et al., 2008; Shi et al., 2009; Li et al., 2010; Ajayi-Oyetunde et al., 2016; Ortega et al., 2016).

The SCN isolates in this study were selected for their ability to overcome several resistance sources that are commonly used by breeders. These isolates were naturally occurring; however, highly virulent SCN populations such as HG 1.2.3.5.6.7, TN21, and TN23 are currently rare in field environments. The widespread dependence on PI 88788 and a handful of other resistance sources increases the likelihood that more nematode field populations will overcome the resistance conferred by these sources. Soybean cyst nematode populations that can reproduce on PI 88788 such as HG 2.5.7 are already common in soybean fields across the United States and Canada (Niblack et al., 2008; Faghihi et al., 2010; Brzostowski et al., 2014). In a survey of 527 soil samples collected across Tennessee, Indiana, Illinois, and Ontario, >61% of the isolates collected had FI >10% on PI 88788 and >56% of the isolates collected within a state had a FI >10 on at least two differentials (Faghihi et al., 2010). This distribution of nematode isolates demonstrates the need to develop cultivars with more broad and durable resistance to SCN.

It will be important to evaluate the effect of stacking resistance QTL on agronomic traits including yield. One of the reasons for the widespread use of PI 88788 as a resistance source is that it has been combined with high yield and other good agronomic traits. In some genetic backgrounds, *rhg1* has been associated with yield drag in environments with low SCN pressure; however, there is evidence that the genetic linkage between *rhg1* and yield depression can be broken (Mudge et al., 1996, Kopisch-Obuch et al., 2005). Kabelka et al. (2006) evaluated the yield of one population segregating for resistance at cqSCN-006 and cqSCN-007 and another segregating for resistance at cqSCN-006, cqSCN-007, and *rhg1* from PI 88788 in environments with varying SCN pressure. Overall, the resistance alleles did not have a negative effect on yield and in some cases enhanced yield. For cultivars with stacked resistance sources to be truly effective, economic yields must be maintained.

Phenotyping for SCN resistance is costly and laborious; therefore, MAS for SCN resistance is of great interest to breeders (Concibido et al., 2004). Although SSR markers linked to SCN resistance QTL were used in this study and have been used in breeding for almost two decades, rapid advances and decreasing costs in sequencing technologies have led to the recent development of low-cost, high-throughput single-nucleotide polymorphism (SNP) marker-based assays for SCN resistance (Kadam et al., 2016). These SNP assays are not only more efficient with a lower error rate than previous genotyping methods, they can also identify the copy number variation at the *rhg1* locus (Lee et al., 2016).

It is expected that the next generation of breeding will lead to improved SCN resistance. High-throughput genotyping platforms could identify new alleles and genes that can be stacked using MAS to create cultivars with broad-based resistance to SCN. The combination of predictive modeling with SNP data could also reduce the time needed to stack multiple resistance QTLs into elite cultivars and to select for these QTLs in breeding programs (Bao et al., 2014; Shi et al., 2015). Population 1 and Population 2 took several years to develop, and although pyramiding resistance is often suggested as a means to improve resistance to pests and pathogens, often the time needed discourages breeders from doing so.

Current genetic resistance to SCN is narrow, and breeders must implement new strategies to effectively manage this pathogen. The objective of our study was to evaluate the effect of stacking novel and common SCN resistance alleles on the reproduction of nematode isolates that can overcome multiple resistance sources. These isolates could not be controlled by *rhg1-b*-mediated resistance alone. Our study indicates PI 468916 and PI 567516C are alternative sources that breeders can use to enhance and diversify SCN resistance in their programs. Combining resistance alleles from PI 88788, PI 468916, and PI 567516C conferred resistance or partial resistance to highly virulent nematode isolates. This suggests that stacking resistance sources improves resistance to SCN and can be a useful strategy for future breeding. Genetic resistance remains important to controlling losses to SCN. Incorporating new resistance sources into breeding programs and stacking resistance sources will provide the necessary options needed for producers to protect their yields from SCN damage.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

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