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ORIGINAL ARTICLE

Genetic characterization of *qSCN10* from an exotic soybean accession PI 567516C reveals a novel source conferring broad-spectrum resistance to soybean cyst nematode

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Abstract

Key message **The qSCN10 locus with broad-spectrum SCN resistance was fine-mapped to a 379-kb region on chromosome 10 in soybean accession PI 567516C. Candidate genes and potential application benefits of this locus were discussed.**

Abstract Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is one of the most devastating pests of soybean, causing significant yield losses worldwide every year. Genetic resistance has been the major strategy to control this pest. However, the overuse of the same genetic resistance derived primarily from PI 88788 has led to the genetic shifts in nematode populations and resulted in the reduced effectiveness in soybean resistance to SCN. Therefore, novel genetic resistance resources, especially those with broad-spectrum resistance, are needed to develop new resistant cultivars to cope with the genetic shifts in nematode populations. In this study, a quantitative trait locus (QTL) *qSCN10* previously identified from a soybean landrace PI 567516C was confirmed to confer resistance to multiple SCN HG Types. This QTL was further fine-mapped to a 379-kb region. There are 51 genes in this region. Four of them are defense-related and were regulated by SCN infection, suggesting their potential role in mediating resistance to SCN. The phylogenetic and haplotype analyses of *qSCN10* as well as other information indicate that this locus is different from other reported resistance QTL or genes. There was no yield drag or other unfavorable traits associated with this QTL when near-isogenic lines with and without *qSCN10* were tested in a SCN-free field. Therefore, our study not only provides further insight into the genetic basis of soybean resistance to SCN, but also identifies a novel genetic resistance resource for breeding soybean for durable, broad-spectrum resistance to this pest.

Introduction

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most damaging pathogen of soybean, causing over $1 billion in yield losses annually in the USA alone (Allen et al. 2017). Furthermore, the nematode continues to spread to other soybean-growing areas in the North America (Tylka and Marett 2017). Therefore, more yield losses are possible. Non-host crop rotation, resistant cultivars, and nematicide applications are conventional strategies for controlling SCN. Among them, the deployment of cultivars with genetic resistance to SCN is the most cost-effective and environment-friendly method to combat this pest. Genetic resistance to SCN in soybean is a multi-genetic and quantitatively inherited trait (Arelli et al. 2009; Vuong et al. 2010; Liu et al. 2012; Yu and Diers 2017). Two major QTL, *rhg1* (*for resistance to Heterodera glycines 1*) and *Rhg4,* responsible for SCN resistance in numerous soybean germplasm and cultivars, have been precisely localized and extensively

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characterized (Kim et al. 2010; Cook et al. 2012; Liu et al. 2012, 2017; Lakhssassi et al. 2019). For more than 2 decades, *rhg1* from plant introduction (PI) PI 88,788 has been the major resistance introgressed into modern soybean varieties for resistance to SCN (Concibido et al. 2004). Approximately 95% of soybean resistant varieties carry this *rhg1* resistance. Unfortunately, the overuse of the same resistance has led to the genetic shifts in nematode populations and resulted in the reduced effectiveness in soybean resistance to SCN (Niblack et al. 2008; Mulrooney et al. 2010; Lian et al. 2016, 2017; Howland et al. 2018). Therefore, other genetic resistance resources or genes are needed for sustainable soybean production for an ever-growing world population. For this purpose, in the past two decades, a large number of soybean accessions have been screened for novel resistance to SCN (Arelli et al. 1997, 2000; Han et al. 2015; Kadam et al. 2016; Zhang et al. 2016; Hua et al. 2018; Klepadlo et al. 2018). As a result, a number of exotic soybean germplasm, such as PI 90763, PI 437654, PI 438489B, PI 464925B, and PI 567516C, have been revealed to be resistant to multiple SCN races or HG types. Furthermore, QTL responsible for the observed resistance have been mapped in some of these accessions (Guo et al. 2005; Winter et al. 2006; Wu et al. 2009; Vuong et al. 2010, 2011).

Among these accessions, PI 567516C was shown to be resistant to multiple SCN HG Types, including 2.5.7 (race 1), 1.2.5.7 (race 2), 0 (race 3), 2.5.7 (race 5), 1.3.6.7 (race 14), and a synthetic nematode population LY1 (HG Type 1.2.3.4.5.6.7) (Young 1998; Vuong et al. 2010). The previous mapping study for this accession using $F_{2:3}$ progeny derived from a Magellan × PI 567516C cross -identified 2 novel QTL on Chr. 10 (*qSCN10*) and Chr. 18 (*qSCN18*), respectively (Vuong et al. 2010). The *qSCN10* locus was associated with resistance to 5 HG Types: 2.5.7 (race 1), 0, 2.5.7 (race 5), 1.3.6.7, and 1.2.3.4.5.6.7, with the likelihood of odds (LOD) scores ranging from 6.6 to 13.2 and explained 7.9% to 21.7% of the phenotypic variation. *qSCN10* was mapped to a 15.3 cM interval (2,542 kb) between SSR markers Satt592 and Sat_038 on Chr. 10 (Vuong et al. 2010). This locus was also detected in a genome-wide association study (GWAS) using a diverse panel of 553 soybean germplasm (Vuong et al. 2015).

PI 567516C was reported to be genetically different from major resistant sources, such as PI 88788 (Chen et al. 2006; Arelli et al. 2009). According to whole-genome resequencing (WGRS) and haplotype analysis, PI 567516C carries three copies of *rhg1*, similar to Peking and PI 437654 (Jiao et al. 2015); however, PI 567516C does not contain the specific *Rhg4* haplotype that is present in Peking-type resistant accessions (Lakhssassi et al. 2019). The initial mapping showed that *qSCN10* was a different locus from the known resistance loci, *rhg1* and *Rhg4,* since it is located on a different chromosome (on chr. 10) from *rhg1* (on chr. 18) and *Rhg4* (on chr. 8) (Vuong et al. 2010). Therefore, the *qSCN10* locus very likely harbors gene(s) with a unique defense mechanism against SCN and shall be a good resistance source for breeding soybean varieties with durable, broad-spectrum resistance to SCN. To ensure the proper application of this unique QTL in breeding, *qSCN10* needs to be further confirmed and fine-mapped to a reasonably small region, and to be shown that there are no adverse agronomic traits, especially yield drag, associated with it. For these purposes, our present study not only confirmed the effectiveness of the *qSCN10* locus in conferring resistance to multiple SCN HG types, but also narrowed down the QTL interval to a 379-kb region for future breeding application. Furthermore, our study also demonstrated that there was no yield drag or other adverse agronomic traits associated with this QTL. Thus, *qSCN10* is an excellent novel genetic resource for breeding soybean for durable, broad-spectrum resistance to SCN.

Materials and methods

Plant materials

An $F_{6:7}$ recombinant inbred line (RIL) population was previously developed from a cross between the cultivar (cv.) Magellan (PI 595362, SCN susceptible) and PI 567516C (SCN resistant) for QTL mapping study (Vuong et al. 2010). A RIL line MPC2090 was selected as a donor parent based on the homozygous genotype at the target region and the multi-SCN resistance phenotype. In this study, MPC2090 was crossed with Magellan at the Bradford Research and Extension Center (BREC), the University of Missouri, Columbia, MO, USA. Heterozygous F1 plants were backcrossed with the recurrent parent, Magellan, for 4 generations (BC_4F_1) using marker-assisted backcrossing (MABC) method. Heterozygous BC_4F_1 plants were self-pollinated to advance the BC_4F_2 population. Over 900 BC_4F_2 seedlings planted in BREC and screened for recombinants containing breakpoints in the target *qSCN10* region with 2 single nucleotide polymorphism (SNP) markers labeled SNP1 and SNP-O8 (Supplementary Table S1). Out of 972 BC_4F_2 plants, 159 were identified as recombinants and self-pollinated seeds ($BC_4F_{2:3}$) were harvested. Four pairs of independent segregating lines ($BC_4F_{2:3}$) derived from BC_4F_2 plants with different heterozygous segments for the target QTL region were selected to develop $BC_4F_{3:4}$ progeny lines of the recombinants (Q041, Q150, Q543, Q660) in a greenhouse, the University of Missouri. These progeny lines were utilized to conduct progeny test of SCN phenotyping for fine-mapping (Fig. 1). Another set of $BC_4F_{3:4}$ progeny lines, Q713-Mgl and Q713-516C, was also developed as described above but carry entire Magellan and PI 567516C segments without

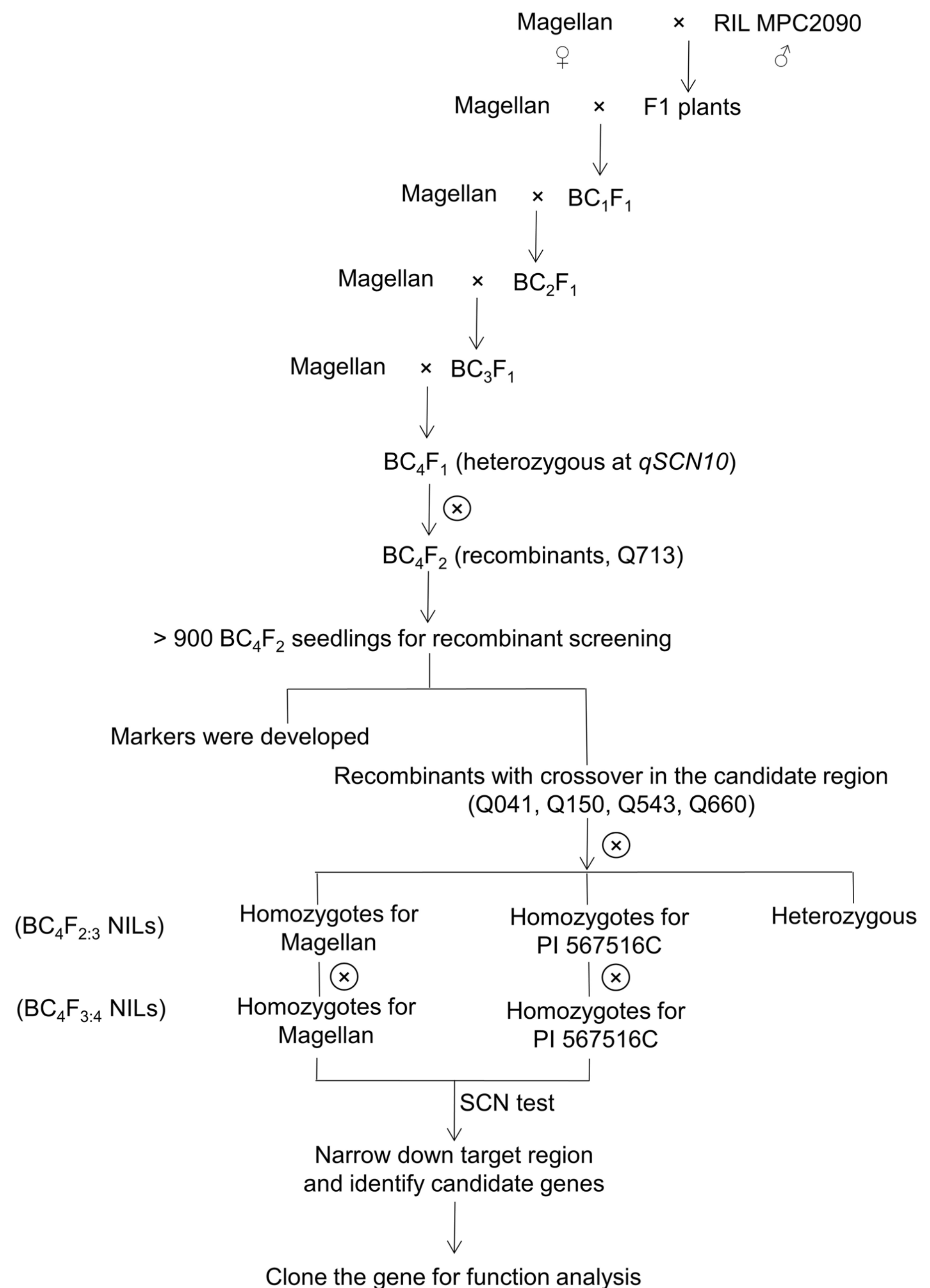


Fig. 1 A flowchart of *qSCN10* fine-mapping and development of near-isogenic lines (NILs) in Magellan background. The construction of recombinant inbred line (RIL) MPC2090 was selected from the previously reported cross of Magellan × PI 567516C (Vuong et al. 2010)

a breakpoint at the *qSCN10* locus, respectively. Q713-Mgl and 516C were used as near-isogenic lines (NILs) to evaluate SCN resistance of the *qSCN10* locus and to test yield drag effects.

Phenotypic evaluation of SCN resistance

Different SCN populations [HG Types 2.5.7 (race 1), 1.2.5.7, 0, 2.5.7 (race 5), 1.3.6.7, and 1.2.3.4.5.6.7] have been separately maintained in a greenhouse at the University of Missouri–Columbia by reproduction in small population sizes and believed to be near-homogeneous (Arelli et al. 2000). SCN resistance bioassays were conducted following an established method (Arelli et al. 2000; Niblack et al. 2009; Brown et al. 2010). Briefly, seeds were germinated at room temperature for 4 days in paper germination pouches. Seedlings were then transplanted into crocks containing micropots filled with sterilized sandy loam soil. Crocks were placed in water bath tanks with the temperature maintained at 27 °C. Plants were inoculated with about 2000 SCN eggs at 3 days after transplanting and watered daily. After 30 days, the root samples were harvested. During the harvesting procedure, seedling roots were gently swirled in water to remove soil, leaving roots undisturbed. The cysts

from each root sample were then flushed with high-pressure water, transferred to a petri dish, scanned, and counted using a fluorescence-based imaging system (Brown et al. 2010). Indicator soybean lines ('Peking', PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, PI 54831, and 'Pickett') and two standard susceptible controls 'Hutcheson' and 'Lee 74′ were simultaneously evaluated for HG Types (Niblack et al. 2002) and race designation (Schmitt and Shannon 1992). Tested soybean plants were arranged in a randomized complete block design (RCBD) with over ten technical replicates per soybean line for each SCN population.

The female index (FI%) was calculated to evaluate the response of plants to SCN populations: FI (%) = (Number of female cyst nematodes on a given individual/Average number of female nematodes on the susceptible 'Lee 74′) × 100. Indicator and test lines were scored according to their FI: FI < 10 = resistant (R); FI 10 to < 30 = moderately resistant (MR); FI 30 to 60 = moderately susceptible (MS); and FI > 60 = susceptible (S).

Observation of nematode development in Magellan and PI 567516C roots

To observe the nematode invasion and development in the two parental lines, their seedlings were inoculated with SCN HG Type 0 as described above. Roots were carefully removed from the soil at 2, 4, 6, and 8 days after inoculation (dai). Five root samples of each parent were harvested at each time point. Fresh roots were stained with acid-fuchsin (Byrd et al. 1983). Briefly, roots were washed with tap water and cleared by soaking roots in 5.25% sodium hypochlorite for 4 min. Roots were completely rinsed with running tap water and then boiled in the 37% acid-fuchsin staining solution for about 30 s in a microwave followed by rinsing with running tap water. The roots were then de-stained with boiling glycerol. After staining, the SCN juveniles from the J2–J4 stages inside the roots were morphologically examined with a dissecting microscope (Cook et al. 2012).

DNA extraction, SNP marker and Kompetitive allele-specific PCR (KASP) assay development

Genomic DNA (gDNA) was extracted from two parental lines, Magellan and PI 567516C, and from individual plants of NILs using the CTAB (cetyl trimethyl ammonium bromide) method (Murray and Thompson 1980).

KASP assays (LGC Genomics, UK) were utilized for genotyping of recombinants and NILs. Genome sequence information in the *qSCN10* region was obtained from the WGRS data for Magellan (unpublished data) and PI 567516C (Valliyodan et al. 2016). The SNPs identified at this locus using Genome Analysis Toolkit (GATK) (McKenna et al. 2010; https://gatk.broadinstitute.org) software were reconfirmed by examining read alignments in the Integrative Genomics Viewer (IGV) tool (Robinson et al. 2011; https://igv.org/). A set of SNPs were selected, and corresponding primers (2 allele-specific forward primers, along with tail sequences and one common reverse primer) were designed for the development of KASP assays (Supplementary Table S1). KASP reactions were run in 10 µl reaction volume, which included 5 µl of 2 × premade KASP master mix, 5 µl of 10–25 ng/µl gDNA, and 0.14 µl of primers mix. The following cycling conditions were used: 15 min at 95 °C, followed by 10 touch-down cycles of 20 s at 94 °C, 1 min at 65–57 °C (dropping 0.8 °C per cycle) and then 23 cycles of 20 s at 94 °C, 1 min at 57 °C. The fluorescent end-point genotyping method was carried out using a Roche LightCycler 480 instrument (Roche Applied Sciences, Indianapolis, IN, USA).

Expression analysis from RNA-Seq and microarray profiles

Expression patterns of candidate genes in different tissues and under presence/absence of SCN infection in different soybean lines were derived from published RNA-Seq (Severin et al. 2010) and microarray profiles (Wan et al. 2015). In microarray assay by Wan et al. (2015), samples were collected from roots of Magellan and PI 567516C at 0, 3, and 8 days after inoculation with SCN HG Type 0. In RNA-Seq assay by Severin et al (2010), root samples were collected from SCN susceptible cultivar Williams 82 seedlings at 5 and 30 days after inoculation with SCN HG Type 0 or water (mock). Expression profiles were downloaded from the Gene Expression Omnibus (GEO) database in series GSE64492 and GSE125103. The average gene expression levels from three replicates were analyzed through Microsoft Excel and shown as normalized RPKM (reads/Kb/Million) data in RNA-Seq and unscaled expression levels in microarray.

Phylogenetic analysis

A panel of 106 diverse soybean genotypes, including 56 *G. max* elite lines, 43 *G. max* landraces, and 7 *G. soja* accessions (Valliyodan et al. 2016), were explored to understand the origin and evolution of the *qSCN10* region. A total of 2,336 SNPs in the delimited 379-kb region were used to build the phylogenetic tree by MEGA X (Kumar et al. 2018). The statistical method was inferred by using the maximum likelihood method and the Tamura-Nei model (Tamura et al. 1993). The tree with the highest log likelihood (− 26,052.38) was shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with superior log

likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site.

Yield test of the near-isogenic lines (NILs)

For yield drag test of the *qSCN10* locus, abundant seeds were harvested from homozygous $BC_4F_{3:4}$ Q713 NIL plants grown at the BREC, the University of Missouri–Columbia, MO, USA, in 2017. Yield test was conducted under normal growth conditions without SCN stress at BREC in 2018. Five replicates for each line were planted using a randomized complete block design. Around 200 seeds of each replicate were planted in the 2-row plots with 0.76 m row spacing and 3 m length. Seeds were harvested at maturity for yield analysis.

Results

SCN growth and development was restricted in PI 567516C

To reveal any possible differences in SCN infection efficiency between PI 567516C and Magellan, nematode growth and development were monitored over time after inoculation. As shown in Fig. 2, no significant difference was observed between 2 parental lines in the number of nematodes (J2 stage) inside the roots at 2 days after inoculation (dai), indicating that the resistance in PI 567516C did not affect the penetration of nematodes in soybean roots. However, an obvious delay of nematode development was observed in PI 567516C at 4 dai: The nematodes were thinner (in diameter) in PI 567516C than in Magellan. The delay was even more obvious at 8 dai: Approximately half of the nematodes were at the J4 stage in Magellan, but majority (80%) of nematodes were at the J3 stage and only a few early J4 nematodes were found in PI 567516C. Clearly, the growth of nematodes in PI 567516C was restrained, similar to the phenomenon observed in other SCN resistant soybean lines, such as Peking and PI 88788 (Mitchum 2016; Chen et al. 2020).

qSCN10 was confirmed to confer resistance to multiple SCN HG types

To validate the *qSCN10* locus, the $BC_4F_{3:4}$ Q713 NIL pair along with the 2 parental lines were examined for their response to six SCN HG Types [2.5.7 (race 1), 1.2.5.7, 0, 2.5.7 (race 5), 1.3.6.7, and 1.2.3.4.5.6.7] in a greenhouse. As shown in Fig. 3b, Magellan, as expected, showed susceptibility (S) to all 6 SCN HG Types; meanwhile, PI 567516C, as the source of the *qSCN10* locus, showed different levels of resistance to different HG Types: resistant (R) to HG Type 2.5.7 (race 5); moderately resistant (MR) to HG Type 2.5.7 (race 1), 0, and 1.2.3.4.5.6.7; and moderately susceptible (MS) to HG Types 1.2.5.7 and 1.3.6.7. Although NIL Q713-516C did not show strong resistance to these SCN HG Types (MS to HG Type 0 and S to other populations) when compared with Magellan, it showed significant resistance to all these six HG types when compared with NIL Q713-Mgl, because significant reductions of the female index (FI) were observed in Q713-516C when compared with Q713-Mgl: 29, 26, 45, 14, 32, and 26% for HG Type 2.5.7 (race 1), 1.2.5.7, 0, 2.5.7 (race 5), 1.3.6.7, and 1.2.3.4.5.6.7, respectively. These results suggest that the *qSCN10* locus confers broad-spectrum and partial resistance to SCN.

No yield drag or other unfavorable traits associated with *qSCN10*

To examine whether there are any adverse agronomic traits, especially yield drag, associated with *qSCN10,* Q713-516C and Q713-Mgl, a pair of NILs, were tested in a field without the presence of SCN. As shown in Fig. 3c, no significant difference was observed in yield between Q713-516C (4.32 t ha^{-1}) and Q713-Mgl (4.29 t ha^{-1}), indicating there was no yield drag associated with the QTL. Additionally, no other obvious undesirable traits were found associated with the QTL. Therefore, the *qSCN10* locus is suitable for application in breeding soybeans for resistance to SCN by introgression.

qSCN10 was fine-mapped to a 379-kb region

To further narrow down the *qSCN10* region for future application in breeding and the cloning of the underlying gene(s), 159 individual BC_4F_2 plants were identified as cross-recombinants in the target *qSCN10* region by two SNP markers (SNP1 and SNP-O8). Four more SNP markers (SNP1.1, SNP2, SNP3, and SNP4) were designed between these two SNP markers to further genotype these recombinants (Supplementary Table S1). Eventually, 4 pairs of NILs (Q041, Q150, Q543, and Q660) were selected for SCN phenotyping due to their different crossover sites within the QTL region (Fig. 4a).

Progeny tests were performed for these four pairs of NILs in response to 6 SCN HG Types in the greenhouse. The progeny lines with the genomic fragment between SNP2 and SNP-O8 from PI 567516C showed significant reductions in female indexes (FIs): The Q041 line showed significant reductions of 32 and 38% in response to SCN HG Type 2.5.7 (race 1) and 1.3.6.7, respectively; the Q660 line showed significant reductions of 44, 47, 49, 35, and 51% in response to HG Type 2.5.7 (race 1), 1.2.5.7, 2.5.7 (race 5), 1.3.6.7, and 1.2.3.4.5.6.7, respectively. No significant difference was found between progeny lines of Q150 and Q543. Hence, the *qSCN10* locus can be narrowed down to a 379-kb interval between markers

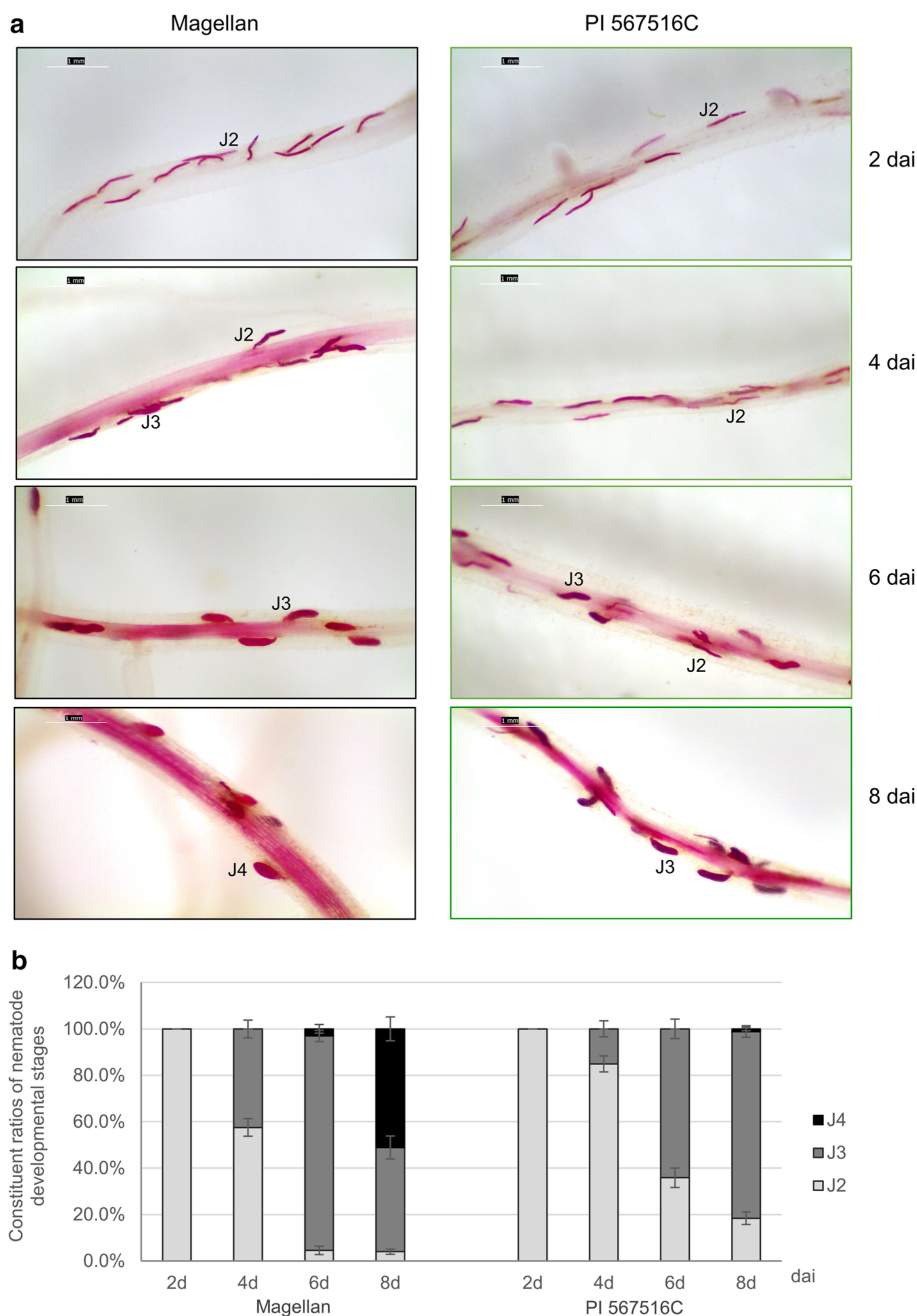


Fig. 2 Observation of SCN (HG Type 0) development in roots of Magellan and PI 567516C. **a** Representative images of nematode-infected roots at 2, 4, 6, and 8 days after inoculation (dai) were showed. Different developmental stages of SCN in the roots were indicated in the images. J2, second-stage juvenile; J3, third-stage juvenile; and J4, fourth-stage juvenile. **b** Statistics of constituent ratios of each nematode developmental stage at 2, 4, 6, and 8 dai. Data are represented by the means ± SE

SNP2 (Gm10:42,430,713) and SNP-O8 (Gm10:42,809,800) based on the above phenotypic as well as genotypic information (Fig. 4). Additional SNP markers (Fig. 4b, Supplementary Table S1) developed from the fine-mapped region were used to genotypes these NILs. Although these markers did not reduce the target interval any further, they can be used in the next round of fine-mapping.

Analysis of candidate genes in the fine-mapped QTL region

There are 51 genes in the delimited 379-kb interval according to the gene models Glyma. Wm82.a2.v1 (Table 1). DNA sequence variations within the QTL region were acquired from of our previous WGRS studies (Valliyodan et al. 2016;

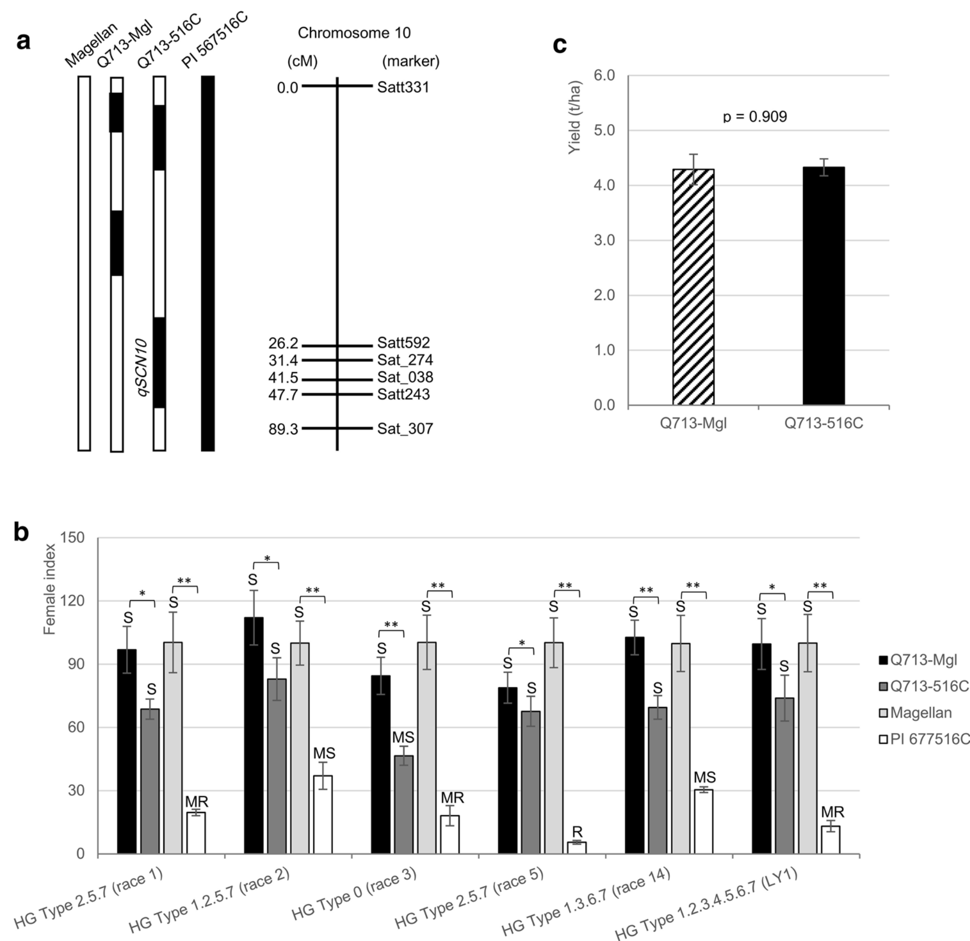
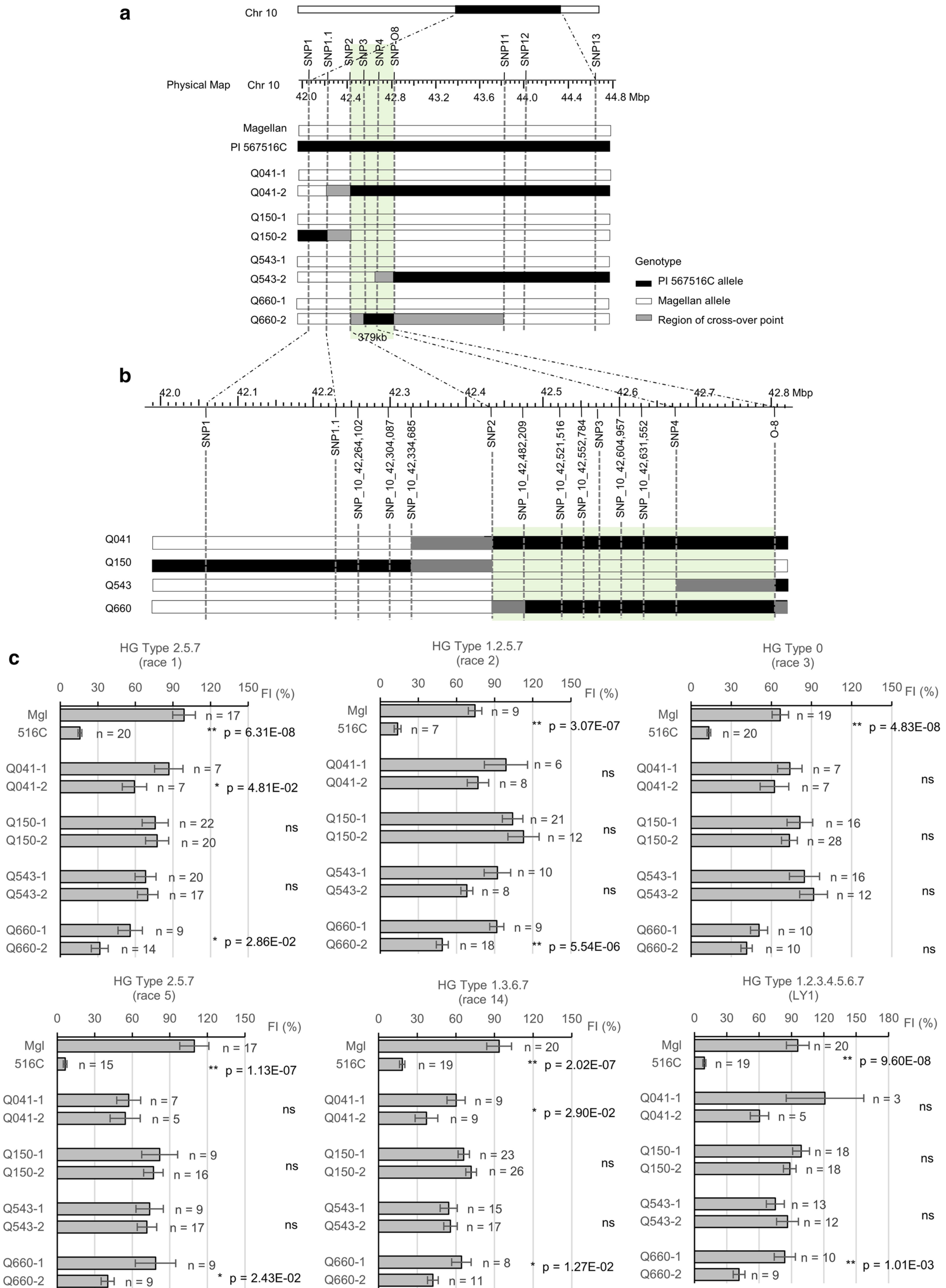


Fig. 3 SCN resistance evaluation of the pair of near-isogenic lines (NILs) (Q713) and parents. **a** Schematic graph of chromosome 10 of a pair of Q713 NIL lines and the 2 parents. The name and genetic locus of the SSR markers and the position of *qSCN10* are indicated as described previously (Vuong et al. 2010). **b** Responses of Q713 NILs and their parents to 6 SCN populations. Q713-Mgl and Q713-516C comprise homozygous Magellan and PI 567516C allele in the *qSCN10* region, respectively. The female index (FI) is shown as mean ± SE ($n > 10$). According to the Student's t test, * and ** indicate 5% and 1% significance level, respectively. Response scores of NILs and 2 parents to each HG Type based on FI scores were labeled on top of each bar. *R*, resistant (FI < 10); MR, moderately resistant (10 < FI < 30); MS, moderately susceptible (30 < FI < 60); and *S*, susceptible (FI > 60). **c** Yield drag test for NILs. The NILs were planted in the field under normal growth conditions (none-SCN stress field). Data shown are means ± SE of yield estimated from 3-m rows with 5 replications

Xu et al. 2013). Sequence variations in annotated genes between Magellan and PI 567516C, including synonymous/non-synonymous changes in exons and nucleotide insertion/deletion/substitutions in non-coding regions (including introns, 5′UTR and 3′UTR), are shown in Supplementary Table S2. A total of 700 variations were detected in 38 genes, including 91 non-synonymous variations in the exons of 24 genes. A 5-bp deletion in *Glyma.10G195000* encoding a cytosolic 5′-nucleotidase III-like protein was identified in the susceptible parent (Magellan). This deletion was found to cause open-reading frame shift and therefore a possible functional change.

Expression profiles of the 51 annotated genes in response to SCN and in different tissues were obtained from the published microarray and RNA-Seq transcriptomes that have been deposited in the Gene Expression Omnibus (GEO) database and the Soybase website (GSE64492, Wan et al. 2015; GSE1251033, Neupane et al. 2019; www.soybase.org). Average expression levels of three replicates for each sample treatment were shown (Fig. 5). Over half of the genes were expressed in the soybean roots, where they may play a role in mediating resistance to SCN. Among them, *Glyma.10G192500*, *Glyma.10G193200*, *Glyma.10G193500*, *Glyma.10G193600*, *Glyma.10G194800*, and *Glyma.10G195600* were up-regulated under SCN infection; *Glyma.10G193400*, *Glyma.10G194500*, and *Glyma.10G195700* were down-regulated; and *Glyma.10G191700* and *Glyma.10G196700* were induced at 5 dai and suppressed at 30 dai. Notably, *Glyma.10G194800*, *Glyma.10G195600*, *Glyma.10G195700*,


a
Chr 10
SNP1
SNP1.1
SNP2
SNP3
SNP4
SNP-O8
SNP11
SNP12
SNP13
Physical Map Chr 10
42.0
42.4
42.8
43.2
43.6
44.0
44.4
44.8 Mbp
Magellan
PI 567516C
Q041-1
Q041-2
Q150-1
Q150-2
Q543-1
Q543-2
Q660-1
Q660-2
379kb
Genotype
PI 567516C allele
Magellan allele
Region of cross-over point
b
42.0
42.1
42.2
42.3
42.4
42.5
42.6
42.7
42.8 Mbp
SNP1
SNP1.1
SNP_10_42,264,102
SNP_10_42,304,087
SNP_10_42,334,685
SNP2
SNP_10_42,482,209
SNP_10_42,521,516
SNP_10_42,552,784
SNP3
SNP_10_42,604,957
SNP_10_42,631,552
SNP4
O-8
Q041
Q150
Q543
Q660
c
HG Type 2.5.7 (race 1)
HG Type 1.2.5.7 (race 2)
HG Type 0 (race 3)
HG Type 2.5.7 (race 5)
HG Type 1.3.6.7 (race 14)
HG Type 1.2.3.4.5.6.7 (LY1)
FI (%)
Mgl
516C
** p = 6.31E-08
* p = 4.81E-02
* p = 2.86E-02
** p = 3.07E-07
** p = 5.54E-06
** p = 4.83E-08
** p = 1.13E-07
* p = 2.43E-02
** p = 2.02E-07
* p = 2.90E-02
* p = 1.27E-02
** p = 9.60E-08
** p = 1.01E-03
ns

◂**Fig. 4** Fine-mapping of *qSCN10* for multiple SCN populations. **a** The *qSCN10* locus was delimited into a 379-kb region by the progeny test of four recombinants in the *qSCN10* region. **b** Zoomed-in physical map and recombinants at the delimited region. **c** Responses of the progeny lines of the recombinants to six SCN HG Types. The female index (FI) is shown as means ± SE. The numbers of homozygous plant individuals used for phenotyping analysis were indicated in the charts. According to the Student's t test, * and ** indicate 5% and 1% significance level, respectively, and "ns" represents no significant difference

and *Glyma.10G196700,* encoding a bZIP transcription factor, a SNF7 family protein, a receptor-like kinase, and a CC-NBS-LRR protein, respectively, were annotated to be involved in defense responses. Therefore, they were considered to be potential candidate genes underlying the *qSCN10*.

Phylogenetic and haplotype analyses of the *qSCN10* locus

To understand the evolution and distribution of the *qSCN10* locus, a phylogenetic tree was constructed using 2,336 SNPs within the delimited 379-kb interval from 106 soybean lines, including seven wild soybean relatives (non-domesticated), 43 landraces (semi-domesticated), and 56 elite lines (Valliyodan et al. 2016; Lakhssassi et al. 2019) (Fig. 6). Based on the constructed tree, PI 567516C and 5 more landraces (PI 567,305, PI 567336B, PI 567,343, PI 567,357, PI 567,387) originating from China were clustered into a small separate group located at the end of the tree. Interestingly, neither do the elite breeding lines nor do the wild relatives in this diverse panel carry this locus. This result suggests the *qSCN10* locus originated from an ancestor soybean in China. Due to its absence in the elite lines, this unique locus can be introgressed into elite breeding lines to increase genetic diversity for resistance to SCN.

Haplotypes and phenotypes of the six clustered landraces were further analyzed to investigate the relationship between the known *rhg1* and *Rhg4* loci and the *qSCN10* locus (Supplementary Table S3). Based on a previous analysis (Kadam et al. 2016), 5 SNP markers identified for KASP assays were used to differentiate different alleles of *rhg1*, *Rhg4* and *qSCN10*: Rhg1-2 and Rhg1-5 for *rhg1*; Rhg4-3 and Rhg4-5 for *Rhg4*; and SNP-O8 for *qSCN10.* The Rhg1-5 marker was used to select the *rhg1* resistance allele, and the Rhg1-2 marker was employed to differentiate Peking and PI 88788-type resistance (Kadam et al. 2016). The WGRS data and the KASP assays revealed that PI 567305, PI 567516C, and PI 567336B carry the same genotype at five SNPs positions in the following loci or genes: Peking-type *rhg1*, PI 88,788/Williams 82-type *Rhg4*, and PI 567516C type *qSCN10*. The genotype is consistent with the closest clustering of these three PIs in the phylogenetic tree. Correspondingly, these three lines showed notable resistance to all the tested SCN HG Types. PI 567357 possesses susceptible haplotypes in all three genes, consistent with its susceptibility to SCN. PI 567343 showed opposite haplotypes from PI 567387 at the *rhg1* and *qSCN10* locus*,* and correspondingly the opposite response to all the tested HG Types from PI 567387. Based on this result, we speculated that the *qSCN10* locus may need to work together with the Peking-type *rhg1* to fulfill strong resistance to multiple SCN HG types, similar to *Rhg4*, which also requires *rhg1* for resistance to SCN, because the *qSCN10* locus alone only showed evident resistance to HG Type 0 and 1.3.6.7.

Discussion

qSCN10 confers broad-spectrum resistance to SCN

Previously, the *qSCN10* locus was shown to confer resistance to SCN HG Types 2.5.7 (race 1), 0, 2.5.7 (race 5), 1.3.6.7, and 1.2.3.4.5.6.7 (Vuong et al. 2010). Our present study not only confirmed its resistance to these SCN HG types, but also identified additional resistance to HG Type 1.2.5.7. Thus, the *qSCN10* locus is capable of conferring resistance to all the SCN HG types that have been tested. However, the resistance conferred by *qSCN10* alone was moderate to low when compared with the resistance observed in the original resistant parent, PI 567516C. The reason that PI 567516C confers strong resistance to these HG types is that, in addition to *qSCN10*, it also contains *qSCN18* as well as three copies of the Peking-type rhg1, although this *rhg1* was not mapped as a resistance QTL in this accession in the previous study (Vuong et al. 2010). Therefore, *qSCN10* very likely needs to interact with other QTL or genes, such as *qSCN18* and *rhg1*, to fulfill strong broad-spectrum resistance to SCN.

qSCN10 is a unique genetic resource different from any known QTL or genes

Two pieces of evidence support that *qSCN10* is a unique genetic resource different from any known QTL or genes. First, *qSCN10* was mapped to chr. 10, different from any known QTL or genes, e.g., *rhg1* (on chr. 18), *Rhg4* (on chr. 8), and *qSCN18* (on chr. 18). Second, phylogenetic analysis showed that *qSCN10* locus existed only in 6 exotic landraces originating from China and was absent in both the current US elite lines and wild races. This data suggests that the *qSCN10* locus may have originated from an ancestor soybean in China and evolved to become a resistance QTL due to nematode stress or domestication.

Table 1 Candidate genes harbored in a delimited *qSCN10* region using the whole-genome resequencing (WGRS) data and annotated based on the reference assembly Glyma.Wm82.a2.v1

No	Gmax 2.0 Gene ID	Genome start	Genome end	Protein length	Arabidopsis homologs	Annotations
1	Glyma.10G191700	42,434,017	42,435,482	329	AT4G11290.1	Peroxidase
2	Glyma.10G191800	42,439,119	42,442,119	83	AT3G22980.1	Elongation factor EF-2
3	Glyma.10G191900	42,443,011	42,446,793	586	AT5G46910.1	Lysine-specific demethylase 5D
4	Glyma.10G192000	42,450,626	42,455,192	175	AT2G37060.3	CCAAT-binding transcription factor family protein
5	Glyma.10G192100	42,455,848	42,459,836	815	AT5G02950.1	Serine/threonine protein kinase ATM
6	Glyma.10G192200	42,461,214	42,465,402	581	AT1G10580.1	Pre-mRNA-processing factor
7	Glyma.10G192300	42,467,953	42,472,437	402	AT4G13670.1	Peptidoglycan-binding domain-containing family protein
8	Glyma.10G192400	42,475,154	42,487,321	953	AT3G24180.2	Non-lysosomal glucosylceramidase
9	Glyma.10G192500	42,482,208	42,482,583	72	N.A.	
10	Glyma.10G192600	42,491,429	42,495,025	231	AT4G13690.1	
11	Glyma.10G192700	42,501,174	42,509,646	1179	AT1G04700.1	Mitogen-activated protein kinase kinase kinase 13-A
12	Glyma.10G192800	42,511,753	42,518,160	1012	AT1G04650.1	T1G11.9 protein
13	Glyma.10G192900	42,520,164	42,521,317	224	AT3G09270.1	Glutathione S-transferase 103-1A
14	Glyma.10G193000	42,527,991	42,540,681	588	AT1G55750.1	General transcription factor IIH subunit
15	Glyma.10G193100	42,542,002	42,544,975	814	AT3G23020.1	Pentatricopeptide repeat-containing protein
16	Glyma.10G193200	42,545,555	42,550,179	578	AT3G23990.1	Chaperonin CPN60-like protein
17	Glyma.10G193300	42,554,568	42,554,789	74	N.A.	
18	Glyma.10G193400	42,562,106	42,563,793	388	AT4G13620.1	Ethylene-responsive transcription factor RAP2-3
19	Glyma.10G193500	42,564,218	42,568,587	245	AT5G51100.1	Superoxide dismutase
20	Glyma.10G193600	42,570,070	42,571,181	186	AT5G07475.1	Blue copper protein
21	Glyma.10G193700	42,575,741	42,576,665	143	AT5G07490.1	Unknown protein
22	Glyma.10G193800	42,580,656	42,599,131	785	AT5G51230.1	Embryonic flower 2
23	Glyma.10G193900	42,600,687	42,601,680	148	AT4G25140.1	Oleosin
24	Glyma.10G194000	42,617,613	42,619,466	465	AT5G54010.1	Flavonol 3-O-glucoside (1->6) rhamnosyltransferase
25	Glyma.10G194100	42,625,670	42,626,996	387	AT5G54010.1	Flavonol 3-O-glucoside (1->6) rhamnosyltransferase
26	Glyma.10G194200	42,630,111	42,632,551	202	AT5G07580.1	Ethylene-responsive transcription factor
27	Glyma.10G194300	42,632,925	42,642,953	523	AT5G61580.1	6-Phosphofructokinase
28	Glyma.10G194400	42,644,023	42,649,627	459	AT5G07590.1	WD-repeat protein
29	Glyma.10G194500	42,656,926	42,664,986	1482	AT3G06880.2	U-box domain-containing protein
30	Glyma.10G194600	42,666,614	42,669,445	306	AT1G14140.1	Mitochondrial uncoupling protein
31	Glyma.10G194700	42,670,924	42,678,035	1288	AT3G10650.1	F18K10.27 protein
32	Glyma.10G194800	42,686,749	42,691,742	232	AT5G10030.2	Transcription factor bZIP
33	Glyma.10G194900	42,694,105	42,699,129	711	AT3G11760.1	K4BPD4 MATE efflux family protein
34	Glyma.10G195000	42,702,305	42,705,748	251	AT2G38680.1	Cytosolic 5′-nucleotidase III-like protein
35	Glyma.10G195100	42,705,870	42,706,336	91	N.A.	
36	Glyma.10G195200	42,708,491	42,710,238	370	AT5G01420.1	Glutaredoxin domain-containing cysteine-rich protein
37	Glyma.10G195300	42,711,052	42,712,419	228	AT2G31050.1	Blue copper protein
38	Glyma.10G195400	42,713,356	42,713,802	149	AT5G06490.1	Zinc finger family protein
39	Glyma.10G195500	42,720,899	42,721,369	157	AT5G06490.1	Ring finger protein
40	Glyma.10G195600	42,722,946	42,727,022	238	AT3G10640.1	SNF7 family protein
41	Glyma.10G195700	42,731,233	42,735,014	1084	AT1G73080.1	Receptor-like protein kinase
42	Glyma.10G195800	42,735,927	42,737,795	485	AT3G10630.1	Glycosyl transferase family 1 protein
43	Glyma.10G195900	42,743,520	42,746,720	202	AT3G10620.1	Nudix hydrolase
44	Glyma.10G196000	42,749,400	42,751,583	142	N.A.	

Table 1 (continued)

No	Gmax 2.0 Gene ID	Genome start	Genome end	Protein length	Arabidopsis homologs	Annotations
45	Glyma.10G196100	42,752,699	42,761,978	642	AT5G03540.1	Exocyst complex component EXO70
46	Glyma.10G196200	42,765,160	42,768,645	181	AT5G04830.2	Nuclear transport factor 2 (NTF2) family protein
47	Glyma.10G196300	42,778,690	42,782,165	866	AT3G52490.1	Heat shock protein
48	Glyma.10G196400	42,788,040	42,790,903	438	AT3G10440.1	Shugoshin-1
49	Glyma.10G196500	42,797,101	42,798,670	261	AT5G22930.1	Unknown protein
50	Glyma.10G196600	42,802,270	42,805,638	388	AT3G24120.1	Myb family transcription factor
51	Glyma.10G196700	42,807,536	42,810,483	691	AT5G35450.1	NBS-LRR protein

N.A. indicates no homologous Arabidopsis gene (TAIR10 genome release) available

DNA sequence variations in the fine-mapped region

In the present study, the *qSCN10* locus was narrowed down to a 379-kb region on chr. 10. Fifty-one genes are present in this region based on the Williams 82 reference genome (version 2). By analyzing the DNA sequence variations within the *qSCN10* locus, no large DNA fragment insertion or deletion (> 50 bp) was detected, which may lead to introgression or deletion of any gene/genes other than the indicated 51 genes. Among these genes, 24 genes were found to have non-synonymous variations in the exons that may cause function changes of their coded proteins. Notably, a 5-bp deletion in *Glyma.10G195000*, which encodes a cytosolic 5′-nucleotidase III-like protein, caused a frameshift and therefore a possible functional change. In addition to these non-synonymous variations, sequence variations in the intergenic regions, introns, and 5′-and3′-UTRs may also affect gene functions by regulating their expressions.

To further narrow down the QTL region and eventually clone the underlying gene(s), another round of fine-mapping, which requires new progeny with recombinant haplotypes between marker SNP2 and SNP-O8, is needed. For this purpose, a large set of BC_4F_4 progeny will be generated and screened with KASP assays for SNPs that have been discovered. New pairs of NILs will be developed for fine-mapping. After the second round of fine-mapping, possible candidate genes will be selected and functionally validated using transgenic approaches for their possible role in conferring resistance to SCN.

Possible candidate genes and defense mechanism underlying the resistance of *qSCN10* to SCN

Among the 51 annotated genes, none of them show significant similarities to *rhg1* or *Rhg4* genes, further supporting the genetic novelty of this locus. Of these genes, 11 were regulated by SCN infection. Among them are the following four defense-related genes. *Glyma.10G194800* encodes a bZIP transcription factor. bZIP transcription factors have been indicated in defense against bacterial pathogens in some plants (Li et al. 2017; Lim et al. 2015). *Glyma.10G195600* encodes a SNF7 family protein. A recent study showed that a SNF7 family protein, together with other proteins, was recruited to repair the membrane damage caused by pathogen infection in *Dictyostelium discoideum* (López-Jiménez et al. 2018). *Glyma.10G195700* encodes a receptor-like kinase. Receptor-like kinases are well known to be important in both plant biotic and abiotic responses (Tang et al. 2017; Ye et al. 2017). *Glyma.10G196700* encodes a CC-NBS-LRR protein. NBS-LRR proteins are typical plant disease resistance proteins important in defending plants against infection by various pathogens, such as viruses, bacteria, and fungi (Eitas et al. 2010; McHale et al. 2006). Multiple non-synonymous variations were found in *Glyma.10G194800*, *Glyma.10G195700*, and *Glyma.10G196700*. Except some sequence variations in the non-coding regions, there were no variations in the exons of *Glyma.10g195600*. Due to their potential role in defense, these four genes are good candidates for the *qSCN10* QTL. However, other non-traditional defense-related genes cannot be completely overlooked. Since nematodes are different pathogens from bacteria and fungi, soybean plants may use a different mechanism to combat them. For example, the cloned *Rhg4* gene is not a traditional defense-related gene: the gene encodes a serine hydroxy methyltransferase, responsible for interconversion of serine and glycine and essential for cellular one-carbon metabolism (Liu et al. 2012). Additionally, it is possible that more than one gene is involved in mediating the resistance to SCN by *qSCN10*, as in the case of *rhg1*, which requires the contributions from three neighboring genes to achieve resistance (Cook et al. 2014). Further work is needed to eventually identify the gene(s) and the corresponding mechanism that are responsible for the resistance to SCN conferred by *qSCN10*.

Our present work also suggests that defense against SCN in PI 567516C occurred after SCN entered the roots. The nematodes appeared to be able to enter the root normally to establish a feeding site (syncytium) in PI 567516C. However, their growth and development in PI 567516C were delayed or restricted compared to that

Wm82.a2.v1 Gene ID	Microarray in response to SCN[a]										RNA-seq in response to SCN[b]				RNA-seq in different tissues[c]								
	Mgl-mock-0d	Mgl-mock-3d	Mgl-SCN-3d	Mgl-mock-8d	Mgl-SCN-8d	516C-mock-0d	516C-mock-3d	516C-SCN-3d	516C-mock-8d	516C-SCN-8d	W82_mock_5d	W82_SCN_5d	W82_mock_30d	W82_SCN_30d	Root	Nodule	Young_leaf	Flower	Pod-1cm	Pod shell 10DAF	Seed 10DAF	Seed 21DAF	Seed 35DAF
* Glyma.10G191700	9	9	8	8	9	9	20	14	27	5	0.4	13.5	77.5	33.4	0	0	0	0	0	0	0	0	0
Glyma.10G191800	.	.	.	.	.	.	.	.	.	.	0.3	0.8	1.7	0.9	0	1	0	0	0	0	0	2	0
Glyma.10G191900	3	2	2	3	2	2	2	2	3	3	0.0	0.0	0.0	0.0	0	0	0	1	0	0	0	0	0
Glyma.10G192000	21	27	29	27	21	15	29	29	30	15	16.6	15.8	16.6	19.3	5	8	8	10	16	11	4	4	4
Glyma.10G192100	20	57	26	25	28	33	56	46	40	31	5.0	4.6	2.0	2.5	2	1	1	3	1	1	1	1	2
Glyma.10G192200	59	61	59	72	83	63	56	77	96	89	9.6	8.5	8.4	6.9	9	11	10	7	11	7	3	11	10
Glyma.10G192300	11	23	16	15	19	9	14	20	26	17	2.3	4.4	3.8	3.7	2	2	13	5	6	4	1	2	3
Glyma.10G192400	19	32	47	28	34	26	53	39	62	28	.	.	.	.	9	4	2	4	2	2	1	1	4
* Glyma.10G192500	.	.	.	.	.	.	.	.	.	.	0.6	1.8	1.0	2.2	.	.	.	.	.	.	.	.	.
Glyma.10G192600	.	.	.	.	.	.	.	.	.	.	0.3	0.3	1.4	0.7	1	0	16	2	4	1	2	13	0
Glyma.10G192700	3	4	4	4	4	4	4	4	4	3	0.1	0.4	0.3	0.3	0	0	0	2	0	0	0	0	0
Glyma.10G192800	7	5	8	6	8	5	9	10	5	6	0.1	0.2	0.3	0.3	0	0	2	1	1	0	1	1	0
Glyma.10G192900	129	101	80	85	140	74	96	113	93	105	.	.	.	.	104	2	0	21	0	0	1	0	1
Glyma.10G193000	53	77	75	47	57	57	76	91	78	63	5.2	7.6	7.0	7.2	3	3	2	3	2	3	2	3	2
Glyma.10G193100	5	9	9	6	12	8	11	8	8	5	1.1	0.8	0.7	0.9	0	0	1	1	0	0	0	0	1
* Glyma.10G193200	22	17	20	18	20	12	17	26	15	29	.	.	.	.	20	9	11	12	10	14	6	17	18
Glyma.10G193300	.	.	.	.	.	.	.	.	.	.	0.0	0.0	0.0	0.0	.	.	.	.	.	.	.	.	.
* Glyma.10G193400	44	12	10	10	8	15	24	26	13	16	0.9	0.3	1.0	0.4	4	0	0	1	0	0	0	1	1
* Glyma.10G193500	.	.	.	.	.	.	.	.	.	.	46.9	202	79.3	227	71	107	2	4	0	3	6	84	10
* Glyma.10G193600	46	101	148	166	213	15	46	57	64	55	.	.	.	.	4	1	0	0	0	0	0	0	0
Glyma.10G193700	4	3	6	4	3	3	4	4	4	4	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
Glyma.10G193800	.	.	.	.	.	.	.	.	.	.	0.0	0.1	0.1	0.1	0	0	0	0	0	0	2	1	0
Glyma.10G193900	25	38	31	25	31	28	49	33	35	20	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	26	2088
Glyma.10G194000	4	6	11	7	6	11	7	11	17	9	0.1	0.1	0.3	0.1	0	0	75	22	20	26	1	0	0
Glyma.10G194100	3	4	5	3	3	3	4	6	3	3	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
Glyma.10G194200	18	28	20	10	8	22	28	14	7	5	23.6	19.3	13.3	17.1	4	0	0	4	0	0	0	1	0
Glyma.10G194300	15	18	15	11	14	13	13	16	14	11	1.7	2.5	2.6	2.9	1	0	9	3	1	2	1	1	0
Glyma.10G194400	99	89	74	46	53	56	80	75	97	38	5.5	6.4	6.5	5.9	3	2	7	7	6	8	3	2	2
* Glyma.10G194500	.	.	.	.	.	.	.	.	.	.	27.3	12.6	9.9	9.7	12	20	0	0	0	0	0	0	0
Glyma.10G194600	34	32	28	32	39	28	28	32	29	21	1.1	2.0	1.9	1.9	1	1	4	2	3	1	1	2	1
Glyma.10G194700	66	120	89	92	101	81	103	90	97	85	8.6	6.5	4.4	4.8	3	4	6	8	6	6	3	4	3
** Glyma.10G194800	4	9	6	5	7	8	9	13	9	8	0.3	3.5	1.5	6.1	1	5	0	0	0	0	0	0	0
Glyma.10G194900	5	8	7	12	10	5	6	10	7	7	0.2	0.8	1.0	1.3	0	0	4	1	3	1	1	1	0
Glyma.10G195000	.	.	.	.	.	.	.	.	.	.	0.4	0.8	0.4	0.6	1	0	5	12	5	3	4	7	10
Glyma.10G195100	.	.	.	.	.	.	.	.	.	.	0.1	0.2	0.2	0.5	.	.	.	.	.	.	.	.	.
Glyma.10G195200	3	3	3	3	4	3	3	5	3	3	0.4	0.7	0.9	1.2	2	0	6	1	3	4	1	2	1
Glyma.10G195300	3	2	3	2	2	2	2	3	2	2	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
Glyma.10G195400	.	.	.	.	.	.	.	.	.	.	0.0	0.1	0.4	0.4	0	0	0	0	0	0	0	0	0
Glyma.10G195500	15	26	21	17	13	5	15	7	9	8	.	.	.	.	3	1	0	0	0	0	0	0	0
** Glyma.10G195600	4	4	4	4	3	3	2	4	2	2	34.8	54.5	42.4	52.0	12	8	15	14	9	7	6	8	5
** Glyma.10G195700	4	3	4	3	4	5	3	6	4	2	2.3	0.1	0.1	0.2	0	0	0	0	0	0	0	1	0
Glyma.10G195800	7	12	8	7	7	5	8	11	13	5	1.7	2.7	3.1	3.0	2	1	6	3	7	5	1	5	1
Glyma.10G195900	73	72	51	53	56	51	60	77	61	42	13.8	21.1	27.3	25.6	9	9	14	10	14	11	8	11	10
Glyma.10G196000	.	.	.	.	.	.	.	.	.	.	0.0	0.0	0.0	0.0	.	.	.	.	.	.	.	.	.
Glyma.10G196100	10	13	8	6	10	7	13	13	14	12	9.3	10.2	10.8	8.5	9	2	5	4	6	5	1	2	2
Glyma.10G196200	3	6	6	4	7	6	9	11	9	8	0.0	0.1	0.1	0.4	0	4	3	4	2	2	1	1	3
Glyma.10G196300	3	4	3	3	4	2	4	12	3	3	2.0	2.5	1.3	1.4	1	0	2	1	2	2	2	1	0
Glyma.10G196400	5	14	12	8	11	22	22	29	25	20	.	.	.	.	3	0	13	3	6	3	3	3	1
Glyma.10G196500	4	6	7	8	8	5	6	9	12	6	0.2	0.2	0.3	0.3	0	0	1	1	1	2	0	0	0
Glyma.10G196600	18	25	23	25	22	12	28	26	30	24	7.3	6.9	5.9	7.8	9	6	44	11	15	18	6	18	11
** Glyma.10G196700	.	.	.	.	.	.	.	.	.	.	0.1	0.5	1.3	0.9	.	.	.	.	.	.	.	.	.

Expression value in microarray: 2, 12, 200

RPKM value in RNA-seq: 0, 1, 200

Fig. 5 Heat map of candidate gene expressions. Raw data from three sets of transcriptome profiles were downloaded from published database and analyzed in Excel: **a** Microarrays from Gene Expression Omnibus (GEO) database GSE64492 (Wan et al. 2015); **b** RNA-Seq from GEO database GSE1251033 (Neupane et al. 2019); **c** RNA-Seq from Soybase database (Severin et al. 2010). Average expression values of 3 replicates for datasets **a** and **b** were shown. Dots indicate no available probe-sets in microarray or no corresponding genes in RNA-Seq; red asterisks close to Gene IDs indicate genes that are regulated by SCN infection; two red asterisks indicate defense-related genes. Genes expressing in the roots (RPKM ≥ 1) are highlighted in blue font. Mgl, Magellan; 516C, PI567516C; W82, Williams 82; mock, control treatment with water; SCN, samples with SCN inoculation (color figure online)

Fig. 6 Phylogenetic analysis for the *qSCN10* locus. The dendrogram of 106 soybean lines was analyzed based on 2,336 SNPs derived from Valliyodan et al. (2016) within the delimited 379-kb *qSCN10* region. Blue circle-shaped bullets show 56 elite lines; yellow triangle-shaped bullets show 43 landraces; and red diamond-shaped bullets show 7 wild races. Subtree containing PI 567516C is highlighted with green branches (color figure online)

in Magellan (Fig. 2). The previous transcriptome analysis in PI 567516C and Magellan with/without SCN infection showed that genes involved in ethylene and protein degradation were significantly enriched in PI 567516C over Magellan and regulated by SCN infection (Wan et al. 2015). Studies suggest that ethylene signaling can induce programmed cell death, and protein degradation is an important part of cell signaling and cell death (Bouchez et al. 2007; Steffens et al. 2009; Kidd et al. 1998). Therefore, the suppressed development of nematodes in PI 567516C may be due to their inability to properly maintain the feeding sites (syncytia) (syncytium degeneration) to provide sufficient nutrients for their growth and development. Although the growth of nematodes in PI 567516C was slowed down, a small portion of nematodes were still able to fully develop, unlike the complete resistance that observed in Peking and PI 88788 (Mitchum 2016; Chen et al. 2020). The previous transcriptomic studies also support this suggestion, as different gene expression profiles were observed in PI 567516C and PI 437654 (Peking-type *rhg1*) during SCN infection. Therefore, the cloning of the gene(s) underlying the *qSCN10* QTL in PI 567516C will likely discover a novel SCN defense mechanism.

Application of *qSCN10* in breeding soybean for broad-spectrum resistance to SCN

Our present study not only confirmed that the *qSCN10* QTL is a novel source conferring resistance to multiple SCN HG types, but also narrowed the QTL down to a 379-kb region. Furthermore, there was no yield drag or other unfavorable traits associated with this QTL. Therefore, *qSCN10* shall be an excellent resistance source to be introgressed into elite soybean varieties for broad-spectrum resistance to SCN via marker-assisted selection (MAS). However, *qSCN10* alone only conferred moderate-to-low resistance to different HG types, in comparison with the original resistant parent, PI 567516C, which confers much stronger resistance to different SCN HG types. As discussed afore, PI 567516C contains not only *qSCN10*, but also *qSCN18* as well as three copies of the Peking-type *rhg1* (Vuong et al. 2010; Patil et al. 2019), suggesting that the strong broad-spectrum resistance observed in PI 567516C is due to the synergistic interactions among these QTLs or genes. Therefore, to achieve strong broad-spectrum resistance to different HG types, *qSCN10* needs to be introgressed into elite soybean varieties with other resistance QTL or genes, such as *qSCN18* and *rhg1*.

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Author contribution statement LZ, LS, HY, and HTN conceived and designed the project; LS and TV contributed to the development of the mapping populations; LZ, LS, YL, and MK collected plant materials and performed genotyping and phenotyping experiments. LZ and LS contributed to the data analysis; LZ wrote the manuscript; HY, TV, and JW contributed to discussion of the manuscript; and all authors critically revised the paper.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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