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Characterizing resistance to soybean cyst nematode in PI 494182, an early maturing soybean accession

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Abstract

The soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) generates more damage to soybean [*Glycine max* (L.) Merr.] than any other parasite in most soybean-producing countries. The use of SCN-resistant cultivars remains the most effective method to limit losses caused by SCN. The SCN-resistant accession PI 88788 has been used almost exclusively to control SCN over the past decades, inducing a shift in nematode virulence to overcome the resistance. Furthermore, PI 88788 and other sources of resistance characterized to date belong to maturity groups (MGs) III and higher, making them less attractive to develop early maturing soybean varieties (MGs 0-000). In this work, we performed a quantitative trait loci (QTL) analysis of the SCN-resistant soybean accession PI 494182 (MG 0). A recombinant inbred lines (RILs) population ('Costaud' × PI 494182) segregating for SCN resistance was challenged with SCN (*H. glycines* [HG] type 0) and genotyped via genotyping-by-sequencing (GBS) to produce a genetic map. Six resistance QTL were identified, including a potentially new resistance locus on chromosome 07. A subset of the RIL population was confronted to a HG type 2.5.7 SCN population and some of these exhibited resistance toward this type. Whole-genome sequencing of PI 494182 and Costaud allowed us to determine the alleles and their copy number for three candidate genes: *GmSNAP11*, *GmSNAP18* (*Rhg1*), and *GmSHMT08* (*Rhg4*). Finally, we determined that selecting for PI 494182 alleles at some SCN-resistance QTL could entail linkage drag (decrease in protein concentration and 100-seed weight, increase in oil concentration). This work provides useful markers for introgressing SCN resistance in early maturing soybean varieties.

1 | INTRODUCTION

Over the past decades, high demand for oil and protein meal has increased as consumer habits have changed in many countries (Delgado, 2003; Henchion, Hayes, Mullen, Fenelon, & Tiwari, 2017). Of all crops, soybean offers the highest protein yield per surface cultivated (Kaldy, 1972) and is the most

Abbreviations: CNV, copy number variation; FI, female index; GBS, genotyping-by-sequencing; GWAS, genome-wide association studies; HG, *Heterodera glycines*; ICIM, inclusive composite interval mapping; LOD, logarithm of the odds; MG, maturity group; PI, plant introduction; PVE, phenotypic variance explained; QTL, quantitative trait loci; RIL, recombinant inbred line; SCN, soybean cyst nematode; SMA, single-marker analysis; WGS, whole-genome sequencing.

important oilseed crop worldwide (SoyStats, 2020). As global food demand is expected to double by 2050 (Hunter, Smith, Schipanski, Atwood, & Mortensen, 2017), the soybean production will remain an important contributor to meet nutritionnal needs. Plant pests constitute a major obstacle to realizing the full yield potential of a crop and the SCN is the most damaging pest of soybean in most producing regions of the world (Riggs, 1977). In the United States, SCN has spread to nearly all soybean-producing regions (Tylka & Marett, 2017) and losses resulting from SCN are estimated at US$1.2 billion annually (Koenning & Wrather, 2010), equivalent to 10–20% in yield losses (Winter, Rajcan, & Shelp, 2006). Together with nonhost crop rotations, one of the main methods to control SCN is the use of resistant cultivars. The use of resistant cultivars hinders the parasite's reproduction and constitutes an economically profitable and environmentally friendly approach.

The SCN is a soil-borne obligate plant parasite that requires infection of the host plant to complete its life cycle and accomplish reproduction. After hatching, the nematodes will penetrate the soybean roots up to the stele were it will begin the establishment of a permanent feeding site. To do so, the nematode targets a single soybean cell and injects effector proteins that modify the cell's metabolism, turning it into an enriched nutrient sink named the syncytium (Masonbrink et al., 2019; Yan & Baidoo, 2018). To support the nematode growth, more surrounding plant cells will be merged to the syncytium to form a larger feeding structure. Survival of the SCN is dependent on the succesfull establisment and maintenance of the syncytium. In resistant soybean plants, the SCN infection triggers an hypersensitive response leading to apoptosis of the syncytium and starvation of the nematode (Kandoth et al., 2011).

Different levels of virulence are encountered among SCN populations and are categorized using the HG-type classification system. The reproductive capability of a SCN population on a set of seven indicator lines (plant introduction [PI] 548402, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316) defines its HG type and thus its virulence (Niblack et al., 2002).

To better understand the molecular mechanism of resistance and to increase efficiency in the development of new resistant lines, resistance to SCN has been mapped via genome-wide association studies (GWAS) or biparental QTL analysis. Such studies have identified several resistance loci and these can be found on almost all soybean chromosomes (Concibido, Diers, & Arelli, 2004; Kim et al., 2016). The list of resistance to SCN QTL identified in biparental populations reported on the SoyBase database (SoyBase, 2020) now comprises 216 loci, of which, six major loci (cqSCN-001, cqSCN-002, cqSCN-003, cqSCN-005, cqSCN-006, and cqSCN-007) were predominant in different mapping populations or environments and designed as confirmed QTL. Among these QTL, cqSCN-001 and cqSCN-002, also designated *Rhg1* and *Rhg4*, respectively, were identified as majors contributors to SCN resistance in several sources studied to date. However, despite the prevalence of *Rhg1* and *Rhg4* in the sources that have been characterized, other loci can provide important contributions to resistance. This fact was highlighted in a recent study by Tran, Steketee, Boehm, Noe, and Li (2019), where 58 new soybean accessions with resistance or moderate resistance to SCN that did not rely on *Rhg1* and *Rhg4* for SCN resistance were identified.

As the *Rhg1* and *Rhg4* loci were repeatedly identified in most SCN resistance sources, they were subjected to extensive characterisation. The *Rhg1* locus is a 31.2-kb region comprising four genes: *Glyma.18G022400*, *Glyma.18G022500*, *Glyma.18G022600*, and *Glyma.18G022700*. However, the only nucleotide variants differing between resistant and susceptible lines in populations segregating for *Rhg1* are located in *Glyma.18G0022500* (*GmSNAP18*), making it the major gene controlling resistance at this locus (Liu et al., 2017). The *GmSNAP18* gene encodes an α-SNAP (α-soluble NSF attachment protein) whose hyperaccumulation can be cytotoxic, leading to apoptosis of the syncytium in which the SCN is feeding (Bayless et al., 2016). On chromosome 08, the *Rhg4* locus is a ∼35.7-kb genomic segment containing four genes (*Glyma.08G108800*, *Glyma.08G108900*, *Glyma.08G109000*, and *Glyma.08G109100*) (Patil et al., 2019). The *Glyma.08G108900* (*GmSHMT08*) gene encodes a serine hydroxymethyltransferase (SHMT) and was confirmed as the resistance gene at this locus (Kandoth et al., 2017; Liu et al., 2012). In most resistant lines, SCN control is provided mainly by *Rhg1* and *Rhg4* with additional contributions from other minor QTL (Tylka & Mullaney, 2019).

Generally, SCN-resistant lines are classified in two main haplotypic groups based on their allelic profiles at the *Rhg1* and *Rhg4* loci. In the 'Peking' type of resistance, lines carry the *Rhg1-a* allele and the *Rhg4-a* allele, with a strong epistatic interaction being reported between these loci (Liu et al., 2017). In the PI 88788 type, resistance relies only on the presence of the *Rhg1-b* allele as the *Rhg4-b* allele does not contribute to resistance.

Copy number variations (CNVs) also play an important role in regulating resistance to SCN. The *Rhg1* locus presents a large variability in copy number, with some lines carrying a single copy of the 31-kb region and others up to 10 tandem repeats of the locus (Cook et al., 2012). A higher copy number at this locus was correlated with a more abundant transcript leading to a toxic accumulation of α-SNAP in the syncytium (Cook et al., 2014). Recently, it was shown that *Rhg4* is also prone to CNVs with some lines carrying up to 4.3 repeats of the *Rhg4-a* allele (Patil et al., 2019). Additionally, transcript abundance was correlated to copy number, where more copies of the locus increased the resistance to SCN.

Moreover, different CNV patterns have been observed at *Rhg1* and *Rhg4* depending on the type of resistance (Patil et al., 2019). For lines carrying PI 88788-type resistance, a single copy of the *Rhg4* locus is observed and a high number of repeats of the *Rhg1-b* allele—5.6 copies being the minimum needed to obtain resistance. Inversely, for lines with the Peking-type resistance, few copies of the *Rhg1-a* allele (1–3.5) are enough to confer resistance as long as these are accompanied by the *Rhg4-a* allele, of which 1–4.3 copies have been reported.

Over the last two decades, PI 88788-type resistance has been used extensively, and today, over 95% of the commercially available SCN-resistant varieties in the United States have this type of resistance (Tylka & Mullaney, 2019). The selection pressure exerted by the continuous use of PI 88788-type resistance has led to the indirect selection of virulent SCN populations. For example, a recent evaluation of SCN virulence in Missouri revealed that this source of resistance was overcome in 100% of the fields (Howland, Monnig, Mathesius, Nathan, & Mitchum, 2018). Few commercial lines have been developed with other sources of resistance (Peking or PI 437654) because of their detrimental effect on yield (Lee, Diers, & Hudson, 2016) or, in the case of PI 437654, the complex genetic basis of resistance. Thus, the identification of new sources of resistance remains necessary for the control of SCN populations.

Another important trait to consider in soybean breeding is maturity. In North America, varieties are categorized into one of 13 MGs, ranging from group 000 to X. The most common sources of resistance to SCN (PI 88788, Peking, PI 90763, and PI 437654) belong to MGs III, IV, IV, and III, respectively (Tylka & Marett, 2017). However, SCN has recently spread to northern regions growing early MG (Mimee et al., 2013) and if the impact on soybean appear limited in these areas currently (Mimee, Gagnon, Colton-Gagnon, & Tremblay, 2016), modelling analyses predict a rapid increase in yield losses (Gendron St-Marseille, Bourgeois, Brodeur, & Mimee, 2019) and dissemination of virulence (Gendron St-Marseille, Lord, Véronneau, Brodeur, & Mimee, 2018). When breeding extremely early varieties (MGs 0 to 000), such as are needed in Canada, making crosses with lines of a much later MG is not trivial and leads to a broad segregation for maturity with only a small subset of the progeny offering the required earliness. Young (1995) reported the early MG0 soybean accession PI 494182 ('Suzuhime') as resistant to HG types 0 (SCN race 3) and 2.7 (SCN race 5). It was later reported to confer resistance against HG type 2.5.7 (SCN race 1) (Arelli & Wang, 2008).

The main objective of this work was to discover the underlying QTL explaining resistance to SCN HG type 0 in PI 494182. In addition, we used whole-genome sequencing (WGS) data to determine nucleotide variation and CNV at known resistance genes in the parental lines. Finally, key agronomic and end-use quality traits were also examined to see if resistance from PI 494182 was associated with undesirable alleles at loci controlling these traits.

2 | MATERIALS AND METHODS

2.1 | Plant material

The soybean accession PI 494182 (Suzuhime) was selected as a donor parent because of its reported resistance against SCN, including to the virulent HG type 2.5.7 (Arelli & Wang, 2008), as well as for its early maturity (MG 0). The second parent was an older early Canadian variety named Costaud, showing extra-early maturity (MG 000), good agronomic qualities, and no known resistance to SCN. A RIL population (named QS13002) resulting from the biparental cross of Costaud × PI 494182 was developed at the Centre de recherche sur les grains (CÉROM) in Saint-Mathieu-de-Beloeil (Canada). The $F_{4:5}$ seed from 372 F_4 plants were harvested individually in 2016. The $F_{4:5}$ lines were grown in individual rows the following year, and 149 randomly selected lines were grown in parallel under greenhouse conditions for the QTL analysis. A single $F_{4:5}$ plant from each of the 149 lines was grown in a greenhouse (Université Laval) to produce $F_{4:6}$ seed on which SCN resistance could be assayed and DNA extracted.

2.2 | DNA isolation and sequencing

For each of 149 RILs, eight $F_{4:6}$ plants were grown, and a 4-mm leaf punch was collected from each. Punches from the different plants of the same RIL were pooled, dried for 4 d using a desiccating agent (W. A. Hammond DRIERITE Co. LTD), and then crushed with metallic beads in a mixer mill (Fisher Scientific, RETSCH MM 400). DNA extraction was performed as per Fulton, Chunwongse, and Tanksley (1995). After the ethanol wash, samples were dried at 4 °C, DNA was suspended in 50 µl of QIAGEN elution buffer, and integrity was verified on a 0.8% agarose gel. DNA quantification was done with a Spark 10-M absorbance microplate reader and the concentrations brought to 10 ng μl^{-1} for each sample. The samples were transferred to the Genomic Analysis Platform at the Institute for Integrative and Systems Biology at Université Laval for GBS library preparation and sequencing. DNA was digested with the *Ape*KI restriction enzyme and prepared for GBS according to the protocol described by Elshire et al. (2011) with minor modifications. Sequencing was done on an Ion Proton System (Thermo Fisher Scientific). Each of the two GBS libraries (76- and 75-plex) was sequenced on a single Ion Proton P1 v3 chip. The entire set of

372 RILs had also been genotyped at the F_4 generation using a similar protocol.

2.3 | Resistance to soybean cyst nematode

To evaluate the resistance of the RILs and parental lines toward SCN, we used a standard greenhouse test quantifying SCN development on each line (Niblack et al., 2002). A SCN population (named IL4) of HG type 0 initially isolated from Illinois, USA, and multiplied several times on the susceptible cultivar Essex, was used as inoculum. The virulence profile of this SCN population was confirmed as HG type 0 (SCN race 3) by inoculating seven indicator lines (Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316) and the susceptible cultivar Essex to evaluate cyst development (Niblack et al., 2002). All the resistance tests took place in a confined greenhouse at the Agriculture and Agri-Food Canada research station in Saint-Jean-sur-Richelieu, Quebec. The parental lines and a total of 149 soybean RILs were tested. Each line was evaluated using six replicates in a randomized complete block design. The commercial resistant line P19T39R2 (Pioneer Seeds) harboring PI 88788-derived resistance was included in each block as a positive check as well as the susceptible line Essex as negative check. The seven indicator lines were also included to validate the HG type at the time of the experiment. The seeds were germinated for 2 d in vermiculite and the seedlings were placed in cone-tainers (Stuewe & Sons) filled with pasteurized sandy soil. At the time of transplantation, each plant received an inoculum of 2,000 SCN eggs applied directly in the planting hole. The cone-tainers were placed in 3-L pails submerged in a heated water table with the temperature set at 27 °C. The cysts were collected 35 d after inoculation by washing the roots on nested 850-µm and 212-µm sieves. Cysts were counted under a microscope for each line and compared with the count on the susceptible line to establish a female index (FI) (Schmitt & Shannon, 1992). All the RILs with a FI < 10% were considered to be resistant to this HG type 0 SCN population:

$$\begin{aligned}\text{FI}\,(\%) &= (\text{number of cysts on tested line}) \\ &\quad / (\text{number of cysts on susceptible check}) \times 100\end{aligned}$$

A subset of the RIL population ($n = 23$) and the parental lines were also confronted to a more virulent SCN population (HG type 2.5.7; SCN race 1) initially isolated from Minnesota using the same methodology. These lines were selected based on their high level of resistance to the HG type 0 SCN population, agronomic potential, and genotype (presence of resistance alleles from PI 494182 at CSqSCN-1/*Rhg1* and CSqSCN-3/*Rhg4*).

2.4 | Phenotyping of agronomic and end-use quality traits

In the summer of 2017, 372 lines (at the $F_{4:5}$ generation) including the 149 lines used for SCN phenotyping and genotyping from the QS13002 population were grown in single-row plots in a modified augmented design (Lin & Poushinsky, 1983, 1985) in Saint-Mathieu-de-Beloeil, Québec, Canada. Four traits were measured: days from sowing to physiological maturity, 100-seed weight, oil concentration, and protein concentration. Oil and protein concentration were measured using near-infrared spectroscopy on a Perten DA7250 instrument. The calibration curve for this analysis was provided by Perten Instruments AB and is based on 2,740 North American soybean samples.

2.5 | Genetic map construction, quantitative trait loci analysis, and *Rhg1* and *Rhg4* confirmation

Sequencing reads obtained from the GBS libraries were processed with the Fast-GBS pipeline (Torkamaneh, Laroche, Bastien, Abed, & Belzile, 2017) to identify SNP variants and small indels among the RIL population using 'Williams 82' (*Glyma.Wm82.a2*) as reference genome. Individual SNP genotypes called with fewer than five reads were replaced with missing data and SNP loci with >80% missing data were removed. Heterozygosity at each SNP locus was determined and loci with >18% heterozygous calls were deemed outliers {calculated on the basis of the interquartile range $[Q_1-k(Q_3-Q_1), Q_3+k(Q_3-Q_1)]$, $k = 3$, as per Tukey (1977)} and were removed. Single nucleotide polymorhisms with a minor allele frequency <30% were also removed on the basis of biased segregation. Imputation of missing genotypes was realized with Beagle version 4.1.0 (Browning & Browning, 2016) using default parameters and redundant markers showing identical segregation patterns were binned. The construction of the genetic map was performed using QTL IciMapping version 4.1.0.0 (Meng, Li, Zhang, & Wang, 2015). Linkage groups were assembled and markers were ordered by anchoring the markers to their physical positions. The distance between markers was estimated using the Kosambi mapping function and linkage groups were split when gaps exceeded 30 cM. Both inclusive composite interval mapping (ICIM) and single-marker analysis (SMA) were performed. The significance level was set to $\alpha = .05$ and the logarithm of the odds (LOD) threshold was calculated by performing a 1,000-permutation test. The scanning interval step to test marker significance was set to 1.0 cM. For the ICIM and SMA analyses, FI (%) values from the SCN assay were used without transformation. The presence of epistatic interactions was verified using the ICIM–EPI function (Meng

et al., 2015). To further assess the impact of each resistance QTL identified in this study, different subgroups of population QS13002, contrasting in their allelic profiles, were tested to identify significant differences in the FI (%) values. For each QTL and RIL, the parental allele was determined. The allele call for each line at each QTL was made only when all the significant markers (including flanking markers) had the same parental genotype, otherwise, the allele was declared not available.

In order to confirm the genotypes obtained by GBS at QTL identified in the *Rhg1* and *Rhg4* regions, all RILs and parental lines were also genotyped with the KASP markers SCN1_1Rhg1 and SCN4-1Rhg4-1 developed by A. Passianotto and I. Rajcan (personal communication, 2020) based on a previous report by Kadam et al. (2016)

2.6 | Statistical analyses

The distribution of values obtained for resistance, end-use quality, and agronomic traits (days to maturity, 100-seed weight, oil and protein concentration) were verified for normality with the Shapiro–Wilk test. Skewness and kurtosis values were also calculated. Student's *t*-test was used to test the differences between means for each trait in the two groups contrasting for their alleles at specific SCN QTL. The threshold *P*-values were adjusted by dividing them by the number of comparisons as per the Bonferroni method. For resistance to SCN, because of non-normality and great disparity in sample sizes, the nonparametric Kruskal–Wallis test was used to identify significant differences in FI values between the contrasting allelic groups. All parameters were calculated using the R program (R Core Team, 2018).

2.7 | Whole-genome sequencing of PI 494182, Costaud, and identification of alleles

Seeds of accession PI 494182 and Costaud were planted in individual two-inch pots containing a single Jiffy peat pellet (Gérard Bourbeau & Fils inc.). The first trifoliate leaf from a single 12-d-old plant was harvested, immediately frozen in liquid nitrogen, and ground using a Qiagen TissueLyser. DNA was extracted from ∼100 mg of ground tissue using the Qiagen Plant DNeasy Mini Kit according to the manufacturer's protocol. DNA was quantified on a NanoDrop spectrophotometer. An Illumina paired-end library was constructed using 500 ng of DNA and the KAPA Hyper Prep Kit (Kapa Biosystems) following the manufacturer's instructions (KR0961 v5.16). DNA library quality was verified on an Agilent Bioanalyzer with a high-sensitivity DNA chip. The library was sequenced using one-sixth of a lane of an Illumina HiSeq XTen sequencer at the McGill University-Génome Québec Innovation Center in Montreal, QC, Canada. Illumina paired-end reads (81 M, 2 × 150 bp) were aligned onto the soybean reference genome (Glyma.Wm82.a2) (Schmutz et al., 2010) and processed using the Fast-WGS pipeline (Torkamaneh, Boyle, & Belzile, 2018). Variants were removed if (a) two or more alternate alleles were seen; (b) observation of the alternate allele was limited to a single strand; (c) an overall read quality (QUAL) score < 20; (d) a mapping quality score < 30; (e) a read depth of < 2; or (f) were suspected of representing false heterozygotes (based on unequal read depth of the two alleles). The variant catalogs of PI 494182 and Costaud were analyzed using the SnpEff program (Cingolani et al., 2012) to determine the predicted effect of SNPs and small indels on the coding sequences. For known SCN-resistance loci, CNVs were predicted using the CNVnator program (Abyzov, Urban, Snyder, & Gerstein, 2011).

3 | RESULTS

3.1 | Resistance to soybean cyst nematode HG type 0

To evaluate SCN resistance in the RIL population (Costaud × PI 494182), we tested the response of $F_{4:6}$ lines to SCN infection in a controlled greenhouse assay. The virulence profile of the IL4 SCN population used in this study was confirmed as HG type 0 (SCN race 3) by measuring its multiplication on the seven indicator lines (Table 1). The FI values of this SCN population on the 149 RILs ranged from 0.8 to 151.1% with a mean value of 63.2%. The distribution of FI values had a bimodal component (Figure 1) and the nonnormality of the distribution was confirmed by the Shapiro–Wilk and symmetry tests ($P = .00003$, $w = 0.942$, skewness = −0.187, kurtosis = −0.385). The susceptible check Essex had an average count of 245 cysts per plant. The FI of the resistant parent PI 494182 was 1.3%, while it was 122.6% for the susceptible parent Costaud. The test identified 19 resistant RILs (FI < 10%). The resistant commercial check (cv. P19T39R2) obtained a FI of 13.2%. Overall, the phenotypic distribution of the RIL population showed a wide range of FI scores, providing sufficient phenotypic variation to undertake QTL analysis.

3.2 | Genetic linkage map

In view of a QTL analysis, we constructed a genetic map based on the segregation of GBS-derived SNP markers. A total of 219 million reads were obtained following the sequencing of two GBS libraries (76- and 75-plex). After filtering of the raw SNP catalog, we retained 7,342 high quality markers. Redundant markers were binned, resulting in a set of 967

TABLE 1 The seven indicator lines and their reaction to soybean cyst nematode (SCN) *Heterodera glycines* (HG) type 0 and HG type 2.5.7

HG type	Soybean line							
	'Essex'	PI 548402	PI 88788	PI 90763	PI 437654	PI 209332	PI 89772	PI 548316
	Female index[a] %							
0	100	0	3	1	0	8	0	8
2.5.7	100	3	27	0	0	22	0	18

[a]Calculated as follows: FI (%) = (number of cysts on tested line)/(number of cysts on susceptible Essex) × 100

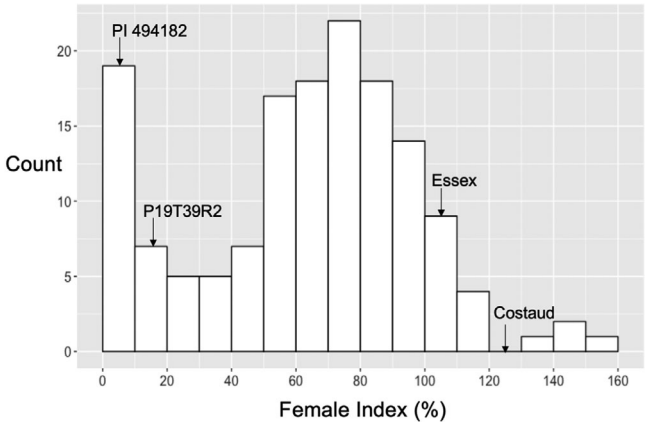

FIGURE 1 Distribution of female index values for 149 $F_{4:6}$ recombinant inbred lines (RIL) generated from the biparental cross 'Costaud' × PI 494182 challenged with soybean cyst nematode (SCN) HG type 0. Relative position of parents, 'Essex' the susceptible check and P19T39R2, a PI 88788-derived resistant line are pointed by black arrows

polymorphic markers showing a distinct segregation pattern. Initially, 20 linkage groups, corresponding to the 20 chromosomes of the soybean genome, were formed by anchoring the markers to their physical positions. Seven gaps located on seven different chromosomes were >30 cM, with the largest one (57 cM) being located on chromosome 20. After splitting linkage groups at these large gaps, we obtained 27 linkage groups (Table 2) spanning a total map length of 1,894 cM. The average distance between markers was 2 cM, and 26 cM was the largest intermarker distance within a linkage group. With close to 1,000 nonredundant informative loci, the resulting map provided excellent coverage of the regions of the genome that were segregating in this cross.

3.3 | Quantitative trait loci associated with resistance to soybean cyst nematode HG type 0

Using the genetic map and FI values for the RIL population, a total of six significant QTL associated with resistance to SCN HG type 0 were found using the ICIM (five QTL) and SMA (four QTL) algorithms, three of which (CSqSCN-1, -2, and -3) were identified by both approaches (Table 3, Figure 2). The QTL CSqSCN-1 had the largest phenotypic

TABLE 2 Properties of the 27 linkage groups obtained for the QS13002 ('Costaud' × PI 494182) population

Chr[a]	No. of markers	Length	Average interval	Chr[a]	No. of markers	Length	Average interval
		cM				cM	
1	48	112.26	2.39	12b	21	54.16	2.71
2	60	138.94	2.36	13a	4	8.11	2.70
3	64	103.32	1.64	13b	33	103.34	3.23
4	61	91.97	1.53	14	45	106.91	2.43
5	50	104.08	2.12	15	42	73.34	1.79
6	51	139.95	2.80	16	34	86.48	2.62
7	44	96.23	2.24	17a	24	29.27	1.27
8	89	134.56	1.53	17b	23	45.79	2.08
9a	36	45.57	1.30	18	45	96.88	2.20
9b	22	24.16	1.15	19a	7	20.43	3.41
10	71	111.49	1.59	19b	30	61.68	2.13
11a	6	4.84	0.97	20a	3	1.52	0.76
11b	20	81.58	4.29	20b	22	47.86	2.28
12a	12	8.79	0.79				

[a]Chr, chromosome. Split chromosomes are annotated a and b.

TABLE 3 Significant quantitative trait loci (QTL) according to inclusive composite interval mapping (ICIM) and single marker analysis (SMA) statistical approaches for resistance to soybean cyst nematode (SCN) *Heterodera glycines* (HG) type 0 in a 149 recombinant inbred lines (RILs) population ('Costaud' × PI 494182) phenotyped using a standard greenhouse test and genotyped using genotyping-by-sequencing (GBS)

Approach	QTL	Chr[a]	Position of flanking markers		Peak marker[c]	Alleles[d]	LOD[e]	PVE[f]	a[g]
			cM	bp[b]				%	
ICIM	CSqSCN-1	18	5.4..9.1	1,543,178..1,867,679	1,748,739	A/G/A	15.23	22.66	−16.74
	CSqSCN-2	20	24.8..26.2	42,805,943..44,119,797	44,119,797	T/C/T	10.26	14.12	−13.07
	CSqSCN-3	08	31.5..34.6	8,267,935..8,771,172	8,267,935	T/C/C	8.25	11.43	−11.7
	CSqSCN-4	07	19.8..22.9	3,432,131..3,985,261	3,625,902	C/G/C	5.52	7.23	−9.31
	CSqSCN-5	06	–	–	–	–	–	–	–
	CSqSCN-6	11	48.6..52.6	32,702,232..32,965,055	32,763,268	A/T/T	3.16	3.93	−6.91
SMA	CSqSCN-1	18	2.9..17.9	685,324..3,442,339	1,748,739	A/G/A	9.67	25.66	−17.81
	CSqSCN-2	20	18.6..26.2	40,613,194..44,119,797	41,978,472	T/C/T	6.00	16.83	−14.19
	CSqSCN-3	08	13.8..37.1	4,620,261..9,500,023	7,780,496	T/C/C	6.26	17.47	−14.46
	CSqSCN-4	07	–	–	–	–	–	–	–
	CSqSCN-5	06	95.8..96.9	15,331,193..16,109,961	15,331,193	G/A/G	4.63	13.24	12.62
	CSqSCN-6	11	–	–	–	–	–	–	–

[a]Chr, chromosome.

[b]Positions according to reference genome Wm82.a2. (Song et al., 2016).

[c]Position for the marker with the highest logarithm of odds (LOD) score at QTL.

[d]Alleles at the peak SNP marker (PI 494182/'Costaud'/'Williams 82').

[e]LOD score for the peak marker. The significance threshold was set to 3.21 by permutation test.

[f]Phenotypic variance explained for peak marker.

[g]Additive effect value of peak marker.

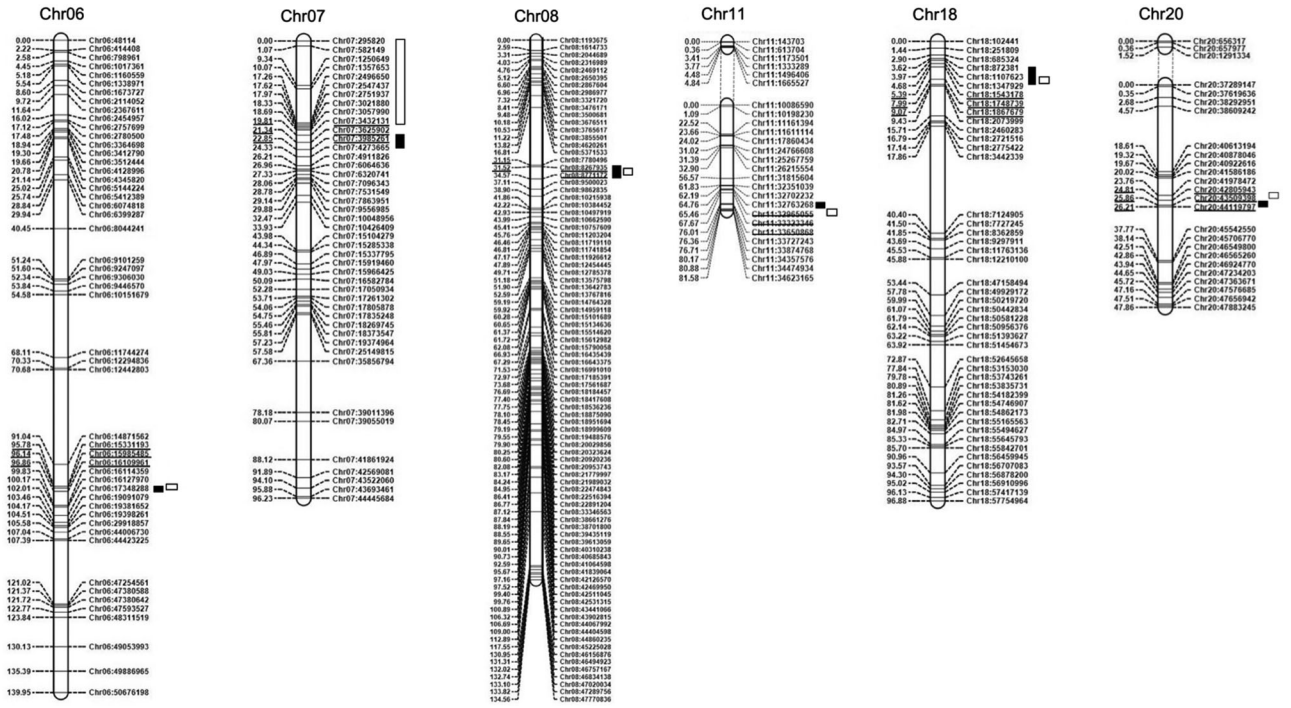

FIGURE 2 Genetic linkage map of population QS13002 and significant quantitative trait loci (QTL) identified for resistance to soybean cyst nematode (SCN) HG type 0. Genetic positions of markers in centimorgans (cM) on the left side of chromosomes (Chr) and physical positions of markers on the right side. Black boxes represents QTL identified in this study, white boxes represent QTL reported in other studies

TABLE 4 Quantitative trait loci (QTL) for resistance to soybean cyst nematode (SCN) *Heterodera glycines* (HG) type 0 identified in population QS13002 ('Costaud' × PI 494182) and overlapping or nearby reported resistance loci

QS13002 QTL	Chromosome	Position	Reported markers or genes	Position	Reference
		Mb		Mb	
CSqSCN-1	18	1.5..1.9	*GmSNAP18* (*Glyma.18G022500*)	1.6..1.7	SoyBase[a]
CSqSCN-2	20	42.8..44.1	Satt148..Satt102	43.4..44.8	Winter et al. (2007)
			Sat_299..Sct_189	43.6..46.7	Wu et al. (2009)
			BARC-060361-16629	42.4..42.4	Jiao et al. (2015)
			Satt330..Satt162	40.2.. 41.4	Winter et al. (2007)
CSqSCN-3	08	8.3..8.8	*GmSHMT08* (*Glyma.08G108900*)	8.4..8.4	SoyBase[a]
CSqSCN-4	07	3.4..4.0	ss107925701..ss107918678	0.2..2.5	Abdelmajid et al. (2014)
CSqSCN-5	06	15.3..16.1	Satt376	15.5..15.5	Ferreira et al. (2011)
CSqSCN-6	11	32.7..33.0	*GmSNAP11* (*Glyma.11G234500*)	33.0..33.0	SoyBase[a]

[a]Positions according to the reference genome Wm82.a2. (Song et al., 2016).

variance explained (PVE) value with 22.7% and an additive effect of −16.7% on the FI value. The locus CSqSCN-2 explained 14.1% of variation with an effect of −13.1%, while the QTL CSqSCN-3 and CSqSCN-4, respectively, explained 11.4 and 7.2% of phenotypic variation with additive effects of −11.7 and −9.3%. Markers flanking CSqSCN-1 and CSqSCN-3 defined genomic intervals that overlapped with two loci known to confer resistance to SCN, *Rhg1* and *Rhg4*, respectively (see Table 4). With the SMA approach, CSqSCN-1 explained 25.7% of phenotypic variation and showed an additive effect of −17.8%. The QTL CSqSCN-2 and CSqSCN-3 were also identified by SMA, with 16.8 and 17.5% of PVE and an additive effect of −14.2 and −14.5%, respectively. The QTL CSqSCN-4, identified only by ICIM, did not show any association with the phenotype using the SMA approach. However, SMA detected CSqSCN-5 on chromosome 06 with a PVE of 13.2% and an additive effect of 12.6%, a region not detected by ICIM. A sixth locus on chromosome 11, CSqSCN-6 (PVE = 3.9%; $a = -6.9\%$) obtained a LOD score of 3.16 by ICIM, just below the significance threshold of 3.21. This region was considered as relevant because of its proximity (~3 kb) to the *GmSNAP11* (*Glyma.11G234500*) gene. All QTL but one (CSqSCN-5) yielded negative additive effects, indicating that the alleles inherited from PI 494182 at these loci contributed to a lower FI, therefore, an increased resistance to SCN. One significant epistatic interaction (LOD = 10.9, PVE = 50.2%) was identified between CSqSCN-1 and CSqSCN-3. Overall, the ICIM method identified five HG type 0 resistance QTL with high resolution on chromosomes 07, 08, 11, 18, and 20, while SMA confirmed three of these QTL and allowed the identification of a sixth resistance QTL located on chromosome 06. All loci identified in this work overlapped or were close (< 1.5 Mb) to previously reported QTL for resistance to SCN (Table 4).

3.4 | Resistance to HG type 2.5.7

Based on the GBS data for the entire Costaud × PI 494182 population (372 RILs), we selected 17 RILs that possessed the PI 494182 alleles at the *Rhg1* and *Rhg4* loci but that had not previously been tested for SCN resistance. To this, we added six lines identified as resistant to HG type 0 in this study and tested this subset of 23 RILs with a HG type 2.5.7 (SCN race 1) population of nematodes known to be more virulent. The virulence profile of this SCN population was confirmed by measuring its reproduction on the seven indicator lines (Table 1). The FI value for the resistant parent PI 494182 was 1.8% while the susceptible parent Costaud obtained a score of 70.9%. The FI values obtained for the 23 RILs ranged from 3.2 to 88.1%. Of the 23 RILs tested for resistance to HG type 2.5.7, 13 proved resistant, confirming the resistance of PI 494182 to a more virulent SCN population.

3.5 | Allelic effects of soybean cyst nematode resistance quantitative trait loci

To confirm the effects of each individual QTL identified in this study, we formed different subgroups of RILs contrasting for their allelic state at the different QTL and compared their average FI values (Figure 3). The subgroups A, B, C, and D were formed to test the contributions of *Rhg1* (CSqSCN-1) and *Rhg4* (CSqSCN-3). Considering the important contribution (50.2% PVE) made by *Rhg1* and *Rhg4*, we chose to include only lines that were fixed for the favorable alleles ($Rhg1^+$ and $Rhg4^+$) at these two loci to test the effect of adding further QTL with lesser effects (CSqSCN-2, CSqSCN-4, CSqSCN-5, and CSqSCN-6; pairs E to L). The greatest difference was observed between group A ($Rhg1^+/Rhg4^+$) and B ($Rhg1^-/Rhg4^-$). This difference was declared highly

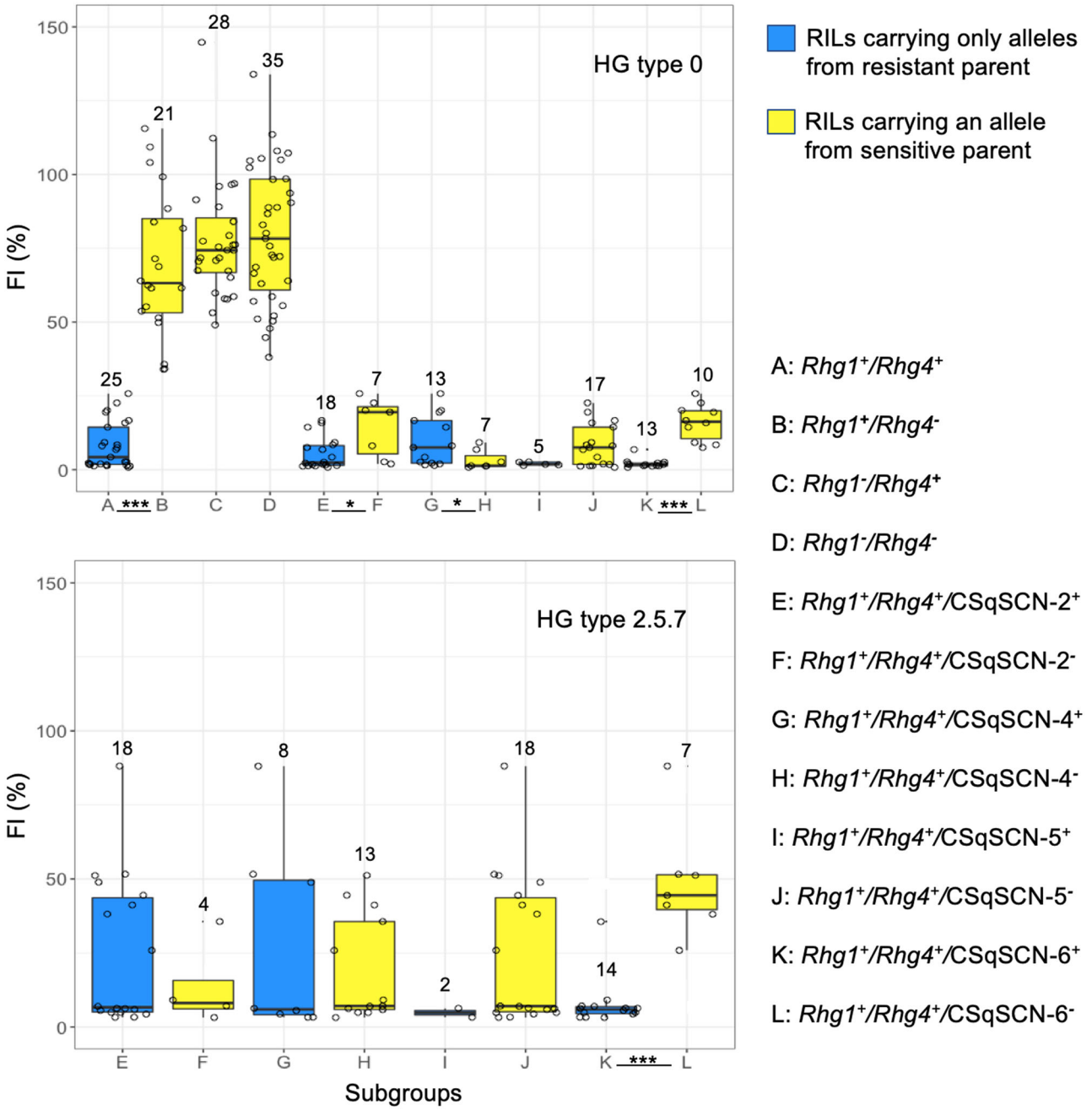

FIGURE 3 Different genotypic subgroups of the recombinant inbred line (RIL) population QS13002 ('Costaud' × PI 494182) and their female index (FI) values for resistance to soybean cyst nematode (SCN) HG type 0 (SCN race 3) and to HG type 2.5.7 (SCN race 1). The RILs were genotyped using genotyping-by-sequencing (GBS) and their resistance evaluated in a greenhouse. The quantitative trait loci (QTL) CSqSCN-1 and CSqSCN-3 were labeled as *Rhg1* and *Rhg4*, respectively. Alleles were marked with a plus (+) sign when the genotype from the parent PI 494182 is present for the subgroup, and marked with a minus (−) sign when the genotype from the parent Costaud is present for the subgroup. Number of lines in each subgroups is indicated over the boxes. Significant differences between mean values of groups according to the Kruskal–Wallis test are represented by an asterisk, significance levels are coded as follow: *, $P < .05$; **, $P < .01$; ***, $P < .001$

significant ($P < .001$) by the Kruskal–Wallis test and the average FI values for resistance to HG type 0 were 8.0 and 79.4%, respectively. This allelic contrast between *Rhg1*$^{+}$/*Rhg4*$^{+}$ and *Rhg1*$^{-}$/*Rhg4*$^{-}$ could not be tested for resistance to HG type 2.5.7 since the 23 RILs selected for this assay all had the *Rhg1*$^{+}$/*Rhg4*$^{+}$ genotype. The contrast between lines forming group E (PI 494182 allele; CSqSCN-2^{+}) and group F (Costaud allele; CSqSCN-2^{-}) was significant only in the test for resistance to HG type 0, where the average FI values were 5.5 and 14.4%, respectively. The G and H pair contrasting for the presence of CSqSCN-4 was also significantly different for resistance to HG type 0 with mean FI values of 9.7

and 3.3%, respectively. The groups I and J (CSqSCN-5 +/−) had mean FI values of 2.0 and 8.3%, respectively, which were not significantly different, while the mean values of FI (%) for resistance to HG type 0 and HG type 2.5.7 for the groups K and L (CSqSCN-6 +/−) were significantly different. Groups K and L resulted in mean FI values of 2.1 and 16.0%, respectively, for resistance to HG type 0 and mean values of 7.7 and 48.7% for resistance to HG type 2.5.7. Among minor QTL in the $Rhg1^{+}/Rhg4^{+}$ background, the resistance locus CSqSCN-6 was the only locus that presented a significant difference between allelic groups against both SCN HG types.

3.6 | Impact of soybean cyst nematode quantitative trait loci on agronomic and end-use quality traits

Even if based on a limited phenotypic assessment, we wanted to explore the possibility of undesirable associations between the QTL conferring SCN resistance and key agronomic traits or end-use quality traits. For this we compared the mean phenotypic values between groups of lines contrasting for their allele at the position of SCN resistance QTL (Figure 4). For 100-seed weight, the PI 494182 allele at the CSqSCN-3 locus was associated with a significant decrease (0.96 g). The oil concentration mean values were significantly different for the contrasting allelic groups for CSqSCN-1 and CSqSCN-3. The alleles from PI 494182 at CSqSCN-1 and CSqSCN-3 were associated with increased oil concentration (0.44 and 0.68%, respectively). A significant difference was observed in protein concentration between lines contrasting at the CSqSCN-3 locus, where the Costaud allele provided a 1.54% increase. Based on these observations, CSqSCN-1 and CSqSCN-3 do carry the potential for some linkage drag.

3.7 | PI 494182 and Costaud nucleotide variants for known soybean cyst nematode resistance genes

Different genes have been confirmed as being involved in resistance to SCN. To determine their alleles in PI 494182, WGS was used and local variants were identified. The average resequencing depth obtained for PI 494182 and Costaud was 32.5× and 20.9×, respectively. Two QTL identified in this study, CSqSCN-1 and CSqSCN-3, matched the positions of the resistance loci known as *Rhg1* and *Rhg4*. An analysis of the SNP variants in the PI 494182 allele of *GmSNAP18* identified three missense variants (D208E, D286Y, and L288I) in addition to a 3-bp in-frame insertion adding a supplemental valine at position 287 (Table 5); these variants correspond to those reported for the *rhg1-a* allele (Liu et al., 2017). For *GmSHMT08*, two missense variants (P200R and N459R) were found in the sequence of the PI 494182 allele, matching the sequence of the *Rhg4-a* allele. Furthermore, the *Rhg1* and *Rhg4* KASP marker results matched the GBS predictions at 99.2%, the discrepency being due an heterozygous genotype not detected by GBS (data not shown). A third QTL (CSqSCN-6) was located very close to another well-documented resistance gene (*GmSNAP11*) (Lakhssassi et al., 2017). In PI 494182, we identified a G → T mutation in the first position of a splice donor site, leading to a premature stop codon resulting from the translation of the seventh intron. Thus, in this case, the allele present in PI 494182 is predicted to produce a truncated protein in amino acid position 239, while the sequence from the reference Williams 82 is 289 amino acids long. The analysis of the sequences from Costaud indicated that no variants reside in the genic sequences of *GmSNAP18*, *GmSNAP11*, and *GmSHMT08* relative to Williams 82, while nucleotide variants observed for PI 494182 indicate that this accession carries alleles differing from the Williams 82 reference genome.

An analysis of copy number was also carried out using the WGS data for PI 494182. Based on the observed depth of coverage, the CNVnator program predicted the repeat of a 31.2-kb segment present in three copies (normalized average read depth = 3.05) located between the positions 1,632,701 and 1,663,900 on chromosome 18. This genomic region overlapped the *Rhg1* locus. For the *Rhg4* locus, a single copy was detected and we found no evidence of duplication in the region comprising *GmSNAP11*. Our nucleotide variant and copy number analysis showed that PI 494182 carries three copies of the *Rhg1-a* allele for the *GmSNAP18* gene, one copy of a truncated allele of the *GmSNAP11* gene, and one copy of the *Rhg4-a* allele at the *GmSHMT08* gene.

4 | DISCUSSION

In this study, 149 RILs derived from a cross between PI 494182 and Costaud were evaluated for resistance to SCN (HG type 0). We found that 12.8% of the RILs were resistant (FI < 10%). In earlier work, Arelli & Wang (2008) examined the segregation of SCN resistance in an F_3 biparental population derived from PI 494182 and 'Skylla'. Using a similar population of SCN (HG type 0) and a greenhouse bioassay, they obtained 12.4% of resistant lines. Though no genetic mapping was done on the PI 494182 × Skylla population, these segregation results are comparable to our own and show reproducibility in the segregation pattern of resistance.

Our QTL analysis identified a total of six genomic regions associated with resistance to SCN. We first examined if the positions of these QTL matched those of previously identified QTL. The locus CSqSCN-1 overlapped the position of the *Rhg1* locus (Concibido et al., 1994), which has been reported in several resistant soybean accessions, including

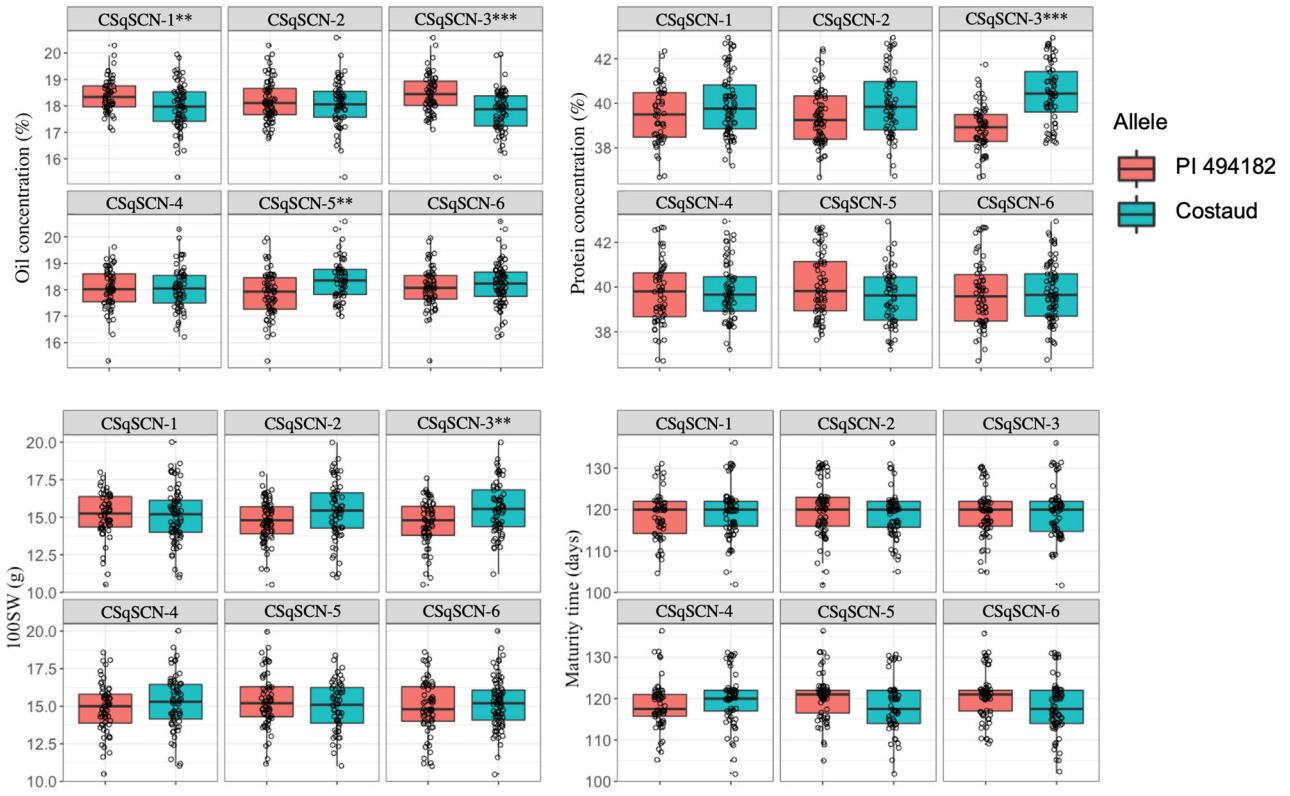

FIGURE 4 Allelic effect of resistance to soybean cyst nematode (SCN) quantitative trait loci (QTL) on agronomic and end-use quality traits. A set of 149 recombinant inbred lines (RILs) ('Costaud' × PI 494182) were genotyped using genotyping-by-sequencing (GBS) and phenotyped for resistance to SCN, 100-seed weight, oil concentration, protein concentration, and days to maturity. The allelic groups comprise RILs with the same parental genotype at SCN resistance QTL. Significant differences between mean values of groups according to the Student's *t*-test are represented by an asterisk, significance levels are coded as follow: *, $P < .05$; **, $P < .01$; ***, $P < .001$

TABLE 5 Nucleotide variants and their corresponding amino acid variants predicted by SnpEff for the soybean accesion PI 494182 and 'Costaud' vs. reported sequences of 'Williams 82' and 'Forrest' for three genes involved in resistance to soybean cyst nematode (SCN)

	Nucleotide variant		Amino acid variant	
Locus, gene	**Position[a]**	**W82[b]/Fo[c]/PI[d]/Co[e]**	**Position[a]**	**W82[b]/Fo[c]/PI[d]/Co[e]**
Rhg1, *GmSNAP18*	Gm18:1,643,660	C/G/G/C	208	D/E/E/D
	Gm18:1,645,403	G/T/T/G	286	D/Y/Y/D
	Gm18:1,645,407	A/AGGT/AGGT/A	287	D/EV/EV/D
	Gm18:1,645,409	C/A/A/C	288	L/I/I/L
Rhg4, *GmSHMT08*	Gm08:8,361,148	C/G/G/C	200	P/R/R/P
	Gm08:8,361,924	A/T/T/A	459	N/Y/Y/N
GmSNAP11	Gm11:32,970,174	G/A/A/G	179	A/T/T/A
	Gm11:32,969,916	G/T/T/G	237..240	DIAA/LGH*/LGH*/DIAA

[a]Position on the 'Williams 82' reference genome Wm82.a2.v1 (Schmutz et al., 2010).
[b]Variant for 'Williams 82'.
[c]Variant for 'Forrest' in gene Glyma.18G02250 (Liu et al., 2017), Glyma.08G108900 (Liu et al., 2012), and Glyma.11G234500 (Lakhssassi et al., 2017).
[d]Variant for PI 494182 according to resequencing data.
[e]Variant for 'Costaud. according to resequencing data.

PI 88788, Peking, PI 90763 (Concibido, Lange, Denny, Orf, & Young, 1997), and PI 437654 (Webb et al., 1995). The resistance locus CSqSCN-2 matched the region reported by Winter, Shelp, Anderson, Welacky, & Rajcan (2007) for resistance to HG type 1.2.5.7 and HG type 0 in a *Glycine soja* accession. The work of Wu et al. (2009) also found a QTL matching the position of CSqSCN-2 in PI 437654. This QTL was reported as conferring resistance to HG types 0, 2.7

and 1.3.5.6.7. More recently, a region close to CSqSCN-2 was reported by Jiao et al. (2015) and was consistently associated with resistance to many SCN types including HG type 1.2.3.4.5.6.7. The resistance locus CSqSCN-3 matched the position of *Rhg4* (Weisemann, Matthews, & Devine, 1992), a resistance QTL that has been associated with resistance to HG types 0 and 2.5.7 in many lines including Peking (Chang et al., 1997) and PI 437654 (Webb et al., 1995). Locus CSqSCN-4 was identified on chromosome 07, and to our knowledge, no QTL conferring resistance to SCN has been reported in this region of the genome. The nearest reported QTL was mapped by Abdelmajid et al. (2014) to a position 1 Mb away from CSqSCN-4. The fifth resistance locus identified in our work, CSqSCN-5, overlapped a QTL that was mapped previously by Ferreira et al. (2011) for resistance to HG type 1.3.5.6.7 in 'Hartwig'. Finally, the locus CSqSCN-6 was very close to *GmSNAP11*, a paralogue of *GmSNAP18*, which has been reported to contribute modestly to SCN resistance in 'Forrest' (Lakhssassi et al., 2017). In addition, we found one epistatic interaction between CSqSCN-1 (*Rhg1*) and CSqSCN-3 (*Rhg4*), as previously reported in many SCN resistant lines (Concibido et al., 2004). Overall, these data indicate that PI 494182 carries useful alleles at many well-known QTL for SCN resistance in addition to a potentially novel QTL (CSqSCN-4 on chromosome 07).

We then asked if the magnitudes of the contributions of these QTL to phenotypic variation in response to SCN infection were comparable to those reported in previous studies. Our analysis showed that the CSqSCN-1 locus (*Rhg1*) was the most impactful in this segregating population. Similarly, in several previous studies focusing on different soybean accessions, the *Rhg1* locus came out as the most significant QTL for resistance against many SCN HG types (Concibido et al., 2004; Kim et al., 2016). As for CSqSCN-2 (second highest PVE), putative corresponding QTL have been reported to be among the most impactful also (Jiao et al., 2015, Winter et al., 2007). The impact of CSqSCN-3 (*Rhg4*) was lower than CSqSCN-1 (*Rhg1*), explaining only half as much of the phenotypic variance in this population. The percentage explained by *Rhg4* (22.7% in ICIM and 25.7% in SMA) falls within the range of values (9–28%) previously reported for this QTL (Concibido et al., 2004). However, the impact of *Rhg4* could have been underestimated by ICIM, which does not consider epistatic interactions. Indeed, in our analysis of allelic classes (Figure 3), *Rhg4* has an impact on resistance only when *Rhg1* is also present. For CSqSCN-4, the PVE was low, showing it has a minor contribution to resistance. Interestingly, the ICIM approach predicted that the allele from PI 494182 at CSqSCN-4 would increase resistance, while the difference in allelic classes for this locus in the $Rhg1^{+}/Rhg4^{+}$ background suggested that it could increase the FI values (i.e. decrease resistance). These results point out that CSqSCN-4 has an effect that remains unclear and it would be important to further explore this effect using a larger sample size for the groups of lines $Rhg1^{+}/Rhg4^{+}/$CSqSCN-$4^{+/-}$. The locus CSqSCN-5 had the lowest PVE in the SMA and this is consistent with Ferreira et al. (2011) who found a low-effect QTL in a position overlapping CSqSCN-5. The additive effect value indicated that the resistance allele came from Costaud, our susceptible parent. The locus CSqSCN-6 had the lowest PVE among all QTL; however, it was the minor QTL with the most significant allelic contrast in the $Rhg1^{+}/Rhg4^{+}$ background. This locus should be favored by breeders. Results from the ICIM model predicted a ~7% FI reduction for the contribution of the allele from PI 494182, similar to the 8% reduction obtained by Lakhssassi et al. (2017) when in presence of the $GmSNAP11^{+}$ allele. Globally, the effect of most QTL found in PI 494182 was confirmed, while the impact of CSqSCN-4 remains to be investigated.

Using WGS data for PI 494182 and Costaud, we analysed the sequences of the well-established resistance genes *GmSNAP18* (*Rhg1*) and *GmSHMT08* (*Rhg4*), both of which are located within CSqSCN-1 and CSqSCN-3, respectively. For *GmSNAP18*, the three SNPs and the 3-bp insertion identified in PI 494182 are identical to those described by Liu et al. (2017) in four resistant lines (Forrest, Peking, PI 437654, and PI 89772); this allele is referred to as *Rhg1-a*. The two SNPs we found in *GmSHMT08* have also been reported by Liu et al. (2012) in the resistant lines Forrest, Peking, PI 90763, PI 437654, and PI 89772, and this allele is designated as *Rhg4-a*. The sequences of Costaud presented no variation relative to the reference Williams 82 at these sites. Data for the genes *GmSNAP18* and *GmSHMT08* indicate that the protein products of these genes in PI 494182 would be identical to those found in resistant lines carrying the Peking-type of resistance. This conclusion is further substantiated by our results with KASP markers for these genes.

In our analysis, CSqSCN-6 was slightly under the significance threshold but because of its proximity to *GmSNAP11*, we verified if the variants associated to resistance in this gene were present in PI 494182. We found two mutations impacting the amino acid sequence of the gene including one inducing the loss of a splice donor site predicted to lead to a truncated protein. These mutations were initially described by Matsye et al. (2012) for a truncated protein product in Peking. Later, the sequence of a 239-amino-acid-truncated protein from *GmSNAP11* was confirmed in Forrest (Lakhssassi et al., 2017); this allele was referred as *GmSNAP11-T1*. Our data for PI 494182 suggest a protein of the same length. The sequence of Costaud was identical to the susceptible reference Williams 82 for this gene. From these results, we concluded that PI 494182 carries the *GmSNAP11-T1* allele.

As resistance to SCN is known to be conditioned not only by the alleles at *Rhg1* and *Rhg4* but also by the number of copies of these genes, we compared the average read depth for these loci with the average local coverage using the

CNVnator program. *Rhg1* was estimated to be present in three copies in PI 494182, while *Rhg4* was present in a single copy. Based on the work of Patil et al. (2019), two to four copies of the *Rhg1-a* locus are sufficient to obtain resistance when in presence of at least one copy of *Rhg4-a*. However, transcriptional analysis showed that a lower copy number of these genes was correlated with reduced expression of *Rhg1* and *Rhg4* and with lower resistance. This could explain the relatively low PVE value obtained for CSqSCN-3 in the QTL analysis. Taken together, the alleles present (*Rhg1-a* and *Rhg4-a*), their copy number (3 and 1), and the epistatic interactions observed between these two loci indicate that PI 494182 possesses all the hallmarks of the Peking-type of resistance.

We also verified if the favorable alleles from PI 494182 could also confer resistance to a more virulent nematode population. For this, we used a HG type 2.5.7 SCN population and inoculated the parental lines and a subset of 23 RILs that were all homozygous for the PI 494182 alleles at the *Rhg1* and *Rhg4* loci. We obtained a FI value of 1.8% for PI 494182. The mean FI value obtained for the RILs was of 23.9%, and 56% of the tested RILs exhibited resistance to this SCN pathotype. This confirmed that PI 494182 can also confer resistance to HG type 2.5.7 as reported previously by Arelli & Wang (2008). Such a resistance is currently desirable since populations of HG type 0 SCN showed their ability to shift in virulence and overcome resistance provided by PI 88788-derived resistant cultivars (McCarville, Marett, Mullaney, Gebhart, & Tylka, 2017).

To evaluate if the use of PI 494182 as a source of resistance would not drag undesirable alleles, impacting key agronomic and end-use quality traits, we compared the mean values of these traits between contrasting allelic groups at the six SCN QTL. The CSqSCN-3 locus showed a significant difference for 100-seed weight values, with lines carrying the PI 494182 allele decreasing the weight by 0.96 g. For the contrasting groups at the CSqSCN-1 locus a significant difference in oil concentration values was observed, where the PI 494182 allele increased the oil concentration by 0.44%. The CSqSCN-3 locus also significantly impacted oil concentration and protein concentration. For oil concentration, lines with the PI 494182 allele at CSqSCN-3 averaged 0.69% more oil, while the same lines suffered an average decrease of 1.54% in protein concentration. Such an inversely correlated relation between oil and protein concentration has been well documented (Lark, Orf, & Mansur, 1994; Qiu, Arelli, & Sleper, 1999). Recently, a SNP linked to *Rhg4* also showed a significant association with total oil concentration (Zhang et al., 2018). However, this marker was not associated with protein concentration. Our results also show that the use of *Rgh4* can be associated with increased oil concentration but it can also lead to lower protein concentration. This association between SCN resistance and important agronomic and end-use quality traits needs to be considered to maintain agronomic performances. Therefore, it is recommended to select appropriate parents and monitor recombinants in the *Rhg4* region when using PI 494182 as a source of resistance. The results from our relatively small population indicate that it is clearly possible to select lines carrying resistance and exhibiting adequate protein levels.

To the best of our knowledge, PI 494182 is the earliest-maturing accession reported to confer SCN resistance to be characterized by QTL mapping. Our results showed that resistance to HG type 0 SCN in PI 494182 is conferred in part by a Peking-type resistance at the *Rhg1* and *Rhg4* loci with additional QTL. Populations of HG type 0 SCN showed their ability to shift in virulence and overcome resistance provided by PI 88788 derived resistant cultivars when exposed to the resistance source consistently (McCarville et al., 2017). Furthermore, a survey conducted from 2006 to 2008 demonstrated that the HG type 2.5.7 SCN population was prevalent when considering the total populations of Tennessee, Illinois, Indiana, and Ontario (Faghihi, Donald, Noel, Welacky, & Ferris, 2010). PI 88788-derived lines offer no resistance to HG type 2.5.7, while the Peking-type lines confer full resistance to this SCN type (Arelli, Sleper, Yue, & Wilcox, 2000). Though recent surveys demonstrated that Peking-type resistant lines yielded more under SCN pressure, the use of this type of resistance is still marginal (Tylka & Mullaney, 2019) compared with PI 88788 derived lines. As SCN populations keep spreading (Tylka & Marett, 2017) and shifting in virulence (Gardner, Heinz, Wang, & Mitchum, 2017; Lee, Kumar, Diers, & Hudson, 2015)), the development of resistant cultivars using the Peking-type resistance remains necessary. Though we showed some negative impact on protein concentration of introgressing the PI 494182 allele at the *Rhg4* locus, we believe that this is not overly detrimental to protein concentration, and overall, this early source of resistance exhibits good potential for breeding resistant cultivars with early maturity. This study provides information about a combination of loci (and associated markers) underlying SCN resistance in PI 494182 including a potentially new locus. These markers will greatly facilitate the use of PI 494182 as a source of resistance in the development of SCN resistant varieties particularly for early maturity regions.

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DATA AVAILABILITY

The genomic datasets generated and analysed during the current study are available in the NCBI's Sequence Reads Archive repository under the Bio project identifier PRJNA579149.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

LO conceived the project; LO designed the genotyping experiments and field experiments and BM designed and supervised the SCN experiments. FB supervised the genotyping experiments; VTBA designed and performed the experiments; VTBA analysed the data with the help of MJ; DT provided whole-genome sequencing data and analysis; VTBA drafted the manuscript with contributions from all the authors; all authors approved the final version of the manuscript.

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REFERENCES

Abdelmajid, K., Ramos, L., Hyten, D., Bond, J., Arelli, P., … Meksem, K. (2014). Quantitative trait loci (QTL) that underlie SCN resistance in soybean [*Glycine max* (L.) Merr.] PI438489B by 'Hamilton' recombinant inbred line (RIL) population. *Agronomy & Horticulture – Faculty Publications*, *809*. Retrieved from http://digitalcommons.unl.edu/agronomyfacpub/809

Abyzov, A., Urban, A. E., Snyder, M., & Gerstein, M. (2011). CNVnator: An approach to discover, genotype, and characterize typical and atypical CNVs from family and population genome sequencing. *Genome Research*, *21*, 974–984. https://doi.org/10.1101/gr.114876.110

Arelli, Prakash R., Sleper, David A., Yue, Pin, & Wilcox, John A. (2000). Soybean reaction to races 1 and 2 of *Heterodera glycines*. *Crop Science*, *40*(3), 824–826. https://doi.org/10.2135/cropsci2000.403824x

Arelli, P. R., & Wang, D. (2008). Inheritance of cyst nematode resistance in a new genetic source, *Glycine max* PI 494182. *Journal of Crop Science and Biotechnology*, *11*, 83–90.

Bayless, Adam M., Smith, John M., Song, Junqi, McMinn, Patrick H., Teillet, Alice, August, Benjamin K., & Bent, Andrew F. (2016). Disease resistance through impairment of α-SNAP–NSF interaction and vesicular trafficking by soybean *Rhg1*. *Proceedings of the National Academy of Sciences*, *113*, E7375–E7382. https://doi.org/10.1073/pnas.1610150113

Browning, Brianâ L., & Browning, Sharonâ R. (2016). Genotype imputation with millions of reference samples. *American Journal of Human Genetics*, *98*, 116–126. https://doi.org/10.1016/j.ajhg.2015.11.020

Chang, S. J. C., Doubler, T. W., Kilo, V. Y., Abuâ-Thredeih, J., Prabhu, R., Freire, V., … Lightfoot, D. A. (1997). Association of loci underlying field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN) race 3. *Crop Science*, *37*, 965–971. https://doi.org/10.2135/cropsci1997.0011183X003700030044x

Cingolani, Pablo, Platts, Adrian, Wang, Le Lily, Coon, Melissa, Nguyen, Tung, Wang, Luan, … Ruden, Douglas M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly*, *6*, 80–92. https://doi.org/10.4161/fly.19695

Concibido, V. C., Denny, R. L., Boutin, S. R., Hautea, R., Orf, J. H., & Young, N. D. (1994). DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). *Crop Science*, *34*, 240–246. https://doi.org/10.2135/cropsci1994.0011183X003400010044x

Concibido, Vergel C., Diers, Brian W., & Arelli, Prakash R. (2004). A decade of QTL mapping for cyst nematode resistance in soybean. *Crop Science*, *44*, 1121–1131. https://doi.org/10.2135/cropsci2004.1121

Concibido, Vergel C., Lange, Douglas A., Denny, Roxanne L., Orf, James H., & Young, Nevin D. (1997). Genome mapping of soybean cyst nematode resistance genes in 'Peking', PI 90763, and PI 88788 using DNA markers. *Crop Science*, *37*, 258–264. https://doi.org/10.2135/cropsci1997.0011183X003700010046x

Cook, David E., Bayless, Adam M., Wang, Kai, Guo, Xiaoli, Song, Qijian, Jiang, Jiming, & Bent, Andrew F. (2014). Distinct copy number, coding sequence, and locus methylation patterns underlie *Rhg1*-mediated soybean resistance to soybean cyst nematode. *Plant Physiology*, *165*, 630–647. https://doi.org/10.1104/pp.114.235952

Cook, D. E., Lee, T. G., Guo, X., Melito, S., Wang, K., Bayless, A. M., … Bent, A. F. (2012). Copy number variation of multiple genes at *Rhg1* mediates nematode resistance in soybean. *Science*, *338*, 1206–1209. https://doi.org/10.1126/science.1228746

Delgado, Christopher L. (2003). Rising consumption of meat and milk in developing countries has created a new food revolution. *Journal of Nutrition*, *133*, 3907S–3910S. https://doi.org/10.1093/jn/133.11.3907S

Elshire, Robert J., Glaubitz, Jeffrey C., Sun, Qi, Poland, Jesse A., Kawamoto, Ken, Buckler, Edward S., & Mitchell, Sharon E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, *6*, e19379. https://doi.org/10.1371/journal.pone.0019379

Faghihi, Jamal, Donald, Patricia A., Noel, Gregory, Welacky, Tom W., & Ferris, Virginia R. (2010). Soybean resistance to field populations of *Heterodera glycines* in selected geographic areas. *Plant Health Progress*, *11*, 1–19. https://doi.org/10.1094/PHP-2010-0426-01-RS

Ferreira, Marcia Flores Da Silva, Cervigni, Gerardo Domingo Lucio, Ferreira, Adésio, Schuster, Ivan, Santana, Fernanda Abreu, Pereira, Waldir Dias, … Moreira, Maurilio Alves (2011). QTLs for resistance to soybean cyst nematode, races 3, 9, and 14 in cultivar Hartwig.

Pesquisa Agropecuária Brasileira, *46*, 420–428. https://doi.org/10.1590/S0100-204X2011000400012

Fulton, Theresa M., Chunwongse, Julapark, & Tanksley, Steven D. (1995). Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Molecular Biology Reporter*, *13*, 207–209. https://doi.org/10.1007/BF02670897

Gardner, Michael, Heinz, Robert, Wang, Jianying, & Mitchum, Melissa G. (2017). Genetics and adaptation of soybean cyst nematode to broad spectrum soybean resistance. *G3: Genes, Genomes, Genetics*, *7*, 835–841. https://doi.org/10.1534/g3.116.035964.

Gendron St-Marseille, A. F., Bourgeois, G., Brodeur, Jacques, & Mimee, Benjamin (2019). Simulating the impacts of climate change on soybean cyst nematode and the distribution of soybean. *Agricultural and Forest Meteorology*, *264*, 178–187. https://doi.org/10.1016/j.agrformet.2018.10.008

Gendron St-Marseille, A. F., Lord, Etienne, Véronneau, Pierre-Yves, Brodeur, J., & Mimee, B. (2018). Genome scans reveal homogenization and local adaptations in populations of the soybean cyst nematode. *Frontiers in Plant Science*, *9*, 987. https://doi.org/10.3389/fpls.2018.00987

Henchion, Maeve, Hayes, Maria, Mullen, Anne, Fenelon, Mark, & Tiwari, Brijesh (2017). Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. *Foods*, *6*, 53. https://doi.org/10.3390/foods6070053

Howland, Amanda, Monnig, Nick, Mathesius, Jeff, Nathan, Manjula, & Mitchum, Melissa G. (2018). Survey of *Heterodera glycines* population densities and virulence phenotypes during 2015–2016 in Missouri. *Plant Disease*, *102*, 2407–2410. https://doi.org/10.1094/PDIS-04-18-0650-SR

Hunter, Mitchell C., Smith, Richard G., Schipanski, Meagan E., Atwood, Lesley W., & Mortensen, David A. (2017). Agriculture in 2050: Recalibrating targets for sustainable intensification. *Bioscience*, *67*, 386–391. https://doi.org/10.1093/biosci/bix010

Jiao, Yongqing, Vuong, Tri D., Liu, Yan, Meinhardt, Clinton, Liu, Yang, Joshi, Trupti, … Nguyen, Henry T. (2015). Identification and evaluation of quantitative trait loci underlying resistance to multiple HG types of soybean cyst nematode in soybean PI 437655. *Theoretical and Applied Genetics*, *128*, 15–23. https://doi.org/10.1007/s00122-014-2409-5

Kadam, Suhas, Vuong, Tri D., Qiu, Dan, Meinhardt, Clinton G., Song, Li, Deshmukh, Rupesh, … Nguyen, Henry T. (2016). Genomic-assisted phylogenetic analysis and marker development for next generation soybean cyst nematode resistance breeding. *Plant Science*, *242*, 342–350 https://doi.org/10.1016/j.plantsci.2015.08.015

Kaldy, M. S. (1972). Protein yield of various crops as related to protein value. *Economic Botany*, *26*, 142–144. https://doi.org/10.1007/BF02860775

Kandoth, Pramod Kaitheri, Ithal, Nagabhushana, Recknor, Justin, Maier, Tom, Nettleton, Dan, Baum, Thomas J., & Mitchum, Melissa G. (2011). The soybean *Rhg1* locus for resistance to the soybean cyst nematode *Heterodera glycines* regulates expression of a large number of stress- and defense-related genes in degenerating feeding cells. *Plant Physiology*, *155*, 1960–1975. https://doi.org/10.1104/pp.110.167536

Kandoth, Pramod K., Liu, Shiming, Prenger, Elizabeth, Ludwig, Andrew, Lakhssassi, Naoufal, Heinz, Robert, … Mitchum, Melissa G. (2017). Systematic mutagenesis of serine hydroxymethyltransferase reveals an essential role in nematode resistance. *Plant Physiology*, *175*, 1370–1380. https://doi.org/10.1104/pp.17.00553

Kim, Ki-Seung, Vuong, Tri D., Qiu, Dan, Robbins, Robert T., Grover Shannon, J., Li, Zenglu, & Nguyen, Henry T. (2016). Advancements in breeding, genetics, and genomics for resistance to three nematode species in soybean. *Theoretical and Applied Genetics*, *129*, 2295–2311. https://doi.org/10.1007/s00122-016-2816-x

Koenning, Stephen R., & Wrather, J. Allen (2010). Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. *Plant Health Progress*, *11*, 5. https://doi.org/10.1094/PHP-2010-1122-01-RS

Lakhssassi, Naoufal, Liu, Shiming, Bekal, Sadia, Zhou, Zhou, Colantonio, Vincent, Lambert, Kris, … Meksem, Khalid (2017). Characterization of the Soluble NSF Attachment Protein gene family identifies two members involved in additive resistance to a plant pathogen. *Scientific Reports*, *7*, 45226. https://doi.org/10.1038/srep45226

Lark, K. G., Orf, J., & Mansur, L. M. (1994). Epistatic expression of quantitative trait loci (QTL) in soybean [*Glycine max* (L.) Merr.] determined by QTL association with RFLP alleles. *Theoretical and Applied Genetics*, *88-88*, 486–489. https://doi.org/10.1007/BF00223665

Lee, Tong Geon, Diers, Brian W., & Hudson, Matthew E. (2016). An efficient method for measuring copy number variation applied to improvement of nematode resistance in soybean. *Plant Journal*, *88*, 143–153. https://doi.org/10.1111/tpj.13240

Lee, Tong Geon, Kumar, Indrajit, Diers, Brian W., & Hudson, Matthew E. (2015). Evolution and selection of *Rhg1*, a copy-number variant nematode-resistance locus. *Molecular Ecology*, *24*, 1774–1791. https://doi.org/10.1111/mec.13138

Lin, C. S., & Poushinsky, G. (1983). A modified augmented design for an early stage of plant selection involving a large number of test lines without replication. *Biometrics*, *39*, 553–561. https://doi.org/10.2307/2531083

Lin, Chuang-Sheng, & Poushinsky, Greg (1985). A modified augmented design (type 2) for rectangular plots. *Canadian Journal of Plant Science*, *65*, 743–749. https://doi.org/10.4141/cjps85-094

Liu, Shiming, Kandoth, Pramod K., Lakhssassi, Naoufal, Kang, Jingwen, Colantonio, Vincent, Heinz, Robert, … Meksem, Khalid (2017). The soybean *GmSNAP18* gene underlies two types of resistance to soybean cyst nematode. *Nature communications*, *8*, 14822. https://doi.org/10.1038/ncomms14822

Liu, Shiming, Kandoth, Pramod K., Warren, Samantha D., Yeckel, Greg, Heinz, Robert, Alden, John, … Meksem, Khalid (2012). A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. *Nature*, *492*, 256–260. https://doi.org/10.1038/nature11651

Masonbrink, Rick, Maier, Tom R., Muppirala, Usha, Seetharam, Arun S., Lord, Etienne, Juvale, Parijat S., … Baum, Thomas J. (2019). The genome of the soybean cyst nematode (*Heterodera glycines*) reveals complex patterns of duplications involved in the evolution of parasitism genes. *BMC Genomics*, *20*, 119. https://doi.org/10.1186/s12864-019-5485-8

Matsye, Prachi D., Lawrence, Gary W., Youssef, Reham M., Kim, Kyung-Hwan, Lawrence, Katheryn S., Matthews, Benjamin F., & Klink, Vincent P. (2012). The expression of a naturally occurring, truncated allele of an α-SNAP gene suppresses plant parasitic nematode infection. *Plant Molecular Biology*, *80*, 131–155. https://doi.org/10.1007/s11103-012-9932-z

McCarville, Michael T., Marett, Christopher C., Mullaney, Mark P., Gebhart, Gregory D., & Tylka, Gregory L. (2017). Increase in soybean cyst nematode virulence and reproduction on resistant soybean varieties in Iowa from 2001 to 2015 and the effects on soybean yields. *Plant Health Progress*, *18*, 146–155. https://doi.org/10.1094/PHP-RS-16-0062

Meng, Lei, Li, Huihui, Zhang, Luyan, & Wang, Jiankang (2015). QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop Journal*, *3*, 269–283. https://doi.org/10.1016/j.cj.2015.01.001

Mimee, Benjamin, Gagnon, A. È., Colton-Gagnon, Katia, & Tremblay, Éléonore (2016). Soybean cyst nematode (*Heterodera glycines*): An overview of the situation in Quebec (2013-2015). (In French with English abstract) *Phytoprotec*, *96*, 33–42. https://doi.org/10.7202/1038941ar

Mimee, B., Peng, H., Popovic, V., Yu, Q., Duceppe, M. O., Tétreault, M.P., & Belair, G. (2013). First report of soybean cyst nematode (*Heterodera glycines* Ichinohe) on soybean in the province of Quebec, Canada. *Plant Disease*, *98*, 429–429. https://doi.org/10.1094/PDIS-07-13-0782-PDN

Niblack, T. L., Arelli, P. R., Noel, G. R., Opperman, C. H., Orf, J. H., … Tylka, G. L. (2002). A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *Journal of Nematology*, *34*, 279–288.

Patil, Gunvant B., Lakhssassi, Naoufal, Wan, Jinrong, Song, Li, Zhou, Zhou, Klepadlo, Mariola, … Nguyen, Henry T. (2019). Whole genome re-sequencing reveals the impact of the interaction of copy number variants of the *rhg-1* and *Rhg4* genes on broad-based resistance to soybean cyst nematode. *Plant Biotechnology Journal*, *17*, 1595–1611, https://doi.org/10.1111/pbi.13086

Qiu, B. X., Arelli, P. R., & Sleper, D. A. (1999). RFLP markers associated with soybean cyst nematode resistance and seed composition in a 'Peking' × 'Essex' population. *Theoretical and Applied Genetics*, *98*, 356–364. https://doi.org/10.1007/s001220051080

R core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. Retrieved from http://www.R-project.org/

Riggs, R. D. (1977). Worldwide distribution of soybean-cyst nematode and its economic importance. *Journal of Nematology*, *9*, 34–39.

Schmitt, D. P., & Shannon, G. (1992). Differentiating soybean responses to *Heterodera Glycines* races. *Crop Science*, *32*, 275–277. https://doi.org/10.2135/cropsci1992.0011183X003200010056x

Schmutz, Jeremy, Cannon, Steven B., Schlueter, Jessica, Ma, Jianxin, Mitros, Therese, Nelson, William, … Jackson, Scott A. (2010). Genome sequence of the palaeopolyploid soybean. *Nature*, *463*, 178. https://doi.org/10.1038/nature08670bib>

Song, Q., Jenkins, J., Jia, G., Hyten, D. L., Pantalone, V., Jackson, S. A., … Cregan, P. B. (2016). Construction of high resolution genetic linkage maps to improve the soybean genome sequence assembly Glyma1.01. *BMC Genomics*, *17*, 33. https://doi.org/10.1186/s12864-015-2344-0

SoyBase. (2020). SoyBase Database. Retrieved from https://www.soybase.org/search/index.php?searchterm=SCN&list=bi_parental_qtl_listview.

SoyStats. (2020). 2018 Soy Highlights. Retrieved from http://soystats.com/2018-highlights

Torkamaneh, Davoud, Boyle, Brian, & Belzile, François. (2018). Efficient genome-wide genotyping strategies and data integration in crop plants. *Theoretical and Applied Genetics*, *131*, 499–511. https://doi.org/10.1007/s00122-018-3056-z

Torkamaneh, Davoud, Laroche, Jérôme, Bastien, Maxime, Abed, Amina, & Belzile, François. (2017). Fast-GBS: A new pipeline for the efficient and highly accurate calling of SNPs from genotyping-by-sequencing data. *BMC Bioinformatics*, *18*, 5. https://doi.org/10.1186/s12859-016-1431-9

Tran, Dung T., Steketee, Clinton J., Boehm, Jeffrey D., Noe, James, & Li, Zenglu (2019). Genome-wide association analysis pinpoints additional major genomic regions conferring resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). *Frontiers in Plant Science*, *10*, 401–402. https://doi.org/10.3389/fpls.2019.00401

Tukey, J. W. (1977). *Exploratory data analysis*. Boston, MA: Addison–Wesley Publishing Company.

Tylka, Gregory L., & Marett, Christopher C. (2017). Known distribution of the soybean cyst nematode, *Heterodera glycines*, in the United States and Canada, 1954 to 2017. *Plant Health Progress*, *18*, 167–168. https://doi.org/10.1094/PHP-05-17-0031-BR

Tylka, G. L., & Mullaney, M. P. (2019). Soybean cyst nematode-resistant soybean varieties for Iowa. Retrieved from https://store.extension.iastate.edu/product/5154

Webb, D. M., Baltazar, B. M., Rao-Arelli, A. P., Schupp, J., Clayton, K., Keim, P., & Beavis, W. D. (1995). Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean PI 437.654. *Theoretical and Applied Genetics*, *91*, 574–581. https://doi.org/10.1007/BF00223282

Weisemann, J. M., Matthews, B. F., & Devine, T. E. (1992). Molecular markers located proximal to the soybean cyst nematode resistance gene, *Rhg4*. *Theoretical and Applied Genetics*, *85*, 136–138. https://doi.org/10.1007/BF00222850

Winter, Shawn M. J., Rajcan, Istvan, & Shelp, Barry J. (2006). Soybean cyst nematode: Challenges and opportunities. *Canadian Journal of Plant Science*, *86*, 25–32. https://doi.org/10.4141/P05-072

Winter, Shawn M. J., Shelp, Barry J., Anderson, Terry R., Welacky, Tom W., & Rajcan, Istvan (2007). QTL associated with horizontal resistance to soybean cyst nematode in *Glycine soja* PI464925B. *Theoretical and Applied Genetics*, *114*, 461–472. https://doi.org/10.1007/s00122-006-0446-4

Wu, Xiaolei, Blake, Sean, Sleper, David A., Shannon, J. Grover, Cregan, Perry, & Nguyen, Henry T. (2009). QTL, additive and epistatic effects for SCN resistance in PI 437654. *Theoretical and Applied Genetics*, *118*, 1093–1105. https://doi.org/10.1007/s00122-009-0965-x

Yan, Guiping, & Baidoo, Richard (2018). Current research status of *Heterodera glycines* resistance and its implication on soybean breeding. *Engineering*, *4*, 534–541. https://doi.org/10.1016/j.eng.2018.07.009

Young, Lawrence D. (1995). Soybean germplasm resistant to races 3, 5, or 14 of soybean cyst nematode. *Crop Science*, *35*, 895–896. https://doi.org/10.2135/cropsci1995.0011183X003500030044x

Zhang, J., Wang, X., Lu, Y., Bhusal, S. J., Song, Q., Cregan, P. B., … Jiang, G. L. (2018). Genome-wide scan for seed composition provides insights into soybean quality improvement and the impacts of domestication and breeding. *Molecular Plant*, *11*, 460–472. https://doi.org/10.1016/j.molp.2017.12.016

SUPPORTING INFORMATION

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