Trends in Plant Science

CellPress

Spotlight

Fortifying nematode resistance through susceptibility gene inactivation

Huan Wang,[1] Ziyue Li,[1] Daowen Wang,[1,*] and Zheng Qing Fu[2,*]

The predominant genetic defense mechanism against soybean cyst nematode (SCN) in 95% of the North America market is under threat by virulent SCN populations. Usovsky *et al*. identified *GmSNAP02* as an SCN susceptibility gene through fine-mapping of unique bi-parental populations. Loss-of-function of *GmSNAP02* confers enhanced resistance to more virulent SCN.

SCN stands as the most devastating pest affecting soybean crops globally, leading to annual losses totaling $1.5 billion in the USA alone [1]. Harnessing natural plant resistance emerges as the most effective method to manage plant-parasitic nematodes. Native SCN resistance identified through screening of soybean germplasm has been primarily used to manage SCN (Figure 1A) [2]. For example, the *rhg1-b* resistance allele found in PI 88788-type plants is attributed to the presence of nine to ten tandem repeats of a 31-kb segment containing three distinct genes that encode an amino acid transporter, an α-soluble NSF attachment protein (α-SNAP; also known as *GmSNAP18*) in vesicle trafficking, and a WI12 (wound-inducible domain) protein implicated in resistance [3,4]. However, most SCN-susceptible soybean germplasm usually only contains one copy of the *Rhg1* genome segment. Additionally, the *rhg1-a* resistance allele from Peking is characterized by three copies and a slightly altered α-SNAP protein compared with the one found in *rhg1-b* [5]. The *Rhg2* (*GmSNAP11*) locus harbors a nonsense splice site mutation that causes mRNA to be improperly spliced. This results in intron retention and translational termination, ultimately leading to the truncation of the protein and an increase in resistance against SCN [6]. *Rhg4* encodes a serine hydroxymethyltransferase (SHMT) that differs by two amino acids in the pocket binding site of the enzyme from the susceptible isoform. This polymorphism results in a gain-of-function in resistance to SCN [7,8]. The epistatic interaction between *rhg1-a* and *Rhg4* confers resistance to *Heterodera glycines* (HG) type 0/7 (Race 3) populations, while the interplay between *rhg1-a* and *rhg2* provides protection against HG type 2.5.7 (Races 1 and 5) as well as HG type 1.2.5.7 (Race 2) [9].

Importantly, *rhg1-b* has been found to provide excellent resistance to many SCN populations without the need for other *rhg* loci [5]. For a few decades, the *rhg1-b* haplotype has served as a remarkable success story in disease resistance breeding. In fact, the predominant genetic mechanism conferring SCN resistance in 95% of the North American market relies on *GmSNAP18-b or rhg1-b*, originating from PI 88788 (Figure 1A). Regrettably, the efficacy of *GmSNAP18-b* has diminished due to the emergence of more virulent SCN populations. Compelling evidence has demonstrated that SCN has the capability to overcome PI 88788-based resistance, Peking-based resistance, or both (sequentially) [5]. Consequently, there is an urgent need for scientists to identify additional sources of resistance.

Recently, Usovsky *et al*. unveiled *GmSNAP02* as a novel susceptibility gene to SCN through meticulous fine-mapping of a quantitative trait locus (QTL) on chromosome 2 linked to heightened resistance against a virulent population of SCN [2]. Intriguingly, the loss-of-function of *GmSNAP02* was found to confer resistance against more virulent SCN.

Usovsky *et al*. employed distinct bi-parental populations in their study, with the aim of mapping the gene that explains the observed difference in resistance to HG type 1.2.5.7 (Race 2) between PI 90763 [~21% female index (FI)] and Peking (0% FI) [2]. A HG type test evaluates the reproductive capacity (FI) of SCN field populations using a specific array of soybean indicator varieties or lines (Figure 1B) [10]. For instance, HG type 1 means the SCN population has an FI ≥ 10% on PI 548401 (Peking), while HG type 7 means the SCN population has an FI ≥ 10% on PI 548316 (Cloud). Notably, the highly virulent HG type 1.2.5.7 SCN population is capable of reproducing on several soybean indicator lines, including PI 548402, PI 88788, PI 209332, and PI 548316. Using MapQTL and RQTL, the authors successfully pinpointed a significant QTL located on chromosome 2 (QTL02) in the population PI 90763 × Peking (Figure 1C). The authors then fine-mapped QTL02 to a 218-kb region housing 34 candidate genes, including the *GmSNAP02* gene encoding an *α-SNAP* protein. The *GmSNAP02* gene is a paralog of *GmSNAP18* at *Rhg1* and *GmSNAP11* at *Rhg2*, both of which contribute significantly to resistance against SCN. Whole-genome sequencing unveiled that two haplotypes of *GmSNAP02*, known for their resistance to SCN, exhibit either a deletion or insertion within this gene. A DNA fragment of the anticipated size (1095 bp) was successfully amplified from the genomic DNA of both Peking and PI 437654. However, a significantly larger fragment of approximately 7 kb was amplified from PI 90763, providing conclusive evidence of an insertion of approximately 6 kb within the *GmSNAP02* gene. Furthermore, full-length cDNA was successfully amplified from

(A)

Locus	Resistance gene	Origin	Growing area	Resistance	Susceptibility
Rhg1-b	*GmSNAP18-b*	PI 88788	95% of North American market	HG 7 (Race 3)	HG 2.5.7 (Race 1, Race5) HG 1.2.5.7 (Race 2)
Rhg1-a ***Rhg4***	*GmSNAP18-a* *GmSHMT08*	Peking	5% of North American market	HG 7 (Race 3)	HG 2.5.7 (Race 1, Race5) HG 1.2.5.7 (Race 2)

(B)

HG Type Classification (Niblack *et al.*, 2002)								
Indicator lines	0	1	2	3	4	5	6	7
1. PI 548402 (Peking)		+						
2. PI 88788			+					
3. PI 90763				+				
4. PI 437654					+			
5. PI 209332						+		
6. PI 89772							+	
7. PI 548316 (Cloud)								+

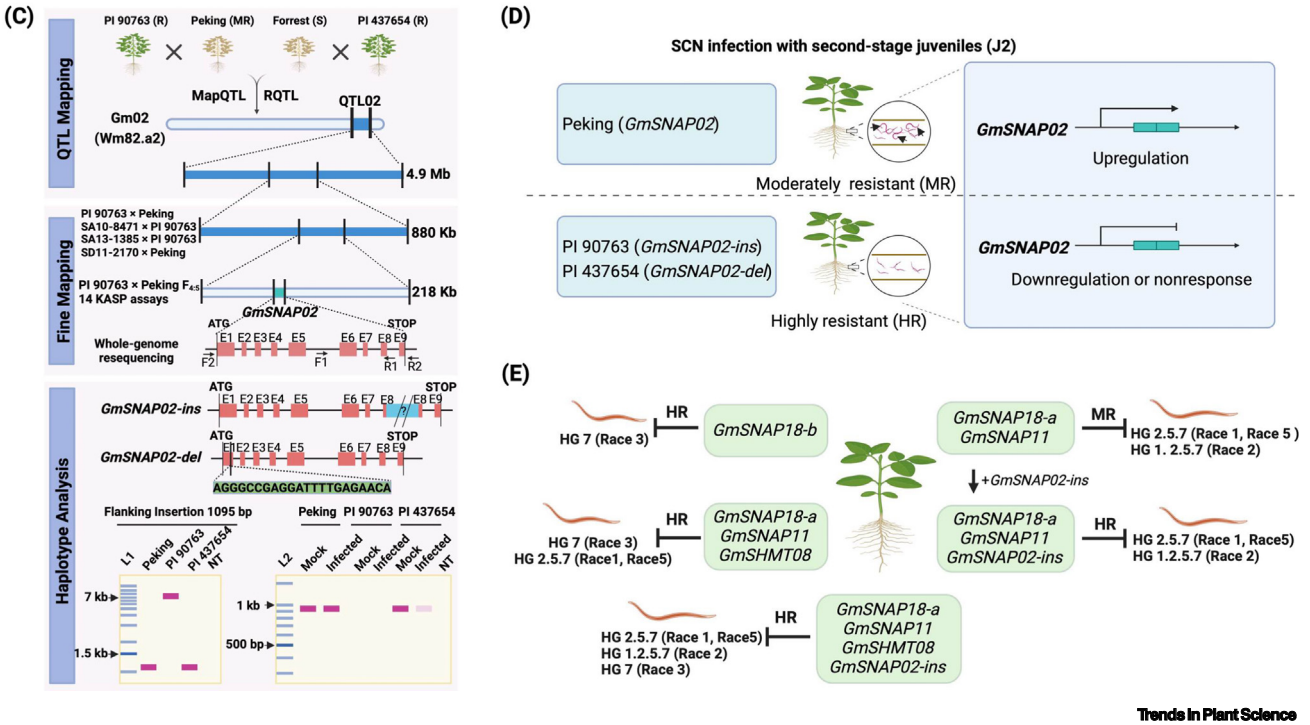

Figure 1. Identification of deletion and insertion in QTL02 through fine-mapping and whole-genome sequencing and enhanced resistance to soybean cyst nematode (SCN) through loss-of-function of *GmSNAP02*. (A) Major SCN resistance genes in soybeans from the North American market. (B) *Heterodera glycines* (HG) type classification system. The interpretation of the HG type test is summarized in the table provided [10]. A '+' signifies the ability of an SCN population to reproduce on a particular indicator line (≥10% female index). While HG type 0 populations cannot reproduce on any indicator lines, HG type 1 populations only reproduce on PI 548402 (Peking), and HG type 7 populations reproduce exclusively on PI 548316 (Cloud). Typically, multiple SCN populations can reproduce on several indicator lines and HG type numbers may overlap. An HG type 5.7 population, for instance, is one that can reproduce on both PI 209332 (indicator line 5) and PI 548316 (Cloud) (indicator line 7). (C) Positional cloning and identification of deletion and insertion associated with QTL02. This figure is adapted from Figure 2 by Usovsky *et al*. (2023) [2]. A quantitative trait locus (QTL02) was identified on Chromosome 02, spanning a region of 4.9 Mb using MapQTL and RQTL analysis in populations derived from crosses involving PI 90763 × Peking and Forrest × PI 437654. Recombination events surrounding QTL02 were investigated across four populations derived from crosses with PI 90763 as one parent, successfully narrowing down the interval to an 880-kb region containing 112 genes. In the subsequent step, F4:5 sister lines were generated from heterozygous lines resulting from the cross between PI 90763 and Peking, which exhibited recombination events within QTL02. These backcross progenies were genotyped using 14 Kompetitive Allele Specific PCR (KASP) markers spanning the 880-kb region, leading to further fine-mapping of QTL02 to a 218-kb region containing 34 candidate genes, including the *GmSNAP02* gene encoding an α-SNAP protein. Whole-genome sequencing revealed a possible insertion in PI 90763 and a deletion in PI 437654. The *GmSNAP02-ins* haplotype was confirmed by amplifying the corresponding GmSNAP02 sequence using gene-specific primers flanking the insertion in exon 8. A product of the expected size (1095 bp) was amplified from genomic DNA of Peking and PI 437654, while a larger product of approximately 7 kb was amplified from PI 90763, indicating the presence of a 6-kb insertion. Furthermore, full-length *GmSNAP02* cDNA could be amplified using gene-specific primers designed immediately upstream of the start and downstream of the stop codons from mock-inoculated and SCN-infected [3 days post-inoculation (dpi)] roots of Peking and PI 437654, but not from PI

(Figure legend continued at the bottom of the next page.)

both Peking and PI 437654, underscoring the absence of the insertion in their transcribed sequences. However, no full-length cDNA was obtained from PI 90763, providing additional support for the presence of the insertion. Sequencing of the full-length cDNA product amplified from PI 437654 confirmed the presence of this 22-nucleotide deletion, which induces a frameshift mutation ultimately resulting in a premature stop codon.

Based on qRT-PCR data, Usovsky *et al*. concluded that an increased expression of *GmSNAP02* in Peking plants in response to SCN infection probably makes them more vulnerable to the disease (Figure 1D). However, the detrimental impact of *GmSNAP02-ins* and *GmSNAP02-del* on the expression of *GmSNAP02* in PI 90763 and PI 437654 in response to SCN infection allows the plants to resist the disease. *GmSNAP02* is induced to a high level only in a compatible interaction, suggesting that *GmSNAP02* functions as a susceptibility gene. It remains to be determined how SCN induces the expression of *GmSNAP02* to favor parasitism.

By utilizing CRISPR-Cas9 genome editing, the authors confirmed that the resistance of PI 90763 against the virulent SCN HG type 1.2.5.7 is significantly enhanced by the nonfunctionality of *GmSNAP02* [2]. Usovsky *et al.* discovered that the utilization of solely *GmSNAP18-b* only provides resistance to PA3 (HG type 0 and 7, Race 3), but did not prove effective in providing resistance against the three virulent populations of SCN:TN7 (HG 2.5.7, Race 1), TN22 (HG 1.2.5.7, Race 2), and MM4 (HG 2.5.7, Race 5) (Figure 1E) [2]. However, the *GmSNAP18-a* allele demonstrated a notable influence on resistance when it was used in conjunction with other alleles. Specifically, the *GmSNAP18-a* + *GmSNAP11* gene exhibits resistance to HG 2.5.7 and HG 1.2.5.7, while the *GmSNAP18-a* + *GmSHMT08* gene stack confers robust resistance to HG type 0 and 7. Moreover, the triple gene stack *GmSNAP18a* + *GmSNAP11* + *GmSNAP02-ins* gene stack was found to provide enhanced resistance to HG 1.2.5.7. Strikingly, the quadruple *GmSNAP18-a* + *GmSNAP11* + *GmSHMT08* + *GmSNAP02-ins* gene stack has been shown to provide effective and broad-spectrum resistance to HG type 0 and 7, HG 2.5.7, and HG 1.2.5.7. For virulent populations such as SCN HG type 1.2.5.7, the introduction of *GmSNAP02-ins* into the gene stack *GmSNAP18-a* + *GmSNAP11* triggered a shift from moderate resistance to a significantly heightened resistance level, as indicated by an FI reaching or approaching zero. These data indicate the functionality of the *GmSNAP02-ins* allele in SCN HG type 1.2.5.7 resistance is contingent upon the presence of the gene stack comprising *GmSNAP18a* and *GmSNAP11*. The utilization of *GmSNAP02* presents a promising avenue for enhancing soybean plant resistance to SCN through refined breeding and biotechnology strategies. The integration of native mutant haplotypes of *GmSNAP02-ins* from PI 90763 and *GmSNAP02-del* from PI 437654 into elite soybean cultivars, already possessing the *GmSNAP18-a* + *GmSNAP11* gene stack, offers a straightforward and readily achievable approach. Unlike resistance genes that can be overcome by plant-parasitic nematodes over time, gaining resistance through loss-of-function of a susceptibility gene is likely to provide soybean plants with durable resistance.

Additional studies will be necessary to elucidate the functioning of *GmSNAP02* as a susceptibility gene and the reasons behind how loss-of-function mutations in *GmSNAP02* contribute to increased resistance against SCN. Further research aimed at understanding how SCN bypasses *rhg*-based resistance could yield valuable insights into the mechanisms underlying SCN-induced diseases. As an obligate sedentary endoparasite, SCN secretes a diverse array of effectors into specific host cells to suppress host immunity [11]. Investigating whether and how these effectors target *GmSNAP02* to enhance host susceptibility would be particularly intriguing [2].

Acknowledgments

Z.Q.F. was supported by National Science Foundation (EAGER grant number IOS-2207677).

Declaration of interests

The authors declare no competing interests.

[1]State Key Laboratory of Wheat and Maize Crop Science, College of Agronomy and Center for Crop Genome Engineering, Henan Agricultural University, Longzi Lake Campus, Zhengzhou 450046, China
[2]Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

*Correspondence:
dwwang@henau.edu.cn (D. Wang) and
zfu@mailbox.sc.edu (Z.Q. Fu).

90763. (D) The expression of *GmSNAP02* in response to SCN infection. Three days after inoculation, the expression of *GmSNAP02* was evaluated in the roots of Peking, PI 90763, and PI 437654 both in the presence and absence of SCN infection. In moderately resistant Peking, *GmSNAP02* was found to be upregulated at 3 dpi. However, highly resistant PI 90763 and PI 437654 either showed significant downregulation or no differential regulation of *GmSNAP02* in response to SCN infection. Black arrowheads signify the presence of engorged parasitic juvenile nematodes in Peking, indicating the successful establishment and growth of feeding sites. (E) Response of specific allele combinations to SCN infection. The allele *GmSNAP18-b* has been found to effectively combat SCN HG type 0 and 7 (Race 3) populations, while the gene stack *GmSNAP18-a* + *GmSNAP11* is highly resistant to HG type 2.5.7 (Races 1 and 5) and HG type 1.2.5.7 (Race 2) and the gene stack *GmSNAP18-a* + *GmSHMT08* has been found to confer resistance against HG type 0 and 7 (Race 3). The triple gene stacks *GmSNAP18-a* + *GmSNAP11* + *GmSNAP02-ins* confers resistance to HG type 2.5.7 (Races 1 and 5) and enhanced resistance to HG type 1.2.5.7 (Race 2). For maximum protection, the quadruple gene stack *GmSNAP18-a* + *GmSNAP11* + *GmSHMT08* + *GmSNAP02-ins* provides a high level of resistance to HG type 0 and 7 (Race 3), HG type 2.5.7 (Races 1 and 5), and HG type 1.2.5.7 (Race 2). The allele combinations depicted in panel E are based on PI 88788 for *GmSNAP18-b* and PI 90763 for all other alleles. The figure was created with the software BioRender (BioRender.com).

https://doi.org/10.1016/j.tplants.2024.04.005

Published by Elsevier Ltd.

References

1. Bandara, A.Y. *et al.* (2020) Dissecting the economic impact of soybean diseases in the United States over two decades. *PLoS One* 15, e0231141
2. Usovsky, M. *et al.* (2023) Loss-of-function of an α-SNAP gene confers resistance to soybean cyst nematode. *Nat. Commun.* 14, 7629
3. Cook, D.E. *et al.* (2012) Copy number variation of multiple genes at Rhg1 mediates nematode resistance in soybean. *Science* 338, 1206–1209
4. Nissan, N. *et al.* (2022) A broad review of soybean research on the ongoing race to overcome soybean cyst nematode. *Biology (Basel)* 11, 211
5. Bent, A.F. (2022) Exploring soybean resistance to soybean cyst nematode. *Annu. Rev. Phytopathol.* 60, 379–409
6. Matsye, P.D. *et al.* (2012) The expression of a naturally occurring, truncated allele of an α-SNAP gene suppresses plant parasitic nematode infection. *Plant Mol. Biol.* 80, 131–155
7. Liu, S. *et al.* (2012) A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. *Nature* 492, 256–260
8. Shaibu, A.S. *et al.* (2020) Soybean cyst nematode-resistance: gene identification and breeding strategies. *Crop J.* 8, 892–904
9. Basnet, P. *et al.* (2022) Epistatic interaction between Rhg1-a and Rhg2 in PI 90763 confers resistance to virulent soybean cyst nematode populations. *Theor. Appl. Genet.* 135, 2025–2039
10. Niblack, T.L. *et al.* (2002) A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *J. Nematol.* 34, 279–288
11. Vieira, P. and Gleason, C. (2019) Plant-parasitic nematode effectors - insights into their diversity and new tools for their identification. *Curr. Opin. Plant Biol.* 50, 37–43