Review

Resistance Components and Interactions of *Aphis glycines* (Hemiptera: Aphididae) and *Heterodera glycines* (Tylenchida: Heteroderidae) in Soybean

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ABSTRACT

The soybean aphid (SBA; *Aphis glycines* Matsumura) and the soybean cyst nematode (SCN; *Heterodera glycines* Ichninohe) are major pests of the soybean (*Glycine max* (L.) Merr.). Independent studies on these pests have made substantial progress in understanding the genetic basis of limiting these pests in both model and non-model plant systems. Classical linkage mapping and genome-wide association studies (GWAS) have identified some major and minor QTLs in soybean. The studies on interactions of aphid and nematode effectors with host proteins allow us to identify molecular cues in various signaling components, including plant disease resistance and phytohormone genes. Here we review various resistance components of both SBA and SCN in terms of their effectors, their role in eliciting or attenuating host defense and update on findings on key quantitative trait loci (QTLs)/genes in soybean that could help in mitigating these widespread shifted virulent soybean aphid and SCN populations. We also provide an update on recent interaction studies of soybean-soybean aphid-SCN as well as interaction studies involving different aphids/nematodes and their host systems. *PHYTOALEXIN DEFICIENT4* (*PAD4*) in soybean could possibly play a major role in providing resistance against both soybean aphid and SCN.

Keywords: effectors, QTLs, GWAS, resistance, soybean-soybean aphid-soybean cyst nematode interactions

Introduction

Soybean [*Glycine max* (L.) Merr.], a source of high-quality sugar, protein, and oil, is one of the most important crops worldwide [1]. According to the United States Department of Agriculture, soybean has become the second major crops of the US in terms of production and acreage (https://www.ers.usda.gov/data-products/oil-crops-yearbook/oil-crops-yearbook). In 2017, the US alone produced 119.5 million metric tons (MMT) of soybean with an estimated worth of $41.01 billion and contributed to 35% of the world soybean production (http://soystats.com). The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) and soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe are common pests that cause a

significant loss in soybean production [2, 3]. The soybean aphid, an aboveground pest, nourishes on phloem sap, whereas the underground SCN infects the soybean roots. The two pests can co-occur and cause a significant reduction in soybean yield [4, 5, 6]. The US annual economic losses in soybean yield due to soybean aphid and SCN are estimated to be approximately $4 billion and $1.3 billion, respectively [5, 7, 8]. To counteract these devastating pests, farmers have been relying on various chemical measures, such dependency of which has developed an imminent threat to the environment and beneficial organisms [9, 10, 11]. At the same time, the chemical measures are not economical, and their use might threaten the sustainability of host plant resistance for these two pests [12, 13, 14, 15]. In addition, increased use of aphid biotypes and SCN populations with virulent characteristics can cause a serious problem on the resistant cultivars sustainably [12, 13]. Understanding of various resistance components soybean aphid and SCN to employ durable host resistance is necessary. The major objective of this research is to provide a thorough review on the resistance components of soybean aphid and SCN in terms of their biology, the discovery of effectors, quantitative trait loci (QTLs), gene functions and resistance mechanisms, and their interactions with each other.

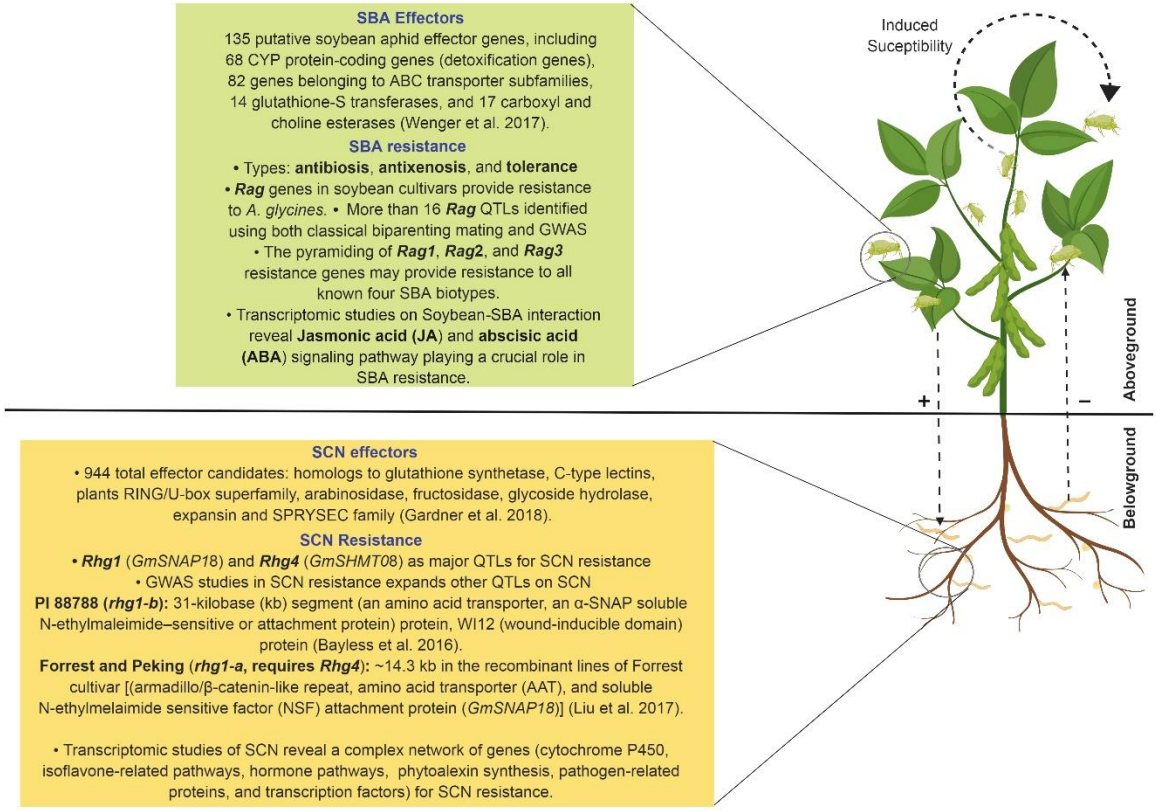

Figure 1. Overview of the resistance components and their interactions between SBA and SCN in soybean. Various SBA and SCN resistance components (effectors and resistance in the host) are shown in a projected green and yellow rectangular box, respectively. The circular arrow represents the process of induced susceptibility within conspecifics of SBA. The dashed arrows represent the interaction between SBA and SCN shown by various studies discussed in this review. The (+) and (-) signs represent the positive and negative effect respectively. The

illustration is created with Biorender (https://app.biorender.com/).

1. Soybean Aphid

1.1. The soybean aphid utilizes soybean as a secondary host

The soybean aphid is a heteroecious, holocyclic species [17] that uses various species of buckthorn (genus *Rhamnus* L.) as its primary host and utilizes soybean as its secondary host [13]. It overwinters on buckthorn before emerging in the spring to produce several generations via sexual reproduction [18]. In the late spring or early summer, the aphids develop into alates (winged morphs) and migrate to soybean plants, where they feed primarily upon the ventral surfaces of young leaves [4, 19, 20]. This results in plant stunting, leaf yellowing and wrinkling with reduced photosynthesis, poor pod fill, reduced seed size and quality, and yield reductions of up to 40% [4, 16, 17]. The aphids also act as a vector for various viruses, including soybean mosaic virus, alfalfa mosaic virus, and bean yellow mosaic virus, and facilitate sooty mold infections via the deposition of honeydew [7, 23]. The soybean aphid is native to East Asia and is considered to be a major pest in China, Japan, the Philippines, South Korea, Indonesia, Malaysia, Thailand, Vietnam, and Russia [18]. It has been present in North America since at least 2000, when it was first reported in the state of Wisconsin [2], and has also been introduced to Australia [19]. By 2009, the species had spread to the northeastern and Midwestern regions of the United States [20] and has since spread to thirty states and three Canadian provinces [11].

1.2. Soybean aphids have developed different biotypes

A biotype is an insect population that can reproduce and survives in cultivars developed for resistance to that same population [52]. It is a pseudo-taxonomic unit that classifies insect populations according to their virulence to specific cultivars and shared phenotype (reviewed in [21]). This term has been used for various insect species, including *Mayetiola destructor*, *Schizaphis graminum, Nilaparvata lugens*, and *Bemisia tabaci* [22]. The insect subpopulations capable of surviving in resistant crop lines, including wheat, barley, melon, and apple, have been studied (reviewed in [23]). Soybean aphids that are avirulent on any soybean plant that contains the *Rag* gene are attributed to biotype 1 [23]. Biotype 1 is the predominant biotype in North America [24]. Biotype 2 (*Rag1* virulent) was discovered in Ohio in 2005, five years before the release of commercial *Rag1* cultivars [25]. Biotype 2 aphids were thought to be the predominant biotype in eastern North America [25], but various field tests found that they were prevalent only in Ohio [13]. Since then, four biotypes of soybean aphid have been discovered in the U.S., suggesting the North American populations possess sufficient genetic variability to adapt to the resistant hosts [25]. Biotype 3 aphids discovered in Indiana were able to reproduce on *Rag2* soybean plants but were poorly adapted to *Rag1* soybean plants [26]. Later, biotype 4 aphids were found in Wisconsin that can reproduce in both *Rag1* and *Rag2* soybean plants [27]. Cooper et al. 2015 [23] studied the geographic distribution of the soybean aphid biotypes across 11 states and one Canadian province between 2008 and 2010. The frequency of aphid populations belonging to biotypes 2, 3, and 4 was 54, 18, and 7%, respectively. The aphid populations from Wisconsin, the state where the soybean aphid was first reported in the U.S. in 2000, showed higher virulence variability [23]. Additionally, Zhong et al. 2014 [28] reported at least four biotypes of soybean aphid in China. These biotypes were named as China Biotype 1 (virulence on host plants with *Rag5* or *Rag6*), China Biotype 2 (virulence on host plants with *Rag1*, *Rag3* or *Rag5*), China Biotype 3 (virulence on host plants with *Rag1*, *Rag3*, or *Rag6*), and China Biotype 4 (virulence on host plants with *Rag1*, *Rag2*, *Rag3*, or *Rag5* genes) [28].

1.3. *Aphid effectors are host specific and undergo selection pressure*

The soybean aphid uses two types of saliva, gelling and watery saliva when feeding. The aphid injects the gelling saliva during the early stages of feeding to form sheaths around the stylets [29] and later injects effector molecules through watery saliva into both the intra- and intercellular spaces of mesophyll cells or directly into phloem cells [30]. Since the effector molecules allow each aphid to sustain and modulate its host plant's immune reaction, they are subject to the scrutiny of host defense mechanisms and undergo natural selection [31]. Such selection helps effectors evade the host defense system, maintain their virulence, and evolve new functions [32].

Transcriptomic and proteomic studies of the pea aphid (*Acyrthosiphon pisum* Harris) found many enriched salivary proteins undergoing positive selection [33]. Aphid effectors are host specific so that they can effectively interact with the host proteins for their virulence [34, 35]. Rodriguez et al. 2017 [34] reported that Mp1, an effector molecule produced by the green peach aphid (*Myzus persicae* Sulzer), specifically targets Vacuolar Protein Sorting-Associated Protein 52 (VPS52) proteins in their strong hosts, while this interaction was absent in the green peach aphid's poor-hosts. Furthermore, the reproduction of the green peach aphid did not increase in *Arabidopsis* that expressed the orthologs of the pea aphid's effectors, including C002, PIntO1 (Mp1), and PIntO2 (Mp2) [35]. Since the identification and functional characterization of the first aphid effector molecule, C002 in the pea aphid [36], significant progress has been made in identifying a wide range of effector molecules in different aphids. The availability of the whole genome sequences of several aphid species, including the pea aphid [37], the Russian wheat aphid (*Diuraphis noxia* Kurdjumov)[38], the green peach aphid [39], and the soybean aphid [40], have allowed to study of various gene families of aphid salivary effectors. Carolan et al. 2011 [41] identified 324 secretory proteins in the salivary gland in the pea aphid. Some, including Glucose dehydrogenase, Glutathione peroxidase, putative sheath protein of aphids, and Angiotensin-converting enzyme-like, showed similarity to known aphid effectors [42, 43, 44], while others were more similar to nematode effectors, including M1 zinc metalloprotease, Disulfide isomerase, Calreticulin, ARMET, Glutathione peroxidase, and CLIP-domain serine protease [41, 45, 46]. Pea aphid effector proteins were further expanded to 3,603 genes expressed in salivary glands, 740 of which were up-regulated in salivary glands [33]. Thirty-four salivary genes were identified in the Russian wheat aphid similar to the most commonly expressed genes in other aphids, including glucose dehydrogenase and trehalase [38]. An intensive analysis of the genome of the green peach aphid, which can infest plant species belonging to 40 families, demonstrated the role of multigene clusters in colonizing distant plant species [39]. The authors suggested genes belonging to cathepsin B and RR-2 cuticular protein gene families undergo rapid transcriptional plasticity so that the aphids can infest a wide range of plant species belonging to the Brassicaceae and Solanaceae families.

RNA-Sequencing (RNA-Seq) has been a standard tool for studying qualitative and quantitative gene expression [47, 48]. In the context of the soybean aphid, Bansal et al. 2014 [49] studied xenobiotic stress response in the soybean aphid using RNA-Seq. The authors found 914 significantly expressed genes in the soybean aphid, most of which were related to stress, detoxification [cytochrome p450s (CYPs), glutathione-S-transferases, carboxyesterases], and ABC transporters. Wenger et al. 2017 [40] identified 135 putative soybean aphid effector genes,

including 68 CYP protein-coding genes (detoxification genes), 82 genes belonging to ABC transporter subfamilies, 14 glutathione-S transferases, and 17 carboxyl and choline esterases. The detoxification genes help aphids adapt to host plants [49]. The small number of CYP genes found in the soybean aphid, the pea aphid (83 CYP genes), and the Russian wheat aphid (48 CYP genes) might explain why these species are adapted to a limited range of hosts, while the green peach aphid (115 CYP genes) is adapted to wide host ranges [50]. The availability of genome sequences of the soybean aphid might explain the species' rapid adaptation to resistant soybean cultivars despite the lack of both genetic differentiation and selection pressure between avirulent and virulent biotypes [51].

1.4. ***Soybean cultivars exhibiting antibiosis, antixenosis, and tolerance as a resistance response to soybean aphids***

According to Smith 1989, 2005 [52, 53], plant resistance mechanisms to insects can be grouped into three categories, including antibiosis, antixenosis, and tolerance. Antibiosis resistance affects the biology, including the mortality or fecundity, of the insect. The soybean cultivar 'Dowling' exhibits antibiosis, and resistance factors are present in the phloem cells [54]. Antixenosis resistance affects the behavior of the insect. The soybean cultivar PI200538 exhibits antixenosis [54]. Jesus et al. 2018 [55] studied the physiological responses of 14 soybean genotypes to aphid infestation in terms of total protein, peroxidase and chlorophyll and resistance mechanisms. The genotypes UX 2569-1592-01 (*Rag2* gene; PI243540) and UX 2570-171-04 showed the highest and moderate level of antibiosis and/or antixenosis respectively. The chlorophyll content in UX 2569-159-2-01 was reduced at 5 and 15 days after infestation. Total protein content remained unchanged between the infested and control plants. Peroxidase activity in UX 2570-171-04 was higher at 5 and 10 days after infestation, and this cultivar showed a moderate level of antibiosis and/or antixenosis. Tolerance resistance is the ability of the plant to endure the presence of the insect without significant impacts on the pest's biology or behavior [56]. The KS4202 cultivar is tolerant of aphids [57]. The tolerance effect in KS4202 may be attributable to the quick regulation of RuBP (ribulose-1,5-biphosphate) and the upregulation of detoxification genes [58].

1.5. ***Rag genes in soybean cultivars provide resistance to A. glycines***

Rag (resistance to *Aphis glycines*) loci were first discovered in Dowling, PI71506, and Jackson cultivars [59] and have since been identified in other soybean lines. The mapping and inheritance mechanism of the *Rag1* gene has been documented in multiple soybean cultivars [60, 61, 62, 63]. *Rag1* loci were mapped as a 115 kb interval on chromosome 7 using the Dowling (PI548663; donor parent of *Rag1*) and Dwight (PI587386; aphid-susceptible parent) cultivars [64]. *Rag2* loci were finely mapped as a 54 kb interval on chromosome 13 in the antixenotic PI200538 cultivar[54, 65]. *Rag3* loci were mapped on chromosome 16 (LG J) using PI567543C [66]. The recessive *rag4* loci were mapped on chromosome 13 (LG F) between markers in PI567541B [67]. The authors also mapped *rag1* provisional (*rag1c*) in chromosome 7 (LG M). *Rag5* (proposed) and *Rag6* have been identified in PI567301B and PI567598B, respectively [68, 69]. Bhusal et al. 2017 [70] identified two major and two minor loci. The major loci were located on chromosome 7 (*qChrom.07.1*) (1Mb distant from *Rag1*) and chromosome 16 (*qChrom.16.1*) (near *Rag3*); the minor loci were located on chromosome 13 (*qChrom.13.1*) (near *Rag4*) and chromosome 17 (*qChrom.17.1*) and were associated with aphid resistance in PI603712. Hill et al. 2017 [71] characterized multiple *A. glycines* biotype resistances in five cultivars.

More than half of the genetic diversity has been lost in the cultivated soybean [72], but its closest wild relative, *Glycine soja* Siebold & Zucc., may be useful for identifying aphid-resistance

genes, studying inheritance patterns, and mapping important resistance loci [84]. Hesler and Tilmon 2018 [73] reported PI135624 and PI65549 to be resistant to aphids, and Conzemius 2018 [74] reported PI101404A and PI65549 showing significant high resistance to Biotype 4 colonies. *Rag6* and *Rag3c* were mapped in 49-kb (42,146,252–42,195,720 bp) and 150-kb intervals (6,621,540–6,771,675 bp) on chromosome 8 and chromosome 16, respectively, in *G. soja* [75]. The 49–kb interval where *Rag6* was mapped contained three clustered NBS–LRR genes (*Glyma.08g303500*, *Glyma.08g303600*, and *Glyma.08g303700*) and one amine oxidase gene (*Glyma.08g303800*). The 150-kb interval where *Rag3c* was mapped contained one LRR gene (*Glyma.16g066800*) and other ten genes belonging to lipase, cytochrome P450, methyltransferases, hydrolases, and Ku70-binding gene families. All identified *Rag* QTLs in various soybean plant introductions (PI) are presented in Table 1.

The 1,691 non-redundant genes assessed from the Rag QTLs, including *Rag1*[64], *rag1b* [69], *rag1c* [67], *Rag2* [76], *Rag3* [66, 77] , *Rag4* [77], *rag3* [69] , *rag3b* [78], *Rag3c* [75], *rag4* [67], *Rag5* [68] , *Rag6* [75]; *qChrom.07.1*, *qChrom.16.1*, *qChrom.13.1*, and *qChrom.17.1* [70] are significantly associated with nutrient reservoir activity (GO:0045735) and binding (GO:0005488). The 'nutrient reservoir activity' molecular function is important in protecting plant tissues that produce surface waxes [79]. Similarly, the 'binding' molecular function that constitutes the higher proportion suggests their important role in signaling and stress responses. The genes engaged in the process of binding (GO: 0005488) belong to ADP binding (GO: 0043531), adenyl ribonucleotide binding (GO: 0032559), calcium-dependent phospholipid binding (GO: 0005544), adenyl nucleotide binding (GO: 0030554), purine nucleoside binding (GO: 0001883), nucleoside binding (GO: 0001882), pattern binding (GO: 0001871), and polysaccharide binding (GO: 0030247) gene families (Figure 2).

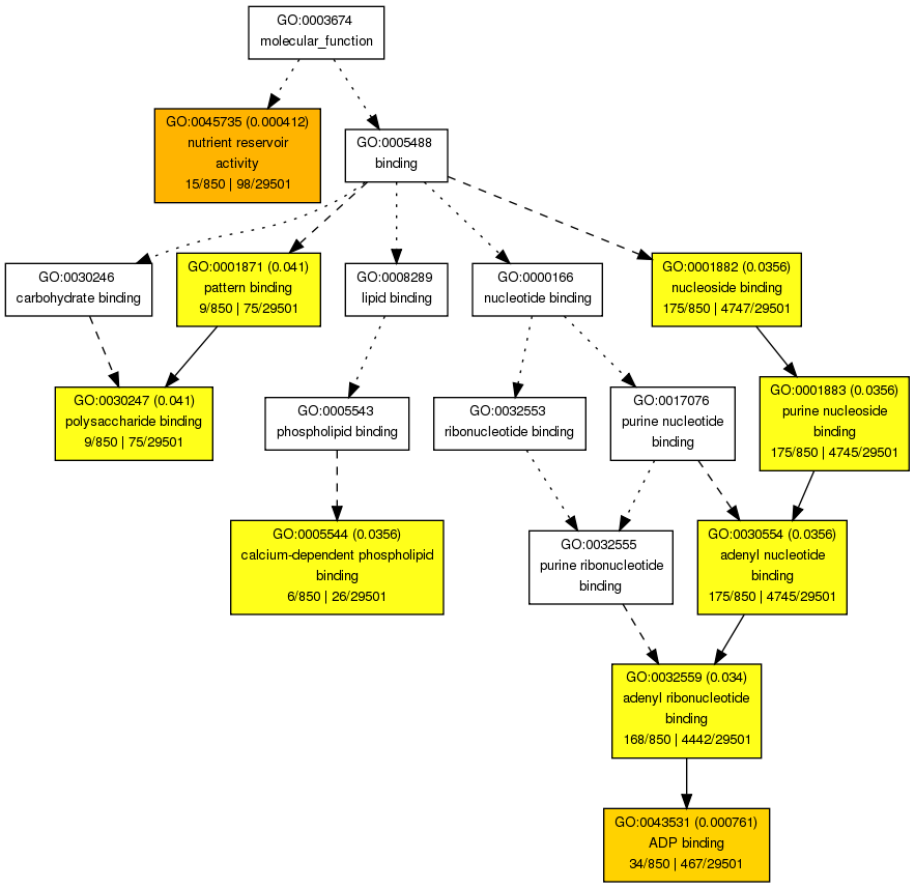

Figure 2. Significantly enriched GO molecular function terms of non-redundant 1,691 genes in the *Rag* QTLs: *Rag1* [64], *rag1b* [69], *rag1c* [67], *Rag2* [76], *Rag3* [66, 77] , *Rag4* [77], *rag3* [69] , *rag3b* [78], *Rag3c* [75], *rag4* [67], *Rag5* [68] , *Rag6* [75]; *qChrom.07.1*, *qChrom.16.1*, *qChrom.13.1*, *qChrom.17.1* [70] as determined by Fisher's exact test using AgriGO [80]. The same gene can be associated with multiple GO annotations. Only significantly ($P < 0.05$) over-represented GO categories are shown. The stronger color represent the lower *P* value. The box consists of the following information: GO term, adjusted *P* value, GO description, number of query list and background mapping GO, and total number of query list and background.

Table 1. List of soybean cultivars for mapping *Rag* genes with chromosome location, markers associated, and type of resistance. (ϒ = The position of the markers are based on Glyma 2.0 as of [81]).

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QTLs	Soybean Plant Introductions	Chromosome (Linkage group)	Markers associated (Location[Y])	Type of resistance	References
Rag1	Dowling (PI548663)	7 (M)	46169.7and 21A (5,529,532-5,770,718 bp)	Antibiosis	[64]
	PI71506	7 (M)		Antixenosis	[63]
	PI548663 (cultivar Dowling)	7 (M)	Satt435 and Satt463		[62]
	PI548657 (cultivar Jackson)	7 (M)	Satt435 andSatt463		
	PI587663	7 (M)	Satt567 and Satt245	Antibiosis	[71]
	PI587677	7 (M)	Satt540	Antibiosis	[71]
	PI587685	7 (M)	Satt540	Antibiosis	[71]
	PI594592	7 (M)	Satt540	Antibiosis	[71]
rag1c	PI567541B	7 (M)	sat229-satt435 (2,434,259-8,234,168 bp)		[67]
rag1b	PI567598B	7 (M)	Satt567 and Satt435 (5,523,128-5,909,485 bp)		[69]
Rag2	PI243540	13 (F)	Satt334 and Sct_033(28,415,888–30,739,587 bp)	Antibiosis	[76]
	PI200538	13 (F)	Satt510, Soyhsp176, Satt114, and Sct_033 (29,609,521– 31,802,676 bp)	Antibiosis	[65]
	PI587663, PI587685	13 (F)	Satt114, SNP2, Satt335		[71]
	PI587677	13 (F)	Satt335		[71]
	PI587972	13 (F)	Satt114, Satt510		[71]
	PI594592	13 (F)	Satt114		[71]
Rag3	PI567543C	16 (J)	Sat_339 and Satt414 (4,964,852-7,212,164 bp)	Antixenosis	[66]
	PI587663	16 (J)	Satt285	Antibiosis	[71]
	PI594592	16 (J)	Satt654	Antibiosis	[71]
	PI567543C	16 (J)	ss715625290 and ss715625308 (6,314,060-6,571,305 bp)		[77]
rag3	PI567598B	16 (J)	Satt285 and Satt414 (6,314,120-6,570,336 bp)		[69]
rag3b	PI567537	16 (J)	4,964,852- 7,957,026 bp	Antibiosis	[78]
Rag3c	E12901	16 (J)	Gm16-3 and Gm16-5 (6,621,540–6,771,675 bp)	Antibiosis	[75]
rag4	PI567541B	13 (F)	Satt649-Satt343 (1,225,665-16,340,514 bp)	Antibiosis	[67]
	PI587677	13 (F)	Satt586		[71]
Rag4	PI567543C	13(F)	MSUSNP13-29-ss247923149 (13,691,537-13,626,971 bp)		[77]
Rag5 **Proposed**	PI567301B	13	4 SSR markers (30,236,183-30,749,047 bp)	Antixenosis	[68]
Rag6	E12901	8	Gm08-15 and Gm08-17 (42,146,252–42,195,720 bp)	Antibiosis	[75]
qChrom.07.1	PI603712	7(M)	ss715598483-ss715598534 (6,444,246-6,819,959 bp)		[70]
qChrom.16.1	PI603712	16(J)	ss715625261-ss715625278 (6,105,250-6,222,257 bp)		[70]
qChrom.13.1	PI603712	13(F)	ss715613721-ss715617240 (13,691,537-13,626,971 bp)		[70]
qChrom.17.1	PI603712	17(D2)	ss715627556-ss715627637 (39,019,814-39,521,449 bp)		[70]

1.6. *GWAS studies on A. glycines resistance on a soybean expanding number of QTLs*

Genome-Wide Association studies (GWAS) have been an important alternative to classical bi-parental QTL mapping [82] for understanding the genetic basis of diseases linked to complex, polygenic traits. Classical QTL mapping is limited in its ability to identify allelic diversity and resolve genomes [83], but GWAS can capture all the recombination events undergone during the evolution of sampled genotypes [84]. Different kinds of phenotypes, including quantitative, binary, and ordinal phenotypes, can be studied using GWAS [85], and these phenotypes can be correlated with genotypes using mixed linear models [86]. Chang and Hartman 2017 [87] reported the first GWAS study for aphid-resistance using USDA soybean germplasms. The authors suggested that ss715596142 may be a significant SNP marker and found three LRR domain containing genes (*Glyma07g13440*, *Glyma07g14810*, and *Glyma07g14791*) and one MYB transcription factor (*Glyma07g14480*). This marker is close to the *rag1c* gene that was reported in PI567541B [67], but not the *Rag1* gene that contains the candidate LRR genes (*Glyma07g06890* and *Glyma07g06920*) [64]. Recently, Hanson et al. 2018 [81] reported significant SNPs on chromosomes 7, 8, 13, and 16 where *Rag* genes have been mapped previously, for multiple aphid biotypes. Additionally, they reported markers on chromosomes 1-2, 4-6, 9-11, 12, 14, and 16-20 where *Rag* genes had not been previously reported.

1.7. *Rag genes pyramiding provides resistance to all soybean aphid biotypes*

The presence of fitness costs associated with aphid virulence in the *Rag* soybean cultivars could be used to preserve the efficacy of resistance genes in *Rag* soybean cultivars [88, 89]. In addition, the use of refuge susceptible soybean plants might limit the frequency of virulent biotypes [88]. It has been proven that soybean aphids are more virulent in cultivars with a single *Rag* gene than those with pyramided genes [52]. The pyramiding of resistance genes in the soybean cultivars protects the plants from the various aphid biotypes [90, 91]. The first soybean cultivar with both *Rag1* and *Rag2* genes became commercially available in 2012 and was resistant to aphid biotypes 2 and 3 [92]. After aphid Biotype 4 was found, the need for pyramiding more genes became imminent. The pyramiding of *Rag1*, *Rag2*, and *Rag3* resistance genes may provide resistance to all known aphid biotypes [89, 91].

1.8. *Transcriptomic studies on Soybean-A. glycines interaction: Jasmonic acid (JA) and abscisic acid (ABA) signaling pathway plays a crucial role in plant resistance*

Several studies have described the differential changes in phytohormones that occur during aphid-feeding in resistant, tolerant, and susceptible cultivars [93, 94, 95, 96, 97]. The cyclical expression patterns of the different markers and responsive genes for salicylic acid (SA) observed in aphid-infested plants suggests the role of SA in soybean resistance to aphid feeding [94]. Furthermore, the application of methyl jasmonate (MeJA) on infested plants significantly decreased soybean aphid populations, but similar salicylic acid applications did not; this suggests MeJA may be the elicitor to induce plant defenses [94]. Thus, the JA signaling pathway that assists the induction of other enzymes, including polyphenol oxidase (PPO), lipoxygenases, peroxidases, and proteinase inhibitors, appears to play a crucial role in aphid-resistance against susceptible soybean cultivar [94, 98].

Brechenmacher et al. 2015 [56] used two *Rag2* and/or *rag2* near-isogenic lines of soybean to identify 396 proteins and 2,361 genes that were differentially regulated in response to soybean

infestation. Several genes mapped within the *Rag2* locus, including a gene of unknown function (*Glyma13g25990*), a mitochondrial protease (*Glyma13g26010*), and a NBS-LRR (*Glyma13g25970*), were significantly upregulated in the presence of aphids. Prochaska et al. 2015 [57] identified 3 and 36 differentially expressed genes (DEGs) at 5 and 15 days after infestation, respectively, in the resistant (tolerant) KS4202 cultivar but found only 0 and 11 DEGs at 5 and 15 days after infestation, respectively, in the susceptible K-03-4686 cultivar. Most of the DEGs were related to WRKY transcription factors (such as WRKY60), peroxidases [*Peroxidase 52* (*PRX52*) and *Ascorbate peroxidase 4* (*APX4*)], and cytochrome p450s. Aphid-tolerance mostly depended on the constitutive levels of abscisic acid (ABA) and jasmonic acid (JA) and the basal expression of ABA (*NAC19* and *SCOF-1)* and JA [*LOX10*, *LOX2* (a chloroplastic-like linoleate 13S-lipoxygenase 2), *OPDA-REDUCTASE 3* (*OPR3*)]-related transcripts [93]. In addition, the genes *PRX52*, *WRKY60*, and *PATHOGENESIS-RELATED1* (PR1; SA-responsive transcript) were found to be induced by aphid infestation in the tolerant KS4202 cultivar [93]. Lee et al. 2017 [99] evaluated the transcriptomic dynamics of soybean near-isogenic lines (NILs) with either the *Rag5* allele for resistance or the *rag5* allele for susceptibility to the aphid biotype 2. Three genes located near the *Rag5* locus, including *Glyma.13 g190200*, *Glyma.13 g190500*, and *Glyma.13g190600*, were reported to be strong candidate genes for imparting soybean aphid resistance. Li et al. 2008 [96] studied soybean responses to aphid infestation by using cDNA microarrays to generate transcript profiles and identified 140 genes related to the cell wall, transcription factors, signaling, and secondary metabolism. Studham and MacIntosh 2013 [97] utilized oligonucleotide microarrays to study soybean-aphid interactions in the aphid-resistant cultivar LD16060 with *Rag1* gene and aphid-susceptible cultivar SD01-76R. They identified 49 and 284 differentially expressed genes (DEGs) at 1 and 7 days after infestation, respectively, in the susceptible cultivar and found only 0 and 1 DEGs at 1 and 7 days after infestation, respectively, in the resistant cultivar. They suggested that the expression of defense genes in resistant plants is constitutive, whereas the defense genes in susceptible plants are expressed only after aphid infestation. A recent study by Hohenstein et al. 2019 [100], have shown reduced response and decreased aphid growth in resistant (*Rag1*) plants upon long term aphid colonization for 21 days. Whereas, upon long term-feeding, susceptible plants showed a strong response in the phenylpropanoid pathway, specifically isoflavonoid biosynthesis.

2. Soybean Cyst Nematode

2.1. The intimate relationship between SCN and soybean

SCN is an obligate, sedentary endoparasite that completes its life cycle in 3 to 4 weeks [101]. Organic molecules secreted by host plants signal key events, including egg hatching and second-stage juvenile (J2) dispersal, in the nematode life cycle. In soybean, these molecules include eclepsins and glycinoeclepin A [101, 102, 103]. Other compounds, such as solanoeclepin A, picloronic acid, sodium thiocyanate, alpha-solanine, and alpha-chaconine, have also been found to initiating the egg hatching process in most of the nematodes [104, 105]. *Gro-nep-1* has been recently identified as the first gene to be upregulated in eggs treated with host root exudate in the golden nematode (*Globodora rostochiensis* Wollenweber) [106]. The exudates are used by the J2 nematodes to find the host plant's root system [14, 107] and nematodes that fail to enter a host plant die of starvation [108]. Once a J2 nematode locates a host, it infects the root cells using its stylet and secretes digestive enzymes, such as cellulase, to facilitate its movement through the epidermal and cortical cells as it moves towards a vascular cylinder [14, 109]. At the vascular cylinder, a J2 nematode induces a single cell to undergo morphological changes in order to form

a permanent feeding site called a syncytium [14, 109]. The syncytium remains intact throughout the remainder of the nematode's life cycle [14]. The nematode then molts into the third juvenile stage (J3) and undergoes sexual differentiation [110]. The ratio of female to male J3 nematodes is generally 1:1 but is sometimes affected by the milieu and resistance of the host plant [111]. The feeding site swells longitudinally throughout the root as it dissolves and incorporates numerous cells with dense cytoplasm, hypertrophied nuclei, and increased organelle content [14]. The J3 male metamorphoses to a vermiform-shape, leaves the root to locate females, and dies after mating [112, 113]. Concurrently, the J3 female molts to form an adult female which changes into a lemon-shaped cyst that extrudes from the root surface. Each female in a cyst produce 40-600 eggs with an average of approximately 200 eggs and sometimes eggs are outside in the gelatinous secretions [114, 115]. Cysts produce compounds such as chitinase and polyphenol oxidase to protect eggs from desiccation and microbial infection [5] and can remain viable for up to nine years [5].

2.2. *CN effectors interact with host proteins and enhance either susceptibility or resistance in the host plants*

Nematode effector molecules are produced in a nematode's esophageal gland before being released into the stylet [116]. The effectors evade and suppress the host plant's defense and reprogram the host cell nucleus, as well as a various cellular process for their suitability [117, 118]. These effector molecules reach into the host cell after dissolving the cell wall through various enzymes and proteins that bind to the components of the cell wall such as cellulose binding proteins and expansins [117]. The successful parasitism of the nematodes in the plants involves via direct or indirect interaction with the host plant targets or immune regulators, cell wall modifications, mimicry of plant peptide hormones, or manipulating hormone transport [reviewed in [119]]. Various nematode effector molecules, including Gr-SPRYSEC (-4,-5, -8, -15, -18, -19), Gp-RBP-1, Gr-VAP1, Hg30C02, Hs10A06, Hs4F01, and Mi-CRT, have been already characterized in different nematodes and hosts [120, 121, 122, 123, 124, 125, 126, 127]. These effectors affect the host immune system by enhancing susceptibility or resistance.

The characteristic cyst nematode effectors, including those found in SCN, are presented in Table 2. Gao et al. 2003 [128] identified 51 effector molecules from the esophageal gland of the *H. glycines*. Most of the effector molecules belonged to cellulose genes, pectate lyases, an enzyme in the shikimate pathway, and ubiquitin proteins. The ortholog of *H. glycines* cellulose binding protein (HgCBP) in *H. schachtii* (HsCBP) interacts with the pectin methyltransferase protein (PME3) of *Arabidopsis* during the early feeding stage and exhibits enhanced susceptibility [129]. The function of an ortholog of 25A01-like effector family was studied in *H. schachtii* (Hs25A01) in *Arabidopsis* system [130]. Hs25A01 interacts with *Arabidopsis* F-box-containing protein, chalcone synthase and the translation initiation factor eIF-2 b subunit to increase both root length and susceptibility to *H. schachtii*. Further, 18 more effector molecules were reported that showed high similarity to N-acetyltransferases, β-fructofuranosidases, serine proteases, cysteine proteases, an effector for protein degradation in the syncytium, cellulose binding protein, chorismate mutase, and glycosyl hydrolase [119]. Among them, HgGLAND18 secreted in the dorsal gland cell, suppresses basal and hypersensitive cell death innate immune responses in *Nicotiana benthamiana* [131]. The similarity of the N-terminal domain of HgGLAND18 to the domain of effector of *Plasmodium spp.* strongly suggest the role of convergent evolution of the effector molecules in diverse parasites [131]. Another effector, biotin synthase (HgBioB) and protein containing protein SNARE domain (HgSLP-1) effector molecules were reported recently employing allelic imbalance

analysis to associate SCN SNPs [132]. HgSLP-1 interacts with Rhg1 α-SNAP evading the host defense [132]. However, the host defense is evaded on the absence of the HgSLP-1 because of its avirulence nature like map-1 protein and Mj-Cg-1 effectors [132, 133, 134]. Until effectors search through *de novo* transcriptome assembly of the second stage juvenile *H. glycines* [135], only 71 effector molecules were reported that were secreted only from the esophageal glands. Upon use of the joint pipeline that utilizes presence or absence of signal peptides, 944 total effector candidates were predicted many of which were homologs to glutathione synthetase, C-type lectins, plants RING/U-box superfamily, arabinosidase, fructosidase, glycoside hydrolase, expansin and SPRYSEC family [135].

Table 2. List of characterized cyst nematode effectors in different plant systems with their targets and susceptibility/resistance effects.

CN effectors	Cyst Nematode	Targets	Host	Susceptibility/Resistance	References
HsCBP	*H. schachtii*	pectin methyltransferase protein (PME3)	*Arabidopsis thaliana*	susceptibility	[129]
Gp-Rbp-1	*G. pallida*	Gpa2	*Nicotiana benthamiana*	hypersensitive response (HR)	[126]
Gr- SPRYSEC (4,5,8,15,18,19)	*G. rostochiensis*	NB-LRR proteins	*Nicotiana benthamiana*	Suppress host defense	[127]
Hs19C07	*H. schachtii*	auxin influx transporter LAX3	*Arabidopsis thaliana*	susceptibility	[136]
Gr-VAP1	*G. rostochiensis*	apoplastic cysteine protease Rcr3pim	*Solanum lycopersicum*	programmed cell death	[125]
Hg30C02	*H. schachtii*	β-1,3-endoglucanase	*Arabidopsis thaliana*	susceptibility	[121]
Hs4D09	*H. schachtii*	14-3-3ε	*Arabidopsis thaliana*	resistance	[137]
Hs10A07	*H. schachtii*	interacting plant kinase (IPK) and IAA16 transcription factor	*Arabidopsis thaliana*	hypersusceptible	[138]
Hs25A01	*H. schachtii*	F-box-containing protein, a chalcone synthase and the translation initiation factor eIF-2 b subunit (eIF-2bs)	*Arabidopsis thaliana*	susceptibility	[130]
Hs30D08	*H. schachtii*	SMU2 (homolog of suppressor of mec-8 and unc-52 2)	*Nicotiana benthamiana*	susceptibility	[139]
Hs10A06	*H. schachtii*	Spermidine Synthase2 (SPDS2)	*Arabidopsis thaliana*	susceptibility	[122]
HgGLAND18	*H. glycines*	---	*Nicotiana benthamiana*	suppresses both canonical basal and HR immune responses	[131]
HgSLP-1	*H. glycines*	*Rhg1* α-SNAP	*Glycine max*	avirulence protein	[132]

2.3. *Rhg1 and Rhg4 as major QTLs for SCN resistance*

SCN gets into the roots of susceptible and resistant soybean cultivars equally [140]. Resistant cultivars prevent SCN infection by disrupting syncytium formation interfering its life cycle. Histological experiments have unraveled that syncytia forming in resistant plants undergo a hypersensitive like response [141]. The sources for the SCN resistance in the commercial soybean cultivars are predominantly Peking (PI548402), PI88788, and PI437654 that carry resistance loci

effective against various races of SCN [142, 143]. Till date, 40 QTLs have been reported in a diverse group of resistant cultivars which are mapped in 17 of 20 chromosomes [143]. Three recessive resistance *rhg1-rhg3* were initially assigned in the Peking plant introduction [144]. *rhg1* confers resistance to SCN in all germplasms with resistance to SCN and is regarded to be significant resistance gene to SCN in soybean cultivars [143]. Moreover, PI437654 and PI88788 each have a different functional SCN resistance allele at or close to *rhg1* [142]. *rhg1* was initially reported as the recessive locus, however, recent studies have shown exhibiting incomplete dominance [145]. The *rhg1* locus has been present in various resistance plant introductions PI209332, PI437654, PI90763, PI209332, PI89772, PI90763, including Peking (PI548402), PI88788, and PI437654 [143]. *Rhg1* locus has been mapped to chromosome 18's subtelomeric region [146, 147, 148, 149]. *Rhg4,* a dominant locus, is present in PI54840 (Peking) and PI437654 but not in PI88788 or PI209332 [142, 143, 150]. *Rhg4* locus has been mapped to on chromosome 8 (linkage group A2) for SCN resistance [143, 151].

2.4. *LRR-RLK genes were considered as the resistance genes against H. glycines until 2010*

Rhg1 and *Rhg4* genomic regions in the soybean and two leucine-rich repeat transmembrane receptor-like kinase (LRR-RLK) genes were patented by two groups [146, 147, 152, 153]. Such claims were based on the similarity of the genes with rice bacterial blight resistance gene *Xa21* [154]. Their claims were accepted by the soybean community but their functional aspect was not studied until 2010. Melito et al. 2010 [145] used artificial microRNA (amiRNA) to study the function of *Glyma18g02680.1* gene (LRR-RLK) at the *Rhg1* locus. The amiRNA used for the reduction of expression of *Glyma18g02680.1* gene from the *Rhg1* locus of Fayette (PI88788 source of *Rhg1*) did not alter the resistance to SCN but instead affected the root development. Later Liu et al. 2011 [155], used the Targeting Induced Local Lesions In Genomes (TILLING) approach to study the role LRR-RLK at the *Rhg4* locus developing EMS-mutants from the SCN-resistant soybean cultivars Forrest and Essex. The TILLING tool is the reverse genetic tool to the function of the gene [156]. They concluded that the *Rhg4 LRR-RLK* gene is not a gene for SCN resistance. After the availability of the complete genome sequence of soybean, it has been easier to narrow down these loci regions and find the additional and particular candidate genes that can potentially be involved in the SCN resistance [157].

2.5. *Role of GmSNAP18 (Rhg1) and GmSHMT08 (Rhg4) uncovered for SCN resistance*

The study by Kim et al. 2010 [158] showed *rhg1-b* within a 67-kb region in PI88788 genotype. Because of the existence of allelic variants of *rhg1* in the different soybean genotypes, the *rhg1* in PI88788 was named as *rhg1-b* [142, 158]. This 67-kb interval from PI88788 does not include the LRR-RLK gene candidate for *rhg1* from Peking cultivar that was previously patented. Matsye et al. 2011 [159] studied the expression of the genes within the 67 kb interval of the *rhg1-b* locus. The genes amino acid transporter (*Glyma18g02580*) and a soluble NSF attachment protein (α –SNAP; *Glyma18g02590*), were specifically expressed in syncytia during the SCN defense in both Peking (PI548402) and PI88788 genotypes. α –SNAP coding regions are identical in resistant genotypes Peking (PI548402) and PI437654 but differ by numbers in single nucleotide polymorphisms (SNPs) with Williams 82 (PI518671) genotype [160]. Later, in a 31-kilobase (kb) segment at *rhg1-b* loci, genes *Glyma.18G022400* formerly *Glyma18g02580*, *Glyma.18G022500* formerly *Glyma18g02590*, *Glyma.18G022700* formerly *Glyma18g02610* that encodes an amino acid transporter, an α-SNAP (soluble N-ethylmaleimide–sensitive factor attachment protein) protein, and a WI12 (wound-inducible domain) protein, respectively were identified that play a significant role in SCN resistance [161, 162]. The WI12 protein may involve in producing phenazine like compounds that can be toxic to the nematodes [161, 163]. α -SNAP protein, involves in vesicle trafficking that

affects the exocytosis of food in syncytium which in turn affects the nematode physiology [161]. The plant transporter protein, *Glyma18g02580* consists of tryptophan/tyrosine permease family domain [161]. Tryptophan upon catalysis by Trp aminotransferases such as AtTAA1 and PsTAR1 and subsequent flavin mono-oxygenase such as YUC forms indole-3-acetic acid which is a precursor of the hormone auxin [164]. This suggests that *Glyma18g02580* may affect the auxin distribution in the soybean plants [161]. Based on *Glyma18g02590* (*GmSNAP18*) gene, the cultivars Peking-type and PI88788 type can be differentiated upon selecting the *rhg1* resistance alleles using two specific KASP (kompetitive allele-specific PCR) SNP markers. [165]. The 31 kb segment is present as a single copy in the susceptible cultivar, whereas, the resistant variety, PI88788, and Peking (PI548402) possess 10 and three tandem copies, respectively [161]. Additionally, Cook et al. 2014 [166] tested *Rhg1* across 41 diverse soybean cultivars using whole-genome sequencing, fiber-FISH (fluorescence in situ hybridization) methods. The study showed seven *Rhg1* copies in PI548316, nine copies in PI88788, and 10 copies in PI209332 whereas, both PI437654 and PI548402 (Peking), which show a high level of SCN resistance contain three copies of the *Rhg1* with α-SNAP allele [166]. Lee et al. 2015 [167] genotyped the *Rhg1* locus in 106 SCN-resistant *G. max* and *G. soja* genotypes developing genomic qPCR assay for the identification of copy number of *Rhg1* locus and found 2–4, 6, 7, 9 and 10 copies in *G. max* and one three-copy variant in a *G. soja* genotype. Fayette, derived from PI88788, has 10 copies of the repeat that suggested an increased copy number by a single unit during the process of selection.

The use of forward genetics and functional genomics approaches showed the Peking-type *rhg1* resistance in Forrest cultivar depends on the *Rhg4* (*GmSHMT08*) gene, SCN-resistant allele [168]. Such resistance in Forrest cultivar (resistance to SCN requires both *rhg1* and *Rhg4*) differs from the PI88788-type of resistance that only requires *rhg1* [151, 168]. *GmSHMT08* gene was emerged because of the artificial selection during the soybean domestication process accumulating a higher number of non-synonymous mutations [169]. A recent study by Liu et al. 2017 [170] narrowed down the interval to ~14.3 kb in the recombinant lines of Forrest cultivar that contained three genes in three tandem repeats with in *rhg1-a* locus. These genes encode armadillo/β-catenin-like repeat, amino acid transporter, and soluble N-ethylmelaimide sensitive factor (NSF) attachment protein (*GmSNAP18*). The mapping results and based on SNPs and InDels in Forrest, Peking, and PI88788 cultivars, *GmSNAP18* was identified as a *rhg1* candidate gene for SCN resistance. Additionally, genetic complementation analyses of *GmSNAP18* revealed its different role in PI88788-type *GmSNAP18* and Peking type *GmSNAP18*. Thus both Peking type *GmSHMT08* (*Rhg4*) and *GmSNAP18* (*Rhg1*) play a different role from PI88788-type *GmSHMT08* and *GmSNAP18*. Bayless et al. 2016 [162] confirmed the presence of a dysfunctional variant of resistance-type α-SNAP in the resistant cultivars that impairs the NSF function reducing its interaction during 20S complex formation. This leads to disruption in vesicle trafficking causing an abundance of NSF protein in the syncytium which is cytotoxic. However, because of the two duplication events that occurred 13 and 59 million years ago (mya) in William 82 soybean genome [157], soybean encodes other four α-SNAPs *GmSNAP02*, *GmSNAP09*, *GmSNAP11*, and *GmSNAP14*, known as wild-type α-SNAPs [162, 171]. Among them, *GmSNAP11* is a minor contributor to SCN resistance but not *GmSNAP14* and *GmSNAP02* [171]. These wild-type α-SNAPs counteract the cytoxicity for the viability of soybeans that carry haplotypes of *Rhg1* for SCN resistance [162]. In the presence of SCN, the ratio of resistance-type to wild-type α-SNAP increases leading to the hyperaccumulation of resistance-type α-SNAP that reduces the viability of the syncytium [162] (Figure 3). Also, some other genes such as ascorbate peroxidase 2, β-1,4-endoglucanase, soybean momilactone A synthase-like, cytochrome b5, DREPP membrane

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protein-family, plastocyanin –like including serine hydroxymethyltransferase decreased female index of SCN by 50 % or more in SCN susceptible cultivar William 82 upon overexpression [172].

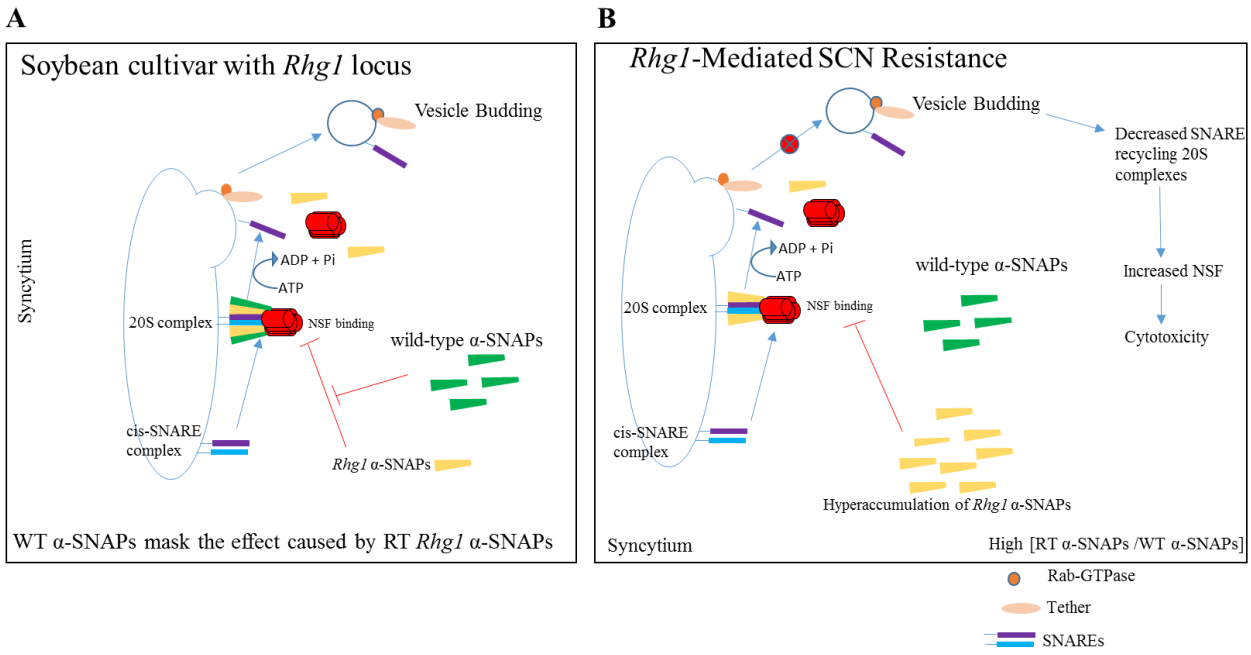

Figure 3. Role of α-SNAP in vesicular trafficking **A.** The wild-type α-SNAPs counteract the cytoxicity for the viability of soybeans that carry haplotypes of *Rhg1* for SCN resistance **B.** In presence of SCN, the ratio of resistance-type to wild-type α-SNAP increases leading to the hyperaccumulation of resistance-type α-SNAP. The presence of high RT α-SNAPs dysfunctional variants in the resistant cultivars impair the NSF function and reducing its interaction during 20S complex formation. This leads to disruption in vesicle trafficking causing an abundance of NSF protein in the syncytium which is cytotoxic. Concept adapted from [162] and [173].

Liu et al. 2012 [174] used two recombinants that carry resistance allele at the *rhg1* and *Rhg4* loci, to study a gene at the *Rhg4* loci. The cultivars used in the study were double recombinants for an 8-kilobase (kb) interval carrying the *Rhg4* resistance allele that carries two important genes, serine hydroxymethyltransferase (SHMT) and the other a subtilisin-like protease (SUB1). *SHMT* (*GmSHMT08*) gene was confirmed as the resistance gene at the *Rhg4* locus that catalyzes methylene carbon of glycine to tetrahydrofolate (THF) to form methyleneTHF, that reacts the second glycine to form L-Ser in the glycolate pathway [175]. This reaction produces S-adenosyl-Met (SAM) which is the precursor for the polyamines and plant hormone ethylene [168]. *GmSHMT08* changes its enzymatic properties because of the changes in two amino acids (P130R and N385Y) in the resistant allele that negatively affects the folate homeostasis in the syncytium resulting hypersensitive responses (HR) leading to programmed cell death (PCD) [169, 174] (Figure 4). The alleles of *GmSHMT08* are different between resistant and susceptible plants [174].

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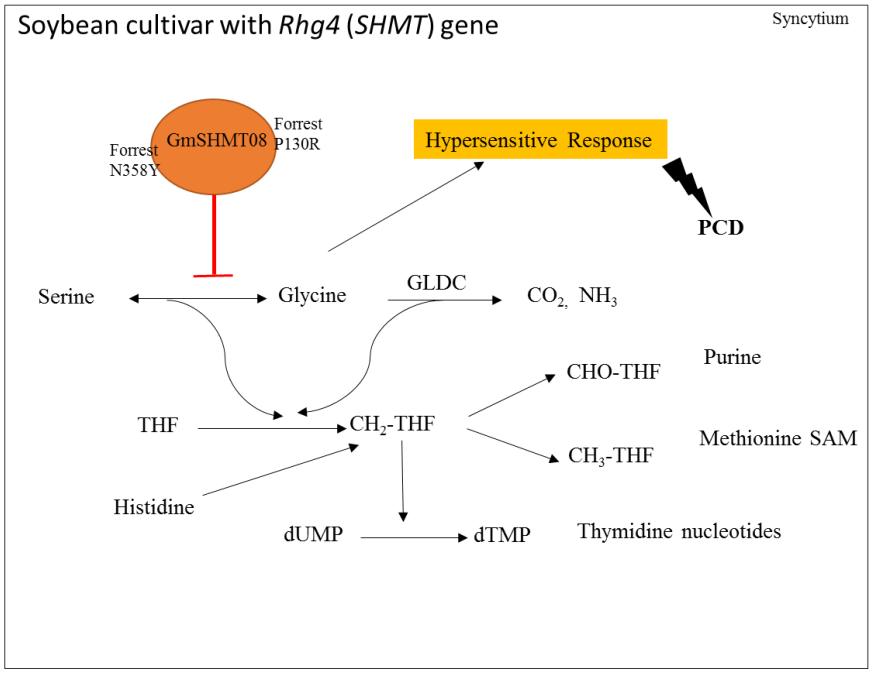

Figure 4. Schematic overview of *GmSHMT08* function and C1 metabolism. SHMT, GLDC, and degradation of histidine feed into the pool of C1 units bound by THF. GmSHMT08 with changes in two amino acids (P130R and N385Y) in Forrest cultivar negatively affects the folate homeostasis in the syncytium resulting in hypersensitive responses (HR) leading to programmed cell death (PCD). dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; GLDC, glycine decarboxylase; SAM, S-adenosyl methionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate. Concept adapted from [169, 174] and pathway adopted from [176].

2.6. *Minor QTLs/genes for SCN resistance*

Apart from the major QTLs identified in *Rhg1* and *Rhg4* loci, there are some minor genes or QTLs identified such as *qSCN10* on chromosome 10 in PI567516C cultivar [177]. PI567516C cultivar lacks two major loci *Rhg1* and *Rhg4* and is SCN resistant that implies the importance of other minor genes for SCN resistance [178]. The resistance acquired by the major genes is sometimes not durable and necessitates the use of horizontal or quantitative resistance acquired from the minor genes [179]. Other minor QTLs are *qSCN-003* in PI88788 [180], *qSCN-005* in Hartwig, which has SCN resistance from PI437654 and Peking [181], and *qSCN-11* in PI437654 and PI90763 [182, 183]. The most recent QTLs reported are cqSCN-006 and cqSCN-007 in *Glycine soja* PI468916 [184] which was mapped finely by Yu and Diers 2017 [185] where *cqSCN-006* was mapped into a 212.1 kb interval and *cqSCN-007* to a 103.2 kb interval on the Williams 82 reference genome in chromosome 15 and 18 respectively. *cqSCN-006* QTL consists of three major potential candidate genes: *Glyma.15g191200* (Soluble NSF attachment protein), *Glyma.15g191300* (BED-zinc finger related), *Glyma.15g191400* (BED-zinc finger related). *Glyma.15g191200* is predicted to encode soluble N-ethylmaleimide–sensitive factor attachment protein (γ-SNAP) that involves in the same function as α-SNAP, which is one of the important genes in *Rhg1* mediated SCN resistance. Likewise, the potential genes identified in the region of *cqSCN-007* are: *Glyma.18g244500* (Lecithin-cholesterol acyltransferase), *Glyma.18g244600* (Apetala 2 transcription factor), *Glyma.18g244700* (Calcineurin-like phosphoesterase), *Glyma.18g244800* (Chromatin

565 assembly factor 1 subunit A), *Glyma.18g244900* (p-Nitrophenyl phosphatase), *Glyma.18g245000*
566 (Rad21/Rec8-like protein), *Glyma.18g245200* (LETM1-like protein) which are mainly involved in
567 signaling pathways, such as transcription, euchromatin expression, and membrane receptor
568 detection. These identified potential candidate genes might be novel SCN resistance genes that
569 should be functionally characterized in the future [185].
570
571 **Table 3**: List of SCN resistance QTLs soybean cultivars with chromosome location, markers
572 associated against SCN HG types or races.

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QTLs	Chromosome and markers associated	SCN HG type or races	Soybean Plant Introductions	References
cqSCN-001 (*Rhg1*)	18	Race 3	PI437654	[186]
		Race 1a, 3a, 3b, 1b, 6	PI209332	[187]
		Races 2, 3 and 5	PI90763	[183, 188]
		Races 1, 3, and 6	PI88788	[188]
		Races 1, 2, were verified in Peking conditioning resistance to SCN 3, 5	PI89772	[189]
		Races 2, 3 and 5	PI404198A	[190]
rhg1-b	18	PA3 (HG type 7) and TN14 (HG type 1.2.5.7)	PI88788	[142]
	18; 67-kb region of the 'Williams 82' genome between BARCSOYSSR_18_0090 and BARCSOYSSR_18_0094	PA3, which originally had an HG type 0 phenotype	PI88788	[158]
cqSCN-002 (*Rhg4*)	8	Race 3	Peking	[140, 151]
		Race 3	PI437654	[186]
cqSCN-003	16	PA3 (HG type 7, race 3) and PA14 (HG type 1.3.5.6.7, race 14)	PI88788	[180]
cqSCN-005	17	HG Type 1.3 (race 14) and HG Type 1.2.5 (race 2)	Hartwig (PI437654 and Peking)	[181]
cqSCN-006	15; (803.4 kb region between SSR markers BARCSOYSSR_15_0886 And BARCSOYSSR15_0903)	HG type 2.5.7 (SCN isolate PA5)	*G. soja* PI468916	[184]
	15; 212.1 kb interval between ss715621232 and ss715621239.	HG type 2.5.7 (SCN isolate PA5)	*G. soja* PI468916	[185]
cqSCN-007	18; (146.5 kb region between the SSR markers BARCSOYSSR_18_1669 and BARCSOYSSR_18_1675)	HG type 2.5.7 (SCN isolate PA5)	*G. soja* PI468916	[184]
	18; 103.2 kb interval between BARCSOYSSR_18_1669 and ss715631888.	HG type 2.5.7 (SCN isolate PA5)	*G. soja* PI468916	[185]
cqSCN 10	10 (Satt592, Satt331, and Sat_274)	LY1 nematode from a mass mating of SCN Race 2 (HG Type 1.2.5) females with Race 5 (HG Type 2.5)	PI567516C	[177]
cqSCN11	11	HG types 0, 2.7, and 1.3.5.6.7 (race 3, 5, and 14)	PI437654	[182]
		Races 2 (HG type 1.2.5.7), 3 (HG type 0) and 5 (HG type 2.5.7)	PI90763	[183]

573

574 *2.7. **GWAS Study in SCN Resistance expands other QTLs on SCN***

575 The GWAS technique has also been used to identify candidate genes for SCN resistance in
576 relatively less time and simultaneously verifying QTLs identified by classical bi-parental mating
577 [82, 83, 84, 191, 192, 193, 194]. Wen et al. 2014 [192] reported 13 GWAS QTLs for SCN resistance
578 associated with the sudden death syndrome (SDS) QTLs spanning a physical region of 1.2 Mb
579 (1.2-2.4 Mb) around three *Rhg1* genes. This might be because of the close linkage of *Rfs2* and *Rhg1*
580 genes that provide resistance to SDS and SCN resistance, respectively [195]. Han et al. 2015 [194]
581 reported 19 significant QTLs related to resistance to both SCN HG Type 0 (race 3) and HG Type
582 1.2.3.5.7 (race 4) using 440 soybean cultivars. Of the reported SNPs, eight overlapped to QTLs
583 with *Rhg1* and *Rhg4* genes, eight to other known QTLs and three were the novel QTLs (on
584 chromosome 2 and 20). The gene, *Glyma.02g161600*, which encodes the RING-H2 finger domain
585 nearest to the novel loci could be the new source of SCN resistance. Vuong et al. 2015 [83] utilized
586 553 soybean PIs and SoySNP50K iSelect BeadChip (with 45,000 SNP markers) to detect the QTL
587 or genes for HG Type 0 SCN resistance using GWAS study. Fourteen loci with 60 SNPs were
588 significantly associated with the SCN resistance. Of the 14 detected loci, six QTL that was
589 identified using bi-parental mapping including *Rhg1* and *Rhg4* were also verified. These GWAS
590 QTLs contained 161 candidate genes located at significant GWAS loci for SCN resistance in
591 soybean. Among them, 26 genes belonged to NBS encoding genes that contained PF90031
592 domain in the proteins. Chang et al. 2016 [84] reported significant loci to multiple races of SCN
593 using GWAS of which one SNP was within *Rhg1* locus for SCN races 1, 3 and 5. Among the five
594 LRR-RLK genes, *Glyma18g02681* and *Glyma20g33531* were nearest to two significant SNPs
595 s715629308 and ss715638409, respectively. Additionally, they reported significant SNPs on
596 chromosomes 4, 7, 10, 15, 18, and 19 for SCN races 1 and 5 (HG type 2). However, Li et al. 2016
597 [191] employed joint linkage mapping and association mapping using 585 informative SNPs
598 across recombinant inbred lines (RILs) bred from the cross Zhongpin03-5373 (ZP; resistant to
599 SCN) × Zhonghuang13 (ZH; susceptible to SCN) to detect alleles associated with SCN race 3.
600 Association mapping revealed three quantitative trait nucleotides (QTNs): *Glyma18g02590*
601 (belonged to locus *rhg1-b*), *Glyma11g35820* and *Glyma11g35810* (a *rhg1-b* paralog). Whereas,
602 linkage mapping revealed two QTLs (one mapping to *rhg1-b* and another to *rhg1-b* paralog).
603 Upon combining both linkage and association mapping, six significant markers were detected.
604 Among them, four (Map-5118, Map-5255, Map-5431, and Map-5432) of the significant markers
605 were not identified in the independent study. Map-5431 lies between *rhg1-a* and *rhg1-b*
606 (*Glyma18g02650*) and Map-5432 lies adjacent to *rhg1-a* (*Glyma1802690*) [195].
607
608 Zhang et al. 2016 [193] utilized 235 wild soybean (*G. soja* Sieb. & Zucc.) accessions to
609 unravel the genetic basis for HG Type 2.5.7 (race 5). GWAS revealed 10 significant SNPs
610 associated with SCN resistance, among which four SNPs were linked to known QTL, *rhg1* on
611 chromosome 18. Remaining four were linked to race 5 resistance QTL [196] and two to 35.5 to
612 37.8Mb region that overlaps some region identified by Vuong et al. 2015 [83]. Additionally, 58
613 potential gene candidates were suggested that belonged to genes encoding NBS-LRR proteins
614 (*Glyma.18G078000*, *Glyma.18G077900*), MAPK proteins (*Glyma.18G106800*) RLPs
615 (*Glyma.18G193800*), a RING/U-box protein (*Glyma.18G063500*), and MYB family transcription
616 factors (*Glyma.19G119300*). Recently, Zhang et al. 2017 [82], performed GWAS in 1032 on *G. soja*
617 with 42,000 SNPs to dissect the genetic basis for resistance to race 1. Ten significant SNPs were
618 identified in chromosomes 2, 4, 9, 16, and 18, among which two were within the previously
619 identified QTLs (SCN 18-5 and SCN 19-4; [196] in chromosome 4, one within QTL SCN 37-2 [178].
620 This study strongly suggests *R* gene, *Glyma.18G102600*, to be the promising candidate gene for
621 the SCN resistance because of its location in strong linkage disequilibrium block.

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The non-redundant 249 genes assessed from the GWAS SCN QTLs [82, 83, 84, 191, 192, 193, 194] showed most of the genes enriched to binding (GO: 0005488), and catalytic activity (GO: 0003824). The binding category includes nucleoside binding (GO: 0001882), nucleotide binding (GO: 0000166), purine ribonucleotide binding (GO: 0017076), purine nucleoside binding (GO: 0001883), ribonucleotide binding (GO: 0032553), adenyl nucleotide binding (GO: 0030554), adenyl ribonucleotide binding (GO: 0032559), ATP binding (GO: 0005524), and ADP binding (GO: 0043531). Similarly the catalytic category includes transferase activity (GO: 0016740), transferase activity-transferring phosphorus-containing groups (GO: 0016772), phosphotransferase activity-alcohol group as acceptor (GO: 0016773), kinase activity (GO: 0016301), protein kinase activity (GO: 0004672), exopeptidase activity (GO: 0008238), serine-type exopeptidase activity (GO: 0070008) (Figure 5).

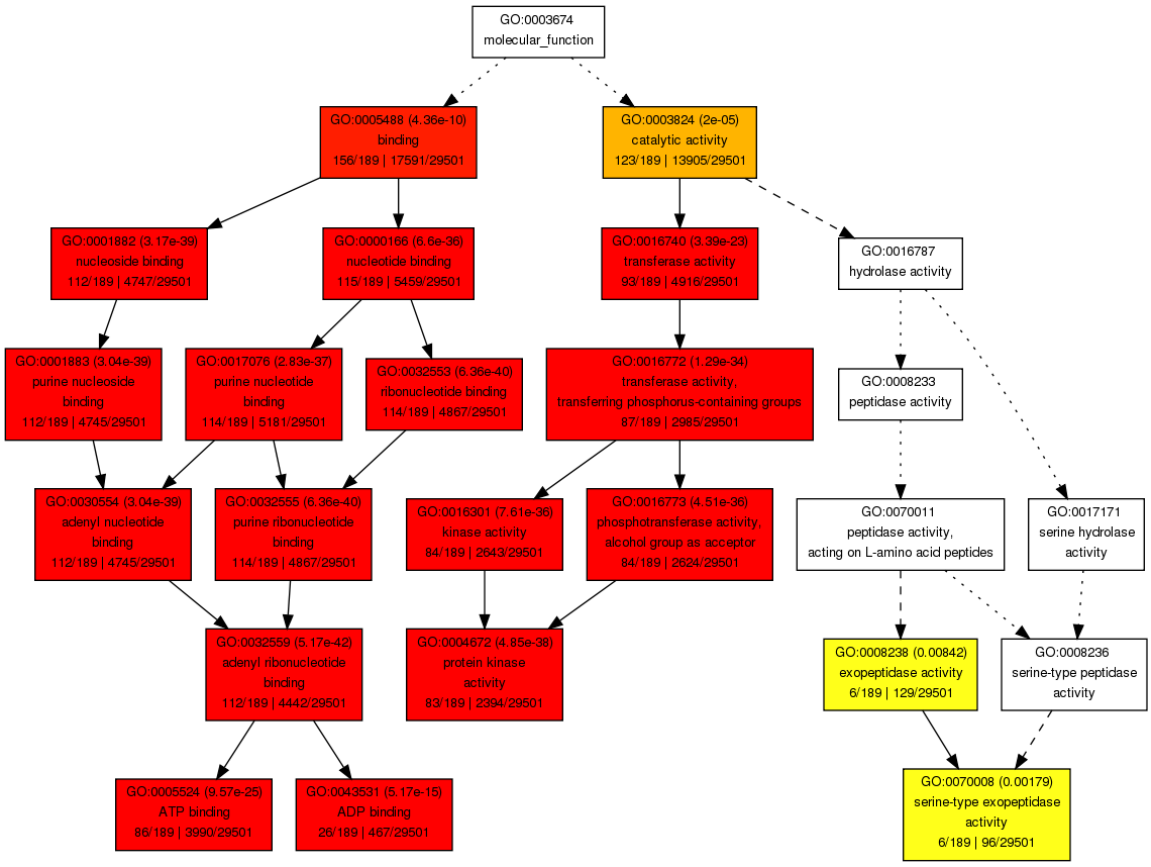

Figure 5. Significantly enriched GO molecular function terms of non-redundant 249 genes in the GWAS SCN QTLs [82, 83, 84, 191, 192, 193, 194] as determined by a hypergeometric test using AgriGO [80]. The same gene can be associated with multiple GO annotations. Only significantly ($P < 0.01$) over-represented and Bonferroni adjusted GO categories are shown. The stronger color represent the lower *P* value. The box consists of the following information: GO term, adjusted P value, GO description, a number of query list and background mapping GO, and the total number of query list and background.

643
644 *2.8.* ***Transcriptomic studies of SCN reveal a complex network of genes for SCN resistance***
645 Hosseini and Matthews 2014 [197] used transcriptomic and regulatory analyses to investigate the
646 effects of NH1–RHg (Race 3) and TN8 (Race 14) SCN populations on soybean roots (Peking
647 cultivar) at 6 and 8 days after inoculation (DAI). The authors found that *β*–1, 4-glucanase,
648 chalcone synthase, and superoxide dismutase, as well as genes for heat shock proteins (HSPs)
649 and isoflavonoids, exhibited race-specific expression in the roots. Additionally, 30 of 46
650 transcription factor binding sites (TFBS), including HAHB4, MYB77, and OsCBT, were either
651 over- or underrepresented in both races. Li et al. 2018 [198] studied time course (5, 10, 15 DAI)
652 transcriptomic changes in SCN-resistant and SCN-susceptible lines of soybean (ZDD2315-
653 resistant, Liaodou15-susceptible) infected by SCN race 3. *Rhg1* and genes related to cytochrome
654 P450, isoflavone-related pathways, phytoalexin synthesis, pathogen-related proteins, and
655 transcription factors, including bHLH, MYB, LOB, bZIP, WRKY, C2H2, and NAC, were
656 differentially expressed in these cultivars.
657
658 Recent research on transcriptomic of SCN has been carried out in wild relatives of soybean or
659 other hosts. Zhang et al. 2017 [199] performed RNA seq analysis in two different cultivars of *G.*
660 *soja*, resistant genotype (PI424093) and a susceptible genotype (PI468396B) using SCN HG type
661 2.5.7. The differentially expressed genes in resistant cultivar (2, 290 genes) were higher than the
662 susceptible cultivar (555 genes) among which genes related to pathogen recognition, calcium-
663 mediated defense, hormone signaling, MAPK signaling, and WRKY transcription factors were a
664 prominent one. Interestingly, they found 16 NBS-LRR genes that showed significant expression
665 upon SCN infection among which *Glyma.17G180000* was strongly induced in PI424093 cultivar.
666 Jain et al. 2016 [200] studied the effect of SCN HG Type 0 in resistant (PI533561) and susceptible
667 cultivar (GTS-900) of the common bean after 8 days post infection. The authors reported
668 successful infection by SCN in common bean for the first time. Various TFs, protein kinases and
669 other important genes such as NBS encoding genes, WRKY transcription factors, pathogenesis-
670 related (PR) proteins, and heat shock proteins were differentially expressed as in interaction
671 between common bean and SCN. A recent study by Tian et al. 2017 [201] utilized the role of small
672 RNAs in defense against pathogens in soybean-SCN interaction study. Among small RNAs,
673 mostly microRNAs (miRNAs) play a crucial role in regulating various genes in their transcription
674 (mRNA) or translation (proteins) processes [202]. The authors utilized two soybean genotypes,
675 susceptible (KS4607), and resistant (KS4313N) and SCN HG type 7 to study the effect of soybean
676 miRNAs during SCN infection. Both conserved (gma-miR159, gma-miR171, gma-miR398, gma-
677 miR399, and gmamiR408) and legume-specific miRNAs (gma-miR1512, gma-miR2119, and gma-
678 miR9750) were suggested to be the potential candidates for the manipulation of SCN infection.
679
680 ***3. Aphid-nematode interactions in the host plant reveal communication via systemic***
681 ***tissues: Soybean-Soybean aphid - SCN relationship***
682 The series of cell signaling events such as plasma membrane potential variation, calcium
683 signaling and generation of reactive oxygen species leads to the production of hormones and
684 metabolites upon infection by pests [203]. In most cases, the release of hormones are specific such
685 as jasmonic acid (JA) in response to chewing herbivores, cell content feeders and necrotrophic
686 pathogens whereas salicylic acid (SA) in response to piercing-sucking herbivores [204]. However,
687 ethylene (ET) is produced synergistically with JA and modulate in JA and SA signaling pathway
688 [205]. The change in metabolite products during the herbivore feeding occurs both in local and
689 systemic tissues [206]. Both above- and belowground herbivores though segregated share the
690 host plant through the systemic tissues and influence each other [207]. Such herbivory has

increased diversification across the insects [208]. Numerous belowground organisms that feed on plant roots such as nematodes, pathogens, fungi, and insects can fluctuate the concentration of defense compounds such as phenolics, terpenoids or glucosinolates, both in belowground and aboveground plant tissues [209]. The impact of root-feeders on shoot defense and the effects of aboveground herbivory on root defense was remained unnoticed for a long time [210]. Till date, many studies to understand relationship between plant-aphid-nematode interactions have been done [6, 118, 209, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222] (Table 4).

The interaction between insect herbivores with their own host creates the condition called induced susceptibility that assists other subsequent herbivores [223] and this type of susceptibility takes place between conspecifics on susceptible as well as resistant plants [223, 224]. The phenotype of conspecific can be both virulent and avirulent biotype. This can be explained by the increased survival capacity of avirulent *Myzus persicae* (Sulzer) on the initially fed resistant plant by avirulent *M. persicae* [225]. Hence the diverse population of both virulent and avirulent that appear phenotypically similar can stimulate induced susceptibility on the resistant plants [226]. Varenhorst et al. 2015 [227] concluded, virulent soybean aphid increases the suitability of resistant soybean for avirulent conspecifics. This implies of lack of genetic differentiation seen in North America between soybean aphid populations on resistant and susceptible soybean. Induced susceptibility arises through two different ways in *A. glycines:* feeding facilitation and obviation of resistance [224, 228]. The influence of soybean cyst nematode, *Heterodera glycines* on aphid, *Aphis glycines* infestation or *vice versa* has been studied on soybean [213, 214, 222, 229, 230]. The study of the interaction effect of SCN and soybean aphid on 'Williams' soybean cultivar revealed that soybean aphid chooses the plants that are uninfected with SCN and the population growth of aphids remained unaffected by SCN infection [213] in laboratory conditions. Further, this study was validated in the natural field conditions (both open plots and experimental cages) where aphids preferably colonized uninfected soybean plants with SCN. Also, the population growth of the aphids remained almost the same in SCN infected and uninfected soybean plants. Further, the independent effect was observed in soybean yield in the presence of soybean aphid and SCN in the field. The effect of SCN was related to a decline in soybean yield, whereas soybean aphid was related to a decline in seed weight depending on their respective population densities. Heeren et al. 2012 [230] utilized resistant and susceptible lines with respect to both soybean aphid and SCN in order to study the interaction effect of soybean aphid and SCN in the field conditions. The effect of soybean aphid feeding on soybean on the SCN reproduction was not observed in any of the soybean cultivars as the SCN eggs and aphid densities, less than 100 SCN eggs per 100 cc of soil and less than 10 aphids per plant for <10 days, respectively, were too low in some of the cultivars. McCarville et al. 2012 [222] conducted experiment on different SCN susceptible (DK 28-52, IA 3018, IA 3041) and SCN resistant (DK 27-52, AG 2821 V, IA 3028) soybean cultivars to understand the effect of multiple pests/pathogens (soybean aphid, SCN, and the fungus *Cadophora gregata*) interaction. The study showed that the SCN reproduction was increased (5.24 times) in the presence of soybean aphid and *C. gregata*. In contrast, the aphid population decreased by 26.4% in the presence of SCN and *C. gregata* and the SCN resistant cultivars (derived from PI88788) reduced apid exposure by 19.8%. Later, McCarville et al. 2014 [214] demonstrated the relationship between the aboveground feeding of soybean aphid and reproduction belowground of SCN in the SCN resistant (Dekalb 27-52, PI88788 derived) and SCN susceptible (Kenwood 94) soybean cultivars. The authors concluded that soybean aphid feeding improved the quality of soybean as a host for SCN but this result was varied significantly with the cultivar and length of the experiment. In 30- days, the SCN eggs and females increased by 33% (1.34 times) in SCN-resistant cultivar and reduced by 50% in the SCN-

susceptible cultivar. In 60-days, the numbers of SCN eggs and females remained unaffected in the resistant cultivar but decreased in the susceptible cultivar.

4. *PAD4* is involved in both SBA and SCN interactions in soybean

The *PHYTOALEXIN DEFICIENT4* (*PAD4*) gene encodes a lipase-like protein [236] and interacts with *ENHANCED DISEASE SUSCEPTIBILITY 1* (*EDS1*) and *SAG101* (*SENESCENCE ASSOCIATED GENE101*) [237, 238] to promote the accumulation of salicylic acid in response to aphid infestation [239]. Extensive research in AtPAD4 has revealed its role in resistance to the green peach aphid, *M. persicae* [239, 240, 241, 242, 243]. *PAD4* gene is expressed at the site of insect feeding and induces antibiotic and antixenotic defenses against aphids [239]. Though the *PAD4* gene requires *EDS1* to provide resistance against various pathogens such as bacteria and fungi, however, the *M. persicae* mediated plant defense do not require both *EDS1* and SA [240, 241]. However, the *PAD4* gene was associated with *SAG13*, *SAG21*, and *SAG27* genes to cause premature leaf senescence of *M. persicae* infested leaves as a basal resistance in Arabidopsis [240]. Although the function of the *PAD4* gene is widely studied in *M. persicae* and *Arabidopsis* system, there are few studies in the *A. glycines* and host soybean system. In resistant cultivar such as *Rag1* cultivar Dowling, *GmPAD4*, a gene induced by the soybean aphid contributes to antibiosis [244]. Also, the high expression of splice variant *GmPAD4*, *GmPAD4-AS1* in *Rag1* Dowling cultivar suggested a role in defense against aphid infestation [245]. In the context of nematode, *H. glycines*, a study on the expression of gene encoding AtPAD4 in soybean roots revealed the negative effect on SCN populations [246]. This study also showed no influence on the *GmEDS1* transcripts but significant increase of *GmPR1* transcripts. The expression of PR1 depends on the accumulation of SA which is downstream in the SA pathway [247]. The infestation by *M. persicae* has shown the accumulation of *LIPOXYGENASE 5* (*LOX5*) transcripts in roots which is the important enzyme in oxygenating α-linolenic acid in the jasmonic acid pathway [248, 249]. Also, *LOX5* upregulates the *PAD4* expression upon *M. persicae* infestation [249]. This leads to the production of *cis*-(+)-12-oxo-phytodienoic acid (OPDA) and dinor-12-oxo-phytodienoic acid (dn-OPDA) [250]. This system has also worked in *Solanum lycopersicum* in providing resistance against *M. persicae* when *SlPAD4*, the tomato homolog of *Arabidopsis PAD4* was expressed [251]. A recent study has shown the constitutive expression of *LOX2*, *LOX10* and *OPDA-REDUCTASE 3* (OPR3) in the tolerant soybean cultivar KS4202 upon *A. glycines* infestation which suggests the role of lipooxygenases and OPDA in soybean- *A. glycines* system [93]. Also, the role of OPDA and dn-OPDA in nematode resistance has been studied in *Arabidopsis* and root-knot nematode, *M. hapla* system using mutants in the JA-biosynthetic pathway [252]. Altogether, these studies suggest a crucial role of *PAD4* and enzymes involved in the JA pathway in defense against both aphid and nematode. Thus, targeting *GmPAD4* gene and modulating lipoxygenases and OPDA concentration in the soybean plant could possibly play a crucial role in defense against above-ground *A. glycines* and below ground *H. glycines*. The role of *PAD4* in SBA and SCN resistance is shown in Figure 6.

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Table 4 List of host-nematode-aphid interaction studies in diverse host systems.

Host	Nematode	Aphids	Effect	Chemistry	References
Brassica nigra	*Pratylenchus penetrans*	*Pieris rapae*	Negative effect on aphids	Increased phenolics and glucosinate levels	[209]
Agrostis capillaris, *Anthoxanthum odoratum*	Paratylenchidae, Pratylenchidae, and Dolichodoridae	*Rhopalosiphum padi* plus *Aphidius coleman*	Negative effect on aphid population/Reduced parasitoid mortality	Decreased foliar phenolic content and amino acid in phloem sap	[218]
Plantago lanceolata	*Pratylenchus Penetrans*	*Myzus persicae*	Negative effect on aphid population	-	[219]
Brassica oleracea	*Heterodera Schachtii*	*Brevicoryne brassicae*	Reduced body size of aphids	-	[231]
Ammophila arenaria	*Pratylenchus*, *Meloidogyne*, and *Heterodera spp*	*Schizaphis rufula*	Nematodes and aphids negatively affect each other	Reduction of foliar nitrogen and amino acid	[232]
Nicotiana tabacum	*Meloidogyne incognita*	*Trichoplusia ni* and *Manduca sexta*	Positive effects on aboveground aphids	Change of foliar nicotine dynamics	[210]
Brassica oleracea	Nematode species dominant of Cephalobidae and Rhabditidae families	*Brevicoryne brassicae*	Negative effect on aphid density	-	[233]
Arabidopsis thaliana	*Heterodera schachtii*	*Brevicoryne brassicae*	No effect on aphid growth in presence of nematode/reduced number of nematodes in presence of aphids	Reduced glucosinolates in shoots	[217]
Brassica oleracea	*Heterodera schachtii*	*Brevicoryne brassicae*	Increase in aphid doubling time from 3.8 to 6.7 days	Reduced glucapin /Increased gluconapoleiferin and 4-methoxyglucobrassicin in leaves/Decreased amino acid and sugar in phloem	[212]
Solanum tuberosum	*Globodera pallida*	*Myzus persicae*	Positive effect on the reproduction of aphids	Increased SA in the leaves and suppression of JA	[211]
Brassica nigra	*Heterodera schachtii*	*Brevicoryne brassicae*	Lower preference of aphids/ lower reproduction of aphids	Induced PR1 (SA pathway) Reduced VSP2 and MYC2 (JA pathway)	[215]
Brassica nigra	*Meloidogyne hapla*	*Brevicoryne brassicae*	Higher preference of ahids/higher reproduction	No PR1 expression/ High VSP2 and MYC2 expression	[215]
Nicotiana tabacum	*Meloidogyne incognita*, *Tylenchorhynchus* and *Pratylenchus*	*Myzus persicae*	Reduced the abundance of aphids/ *Tylenchorhynchus* was decreased on aphid infested plants/no effect on *Pratylenchus*	-	[234]
Zea mays	*Meloidogyne incognita*	*Ostrinia nubilalis*	Reduced nematode reproduction	-	[221]
Solanum tuberosum	*Globodera pallida*	*Myzus persicae*	Inhibited the hatching of eggs of nematode	Decreased fructose and glucose in the root exudates	[235]
Solanum tuberosum	*Meloidogyne incognita*	*Myzus persicae*	No effect on the nematodes	Decreased the root SA content	[216]
Glycine max	*Heterodera glycines*	*Aphis glycines*	Aphids unaffected/aphid preference	-	[213, 229]
Glycine max	*Heterodera glycines*	*Aphis glycines*	No effect of aphid on SCN reproduction	-	[230]
Glycine max	*Heterodera glycines* plus *Cadophora gregata*	*Aphis glycines*	SCN reproduction increased (5.24 times) in presence of soybean aphid and *C. gregata*/ aphid population decreased by 26.4% in presence of SCN and *C. gregata* in PI88788 derived cultivar	-	[222]
Glycine max	*Heterodera glycines*	*Aphis glycines*	SCN eggs and females increased by 33% (1.34 times) in SCN-resistant cultivar/reduced by 50% in the SCN-susceptible cultivar.	-	[214]

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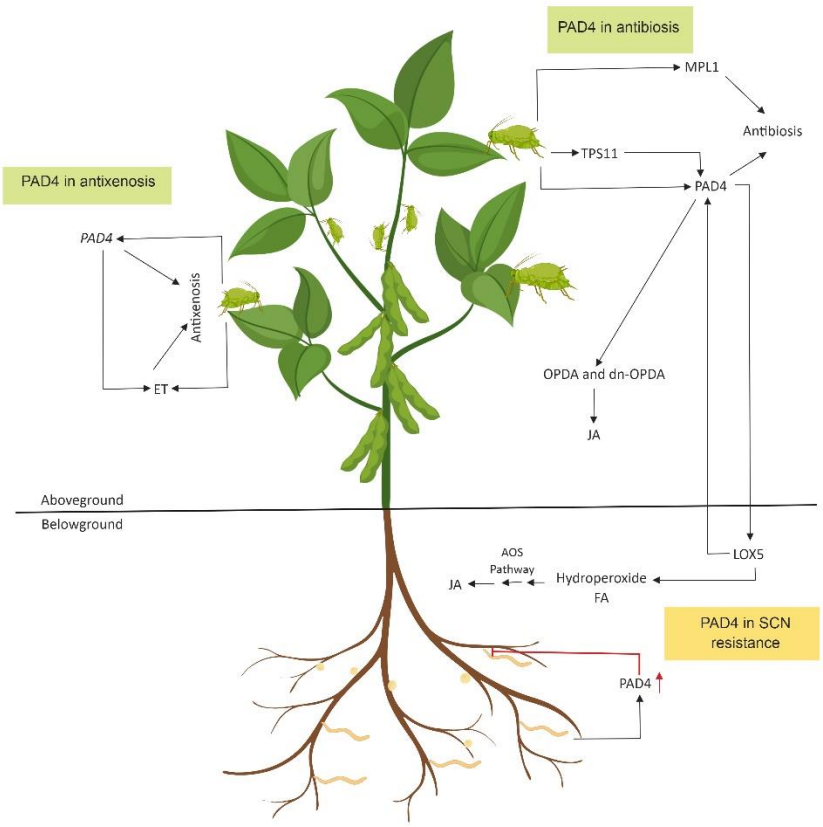

780

781 **Figure 6.** The role of *PAD4* in SBA and SCN resistance. The pathways involved are adapted from
782 various studies [239, 240, 241, 242, 244, 245, 246, 247, 248, 249, 250, 251, 252]. In the shoot, PAD4
783 induced by soybean aphid feeding provide both antibiosis and antixenosis mode of resistance
784 against soybean aphid. The function of PAD4 is well studied in both *M. persicae-Arabidopsis* and
785 soybean-SBA system [243, 244]. The involvement of TPS11 and MPL1 (regulators of PAD4) in
786 aphid resistance (antibiosis) is well studied in *M. persicae-Arabidopsis* system [253, 254, 255]. The
787 antixenosis mode of resistance against aphid is caused by the accumulation of ethylene. In *M.*
788 *persicae-Arabidopsis* system, aphid feeding causes accumulation of LOX5 in the root which is the
789 crucial enzyme in the jasmonic acid pathway [248, 249]. In addition, LOX5 upregulates PAD4 in
790 the shoot leading to the production of *cis*-(+)-12-oxo-phytodienoic acid (OPDA) and dinor-12-oxo-
791 phytodienoic acid (dn-OPDA) [250]. In the root, expression of PAD4 causes negative effect on
792 SCN [246]. Altogether, these studies suggest PAD4 as a key protein in interactions among SBA
793 and SCN. TPS11: *TREHALOSE-6-PHOSPHATE SYNTHASE 11*; MPL1: *MYZUS PERSICAE-*
794 *INDUCED LIPASE 1*, PAD4: *PHYTOALEXIN DEFICIENT4*, FA: Fatty Acids, AOS: allene oxide
795 synthase, JA: Jasmonic acid, ET: Ethylene, LOX5: *LIPOXYGENASE 5*, OPDA: *cis*-(+)-12-oxo-
796 phytodienoic acid, dn-OPDA: dinor-12-oxo-phytodienoic acid. The illustration is created with
797 Biorender (https://app.biorender.com/).

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801

5. Conclusions and Future Directions

Resistance to the soybean aphid and SCN is in each case mediated by several genes, including *Rag* genes for the soybean aphid and *Rhg* genes for the SCN. While significant progress has been made towards identifying the genes for SCN resistance, the genes responsible for soybean aphid resistance remain largely obscure. The advent of sequencing technologies has made now the availability of soybean, soybean aphid, and SCN (*de novo* assembly) genomes. This should speed the discovery of particular molecular cues in terms of effector and host resistance components. With the development of various gene editing tools such as CRISPR/Cas9 system and advancement in producing various mutant hosts could help on understanding the function of the genes. In addition, since soybean aphid and SCN have been co-existed in many soybean fields we should target the common pathway so that these pests could be mitigated. *GmPAD4*, lipoxygenases, and OPDA could possibly provide the resistance against both soybean aphid and SCN when expressed in the soybean plant.

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