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Identification of Adzuki Bean Quantitative Trait Loci Associated With Resistance to Soybean Cyst Nematode

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ABSTRACT

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) infection has recently been shown to cause yield loss in adzuki bean (*Vigna angularis* [Willd.] Ohwi & Ohashi). Development of SCN-resistant cultivars is used extensively to manage SCN in soybean (*Glycine max* [L.] Merr.). It is considered the most effective way to manage SCN in adzuki bean. Adzuki bean germplasm "Acc2766" shows high resistance to SCN Races 1 and 3, but it is poorly adapted to the environment of Hokkaido because of late maturity. To enable cultivation in this region, genetic improvement is necessary, and development of DNA markers is expected to accelerate breeding for SCN resistance in adzuki bean. Using GRAS-Di technology, 491 single nucleotide polymorphism markers were identified between "Acc2766" and the susceptible cultivar "Shumari." QTL analysis was performed using individual F_2 plants derived from a cross between "Shumari" and "Acc2766." Three QTLs associated with SCN resistance were detected: *Qrhgaz-1* on Chromosome 1, *Qrhgaz-8* on Chromosome 8, and *Qrhgaz-9* on Chromosome 9. The results of tests using BC_3F_3 lines indicated that the introduction of all three QTLs was necessary to confer high resistance. DNA markers closely linked to these QTL regions may be useful for the selection of SCN-resistant lines in future adzuki bean breeding programs.

1 | Introduction

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is a devastating pest of soybean [*Glycine max* (L.) Merr.] worldwide (Matsuo et al. 2017). In Japan, SCN Races 1 (HG Type 2.5.7), 3 (HG Type 0), and 5 (HG Type 2.7) (Arelli and Wang 2008; Young 1995) have been identified, and SCN Races 1 and 3 are dominant (Inagaki 1979; Shimizu and Momota 1992). To control SCN infestation, the breeding of resistant soybean cultivars has been promoted, and cultivars resistant to SCN Race 3 were released in 1965. Since then, many additional SCN-resistant soybean cultivars have been developed. In 2019, about 84.4% of the soybean cultivation areas were planted with cultivars resistant to SCN Race 3, and in Hokkaido, about 14.4% were planted with cultivars resistant to SCN Race 1 (Hokkaido Government 2021). In recent years, cases have been identified in which soybean cultivars resistant to SCN Race 3 were infected by SCN Race 1. Developing cultivars resistant to SCN Race 1 is becoming very important in soybean breeding in Hokkaido.

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Adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] is another important crop in Hokkaido, accounting for about 93% of the total harvest in Japan. Adzuki bean is also susceptible to SCN, but no action has been taken, as it was not recognized that adzuki bean was damaged as badly as soybean. However, SCN has been found in approximately 20% of the fields in Hokkaido (Todai 2010), and cultivation of adzuki bean in soils containing more than 100 eggs per gram (dry weight) can result in a 46% yield reduction (Todai 2014).

The adzuki bean germplasm "Acc2766" shows high resistance to SCN Races 1 and 3. Growing this germplasm reduces the population density of SCN in soil as much as growing the SCN-resistant soybean cultivar "Suzuhime" (Kousaka 2015; Todai and Tazawa 2010, 2013). Unfortunately, "Acc2766" is poorly adapted to the environment of Hokkaido because of late maturity and other agronomic traits. Therefore, the breeding of adzuki bean using "Acc2766" as a donor parent for SCN resistance was initiated at Tokachi Agricultural Experiment Station (AES). However, the development of resistant lines while retaining excellent agronomic traits is a complex task.

In soybean, one dominant gene and two recessive genes were found to be involved in SCN resistance in an analysis using "Peking" and "PI 90763" (Rao-Arelli et al. 1992). SCN Race 3 is effectively controlled by a combination of the *rhg1* and *rhg2* genes, whereas SCN Race 1 is controlled by *rhg1*, *rhg2*, and *Rhg*4 (Suzuki et al. 2020; Suzuki, Tanaka, et al. 2012). In common bean (*Phaseolus vulgaris* L.), several genome regions involved in SCN resistance were identified in genome-wide association studies (GWASs) by Jain et al. (2019) and Shi et al. (2021). However, no genetic information is available about SCN resistance in adzuki bean.

Han et al. (2005) constructed a comprehensive genetic linkage map of adzuki bean from simple sequence repeat (SSR) markers, amplified fragment length polymorphism markers, and restriction fragment length polymorphism markers. The map was constructed using a backcross population of (*Vigna nepalensis* × *V. angularis*) × *V. angularis*. When adzuki bean cultivars "Shumari" and "Acc2766" were compared in this way in preliminary investigations for the work reported here, 66 out of 205 SSR markers (32.2%) did not show polymorphism, probably due to their similar genetic backgrounds. Therefore, new methods to detect a large number of polymorphisms were needed. In recent years, various technologies for high-throughput genotyping have been developed, including genotyping by random amplicon sequencing-direct (GRAS-Di) technology, which easily acquires a large number of single nucleotide polymorphisms (SNPs) in any species (Ezeah et al. 2023; Umeda et al. 2021).

To develop adzuki bean cultivars with resistance to SCN Race 1, GRAS-Di technology was applied. The aims of the study reported here were to identify quantitative trait loci (QTLs) associated with resistance to SCN Race 1 and to develop flanking PCR-based DNA markers. SNPs were detected in an F_2 population from a cross between SCN-resistant and susceptible adzuki bean cultivars, and a linkage map was constructed. Then QTL analysis was performed using SCN inoculation test results from individual F_2 plants to identify loci conferring resistance.

2 | Materials and Methods

2.1 | Plant Materials

"Acc2766" is resistant to SCN Race 1 and is maintained at the Tokachi AES of the Hokkaido Research Organization. "Shumari" is an SCN-susceptible cultivar. The F_2 plants were derived from one individual F_1 plant from a single cross between "Acc2766" and "Shumari," and 85 F_2 plants were used for the SCN Race 1 inoculation tests. To evaluate detected QTLs, backcrossed (BC_3) lines were developed using "Acc2766" as the resistance donor and the susceptible cultivar "Erimo-167" as the recurrent parent. We used "Erimo-167" as the recurrent parent because it is one of the most widely grown adzuki bean cultivars in Hokkaido; it was registered in 2022, and our breeding goal is to provide SCN resistance to "Erimo-167" as soon as possible.

2.2 | SCN Population and Preparation of Inoculum

The SCN population was collected from a field at Tokachi AES. To propagate this population, a susceptible adzuki bean cultivar ("Kita-roman") was grown in the SCN-infested field. Using four standard soybean SCN race differentials (Riggs and Schmitt 1988), the SCN population was identified as SCN Race 1 (data not shown). Cysts were collected from the infested soil and crushed, and the released eggs were suspended in sterile distilled water. The supernatant was decanted and discarded, and the remainder was resuspended in sterile distilled water; this was repeated several times. Finally, the collected eggs were resuspended in 3-mM $ZnSO_4$ and held at 25°C until hatching. The hatched second-stage juveniles were used for evaluation of SCN resistance. The numbers of second-stage juveniles in 1 mL of the suspension were counted using a nematode counting plate (Fujiwara Scientific, Tokyo, Japan) under a stereoscopic microscope at 20× magnification (SZX12, Olympus, Tokyo, Japan).

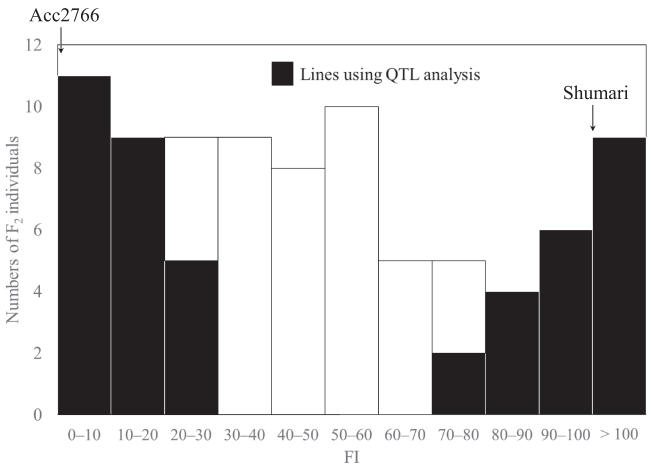


FIGURE 1 | Frequency distribution of soybean cyst nematode female index (FI) in individual F_2 adzuki bean plants from a cross between "Acc2766" and "Shumari" ($n = 85$). The mean FI of the parents is indicated by arrows. Black bars indicate the numbers of F_2 individuals used for the QTL analysis.

TABLE 1 | Primers used in this study.

	QTL name	Marker	Position (Mbp)	Marker type	Primer	Sequence (5′ to 3′)[a]	SNP source or fragment size (bp)[b]	
Chromosome 1	*Qrhgaz-1*	Vi01G007200	0.424	KASP	Primer_AlleleX	TCCATGCCTAGCATAACCAGATAACA-FAM	Acc2766	T
					Primer_AlleleY	CATGCCTAGCATAACCAGATAACG-HEX	Shumari	C
					Primer_Common	AACATAAAGATATCGTCTCATGGAACACAA		
		CEDG149	0.590	SSR	CEDG149F-M13	cacgacgttgtaaaacgacGGCTGAAGGTGATGACAGAAG	Acc2766	208
					CEDG149R	GGCACTGGTTTTCTAAGGTTGTTG	Shumari	206
Chromosome 8	*Qrhgaz-8*	Vi08G060800	4.673	KASP	Primer_AlleleX	CTCATGGATTGGCATCACATGTTCTA-FAM	Acc2766	A
					Primer_AlleleY	TCATGGATTGGCATCACATGTTCTG-HEX	Shumari	G
					Primer_Common	TCACTACACGGATACGTGGTCCTT		
		CEDG271	7.443	SSR	CEDG271F-M13	cacgacgttgtaaaacgacGCACTAAAGTTAGACGTGGTTC	Acc2766	243
					CEDG271R	CACTCCCACTGCCAAACAAGG	Shumari	241
Chromosome 9	*Qrhgaz-9*	Vi09G030400	3.465	SCAR	Vi09G030400_01	TGAAATGCATTTGGTGTATG	Acc2766	609
					Vi09G030400_02	CCTCAACACTTTCTTGCTG	Shumari	1088
					Vi09G030400_03	GGTCGCTATCGGAGTATCC		
					Vi09G030400_04	GTATTGTACAAATGTCTTGAGC		
		Vi09G051700	6.810	SCAR	Vi09G051700_01	CTAAGCTTGTACATGTAGTATATC	Acc2766	399
					Vi09G051700_02	GTGCTTTAGATCAACATTGC	Shumari	695
					Vi09G051700_03	GTGAAAAACTTTCTTTTTCTGGG		
					Vi09G051700_04	CCTTTGTACAATCCCTCCCC		

[a]The sequence "cacgacgttgtaaaacgac" is the M13 tail.
[b]Fragment size differs from the actual size because of the analysis method used.

2.3 | Evaluation of SCN Resistance

Tests were conducted in a growth chamber (model MLR-352, PHC, Tokyo, Japan). F_2 adzuki bean seeds were sown individually in four seedling plug trays (plug size 43 × 43 × 43 mm; 30 plugs per tray). Each plug was filled with 50 mL of a mixture of granular culture soil and organic matter-rich culture soil, with pH adjusted to 6. The susceptible control "Shumari" was sown at four or five random locations in each tray. The resistant control "Acc2766" was sown at four random locations in Tray 1. The trays were held at 32°C in the dark until germination, and then the plants were grown at 24°C under 24-h lighting. When the main leaves had expanded, a suspension containing over 2000 second-stage SCN juveniles was poured onto each plug. The white to yellow females, which appeared on the root surface, were counted 30–35 days after inoculation. Because of some variability in the number of female parasites (NFP) among the trays, the number of females associated with individual F_2 plants was evaluated by female index (FI; Niblack 2005). The FI was calculated for the plants in each tray as follows: [(the number of females on an individual F_2 or "Acc2766" plant)/(the mean number of females on a "Shumari" plant)] × 100.

2.4 | QTL Analysis

QTL analysis was performed using 25 F_2 plants with FI < 26.4 and 21 F_2 plants with FI > 75.3 (Figure 1). Total DNA was extracted from young leaves by a modified CTAB method (Suzuki, Sato, and Takeuchi 2012) and treated with RNase (Qiagen, Hilden, Germany). DNA concentration was determined using the Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA) with a Qubit Fluorometer 2.0 (Invitrogen, Grand Island, NY, USA), and at least 15 ng μL^{-1} was used for analysis. The GRAS-Di, marker linkage analyses, and identification of SNPs were outsourced to Bioengineering Lab Co. Ltd. (https://gikenbio.com). To discard low-quality reads, the raw data were cleaned using Sickle v.1.33 software (https://github.com/najoshi/sickle). The cleaned reads were mapped to the *V. angularis* v.1.0 reference genome (https://viggs.dna.affrc.go.jp/download) using Bowtie 2 v.2.3.2 software (https://sourceforge.net/projects/bowtie-bio/files/bowtie2/). The QTL analysis used physical distance based on the *V. angularis* v.1.0 reference genome. To output SNPs and insertions and deletions, mpileup and BCFtools of Samtools v.0.1.19 (https://www.htslib.org) were used. Then, using VCFtools (http://vcftools.sourceforge.net), quality filtering was performed with the minimum quality of coverage 10 and minimum depth of coverage 10. QTL analysis was performed using the R/qtl program with the composite interval mapping model (Broman et al. 2003). The following parameters were used: interval = 1 cM, window size = 10 cM, method = Haley–Knott regression, and number of marker covariates = 3.

2.5 | Development of PCR-Based Markers

Kompetitive allele-specific PCR (KASP) and sequence-characterized amplified region (SCAR) markers flanking each QTL peak were designed from the sequences of the surrounding regions (Table 1). SSR markers (Han et al. 2005) were genotyped using Taq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA). PCR products were analyzed on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems) with GeneMapper software as described by Suzuki et al. (2015). KASP markers were genotyped using KASP Assay Mix and KASP v.4.0 2X Master Mix (LGC Biosearch Technologies, Teddington, Middlesex, England). PCR and endpoint fluorescence readings were carried out using QuantStudio 3 (Applied Biosystems). Two SCAR markers were genotyped using a HotStarTaq Master Mix Kit (Qiagen). PCR was performed following the manufacturer's instructions; the annealing temperature was 55°C. PCR products were separated on 2% agarose gels and stained with SYBR Gold Nucleic Acid Gel Stain (Invitrogen).

2.6 | Evaluating Detected QTLs

Individual BC_3F_2 seeds (196 in total) derived from a single BC_3F_1 plant were sown, and the plants were selected using six DNA markers linked to the detected QTLs (Table 2). Two BC_3F_2 plants were randomly selected for each of eight genotypes, and BC_3F_3 seeds of the selected plants were harvested. The resulting BC_3F_3 lines were evaluated for SCN resistance by randomly sowing 5–10 individual plants of each line in multiple trays. We compared the NFP infesting each line by the Tukey–Kramer test in EZR software (Kanda 2013).

TABLE 2 | QTL analysis for soybean cyst nematode Race 1 resistance in adzuki bean based on F_2 population ("Acc2766" × "Shumari").

QTL	Chromosome	LOD peak position (cM)	LOD[a]	PVE[b] (%)	Additive effect[c]	Dominance effect	Flanking markers[d]
Qrhgaz-1	1	0.43	7.61	19.8	−81.6	35.3	Vi01G007200–CEDG149
Qrhgaz-8	8	6.56	6.15	20.2	−56.4	−64.4	Vi08G060800–CEDG271
Qrhgaz-9	9	5.14	9.60	30.9	−88.7	−50.3	Vi09G030400–Vi09G051700

[a]Logarithm of the odds score of the nearest marker.
[b]Percentage of phenotypic variation explained by the nearest marker.
[c]Values indicate the effect of the "Acc2766" allele on the QTL.
[d]Designed across the LOD peak position.

TABLE 3 | Number of female parasites (NFP) found on adzuki bean plants grown from seeds of a cross between "Shumari" (susceptible) and "Acc2766" (resistant) after artificial soybean cyst nematode inoculation.

	NFP per F_2 plant					NFP per "Shumari" plant					NFP per "Acc2766" plant				
	Number of plants	Min	Max	Mean	SD	Number of plants	Min	Max	Mean	SD	Number of plants	Min	Max	Mean	SD
Tray 1	12	2	315	156	96.3	5	200	300	270	17.9	4	0	5	1.3	2.5
Tray 2	24	2	265	112	84.1	5	223	270	250	9.8	—	—	—	—	—
Tray 3	24	4	315	134	81.0	4	275	368	312	20.4	—	—	—	—	—
Tray 4	25	4	376	144	111.1	4	223	255	240	7.7	—	—	—	—	—

3 | Results

3.1 | Segregation of SCN Race 1 Resistance

The mean number of SCN female parasites per "Shumari" plant ranged from 240 (SD 7.7) to 312 (SD 20.4), whereas that per "Acc2766" plant ranged from 0 to 5 (Table 3). The FI values of individual F_2 plants were continuously distributed from 0.7 to above 100 (Figure 1). Low FI (below 10) was observed in 11 F_2 plants, whereas FI higher than that of the susceptible parent (i.e., above 100) was observed in 9 plants.

3.2 | QTL Analysis for SCN Race 1 Resistance

A total of 491 SNPs between the parents were reported by the outsourcing lab. The linkage map for QTL analysis was constructed using SNP markers identified by the GRAS-Di technique (Figure S1). The total length of the map was 453.8 Mbp, with an average marker interval of 0.9 Mbp (range, 0.0–12.6 Mbp). A minimum of 26 SNP markers per chromosome was mapped. A logarithm of odds (LOD) score threshold was not set because of the small number of individual F_2 plants. Instead, we defined the QTL region by an LOD score of 3.0 or higher, and then we evaluated the detected QTLs with BC_3 lines. Three QTLs were detected on Chromosomes 1, 8, and 9, which were named *Qrhgaz-1*, *Qrhgaz-8*, and *Qrhgaz-9*, respectively (Figure 2). Peak LOD scores were 7.61 (0.43-cM position on Chromosome 1), 6.15 (6.56-cM position on Chromosome 8), and 9.60 (5.14-cM position on Chromosome 9), respectively (Table 2). The phenotypic variation explained by the nearest markers of these QTLs in 46 individual F_2 plants ranged from 19.8% to 30.9%. The additive effects of these three QTLs were all negative (−56.4 to −88.7), which indicated that the "Acc2766" alleles at these QTLs decreased the FI. The dominance effect of *Qrhgaz-1* was positive (35.3), whereas those of *Qrhgaz-8* and *Qrhgaz-9* were both negative (−64.4 and −50.3, respectively).

The genotypes of individual F_2 plants at the extremes of the FI range are shown in Table 4. In the low-FI group (4.4 and lower), the numbers of plants with the "Acc2766" genotype (A), heterozygous genotype (H), and "Shumari" genotype (B) were 9:2:0, respectively, at marker CEDG149, 4:5:2 at CEDG271, and 4:7:0

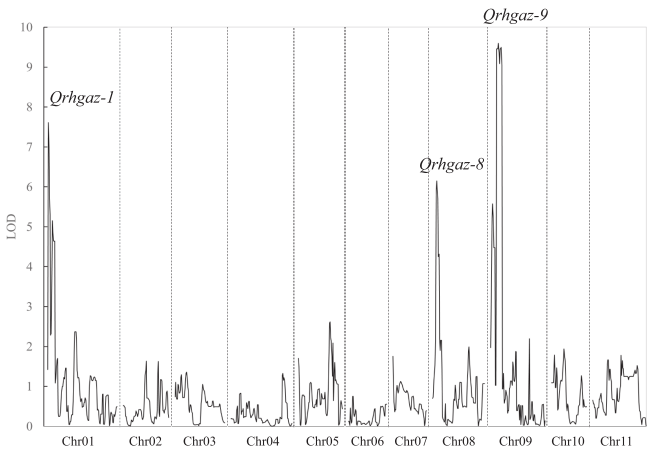


FIGURE 2 | Logarithm of odds (LOD) score for each SNP marker.

at Vi09G030400. In the high-FI group (100.9 and higher), these numbers were 2:3:4 at marker CEDG149, 0:2:7 at CEDG271, and 0:2:7 at Vi09G030400 (Table 4).

3.3 | Evaluation of Detected QTLs in Different Genetic Background

The mean NFP values of the two BC_3F_3 lines with the "Acc2766" alleles at all three QTLs (designated genotype AAA) were 0.7 and 1.0 (Table 5). In contrast, the mean NFP values of the two genotype BBB lines were 141.7 and 160.3. Genotype AAA lines clearly had the lowest NFP, followed by genotype AAB and BAA lines (i.e., with "Acc2766" alleles at two of the QTLs). The lines with genotypes ABA, ABB, and BAB all had intermediate NFP (Table 5). The mean NFP for genotype BBA lines was similar to that for BBB lines (including the "Erimo-167"; data not shown).

4 | Discussion

We initially planned to use more than 100 individual F_2 plants, but only 85 individuals grew normally prior to artificial inoculation with SCN. Based on our inoculation assay results (Figure 1), we hypothesized the presence of multiple loci associated with SCN resistance. Because QTL analysis with a small population size carries a high risk of false positives and false negatives, we dared to exclude intermediate phenotypes from the analysis. Then we validated the effect of the detected QTLs with different materials. For these reasons, only 46 individual F_2 plants were used in the QTL analysis, and

TABLE 4 | Genotypes of flanking markers at the QTLs in individual F_2 adzuki bean plants with low or high soybean cyst nematode female index (FI).

		Genotype of DNA markers					
		Qrhgaz-1		*Qrhgaz-8*		*Qrhgaz-9*	
		Vi01G	CEDG	Vi08G	CEDG	Vi09G	Vi09G
Individual no.	FI	007200	149	060800	271	030400	051700
2	0.7	A	A	A	A	H	H
32	0.8	A	A	H	H	A	A
59	1.3	A	A	A	A	A	A
70	1.7	H	H	H	H	A	A
21	2.8	A	A	B	B	H	H
22	3.2	A	A	H	H	H	H
67	3.3	A	A	B	B	A	A
81	3.3	A	A	H	H	H	H
23	3.6	A	A	H	H	H	H
73	3.8	A	A	A	A	H	H
36	4.4	H	H	A	A	H	H
54	100.9	H	H	H	H	B	B
12	100.9	A	A	B	B	B	B
75	109.6	B	B	B	B	H	H
85	115.0	B	B	B	B	B	B
80	116.3	A	A	B	B	B	B
4	116.8	B	B	H	H	B	B
90	119.2	B	B	B	B	H	H
78	137.5	H	H	H	B	B	B
84	156.7	H	H	B	B	B	B
Acc2766	0.0	A	A	A	A	A	A
Shumari	100.0	B	B	B	B	B	B

Note: "A" (black boxes) indicates homozygous "Acc2766" genotype, "B" (white boxes) indicates homozygous "Shumari" genotype, and "H" (gray boxes) indicates heterozygous genotype.

three QTLs for resistance to SCN Race 1 in adzuki bean were detected. The difference in SCN resistance between the parents was very clear: At least 200 SCN females were observed on plants of the susceptible parent "Shumari," but only 5 or fewer females were observed on plants of the resistant parent "Acc2766" (Table 3). Using progeny of these parents allowed evaluation of F_2 plants with a wide range of FI, enabling accurate phenotyping. This was one of the factors that enabled detection of QTLs despite the limited sample size.

We used a physical map for QTL analysis. In rice (*Oryza sativa*), the genome exhibits an average of approximately 290 kb per 1 cM (Kurata et al. 2002). However, because of the limited information available for adzuki bean, we approximated genetic distance by assuming 1 Mb = 1 cM for convenience. DNA markers were developed using sequences of the detected QTL regions. The BC_3 lines developed using these DNA markers and SSR markers confirmed the validity of the detected QTLs. These QTLs had an additive effect, and the presence of all three QTLs was found to be necessary to confer high resistance. These lines possessing all three QTLs and exhibiting high levels of resistance could be immediately usable in SCN-resistance breeding programs of adzuki bean, for example, to overcome the late maturity of the SCN-resistant parent "Acc2766."

Among 11 F_2 plants in the low-FI group, 9 plants (82%) had a homozygous "Acc2766" genotype at CEDG149 (Table 4). Also in the low-FI group, 5 of 11 plants (45%) and 7 of 11 plants (64%) had heterozygous genotypes at CEDG271 and Vi09G030400, respectively. On the basis of these results and the dominance effect values (Table 2), *Qrhgaz-1* was judged to be recessively inherited, whereas *Qrhgaz-8* and *Qrhgaz-9* were apparently dominantly inherited.

It is widely known that three resistance genes are involved in SCN resistance in soybean. Using soybean cultivars "Peking" and "PI 90763," Rao-Arelli et al. (1992) found that two recessive genes and one dominant gene (*rhg1*, *rhg2*, and *Rhg4*) are involved in SCN Race 1 resistance. These three loci are located in soybean on Chromosomes 8, 11, and 18, respectively (Dong et al. 2020; Kandoth et al. 2017; Suzuki et al. 2020; Suzuki, Tanaka, et al. 2012). In adzuki bean, regions homologous to *rhg1* and *rhg2* were identified near 6.4 Mbp on Chromosome 7, and a region homologous to *Rhg4* was identified near 27 Mbp on Chromosome 1, in a database of the whole genome sequence of "Shumari" (Sakai et al. 2015). However, the QTLs detected

TABLE 5 | Number of female parasites (NFP) on BC_3F_3 adzuki bean lines.

			Genotype of DNA markers			NFP				
			Qrhgaz-1	*Qrhgaz-8*	*Qrhgaz-9*					
			CEDG	CEDG	Vi09G					
Line		Number of plants	149	271	030400	Min	Max	Mean	SD	
TC2041④-20-2-3	BC_3F_3	10	A	A	A	0.0	3.0	0.7	1.2	a
TC2041④-20-2-14	BC_3F_3	10	A	A	A	0.0	5.0	1.0	1.6	a
TC2041④-20-2-21	BC_3F_3	10	A	A	B	22.0	80.0	43.7	19.3	ab
TC2041④-20-2-52	BC_3F_3	10	A	A	B	7.0	51.0	22.8	14.0	a
TC2041④-20-2-83	BC_3F_3	10	B	A	A	24.0	81.0	46.9	19.5	abc
TC2041④-20-2-110	BC_3F_3	10	B	A	A	13.0	56.0	44.0	12.6	ab
TC2041④-20-2-112	BC_3F_3	10	A	B	A	20.0	185.0	105.9	55.1	cd
TC2041④-20-2-189	BC_3F_3	10	A	B	A	80.0	162.0	122.5	26.6	d
TC2041④-20-2-93	BC_3F_3	10	A	B	B	58.0	196.0	111.8	42.5	cd
TC2041④-20-2-169	BC_3F_3	10	A	B	B	45.0	159.0	96.2	34.2	bcd
TC2041④-20-2-44	BC_3F_3	5	B	A	B	91.0	152.0	116.6	23.1	cd
TC2041④-20-2-95	BC_3F_3	10	B	A	B	77.0	201.0	143.7	37.1	cd
TC2041④-20-2-5	BC_3F_3	10	B	B	A	117.0	253.0	174.2	42.3	de
TC2041④-20-2-87	BC_3F_3	10	B	B	A	127.0	181.0	153.5	19.6	de
TC2041④-20-2-88	BC_3F_3	10	B	B	B	93.0	205.0	141.7	39.1	d
TC2041④-20-2-114	BC_3F_3	10	B	B	B	116.0	225.0	160.3	33.0	de
Acc2766		10	A	A	A	0.0	1.0	0.2	0.4	a
Shumari		10	B	B	B	62.0	274.0	176.0	67.8	e

Note: "A" (black boxes) indicates homozygous "Acc2766" (resistant) genotype, and "B" (white boxes) indicates homozygous "Shumari" or "Erimo-167" (susceptible) genotypes. Different lowercase letters represent statistically significant differences at the 5% level by the Tukey–Kramer method.

in this study did not match these homologous regions, and the mode of expression of resistance was different from that in soybean. In addition, the three genes involved in SCN resistance in soybean do not work in an additive manner: If any one of them is lacking, the plants are susceptible (Suzuki et al. 2020). In contrast, the QTLs detected in this study apparently function in an additive manner. Therefore, the mechanisms of SCN resistance in adzuki bean likely differ from those in soybean.

In soybean, the *rhg1* gene, Glyma.18G022500, and the prime candidate gene for *rhg2*, Glyma.11G234500, encode α-SNAP (soluble *N*-ethylmaleimide-sensitive factor [NSF] attachment protein), and the hyperaccumulation of α-SNAP inhibits its infection by the SCN (Bayless et al. 2016). The *Rhg4* gene, Glyma.08G108900, encodes serine hydroxymethyltransferase (SHMT) and reportedly contributes to soybean resistance against SCN by triggering a hypersensitive response that restricts nematode infection (Liu et al. 2012). In common bean, an ortholog of soybean *rhg1* is located near an SNP identified through GWAS associated with SCN resistance (Wen et al. 2019). Based on annotation analysis using Vigna Genome Server (https://viggs.dna.affrc.go.jp), the *Qrhgaz-1* region in adzuki bean was anticipated to code for a plant disease resistance response protein (IPR004265). The *Qrhgaz-8* region was estimated to encode five leucine-rich repeat proteins (IPR001611) as well as a protein with cysteine-type peptidase activity (GO:0008234), which has been reported to kill nematodes by degrading their cuticle layer (Njom et al. 2021). The *Qrhgaz-9* region was expected to code for 20 disease resistance proteins (IPR000767). Together, these findings support the suggestion that "Acc2766" confers resistance through a mechanism that is distinct from that in soybean. The lines carrying *Qrhgaz-1* and *Qrhgaz-8* (designated as genotype AAB) or *Qrhgaz-8* and *Qrhgaz-9* (designated as genotype BAA) had lower NFP values than those carrying *Qrhgaz-1* and *Qrhgaz-9* (designated as genotype ABA). This suggests that the contribution of *Qrhgaz-8* to SCN resistance is substantial and implies that cysteine-type peptidase activity may play an important role in this resistance. Future GWAS using a larger number of genetic resources, as was done for common bean, may identify other novel resistance loci. Perhaps these loci harbor orthologs of *rhg1*, *rhg2*, or *Rhg4*. Further research is needed to explore these exciting possibilities.

We believe this is the first report of SCN-resistance QTLs in adzuki bean. The DNA markers described in this report may be useful for selection of SCN-resistant lines in adzuki bean breeding and could lead to development of SCN-resistant adzuki bean cultivars in the near future. In addition, clarification of the functions of the three SCN-resistance QTLs may be useful for resistance analysis in other legumes.

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Ethics Statement

No ethical review was required because this research did not involve humans and animals.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are stored according to the guidelines for keeping research records at the Hokkaido Research Organization Central Agricultural Experiment Station.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The SNP-Based genetic linkage map. Marker names correspond to chromosome number and physical position.