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Research Paper

Mapping soybean *rhg2* locus, which confers resistance to soybean cyst nematode race 1 in combination with *rhg1* and *Rhg4* derived from PI 84751

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The soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is a devastating pest of soybean (*Glycine max* (L.) Merr.) in the world. Three soybean QTLs for resistance to SCN race 1 were detected through QTL analyses using recombinant inbred lines (RILs) derived from a cross between 'Tokei 758' (susceptible) and 'To-8E' (resistant to races 1 and 3, derived from 'PI 84751' and 'Gedenshirazu'). Two of the three QTLs appear to be *rhg1* and *Rhg4* from their locations on the linkage map. The third QTL, detected around Satt359 on chromosome 11, was tentatively identified as *rhg2*. All RILs resistant to race 1 had all three QTLs. We developed lines carrying the three loci in various combinations, including all and none, from descendants of a cross between 'NIL-SCN' (with resistance derived from 'PI 84751' in the 'Natto-shoryu' background) and 'Natto-shoryu'. Evaluating these lines in a race 1-infected field in Mito, Ibaraki, showed that resistance to race 1 required all three loci. Through field evaluation of 10 recombinant fixed pairs that we developed, we located the *rhg2* locus to an 821 kb-region between SSR markers Sat_123 (=WGSP11_0140) and BARCSOYSSR11_1420 on chromosome 11.

Key Words: soybean cyst nematode resistance, *rhg2*, SCN, QTL, marker-assisted selection, soybean, *Glycine max* (L.) Merr..

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 the USA, it is considered the most economically damaging pest of soybean (Allen *et al.* 2017). By infecting soybean roots, SCN suppresses plant growth, causing stunting and chlorosis (Noel 1999). The female's body develops into a tough, lemon-shaped brown "cyst", which protects the eggs for several years in the soil (Jackson 2014). SCN can enter the roots of both susceptible and resistant soybeans, but resistant soybean disrupts syncytium formation (Mahalingam and Skorupska 1996).

In Japan, SCN races 1, 3, and 5 were identified (Inagaki 1979) in a four-cultivar race test (Golden *et al.* 1970). Races 1 and 3 were dominant (Inagaki 1979, Shimizu and Momota 1992). Their HG (="*H. glycines*") types as determined by a seven-cultivar test (Niblack *et al.* 2002) are unknown. As donors of resistance to SCN, 'Gedenshirazu' (resistant to race 3) and 'PI 84751' (resistant to races 1 and 3) have been used in breeding programs. 'Gedenshirazu', a local cultivar from Akita, has been used since the 1950s, and its descendants include cultivars of the Toyomasari brand in Hokkaido ('Yukihomare', 'Toyomusume', 'Toyokomachi', 'Toyoharuka', and 'Toyomizuki') and 'Ryuhou', a main cultivar in the Tohoku region. 'PI 84751', from Korea, has been used as a donor of resistance to SCN races 1 and 3 since the 1960s. In 1980, 'Suzuhime', which own resistance derived from 'PI 84751', was released for natto production. 'PI 84751'-type resistance was

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also introduced into 'Yukihomare', and 'Yukihomare R' was released in 2010.

Genes involved in resistance are still not clear, but some major genes have been identified. In the USA, where race 3 is predominant (Jackson 2014), resistance loci *rhg1*, *rhg2*, *rhg3* (Caldwell *et al.* 1960), *rhg4* (Matson and Williams 1965), and *Rhg5* (Rao-Arelli *et al.* 1992) were reported. Those on chromosomes (Chrs.) 18 (*rhg1*) and 8 (*Rhg4*) have been consistently mapped as major quantitative trait loci (QTLs) (Concibido *et al.* 2004). The *rhg1* type was classified as *rhg1*-a in Peking-type soybeans and *rhg1*-b in PI 88788-type soybeans (Brucker *et al.* 2005). *rhg1*-b can confer resistance to race 3 by itself (Concibido *et al.* 2004). In cultivars such as 'Forrest', with *rhg1*-a resistance, resistance to race 3 requires both *rhg1* and dominant *Rhg4* (Meksem *et al.* 2001). In *rhg1*-b cultivars, a high copy number of three genes (Glyma18g02580, Glyma18g02590, and Glyma18g02610 in Wm82.a1; Glyma.18g022400, Glyma.18g022500, and Glyma.18g022700 in Wm82.a2) in a 31-kb segment from PI 88788 contributes to PI 88788-type resistance (Cook *et al.* 2012). In *rhg1*-a cultivars, only one of the three genes is needed: Peking-type GmSNAP18 (Glyma18g02590 in Glyma1.0; Glyma.18g022500 in Glyma2.0) was enough to confer resistance in combination with *Rhg4*, which encodes a serine hydroxymethyltransferase (Liu *et al.* 2017).

In Japan, resistance derived from 'PI 84751' was due to three or four loci including *rhg1*, *rhg2*, (*rhg3*), and *Rhg4* (Suzuki *et al.* 2012). Resistance to race 1 was due to *rhg1* and *Rhg4* derived from 'PI 84751' in progeny derived from crosses of lines resistant to race 3 (donor: 'Gedenshirazu') and lines resistant to races 1 and 3 (donor: 'PI 84751'). Resistance to race 3 was stronger in 'PI 84751' than in 'Gedenshirazu' (Suzuki *et al.* 2012).

To identify more resistance genes, we performed QTL analysis using a hybrid population derived from a cross between a susceptible line and a resistant line developed from 'PI 84751'. We detected three QTLs, two near *rhg1* and *Rhg4*. Resistance to race 1 required all three QTLs. Using recombinant fixed pairs, we located the new QTL, tentatively identified as *rhg2*, in an 821-kb region on Chr. 11.

Materials and Methods

Plant materials

For QTL analyses, we used 199 RILs ('Tokei 758'/'To-8E') derived from a cross between susceptible 'Tokei 758' and resistant 'To-8E' developed in Tokachi, Hokkaido, by the single-seed-descent method. Both parents were distributed by the Hokkaido Research Organization.

To develop recombinant fixed pairs rapidly, we used 'NIL-SCN' and 'Natto-shoryu', distributed by the Ibaraki Agricultural Center (Ibaraki Prefecture, Japan). 'NIL-SCN' is a near-isogenic line of 'Natto-shoryu' with SCN resistance from 'PI 84751' and Soybean mosaic virus (SMV) resistance from 'Seiken 2'. 'NIL-SCN' was surveyed its background using 349 SSR markers distributed in whole genome, and 41 out of 349 (11.7%) showed genotype which was different from 'Natto-shoryu'. Because of its small seed size, 'Natto-shoryu' produces more seeds than cultivars of the usual seed size. So even in winter, it is possible to harvest enough number of seeds in greenhouse. In field and a greenhouse, we grew three generations per year. In August 2017, 'NIL-SCN' was crossed with 'Natto-shoryu', and eight resultant F_1 seeds were planted in the greenhouse in November and selfed. In January 2018, we harvested F_2 seeds and selected 44 out of 478 seeds carrying *rhg1* and a recombination in the *rhg2* region using DNA extracted from cotyledon of seed. We also selected F_2 seeds carrying *rhg1*, *rhg2*, and *Rhg4* in various combinations. In March, the selected F_2 seeds were planted in the greenhouse (13 h dark/11 h light for 6 weeks) and the flowers were selfed. F_3 seeds were harvested in May. From 40 F_3 seeds, we selected fixed genotypes to make two recombinant fixed pairs, which we grew in the 2018 field test. From 368 F_3 seeds, we selected eight pairs and planted them in a field in Tsukuba. In November 2018, F_4 seeds were harvested, and eight pairs were obtained as lines.

QTL analysis and SSR markers

A linkage map of the 199 F_9 ('Tokei 758'/'To-8E') RILs carrying 162 SSR markers is described in Sayama *et al.* (2010). QTL analyses were performed by composite interval mapping as implemented in QTL Cartographer 2.5 software (Wang *et al.* 2005). Genome-wide threshold values ($\alpha = 0.05$) were used to detect putative QTLs (1000 permutations) (Churchill and Doerge 1994), and markers with LOD > 2.5 were considered significant.

On the linkage map, and to develop recombinant fixed pairs, we used candidate polymorphic SSR markers (Song *et al.* 2004), GMES markers (Hisano *et al.* 2007), BARCSOYSSR markers (BARCSOYSSR_1.0; Song *et al.* 2010), WGSP markers (Fujii *et al.* 2018), and T markers (Watanabe *et al.* 2018). In developing F_2 ('NIL-SCN'/'Natto-shoryu') lines, we used 'SCN_Res Bridge' (Cook *et al.* 2012), which detects *rhg1*-b, to select seeds with *rhg1*, and 'T000808358s', which detects a 2-bp indel in intron 3 of *Rhg4*, to select seeds with *Rhg4*.

The locations of the markers were based on the genomic sequence of *G. max* 'Williams 82' assembly 2 pseudomolecules (Wm82.a2; Department of Energy Joint Genome Institute Community Sequencing Program 2013).

Evaluation of resistance to SCN race 1 in phytotron

The seeds of the 199 F_{11} ('Tokei 758'/'To-8E') RILs, their parents, and the susceptible control cultivar 'Suzumaru' were sown in plastic trays (5 × 10 cells; 28 cm × 45 cm) filled with soil infested with a race 1 population (Golden *et al.* 1970). After 34 days, we counted the cysts on the roots of three plants. Numbers of cysts were indexed to that of 'Suzumaru' = 100.

Field trials and evaluation of resistance to SCN race 1

We planted the 10 recombinant fixed pairs and their parents, 'NIL-SCN' and 'Natto-shoryu', in a race 1-infested field at the Ibaraki Agricultural Center, Mito, in late June 2018 and 2019. We evaluated SCN resistance in 5 to 7 plants of each line, with two replicates. The numbers of cysts on roots were evaluated in late August to early September. We scored replicates in which two control lines with or without resistance to race 1 was significantly different at least at 1% level.

Cysts on soybean roots were scored from 0 (resistant) to 5 (susceptible) thus: 0, no cysts; 1, 0–10 cysts; 2, 11–30 cysts; 3, 31–50 cysts; 4, 51–100 cysts; and 5, >100 cysts. Scores of five plants were averaged for each line or cultivar.

We compared the two lines in each recombinant fixed pair and compared 'Natto-shoryu' and the F_2 lines by *F*-test and *t*-test in JMP 13.2.1 software (SAS Institute, Cary, NC, USA). We also compared the numbers of cysts of two groups of RILs which genotype was 'To-8E'-type or 'Tokei 758'-type in each QTL region in the same way. We compared the numbers of cysts of each genotype (defined by the three resistance genes) with the 'Natto-shoryu' control by Tukey's multiple comparison test in JMP 13.2.1.

Results

QTL analyses for resistance to SCN race 1

In the 199 RILs, we detected three QTLs on Chrs. 4 and 18 (**Table 1**, top). That with the largest effect was detected around Sat_210 (=WGSP18_0020, 1.6 Mb on the physical map) on Chr. 18. It accounted for 29% of the total variation, and its 'To-8E' allele conferred resistance to SCN race 1. We considered this QTL to be *rhg1*. The other two QTLs accounted for only 4% and 7% of the total variation. RILs were grouped according to genotype of the nearest marker in each QTL and compared by *t*-test (**Supplemental Table 1**). The number of cysts was significantly different between 'To-8E'-type and 'Tokei 758'-type only in *rhg1*.

To detect QTLs which confer resistance in combination with *rhg1*, we selected RILs with *rhg1* and performed QTL analysis again. In 58 such RILs, we detected two QTLs on Chrs. 8 and 11, which accounted for 26% and 30% of the total variation (**Table 1**, bottom). We considered the QTL near Sat_162 (=WGSP08_0060, 8.3 Mb) on Chr. 8 to be *Rhg4*. We tentatively designated that near Satt_359 (32.4 Mb) on Chr. 11, with a larger effect than *Rhg4*, as *rhg2*.

Alleles of rhg2 locus

The genotype of the three loci in the parents of the RILs and the donors of resistance showed that the resistant parent, 'To-8E', had PI 84751-type *rhg1* and *Rhg4* and Gedenshirazu-type *rhg2* (**Table 2**). So 'To-8E', which is resistant to race 1, was found to have Gedenshirazu-type *rhg2* and PI 84751-type *rhg1* and *Rhg4*. The susceptible parent, 'Tokei 758', had non-PI 84751-type, non-Gedenshirazu-type *rhg1*, *Rhg4*, and *rhg2*.

Segregation distortion in RILs

QTL analyses detected three QTLs, but the segregation ratios of the resistant RILs did not fit the expected ratio of 1:7: only eight RILs were resistant among the 199 RILs (**Table 3**), fitting the segregation ratio of 1:15 (resistant:susceptible), at which four loci are expected (**Supplemental Table 2**). This result suggests segregation distortion.

Resistance in genotypes with three resistance loci in various combinations

All RILs were grouped into eight genotypes by three markers, Sat_210 (*rhg1*), Sat_162 (*Rhg4*), and Satt_359 (*rhg2*). The eight RILs with the 'To-8E' allele at all three markers (**Table 3**, top row) had significantly fewer cysts than all other RILs. Thus, soybean resistance to SCN race 1 requires all three loci.

We evaluated F_2 lines derived from 'NIL-SCN' × 'Natto-shoryu' in the race 1 field. Of the two replicates, we scored only one replicate since two control lines ('Natto-shoryu' and 'NIL-SCN') was not significantly different at 1% level. Only one F_2 line, F_2-1, having all three loci, had a lower cyst number score than 'Natto-shoryu' (**Table 4**).

Table 1. QTLs for resistance to soybean cyst nematode race 1 detected in RILs ('Tokei 758'/'To-8E') (LOD > 2.5)

Chromosome	Marker interval[a]	cM	LOD[b]	R^2 (%)[c]	Additive effect[d]	Resistance allele	Reported resistance gene/ QTL at the location
Using all 199 RILs							
4	Satt607–AW277661	33.4–49.0	4.9	4	10	To-8E	–
18	Sat_210–Satt235	0.0–36.6	18.1	29	27	To-8E	*rhg1*
18	Satt199–Satt288	77.4–103.4	4.2	7	–12	Tokei 758	–
Using 58 RILs with *rhg1*							
8	Sat_162–Sat_215	10.5–15.9	5.6	26	20	To-8E	*Rhg4*
11	Satt359–Satt453	130.7–149.7	5.5	30	23	To-8E	*QTL on linkage group B1*

[a] Nearest marker is underlined.
[b] LOD score of the nearest marker.
[c] Percentage of phenotypic variation explained by the nearest marker.
[d] Additive effect = (('P1' allele effect) – ('P2' effect))/2.

Table 2. Genotypes of three resistance loci in parents of RILs and donors of resistance

Cultivar/line	SCN resistance		*rhg1*	*Rhg4*	*rhg2*	
	race 1	race 3	Sat_210	Sat_162	Sat_123	Satt453[a]
PI 84751	R	R	P	P	P	P
Gedenshirazu	S	R	G	G	G	G
P1: Tokei 758	S	S	other	other	other	other
P2: To-8E	R	R	P	P	G	G

P, PI 84751-type; G, Gedenshirazu-type. Note that PI 84751 (resistant to SCN race 1) own *rhg1*, *Rhg4* and *rhg2*, and that Gedenshirazu (resistant to race 3) own only *rhg1* and *rhg2*.
[a] Satt453 (34.2 Mb on Chromosome 11) is 348 kb downstream of Satt484.

Table 3. Genotypes of three loci for resistance to SCN and phenotype of RILs ('Tokei 758'/'To-8E')

Marker[a]	Resistance genes		QTL	Number of RILs[b]	Number of cysts[c]	
	rhg1	*Rhg4*	*rhg2*			
	Sat_210	Sat_162	Satt359		Average SD[d]	
	+	+	+	8	4.9 ± 7.0	a
	+	+	–	25	41.2 ± 9.6	b
	+	–	+	8	40.9 ± 13.3	bc
	–	+	+	26	47.7 ± 5.0	c
	+	–	–	17	43.1 ± 12.7	bc
	–	+	–	41	49.2 ± 5.1	c
	–	–	+	25	47.4 ± 4.9	bc
	–	–	–	47	48.8 ± 5.3	c

[a] + 'To-8E'-type (resistant to race 1); – 'Tokei 758'-type (susceptible).
[b] RILs heterozygous or not detected at any marker are not shown.
[c] Number of cysts was indexed that of 'Suzumaru' = 100.
[d] Tukey's multiple comparison test.

Mapping rhg2 using recombinant fixed pairs in race 1 field

To locate the QTL on Chr. 11, we evaluated the recombinant fixed pairs in the race 1 field in Mito (**Fig. 1**). We evaluated two recombinant fixed pairs (selected from F_3 ('NIL-SCN'/'Natto-shoryu')) in 2018 (**Fig. 1**, top) and eight pairs (F_4 lines) in 2019 (**Fig. 1**, bottom). An 821-kb region between Sat_123 (WGSP11_0140) and BARCSOYSSR11_1420 on Chr. 11 contained the resistance QTL.

Discussion

We identified three QTLs for race 1 resistance in RILs derived from a cross between a susceptible parent and the resistant line 'To-8E', with 'PI 84751' and 'Gedenshirazu' ancestors (**Table 1**, **Supplemental Fig. 1**). We identified two of the three QTLs as *rhg1* and *Rhg4* from their locations. All three QTLs were needed for resistance: if any one of them was lacking, the plant was susceptible (**Tables 3**,

Table 4. Genotypes of three loci for resistance to SCN and phenotype of F_2 ('NIL-SCN'/'Natto-shoryu') lines

Cultivar or line[a]		Marker	Resistance genes		QTL	Number of plants	Score of cyst number	
			rhg1	*Rhg4*	*rhg2*		Average SD	*P*[b]
			SCN_Res Bridge	T000808358s	Satt359 (WGSP11_0150)			
P1	Natto-shoryu		–[c]	–	–	5	4.2 ± 0.4	
P2	NIL-SCN[d]		+	+	+	5	0.8 ± 0.4	<0.0001***
F2	F_2-1		+	+	+	5	1.2 ± 0.4	0.0018**
	F_2-2		+	+	–	5	3.0 ± 1.0	ns
	F_2-3		+	+	–	4	3.5 ± 0.9	ns
	F_2-4		+	+	–	5	3.9 ± 0.2	ns
	F_2-5		+	+	–	5	4.1 ± 0.5	ns
	F_2-6		+	–	+	5	3.5 ± 0.3	ns
	F_2-7		+	–	–	5	3.2 ± 1.1	ns
P1	Natto-shoryu		–	–	–	5	3.6 ± 0.8	
P2	NIL-SCN[d]		+	+	+	5	1.2 ± 0.4	0.0018**
F2	F_2-8		–	+	–	5	3.7 ± 0.2	ns
	F_2-9		–	–	+	5	3.2 ± 1.1	ns
	F_2-10		–	–	–	5	3.7 ± 0.4	ns

[a] F_2 lines from a cross between 'NIL-SCN' and 'Natto-shoryu'. 'NIL-SCN' is a near-isogenic line with resistance from 'PI 84751' in the 'Natto-shoryu' background.
[b] 'NIL-SCN' and all F_2 lines were compared with 'Natto-shoryu', their background.
[c] + 'NIL-SCN'-type (resistant to race 1); – 'Natto-shoryu'-type (susceptible).
[d] 'NIL-SCN' (resistance from 'PI 84751') was compared with 'Natto-shoryu' to verify the range of scores of cyst number.

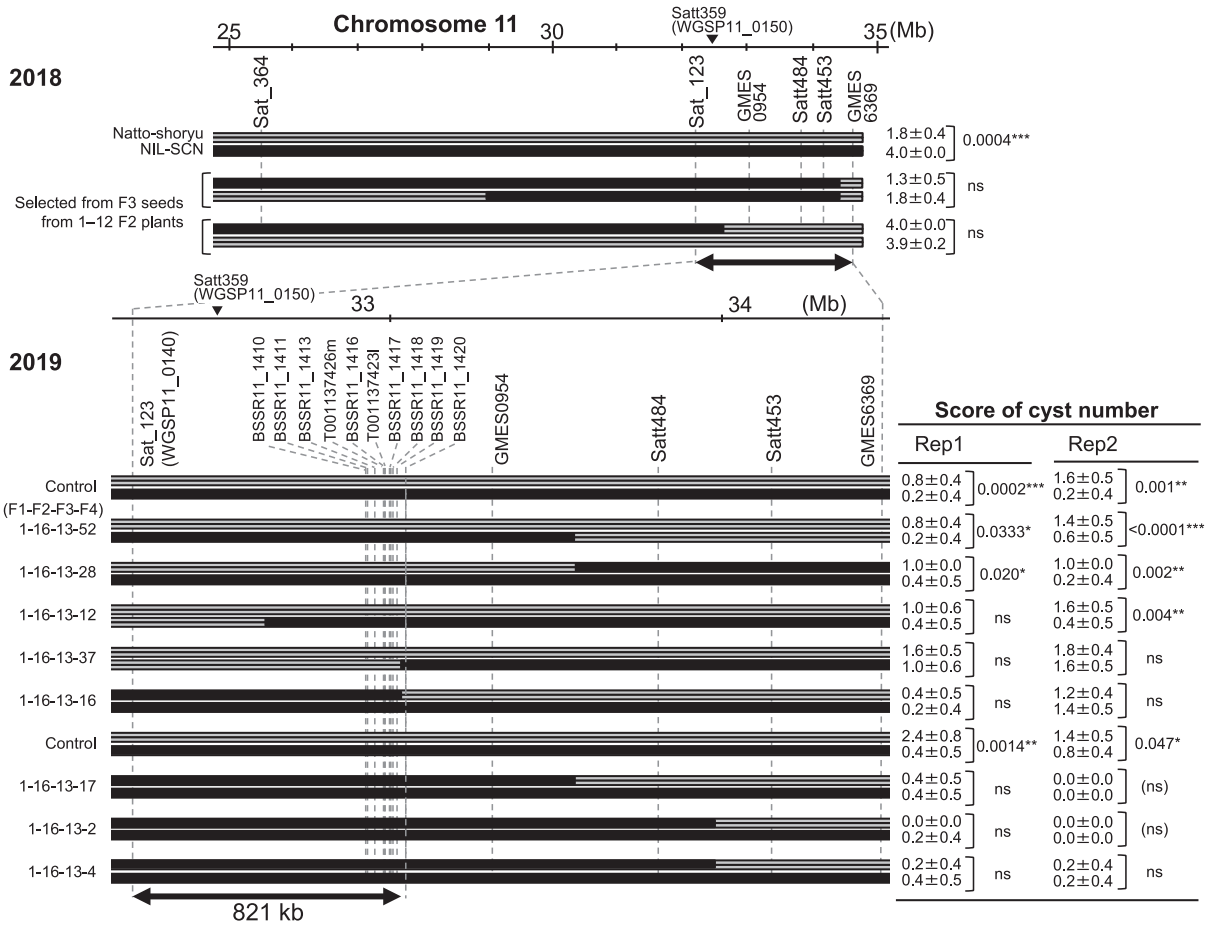

Fig. 1. Graphical genotypes of recombinant fixed pairs carrying 'NIL-SCN' fragments around the QTL regions on chromosomes 11. Except for 'Natto-shoryu', all lines and 'NIL-SCN' have *rhg1* and *Rhg4* in common. Top: F_3 seeds selected in 2018. Bottom: F_4 lines in 2019. Controls in 2019 were F_4 lines with or without an 'NIL-SCN' fragment that covers this QTL region. ¦ Positions of SSR markers. 'BARCSOYSSR' (part of the name of some markers) is abbreviated 'BSSR'. □ 'Natto-shoryu' fragments; ■ 'NIL-SCN' fragments. ▼ Nearest marker in QTL analysis. Scales (in Mb) are based on the 'Williams 82' genome (Wm82.a2). The significance of differences in scores of cyst number of recombinant fixed lines is shown on the right; $N = 5$.

4). The third QTL, which we tentatively identified as *rhg2*, was detected in the marker interval Satt359–Satt453 and the nearest marker was Satt359 (WGSP11_0150) (**Table 1**). Since 'To-8E' had Gedenshirazu-type *rhg2* (**Table 2**), this *rhg2* was derived from 'Gedenshirazu'.

In the QTL analysis using 199 RILs, *rhg2* and *Rhg4* were not detected (**Table 1**, above). The resolution power would be insufficient since only eight out of 199 RILs had all three loci (**Table 2**). We solved this problem by another QTL analysis using 58 RILs which had *rhg1* (**Table 1**, bottom).

Suzuki *et al.* (2012) reported that *rhg2* derived from either 'PI 84751' or 'Gedenshirazu' would suffice to make soybean plants resistant to race 1, as both *rhg1* and *Rhg4* were derived from 'PI 84751'. This statement is consistent with the fact that 'To-8E', which is resistant to race 1, has Gedenshirazu-type *rhg2* and PI 84751-type *rhg1* and *Rhg4* (**Table 2**). 'To-8E' has both 'PI 84751' and 'Gedenshirazu' as ancestors (**Supplemental Fig. 1**) (Tanaka and Yumoto 2002, Tanaka *et al.* 2015). In its pedigree, race 1 resistance was continuously selected, so plants with PI 84751-type *rhg1* and *Rhg4* and PI 84751- or Gedenshirazu-type *rhg2* would have been selected.

We also located *rhg2* derived from 'PI 84751' in an 821-kb region between SSR markers Sat_123 (=WGSP11_0140) and BARCSOYSSR11_1420, on Chr. 11, as shown through evaluation of recombinant fixed pairs with the 'Natto-shoryu' background (**Fig. 1**). This 821-kb region overlapped the region which QTL was detected although *rhg2* derived from 'Gedenshirazu' and that derived from 'PI 84751' have not confirmed to be the same gene. The location of *rhg2* lay within the region in which 'QTL on linkage group B1' associated with resistance to races 2 and 5 was detected (**Table 1**, right, Guo *et al.* 2005). Further research is needed to clarify whether they are the same QTL or not.

Race 1 resistance in PI 84751 is independently controlled by *rhg1*, *Rhg4*, and *rhg2* (and possibly *rhg3*) (Suzuki *et al.* 2012). We demonstrated that race 1 resistance is controlled by three genes, two of which are apparently *rhg1* and *Rhg4*

(**Table 1**). Segregation distortion of the ratio of resistant plants in hybrid populations might explain why more than three genes were reported in the 1960s (Caldwell *et al.* 1960, Matson and Williams 1965). Here, we observed segregation distortion in the hybrid population used in the QTL analyses (**Supplemental Table 2**): the observed segregation ratio was lower than expected, and fitted the hypothesis in which not three but four loci were involved.

In the USA, PI 88788-type *rhg1* confers resistance to SCN race 3. The combination of Peking-type *rhg1* and *Rhg4* confers resistance to races 1 and 3. Our results show that the combination of PI 84751-type *rhg1* and *Rhg4* and Gedenshirazu-type *rhg2* is needed to confer resistance to race 1. Since PI 84751 is resistant to race 1, the combination of PI 84751-type *rhg1* and *Rhg4* and PI 84751 or Gedenshirazu-type *rhg2* can confer resistance to race 1. This combination would confer resistance to race 3 also since all cultivars which are resistant to race 1 are resistant to race 3 also (Suzuki *et al.* 2012). The reason why *rhg2* is also needed is unknown: The *rhg1* genes in PI 88788, Peking, and PI 84571 might be different from each other. The SCN race 1 population in Japan might not be the same as that in the USA, but races in Japan have not been classified by HG type (Niblack *et al.* 2002). Further research is needed to identify why resistance derived from 'PI 84751' needs *rhg2* in addition to *rhg1* and *Rhg4*.

In conclusion, our detection of a third SCN resistance QTL, tentatively designated *rhg2*, in addition to *rhg1* and *Rhg4*, through QTL analyses using RILs, one parent of which had race 1 resistance derived from 'PI 84751', shows that all three QTLs are needed to confer resistance to race 1. By evaluating recombinant inbred pairs, we located *rhg2* to an 821-kb region on Chr. 11. For the selection of plants with *rhg2* for breeding, further mapping is needed to obtain closer markers to *rhg2* than ours at 821 kb.

Author Contribution Statement

CS, YY and RO performed the QTL analyses. FT-S designed the experiments and developed recombinant inbred pairs, which CI, MI and TM evaluated in the field. FT-S wrote the paper.

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