

Novel sources of resistance to the soybean cyst nematode (*Heterodera glycines*) found in wild relatives of azuki bean (*Vigna angularis*) and their characteristics of resistance

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Abstract Soybean cyst nematode (*Heterodera glycines*; SCN) is becoming a serious problem in azuki bean (*Vigna angularis* var. *angularis*) production in Hokkaido, Japan. To find sources of resistance to SCN, we screened wild relatives of azuki bean and calculated “female indices” (FIs) of 342 accessions from 8 *Vigna* species that are cross-compatible with azuki bean. Twenty-three accessions belonging to *V. hirtella*, *V. minima*, *V. nakashimae*, *V. riukiensis*, and

V. tenuicaulis were resistant to the most prevalent SCN race, race 3, but most of them were only moderately resistant or susceptible to race 5. Four promising accessions (*V. minima* JP205886, JP205891, and JP210806, and *V. nakashimae* JP107879) showed a high level of resistance to all SCN races found in Japan (1, 3, and 5) and race 2 (an experimentally derived race from race 5). Since the SCN-resistant soybean cultivars released in Japan are not resistant to races 2 and 5, these wild *Vigna* accessions may have resistance mechanisms different from that of soybean. Continuous culture of race 3 on promising accessions showed that it might be difficult to overcome the resistance of these accessions. The resistance sources found in this study will be useful in controlling SCN through the breeding of SCN-resistant azuki bean cultivars. This study also showed the effectiveness of using wild genetic resources for identifying novel resistance sources.

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Introduction

Azuki bean (*Vigna angularis* (Willd.) Ohwi et Ohashi var. *angularis*) is one of the important legume crops in Hokkaido, which produces 90 % of all Japanese azuki bean production. In 2010, the total area planted to azuki bean in Hokkaido was 23,800 ha, slightly more than the area planted to soybean (MAFF 2011). Azuki bean is a

suitable host plant for the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, and permits copious reproduction of SCN (Ichinohe 1953; Ozaki and Asai 1963; Watanabe and Saito 1993). However, as yield losses of azuki bean caused by SCN are not as devastating as those of soybean, little effort has been made to control SCN in azuki bean. Recently, however, damage has started to increase because the population density of SCN in Hokkaido fields has increased owing to the short-term rotation of legumes (Aoyama and Kushida 2011). Consequently, the yield loss of soybean, which is one of the rotation crops, is increasing. Thus, controlling SCN in azuki bean production is becoming increasingly important. Although the use of resistant cultivars is an effective practice for managing SCN, genetic sources of resistance to SCN have not yet been identified in azuki bean.

The numbers of female SCN on the roots of about 30 azuki bean cultivars were investigated and compared at the Tokachi Agricultural Experiment Station, but no cultivars with few females were found (unpublished data). Shimizu and Mitsui (1987) reported differences in the rate of SCN reproduction among some azuki bean cultivars, but cultivars with a low rate were not sufficiently resistant to control the SCN population. In contrast, we recently identified an accession (JP235878) of *Vigna riukiensis* with a very low female index (FI; see Materials and Methods for definition) value (Aoyama and Kushida 2011). This accession proved to be resistant to a number of diseases, including brown stem rot, phytophthora stem rot, and fusarium wilt, and it was crossed with azuki bean to introduce these resistance genes in the Tokachi Agricultural Experiment Station. The SCN resistance (low FI) of this accession was inherited in some derivative lines (Aoyama and Kushida 2011). Since other genetic resources of resistance are likely to be present among other wild relatives of azuki bean, we investigated these relatives. The objectives of this study were to identify sources of resistance to SCN by screening a large number of accessions of *Vigna* species that are cross-compatible with azuki bean, and to determine the characteristics of the resistance.

Materials and methods

Plant materials

We screened 342 accessions from 8 *Vigna* species for SCN resistance: *Vigna angularis* (Willd.) Ohwi et

Ohashi var. *nipponensis* (Ohwi) Ohwi et Ohashi (112 accessions), *V. hirtella* Ridley (23), *V. minima* (Roxb.) Ohwi et Ohashi (25), *V. nakashimae* (Ohwi) Ohwi et Ohashi (31), *V. nepalensis* Y. Tateishi et N. Maxted (6), *V. riukiensis* (Ohwi) Ohwi et Ohashi (27), *V. tenuicaulis* N. Tomooka et N. Maxted (28), and *V. umbellata* (Thunb.) Ohwi et Ohashi (90). The passport information of these accessions is provided in Supplementary Table 1. These wild *Vigna* species are cross-compatible with azuki bean and are primarily distributed in Southeast and East Asia (Tomooka et al. 2002). The accessions were provided by the Genebank of the National Institute of Agrobiological Sciences (NIAS) in Tsukuba, Japan.

Nematode populations

Three races of SCN (races 1, 3, and 5) were collected from fields in Hokkaido, and one race (race 2) was experimentally obtained from continuous culture of race 5 on the resistant soybean cultivar ‘Suzuhime’. Race 3 was maintained on the susceptible soybean cultivar ‘Kitamusume’. Since races 1 and 5 are more virulent than race 3, and so seriously damages ‘Kitamusume’ that they could not increase their population size on ‘Kitamusume’. Therefore, races 1 and 5 were maintained on the resistant soybean cultivar ‘Toyomusume’, which shows a suitable level of resistance to enable the population size of these races to increase sufficiently. The populations were maintained at 4 °C.

Calculation of the female index (FI)

Two seeds from each accession were sown in a pot (9-cm diameter and 7.5-cm height) filled with approximately 250 mL of autoclaved soil and maintained in a greenhouse at 20–25 °C. After germination, seedlings were reduced to 1 per pot. When each seedling had produced 5 or 6 foliage leaves, 1,000 second-stage juveniles (J2) were inoculated into each pot. J2s were prepared as follows. Cysts of SCN were isolated from soil using a method described by Yoshihara and Kegasawa (1989) and crushed to release eggs. Eggs suspended in water were passed through a sieve with a pore size of 64 μm to remove debris. The eggs were rinsed 3 times with distilled water and resuspended in distilled water and were then incubated at 25 °C for 1 week. Then they were suspended in root leachate

obtained from kidney bean seedlings according to the method described by Kushida et al. (2003) and incubated at 27 °C. After 1 week, hatched J2s were collected by the Baermann method.

At 30–35 days after J2 inoculation, we dislodged SCNs from the soil and roots by rubbing the roots gently in water, then collected them on a 250- μ m-pore sieve and counted the females under a stereomicroscope. The wet roots were patted dry with a paper towel and weighed.

The azuki bean cultivar ‘Erimo-shozu’ was grown as a control. ‘Erimo-shozu’ is the most popular azuki bean cultivar in Hokkaido and is highly susceptible to all SCN races. The resistance of each accession was evaluated as the FI, which was calculated as (average number of females on test accession/average number of females on ‘Erimo-shozu’) \times 100.

First and second screenings

In the first screening, FIs of all accessions against SCN race 3 were measured with no replication. Accessions with $FI \leq 25$ were selected and used in the second screening, in which FIs against SCN races 3 and 5 were determined with 1–3 replications. An accession of *V. riukiensis*, JP235878, which showed a low FI against SCN (Aoyama and Kushida 2011), was included in the second screening.

Testing of resistance of selected accessions to SCN races 1, 2, 3, and 5

We used 4 SCN-resistant accessions (*V. minima* JP205886, JP205891, JP210806, and *V. nakashimae* JP107879) from the second screening and 2 susceptible accessions (*V. minima* JP210821 and *V. nakashimae* JP212341) from the first screening showing vigorous growth to investigate FIs against SCN races 1, 2, 3, and 5 with 4 replications. Differences among FIs against each SCN race were analyzed by the Kruskal–Wallis test followed by Scheffé’s multiple comparison test. Values were log-transformed ($\log_{10}[FI + 1]$) for analysis.

Effect of continuous culture of SCN race 3

To investigate whether the resistance of selected *Vigna* accessions could be overcome, we continuously cultured SCN race 3 on the roots of resistant

accessions. Two resistant accessions (*V. minima* JP205891 and *V. nakashimae* JP107879) were planted in plastic pots (16-cm diameter and 20-cm height). When each seedling had produced 5 to 8 leaves, we inoculated approximately 8,000 J2s of race 3 into each pot. Plants were maintained in a greenhouse for 4 months. Cysts were isolated from the soil and used to prepare J2 inoculum for the next culture. After a second culture, all cysts in the pot were isolated and counted. J2s that had hatched were gathered and inoculated onto 5 replicated seedlings of the 2 resistant accessions, the 2 susceptible control accessions, and ‘Erimo-shozu’, and FIs were calculated as above. FIs of the original population, which had not been cultured on resistant accessions, were also examined at the same time and compared. To analyze the differences in FIs between the original population and the cultured population, we compared log-transformed FIs with the Mann–Whitney *U* test.

Development of juveniles in the roots of resistant and susceptible accessions

Seeds of the 2 selected resistant and 2 selected susceptible accessions were sown in 16 pots each and grown as in the first screening. When each seedling had produced 3 or 4 leaves, approximately 500 J2s of race 5 were inoculated into each pot, and plants were maintained in a greenhouse (22–26 °C). Seedlings from 4 pots were harvested 3, 14, and 21 days after inoculation, and soil was gently washed off the root systems with tap water. Roots were soaked in 3 % formaldehyde solution for a few days at room temperature, washed in tap water, and stained by boiling in lactoglycerol containing 0.05 % acid fuchsin (Hooper 1986). On day 21, females that came out from the roots during the washing process were collected with a 212- μ m-pore sieve and counted. Stained roots were homogenized in 30–50 mL of distilled water, and the J2s, J3s, J4s, and adult females in 1 mL of homogenate were counted in 5 replicates. The sex of J4s was determined from the internal form or shape (shown later in Fig. 5). J2s and J3s were discriminated by tail form. J3s and J4s (female) were roughly discriminated by size. On day 35, females were collected from the soil and roots, as described above, and counted. Differences in the numbers of nematodes of each stage between the resistant and

Table 1 Classification of the *Vigna* accessions based on their female index (FI) values in the first screening against SCN race 3

Range of FI	<i>V. angularis</i> var. <i>nipponensis</i>	<i>V. hirtella</i>	<i>V. minima</i>	<i>V. nakashimae</i>	<i>V. nepalensis</i>	<i>V. rikiuensis</i>	<i>V. tenuicaulis</i>	<i>V. umbellata</i>
0–25	87903, 87906, 87909, 217477, 217479, 217481, 220093, 225941, 226013, 226056, 226665, 226667, 226676	108551, 205885, 217435, 217491, 220133, 220134, 220137, 226687	108129, 202289, 205886, 205887, 205888, 205890, 205891, 210655, 210669, 210682, 210683, 210806, 210824, 218938, 220136, 220139, 220140, 220141, 222402, 226678, 226877	107879	110827	201446, 201476, 201479, 201499, 201500, 201502, 201508, 201514, 201521, 201522, 201526, 201532, 201533, 201542	205883, 210822, 217465, 217475, 217486	210677, 211788, 217461
26–50	87897, 87899, 87901, 87910, 87911, 87915, 87918, 90790, 90791, 90797, 90808, 90813, 90834, 90835, 90838, 90842, 90845, 90847, 90849, 90853, 90855, 90865, 90867, 91231, 110658, 110662, 110675, 110683, 110688, 110691, 110694, 110699, 110703, 201454, 201460, 217490, 217495, 220099, 220101, 224735, 225140, 225167, 226779, 226816, 231271, 231282, 231287	109681, 217447, 217451, 224443, 226680	107869, 210821, 220142, 224430	81231, 212325, 212330, 212332, 212333, 212334, 212335, 212336, 212339, 212343, 212352	107881, 109688, 109689, 110826	107884, 201477, 201490, 201495, 201498, 201503, 201513	108552, 210671, 210795, 210807, 210818, 217452, 217471, 226603, 226636, 226648, 226651, 226684, 226690, 226696	85358, 100288, 207936, 217439, 217454, 217455, 222400, 207982, 207985, 210644, 210665, 210667, 210672, 210673, 210675, 210676, 211786, 226596, 226603, 226636, 226648, 226651, 226684, 226690, 226696
51–75	81646, 87913, 90796, 90803, 90806, 90807, 90809, 90816, 90818, 90832, 90836, 90861, 90871, 110659, 110681, 110686, 201207, 201458, 211958, 217489, 220089, 220102, 225101, 225121, 225146, 226746, 226802, 231277, 231289, 231293, 231295, 231296, 231297	210823, 220131, 224432, 224436, 226637, 226663	212326, 212329, 212338, 212340, 212342, 212344, 212345, 212347, 212348, 212349, 212350, 212351, 212353	108810, 201442, 201444, 201484	109682, 210808, 210825, 211875, 217444, 217448, 217460, 217496, 217519, 226688	73246, 76523, 81576, 81637, 81639, 100278, 100281, 100285, 100286, 100287, 110715, 205889, 207915, 207931, 217442, 220127, 226588, 226595, 226599, 226615, 109669, 210639, 210659, 210660, 210666, 210668, 210670, 210674, 210679, 210680, 210797, 210800, 210801, 210802, 210803, 211785, 211787, 217417, 217418, 226611, 226623, 226624, 226626, 226647, 226659, 226683		

Table 1 continued

Range of FI	<i>V. angularis</i> var. <i>nipponensis</i>	<i>V. hirtella</i>	<i>V. minima</i>	<i>V. nakashimae</i>	<i>V. nepalensis</i>	<i>V. riukiensis</i>	<i>V. tenuicaulis</i>	<i>V. umbellata</i>
76–100	90792, 90805, 90858, 90863, 90870, 110677, 110678, 110679, 201462, 225095, 225117, 225132, 226753, 226757	108558, 220130, 224435, 226669		212327, 212328, 212337, 212341, 212346	109687	201482, 201504	217446, 217453, 226671	73244, 73245, 81638, 90925, 99485, 100279, 100280, 100282, 100283, 100284, 207938, 220128, 226608, 109675
>100	90794, 90814, 90864, 201463, 226789			212331			217445	31442, 226594
Root weight ^a	10.04 ± 5.56	10.68 ± 2.94	13.62 ± 6.79	15.07 ± 4.13	4.62 ± 3.79	6.95 ± 2.16	3.37 ± 1.29	14.18 ± 5.99

Each accession (identified by JP number) is classified into 1 of 5 categories based on FI (0–25, 26–50, 51–75, 76–100, and >100)

^a Values are fresh root weights (mean ± SD) of wild *Vigna* species at the time of female isolation. The fresh root weight of azuki bean was 12.17 g (±3.99 g)

susceptible accessions were compared by Mann–Whitney *U* test.

Results

First screening

Each accession was classified into 1 of 5 FI categories (0–25, 26–50, 51–75, 76–100, and >100; Table 1). FIs of tested accessions ranged between 3 and 121. All accessions had females. Low FIs were common among *V. minima* accessions. High FIs were generally common among *V. umbellata* and *V. nakashimae* accessions. All *V. nepalensis* accessions showed very poor growth. A positive correlation between FI and fresh root weight ($P < 0.05$, Spearman's rank correlation analysis) suggested that the low FIs of *V. nepalensis* might have been caused by poor growth rather than by resistance. Because it was difficult to accurately judge the resistance of these accessions, we omitted them from the second screening. Although the root weights of *V. tenuicaulis* were also small, there was no correlation between FI and root weight in this species. Therefore, we used accessions of *V. tenuicaulis* in the second screening. We re-examined 65 accessions with $FI \leq 25$ in the second screening.

Second screening

Vigna hirtella JP108551 and *V. tenuicaulis* JP205883 were omitted owing to their very poor growth. Accessions with $FI \leq 10$ against SCN race 3 were found in *V. hirtella*, *V. minima*, *V. nakashimae*, *V. riukiensis*, and *V. tenuicaulis*, but most of them showed $FI > 10$ against race 5 (Table 2). The FIs of 5 accessions of *V. minima* and 1 of *V. nakashimae* against race 5 were ≤ 10 . Since we considered the importance of the resistance against SCN race 5 which can grow normally on SCN-resistant soybean, we selected these 6 accessions first. From these accessions, we selected 3 accessions of *V. minima* (JP205886, JP205891, JP210806) and 1 of *V. nakashimae* (JP107879) on the basis of their resistance to SCN race 3 and the vigor of the plants.

FIs against races 3 and 5 on *V. riukiensis* JP235878, which was found to be resistant to SCN in the previous study, were around 30. Many other accessions of *V. riukiensis* showed lower FIs than JP235878.

Table 2 Female index (FI) values on each accession of *Vigna* against SCN races 3 and 5

JP no.	Population		JP no.	Population		JP no.	Population	
	Race 3	Race 5		Race 3	Race 5		Race 3	Race 5
<i>V. angularis</i> var. <i>nipponensis</i>			<i>V. minima</i>			<i>V. riukiensis</i>		
87903	67 (2)	89 (2)	108129	16 (1)	10 (3)	201446	11 (2)	47 (3)
87906	78 (1)	69 (2)	202289	5 (1)	7 (3)	201476	12 (2)	29 (3)
87909	45 (1)	72 (2)	205886	14 (1)	8 (3)	201479	10 (2)	13 (3)
217477	24 (3)	54 (2)	205887	40 (1)	51 (3)	201499	6 (2)	17 (3)
217479	114 (4)	111 (2)	205888	48 (1)	33 (3)	201500	8 (2)	30 (3)
217481	69 (4)	86 (2)	205890	25 (1)	23 (3)	201502	13 (2)	13 (3)
220093	89 (4)	101 (2)	205891	10 (1)	4 (3)	201508	6 (2)	29 (3)
225941	71 (3)	79 (2)	210655	8 (1)	54 (3)	201514	2 (2)	24 (3)
226013	18 (4)	58 (2)	210669	38 (1)	29 (3)	201521	7 (1)	44 (3)
226056	80 (4)	84 (2)	210682	52 (1)	77 (3)	201522	6 (2)	24 (3)
226665	72 (4)	79 (2)	210683	20 (1)	35 (3)	201526	5 (2)	39 (3)
226667	52 (4)	72 (2)	210806	8 (1)	6 (3)	201532	2 (2)	34 (3)
226676	38 (4)	65 (2)	210824	14 (1)	33 (3)	201533	6 (2)	54 (3)
			220136	94 (1)	43 (3)	201542	7 (2)	25 (3)
<i>V. hirtella</i>			220139	48 (1)	43 (3)	235878	27 (2)	34 (3)
205885	16 (3)	48 (3)	220140	2 (1)	95 (2)			
217435	23 (3)	85 (3)	220141	28 (1)	65 (3)	<i>V. tenuicaulis</i>		
217491	9 (3)	39 (3)	222402	7 (1)	19 (3)	210822	1 (2)	48 (3)
220133	10 (3)	53 (3)	226678	38 (1)	55 (3)	217465	44 (4)	60 (3)
220134	40 (3)	84 (3)	218938	9 (1)	21 (3)	217475	25 (4)	44 (3)
220137	44 (3)	93 (3)	226877	1 (1)	14 (3)	217486	22 (3)	34 (3)
226687	13 (3)	62 (3)						
			<i>V. nakashimae</i>			<i>V. umbellata</i>		
			107879	5 (2)	10 (3)	210677	77 (3)	76 (3)
						211788	70 (3)	104 (3)
						217461	50 (3)	77 (2)

Values in parentheses show the numbers of tested plants

Testing of resistance to various SCN races

The susceptible *V. minima* JP210821 and *V. nakashimae* JP212341 showed high FIs against all 4 SCN races. The mean FIs of all resistant accessions against each SCN race were <10, except for that of *V. minima* JP205886 against race 1 (Fig. 1). In particular, *V. minima* JP205891 and JP210806 showed high resistance to races 2 and 5, which can break down the SCN resistance of soybean cultivars in Japan. Although all FIs were low, those of *V. minima* JP205886 showed significant differences between races 1 and 3, and those of *V. minima* JP210806 showed significant differences between race 3 and all other races ($P < 0.05$).

Effects of continuous culture of SCN on resistant *Vigna* accessions

Two SCN resistant accessions, *V. minima* JP205891 and *V. nakashimae* JP107879, were used for continuous SCN culturing. After the second cycle of cultivation, we isolated 212 cysts from the pot of *V. minima* JP205891 and 2,282 cysts from *V. nakashimae* JP107879. These numbers are both lower than the number of J2s inoculated (ca. 8,000). The FIs of the resistant accessions against the SCN populations raised on resistant accessions were very low and were not significantly different from the FIs against the original SCN populations ($P > 0.05$, Fig. 2). In

Fig. 1 Female index (FI) values of each accession of wild *Vigna* species against 4 SCN races. Error bars indicate standard deviations

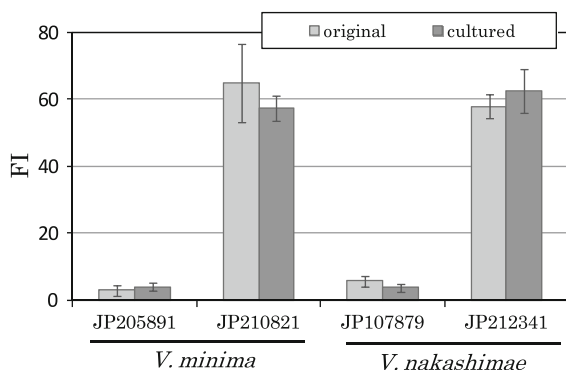
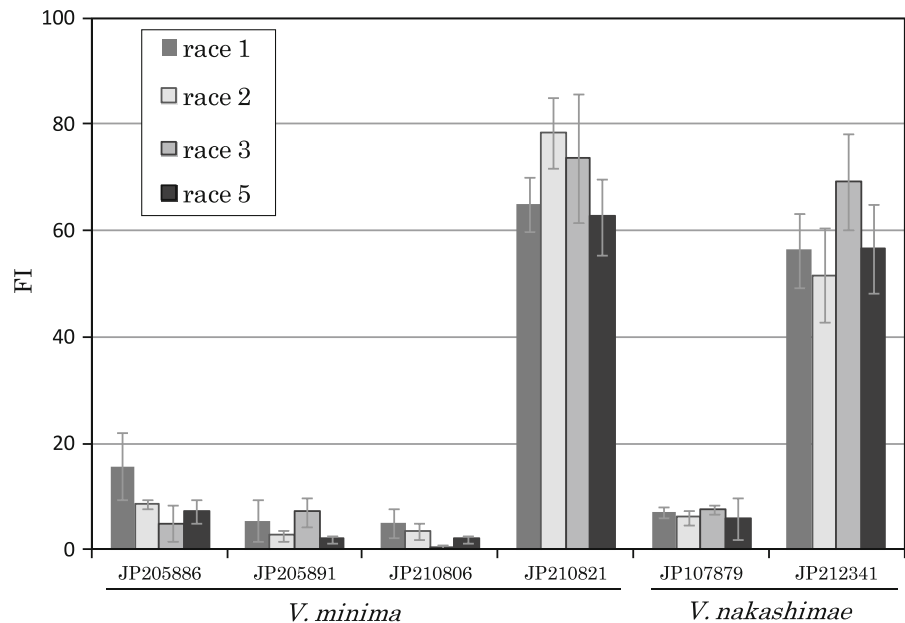


Fig. 2 Female index (FI) values of each accession against original and cultured SCN. Values are means of 5 replications; error bars indicate standard deviation. There were no significant differences between FIs against the original and cultured populations ($P > 0.05$, Mann–Whitney U test, $n = 5$)

contrast, the FIs of the 2 susceptible accessions against both cultured and original SCN were very high.

Development of SCN juveniles on the roots of resistant and susceptible accessions

Only J2s were isolated from the roots of each accession 3 days after inoculation of J2s. The mean number of J2s in the roots of resistant accessions was 360–400, not significantly different from the number on susceptible accessions (data not shown). The mean numbers of SCN juveniles of each stage that were

isolated from the roots of each accession on 14 and 21 days after J2s inoculation were shown in Figs. 3 and 4, respectively. J2s, J3s, and J4s were isolated from the roots of all accessions 14 days after inoculation. Both fat J4s (thought to be females) and thin J4s (males, visible adult male in their body) were present (Fig. 5). There were significant differences between resistant and susceptible accessions of *V. minima* in all stages, and between those of *V. nakashimae* in female J4s (Fig. 3). The mean number of female J4s collected from resistant accessions was significantly lower than those collected from susceptible accessions, whereas the number of J2s collected from resistant accessions was higher (significantly in *V. minima*). Most of J2s were not stained deeply. J3s stained well, but some were shrunken (Fig. 5).

J2s, J3s, J4s, and adults were isolated from the roots of all accessions 21 days after inoculation (Fig. 4). J2s and most of J3s were transparent. The numbers of females (adult + J4) on each accession at 21 days were the same as or slightly higher than the numbers of J4 females at 14 days (Figs. 3 and 4). Moreover, these values were similar to the numbers isolated at 35 days (data not shown). On the susceptible accessions, the numbers of males were nearly identical to or slightly lower than the numbers of females at 14 and 21 days. On the resistant accessions, in contrast, the numbers of males were significantly more than the number of females at both days ($P < 0.05$).

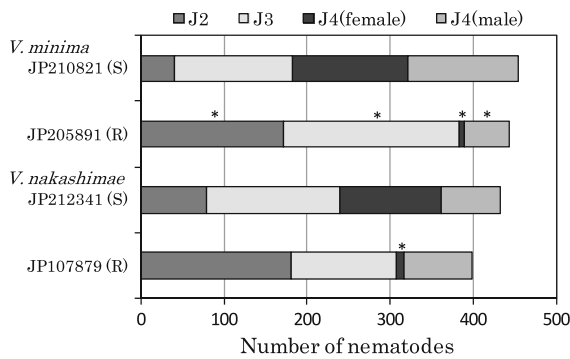


Fig. 3 Mean numbers of SCN juveniles of each stage that were isolated from the roots of each accession 14 days after inoculation of 500 second-stage juveniles ($n = 4$). (R): resistant accession, (S): susceptible accession. *Mean of resistant accession is significantly different from that of the control accession of the same species ($P < 0.05$, Mann–Whitney U test, $n = 4$)

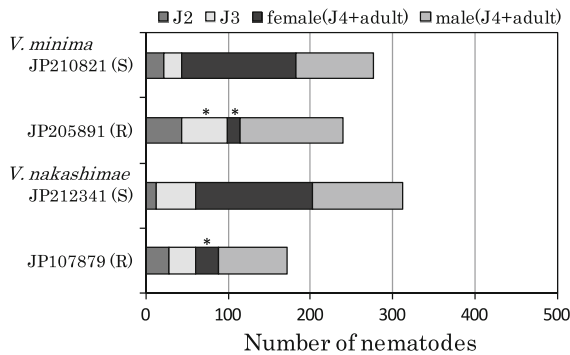


Fig. 4 Mean numbers of SCN juveniles of each stage that were isolated from the roots of each accession 21 days after inoculation of 500 second-stage juveniles ($n = 4$). *Mean of resistant accession is significantly different from that of the control accession of the same species ($P < 0.05$, Mann–Whitney U test, $n = 4$)

Discussion

Since all *Vigna* species investigated held accessions with FIs as high as that of ‘Erimo-shozu’, these wild species might be suitable hosts for SCN (Table 1). Since *V. angularis* var. *nipponensis* and *V. nakashimae* are distributed in northeastern China, Korea, and Japan (Tomooka et al. 2002), where SCN is also distributed, SCN may be maintained on these plants outside of cultivated fields. Although most *V. minima* accessions had low FIs, this species was ranked as a host, because JP210821, which we used as a susceptible control, consistently showed high FIs.



Fig. 5 Juveniles isolated from the roots of *V. minima* JP205891 14 days after J2 inoculation

Plants with $FI < 10$ are considered to be resistant to SCN, and those with FIs between 10 and 30 to be moderately resistant (Schmitt and Shannon 1992). In the first screening, we selected accessions with $FI \leq 25$ as resistant candidates because we tested them without replication. In the second screening, we determined accessions with $FI \leq 10$ to be resistant to SCN.

Accessions with $FI \leq 10$ against race 3 were found in *V. hirtella*, *V. minima*, *V. nakashimae*, *V. riukiensis*, and *V. tenuicaulis* (Table 2). These accessions were classified to be resistant to SCN race 3. *V. riukiensis* JP235878, the first reported resistant resource (Aoyama and Kushida 2011), was only moderately resistant ($FI \approx 30$). Therefore, the accessions we identified here are the first with a high level of SCN resistance. As we found resistant accessions in 5 species, sources of SCN resistance may be widespread among *Vigna* species.

Resistant accessions of *V. minima*, *V. hirtella*, and *V. riukiensis* were collected primarily from Southeast Asia (Thailand, Myanmar, Laos, and Okinawa-Japan), where SCN is not present. In fact, no species of cyst nematodes that parasitize beans have been found in these regions (Evans and Rowe 1998). Therefore, these accessions might have developed resistance to other pests that might coincidentally work against SCN.

Only 1 among 31 *V. nakashimae* accessions (JP107879) was found to be resistant. This accession was collected in the Goto Islands in Nagasaki Prefecture in Japan, whereas the other 30 were collected in

Korea. Therefore, the SCN resistance of this accession might have developed after geographic isolation. In contrast, many *V. minima* accessions had low FIs, suggesting that SCN resistance is widespread among this species. Three promising resistant accessions (JP205886, JP205891, and JP210806) were selected. These accessions were collected in northern Thailand (Nan, alt. 625 m), northeastern Thailand (Surin, 305 m), and central Myanmar (Kalaw, 1,230 m), respectively. Susceptible *V. minima* accessions selected in the first screening (JP107869, JP210821, JP220142, and JP224430) were collected in central Thailand (Uthai Tani, alt. 100 m), southern Myanmar (Setse, 5 m), southern Laos (Pakse, 100 m), northern Laos (Udom Xai, 949 m), and northern Thailand (Nan, 1,400 m), respectively. There was no clear pattern of geographic distribution of resistant or susceptible accessions.

SCN races 1, 3, and 5 are confirmed to be distributed in Japan (Inagaki 1979; Shimizu 1987). Race 2 has not been found in Japan. However, it may appear in the future through the continuous cultivation of resistant soybean cultivars, as it was experimentally selected by the continuous culture of race 5 on SCN-resistant soybean ‘Suzuhime’. Race 3, which is distributed the most widely in Japan, can seldom reproduce on the resistant soybean cultivars grown in Japan. However, races 1, 5, and 2 can reproduce on resistant soybeans, with the ability to reproduce increasing in that order. Race 2 develops well on the roots of the strongest resistant soybean cultivar in Japan (A. Kushida, unpublished). Many accessions showing resistance to race 3 showed moderate or no resistance to race 5 (Table 2). Therefore, the mechanisms of resistance in these *Vigna* accessions are similar to those of soybean. On the other hand, four accessions (*V. minima* JP205886, JP205891, and JP210806, and *V. nakashimae* JP107879) selected from the screening tests showed high levels of resistance to all SCN races in Japan (1, 3, and 5) and race 2. Therefore, the resistance of these *Vigna* accessions may be more effective for controlling SCN than that of resistant soybean cultivars and may have a unique resistance mechanism.

Field populations of SCN are mixtures of parasitic phenotypes. Therefore, continuous planting of resistant soybean cultivars results in the selection of resistance-breaking SCN races and the loss of resistance (Niblack 1992; Riggs and Schuster 1998; Young 1992). Young (1994) cultured SCN race 5 on the

soybean cultivar ‘Cordell’ for 10–14 generations and confirmed that the FIs increased from 10 to 77 and that the race changed to race 14. To test whether the resistance of *V. minima* JP205891 and *V. nakashimae* JP107879 could also be broken down by continuous cultivation, we cultured SCN race 3 on them twice for 4 months each. The FIs did not change significantly (Fig. 2). Very few cysts compared to the number of inoculated J2 (8,000) were isolated after the second culture, but we observed some white females on the roots and cysts with many egg shells at the end of second culture. These clues indicate that SCN underwent several generations on the resistant *Vigna* accessions. In contrast, previous experiments showed that the mean FIs of the same race 3 population that we used here increased significantly after continuous culture on 2 crops of resistant soybeans (A. Kushida, unpublished). Therefore, our results suggest that it might be difficult to overcome the resistance of these promising *Vigna* accessions. However, it will be necessary to culture SCN on them over several crops before we can be sure.

The number of J2s invading the roots of resistant soybeans was not different from the number invading susceptible cultivars, so the resistance was considered to start working after their invasion into the roots (Huang 1998). Similarly, the number of J2s invading the roots of the resistant *Vigna* accession was not significantly different from the number invading the susceptible accession. Therefore, this resistance might also begin to work after invasion. Many J2s were still present on day 14 after inoculation of resistant *Vigna* accessions (Fig. 3), but most of them were thought to be dead because they were not stained deeply, indicating that they had failed to parasitize to the roots. The number of J2s decreased on day 21 (Fig. 4), maybe because their transparency made them hard to see. Although some juveniles developed to J4 or higher on the roots of resistant accessions, most of these were male (Figs. 3,4). Similar phenomena have been observed in resistant soybean cultivars (Colgrove and Niblack 2005; Luedders 1987; Ross 1958; Sato and Omori 1969; Yuhara and Inagaki 1963). Since the resistance of *V. minima* JP205891 and *V. nakashimae* JP107879 was effective against all the SCN races tested, the mechanism of this resistance might be different from that of soybean resistance. However, no clear differences in the developmental pattern of SCN were evident between resistant soybean cultivars and

resistant *Vigna* accessions. To clarify the characteristics of the *Vigna* accessions' resistance, it will be necessary to perform a histological survey of the syncytium in detail.

We found promising genetic resources with resistance to all races of SCN present in Japan in wild *Vigna* species. These resources will find use in the breeding of azuki bean cultivars that are resistant to SCN.

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