Breeding Soybean Cyst Nematode Resistant Azuki Bean using Wild *Vigna* Germplasm

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Abstract

Wild accessions of *Vigna riukiuensis* have been crossed to azuki bean (*V. angularis*) cultivars in a series of breeding programs at Hokkaido Prefectural Tokachi Agricultural Experiment Station aiming to introduce resistance of soil-borne diseases and abiotic stresses. In one of the characterization process, we have found an accession "Acc2482 (JP235878)" of *V. riukiuensis* which was collected from Iriomote island of Okinawa prefecture, Japan showed resistance to Soybean Cyst Nematode (SCN), *Heterodera glycines*. In an evaluation experiment, SCN could produce significantly less number of cysts on the roots of "Acc2482". To confirm SCN resistance of "Acc2482", we re-investigated the level of SCN resistance of this accession and the hybrid progenies. The results showed that the SCN resistance of "Acc2482" was stable and genetically inherited in the hybrid progenies produced from a hybrid using a susceptible azuki bean breeding line as a female parent. This suggested a possibility of introducing SCN resistance gene(s) from *V. riukiuensis* to azuki bean.

Introduction

Hokkaido is the largest beans producing area in Japan. It is located in the northern part of Japan, as illustrated in Fig.1. Soybean Cyst Nematode (SCN), *Heterodera glycines*, is one of the most serious pests that cause yield loss of bean crops such as soybean (*Glycine max*), azuki bean (*Vigna angularis*), common bean (*Phaseolus vulgaris*) (Ichinohe 1953). Until recently, breeding of SCN resistant azuki bean cultivar has not been considered as a high priority objective, since azuki bean is relatively tolerant to the infestation of SCN compared with soybean and yield loss is less prominent. However, the relatively high level of tolerance in azuki bean revealed to cause an adverse effect on the subsequent susceptible crops such as soybean by increasing population density of SCN in the field. In addition, since the population density of SCN in the field of short-term rotation of beans becomes very high recently, yield loss of azuki bean itself has been becoming a serious problem too. At present, use of resistant varieties is the most effective way of controlling SCN damage.

In 1954, screening of SCN resistant varieties in azuki bean germplasm was carried out in Tokachi Agricultural Experiment Station. However, no promising SCN resistant azuki bean variety was found at that time. Shimizu and Mitsui (1987) reported a phenotypic difference in resistance level to SCN among azuki bean varieties. However, the level of resistance detected was insufficient for the breeding of SCN resistant cultivar. On the other hand, Sawa (1973) reported that green gram (*V. radiata*) showed higher level of resistance to SCN. Interspecific hybridization was tried, however to the authors' knowledge, there have been no experimental data on SCN resistance in the hybrid progenies.

Collection, conservation and classification of wild relatives of azuki bean have been conducted in the NIAS genebank, Tsukuba, Japan (Tomooka *et al*., 2002). In addition to the germplasm collection, molecular markers and molecular linkage maps of azuki bean have been developed (Han *et al*. 2005, Isemura *et al*., 2007, Kaga *et al*., 2008, Tomoka *et*

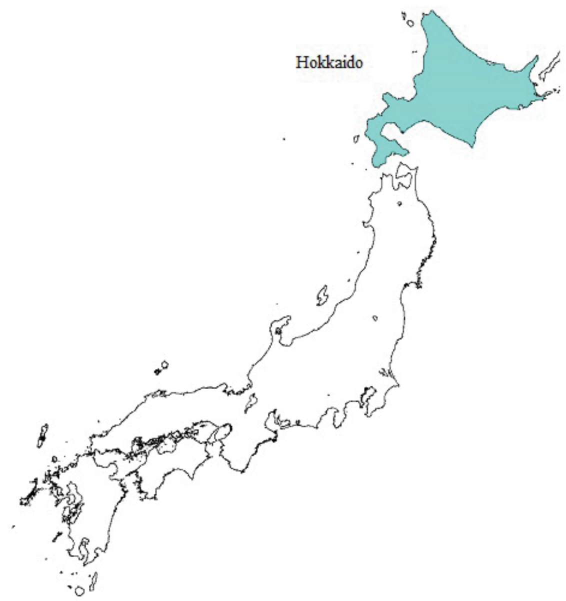

Fig. 1. Hokkaido (shaded) located in northern Japan

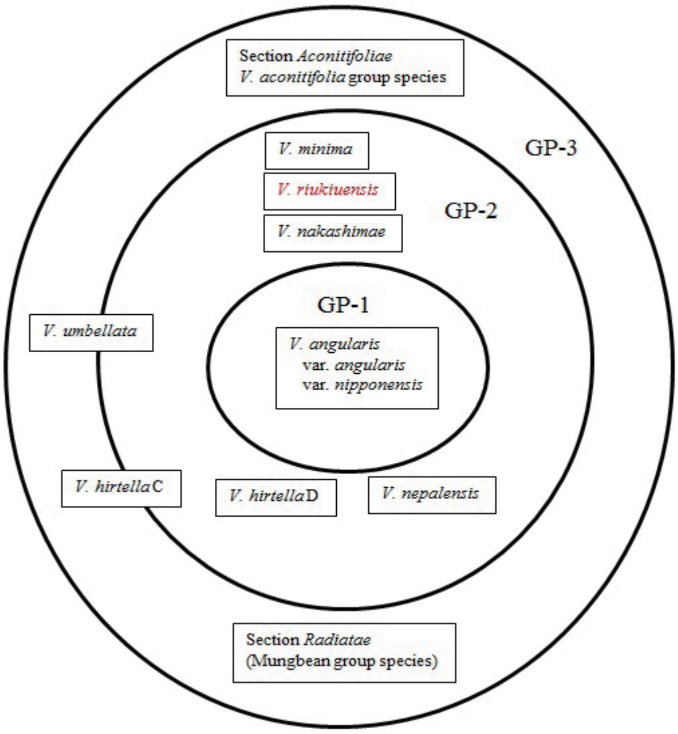

Fig. 2. Gene pool classification of azuki bean (moodified from Tomooka *et al.*, 2000)

al. 2006, Xu *et al*. 2008).

Availability of the wild germplasm collection and molecular tools has encouraged us to use wild genetic resources for the azuki bean breeding. For example, a powdery mildew-resistant accession of *V. hirtella* was found and introduction of resistance gene into azuki bean was attempted (Egawa *et al*., 2003).

Egawa *et al*. (1999) have reported that accessions of *V. riukiuensis*, which belongs to the primary genepool of azuki bean (see Fig. 2, Vaughan *et al*., 2005), showed significantly higher levels of heat tolerance compared with azuki bean. Previous research in Hokkaido Prefecture Tokachi Agricultural Experiment Station (1997) showed that *V. riukiuensis* has resistance to brown stem rot, phytophthora stem rot and fusarium wilt. Therefore, we have crossed *V. riukiuensis* with azuki bean in order to introduce these resistance genes of *V. riukiuensis*. In one of the characterization process, we accidentally found the

Table 1. The number of cysts in breeding line in F_5 generation

Line	Species or Cross	Evaluation of resistance[1)]		No. of cysts (/plant)					Root weight (Fwt/plant)
		F_3 or F_4	F_5	1	2	3	4	Average	
Acc2482	*V.riukiuensis*	R	R	26	21	43	17	27	6.6
Toiku No.150	*V.angularis*	S	S	219	231	197	224	218	8.3
0321-②83-2	Toiku No.150	S	S	256	255	241	260	253	21.1
0321-②85-1-9	×	R	S	229	216	-	-	223	11.4
0321-②85-1-13	Acc2482	R	S	97	153	159	220	157	4.8
0321-②85-2-1		R	R	45	34	34	-	38	16.5
0321-②85-3-5		R	Seg	44	130	41	98	78	16.8
0321-②85-4-2		R	S	190	87	149	102	132	5.6
0321-②85-6-2		S	S	267	216	93	254	208	12.4

1) R : resistance, S : susceptible, Seg : segregating.

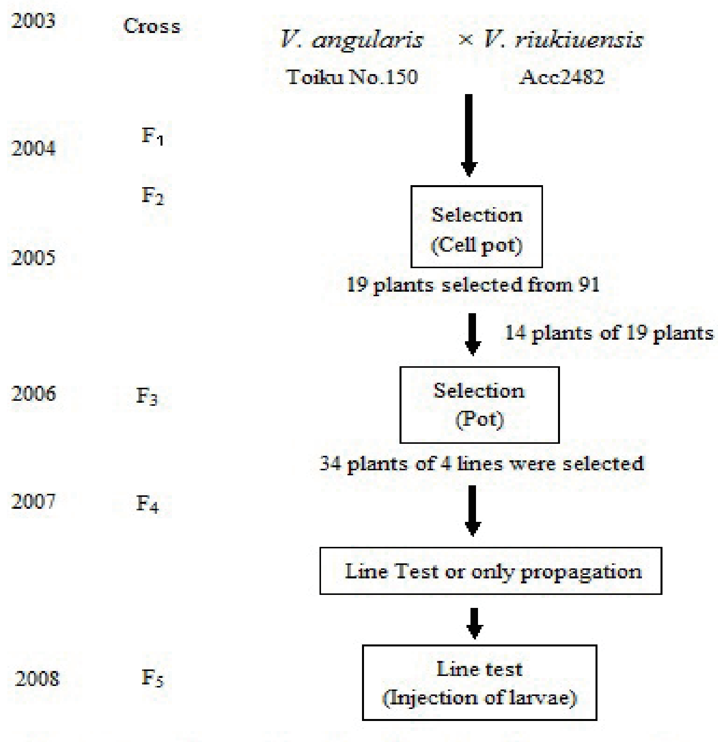

Fig. 3. Procedure used to obtain lines for resistance to SCN

SCN resistance in an accession "Acc2482 (JP235878, NIAS genebank passport number)" of *V. riukiuensis*

The objectives of this study are to confirm SCN resistance found in an accession "Acc2482" of *V. riukiuensis* and to examine the possibility of introducing SCN resistance to azuki bean. Additionally, we attempted to find novel germplasm of SCN resistance by screening larger number of wild *Vigna* germplasm.

Materials and Methods

The procedure to develop azuki bean lines harboring resistance to SCN is shown in Fig. 3. In 2003, an accession "Acc2482" of *V. riukiuensis* was crossed with an azuki bean breeding line "Toiku No.150" (the cross number is "0321"). Selection based on the resistance level to SCN has been performed from F_2 to F_4 generation. In F_5 generation, line test for the SCN resistance was performed using lines which have enough number of seeds.

Searching of novel source of SCN resistance germplasm from wild relatives of azuki bean has been conducted using 9 accessions consist of 4 species (Table 2). Seeds were directly sown in a plastic pot filled with autoclaved soil and maintained in a greenhouse. About 1,000 larvae of SCN were inoculated into each pot about one month after sowing. At 35 days after SCN inoculation, cysts on the roots

Table 2. The number of cysts in wild relatives

Acc No.	Species	Origin	No. of cysts (/pot)	
			Average	S.E.
-	*V.angularis*[1)]	Hokkaido Pref.	198	14
2463	*V.angularis var.nipponensis*	Miyagi Pref.	278	10
2464	*V.angularis var.nipponensis*	Ibaraki Pref.	246	21
2477	*V.riukiuensis*	Okinawa Pref.	124	13
2482	*V.riukiuensis*	Okinawa Pref.	15	3
2513	*V.nepalensis*	Nepal	249	10
2243	*V.nepalensis*	Nepal	318	22
2502	*V.umbellata*	Thailand(Phrae)	144	13
2503	*V.umbellata*	Thailand(Phrae)	117	13

1) Erimoshouzu is a leading variety in Hokkaido Prefecture.

were collected from each pot (or plant) and number of cysts was counted.

Results and Discussion

SCN resistance of F5 hybrid breeding lines and their parents

Number of cysts on the roots of parents is shown (upper 2 rows, Table 1). Average number of cysts detected on the roots of “Toiku No.150” (azuki bean breeding line) was 218 cysts / plant, while that detected on the roots of “Acc2482” (a resistant wild accession of *V. riukiuensis*) was 27 cysts/plant. Although the root mass of “Acc2428” was about 80% of “Toiku No.150”, the number of cysts of “Acc2482” was only 12 % of “Toiku No.150”. Since the tested 4 individuals of “Acc2482” and “Toiku No.150” showed stable response to SCN infestation, resistance of “Acc2482” was confirmed to be genetically stable.

Number of cysts on the roots of F_5 breeding lines is shown (lower 7 rows, Table 1). Among 7 lines tested, 5 lines were evaluated as susceptible (S), 1 as resistant (R) and 1 as segregating (Seg). Three lines formerly evaluated as R in F_3 or F_4 generation were evaluated as S in F_5 generation. One line evaluated as R in F_3 or F_4 generation (0321- ② 85-3-5) was evaluated as segregating and another line evaluated as R in F_3 or F_4 generation (0321- ② 85-2-1) was evaluated as R. These results suggest that SCN resistance of *V. riukiuensis* accession is incorporated, genetically inherited between generations and segregating in the hybrid lines. Judging from the root weight of hybrid lines, level of SCN resistance is not directly related to the amount of roots (Photo 1).

Searching novel source of SCN resistance

Since promising SCN resistance source was found in an accession of cross compatible wild relative of azuki bean (*V. riukiuensis*), we have started a screening using comprehensive collection of wild *Vigna* species which can cross with cultivated azuki bean. Preliminary result was shown in Table 2. A leading azuki bean variety “Erimoshouzu” showing susceptibility (198 cysts / plant) was used as a control (uppermost row in Table 2). Two accessions of wild ancestor of azuki bean (*V. angularis* var. *nipponensis*) collected from Japan showed higher levels of susceptibility. Higher levels of susceptibility were also recorded for *V. nepalensis* accessions collected from Nepal. Two cultivated accessions of rice bean (*V. umbellata*) from Thailand showed similar levels of susceptibility to “Erimoshouzu”.

“ACC2477” of *V. riukiuensis* collected from the same prefecture as “ACC2482” did not show resistance to SCN. It was found that intraspecific variation of SCN resistance exists in *V. riukiuensis*.

Further screening program using larger number of wild accessions is in progress.

Acknowledgments

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References

Egawa, Y., Takeda, H. and Suzuki, K. 1999. Research plan on crop heat tolerance at the crop introduction and cultivation laboratory. JIRCAS Working Report 14: 103-107.

Egawa, Y., Kashiwaba, K., Ohmae, H. and Shono, M. 2003. Development of powdery mildew-resistant adzuki bean using wild adzuki bean germplasm. JIRCAS Research Highlights 2003.

Han, O.K., Kaga, A., Isemura, T., Wang, X.W., Tomooka, N. and Vaughan, D.A. 2005. A genetic linkage map for azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]. Theor. Appl. Genet.111: 1278-1287.

Hokkaido Prefecture, Tokachi Agricultural Experiment Station. 1997. Test for disease resistance of wild relatives in the genus *Vigna*. Heisei 9 Bean Experiment Results Report: 44-45. (in Japanese).

Ichinohe M. 1953. On the parasitism of the soybean nematode, *Heterodera glycines*. Research Bulletin of the Hokkaido National Agricultural Experiment Station 64: 113-124. (in Japanese with English summary).

Isemura, T., Kaga, A., Konishi, S., Ando, T., Tomooka, N., Han, O. and Vaughan, D.A. 2007. Genome dissection of traits related to domestication in azuki bean (*Vigna angularis*) and comparison with other warm season legumes. Annals of Botany 100: 1053-1071.

Kaga, A., Isemura, T., Tomooka, N. and Vaughan D.A.2008. The genetics of domestication of the azuki bean (*Vigna angularis*). Genetics178:1013-1036.

Sawa, M. 1973. On the interspecific hybridization between the adzuki bean, *Phaseolus angularis* (Willd) W. F. Wight and the green gram, *Phaseolus radiatus* L. I. Crossing between a cultivar of the green gram and a semi wild relative of the adzuki bean, in endemic name "Bakaso". Jpn. J. Breed. 23:61-66. (in Japanese with English summary).

Shimizu, K. and Mitsui, Y. 1987. Varietal Difference of Azuki bean and Kidney bean in Susceptibility to the Race of the Soybean Cyst Nematode, *Heterodera glycines* Ichinohe, 1952. Ann. Rept. Plant Prot. North Japan 38: 109-110. (in Japanese with English summary).

Tokachi Agricultural Experiment Station. 1954. Test for soybean cyst nematode resistance in azuki bean varieties. Tokachi Agricultural Experiment Data: 526-531. (in Japanese).

Tomooka, N., Egawa, Y. and Kaga, A. 2000. Biosysematics and genetic resources of the genus *Vigna* subgenus *Ceratotropis*. Proceedings of the 7th MAFF International Workshop on Genetic Resources Part 1. Wild Legumes. Published March 2000 AFFRC and NIAR, Japan.

Tomooka, N., Vaughan, D.A., Maxted, N. and Moss, H. 2002. The Asian *Vigna*. Genus *Vigna* subgenus *Ceratotropis* genetic resources. 270 pages. Kluwer Academic Press.

Tomooka, N., Kaga, A. and Vaughan, D. A. 2006. The Asian *Vigna* (*Vigna* Subgenus *Ceratotropis*) Biodiversity and Evolution. Pages 87-126 in A.K. Sharma and Sharma (eds.) Plant Genome: Biodiversity and evolution. Vol. 1, Part C. Phanerogams (Angiosperms - Dicotyledons). Science publishers, Enfield, New Jersey.

Vaughan, D. A., Tomooka, N. and Kaga, A. 2005. Azuki Bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]. In (R.J. Singh & P.P. Jauhar eds.) Genetic resources, chromosome engineering, and crop improvement. Vol. 1. Grain Legumes. Chapter 11: 347-359. CRC Press.

Xu, X. H., Jing, T., Tomooka, N., Kaga, A., Isemura, T. and Vaughan, D. A. 2008. Genetic diversity of the azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi) gene pool as assessed by SSR markers. Genome 51: 728-738.

Maturing

V. riukiuensis F_2 (0321) *V. angularis*

Seedling

V. riukiuensis F_3 (0321) *V. angularis*

Testing of SCN resistance

V. riukiuensis F_3(0321- ② 85) *V. angularis*

Root

V. riukiuensis F_3 (0321- ② 85) *V. angularis*

Photo 1. Comparison of morphology