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Collection of Wild Ceratotropis Species on the Nansei Archipelago, Japan and Evaluation of Bruchid Resistance

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Abstract
The Nansei Archipelago was explored to collect wild *Ceratotropis* species, which are considered to be a wide range of gene(s) sources of cultivated Ceratotropis species such as azuki bean and mungbean. During the exploration, 12 accessions of Vigna riukiuensis and 4 accessions of *V. reflexo-pilosa* were collected. Evaluation the resistance of these species against the infestation with bruchid beetles was then conducted. *V. riukiuensis* showed higher levels of resistance against Callosobruchus maculatus (cowpea weevil), while *V. reflexo-pilosa* exhibited higher levels of resistance to *C. chinensis* (azuki bean weevil) compared with azuki bean. Therefore, these wild species were considered to be useful gene(s) sources of bruchid resistance for the breeding of azuki bean. In addition to these wild species, rice bean was found to show extremely high levels of resistance to both *C. maculatus* and *C. chinensis*. Gene(s) transfer from rice bean to azuki bean has not been successful due to the difficulty of producing interspecific hybrids and sterility of the hybrids. However, since *V. riukiuensis* could be crossed with both azuki bean and rice bean, it was considered that *V. riukiuensis* could become a bridge species for the incorporation of bruchid resistance gene(s) of rice bean into azuki bean.

Discipline: Genetic resources
Additional key words: azuki bean, Callosobruchus species, insect resistance, Vigna riukiuensis, Vigna reflexo-pilosa

Introduction

Ceratotropis species are naturally distributed in Asia and therefore they are referred to as Asian *Vigna*[13)]. *Ceratotropis* includes 16 species containing 5 food legumes, i.e. mungbean (*V. radiata*), black gram (*V. mungo*), rice bean (*V. umbellata*), azuki bean (*V. angularis*), and moth bean (*V. aconitifolia*)[27)]. Based on the attempts to produce

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This is a report on the results of the collaborative studies undertaken by TARC (Japan) and Chainat FCRC (Thailand), which were conducted to evaluate a wide range of gene sources for the breeding of food legumes belonging to the subgenus *Ceratotropis*.

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interspecific hybrids, it is considered that the *Ceratotropis* species form primary and secondary gene pools and can be utilized for crop improvement programs[1,3,6,14,20,26]. Our project envisioned to cover a wide range of genetic sources which include both cultivated and wild species in *Ceratotropis*. At first, local varieties of mungbean, which is an important food legume throughout Asia, collected from a wide range of geographical regions were studied to determine the center of genetic diversity and the pattern of landrace differentiation[24,25]. To date, however, very few wild species of the subgenus *Ceratotropis* have been collected and evaluated[12,19]. Considering the rapid occurrence of genetic erosion, it is important to collect and evaluate wild species within the subgenus *Ceratotropis*. Therefore, collection and evaluation of wild *Ceratotropis* species were undertaken both in Thailand and Japan[11,23].

Wild species generally exhibit a wide range of genetic diversity in terms of agronomic characteristics involving pest and disease resistance, maturity span, environmental adaptation, and yield potential[9]. Wild relatives of cultivated plants are thus considered to be essential for crop improvement programs. The wild *Ceratotropis* species which occur in Japan consist of *V. angularis* var. *nipponensis, V. nakashimae, V. riukiuensis*, and *V. reflexo-pilosa*[10,15]. These are diploid species ($2n = 22$) except for *V. reflexo-pilosa* which is a tetraploid species ($2n = 44$). Geographical distribution of these species in Japan is as follows. *V. angularis* var. *nipponensis*, which is considered to be an ancestor of azuki bean (*V. angularis* var. *angularis*), is distributed from Iwate and Akita Prefectures (northern part of Honshu Island) to Kagoshima Pref. (southernmost part of Kyushu Island). *V. nakashimae* occurs in Fukuoka, Nagasaki, and Saga Prefs. (northern part of Kyushu). *V. riukiuensis* is restricted to Okinawa Pref., from Okinawa Island to the Yaeyama Islands, which are located in the southernmost part of the Okinawa Pref. *V. reflexo-pilosa* is found in the areas extending from Amami-oshima Island (southern part of Kagoshima Pref., Kyushu) to the Yaeyama Islands of Okinawa Pref.[21]. Among these areas, the Nansei Archipelago, which includes many islands in Kagoshima and Okinawa Prefs., has not been explored systematically for the *Ceratotropis* species to identify useful gene sources for crop breeding. These islands are rich in indigenous flora but under the threat of urbanization. In the present study, an exploration of wild *Ceratotropis* species was organized on these islands, then the evaluation of bruchid resistance was conducted using the germplasm collected.

Materials and methods

1) *Exploration and description of morphological characters*

A total of six islands, i.e. Ishigaki, Iriomote, Yonaguni, Okinoerabu, Tokunoshima, and Tanegashima Islands, on the Nansei Archipelago were explored from 15 to 27 May, 1989 (Fig. 1). The Nansei Archipelago extends from the southern tip of Kyushu to Taiwan, covering a distance of about 1,200 km. The exploration was conducted along roadsides for the collection of the wild species belonging to the subgenus *Ceratotropis*. The flowers of the subgenus *Ceratotropis* species show various shades of yellow color, but are never purple, violet or white as often found in other groups of *Vigna*[2]. Therefore, the plants with trifoliate leaves or yellow flowers were examined for their detailed morphology, and classified based on the key characters proposed by Tateishi (1984)[21]. Seeds were collected wherever possible.

All the accessions were planted in pots at Chainat FCRC (15°10′N, 100°15′E), Thailand, and further examined for the morphological characteristics under more uniform conditions. Basically, the seed weight

Fig. 1. Location of the Nansei Archipelago, Japan

was determined using 100 seeds for each accession, but sometimes a smaller number of seeds were used depending on the availability of seeds. Seed length and seed thickness were measured for 10 seeds with typical size per each accession. Number of seeds per pod and pod length were recorded for five pods per each accession.

2) Test for bruchid resistance

Two species of bruchid beetles, *Callosobruchus chinensis* (azuki bean weevil) and *C. maculatus* (cowpea weevil) were collected at the mungbean seed storage house of Chainat FCRC. The insects were mass-reared in petri dishes (9 cm in diameter) using cultivated mungbean seeds under laboratory conditions.

For the resistance test, 10 seeds were placed in a petri dish (9 cm in diameter) with two replications. The test seeds were infested with two pairs of freshly emerged bruchid adults and kept in an incubator which was maintained at 27°C and 70% relative humidity. The adults were allowed to mate and lay eggs on the seeds for 2 days. The parental insects were then removed from the petri dish. Five days after infestation (DAI), when the eggs became distinctly visible, the number of eggs laid on seeds was counted. The petri dish was then placed in an incubator until the onset of adult emergence. When adults started to emerge the number of adults which emerged was counted daily until 50 DAI. The counting was discontinued thereafter to avoid double count of adults of the second generation. The counted adults were removed from the petri dish each day.

Results

1) Exploration

Twelve accessions of *V. riukiuensis* and four accessions of *V. reflexo-pilosa* were collected during the exploration (Table 1). Among the 12 accessions of *V. riukiuensis*, four were collected on Iriomote Island, and eight on Yonaguni Island. For *V. reflexo-pilosa*, one accession was collected on Iriomote Island, two on Yonaguni Island, and one on Okinoerabu Island. One population of *V. riukiuensis* (near Kabira on Ishigaki Island) and four populations of *V. reflexo-pilosa* (two populations near the top of Mt. Ohyama on Okinoerabu Island, and two populations in the western part of Tokunoshima Island) were also found during the exploration. However, since the plants did not reach the mature stage, no seeds could be collected at these sites.

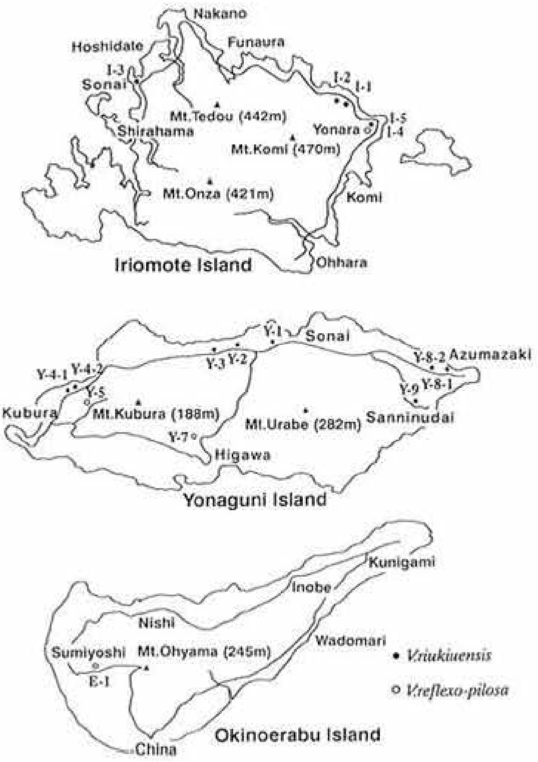

Fig. 2. Location of site for collection of *V. riukiuensis* and *V. reflexo-pilosa*

On Iriomote Island, four accessions of *V. riukiuensis* and one accession of *V. reflexo-pilosa* were collected (Fig. 2). Among them, three accessions of *V. riukiuensis* (I-1, I-2, I-5) and one accession of *V. reflexo-pilosa* (I-4) were collected in the area near Yonara, in the eastern part of Iriomote Island. Especially, in the area near the Yonara bridge, a large number of plants of *V. riukiuensis* and *V. reflexo-pilosa* occurred widely at the edge of pastures along the roadside and were intermingled. In the Sonai area, in the western part of Iriomote Island, *V. riukiuensis* individuals were found sporadically along the path to a small hill, which was used for reaching upland fields. The plants were twining on grasses. One accession was collected at this site (I-3).

On Yonaguni Island, eight accessions of *V. riukiuensis* and two accessions of *V. reflexo-pilosa* were

Table 1. Collection sites and characteristics of *V. riukiuensis* and *V. reflexo-pilosa* collected on the Nansei Archipelago, Japan

Species	Accession name	Collection site*	100-seed weight (g)	Seed length (mm)	Seed thickness (mm)	Seeds/pod	Pod length (cm)
V. riukiuensis	I-1	Yonara, Iriomote Is.	1.2	2.4	1.5	5.6	3.6
	I-2	Yonara, Iriomote Is.	1.1	2.8	2.0	5.2	3.3
	I-3	Sonai, Iriomote Is.	1.2	2.9	2.2	6.6	3.4
	I-5	Near Yonara bridge, Iriomote Is.	1.1	2.7	1.9	7.4	4.0
	Y-1	1 km W of Sonai, Yonaguni Is.	1.0	2.9	2.1	4.8	3.2
	Y-2	4 km W of Sonai, Yonaguni Is.	1.4	3.0	2.4	4.8	3.1
	Y-3	16 km W of Sonai, Yonaguni Is.	1.0	3.1	2.0	5.6	3.7
	Y-4-1	2 km NW of Kubura, Yonaguni Is.	1.1	2.9	2.1	7.0	3.6
	Y-4-2	2 km NW of Kubura, Yonaguni Is.	1.3	3.0	2.3	7.4	3.9
	Y-8-1	Azumazaki, Yonaguni Is.	1.0	3.1	2.1	6.8	4.0
	Y-8-2	Azumazaki, Yonaguni Is.	1.1	2.8	2.1	4.6	3.0
	Y-9	Sanninudai, Yonaguni Is.	1.2	2.9	2.3	-	-
Subtotal	12 accessions	Average	1.1	2.9	2.1	6.0	3.5
V. reflexo-pilosa	I-4	Near Yonara bridge, Iriomote Is.	1.6	3.3	2.6	-	-
	Y-5	5 km W of Kubura, Yonaguni Is.	1.7	3.2	2.4	5.2	3.9
	Y-7	3 km N of Higawa, Yonaguni Is.	1.6	3.2	2.3	6.6	4.0
	E-1	1 km E of Sumiyoshi, Okinoerabu Is.	1.4	3.1	2.4	-	-
Subtotal	4 accessions	Average	1.6	3.2	2.4	5.9	4.0
Total	16 accessions						

* Is. = Island, E = east, NW = northwest, W = west, N = north.

collected (Fig. 2). In open and sunny areas along roadsides on the northern seacoast to the west of Sonai, three accessions of *V. riukiuensis* were collected (Y-1, Y-2, Y-3). A large population of *V. riukiuensis* was found in the area located 2 km northeast from Kubura. Two accessions were collected there, one with black-colored mature pods (Y-4-1) and another with straw-colored mature pods (Y-4-2). In the eastern part of Yonaguni Island, several populations of *V. riukiuensis* were observed in the areas near Azumazaki and Sanninudai. Three accessions were collected there (Y-8-1, Y-8-2, Y-9). *V. reflexo-pilosa* was found and collected along roadsides in the areas located 5 km east from Kubura (Y-5), and 3 km north from Higawa (Y-7).

On Okinoerabu Island, a population of *V. reflexo-pilosa* was found at the site located 1 km east from Sumiyoshi (E-1). The plants were still young, but a few mature pods were collected at this site.

2) *Morphological characters*

V. riukiuensis was characterized by glossy orbicular leaflets with a rounded apex. Stipules of *V. riukiuensis* were smaller and shorter than those of *V. reflexo-pilosa*. The seed morphology of *V. riukiuensis* and *V. reflexo-pilosa* is shown in Plate 1. Seeds of *V. riukiuensis* were oblong and the hilum was linear with a thick rim-aril. Seeds of *V. reflexo-pilosa* were short-cylindrical and the hilum was short with a thin rim-aril. Detailed data of the character-

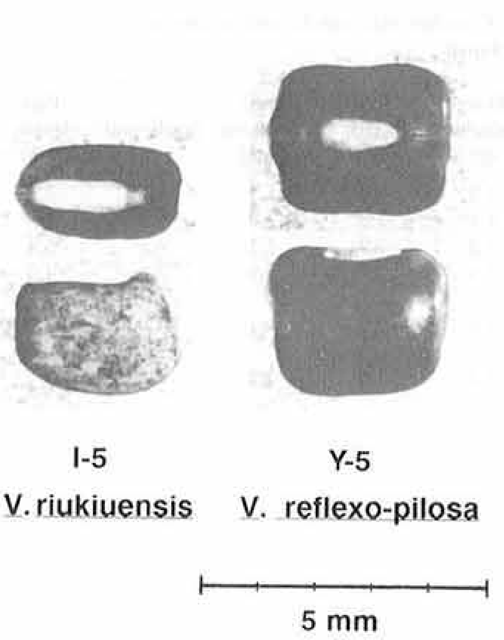

Plate 1. Seed morphology of *V. riukiuensis* and *V. reflexo-pilosa*

istics of seed and pod are presented in Table 1. Seed weight (100-seed weight), seed length, and seed thickness of *V. riukiuensis* ranged from 1.0 to 1.4 g (average = 1.1 g), from 2.4 to 3.1 mm (ave. = 2.9 mm), and from 1.5 to 2.4 mm (ave. = 2.1 mm), respectively. The values of the corresponding parameters for *V. reflexo-pilosa* ranged from 1.4 to 1.7 g (ave. = 1.6 g), from 3.1 to 3.3 mm (ave. = 3.2 mm), and from 2.3 to to 2.6 mm (ave. = 2.4 mm), respectively. Average number of seeds per pod was 6.0 in *V. riukiuensis*, while that in *V. reflexo-pilosa* was 5.9. Pod length of *V. riukiuensis* was shorter (ave. = 3.5 cm) than that of *V. reflexo-pilosa* (ave. = 4.0 cm). Pods of *V. riukiuensis* were glabrous and those of *V. reflexo-pilosa* were glabrous or scabrous.

3) Bruchid resistance

Since *V. riukiuensis* and *V. reflexo-pilosa* are considered to be closely related to *V. angularis* (azuki bean) and *V. umbellata* (rice bean), the levels of resistance of *V. riukiuensis* and *V. reflexo-pilosa* to *C. chinensis* and *C. maculatus* are indicated together with those of azuki bean and rice bean in Tables 2 and 3.

Against the infestation by *C. chinensis*, the accessions of azuki bean were highly susceptible (emergence(%) = 56.1, damaged seeds(%) = 100.0 on the average) (Table 2). The accessions of *V. riukiuensis* showed a slightly lower percentage of emergence (45.4%) and a significantly lower percentage of damaged seeds (61.4%). *V. reflexo-pilosa* and rice bean exhibited significantly higher levels of resistance (emergence(%) = 15.1, damaged seeds(%) = 18.3 for *V. reflexo-pilosa*, and emergence(%) = 0.2, damaged seeds(%) = 1.4 for rice bean) compared with azuki bean. Especially, rice bean showed very high levels of resistance. Among the seven accessions of rice bean tested, five showed a complete resistance to *C. chinensis*. Although two accessions of rice bean were found to be infested by *C. chinensis*, only one adult managed to emerge from each of these two accessions, showing extremely low emergence percentages (0.5 and 1.0%, respectively). Furthermore, the emergence was significantly delayed in rice bean (developmental period = 42.5 days) compared with that in azuki bean (29.4 days).

Against the infestation by *C. maculatus*, azuki bean and *V. reflexo-pilosa* were highly susceptible (emergence(%) = 69.4, damaged seeds(%) = 79.0 for azuki bean, and emergence(%) = 53.7, damaged seeds(%) = 95.0 for *V. reflexo-pilosa*) (Table 3). *V. riukiuensis* showed significantly higher levels of resistance to *C. maculatus* (emergence(%) = 13.4, damaged seeds(%) = 25.5) compared with azuki bean and *V. reflexo-pilosa*. Against the infestation by *C. maculatus*, all the accessions of rice bean showed complete resistance (emergence(%) = 0.0, damaged seeds(%) = 0.0).

Discussion

Rice bean has been reported to show higher levels of resistance to *C. chinensis* compared with azuki bean[17]. Higher levels of resistance of rice bean against *C. chinensis* were confirmed (Table 2), and complete immunity of rice bean against *C. maculatus* was revealed (Table 3) in the present study. In addition to the resistance against bruchid beetles, rice bean was reported to show higher levels of resistance to AMV (azuki bean mosaic virus)[18] and to root-knot nematode (*Meloidogyne javanica*)[22] compared with azuki bean. However, since hybrids between azuki bean and rice bean are very difficult to

Table 2. Levels of resistance of *V. riukiuensis*, *V. reflexo-pilosa*, azuki bean (*V. angularis*) and rice bean (*V. umbellata*) against azuki bean weevil (*C. chinensis*)

Legume species	Accession name	100-seed weight (g)	Eggs/rep. (no.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
V. riukiuensis	I-2	1.1	11.5 a*	57.5 a	65.0 a	30.3 ab
	I-3	1.2	21.5 b	43.5 a	85.0 a	29.1 a
	I-5	1.1	11.5 a	39.0 a	65.0 a	35.7 b
	Y-1	1.0	14.0 ab	61.0 a	75.0 a	29.2 a
	Y-2	1.4	16.0 ab	79.0 a	85.0 a	29.7 a
	Y-3	1.0	15.0 ab	30.0 a	55.0 a	31.3 ab
	Y-4-1	1.1	17.5 ab	34.0 a	60.0 a	30.5 ab
	Y-4-2	1.3	13.5 ab	25.0 a	30.0 a	29.8 ab
	Y-8-1	1.0	12.0 a	60.0 a	70.0 a	28.9 ab
	Y-8-2	1.1	17.0 ab	23.5 a	30.0 a	33.1 ab
	Average	1.1	14.8 a**	45.4 b	61.4 b	31.1 a
V. reflexo-pilosa	I-4	1.6	12.0 a	25.0 a	20.0 a	30.2 a
	Y-5	1.7	14.0 a	10.5 a	15.0 a	32.0 a
	Y-7	1.6	15.0 a	10.0 a	20.0 a	29.3 a
	Average	1.6	13.7 a	15.1 a	18.3 a	30.5 a
V. angularis (azuki bean)	Kyoto dainagon	22.4	37.5 b	66.0 abc	100.0 a	28.7 a
	Omuta	18.5	32.0 b	55.0 abc	100.0 a	28.7 a
	101	7.8	43.5 bc	49.5 abc	100.0 a	28.5 a
	102	6.8	34.0 b	34.5 a	100.0 a	29.7 a
	104	7.7	31.5 ab	48.0 abc	100.0 a	30.3 a
	105	8.6	30.5 ab	54.5 abc	100.0 a	29.2 a
	108	9.8	14.0 a	78.5 c	100.0 a	30.9 a
	118	10.6	61.0 c	46.5 ab	100.0 a	29.2 a
	119	11.1	39.0 b	55.0 abc	100.0 a	29.4 a
	126	9.8	28.0 ab	73.0 bc	100.0 a	29.4 a
	Average	11.3	35.1 b	56.1 b	100.0 c	29.4 a
V. umbellata (rice bean)	Chainat	9.3	51.5 a	0.0 a	0.0 a	-
	070001	4.7	57.0 a	0.0 a	0.0 a	-
	220001	8.5	67.5 a	0.0 a	0.0 a	-
	220002	7.3	66.0 a	0.5 a	5.0 a	42.0
	220003	6.4	56.0 a	1.0 a	5.0 a	43.0
	220004	7.5	48.0 a	0.0 a	0.0 a	-
	220005	6.5	68.0 a	0.0 a	0.0 a	-
	Average	7.2	59.1 c	0.2 a	1.4 a	42.5 b

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate.
*,** Mean separation was performed by least significant difference at 99% level.

produce[15,20], attempts to incorporate useful genes of rice bean into azuki bean have not been successful[18]. Using the materials collected in this exploration, it was revealed that *V. riukiuensis* was cross-compatible with both azuki bean and rice bean when crossed as a pollen parent[20]. Therefore, it was suggested that *V. riukiuensis* could act as a bridge species between azuki bean and rice bean. It may be possible to incorporate useful genes from rice bean to azuki bean through *V. riukiuensis*. Since *V. riukiuensis* showed significantly higher levels of resistance to *C. maculatus* compared to azuki bean (Table 3), *V. riukiuensis* itself could also be useful for improving the levels of resistance of azuki bean against *C. maculatus* in addition to the role of genetic bridge.

V. reflexo-pilosa showed significantly higher levels of resistance to *C. chinensis* compared with azuki

Table 3. Levels of resistance of *V. riukiuensis*, *V. reflexo-pilosa*, azuki bean (*V. angularis*) and rice bean (*V. umbellata*) against cowpea weevil (*C. maculatus*)

Legume species	Accession name	100-seed weight (g)	Eggs/rep. (no.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
V. riukiuensis	I-1	1.2	31.0 a*	8.0 a	25.0 a	39.3 a
	I-2	1.1	18.5 a	5.5 a	10.0 a	38.0 a
	I-3	1.2	33.0 a	8.0 a	30.0 a	37.7 a
	I-5	1.1	15.5 a	19.5 ab	30.0 a	37.4 a
	Y-1	1.0	19.0 a	16.5 ab	35.0 ab	32.8 a
	Y-2	1.4	19.0 a	47.5 b	75.0 b	33.7 a
	Y-3	1.0	20.5 a	7.0 a	10.0 a	39.5 a
	Y-4-1	1.1	16.0 a	3.5 a	5.0 a	43.0 a
	Y-4-2	1.3	23.5 a	4.0 a	5.0 a	31.0 a
	Y-8-1	1.0	21.5 a	17.5 ab	40.0 ab	31.8 a
	Y-8-2	1.1	19.0 a	10.5 a	15.0 a	38.0 a
	Average	1.1	21.5 a**	13.4 a	25.5 b	36.5 a
V. reflexo-pilosa	I-4	1.6	19.0 a	47.5 a	90.0 a	31.2 a
	Y-5	1.7	16.5 a	58.0 a	100.0 a	30.3 a
	Y-7	1.6	18.0 a	55.5 a	95.0 a	31.2 a
	Average	1.6	17.8 a	53.7 b	95.0 c	30.9 a
V. angularis (azuki bean)	Kyoto dainagon	22.4	24.5 abc	69.0 ab	45.0 a	36.0 a
	Omuta	18.5	32.5 abcd	100.0 b	100.0 a	35.2 a
	101	7.8	20.0 a	46.0 ab	50.0 a	39.6 a
	102	6.8	43.0 bcd	54.5 ab	80.0 a	38.3 a
	104	7.7	35.0 abcd	46.5 ab	80.0 a	36.2 a
	105	8.6	23.0 ab	26.5 a	70.0 a	36.9 a
	108	9.8	31.5 abcd	94.0 b	65.0 a	34.6 a
	118	10.6	15.5 a	91.5 b	100.0 a	32.5 a
	119	11.1	45.5 cd	70.5 ab	100.0 a	35.4 a
	126	9.8	47.5 d	95.5 b	100.0 a	33.2 a
	Average	11.3	31.8 a	69.4 b	79.0 c	35.8 a
V. umbellata (rice bean)	Chainat	9.3	53.5 a	0.0 a	0.0 a	-
	070001	4.7	58.0 a	0.0 a	0.0 a	-
	220001	8.5	58.5 a	0.0 a	0.0 a	-
	220002	7.3	41.0 a	0.0 a	0.0 a	-
	220003	6.4	54.5 a	0.0 a	0.0 a	-
	220004	7.5	45.5 a	0.0 a	0.0 a	-
	220005	6.5	43.5 a	0.0 a	0.0 a	-
	Average	7.2	50.6 b	0.0 a	0.0 a	-

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate.
*,** Mean separation was performed by least significant difference at 99% level.

bean. However, it is considered to be difficult to incorporate resistance genes of *V. reflexo-pilosa* to azuki bean due to the tetraploid nature (2n = 44) of *V. reflexo-pilosa*. In the subgenus *Ceratotropis*, another tetraploid species, *V. glabrescens*, was also described[13]. *V. glabrescens* shows a vigorous growth and has a great potential for high yield of good quality fodder. In fact, *V. glabrescens* was cultivated under the name of "Lentille de Créole" in Mauritius, and as a forage crop in Haringhata, West Bengal[2]. *V. glabrescens* exhibits pest and disease resistance, especially resistance to beanfly (*Ophiomyia phaseoli*, *O. centrosematis*, *Melanagromyza sojae*), and has been used in the mungbean improvement program at AVRDC in Taiwan[8]. Since gene transfer from tetraploid *V. glabrescens* to diploid mungbean by means of conventional cross breeding methods was difficult, hybrids were obtained with the aid of embryo culture and plans are made to use the backcross progenies as bridging materials[4].

The wild ancestor of *V. glabrescens* has not been identified[5]. Utilizing the materials collected in the present study, it was demonstrated that *V. reflexo-pilosa* was cross-compatible with *V. glabrescens*[7]. These two species readily produced fertile hybrids when cross-pollinated with each other. Judging from the morphological similarities of seeds and primary leaves, the same ploidy level ($2n = 44$, $4\times$), and high level of cross-compatibility, *V. reflexo-pilosa* seemed to be a candidate of the wild ancestral form of *V. glabrescens*. Considering the close relationship between *V. glabrescens* and *V. reflexo-pilosa*, it is anticipated that *V. reflexo-pilosa* also harbors good genes in the same way as *V. glabrescens* does. Therefore, *V. reflexo-pilosa* could become a source of pest and disease resistance for the breeding of *Ceratotropis* cultigens.

V. reflexo-pilosa has been known to occur only in Taiwan and the Ryukyus (Okinawa Pref., the southernmost islands in Japan). According to Tateishi[21], however, many specimens from South China (Hainan), Thailand, the Philippines, Indonesia (Sumatra, Java, Timor), New Guinea, Australia, New Caledonia, New Hebrides and Fiji, which have been identified as *V. mungo*, *V. radiata* var. *sublobata*, or *Phaseolus calcaratus* (= *V. umbellata*) may correspond to *V. reflexo-pilosa*. If so, a still wider range of variations can be expected to occur in *V. reflexo-pilosa*. Therefore, the geographical area of exploration of *V. reflexo-pilosa* should be expanded to these regions.

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