

Collection and Conservation of Legume Genetic Resources in Oita and Miyazaki Prefectures of Japan in 2016

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Summary

We conducted a field survey in Oita and Miyazaki prefectures from October 3 to 7, 2016, to collect and conserve legume genetic resources, especially crop wild relatives. A total of 51 accessions were collected of five species from the genera *Glycine* and *Vigna*, including the wild relatives of soybean, azuki bean, and mung bean. We plan to multiply the seeds collected in this survey and to conserve them as genetic resources for research and crop breeding programs.

KEY WORDS: crop wild relatives, endangered species, genetic resources, legume

Introduction

Collection and conservation of plant genetic resources for food and agriculture are important activities of gene banks around the world. The importance of crop wild relatives has increased recently, mainly because they may be used in crop breeding to counter a variety of biological and environmental stress conditions (Takahashi *et al.* 2016). Wild plants of the leguminous genera *Vigna* and *Glycine* are widely distributed in Japan, and systematic collection of these genetic resources has been conducted through the NARO Genebank project (Tomooka *et al.* 2010a; Vaughan *et al.* 2010; Takahashi *et al.* 2015). Crop wild relatives sometimes include endangered species. The Ministry of the Environment of the Government of Japan described 1,782 species as 'Endangered' in the Red List based on the investigation of about 7,000 vascular plant species (2017 Japanese Red Lists, <http://www.env.go.jp/press/103881.html>, in Japanese). In the Red Lists, three species of *Glycine* and three species of *Vigna* are listed (<http://www.env.go.jp/press/files/jp/105449.pdf>, in Japanese). Therefore, the importance of *ex-situ* conservation of crop wild relatives in the gene bank as a safety backup of endangered species has increased.

In recent years, we focused on the collection and conservation of wild *Glycine* and *Vigna* genetic resources in the northwestern part of Kyushu island, Japan, including small islands, such as Tsushima, Goto, Iki, Hirado, and Amakusa (Baba-Kasai *et al.* 2016; Takahashi *et al.* 2014; Tomooka *et al.* 2010b, 2013, 2015). In these surveys, the main target species has been *Vigna nakashimae* (Ohwi) Ohwi & H. Ohashi and *Vigna vexillata* (L.) A. Rich., which are currently categorized as ‘Endangered.’ Since we obtained internet-based information according to which *V. vexillata* plants were found growing in Oita and Miyazaki prefectures, on the eastern side of Kyushu island, we decided to visit the location. Climate in low altitude areas of Oita and Miyazaki prefectures is classified as Cfa (i.e., humid subtropical) according to Köppen classification scheme. These prefectures also have inland mountainous areas (the highest peak is 1,791 m) with humid continental climate, Dfa and Dfb (Wikimedia file).

Methods

A field survey was conducted in Oita and Miyazaki prefectures from October 3 to 7, 2016 (Table 1). (1) Bungotakada, (2) Hita-Kusu-Yufu, and (3) Oita-Bungoohno-Saiki areas were surveyed in Oita prefecture (Fig. 1), and (4) Nobeoka and (5) Hinokage-Takachiho areas were surveyed in Miyazaki prefecture. Before the survey we consulted the Bungotakada city office of Wild Life Conservation about the availability of detailed information on the habitat of the target wild legumes. Additionally, at the collection sites we interviewed land owners and sought their permission for collection. Thereafter, we collected seeds of wild leguminous plants, and recorded passport data including, latitude, longitude, and altitude using a Garmin GPSMAP 60CSx handheld GPS device (USA). After the field trip, GPS data were corrected by Google Earth Pro software (Google Inc.). Identification of the *Vigna* species is based on taxonomic keys (Tomooka *et al.* 2002; Maxted *et al.* 2004).

Collected samples were classified into two categories, i.e., “cultivated” and “naturally growing” plants (Table 2). Based on morphological traits, “Naturally growing” plants were further classified in two categories, i.e., “intermediate” and “wild” types. Wild-type plants show the typical characteristics of wild *Vigna* plants, such as relatively small and black mottled seeds, easy shattering pods, and slender twining purple stems. Contrairily, the intermediate-type plants show some characteristics of domesticated plants, such as relatively large and light-colored seeds, rarely- or non-shattering pods, and less- or non-twining stems. Such intermediate types potentially include both, (1) hybrid offspring of wild and cultivated forms, and (2) naturally growing populations established by plants that escaped cultivation.

Table 1. Itinerary of the field survey in the Oita and Miyazaki prefectures, 2016

Date	Itinerary	Stay
3-Oct	Tsukuba -- Tokyo Haneda airport - (ANA91) - Oita airport -- survey of Bungotakada area -- Beppu	Beppu, Oita
4-Oct	Beppu -- survey of Hita-Kusu-Yufu area -- Beppu	Beppu, Oita
5-Oct	Beppu -- survey of Oita-Bungoohno-Saiki, Nobeoka and Hinokage-Takachiho areas -- Takachiho	Takachiho, Miyazaki
6-Oct	survey of Takachiho area -- Miyazaki	Miyazaki, Miyazaki
7-Oct	Miyazaki airport - (ANA608) - Haneda airport -- Tsukuba	-

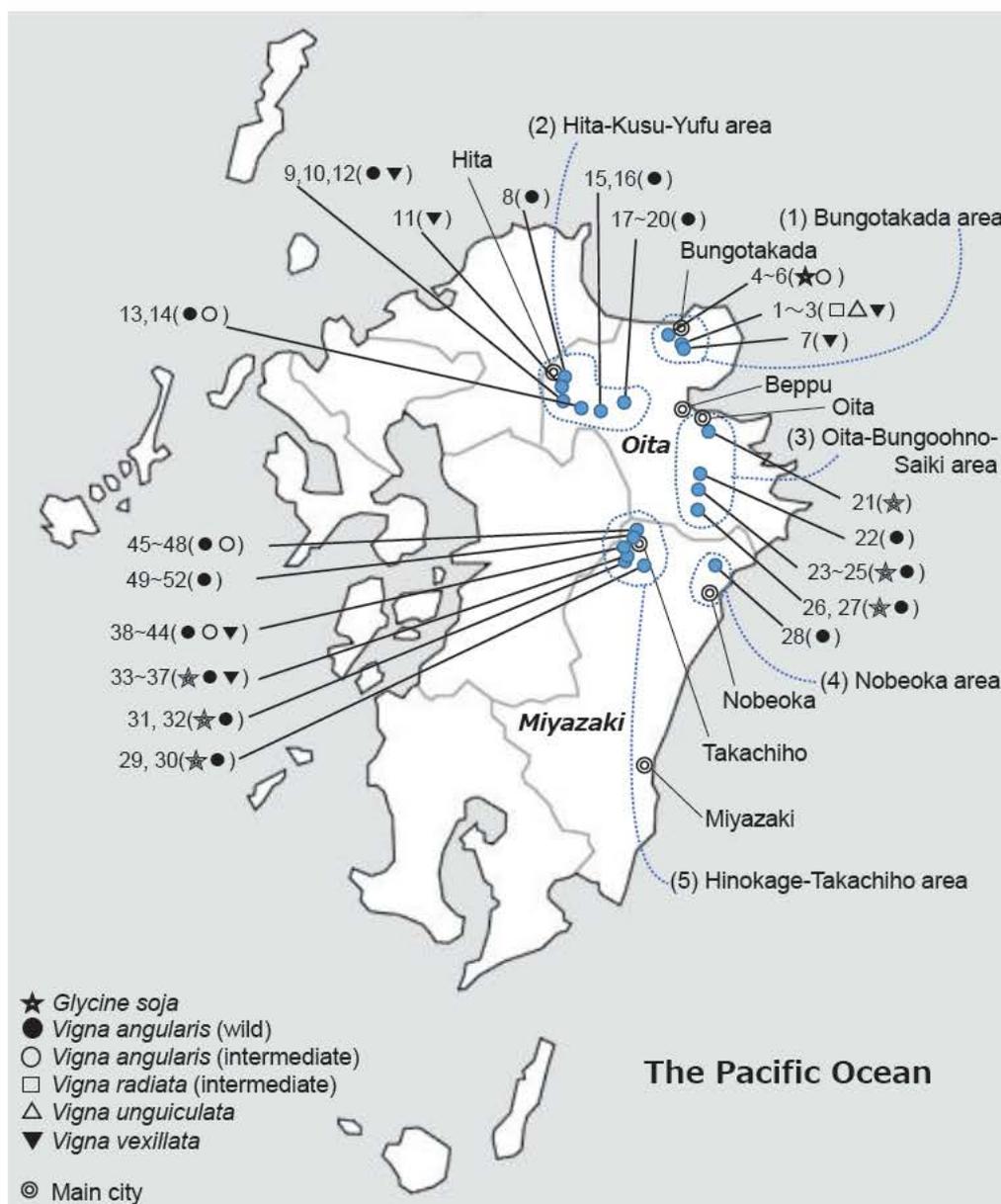


Fig. 1. Collection sites in Oita and Miyazaki prefectures, Kyushu, Japan

Results and Discussion

A total of 51 accessions, including six of *Glycine soja* Siebold & Zucc., 34 of *Vigna angularis* (Willd.) Ohwi & H. Ohashi, one of *Vigna radiata* (L.) R. Wilczek, one of *Vigna unguiculata* (L.) Walp., and nine of *Vigna vexillata* were collected (Table 2, Fig. 1). The collection consists of one cultivated accession, seven intermediate-type accessions, and 43 wild-type accessions. We summarized the passport information of each accession (Table 3), and described the characteristics of plant samples collected of each species.

Wild-type soybean accessions (*Glycine soja*)

Intermediate-type *Glycine* plants are rare in natural environment of Japan (Kaga *et al.* 2005; Kuroda *et al.* 2005). However, SSR profiling for 616 individuals from 77 natural populations collected widely in Japan suggested that 42 individuals (6.8%) have presumed introgressed genomic segments from domesticated soybeans (Kuroda *et al.* 2006a). These authors mention that, “The lack of intermediate

Table 2. A summary of the collections in Oita and Miyazaki prefectures

Species	Cultivated (crops)	Naturally growing		Total
		Intermediate form	Wild form	
<i>Glycine soja</i>	-	-	6	6
<i>Vigna angularis</i>	-	6	28	34
<i>Vigna radiata</i>	-	1	-	1
<i>Vigna unguiculata</i>	1	-	-	1
<i>Vigna vexillata</i>	-	-	9	9
Total	1	7	43	51

morphological characteristics in presumed introgressed samples analyzed suggests that hybrid progenies between wild and cultivated soybeans revert to wild soybean characteristics under natural conditions.” Therefore, wild-type soybean plants collected in the present survey might include introgressed genomic segments from domesticated soybeans.

Seven wild-type soybean populations were found in this survey, and mature seeds could be obtained from six of them (Tables 2 and 3). Four populations were found in Oita and three in Miyazaki prefecture (Fig. 1). They were found around paddy fields and along river banks (Photographs 1-5). In most places, plants for collection were still at the grain-filling stage. Therefore, we collected immature pods together with the plant shoots and keep them in a net to obtain mature seeds. This method allowed us to obtain mature seeds with good germination ability for all collected materials, except for accession OM29 (Table 3).

Wild- and Intermediate-type azuki bean (*Vigna angularis*)

Taxonomically, wild-type azuki bean, *Vigna angularis* (Willd.) Ohwi & H. Ohashi var. *nipponensis* (Ohwi) Ohwi & H. Ohashi is considered a variety. However, in natural environments in Japan, plants of *V. angularis* with intermediate morphology between wild and domesticated types are common, occasionally forming complex populations consisting of wild and intermediate types (Xu *et al.* 2000). Many of these intermediate types, sometimes, called ‘weedy,’ are considered as hybrid derivatives between wild and domesticated types.

Twenty-eight wild-type accessions and six intermediate-type accessions of azuki bean were collected (Table 2). Wild-type azuki bean was more common than wild-type soybean in this survey. Wild- and intermediate-type azuki beans were found on artificially disturbed environments, such as road side, paddy fields, fallow fields, and river banks (Photographs 6-10). Their leaflets are ovate to lanceolate, lobed or entire (Photographs 11 and 12). Among six intermediate-type accessions, ‘OM14’ showed the largest vegetative organs and seed size, although it has shattering pods and dormant seeds (Photograph 13). We found accession ‘OM16’ with small glossy leaves and short pods (Photographs 14 and 15). Superficially, these characteristics are similar to those of *Vigna nakashimae*. However, the accession was identified as wild *V. angularis*, because it has large stipules and non-protruding seed hilum (Seed photograph, OM16).

Intermediate-type Mung bean (*Vigna radiata*)

A natural population of mung bean ‘OM1’ was found growing in soybean crop fields in Bungotakada city, Oita prefecture (Photograph 16). The plants showed twining stems, smaller vegetative organs, shattering pods, and dormant small brown seeds (Seed photograph). The owner of the fields recalled that

mung bean plants began to grow in his fields since he started applying cow manure to it.

According to Kurokawa (2017), alien weeds have been increasingly becoming a serious problem in Japan since the late 1980s, especially in lands cultivated for forage production. He summarized data on alien weed seeds contaminating imported forage crop seeds from 1993 to 1995, and found seeds of 91 weed species, including 12 of legume plants. He suggested that the application of unripe cow dung could be a major driver for the spreading alien weeds in the country. Although mung bean was not in the list prepared by Kurokawa (2017), it is possible that intermediate-type (weedy) mung bean seeds were imported as contaminants of forage seeds and spread through application of cow manure to the fields. We also found natural populations of intermediate-type mung bean in Saga (JP225160, JP226798, JP226803, Kuroda *et al.* 2005, 2006b), Nagasaki (JP247287, JP252171, JP252173, Tomooka *et al.* 2013) and Kumamoto (JP252193, JP252215, JP252220, JP252228, Tomooka *et al.* 2015) prefectures, Kyushu, Japan.

Cowpea (*Vigna unguiculata*)

An accession of red seeded cowpea was collected from a cultivated field along the road in Bungotakada city, Oita prefecture (Photograph 17). Although we could not find naturally growing cowpea populations, plants of this species with small seeds and shattering pods were collected in Tsushima (JP237059, JP237062, Tomooka *et al.* 2010b), Goto (JP247323, JP247339, JP247353, Tomooka *et al.* 2013) and Sasebo (JP254625, JP254635, Baba-Kasai *et al.* 2016) in Nagasaki prefecture, and in Amakusa (JP252189, JP252226, Tomooka *et al.* 2015) in Kumamoto prefectures.

Wild-type Tuber cowpea (*Vigna vexillata*)

Vigna vexillata is an annual or perennial legume distributed in the tropics and temperate regions. It is characterized by its acuminate stipule produced at the base, purple flowers, curved keel, and hairy long pods (Verdcourt 1970; Maxted *et al.* 2004). The species exhibits significant morphological diversity, and seven botanical varieties have been described (Maxted *et al.* 2004). Recently, the existence of a cultivated variety domesticated for its edible roots was reported in Indonesia, for which the name “tuber cowpea” was proposed (Karuniawan *et al.* 2006).

In Japan, two morphologically distinguishable types of *V. vexillata* have been described. First, Matsumura (1902) described *V. vexillata* Benth. var. *tsusimensis* Matsum., known as “Akasasage.” However, he did not select type-specimens, nor did he clarify the distinguishing morphological characters between this variety and *V. vexillata* var. *vexillata*. In addition, *V. vexillata* (L.) A. Rich., should be the correct name. Probably for these reasons, the taxon name *V. vexillata* var. *tsusimensis* has not been listed in major *Vigna* monographs (Maréchal *et al.* 1978; Maxted *et al.* 2004).

Nakajima (1968) noticed that *V. vexillata* plants growing in Okinawa island showed different morphology from *V. vexillata* var. *tsusimensis*, and named it “Sakuya-akasasage.” He noted that “Sakuya-akasasage” showed a similar morphology to *V. vexillata* var. *vexillata*, having smaller ovate leaflets, shorter terminal leaflet petiole, larger flowers, 1 or 2 flowers in a peduncle, shorter (6 - 8 cm), and slender (3 - 4 mm width) pods, each containing 20 seeds; lastly, plant parts are more densely covered with hairs. Isozyme polymorphisms were detected between these morphological types (Yasuda *et al.* 1996).

“Akasasage” as *V. vexillata* var. *tsusimensis* and “Sakuya-Akasasage” as *V. vexillata* var. *vexillata*, are listed in the 2017 Japanese Red Lists (in Japanese, <http://www.env.go.jp/press/files/jp/105449.pdf>). “Akasasage” was categorized as ‘Endangered’ in Nagasaki, Kumamoto, Oita, Miyazaki, and Kagoshima

prefectures (<http://jpnrd.com/search.php?mode=map&q=06030572479>), while “Sakuya-Akasasage” was reported as ‘Critically Endangered’ in Okinawa prefecture and as ‘Vulnerable’ in Kagoshima prefecture (<http://jpnrd.com/search.php?mode=map&q=06030572478>).

In the survey reported here, we could collect nine accessions of *V. vexillata* in Bungotakada and Hita cities, Oita prefecture and in Takachiho city, Miyazaki prefecture. They were considered as *V. vexillata* var. *tsusimensis*, because they showed very similar morphology to the accessions collected from Tsushima island, Nagasaki prefecture (Tomooka *et al.* 2010b). *V. vexillata* plants inhabit artificially disturbed wet environments, such as fallow paddy fields, wet stream side and shady road sides (Photographs 18-21) in hilly area (altitude ranging from 112 to 397 m.a.s.l). In Takachiho city, Miyazaki prefecture, accession ‘OM35’ was found growing together with wild soybean and wild azuki bean on a ridge slope of a terraced paddy field (Photograph 22). Tuber of ‘OM35’ was slightly swollen (Photograph 23).

In Hita city, Oita prefecture, *V. vexillata* var. *tsusimensis* ‘OM09’ plants were grown in front of the Japan Water Agency -Kyusyu Regional Office- Oyama Dam Operation and Maintenance Office (Photograph 24). They found *V. vexillata* var. *tsusimensis* populations during ecological surveys before the Oyama Dam construction and transplanted them around the dam banks and in front of Regional Office for the conservation of ‘Endangered’ plants (Murata and Goshima 2010). When we visited the Oyama Dam Regional Office, we met Mr. Goshima, who is one of the authors of the report. Mr. Goshima kindly guided us to the other population sites, where we collected accessions ‘OM10’ and ‘OM11’.

Future perspectives

We have conducted field surveys in the Kyushu District over the past four years, and collected legume genetic resources including 89 accessions in 2012, 64 accessions in 2013, 91 accessions in 2014, and 71 accessions in 2015 (Tomooka *et al.* 2013; Takahashi *et al.* 2014; Tomooka *et al.* 2015; Baba-Kasai *et al.* 2016). These included *V. vexillata* var. *tsusimensis* in Nagasaki prefecture and *V. nakashimae* in Nagasaki and Kumamoto prefectures, which are classified as ‘Endangered’ in the Red List (in Japan, <http://www.env.go.jp/press/files/jp/105449.pdf>). These valuable genetic resources have been conserved at the NARO Genebank, and multiplied seeds are now available upon request (https://www.gene.affrc.go.jp/index_en.php). We plan to multiply the seeds collected in this survey and to conserve them to distribute for education, research and crop breeding programs.

References

- Baba-Kasai A, Akiba M, Hirashima S, Ohmizu T, Tomooka N (2016) Field survey and collection of *Glycine* and *Vigna* genetic resources in Nagasaki prefecture, Japan, 2015. AREIPGR 32: 1-27.
[https://www.gene.affrc.go.jp/publications_en.php]
- Kaga A, Tomooka N, Phuntsho U, Kuroda Y, Kobayshi N, Isemura T, Miranda-Jonson G, Vaughan DA (2005) Exploration and collection for hybrid derivatives between wild and cultivated soybean: Preliminary survey in Akita and Hiroshima Prefectures, Japan. AREIPGR 21: 59-71 (in Japanese with English summary).
[https://www.gene.affrc.go.jp/publications_en.php]
- Karuniawan A, Iswandi A, Kale PR, Heinzemann J, Gruneberg WJ (2006) *Vigna vexillata* (L.) A. Rich. cultivated as a root crop in Bali and Timor. Genet Resour Crop Evol 53: 213-217.
- Kuroda Y, Kaga A, Apa A, Vaughan DA, Tomooka N, Yano H, Matsuoka N (2005) Exploration, collection

- and monitoring of wild soybean and hybrid derivatives between wild soybean and cultivated soybean: based on field surveys at Akita, Ibaraki, Aichi, Hiroshima and Saga prefectures. AREIPGR 21: 73-95 (in Japanese with English summary).
[https://www.gene.affrc.go.jp/publications_en.php]
- Kuroda Y, Kaga A, Tomooka N, Vaughan DA (2006a) Population genetic structure of Japanese wild soybean (*Glycine soja*) based on microsatellite variation. Mol Ecol 15: 959-974.
- Kuroda Y, Kaga A, Guaf J, Vaughan DA, Tomooka N (2006b) Exploration, collection and monitoring of wild soybean, cultivated soybean and hybrid derivatives between wild soybean and cultivated soybean: based on field surveys at Akita, Ibaraki, Kochi and Saga prefectures. AREIPGR 22: 1-12 (in Japanese with English summary).
[https://www.gene.affrc.go.jp/publications_en.php]
- Kurokawa S (2017) Alien weeds in agricultural land: problems and solutions. J Weed Sci Tech 62: 36-47 (in Japanese).
- Maréchal R, Mascherpa JM, Stainer F (1978) Etude taxonomique d'un groupe complexé d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de données morphologiques et polliniques, traitées par l'analyse informatique. Boissiera 28: 1-273.
- Matsumura J (1902). A conspectus of the Leguminosae found growing wild, or cultivated in Japan, Loochoo Formosa, Botanical Magazine 16: 93.
[<https://archive.org/details/botanicalmagazin16toky>]
- Maxted N, Mabuza-Dlamini P, Moss H, Padulosi S, Jarvis A, Guarino L (2004) African *Vigna*: an ecogeographic study. International Plant Genetic Resources Institute, Rome, p. 454.
- Murata H, Goshima T (2010) Conservation of “Akasasage” (*Vigna vexillata* var. *tsusimensis*) for Oyama dam construction. 大山ダムにおけるアカササゲの保全対策. ダム技術 281: 4-51 (in Japanese).
[<http://www.mlit.go.jp/chosahokoku/h22giken/program/kadai/pdf/ippan/ippan3-08.pdf>]
- Nakajima K (1968) *Vigna vexillata* A. Rich., a new addition to the flora of the Ryukyus. サクヤアカササゲについて. J Jpn Bot 43: 247-248 (in Japanese).
[http://www.jjbotany.com/pdf/JJB_043_247_248_d.pdf]
- Takahashi Y, Akiba M, Iizumi T, Tomooka N (2014) Collection and conservation of wild leguminous crop relatives on Iki Island and Hirado Island, Nagasaki Prefecture, Japan, 2013. AREIPGR 30: 1-27.
[https://www.gene.affrc.go.jp/publications_en.php]
- Takahashi Y, Lay-Heng S, Channa T, Makara O, Tomooka N (2015) Exploration of leguminous crops and their wild relatives in western regions of Cambodia, 2014. AREIPGR 31: 121-149.
[https://www.gene.affrc.go.jp/publications_en.php]
- Takahashi Y, Somta P, Muto C, Iseki K, Naito K, Pandiyan M, Natesan S, Tomooka N (2016) Novel genetic resources in the genus *Vigna* unveiled from gene bank accessions. PLoS One 11: e0147568.
- Tomooka N, Vaughan DA, Moss H, Maxted N (2002) The Asian *Vigna*: Genus *Vigna* subgenus *Ceratotropis* genetic resources. Kluwer Academic Publishers, Dordrecht, p. 270.
- Tomooka N, Kaga A, Isemura T, Vaughan DA, Srinives P, Somta P, Thadavong S, Bounphanousay C, Kanyavong K, Inthapanya P, Pandiyan M, Senthil N, Ramamoorthi N, Jaiwal PK, Jing T, Umezawa K, Yokoyama T (2010a) *Vigna* Genetic Resources. In: Proceedings of the 14th NIAS International Workshop on Genetic Resources “Genetics and Comparative Genomics of legumes (*Glycine* and *Vigna*)”, pp. 11-21.

[https://www.gene.affrc.go.jp/publications_en.php]

Tomooka N, Inoue J, Akiba M (2010b) Collection and conservation of wild leguminous crop relatives on Tsushima Island, Nagasaki, Japan, 2009. AREIPGR 26: 27-34.

[https://www.gene.affrc.go.jp/publications_en.php]

Tomooka N, Fukui K, Chankaew S, Iizumi T, Hirashima S (2013) Collection of wild leguminous crop relatives on Goto islands, Nagasaki, Japan, 2012. AREIPGR 29: 19-43.

[https://www.gene.affrc.go.jp/publications_en.php]

Tomooka N, Iseki K, Naito K, Akiba M, Iizumi T (2015) Collection of *Glycine* and *Vigna* plant genetic resources in Hirado and Shimabara areas of Nagasaki Prefecture and Amakusa area of Kumamoto Prefecture in Japan, from 20th to 24th October, 2014. AREIPGR 31: 1-33.

[https://www.gene.affrc.go.jp/publications_en.php]

Vaughan DA, Tomooka N, Kaga A, Isemura T, Kuroda Y (2010) *Glycine* genetic resources. *In: Proceedings of the 14th NIAS International Workshop on Genetic Resources “Genetics and Comparative Genomics of Legumes (*Glycine* and *Vigna*)”*, pp. 1-9.

[https://www.gene.affrc.go.jp/publications_en.php]

Verdcourt B (1970) Studies in the Leguminosae-Papilionoideae for the flora of tropical east Africa IV. *Kew Bulletin* 24: 507-569.

Wikimedia file: Japan map of Köppen climate classification.svg

[http://commons.wikimedia.org/wiki/File:Japan_map_of_K%C3%B6ppen_climate_classification.svg]

Xu RQ, Tomooka N, Vaughan DA, Doi K (2000) The *Vigna angularis* complex: Genetic variation and relationships revealed by RAPD analysis, and their implications for in situ conservation and domestication. *Genet Resour Crop Evol* 47: 123-134.

Yasuda K, Yamaguchi H (1996) Phylogenetic analysis of the subgenus *Ceratotropis* (genus *Vigna*) and an assumption of the progenitor of azuki bean using isozyme variation. *Breed Sci* 46: 337-342.

大分県および宮崎県におけるマメ科植物遺伝資源の 収集と保全，2016年

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和文摘要

本報告は、2016年10月3日～7日に行った大分県および宮崎県でのマメ科植物遺伝資源の調査報告である。結果として、野生型ダイズ6点、野生型アズキ28点、中間型アズキ6点、中間型リョクトウ1点、栽培ササゲ1点、野生型アカササゲ9点、合計51点のマメ科植物遺伝資源を収集した。この中で、アカササゲは、環境省が指定する絶滅危惧種のリストに記載された希少種である。収集したすべてのマメ科植物遺伝資源は、つくば市にある農業・食品産業技術総合研究機構遺伝資源センター圃場で栽培し、特性評価と種子増殖を行う計画である。増殖種子は、農業・食品産業技術総合研究機構のジーンバンクで保存するとともに、研究や育種あるいは教育目的に利用するために配布可能な遺伝資源とする。

Table 3. A passport data of the collections in Oita and Miyazaki prefectures

Collection No	Accession No	Collection Date (yyyy/mm/d)	Scientific name	Status	Collection Site	Latitude	Longitude	Altitude (m)	Soil	Collection method	100 seed weight (g)	Remarks
OM1	JP257436	10/3/2016	<i>Vigna radiata</i>	intermediate	Tashibumaki, Bungotakada, Oita 大分県 豊後高田市 田染真木, 桂川左岸	N33-30-29 7	E131-31-06 5	88	clay	bulk	1 6	Naturally growing in a soybean field ダイズ畑の雑草として生育
OM2	JP257437	10/3/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Tashibumaki, Bungotakada, Oita 大分県 豊後高田市 田染真木, 荒城屋敷跡地	N33-29-58 7	E131-31-02 9	118	-	bulk	2 1	paddy field, along roadside 荒城家屋敷跡、道路下の水田脇、休耕地に自生
OM3	JP257438	10/3/2016	<i>Vigna unguiculata</i>	cultivated	Tashibumanaka, Bungotakada, Oita 大分県 豊後高田市 田染真中	N33-30-30 6	E131-31-00 5	94	organic soil	bulk	17 1	fields beside a road 県道 655 号沿いの細長い畑に栽培 (赤大粒)
OM4	JP257439	10/3/2016	<i>Vigna angularis</i>	intermediate	Sano, Bungotakada, Oita 大分県 豊後高田市 佐野	N33-32-04 5	E131-28-27 1	37	clay	bulk	2 9	ridge separating paddy fields 道路と水田の間の溝に自生
OM5	JP257440	10/3/2016	<i>Glycine soja</i>	wild	Sano, Bungotakada, Oita 大分県 豊後高田市 佐野	N33-32-04 0	E131-28-37 4	35	clay	bulk	1 7	bank of an agricultural waterway, immature stage 農業用水路の脇に自生、未熟
OM6	JP257441	10/3/2016	<i>Vigna angularis</i>	intermediate	Sano, Bungotakada, Oita 大分県 豊後高田市 佐野	N33-32-04 0	E131-28-37 4	35	clay	bulk	3 5	bank of an agricultural waterway 農業用水路の脇に自生
OM7	JP257442	10/3/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Tashibuhirano, Bungotakada, Oita 大分県 豊後高田市 田染平野, ひらの橋横	N33-29-41 6	E131-30-56 0	120	clay	bulk	3 3	bank of a stream 県道 655 号から横に入ったひらの橋下の小川土手
OM8	JP257443	10/4/2016	<i>Vigna angularis</i>	wild	Hita, Oita 大分県 日田市, 大分高速, 日田料金所	N33-20-10 8	E130-55-59 5	105	-	bulk	2 0	near highway toll gate 高速料金所 (日田) 出たところ
OM9	JP257444	10/4/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Oyamamachi Nishioyama, Hita, Oita 大分県 日田市 大山町西大山, 大山ダム	N33-14-40 22	E130-57-19 89	246	organic soil	bulk	3 4	grown for conservation of "Endangered species" in a planter in front of Oyama Dam Office 大山ダム事務所の前のプランターで絶滅危惧種保存のために栽培
OM10	JP257445	10/4/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Oyamamachi Nishioyama, Hita, Oita 大分県 日田市 大山町西大山, 大山ダム	N33-15-21 5	E130-57-26 2	160	-	individual	4 3	River side ridge 平成 20 年の豪雨の後に作られた吾々路川の堤防上に自生 (1 個体のみ)
OM11	JP257446	10/4/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Hidakamachi, Hita, Oita 大分県 日田市 日高	N33-17-37 8	E130-57-07 0	112	-	individual	4 2	roadside 県道 212 号線、大山川沿いの道脇 (1 莢のみ)
OM12	JP257447	10/4/2016	<i>Vigna angularis</i>	wild	Oyamamachi Nishioyama, Hita, Oita 大分県 日田市 大山町西大山	N33-15-11 7	E130-57-31 52	149	volcanic tuff	bulk	2 1	roadside, in red soil and gravel 大山ダムのすぐ下流、川沿いの集落の空き地
OM13	JP257448	10/4/2016	<i>Vigna angularis</i>	wild	Tobata, Kusugun Kusumachi, Oita 大分県 玖珠郡 玖珠町 戸畑	N33-16-02 3	E131-04-28 8	270	sand	bulk	2 0	forest along a path 国道 210 号沿い、玖珠川沿いの小道に自生
OM14	JP257449	10/4/2016	<i>Vigna angularis</i>	intermediate	Tobata, Kusugun Kusumachi, Oita 大分県 玖珠郡 玖珠町 戸畑	N33-16-02 6	E131-04-27 1	270	sand	bulk	4 1	beside a road, a large number of seedlings observed 国道 210 号線脇の荒れ地に多数自生
OM15	JP257450	10/4/2016	<i>Vigna angularis</i>	wild	Nogami, Kusugun Kokonoemachi, Oita 大分県 玖珠郡 九重町 野上	N33-14-09 2	E131-13-13 8	469	organic soil	bulk	2 9	roadside of R210 国道 210 号道路脇に自生
OM16	JP257451	10/4/2016	<i>Vigna angularis</i>	wild	Nogami, Kusugun Kokonoemachi, Oita 大分県 玖珠郡 九重町 野上	N33-14-12 87	E131-13-03 77	499	clay	bulk	2 5	roadside of small village beside R210 国道 210 号から細い脇道を上った集落の小道脇
OM17	JP257452	10/4/2016	<i>Vigna angularis</i>	wild	Kawanishi, Yufuincho, Yufu, Oita 大分県 由布市 湯布院町 川西	N33-14-43 1	E131-19-47 3	457	volcanic tuff	bulk	2 0	beside paddy field below R210 国道 210 号下の水田脇に自生
OM18	JP257453	10/4/2016	<i>Vigna angularis</i>	wild	Kawanishi, Yufuincho, Yufu, Oita 大分県 由布市 湯布院町 川西	N33-14-44 4	E131-19-44 1	464	-	bulk	2 0	in wet place of road side open space beside R210 国道 210 号脇の空き地 (湿った場所のみ自生)
OM19	JP257454	10/4/2016	<i>Vigna angularis</i>	wild	Kawaminami, Yufuincho, Yufu, Oita 大分県 由布市 湯布院町 川南	N33-14-58 4	E131-20-40 4	465	clay	bulk	1 8	along a trail near a railway 県道 11 号横の線路と川の間の荒れ地
OM20	JP257455	10/4/2016	<i>Vigna angularis</i>	wild	Nakagawa, Yufuincho, Yufu, Oita 大分県 由布市 湯布院町 中川	N33-15-00 5	E131-20-37 6	458	clay	bulk	2 2	along a trail between R11 and railway 県道 11 号と線路との間の道路脇
OM21	JP257456	10/5/2016	<i>Glycine soja</i>	wild	Shimohetsugi, Oita, Oita 大分県 大分市 下戸次	N33-10-13 1	E131-39-54 4	20	sand	bulk	1 7	a dry riverbed, had lanceolate leaflets, immature stage 国道 10 号沿い水田横の大野川河川敷、未熟

Table 3. (Continued).

Collection No	Accession No	Collection Date (yyyy/mm/d)	Scientific name	Status	Collection Site	Latitude	Longitude	Altitude (m)	Soil	Collection method	100 seed weight (g)	Remarks
OM22	JP257457	10/5/2016	<i>Vigna angularis</i>	wild	Asase, Miemachi, Bungoohno, Oita 大分県 豊後大野市 三重町 浅瀬	N33-01-33 8	E131-36-27 3	55	clay	bulk	2 5	on a 60° slope and on a ridge separating paddy fields 国道 326 号から県道 636 に入ってしまった水田脇の急斜面
OM23	JP257458	10/5/2016	<i>Vigna angularis</i>	wild	Uchiyama, Miemachi, Bungoohno, Oita 大分県 豊後大野市 三重町 内山	N32-57-28 2	E131-35-06 1	156	clay	bulk	2 1	a grassy area near a parking lot of "Renjoji temple" 蓮城寺駐車場脇の草地
OM24	JP257459	10/5/2016	<i>Glycine soja</i>	wild	Uchiyama, Miemachi, Bungoohno, Oita 大分県 豊後大野市 三重町 内山	N32-57-28 2	E131-35-06 1	156	clay	bulk	1 4	a grassy area near a parking lot of "Renjoji temple" 蓮城寺駐車場脇の草地
OM25	JP257460	10/5/2016	<i>Vigna angularis</i>	wild	Uchiyama, Miemachi, Bungoohno, Oita 大分県 豊後大野市 三重町 内山	N32-57-30 4	E131-35-06 8	154	-	bulk	2 2	on a river embankment beside "Renjoji temple" 蓮城寺横の三重川の土手
OM26	JP257461	10/5/2016	<i>Glycine soja</i>	wild	Onoichi, Ume, Saiki, Oita 大分県 佐伯市 宇目小野市	N32-51-33 1	E131-37-42 1	186	-	bulk	1 2	a grassy area along a river 国道 326 号沿いの川に沿った荒地
OM27	JP257462	10/5/2016	<i>Vigna angularis</i>	wild	Onoichi, Ume, Saiki, Oita 大分県 佐伯市 宇目小野市	N32-51-33 1	E131-37-42 1	186	-	bulk	2 4	a grassy area along a river 国道 326 号沿いの川に沿った荒地
OM28	JP257463	10/5/2016	<i>Vigna angularis</i>	wild	Nagai, Kitagawamachi, Nobeoka, Miyazaki 宮崎県 延岡市 北川町 長井	N32-39-41 6	E131-41-48 2	6	clay	bulk	2 0	embankment of an agricultural waterway 国道 10 号脇の水田地帯に沿った水路沿い
OM29	No seeds	10/5/2016	<i>Glycine soja</i>	wild	Nagai, Kitagawamachi, Nobeoka, Miyazaki 宮崎県 延岡市 北川町 長井	N32-39-41 6	E131-41-48 2	6	clay	-	-	embankment of an agricultural waterway 国道 10 号脇の水田地帯に沿った水路沿い
OM30	JP257464	10/5/2016	<i>Vigna angularis</i>	wild	Nanaori, Nishiusukigun Hinokagecho, Miyazaki 宮崎県 西臼杵郡 日之影町 七折	N32-39-48 85	E131-23-04 71	268	clay	bulk	1 4	between a stream and a paddy field 国道 218 号日之影集落の水田脇
OM31	JP257465	10/5/2016	<i>Vigna angularis</i>	wild	Nanaori, Nishiusukigun Hinokagecho, Miyazaki 宮崎県 西臼杵郡 日之影町 七折	N32-41-41 1	E131-19-23 3	333	clay	bulk	2 1	in a fallow field 国道 218 号沿いの湿った休耕地
OM32	JP257466	10/5/2016	<i>Glycine soja</i>	wild	Nanaori, Nishiusukigun Hinokagecho, Miyazaki 宮崎県 西臼杵郡 日之影町 七折	N32-41-41 1	E131-19-23 3	333	clay	bulk	1 3	in a fallow field 国道 218 号沿いの湿った休耕地
OM33	JP257467	10/5/2016	<i>Vigna angularis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-13 2	E131-19-02 9	359	-	bulk	2 3	beside an stream "Jindai river" 神代川沿いの小道
OM34	JP257468	10/5/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-13 2	E131-19-02 9	359	-	bulk	3 1	beside an stream "Jindai river" 神代川沿いの小道
OM35	JP257469	10/5/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-16 5	E131-19-03 6	366	clay	bulk	2 8	on a slope between terraced paddy fields 神代川沿いの水田法面に多数自生
OM36	JP257470	10/5/2016	<i>Vigna angularis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-16 5	E131-19-03 6	366	clay	bulk	2 3	on a slope between terraced paddy fields 神代川沿いの水田法面に自生
OM37	JP257471	10/5/2016	<i>Glycine soja</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-16 5	E131-19-03 6	366	clay	bulk	1 3	on a slope between terraced paddy fields 神代川沿いの水田法面に自生
OM38	JP257472	10/5/2016	<i>Vigna angularis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-36 2	E131-18-51 1	406	clay	bulk	2 5	beside stream in a village above "Jindai river" 神代川横上阿床集落最上部の小川沿い
OM39	JP257473	10/5/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-34 2	E131-18-50 3	397	clay	bulk	3 1	beside stream in a village above "Jindai river" 神代川横上阿床集落最上部の小川沿い
OM40	JP257474	10/5/2016	<i>Vigna angularis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-33 4	E131-18-48 3	397	clay	bulk	2 6	on a slope between terraced paddy fields 神代川横上阿床集落の水田脇斜面 (白い家)
OM41	JP257475	10/5/2016	<i>Vigna vexillata</i> var <i>tsusimensis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-31 3	E131-18-47 9	390	clay	bulk	3 5	on a slope between terraced paddy fields 神代川横上阿床集落の水田脇斜面
OM42	JP257476	10/5/2016	<i>Vigna angularis</i>	intermediate	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-27 18	E131-18-48 26	382	clay	bulk	2 7	beside paddy fields 神代川横上阿床集落の水田脇
OM43	JP257477	10/5/2016	<i>Vigna angularis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-24 01	E131-18-49 91	375	clay	bulk	2 6	early maturing, a large number of plants 神代川横上阿床集落の水田脇、早生、大量

Table 3. (Continued).

Collection No	Accession No	Collection Date (yyyy/mm/d)	Scientific name	Status	Collection Site	Latitude	Longitude	Altitude (m)	Soil	Collection method	100 seed weight (g)	Remarks
OM44	JP257478	10/5/2016	<i>Vigna angularis</i>	wild	Mitai, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 三田井	N32-43-16 12	E131-18-52 77	366	clay	bulk	2 1	beside paddy fields 神代川横上阿床集落の水田脇
OM45	JP257479	10/5/2016	<i>Vigna angularis</i>	intermediate	Iwato, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 岩戸	N32-44-43 1	E131-20-50 5	380	clay	bulk	2 1	on a slope between terraced paddy fields 県道 204 号沿い
OM46	JP257480	10/5/2016	<i>Vigna angularis</i>	wild	Iwato, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 岩戸	N32-44-44 8	E131-20-50 4	371	clay	bulk	2 1	embankment near a cowshed beside river, R204 県道 204 号沿いの川横牛舎脇
OM47	JP257481	10/6/2016	<i>Vigna angularis</i>	intermediate	Iwato, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 岩戸	N32-45-04 2	E131-20-31 8	443	clay	bulk	2 7	near a grave, R204 県道 204 号沿いの墓の脇
OM48	JP257482	10/6/2016	<i>Vigna angularis</i>	wild	Iwato, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 岩戸	N32-45-19 6	E131-20-13 9	530	clay	bulk	2 1	in front of a house 上村最上部の家の脇
OM49	JP257483	10/6/2016	<i>Vigna angularis</i>	wild	Iwato, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 岩戸	N32-43-44 1	E131-20-28 3	382	clay	bulk	2 6	on a slope between terraced paddy fields 天岩戸温泉奥の集落の棚田の脇
OM50	JP257484	10/6/2016	<i>Vigna angularis</i>	wild	Iwato, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 岩戸	N32-43-43 8	E131-20-30 0	386	clay	bulk	2 9	ridge separating paddy fields 天岩戸温泉奥の集落の棚田の脇
OM51	JP257485	10/6/2016	<i>Vigna angularis</i>	wild	Iwato, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 岩戸	N32-43-40 9	E131-20-24 0	415	clay	bulk	2 5	in a fallow field 天岩戸温泉奥の集落の寺の奥の畑横
OM52	JP257486	10/6/2016	<i>Vigna angularis</i>	wild	Iwato, Nishiusukigun Takachihocho, Miyazaki 宮崎県 西臼杵郡 高千穂町 岩戸	N32-44-10 1	E131-21-04 8	333	-	bulk	2 0	near Amanoiwato Shrine 天野岩人神社から天安河原へ向かう小道沿い



Photograph 1. Habitat of *G. soja*, OM05 in Bungotakada, Oita.



Photograph 2. Immature *G. soja*, OM05 in Bungotakada, Oita.



Photograph 3. Leaf of *G. soja*, OM21 in Oita, Oita.



Photograph 4. Flower of *G. soja*, OM29 in Nobeoka, Miyazaki.



Photograph 5. Pods of *G. soja*, OM05 in Bungotakada, Oita.



Photograph 6. Habitat of intermediate form of *V. angularis*, OM06 in Bungotakada, Oita.



Photograph 7. Wild form of *V. angularis*, OM08 in Hita, Oita.



Photograph 8. Habitat of wild form of *V. angularis*, OM20 in Yufu, Oita.



Photograph 9. Pods of wild form of *V. angularis*, OM20 in Yufu, Oita.



Photograph 10. Habitat of wild form of *V. angularis*, OM25 in Bungoono, Oita.



Photograph 11. Leaf of wild form of *V. angularis*, OM38 in Takachiho, Nishiusuki, Miyazaki.



Photograph 12. Leaf of wild form of *V. angularis*, OM22 in Bungoono, Oita.



Photograph 13. Leaf of intermediate form of *V. angularis*, OM14 in Kusu, Oita.



Photograph 14. Leaf of wild form of *V. angularis*, OM16 in Kokonoe, Kusu, Oita.



Photograph 15. Pods of wild form of *V. angularis*, OM16 in Kokonoe, Kusu, Oita.



Photograph 16. Intermediate form of *V. radiata*, OM01 in Bungotakada, Oita.



Photograph 17. Cultivated *V. unguiculata*, OM17 in Bungotakada, Oita.



Photograph 18. Habitat of *V. vexillata* var. *tsusimensis*, OM02 in Bungotakada, Oita.



Photograph 19. *V. vexillata* var. *tsusimensis*, OM02 in Bungotakada, Oita.



Photograph 20. Habitat of *V. vexillata* var. *tsusimensis*, OM07 in Bungotakada, Oita.



Photograph 21. Habitat of *V. vexillata* var. *tsusimensis*, OM35 in Takachiho, Nishiusuki, Miyazaki.



Photograph 22. Sympatric habitat of *V. vexillata* var. *tsusimensis*, OM35 and wild form of *V. angularis*, OM36 in Takachiho, Nishiusuki, Miyazaki.



Photograph 23. Tuberous root of *V. vexillata* var. *tsusimensis*, OM35, Takachiho, Nishiusuki, Miyazaki.



Photograph 24. Plants of *V. vexillata* var. *tsusimensis*, OM09, grown for conservation of "Endangered species" in a planter in front of Oyama Dam Office, Oyamamachi Nishioyama, Hita, Oita.



OM1, JP257436, *Vigna radiata* (intermediate)



OM2, JP257437, *Vigna vexillata* var. *tsusimensis* (wild)



OM3, JP257438, *Vigna unguiculata* (cultivated)



OM4, JP257439, *Vigna angularis* (intermediate)



OM5, JP257440, *Glycine soja* (wild)



OM6, JP257441, *Vigna angularis* (intermediate)



OM7, JP257442, *Vigna vexillata* var. *tsusimensis* (wild)



OM8, JP257443, *Vigna angularis* (wild)



OM9, JP257444, *Vigna vexillata* var. *tsusimensis* (wild)



OM10, JP257445, *Vigna vexillata* var. *tsusimensis* (wild)



OM11, JP257446, *Vigna vexillata* var. *tsusimensis* (wild)



OM12, JP257447, *Vigna angularis* (wild)



OM13, JP257448, *Vigna angularis* (wild)



OM14, JP257449, *Vigna angularis* (intermediate)



OM15, JP257450, *Vigna angularis* (wild)

Seed photographs of collected accessions. A division on a ruler indicates one millimeter.



OM16, JP257451, *Vigna angularis* (wild)



OM17, JP257452, *Vigna angularis* (wild)



OM18, JP257453, *Vigna angularis* (wild)



OM19, JP257454, *Vigna angularis* (wild)



OM20, JP257455, *Vigna angularis* (wild)



OM21, JP257456, *Glycine soja* (wild)



OM22, JP257457, *Vigna angularis* (wild)



OM23, JP257458, *Vigna angularis* (wild)



OM24, JP257459, *Glycine soja* (wild)



OM25, JP257460, *Vigna angularis* (wild)



OM26, JP257461, *Glycine soja* (wild)



OM27, JP257462, *Vigna angularis* (wild)



OM28, JP257463, *Vigna angularis* (wild)

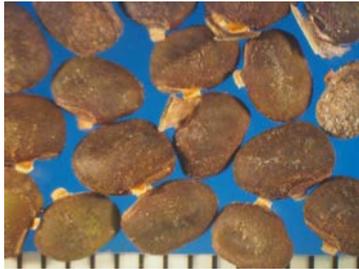


OM30, JP257464, *Vigna angularis* (wild)



OM31, JP257465, *Vigna angularis* (wild)

Seed photographs of collected accessions. A division on a ruler indicates one millimeter.



OM32, JP257466, *Glycine soja* (wild)



OM33, JP257467, *Vigna angularis* (wild)



OM34, JP257468, *Vigna vexillata* var. *tsusimensis* (wild)



OM35, JP257469, *Vigna vexillata* var. *tsusimensis* (wild)



OM36, JP257470, *Vigna angularis* (wild)



OM37, JP257471, *Glycine soja* (wild)



OM38, JP257472, *Vigna angularis* (wild)



OM39, JP257473, *Vigna vexillata* var. *tsusimensis* (wild)



OM40, JP257474, *Vigna angularis* (wild)



OM41, JP257475, *Vigna vexillata* var. *tsusimensis* (wild)



OM42, JP257476, *Vigna angularis* (intermediate)



OM43, JP257477, *Vigna angularis* (wild)



OM44, JP257478, *Vigna angularis* (wild)



OM45, JP257479, *Vigna angularis* (intermediate)



OM46, JP257480, *Vigna angularis* (wild)

Seed photographs of collected accessions. A division on a ruler indicates one millimeter.



OM47, JP257481, *Vigna angularis*
(intermediate)



OM48, JP257482, *Vigna angularis*
(wild)



OM49, JP257483, *Vigna angularis*
(wild)



OM50, JP257484, *Vigna angularis*
(wild)



OM51, JP257485, *Vigna angularis*
(wild)



OM52, JP257486, *Vigna angularis*
(wild)

Seed photographs of collected accessions. A division on a ruler indicates one millimeter.