The ex situ Conservation of Wild Legume Genetic Resources in Shimane Prefecture in 2018

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Original Paper

The *ex situ* Conservation of Wild Legume Genetic Resources in Shimane Prefecture in 2018

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Summary

This is a report describing the *ex situ* conservation activity of the NARO Genebank project for the collection of wild legume genetic resources in Shimane Prefecture from October 16 to 19, 2018. During this survey, we recorded a total of 30 habitats and collected 30 samples, including seven seed samples of wild soybean (*Glycine max* subsp. *soja*, syn. *Glycine soja*), two wild and 20 seed samples of the intermediate form of azuki bean (*Vigna angularis*), and one seedling sample of *Vigna vexillata*. To our knowledge, this is the first scientific report that confirms the habitat of *V. vexillata* on Honshu Island, Japan. After the seeds collected in this survey have been multiplied, we plan to conserve them in the NARO Genebank as genetic resources for education, research, and breeding programs.

KEY WORDS: crop wild relatives, genetic resources, *Glycine*, legume, *Vigna*

Introduction

Conservation of crop wild relatives is one of the most important roles assigned to world gene banks. In recent years, the importance of crop wild relatives has received considerable attention (McCouch *et al.* 2013), because some of them are tolerant or resistant to environmental or biological stresses. Therefore, the NARO Genebank has been continuously collecting crop wild relatives of the genus *Glycine* and *Vigna* (Tomooka *et al.* 2010a; Vaughan *et al.* 2010). However, relatively few Asian wild accessions of the tuber cowpea (*Vigna vexillata* (L.) A. Rich.) were conserved in the NARO Genebank (Takahashi *et al.* 2018). In

Japan, wild tuber cowpea is designated as “Endangered IA” (critically endangered) on the red list issued by the Ministry of the Environment Government of Japan. Therefore, recently, we concentrated on its conservation (Takahashi *et al.* 2017). During information gathering, we realized that wild tuber cowpea was also found in Shimane Prefecture on Honshu Island in addition to Kyushu Island, the original habitat. Shimane Prefecture is one of the important regions for conducting studies to elucidate the domestication origins of soybean (*Glycine max* (L.) Merr.) and azuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi), crops that have been used in Japan since ancient times. The area has been thought to have been inhabited by ancient Japanese, as many archaeological sites of the Jomon period were discovered (https:// iseki. shimane-u.ac.jp/).

This report details the conservation of legume genetic resources in Shimane Prefecture. Shimane Prefecture is classified as humid subtropical climate (Cfa) in the Köppen climate classification. The latitude is 35º north on the central part of the prefecture, which is the same as that for the metropolitan Tokyo, Japan. The region receives considerable rainfall throughout the year, with annual precipitation ranging from 1,600 mm to 2,300 mm. In summer, the nighttime temperature reaches above 30 ºC on some days; in winter, the daytime temperature occasionally reaches below 0 ºC. We conducted a survey in October during the autumn in this region.

Methods

A field survey was conducted in Shimane Prefecture from October 16 to 19 in 2018 (Table 1). We interviewed landowners and asked their permission to collect plant materials. We collected seeds from wild leguminous plants and recorded their passport data, including the latitude, longitude, and altitude of their habitat, by using Google Maps and Google Earth (Google). Identification of *Vigna* species was based on taxonomic keys (Tomooka *et al.* 2002; Maxted *et al.* 2004).

Results and Discussion

In this survey, we collected seed samples from 22 *V. angularis* and 7 *G. max* subsp. *soja* populations, as well as 1 seedling sample from a *V. vexillata* population (Table 2, Fig. 1). The passport data of each sample are shown in Table 3, and the characteristics of each species are described below.

Wild tuber cowpea

***Vigna vexillata* (L.) A. Rich. in Hist. Fis. Polit. Nat. I. Cuba 11:191 (1845)**

***Vigna vexillata* (L.) A. Rich. var. *tsusimensis* Matsum. in Bot. Mag. 16:93 (1902)**

Wild tuber cowpea distributed in Africa, the Americas, Asia, and Oceania, and their cultigens have been collected from West Africa, Central America, and Southeast Asia (Garba and Pasquet 1998). Some hundreds of accessions from Africa and Australia are stored in world gene banks, but relatively few

Table 1. Itinerary of the field survey in Shimane Prefecture in 2018

Date	Itinerary	Stay
16-Oct	Haneda airport - JAL279 - Izumo enmusubi airport - Matsue City	Matsue City
17-Oct	Matsue City - Daikon Island - Unnan City - Matsue City	Matsue City
18-Oct	Matsue City - Izumo City - Matsue City	Matsue City
19-Oct	Matsue City - Izumo enmusubi airport - JAL280 - Haneda airport	-

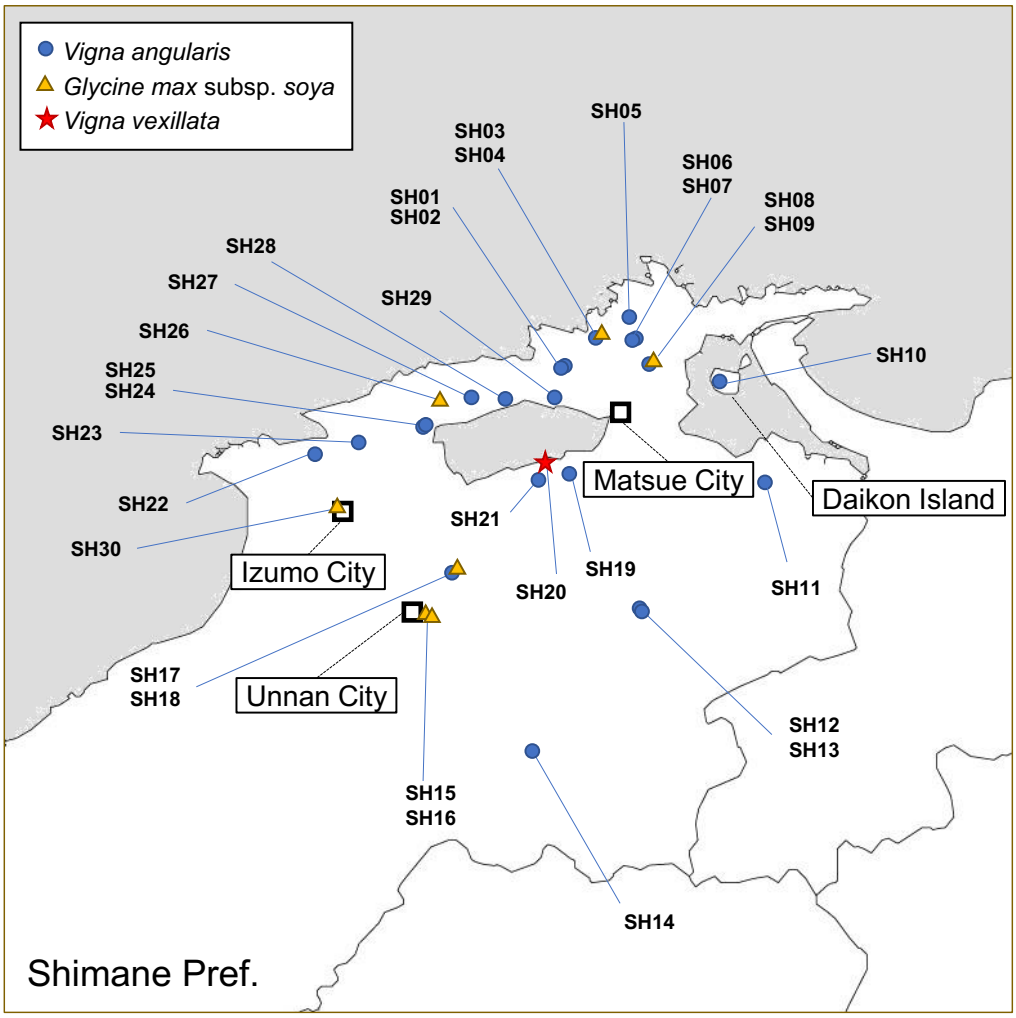

Fig. 1. Collection sites of each accession in Shimane Prefecture.

accessions collected from Asia, including Japan, are currently conserved (Tomooka *et al.* 2010b; Takahashi *et al.* 2017, 2018). Two taxa have been reported for Japanese wild tuber cowpea, "Aka-sasage" (*Vigna vexillata* (L.) A. Rich. var. *tsusimensis* Matsum.), which is distributed in Kyushu Island, and "Sakuya-aka-sasage" (*Vigna vexillata* (L.) A. Rich.), which is found in Okinawa Island. Thus far, no scientific report has described the distribution of wild tuber cowpea on Honshu Island. We obtained information from a webpage suggesting that "Aka-sasage" was found in Matsue City, Shimane Prefecture (Photos 1 and 2). The author of this webpage (Mr. Takaaki Notsu, residing in Matsue City) uploaded the photos of the wild tuber cowpea from flowering (September) to pod-setting stages (November) captured in 2016 (https:// matsue-hana.com/hana/ akasasage.html).

We visited and found wild tuber cowpea plants at the site he mentioned. The site was a relatively new roadside embankment slope, where shrubs and grasses were flourishing (Photo 3). The soil was artificial organic soil introduced for the development of embankments. Although plants at flowering or pod-setting stage could not be found, we noted several seedlings (Photo 4). We identified the seedlings as "Aka-sasage" because of the hairy stems, basifixed stipule, leaflets with white spots, and long petiole of the terminal leaflet. Since seeds could not be obtained, we dug and brought back the seedlings and cultivated them in a greenhouse at the NARO Genebank. Finally, we could successfully obtain several seeds (Seed Photo SH20).

The growth of wild tuber cowpea was confirmed in Shimane Prefecture on Honshu Island. To our knowledge, this is the first scientific report that confirms the habitat of *V. vexillata* on Honshu Island,

Table 2. Summary of the collections in Shimane Prefecture in 2018

Species	Status	No. of seed samples
Vigna angularis (Willd.) Ohwi & Ohashi	wild	2
Vigna angularis (Willd.) Ohwi & Ohashi	Intermediate	20
Glycine max (L.) Merr. subsp. *soja* (Sieb. & Zucc.) Ohashi Syn. *Glycine soja* Sieb. & Zucc.	Wild	7
Vigna vexillata (L.) A. Rich. var. *tsusimensis* Matsum.	Wild	1
Total		30

Japan. The population seemed to have recently established. Although we could not determine how the population was introduced to Shimane Prefecture, we assumed that it was probably from Kyushu Island. There seemed to be two possibilities (1) seeds were brought by birds, and (2) seeds were brought by humans together with soils when roadside embankments were built or renewed. In 2016, several plants at flowering and pod-setting stages were recognized (Photos 1 and 2). However, no plants at flowering or pod-setting stage were observed in 2018 (Photos 3 and 4). In 2016, no trees and shrubs were found at the site where *V. vexillata* plants were growing (Photo 1). In 2018, the site was covered by trees and shrubs. Since *V. vexillata* plants prefer sunny open habitat, its survival might become difficult as the ecological succession of the vegetation progresses. Although this population may be ephemeral, *V. vexillata* could possibly expand their geographical distribution range to Honshu Island in accordance with the progression of global warming.

Wild and intermediate forms of wild and domesticated azuki bean

***Vigna angularis* (Willd.) Ohwi & H. Ohashi in J. Jap. Bot. 44:29 (1969)**

Tomooka *et al.* (2014) suggested that azuki bean were domesticated in Japan, and the wild ancestors are frequently found in Japan. For the intraspecific classification, Ohwi and Ohashi (1969) described domesticated azuki bean as *Vigna angularis* (Willd.) Ohwi & Ohashi and wild azuki bean as *Vigna angularis* (Willd.) Ohwi & Ohashi var. *nipponensis* (Ohwi) Ohwi & Ohashi.

However, in Japan, “weedy azuki bean” or “intermediate form” showing intermediate morphological characteristics between wild and domesticated azuki bean were frequently found (Yamaguchi 1992). Occasionally, determining whether the collected plants are wild or intermediate form is difficult, since intermediate forms show large morphological variations from wild-like to domesticated-like. In addition, most of the intermediate forms are thought to be derived from hybrids between wild and domesticated azuki bean. Hence, in this study, we did not use a botanical variety name (*V. angularis* var. *nipponensis*) for our collections even though they were growing naturally (not cultivated), as the scientific name *V. angularis* var. *nipponensis* was used for weedy azuki bean or intermediate form in our previous studies (https://www.gene. affrc.go.jp/publications.php). The NARO Genebank has recently discontinued using the variety name for the same reason, although its scientific name is still valid.

Instead, we categorized the status of our collection as either “wild” or “intermediate” based on the superficial morphological appearance. Plants having small black mottled seeds with very slender purple twining stems were categorized as “wild.” Conversely, those having light-colored seed coats or large-sized seeds with green or thicker stems and larger leaflets were categorized as “intermediate” (see seed photos, Tables 2 and 3).

In Shimane Prefecture, this species was found mainly in artificially disturbed environments such as

around paddy fields (Photo 5). One of the remarkable characteristics of this species is that it has secondary bracts that are sufficiently large to encase a young flower bud (Photo 6). The leaflets showed continuous variations in shape from ovate to lanceolate and in the periphery from entire to lobed (Photos 7 and 8). At many sites, we found populations with traits that seemed to be derived from domesticated azuki bean, such as determinate growth, large seeds, and light-colored seed coats (Table 3, Photo 9, Seed photos SH01, SH06, SH19, and SH22). Azuki bean have long been cultivated in Shimane Prefecture, and the hybrids of both wild and domesticated azuki bean have been thought to occur frequently. Some of the hybrid derivatives adapted to a specific environment might have been genetically fixed as intermediate forms. We also found populations that retain wild traits such as climbing growth habit, small organs, and dark mottled seeds (Photo 10, Seed Photos SH05 and SH17).

Wild soybean

***Glycine max* (L.) Merr. subsp. *soja* (Sieb. & Zucc.) Ohashi in J. Jap. Bot. 57:30 (1982). Syn. *Glycine soja* Sieb. & Zucc. in Abh. Akad. Muench IV 2:119 (1845)**

Although the NARO Genebank (Japan) and major world gene banks such as the US Department of Agriculture and Consultative Group on International Agricultural Research centers use a scientific name "Glycine soja Sieb. & Zucc." for wild soybean in their databases, the first author (Y. T.) supports the classification system of Ohashi (1982), because it is a wild ancestor of domesticated soybean and can be crossed with each other (Sedivy *et al.* 2017). Ohashi (1982) classified wild soybean as *Glycine max* (L.) Merr. subsp. *soja* (Sieb. & Zucc.) and domesticated soybean as *Glycine max* (L.) Merr. subsp. *max*. Ohashi (1982) described domesticated and wild soybean as follows: domesticated soybean have an erect stem, brown hair, large ovate leaflets, bracteole length of 2.5–3mm (wild soybean, 1–2 mm), pod length of 3–7.5 cm, and seed size of 6–11 mm on the long axis and 5–8mm on the short axis (wild soybean: long axis, 4.5 mm; short axis, 3 mm).

In Shimane Prefecture, wild soybean was found in artificially disturbed environments such as around paddy fields and riverbanks (Photos 11, 12, and 13). Many populations occupied larger areas than those for naturally growing azuki bean. Their seeds were brownish black (Seed Photos SH03, SH08, SH15, SH16, SH18, SH26, and SH30), and the pods were as short as 3 cm (Table 3). Unlike in the case of naturally growing azuki bean populations, no soybean population was judged to be derived from hybrids between wild and domesticated ones. This is because the natural crossing rate is low between domesticated and wild soybean (Kuroda *et al.* 2008), and the progeny of the hybrid would have difficulty in surviving in natural or artificially disturbed environments (Kuroda *et al.* 2007). However, hybrid-derived populations were very occasionally found in Japan, such as in Akita, Hyogo, and Saga Prefectures (Kuroda *et al.* 2007) and in Saitama Prefecture (Ohashi 2014). Based on the herbarium specimens (TUS 55427, TUS 56128, TUS 57844, and TUS 81107) collected from Hannoo City, Saitama Prefecture, Ohashi (2014) proposed a new taxonomic treatment for the hybrid-derived intermediate soybean plants –*Glycine max* (L.) Merr. nothosubsp. *gracilis* (Skvortsov) H. Ohashi.

Future perspectives

Confirming the habitat of wild tuber cowpea in Honshu Island was one of the important results of this study. When we grew all the collected Japanese wild tuber cowpeas together, the flowering time was found to differ depending on the collection sites. These accessions were revealed to be differentiated at the

DNA level (unpublished results). The wild tuber cowpea collected in this survey may have been artificially introduced from Kyushu Island relatively recently, but genetic selection might have already occurred. In the near future, we plan to cultivate wild tuber cowpeas collected from other regions together with SH20 to investigate the phenotypic variation, and to conduct molecular phylogenetic analysis.

In addition to Shimane Prefecture, a person discovered and reported wild tuber cowpea in Aichi Prefecture on his web blog (https://blog.goo.ne.jp/avril_kanabun/e/536e9f6a4f08532f47c23dd88e567984); hence, wild tuber cowpea might have already expanded their geographical distribution to Honshu Island. We intend to continue the investigation of the distribution of wild tuber cowpea in Japan.

After the seeds collected in this survey have been multiplied, we plan to conserve them in the NARO Genebank as genetic resources for education, research, and breeding programs (https://www.gene.affrc.go.jp/index_en.php).

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島根県におけるマメ科遺伝資源の生息域外保全 2018 年

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和文摘要

本報告は島根県におけるマメ科遺伝資源の保全に関する報告書である．我々は 2018 年 10 月 16 日から 10 月 19 日にかけて，マメ科野生種遺伝資源の収集のため島根県の人為攪乱環境および自然環境を探索した．その結果，アカササゲ（*Vigna vexillata*）の実生を 1 サンプル，２サンプルの野生および 20 サンプルの雑草（中間型）アズキ（*Vigna angularis*）種子，および 7 サンプルのツルマメ（*Glycine max* subsp. *soja*, syn. *Glycine soja*）種子，合計 30 サンプルの遺伝資源を収集した．この論文は本州におけるアカササゲの生息を確認した初めての学術的報告である．農研機構ジーンバンクは，本調査で収集した増殖後の種子を，教育・研究・産業利用のために配布する予定である．

Table 3. Passport data of the collected materials

ID	JP No.	Coll. Date	Scientific name	Status	Latitude	Longitude	Altitude (m)	100 seeds weight (g)	Pod length (cm)	Coll. Site	Topography	Soil type	Remarks
SH01	267704	201810/16	*Vigna angularis*	intermediate	35.5070230	133.0092150	3	3.45	7.52	Myoubun, Kashimachou, Matsue, Shimane	Plain	Clay	Pale seed coat
SH02	267705	2018/10/16	*Vigna angularis*	intermediate	35.5069400	133.0095660	5	2.97	6.22	Myoubun, Kashimachou, Matsue, Shimane	Plain	Clay	Dark seed coat
SH03	267706	2018/10/16	*Glycine max* subsp. *soja*	wild	35.5377560	133.0226769	8	2.73	2.82	Mitsu, Kashimachou, Matsue, Shimane	Mountains	Silt	in a fallow field, damaged by wild boars
SH04	267707	2018/10/16	*Vigna angularis*	intermediate	35.5390222	133.0235335	4	2.98	7.30	Mitsu, Kashimachou, Matsue, Shimane	Mountains	Silt	beside a ditch
SH05	267708	2018/10/16	*Vigna angularis*	wild	35.5516067	133.0553542	23	2.24	6.92	Owashi, Shimanechou, Matsue, Shimane	Mountains	Clay	between the stone of a wall, along with Wild *Coix lacryma-jobi*
SH06	267709	2018/10/16	*Vigna angularis*	intermediate	35.5275847	133.0555574	80	4.18	8.42	Kamikoubu, Kashimachou, Matsue, Shimane	Mountains	Silt	Brown seed coat
SH07	267710	2018/10/16	*Vigna angularis*	intermediate	35.5275847	133.0555574	80	3.83	8.38	Kamikoubu, Kashimachou, Matsue, Shimane	Mountains	Silt	Black seed coat
SH08	267711	2018/10/16	*Glycine max* subsp. *soja*	wild	35.5001514	133.0776658	9	2.76	2.34	Higashimochidachou, Matsue, Shimane	Plain	Clay	beside a ditch
SH09	267712	2018/10/16	*Vigna angularis*	intermediate	35.5001514	133.0776658	9	3.09	8.47	Higashimochidachou, Matsue, Shimane	Plain	Clay	beside a ditch
SH10	267713	2018/10/17	*Vigna angularis*	intermediate	35.4909635	133.1628343	1	3.78	8.06	Nyuukou, Yatsukachou, Matsue, Shimane	Plain	Organic soil	in a fallow field on Daikon Island
SH11	267714	2018/10/17	*Vigna angularis*	intermediate	35.4117963	133.2291972	4	3.93	8.20	Kirekawachou, Yasugi, Shimane	Plain	Clay	along with *Solidago canadensis* var. *scabra*
SH12	267715	2018/10/17	*Vigna angularis*	intermediate	35.3084545	133.1548236	107	3.08	6.96	Fube, Hirosechou, Yasugi, Shimane	Plain	Sand	Smaller plants compared with SH13
SH13	267716	2018/10/17	*Vigna angularis*	intermediate	35.3084545	133.1548236	107	4.27	8.26	Fube, Hirosechou, Yasugi, Shimane	Plain	Sand	Larger plants compared with SH12
SH14	267717	2018/10/17	*Vigna angularis*	intermediate	35.1545760	132.9759070	298	4.36	8.74	Shimoai, Okuizumochou, Nitagun, Shimane	Mountains	Organic soil	on a slope
SH15	267718	2018/10/17	*Glycine max* subsp. *soja*	wild	35.3046264	132.8978743	38	3.07	3.14	Shimokumatani, Mitoyachou, Unnan, Shimane	Plain	Sand	Small population, on a dry riverbed
SH16	267719	2018/10/17	*Glycine max* subsp. *soja*	wild	35.3046264	132.8978743	38	2.83	3.12	Shimokumatani, Mitoyachou, Unnan, Shimane	Plain	Sand	Large population, under an elevated road, on a dry riverbed
SH17	267720	2018/10/17	*Vigna angularis*	wild	35.3392609	132.9132746	30	2.64	7.40	Kamonaka, Kamochou, Unnan, Shimane	Plain	Organic soil	in a fallow field, along with *Solidago canadensis* var. *scabra*
SH18	267721	2018/10/17	*Glycine max* subsp. *soja*	wild	35.3376850	132.9109150	29	2.42	3.20	Minamigamo, Kamochou, Unnan, Shimane	Plain	Organic soil	on an embankment between paddy field
SH19	267722	2018/10/18	*Vigna angularis*	intermediate	35.4199836	132.9928158	29	2.81	7.54	Hayashi, Tamayuchou, Matsue, Shimane	Mountains	Clay	in a fallow field in a valley
SH20	267723	2018/10/18	*Vigna vexillata*	wild	35.4244440	132.9761170	7	-	-	Hayashi, Tamayuchou, Matsue, Shimane	Mountains	Organic soil	No mature plants found. Young seedlings were brought back to Tsukuba and seeds were increased
SH21	267724	2018/10/18	*Vigna angularis*	intermediate	35.4193478	132.9721756	10	3.37	8.40	Higashikimachi, Shinjichou, Matsue, Shimane	Mountains	Clay	on an embankment between paddy field
SH22	267725	2018/10/18	*Vigna angularis*	intermediate	35.4436977	132.7566379	18	3.18	6.64	Okuugachou, Izumo, Shimane	Mountains	Organic soil	along with Persicaria thunbergii
SH23	267726	2018/10/18	*Vigna angularis*	intermediate	35.4421170	132.7839914	9	3.44	6.94	Mandachou, Izumo, Shimane	Plain	Organic soil	Large population, on an embankment between paddy field
SH24	267727	2018/10/18	*Vigna angularis*	intermediate	35.4615496	132.8664915	6	3.25	6.26	Sonochou, Izumo, Shimane	Plain	Clay	Small population
SH25	267728	2018/10/18	*Vigna angularis*	intermediate	35.4624376	132.8663758	8	3.16	6.58	Sonochou, Izumo, Shimane	Plain	Organic soil	Lerge population, along the road side
SH26	267729	2018/10/18	*Glycine max* subsp. *soja*	wild	35.4821454	132.8949470	28	1.78	2.94	Nozatochou, Izumo, Shimane	Plain	Clay	on an embankment between paddy field
SH27	267730	2018/10/18	*Vigna angularis*	intermediate	35.4880286	132.9220112	30	4.33	8.80	Kamioonochou, Matsue, Shimane	Mountains	Organic soil	near a soybean field
SH28	267731	2018/10/18	*Vigna angularis*	intermediate	35.4871490	132.9531865	12	3.63	8.64	Aikachou, Matsue, Shimane	Plain	Clay	on a river bank
SH29	267732	2018/10/18	*Vigna angularis*	intermediate	35.4869522	132.9927940	17	3.79	7.10	Kososhichou, Matsue, Shimane	Plain	Organic soil	on a slope
SH30	267733	2018/10/19	*Glycine max* subsp. *soja*	wild	35.3799044	132.7536617	4	3.04	3.00	Ootsukachou, Izumo, Shimane	Plain	Sand	on a river bank, along with *Canna* sp.

Photo 1. Habitat of wild *V. vexillata* in Matsue City, photo by Takaaki Notsu in 2016.

Photo 2. Flower of wild *V. vexillata* in Matsue City, photo by Takaaki Notsu in 2016.

Photo 3. Habitat of wild *V. vexillata*, 2018SH20 in Matsue City.

Photo 4. Seedling plant of wild *V. vexillata*, 2018SH20 in Matsue City.

Photo 5. Habitat of intermediate *V. angularis*, 2018SH21 in Matsue City.

Photo 6. Inflorescence of intermediate *V. angularis*, 2018SH04 in Matsue City.

Photo 7. Leaf of wild *V. angularis*, 2018SH05 in Matsue City.

Photo 8. Leaf of wild *V. angularis*, 2018SH17 in Unnan City.

Photo 9. Plant of intermediate *V. angularis*, 2018SH27 in Matsue City.

Photo 10. Plant of wild *V. angularis*, 2018SH17 in Unnan City.

Photo 11. Habitat of *G. max* subsp. *soja*, 2018SH03 in Matsue City.

Photo 12. Habitat of *G. max* subsp. *soja*, 2018SH30 in Izumo City.

Photo 13 Plant of *G. max* subsp. *soja*, 2018SH30 in Izumo City.

2018SH-01 (JP267704)

2018SH-02 (JP267705)

2018SH-03 (JP267706)

2018SH-04 (JP267707)

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2018SH-06 (JP267709)

2018SH-07 (JP267710)

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2018SH-28 (JP267731)

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2018SH-30 (JP267733)