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Original Paper

Collection and Conservation of Legume Genetic Resources on Okinawa Island, Yagaji Island, Kouri Island and Hamahiga Island in 2017

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Summary

This paper is a report on the collection and conservation of wild relatives of leguminous crops on Okinawa Island, Japan, from July 24th to 28th and October 31st in 2017. As a result of this survey, we recorded a total of 19 habitats, and collected 16 seed samples and 10 soil samples from the habitats of *Canavalia* sp., *Vigna reflexo-pilosa* Hayata, *Vigna marina* (Burm.) Merrill and an endemic taxon, *Vigna vexillata* (L.) A. Richard, called “Sakuya-akasasage” in Japanese. Analysis of the collected soil samples revealed that *V. marina* inhabits alkaline soil while *V. reflexo-pilosa* inhabits neutral to acidic soils. After the multiplication of the seeds collected in this survey, 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, legume, Okinawa Island, *Vigna vexillata* (L.) A. Richard

Introduction

Ex-situ conservation of crop wild relatives supports the development of industry and science, because these can be used to improve the crop or for the production of useful substances. In the NARO Genebank project, we collected crop wild relatives, including endangered species, of the leguminous genera *Vigna* and *Glycine* for the development of stress tolerant crops (Vaughan *et al.* 2010; Tomooka *et al.* 2014; Takahashi *et al.* 2016). The Ministry of the Environment of the Government of Japan described 1,782 taxa as 'Endangered' in the Red List based on the investigations of about 7,000 vascular plant species (2017 Japanese Red Lists, http://www.env.go.jp/press/103881.html). In the Red Lists, three taxa of *Vigna* are listed. Therefore, the importance of *ex-situ* conservation of crop wild relatives in the gene bank as a backup for endangered species has increased.

An endemic and endangered tuber cowpea (*Vigna vexillata* (L.) A. Richard), called "Sakuya-akasasage" in Japanese, was found in the northern part of Okinawa Island. Nakajima (1968) reported the morphological difference between "Sakuya-akasasage" and another taxon "Akasasage" (*Vigna vexillata* (L.) A. Richard var. *tsusimensis* Matsumura), which is found on the Kyushu Island. However, these taxa are not mentioned in any monograph for this species (Garba and Pasquet 1998; Maxted *et al.* 2004). The reason for this is that there is no English description nor is there any living collections (= gene bank accessions). Wild *V. vexillata* is an important genetic resource, because this species contains cultigen and are closely related to cowpea, which are an important source of protein in arid regions, specially Africa.

We report on the survey of legume genetic resources in the northern and southern parts of Okinawa Island and its neighbors; Yagaji Island, Kouri Island and Hamahiga Island. Okinawa Island is located to the southwest of Japan and is classified as having a humid subtropical climate (Cfa), based on Köppen climate classification. Strata in the northern part of the Okinawa Island consists mainly of mudstone, sandstone, and greenstone while the strata in the southern part mainly consists of mudstone and limestone named Ryukyu limestone (Shinjo 2014). In the northern part, some endemic species were found in a forest called "Yanbaru" and on coastal cliffs called "Manzamou". We also report the release of the first living collection of "Sakuya-akasasage" from the NARO Genebank (https://www.gene.affrc.go.jp/index_en.php).

Methods

A field survey was conducted on Okinawa Island, Yagaji Island, Kouri Island and Hamahiga Island from the 24th to 28th of July in 2017 (Table 1, Fig. 1). We interviewed landowners and asked their permission to collect seeds, and collected seeds from wild leguminous plants, and recorded their passport

Table 1. Itinerary of the field survey in Okinawa Island

Date	Itinerary	Stay
2017/7/24	Haneda airport - ANA471 - Naha airport - Tomigusuku City - Nago City	Nago City
2017/7/25	Nago City - Yagaji Island - Kouri Island - Nago City - Ogimi Village - Kunigami Village	Kunigami Village
2017/7/26	Kunigami Village - Azuma Village - Nakijin Village	Nakijin Village
2017/7/27	Nakijin Village - Hamahiga Island - Nago City	Nago City
	A detached force: Nakijin Village - Nanjo City - Nago City	Nago City
2017/7/28	Nago City - Nanjo City - Naha airport - ANA996 - Haneda airport	-
2017/10/31	Onna Village (by two resercaers from the Okinawa Churashima Research Center)	-

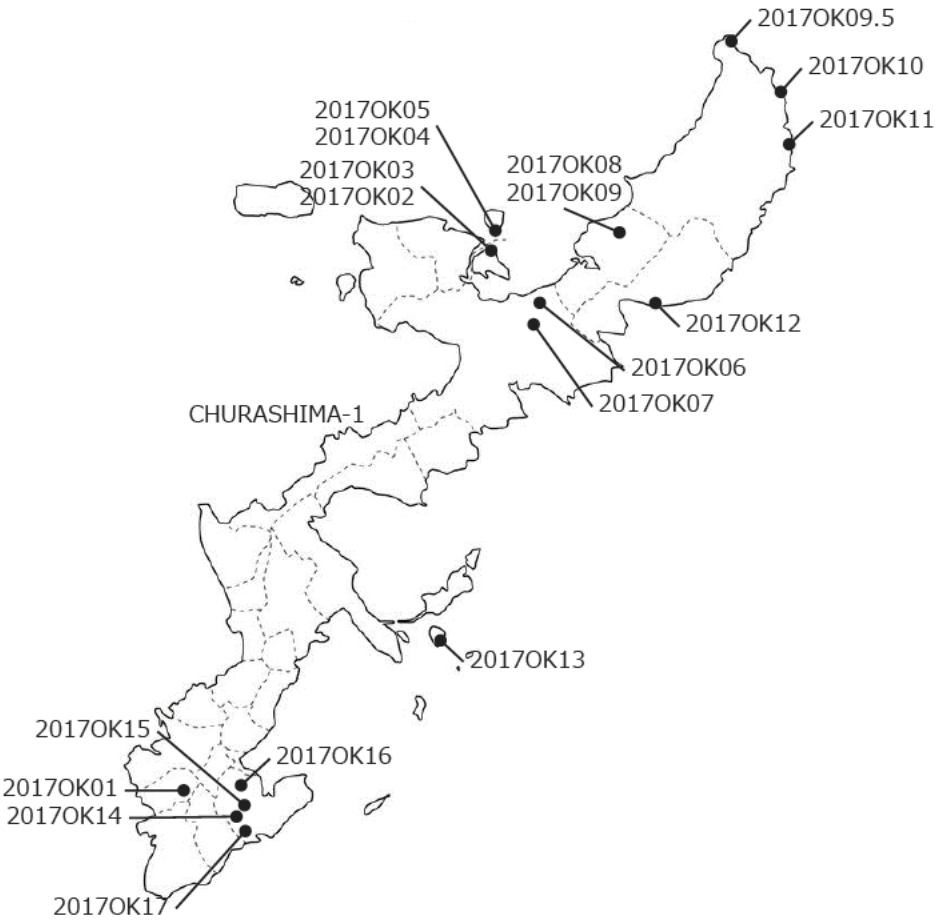

Fig. 1. Collection sites of each accession on Okinawa Island.

data including latitude, longitude, and altitude of their habitats using Google Maps and Google Earth (Google). Identification of the *Vigna* species is based on the taxonomic keys (Tomooka *et al.* 2002; Maxted *et al*. 2004).

Collecting "Sakuya-akasasage" from its natural habitat is legally regulated, so after the survey was completed we received permission from the Okinawa Prefecture to collect seed samples. Two authors from the Okinawa Churashima Research Center (Abe and Akai) collected seeds on October 31st in 2017. The geographical information of the habitat was not listed in this report in order to protect the endangered plants location.

In order to characterize the soil environments of several wild *Vigna* habitats, we collected surface soil samples. Each soil sample was divided into two when we returned to Tsukuba. One sample was directly used to characterize fresh soil properties and the other was used to characterize air-dried soil properties, after air-drying at room temperature. The values of soil pH (H_2O), EC (H_2O) and NaCl concentration were estimated from measuring slurry of soil samples made by mixing 2.5 times the volume of distilled water. The values of soil pH (KCl) were estimated from measuring slurry made by mixing 2.5 times volume of 1M KCl solution. Using the Al^{3+} test paper, Alumi-check (Advantec, Tokyo, Japan), we estimated the concentrations of aluminum trivalent ions (Al^{3+}) in each slurry, which is toxic for plants and inhibits their root elongations.

Table 2. A summary of the collections in Okinawa Island

Species	No. of habitats surveyed	No. of seed samples collected	No. of soil samples collected
Vigna marina (Burm.) Merr.	7	7	3
Vigna reflexo-pilosa Hayata	10	7	7
Vigna vexillata (L.) A. Rich.	1	1	0
Canavalia sp.	1	1	0
Total	19	16	10

Results and Discussion

We recorded 19 habitats of wild leguminous plants and collected 16 seed samples and 10 soil samples from the habitat of *Canavalia* sp., *Vigna marina* (Burm.) Merrill, *Vigna reflexo-pilosa* Hayata and *Vigna vexillata* Rich. (Table 2). We describe the details of the species in each collection (see also Table 3).

Wild tuber cowpea:

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

This wild species inhabits across Africa, the Americas, Asia and Oceania, while the cultigens have been collected in West Africa, Central America and Southeast Asia (Garba and Pasquet 1998). There are substantial numbers of genebank accessions collected from Africa and Australia, but very few from Asia (Dachapak *et al.* 2017). To conserve living samples of this species in the NARO Genebank, we have collected "Akasasage" (*V. vexillata* var. *tsusimensis*) in the Kyushu region (Tomooka *et al.* 2010; Takahashi *et al.* 2017).

An accession CHURASHIMA-1 collected in this survey inhabited the coastal cliffs of Onna Village (Photo 1). Three individuals were found growing on sand that had accumulated among the coral rocks. This place had low humus (poor nutrition) and a low water holding property (arid soil), as well as being affected by seawater (high salinity). CHURASHIMA-1 was characterized by shorter internodes, shorter petiolules, and smaller seeds than those of "Akasasage" (*V. vexillata* var. *tsusimensis*) plants previously collected in the Kyushu region (Photo 2, Seed photo CHURASHIMA-1). These morphological characteristics match well with the description of "Sakuya-akasasage" reported by Nakajima (1968). As Nakajima did not mention the taxonomic treatment of "Sakuya-akasasage", the intraspecific classification of CHURASHIMA-1 or "Sakuya-akasasage" was left as a topic to be addressed in the future, since we have never found the above-mentioned habitat and traits in any other taxon of this species.

The accession CHURASHIMA-1 will become an active collection that can be distributable upon request during 2018, since plants have already been grown and multiplied seeds were harvested in the NARO Genebank. From the environmental conditions of its habitat, we believed that CHURASHIMA-1 must have a tolerance to salt, drought, and a low-nutrient, so the NARO Genebank has a plan to evaluate the various stress tolerances of *V. vexillata* accessions collected from wide geographical sites around the world together with CHURASHIMA-1. The Okinawa Churashima Research Center will continue to observe "Sakuya-akasasage" population in its natural habitat for secure *in-situ* conservation.

Wild creole bean:

***Vigna reflexo-pilosa* Hayata, J. Coll. Sci. Imp. Univ. Tokyo 30:82 (1911)**

Vigna reflexo-pilosa Hayata is a tetraploid (2n = 4x = 44), but most *Vigna* species are diploid (2n = 22). It includes the cultigen called "creole bean" (*Vigna reflexo-pilosa* Hayata var. *glabra* (Maréchal, Mascherpa & Stainer) N. Tomooka & Maxted) and its wild ancestor (*Vigna reflexo-pilosa* Hayata var. *reflexo-pilosa*), both are closely related genetically and mutually cross-compatible (Chankaew *et al.* 2014; Tomooka *et al.* 1991, 2002). The wild ancestor is found in Southeast Asia, East Asia and Oceania, but the collection sites of the NARO genebank accessions were limited to Japan, Malaysia, Laos and Papua New Guinea (Chankaew *et al.* 2014). There is no record of creole bean ever having been cultivated in Japan, but its wild ancestor can be widely found on the Nansei Islands (chain of Islands extending from southwestern Kyushu region to northeast of Taiwan).

On the northern part of Okinawa Island, *V. reflexo-pilosa* populations were found at the edge of a forest and on coastal cliffs. In Nago City, accession 2017OK06 grew on the dark edge of a forest on a north facing slope, 2017OK07 grew with *Alocasia* sp. and *Cyathea* sp. along the edge of a forest, where the site was inundated with water flowing from a mountain (Photos 3‐5). In Ogimi Village, 2017OK08 and 2017OK09 grew on the wet site beside forest (Photos 6‐8). We then surveyed the forest road along the river in the northernmost areas of Okinawa Island, but we could not find *V. reflexo-pilosa* populations. Meanwhile, in Kunigami Village, 2017OK11 grew on bright, dry coastal cliffs (Photo 9). We also investigated other coastal areas, but we were unable to find *V. reflexo-pilosa* populations anywhere else.

On the southern part of Okinawa Island, *V. reflexo-pilosa* populations were found along a river and in fallow fields. Accession 2017OK01 grew along a river embankment in Tomigusuku City (Photo 10), 2017OK14-17 grew in grasslands such as fallow fields in Nanjo City (Photos 11 and 12). Such an environment is similar to that preferred by wild azuki bean ancestors (*Vigna angularis* (Willd.) Ohwi & Ohashi var. *nipponensis* (Ohwi) Ohwi & Ohashi) in Japan.

Wild beach pea:

***Vigna marina* (Burm.) Merrill, Interpret. Rumph. Herb. Amboina:285 (1917)**

Vigna marina is a pantropical plant with sea-drifted seeds and is widely distributed along tropical coastal areas. In addition, *Vigna marina* was cultivated as a feed crop and a green manure crop in both Africa and Asia and was used as a food in the Maldives (Padulosi and Ng 1993). In Japan, *Vigna marina* is commonly found on the coastal areas of the Nansei Islands, but we could not find publications of its use by human.

Vigna marina has a similar morphology to *Vigna luteola* (Jacq.) Benth, but generally *V. marina* inhabits coastal areas that are affected by seawater, and *Vigna luteola* inhabits freshwater riverbanks or lakesides. Compared to *V. luteola*, *Vigna marina* have rounder leaves, hairless pods and larger seeds (Sonnante *et al.* 1997). However, both are closely related genetically, so the morphological gap between the two species is often not clear (Takahashi *et al.* 2016).

On Okinawa Island, most *V. marina* plants inhabited coral-derived sandy beaches (Photos 13‐16), but an accession 2017OK12 inhabited bush land with humus soil (Photo 17). *Vigna marina* plants are generally found growing on sandy beaches, but they sometimes grow also on humus soil sites located inland areas.

Wild jack-bean:

***Canavalia* Adanson, Familles des Plantes 2:325, 531 (1763)**

The genus *Canavalia* consists of 51 species including 4 cultigens used as food, feed, green manure, cover crops, and medicine (Sridhar and Seena 2006; Purseglove 1974; Westphal 1974; Sauer and Kaplan 1969). Three wild *Canavalia* species inhabit the coastal area of Japan (Yamashiro *et al.* 2013; Matsumura *et al.* 2004; Sauer 1964). *Canavalia lineata* (Thunb.) DC. is found in the south of Honshu, *Canavalia maritima* (Aubl.) Thouars is found in the south of Kagoshima Prefecture, and *Canavalia cathartica* Thouars is found on the south end of Okinawa Island (Yamashiro *et al.* 2013; Matsumura *et al.* 2004; Sauer 1964).

We recommend using the species name *C. maritima* instead of *Canavalia rosea* (Swartz) DC., because *Canavalia maritima* (Aubl.) Thouars, Jour. Bot. 1: 80 (1813) has a priority of publication for "International Code of Nomenclature for algae, fungi, and plants for the scientific name", and *Canavalia rosea* (Swartz) DC. Prodromus 2: 404 (1825) should be treated as its synonym. The misunderstanding is found in recently published papers (Snak *et al.* 2016; Vatanparast *et al.* 2011; Matsumura *et al.* 2004).

In this survey, 2017OK09.5 was collected at Cape Hedo in Kunigami Village on the northernmost tip of Okinawa Island (Photos 18‐21). It was found bearing many light purple flowers and pods. We classified 2017OK09.5 as *Canavalia* sp., because we have little experience in the classification of the genus *Canavalia*. However, we thought that 2017OK09.5 might belong to *C. lineata*, because it has two primary leaves, a long hilum against seed length and its habitat is on coastal cliffs. In the future, we will re-examine the classification of the accessions conserved as genus *Canavalia* in the NARO Genebank.

Soil property of habitat for *V. marina* and *V. reflexo-pilosa*

The results of the soil analysis are summarized in Table 4. Each value obtained by using air-dried soil is directly indicated, while the values in parentheses show the results obtained by using fresh soil in Table 4. Comparing the results of fresh soils with those of air-dried soils, we found only a little difference between them. The fact would be valuable for us when we are constrained to analyze soil samples only by on-the-spot. But, we discuss the values obtained from air-dried soil as representative results in the following sentences. The appearance of each air-dried sample is shown in the Photo 22.

EC (Electrical Conductivity) values were measured using a slurry of soils mixed with distilled water. All EC values of soil samples where *V. marina* plants were growing were around 0.1 mS/cm, which are much lower than those of *V. reflexo-pilosa* growing sites. The results indicated that *V. marina* could survive

Table 4. Properties of the soils (0‐5 cm depth) at wild *Vigna* plants habitats on Okinawa Island

Soil Sample ID	Dominant plant	State	Soil type	pH_H_2O	EC_H_2O (mS/cm)	Al^{3+}_H_2O (ppm)	NaCl_H_2O	pH_KCl	Al^{3+}_KCl (ppm)
S_2017OK02	*V. marina*	Coast	Coral sand	7 8 (7.3)	0.12 (0.09)	0 (0)	0	9.3 (8.3)	0 (0)
S_2017OK07-1	*V. reflexo-pilosa*	Inland	Clay	5.9 (5.9)	0.43 (0.19)	0 (0)	0	4.6 (4.8)	slight (slight)
S_2017OK07-2	*V. reflexo-pilosa*	Inland	Clay	6.9 (6.0)	0.24 (0.11)	0 (0)	0	5.4 (5.1)	slight (slight)
S_2017OK08	*V. reflexo-pilosa*	Inland	Clay	7 6 (6.8)	0.75 (0.46)	0 (0)	0	6.6 (6.1)	0 (0)
S_2017OK09	*V. reflexo-pilosa*	Inland	Clay	7.9 (7.2)	0.27 (0.16)	0 (0)	0	6.9 (6.2)	0 (0)
S_2017OK10	*V. marina*	Coast	Coral sand	8.7 (8.6)	0.11 (0.09)	0 (0)	0	9.3 (8.6)	0 (0)
S_2017OK11	*V. reflexo-pilosa*	Coast	Sand	8 0 (7.4)	0 53 (0.35)	0 (0)	0	7.4 (6.8)	0 (0)
S_2017OK13	*V. marina*	Coast	Coral sand	8 8 (8.8)	0.13 (0.14)	0 (0)	0	9.3 (8.8)	0 (0)
S_2017OK14-1	*V. reflexo-pilosa*	Hills	Clay	7.7 (7.5)	0 50 (0.30)	0 (0)	0	7.0 (6.8)	0 (0)
S_2017OK14-2	*V. reflexo-pilosa*	Hills	Clay	7.9 (7.5)	0 55 (0.31)	0 (0)	0	7.2 (7.1)	0 (0)

Each value obtained by using air-dried soil is directly indicated, while the values in parentheses show the results obtained by using fresh soil.

in a nutrient-poor condition, even when the rhizospheres (not only surface soil) were composed of sand (Takahashi *et al.* 2014).

We measured NaCl concentration in same way as EC, only using soils mixed with distilled water. Even for coastal sand samples which were collected at *V. marina* habitats, we could not detect any trace of NaCl. The results were consistent with a previous report (Takahashi *et al.* 2014). These findings further support the hypothesis that NaCl must be rapidly washed away by rainwater on sandy beaches.

Two kinds of pH values, pH (H_2O) and pH (KCl), showed some differences but the overall tendency between the two values was not conflicting. In case of neutral to acidic soil, the pH (KCl) are lower than the pH (H_2O) because KCl is used as an extractant of exchangeable Al. pH (KCl) indicates the pH at which Al is extracted (Science Division Staff. 2017). That can explain the results of S_2017OK07-1 and S_2017OK07-2 in Table 4. The pH (KCl) was 4.6 and 5.4 while the pH (H_2O) was 5.9 and 6.9, respectively, and a trace amount of Al^{3+} was detected only in soils mixed with 1M KCl. Based on the pH results shown in Table 4, we could conclude that *V. marina* inhabits alkaline soil while *V reflexo-pilosa* inhabits neutral to acidic soil.

Future perspectives

Ex-situ conservation of "Sakuya-akasasage" is the most important result of the survey. The Okinawa Churashima Research Center will continue to observe the site and work on its *in-situ* conservation in its natural habitat, and the NARO Genebank will conserve its *ex-situ* living accession.

We collected 135 accessions of legume genetic resources on the Nansei Islands (Muto *et al.* 2015; Takahashi *et al.* 2014; Tomooka *et al.* 2000, 2012, 2013). We have failed in the seed multiplication of *Vigna marina* and *Canavalia* spp. in green house conditions using pots in Tsukuba city. However, we found that they could set pods when they were grown crawling on the soil surface using wide spaces in outside fields. Therefore, we plan to multiply seeds of *Vigna marina* and *Canavalia* spp. using this outside field condition.

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沖縄島・屋我地島・古宇利島・浜比嘉島における マメ科遺伝資源の収集と保全，2017 年

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和文摘要

本報告は沖縄島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 2017 年 7 月 24 日から 7 月 28 日にかけて，マメ科遺伝資源の収集のため沖縄島の自然環境を探索した．その結果，*Canavalia* 属植物，*Vigna reflexo-pilosa* Hayata，*Vigna marina* (Burm.) Merrill，*Vigna vexillata* (L.) A. Rich. を対象に，19 地点の生息環境，16 点の種子サンプル，10 地点の土壌サンプルが記録あるいは収集された．これらは「絶滅のおそれのある野生動植物の種の保存に関する法律」で定められた「国内希少野生動植物種」に指定されるサクヤアカササゲ（*Vigna vexillata*）を含む．土壌分析の結果，*Vigna marina* はアルカリ土壌に，*Vigna reflexo-pilosa* は中性から酸性土壌に生息していることが明らかとなった．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（https://www.gene.affrc.go.jp/index_en.php）．

Table 3. A passport data of the collected materials

Collecting No.	JP No.	Date collected	Scientific name	Status	Location	Latitude	Longitude	Altitude (m)	Locality	Habitat	Soil type	Associated plants	Other
2017OK01	258943	7/24/2017	*Vigna reflexo-pilosa* Hayata	Wild	Tomigusuku City	26.172139	127.699472	11	Riverside	Bushes	Clay	*Arundo* sp., *Albizia* sp.	on a river embankment
2017OK02	258944	7/25/2017	*Vigna marina* (Burman) Merrill	Wild	Yagaji Island	26.678181	128.021042	4	Coast	Sandy beach	Coral sand	*Ipomoea pes-caprae*, *Cassytha filiformis*	a few pod
2017OK03	258945	7/25/2017	*Vigna marina* (Burman) Merrill	Wild	Yagaji Island	26.679181	128.019754	2	Coast	Sandy beach	Coral sand	*Ipomoea pes-caprae*	a few pod
2017OK04	258946	7/25/2017	*Vigna marina* (Burman) Merrill	Wild	Kouri Island	26.694395	128.020457	4	Coast	Sandy beach	Coral sand	*Ipomoea pes-caprae*	sandy beach
2017OK05	258947	7/25/2017	*Vigna marina* (Burman) Merrill	Wild	Kouri Island	26.694891	128.022021	4	Coast	Sandy beach	Coral sand	*Ipomoea pes-caprae*	sandy beach
2017OK06	258948	7/25/2017	*Vigna reflexo-pilosa* Hayata	Wild	Nago City	26.628755	128.062695	13	Inland	Forest	Clay	*Alocasia* sp., *Cyathea* sp.	along cliff
2017OK07	258949	7/25/2017	*Vigna reflexo-pilosa* Hayata	Wild	Nago City	26.622942	128.057517	25	Inland	Forest	Clay	*Grass*, *Cyathea* sp.	in stagnant water
2017OK08	258950	7/25/2017	*Vigna reflexo-pilosa* Hayata	Wild	Ogimi Village	26.695472	128.143038	45	Inland	Forest	Clay	-	no seed
2017OK09	258951	7/25/2017	*Vigna reflexo-pilosa* Hayata	Wild	Ogimi Village	26.695063	128.143475	46	Inland	Forest	Clay	-	a few pod
2017OK09.5	258952	7/25/2017	*Canavalia* sp.	Wild	Kunigami Village	26.872318	128.263144	18	Coast	Coral rock	Sand	*Indigofera trifoliata*	coastal cliffs
2017OK10	258953	7/26/2017	*Vigna marina* (Burman) Merrill	Wild	Kunigami Village	26.821130	128.313682	5	Coast	Sandy beach	Coral sand	*Ipomoea pes-caprae*	sandy beach
2017OK11	258954	7/26/2017	*Vigna reflexo-pilosa* Hayata	Wild	Kunigami Village	26.781295	128.325056	38	Coast	Coral rock	Sand	-	coastal cliffs
2017OK12	258955	7/26/2017	*Vigna marina* (Burman) Merrill	Wild	Higashi Village	26.626944	128.177889	16	Coast	Bushes	Organic	Bamboo, *Alocasia* sp., *Cyathea* sp.	twining to bamboo
2017OK13	258956	7/27/2017	*Vigna marina* (Burman) Merrill	Wild	Hamahiga Island	26.316906	127.960794	3	Coast	Sandy beach	Coral sand	*Ipomoea pes-caprae*	damaged by spider mite
2017OK14	258957	7/27/2017	*Vigna reflexo-pilosa* Hayata	Wild	Nanjo City	26.146949	127.755380	116	Hills	Grassland	Clay	Grass	close to grave
2017OK15	258958	7/27/2017	*Vigna reflexo-pilosa* Hayata	Wild	Nanjo City	26.159330	127.765220	95	Hills	Bushes	Clay	Grass	no seed, in a fallow field
2017OK16	258959	7/27/2017	*Vigna reflexo-pilosa* Hayata	Wild	Nanjo City	26.172307	127.758520	87	Hills	Bushes	Clay	Grass, *Pueraria* sp.	no seed, in a fallow field
2017OK17	258960	7/27/2017	*Vigna reflexo-pilosa* Hayata	Wild	Nanjo City	26.138268	127.761569	38	Hills	Grassland	Clay	Grass	damaged by spider mite
CHURASHIMA-1	259801	10/31/2017	*Vigna vexillata* (L.) A. Richard	Wild	Onna Village	-	-	-	Coast	Coral rock	Sand	-	coastal cliffs

Photo 1 Habitat of *V. vexillata*, CHURASHIMA-1 in Onna Village.

Photo 2 Plant of *V. vexillata*, CHURASHIMA-1 in Onna Village.

Photo 3 Habitat of *V. reflexo-pilosa*, 2017OK06 in Nago City.

Photo 4 Habitat of *V. reflexo-pilosa*, 2017OK07 in Nago City.

Photo 5 Plant of *V. reflexo-pilosa*, 2017OK07 in Nago City.

Photo 6 Habitat of *V. reflexo-pilosa*, 2017OK08 in Ogimi Village.

Photo 7 Leaf of *V. reflexo-pilosa*, 2017OK08 in Ogimi Village.

Photo 8 Pods of *V. reflexo-pilosa*, 2017OK09 in Ogimi Village.

Photo 9 Habitat of *V. reflexo-pilosa*, 2017OK11 in Kunigami Village.

Photo 10 Habitat of *V. reflexo-pilosa*, 2017OK01 in Tomigusuku City.

Photo 11 Habitat of *V. reflexo-pilosa*, 2017OK14 in Nanjo City.

Photo 12 Flower of *V. reflexo-pilosa*, 2017OK14 in Nanjo City.

Photo 13 Habitat of *V. marina*, 2017OK10 in Kunigami Village.

Photo 14 Habitat of *V. marina*, 2017OK13 in Hamahiga Island.

Photo 15 Plant of *V. marina*, 2017OK05 in Kouri Island.

Photo 16 Flower of *V. marina*, 2017OK13 in Hamahiga Island.

Photo 17 Plant of *V. marina*, 2017OK12 in Azuma Village.

Photo 18 Habitat of *Canavalia* sp., 2017OK09.5 in Kunigami Village.

Photo 19 Plant of *Canavalia* sp., 2017OK09.5 in Kunigami Village.

Photo 20 Flower of *Canavalia* sp., 2017OK09.5 in Kunigami Village.

Photo 21 Pod of *Canavalia* sp., 2017OM09.5 in Kunigami Village.

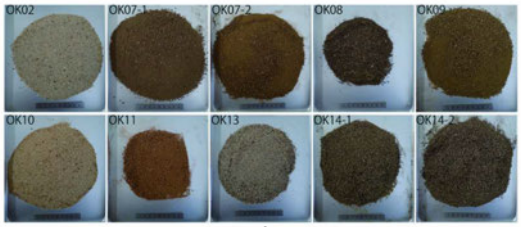

Photo 22 Appearance of each air-dried sample; Each soil sample ID is indicated in the upper left of photo.

Seed photos

2017OK01, *Vigna reflexo-pilosa*

2017OK02, *Vigna marina*

2017OK03, *Vigna marina*

2017OK04, *Vigna narina*

2017OK05, *Vigna marina*

2017OK06, *Vigna reflexo-pilosa*

2017OK07, *Vigna reflexo-pilosa*

2017OK09.5, *Canavalia* sp.

2017OK09, *Vigna reflexo-pilosa*

2017OK10, *Vigna marina*

2017OK11, *Vigna reflexo-pilosa*

2017OK12, *Vigna marina*

2017OK13, *Vigna marina*

2017OK14, *Vigna reflexo-pilosa*

2017OK17, *Vigna reflexo-pilosa*

CHURASHIMA-1, *Vigna vexillata*