

Field Survey and Collection of *Vigna marina*'s Seeds and Root Nodules on Tokunoshima Island, Okinoerabu Island and Yoron Island, Japan in 2019

Akiko BABA-KASAI

*Research Center of Genetic Resources, National Agriculture and Food Research Organization (NARO), 2-1-2
Kannondai, Tsukuba, Ibaraki 305-8602, Japan*

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Corresponding author: A. BABA-KASAI (e-mail: akikoba@affrc.go.jp)

Summary

A field survey was conducted to collect the salt-tolerant Leguminosae genetic resource, *Vigna marina* (Burm.) Merr., and their symbiotic rhizobia on the beaches of Tokunoshima Island, Okinoerabu Island, and Yoron Island, Kagoshima Prefecture, Japan, from November 18 to 22, 2019. In the field survey, 19 accessions of *V. marina* were collected. Root nodules were collected from 9 accessions of *V. marina* in order to isolate their symbiotic rhizobia, and soil samples were collected from the habitats of 6 accessions. All collected materials were conserved at the National Agriculture and Food Research Organization (NARO) Genebank of Japan.

KEY WORDS: Salt-tolerant genetic resources, *Vigna marina*, Root nodule, Rhizobia, Tokunoshima, Okinoerabu, Yoron

Introduction

The National Agriculture and Food Research Organization (NARO) Genebank has been conducting frequent collection trips for the *ex-situ* conservation of crops and their wild relatives in Japan (see Annual Report on Exploration and Introduction of Plant Genetic Resources, https://www.gene.affrc.go.jp/publications.php#plant_report). With regards to leguminous crops, many beans belong to the genus *Vigna*, such as cowpea, mung bean, and azuki bean, and at present, three wild relatives of the azuki bean inhabit Japan. One particular species, *Vigna angularis* var. *nipponensis* (Ohwi) Ohwi & Ohashi, are widely distributed from the Kagoshima Prefecture to Akita Prefecture, whereas two other species, *Vigna nakashimae* (Ohwi) Ohwi & Ohashi and *Vigna riukiensis* (Ohwi) Ohwi & Ohashi, inhabit a limited area in southern Japan. *V. nakashimae* inhabits

Nagasaki and Kumamoto Prefectures, and *V. riukiensis* inhabits the Okinawa Prefecture (see the data of the Genebank database). Similarly, *Vigna marina* (Burm.) Merr. is one of the wild relatives of cowpea inhabiting Japan. It is a pantropical plant with sea-drifted seeds that is widely distributed in tropical and subtropical coastal areas of the world and is also known as the beach pea. In Japan, our field surveys have shown that *V. marina* grows well on the coral sand beaches of Okinawa and Sakishima Island, with distinctive yellow flowers and brown pods (Egawa *et al.* 1990, 1991; Tomooka *et al.* 2000, 2005, 2012, 2013; Takahashi *et al.* 2014, 2018; Muto *et al.* 2015),

In the past, I have been involved in seven field surveys of leguminous plants and their symbiotic rhizobia (Muto *et al.* 2015; Baba-Kasai *et al.* 2016, 2017, 2018, 2019; Takahashi *et al.* 2017, 2018). Through

these field surveys, I confirmed that *V. marina* is the best host plant for identifying salt-tolerant rhizobia. Since *V. marina* can grow in sunny beach front areas and possesses high tolerance to salt stress and drought (Chankaew *et al.* 2014), it is likely that their symbiotic rhizobia also possess high salt tolerance (Elanchezian *et al.* 2009). Another important factor is that *V. marina* prefers sandy beaches as habitats; therefore, it is very easy to collect root nodules without special tools.

Although many collection trips have been previously performed and many habitats of *V. marina* have been recorded on Okinawa Island and Sakishima Island, there have been few surveys conducted on Amami Island and Osumi Island. Therefore, I decided to conduct a field survey of *V. marina* and their symbiotic rhizobia in 2019 on Tokunoshima Island, Okinoerabu Island, and Yoron Island, which collectively belong to the Amami Islands.

Methods

A field survey of the Tokunoshima, Okinoerabu, and Yoron islands in Kagoshima Prefecture, Japan, was conducted by car from November 18 to 22, 2019. The survey began at Tokunoshima Airport and ended at Yoron Airport (the itinerary is shown in Table 1). When naturally growing *V. marina* or a habitat with conditions suitable for *V. marina* was found, the area was searched for natural populations.

Bulk seed samples were collected from each population. Seeds of each morphotype were collected separately when the population contained plants with different traits.

The passport data recorded included the location of collection sites, such as latitude, longitude and altitude. Maps was sketched for each habitat and any

special characteristics of sampled plants were noted, as shown in Table 3. This information was stored in the Genebank database after the sampled plants were registered as accessions. The latitude and longitude were measured using the WGS84 world geodetic system and a Garmin GPSMAP 60sc handheld GPS device (Germin International, Inc., Olathe, KS).

Root nodules were collected from *V. marina*'s roots at nine specific habitats, and they were placed in a screw bottle with desiccant silica gel.

Results and Discussion

For all islands

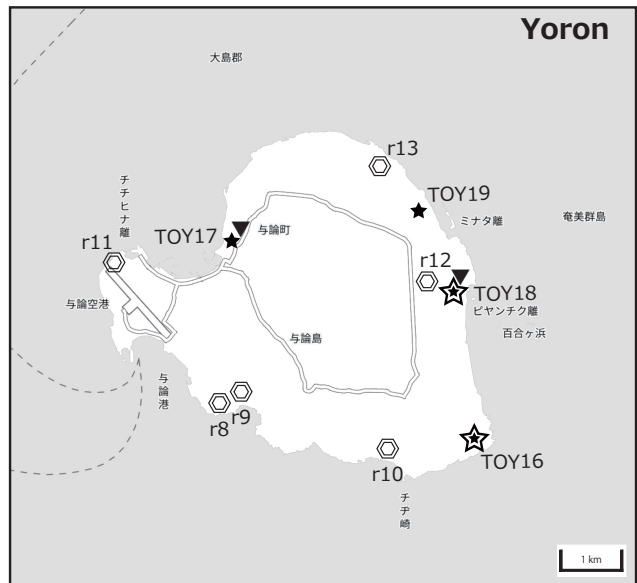
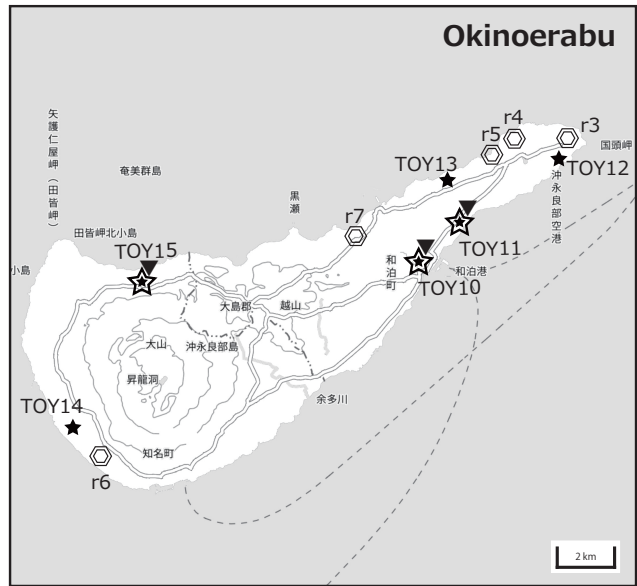
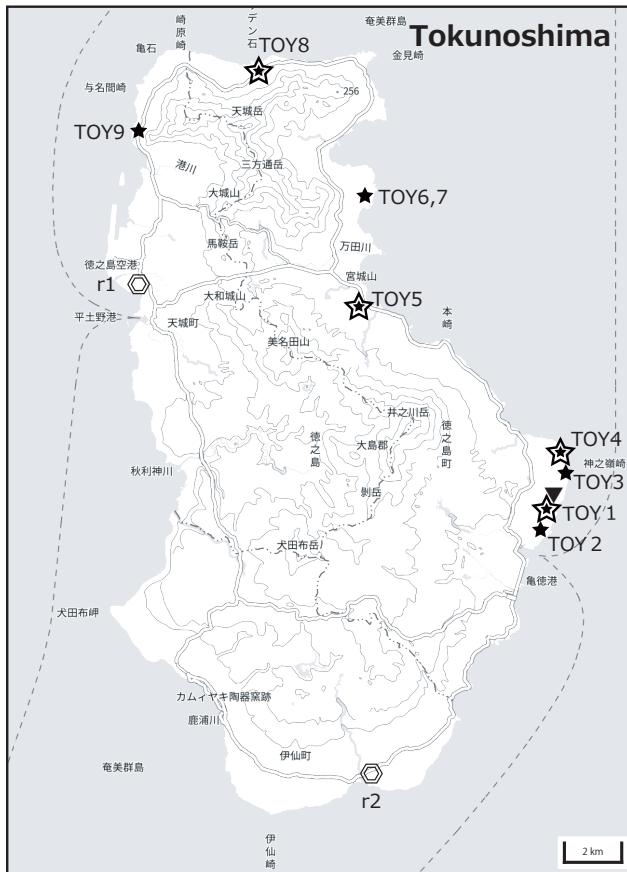
Individuals of *V. marina* were often found in areas slightly inland from the beach, such as parking lots near beaches, at a parking space near Tokunoshima Airport (Photo 1), and along roadsides leading to the ocean, for example, at a roadside leading to Hakibina Beach on Yoron Island (Photo 2). This is characteristic of the



Photo 1. *Vigna marina* grew among other weeds in a parking space near the Tokunoshima Airport. The location is marked as r1 on the map (Fig. 1).

Table 1. Itinerary of the field survey on Tokunoshima Island, Okinoerabu Island and Yoron Island in Japan (November 18–22, 2019)

Date	Itinerary	Stay
2019/11/18	Tsukuba -- Haneda Airport 8:15 -- (JAL643) -- Kagoshima Airport -- (JAL3795) -- Tokunoshima Airport 12:40 -- Tokunoshima-cho	Tokunoshima-cho
2019/11/19	Exploration on Tokunoshima Island	Tokunoshima-cho
2019/11/20	Moved to Okinoerabu Island from Tokunoshima Island by a ferry, then, exploration on Okinoerabu Island	China-cho
2019/11/21	Exploration on Okinoerabu Island and moved to Yoron Island by a ferry, then, exploration on Yoron Island	Yoron-cho
2019/11/22	Exploration Yoron Island until noon; Yoron Airport 13:15 -- (JAC3824) -- Kagoshima Airport -- (JAL650) -- Haneda Airport 17:55 -- Tsukuba	



- ★ Collection sites of *Vigna marina* seeds
- ☆ Collection sites of nodules from *V. marina*
- ☆☆ Both of above
- ▼ Point of soil sampling
- ⊙ Search sites with numbers in chronological order

Fig. 1. The collection and search sites on Tokunoshima Island, Okinoerabu Island and Yoron Island, Kagoshima Prefecture.



Photo 2. *Vigna marina* formed a small bush along with other weeds at a roadside near Hakibina Beach on Yoron Island. The location is marked as r9 on the map (Fig. 1).

habitat distribution of *V. marina* on Tokunoshima Island, Okinoerabu Island, and Yoron Island, and it seems to be a very common seaweed on these three islands. In fact, I observed park maintenance workers weeding *V. marina* at the beach near Tetchama Seaside Park in Tokunoshima due to heavy overgrowth (Photo 3).

V. marina was generally found growing on clean coral sand on all three islands (Photo 4: Tokunoshima, Photo 5: Okinoerabu, and Photo 6: Yoron) even though it was also found in a variety of non-beach soil environments as noted above. On all three islands, *Cassipya filiformis* L., also referred to as love-vine, was often found growing parasitically on *V. marina* and flowering (Photos 7–9) on sandy beaches.

As summarized in Table 2, *V. marina* seeds were collected from 19 sites on three islands, and root nodules were collected from 9 accessions of *V. marina*. Soil

samples were also collected from 6 of the 19 habitat sites. The weather was not favorable, with heavy squalls on Tokunoshima and Yoron Island; therefore, some parts



Photo 3. Large thickets of *Vigna marina*, along with other weeds, were found near Tetehama Seaside Park. The park maintenance workers were busy weeding. The location is marked as TOY8 on the map (Fig. 1).



Photo 6. Typical coral sand was found on the beaches of Yoron Island. The location is marked as TOY18 on the map (Fig. 1).



Photo 4. Typical coral sand was found on the beaches of Tokunoshima Island. The location is marked as TOY5 on the map (Fig. 1).



Photo 7. Love-vine (*Cassytha filiformis*) grows parasitically on *Vigna marina*'s pods. The location is marked as TOY1 on the map (Fig. 1).



Photo 5. Typical coral sand was found on the beaches of Okinoerabu Island. The location is marked as TOY11 on the map (Fig. 1).



Photo 8. *Vigna marina* was covered by overgrown *Cassytha filiformis*. The location is marked as TOY12 on the map (Fig. 1).

Table 2. A summary of the materials collected on Tokunoshima Island, Okinoerabu Island and Yoron Island

Species	Collected number
Seeds of <i>Vigna marina</i>	19
Nodule samples from <i>V. marina</i>	9
Soil samples of <i>V. marina</i> habitat	6
Total	34



Photo 9. *Cassytha filiformis* was flowering over *Vigna marina*. The location is marked as TOY14 on the map (Fig. 1).



Photo 10. *Vigna marina* was found to be overgrown on vacant ground on the road at the Omonawa fishing port. The location is marked as r2 on the map (Fig. 1).

of the coastlines on the three islands were not explored. Because one of the aims of this survey was to visit as many sites as possible, root nodules were not collected from all of the identified *V. marina* habitats. This is because the collection of root nodules, in addition to the collection of seeds, is an extra time-consuming process. Therefore, the number of rhizoids collected was equal to approximately half of the number of seeds collected.

Tokunoshima Island

Tokunoshima Island was started to survey on November 18. On this particular day, it was raining heavily; therefore, the southern half of the island could not be properly surveyed. Nevertheless, *V. marina* was often found on the side of the road in areas near the beach (Photos 1 and 10). Overall, 9 accessions (JP286186–286194) of *V. marina* and 4 samples of *V. marina* root nodules (at TOY-01, 04, 05, and 08) were collected from Tokunoshima Island (detail are shown in Table 3). Root nodules were dug up from the beach with a shovel (Photos 11 and 12) and stored in sealed sample jars filled with silica gel (Photo 13).



Photo 11. *Vigna marina* root nodules were dug from the beach with shovels. The location is marked as TOY10 on the map (Fig. 1).

Okinoerabu Island

Okinoerabu Island was formed by uplifted coral reefs, and the map gave the impression of a flat island surrounded by many sandy beaches. However, the erosion of uplifted coral reefs has created a complex coastline with strange rocky beaches such as Ujiji



Photo 12. Another root nodules that was growing symbiotically with *Vigna marina*. The location is marked as TOY4 on the map (Fig. 1).



Photo 13. *Vigna marina* root nodules were placed in a sealed sample jar filled with silica gel. The collecting location is marked as TOY1 on the map (Fig. 1).

Beach, spouting caves such as Fucha, and cliffs such as Cape Tamina. In addition, some beaches, such as Wanjo Beach, have been so well maintained that there are hardly any wild plants due to recent tourism. Hence, the number of collection points for *V. marina* was less than expected. Overall, 6 accessions (JP286195–286200) of *V. marina* and 3 samples of *V. marina* root nodules (at TOY-10, 11, and 15) were collected from Okinoerabu Island (details are shown in Table 3).

Lablab purpureus (L.) was observed at a roadside near Yakomo, China-cho (Photo 14). Unfortunately, all of the pods were too young to collect seeds. *Glycine tabacina* (labill.) Benth grows in Tamina Cape, which is the northern limit of the *G. tabacina* habitat in Japan (Ooya 2013; Ryukyu Plant Research Group 2018 onward). Similarly, several studies have reported that habitats of *Vigna reflexo-pilosa* Hayata were confirmed



Photo 14. *Lablab purpureus* was flowering at a roadside near Yakomo Beach on Okinoerabu Island. The location is marked as r6 on the map (Fig. 1).

in Okinoerabu, along with Tokunoshima and Yoron (Tomooka *et al.* 1992; Ryukyu Plant Research Group 2018 onward). In the future, I believe that it would be valuable to resurvey the inland areas to collect their seeds.

Yoron Island

On November 21, Yoron Island was started to survey. Due to heavy rain, only 4 beaches on the southern side of the island were explored on that day. Furthermore, 2 of the 4 beaches were undergoing construction of seawalls or breakwaters, making the beaches inaccessible. Nevertheless, it was confirmed that *V. marina* grew at 3 sites; however, only young plants without mature pods were found at 2 of the 3 sites.

On the north side of the island, mature pods of *V. marina* could be collected on 3 beaches of 6 site searched in the morning of November 22. On the remaining 2 beaches, *V. marina* plants were too young to collect seeds. The last beach, Chabana Beach, was over-maintained similarly to Wanjo Beach on Okinoerabu Island; therefore, no *V. marina* plants were found.

As a result, four accessions (JP286201–286204) of *V. marina* and two samples of *V. marina*'s root nodule (at TOY-16 and TOY-18) were collected on Yoron Island (details are shown in Table 3). In general, I found that the *V. marina* plants growing on Yoron Island were substantially younger than those on the other two islands. As indicated by the construction of seawalls and breakwaters, the sandy beaches of Yoron Island are narrow and are easily affected by high waves. Thus, I believe that salt damage caused by exposure to the tides is one of the reasons for the high proportion of young individuals on Yoron Island.

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徳之島，沖永良部島，与論島における
Vigna marina の種子および根粒の探索収集，
2019年11月18日～22日

馬場（笠井）晶子

農業・食品産業技術総合研究機構 遺伝資源研究センター

和文摘要

本報告は，2019年11月18日から22日にかけて，鹿児島県の徳之島，沖永良部島，与論島の海岸で行った耐塩性マメ科遺伝資源 *Vigna marina* とその根粒の探索収集報告である．現地調査の結果，3島から *V. marina* 19点を収集した．また，共生根粒菌を分離するために，*V. marina* の9つのアクセッションから根粒を採取し，さらに6つのアクセッションの生息地からは土壌試料も採取した．収集した材料はすべて農業・食品産業技術総合研究機構（NARO）ジーンバンクに保存し，増殖した種子は，研究，育種，教育目的のために，要望に応じて提供する予定である．

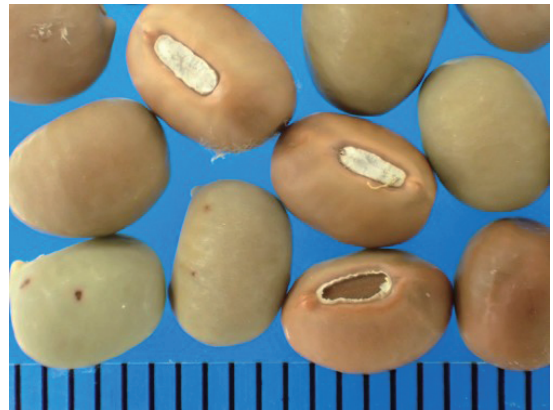
Table 3. Passport information of collected materials

Col. No. / Map ID	JP No.	Scientific name	Col. Date	Status	Collection Site (Address)	Latitude	Longitude	Altitude (m)	Soil	Seed	Herbarium	Nodule	Soil sample	Remarks	100 seed weight (g)
TOY-01	286186	<i>Vigna marina</i>	19 Nov, 2019	Wild	Kametoku, Tokunoshima-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 徳之島町 亀徳	N27°44'43.1"	E129°01'44.2"	11	sandy loam	bulk	no	yes	yes	At a beach near Nagominomisaki Park	1.52
TOY-02	286187	<i>Vigna marina</i>	19 Nov, 2019	Wild	Kametoku, Tokunoshima-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 徳之島町 亀徳	N27°44'41.6"	E129°01'43.1"	10	sandy loam	bulk	no	no	no	At a beach near Nagominomisaki Park	1.76
TOY-03	286188	<i>Vigna marina</i>	19 Nov, 2019	Wild	Shoda, Tokunoshima-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 徳之島町 諸田	N27°45'59.4"	E129°02'12.9"	13	sandy loam	bulk	no	no	no	At Shinde Beach	2.04
TOY-04	286189	<i>Vigna marina</i>	19 Nov, 2019	Wild	Shoda, Tokunoshima-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 徳之島町 諸田	N27°46'03.0"	E129°02'14.4"	10	sandy loam	bulk	no	yes	no	At Shinde Beach	1.60
TOY-05	286190	<i>Vigna marina</i>	19 Nov, 2019	Wild	Kedoku, Tokunoshima-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 徳之島町 花徳	N27°49'00.4"	E128°58'08.1"	4	sandy loam	bulk	no	yes	no	Near Rikubama Beach	1.62
TOY-06	286191	<i>Vigna marina</i>	19 Nov, 2019	Wild	San, Tokunoshima-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 徳之島町 山	N27°50'53.5"	E128°58'14.6"	7	sandy loam	bulk	no	no	no	At Aze Prince Beach, the leaves of TOY-6 are small	0.79
TOY-07	286192	<i>Vigna marina</i>	19 Nov, 2019	Wild	San, Tokunoshima-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 徳之島町 山	N27°50'54.7"	E128°58'15.0"	6	sandy loam	bulk	no	no	no	At Aze Prince Beach, TOY-7 has bigger leaf than TOY-6	0.94
TOY-08	286193	<i>Vigna marina</i>	19 Nov, 2019	Wild	Tete, Tokunoshima-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 徳之島町 手々	N27°53'17.2"	E128°55'39.6"	3	sandy loam	bulk	no	yes	no	At a beach near Tetehama Seaside Park	1.24
TOY-09	286194	<i>Vigna marina</i>	19 Nov, 2019	Wild	Yonama, Amagi-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 天城町 与名間	N27°52'05.7"	E128°53'19.7"	8	sandy loam	bulk	no	no	no	At Yonama Beach	1.22
TOY-10	286195	<i>Vigna marina</i>	20 Nov, 2019	Wild	Tedechina, Wadamari-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 和泊町 手々知名	N27°23'58.7"	E128°39'44.7"	10	sandy loam	bulk	no	yes	yes	At a beach near Wadamari Port Terminal	2.56
TOY-11	286196	<i>Vigna marina</i>	20 Nov, 2019	Wild	Kibiru, Wadamari-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 和泊町 喜美留	N27°24'37.3"	E128°40'20.3"	13	sandy loam	bulk	no	yes	yes	At a beach near Kasaishi Beach Park	1.44
TOY-12	286197	<i>Vigna marina</i>	20 Nov, 2019	Wild	Kunigami, Wadamari-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 和泊町 国頭	N27°25'57.0"	E128°42'34.1"	7	sandy loam	bulk	no	no	no	At a beach beside Kunigami-misaki Lighthouse near Okinoerabu Airport	1.88
TOY-13	286198	<i>Vigna marina</i>	20 Nov, 2019	Wild	Nishibaru, Wadamari-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 和泊町 西原	N27°25'26.8"	E128°39'48.6"	9	sandy loam	bulk	no	no	no	At a beach	1.08
TOY-14	286199	<i>Vigna marina</i>	21 Nov, 2019	Wild	Otsukan, China-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 知名町 大津勘	N27°20'37.9"	E128°32'13.5"	7	sandy loam	bulk	no	no	no	At a bank near Beach Rock	0.96
TOY-15	286200	<i>Vigna marina</i>	21 Nov, 2019	Wild	Shimojiro, China-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 知名町 下城	N27°23'44.0"	E128°33'31.5"	3	sandy loam	bulk	no	yes	yes	At a bank beside Okidomari Seaside Park	2.04
TOY-16	286201	<i>Vigna marina</i>	21 Nov, 2019	Wild	Mugiya 815, Yoron-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 与論町 大字麦屋 815	N27°01'25.1"	E128°27'20.5"	9	sandy loam	bulk	no	yes	no	At a beach near Akazaki Lighthouse	1.04
TOY-17	286202	<i>Vigna marina</i>	22 Nov, 2019	Wild	Chabana, Yoron-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 与論町 大字茶花	N27°03'09.4"	E128°24'56.1"	4	sandy loam	bulk	no	no	no	At a beach	2.20
TOY-18	286203	<i>Vigna marina</i>	22 Nov, 2019	Wild	Furusato, Yoron-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 与論町 大字古里	N27°02'44.4"	E128°27'12.6"	5	sandy loam	bulk	no	yes	yes	At Okaneku (Ubuganiku) Beach between Hatono Mizuumi and Pyanchiku Hanari	0.84
TOY-19	286204	<i>Vigna marina</i>	22 Nov, 2019	Wild	Nama, Yoron-cho, Oshima-gun, Kagoshima 鹿児島県 大島郡 与論町 大字那間	N27°03'42.0"	E128°26'38.6"	1	sandy loam	bulk	no	no	no	At Kurubana Beach	1.25

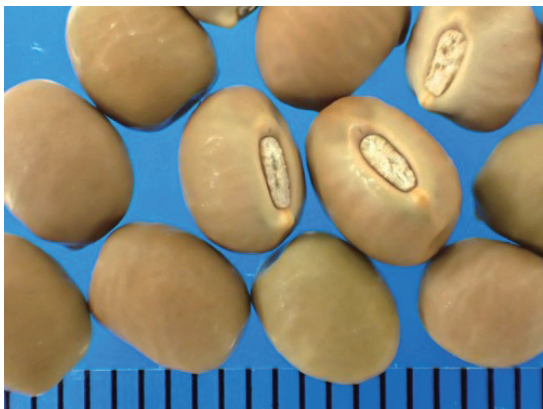
Photos of Seed samples



Sample Photo 1.
TOY-01, JP286186, *Vigna marina*



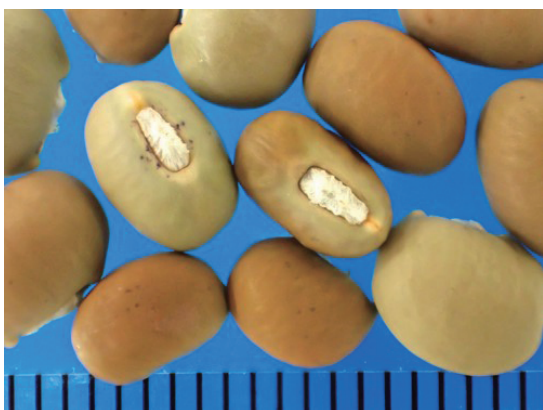
Sample Photo 2.
TOY-02, JP286187, *Vigna marina*



Sample Photo 3.
TOY-03, JP286188, *Vigna marina*



Sample Photo 4.
TOY-04, JP286189, *Vigna marina*



Sample Photo 5.
TOY-05, JP286190, *Vigna marina*



Sample Photo 6.
TOY-06, JP286191, *Vigna marina*

Photos of Seed samples



Sample Photo 7.
TOY-07, JP286192, *Vigna marina*



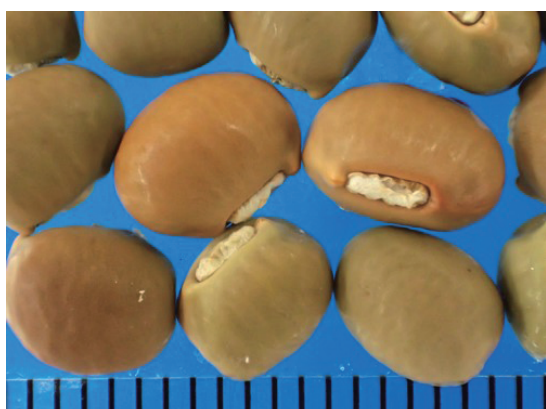
Sample Photo 8.
TOY-08, JP286193, *Vigna marina*



Sample Photo 9.
TOY-09, JP286194, *Vigna marina*



Sample Photo 10.
TOY-10, JP286195, *Vigna marina*



Sample Photo 11.
TOY-11, JP286196, *Vigna marina*



Sample Photo 12.
TOY-12, JP286197, *Vigna marina*

Photos of Seed samples



Sample Photo 13.
TOY-13, JP286198, *Vigna marina*



Sample Photo 14.
TOY-14, JP286199, *Vigna marina*



Sample Photo 15.
TOY-15, JP286200, *Vigna marina*



Sample Photo 16.
TOY-16, JP286201, *Vigna marina*

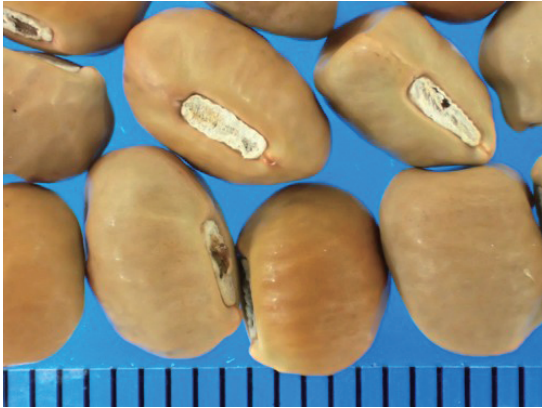


Sample Photo 17.
TOY-17, JP286202, *Vigna marina*



Sample Photo 18.
TOY-18, JP286203, *Vigna marina*

Photos of Seed samples



Sample Photo 19.
TOY-19, JP286204, *Vigna marina*