Original Paper

## Ex-situ Conservation of Crop Wild Relatives and Crop Landraces in the Genera Asparagus, Vitis, Citrus, Dioscorea, Actinidia, and Amorphophallus in Fukuoka, Kumamoto, and Oita Prefectures

Yu TAKAHASHI<sup>1)</sup>, Shin-ichi YAMAMOTO<sup>1)</sup>, Hirotaka ARIGA<sup>1)</sup>,

Takeo SHIMIZU<sup>2)\*</sup>, Hiroyuki IKETANI<sup>3)</sup>

- 1) Research Center of Genetic Resources, National Agriculture and Food Research Organization, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
- 2) Division of Fruit Tree Breeding Research, Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization, 301-2 Mitsu, Akitsu, Higashihiroshima, Hiroshima, 739-2494, Japan
- 3) Faculty of Biosphere and Geosphere, Okayama University of Science, 1-1 Ridai-cho, Kita-ku, Okayama 700-0005, Japan
- \* Present affiliation: Division of Fruit Tree and Tea Pest Control Research, Institute for Plant Protection, National Agriculture and Food Research Organization, 2-1 Fujimoto, Tsukuba, Ibaraki 305-8605, Japan

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### Summary

This paper reports on the conservation of mainly crop wild relatives as plant genetic resources for food and agriculture in Fukuoka, Kumamoto, and Oita Prefectures, Japan, in 2022. We surveyed natural and/or artificially disturbed environments in these prefectures, collecting asparagus (*Asparagus kiusianus* Makino, *Asparagus oligoclonos* Maxim, and *Asparagus cochinchinensis* (Lour.) Merr.), grape (*Vitis kiusiana* Momiy.), kiwifruit (*Actinidia rufa* (Siebold et Zucc.) Planch. ex Miq.), and yam (*Dioscorea japonica* Thunb) wild relatives. In addition, we acquired citrus landrace (*Citrus nobilis* Lour. var. *kunep* Tanaka) and Konnyaku landrace (*Amorphophallus rivieri* Durieu var. *konjac* (K. Koch) Engl.) through the courtesy of local farmers involved in the survey. Notably, we successfully achieved the *exsitu* conservation of endangered asparagus wild relatives (*A. kiusianus* and *A. oligoclonos*). The NARO Genebank will conserve these genetic resources after multiplication and distribute them for research and educational purposes (https://www.gene.affrc.go.jp/index\_en.php).

KEY WORDS: Asparagus, Crop wild relatives, Genebank, Genetic resource

#### Introduction

Conserving crop wild relatives that are potentially tolerant to biotic and abiotic stresses is a challenge faced both by Japan and around the world. The National Agriculture and Food Research Organization (NARO) Genebank is paying renewed attention to the conservation of crop wild relatives (Ariga and Takahashi 2022), prompted by the aim to "maintain the genetic diversity of seeds, cultivated plants and farmed and domesticated animals and their related wild species" of "Goal 2: Zero Hunger" in the Sustainable Development Goals (United Nations, https://sdgs.un.org/goals). The Red List 2020, published by the Ministry of the Environment in Japan (MOE), lists 1,790 species of vascular plants as endangered (https://www.env.go.jp/press/107905.html). Of these, 203 were designated as nationally rare species of wild fauna and flora under the Act on Conservation of Endangered Species of Wild Fauna and Flora (the Act) in January 2023 (Ministry of Justice in Japan, https:// www.japaneselawtranslation.go.jp/en/laws/view/4236). However, no systematic surveys and/or conservation activities have been conducted to evaluate their role as plant genetic resources for food and agriculture in Japan.

Kyushu Island is the southernmost and third largest of four main islands in Japan. While Kyushu Island is a geoscientific term referring to a single island, "Kyushu region" is a geopolitical term used to refer to Kyushu Island, its surrounding islands, and Okinawa Prefecture. There are seven prefectures comprising Kyushu Island: Fukuoka, Saga, Nagasaki, Kumamoto, Oita, Miyazaki, and Kagoshima. Fukuoka, which is the most northerly prefecture of Kyushu Island, had a population of over 5 million people in January 2023, making it the most urbanized prefecture in the Kyushu region (Fukuoka Prefectural Government, https://www.pref.fukuoka.lg.jp/ contents/fukuokakennojinkoutosetai.html). Although Kyushu Island possesses a "Humid subtropical climate (Cfa)" according to the Köppen climate classification, the regions around the Aso mountain in Kumamoto Prefecture (highest altitude: 1,592 m) and the Kuju mountain in Oita Prefecture (highest altitude: 1,791 m)

Table 1. Itinerary of the field survey

have a cooler climate (Kumamoto Local Meteorological Office, https://www.jma-net.go.jp/kumamoto/shosai/ climate.html).

The decline in crop wild relatives due to modernization and environmental change has necessitated their *ex-situ* conservation. The asparagus wild relative "Hama-Tamaboki" (*A. kiusianus*) endemic to Japan is listed as Endangered IB (EN) in the Red List 2020 published by the MOE. The asparagus wild relative "Tamaboki" (*A oligoclonos*), inhabiting East Asia, has only been found in Japan in the region around the Aso and Kuju mountains and is designated as the specified class I nationally rare species of wild fauna and flora under the Act. While *in-situ* conservation is the best policy in terms of environmental conservation, *ex-situ* conservation is the best policy when wild species are used as plant genetic resources for food and agriculture.

This paper reports on the conservation of mainly crop wild relatives in Fukuoka, Kumamoto, and Oita Prefectures as plant genetic resources for food and agriculture in 2022. We conducted two field surveys in the aforementioned prefectures in May (spring) and October (autumn) after obtaining information on the habitats of the endangered species in the genus *Asparagus*.

#### Methods

A field survey was conducted in Fukuoka, Kumamoto, and Oita Prefecture on May 25-30 and October 17-20 (Table 1). We obtained information on each habitat from Mr. Sumio Sei (Aso flower field association), Dr. Tetsukazu Yahara (Kyushu Open University), Mr. Tsuzuki Miyagawa (rare wildlife

Date	Itinerary
25-May-22	Haneda Airport – ANA643 – Kumamoto Airport <sup>1</sup> – Itoshima City <sup>2</sup> – Otsu Twon <sup>1</sup> (Stay)
26-May-22	Otsu Twon <sup>1</sup> – Minamiaso Village <sup>1</sup> – Otsu Twon <sup>1</sup> (Stay)
27-May-22	Otsu Twon <sup>1</sup> – Taketa City <sup>3</sup> – Otsu Twon <sup>1</sup> (Stay)
28-May-22	Otsu Twon <sup>1</sup> – Oguni Twon <sup>1</sup> – Otsu Twon <sup>1</sup> (Stay)
29-May-22	Otsu Twon <sup>1</sup> – Itsuki Village <sup>1</sup> – Otsu Twon <sup>1</sup> (Stay)
30-May-22	Otsu Twon <sup>1</sup> – Kumamoto Airport <sup>1</sup> – ANA2418 – Haneda Airport
17-Oct-22	Haneda Airport – ANA643 – Kumamoto Airport <sup>1</sup> – Itoshima City <sup>2</sup> – Otsu Twon <sup>1</sup> (Stay)
18-Oct-22	Otsu Twon <sup>1</sup> – Itsuki Village <sup>1</sup> – Otsu Twon <sup>1</sup> (Stay)
19-Oct-22	Otsu Twon <sup>1</sup> – Minamiaso Village <sup>1</sup> – Otsu Twon <sup>1</sup> (Stay)
20-Oct-22	Otsu Twon <sup>1</sup> – Kumamoto Airport <sup>1</sup> – ANA644 – Haneda Airport

<sup>1</sup>Kumamoto Prefecture

<sup>2</sup>Fukuoka Prefecture

<sup>3</sup>Oita Prefecture

species conservation promoter appointed by the Minister of the Environment), Mr. Masato Watanabe (Kumamoto University) and Dr. Takayuki Tanaka (Tokai University). We recorded passport data and collected seeds or plants after obtaining permission from landowners. The passport data included the latitude, longitude, and altitude of each habitat, obtained using Google Maps and Google Earth. The latitude and longitude information of the habitats containing endangered species is confidential in this paper. The acquisition of the "rare species of wild fauna and flora" followed the Act and other relevant laws and regulations. Notably, although article 12 of the Act prohibits the transfer of any individual of "rare species of wild fauna and flora," this does not apply to the "specified class I nationally rare species of wild fauna and flora," as noted in item (ii). We performed a classification of the genera based on the Angiosperm Phylogeny Group system IV (APG et al. 2016). For the romanization of Japanese botanical names, we adopted the Hepburn system, ignoring long vowels, enclosing them in double quotation marks, and capitalizing the initials of all words by using hyphens as word separators according to the NARO Genebank method.

#### **Results and Discussion**

We collected 10 genetic resources, including 1 accession of "Hama-Tamaboki" (Asparagus kiusianus Makino), 1 of "Tamaboki" (Asparagus oligoclonos Maxim), 1 of "Kusasugi-Kazura" (Asparagus cochinchinensis (Lour.) Merr.), 1 of "Kumagawa-Budo" (Vitis kiusiana Momiy.), 1 of "Shima-Sarunashi" (Actinidia rufa (Siebold et Zucc.) Planch. ex Miq.), 2 of "Kunebu" (Citrus nobilis Lour. var. kunep Tanaka), 2 of "Yamanoimo" (Dioscorea japonica Thunb.), and 1 of "Konnyaku" (Amorphophallus rivieri Durieu var. konjac (K. Koch) Engl.). The passport data for each accession is shown in Table 2, and the characteristics of each species are described below.

#### Genus Asparagus Tourn. ex L. in Sp. Pl.: 313 (1753)

Over 200 species in the genus Asparagus of the family Asparagaceae have been accepted in the Plants of the World Online (POWO, https://powo.science. kew.org/) and World Flora Online (WFO, http://www. worldfloraonline.org/) sites as of August 2023. Of these, Asparagus officinalis L, which originated in Europe, has been domesticated as asparagus, a vegetable with edible shoots. Asparagus officinalis is a dioecious plant, whose genes for sex determination have been identified for breeding applications (Murase et al. 2017). Four wild species have been observed in Japan:

"Hama-Tamaboki" (A. kiusianus), "Tamaboki" (A. oligoclonos), "Kusasugi-Kazura" (A. cochinchinensis), and "Kijikakushi" (Asparagus schoberioides Kunth). Among them, Asparagus schoberioides is the most widely distributed in mountainous grasslands in the mainland, from Hokkaido to Kyushu Island. We found A. schoberioides without flowers or fruits during the survey in May (Fig. 1). A species similar to A. schoberioides, "Oobasugi-Kazura" (Asparagus rigidulus Nakai), has been described as a coastal species. Although POWO and WFO list A. rigidulus as a synonym of A. schoberioides, two plant types classified as key character pedicel length collected in Korea were located in different clusters on a phylogenetic tree based on ITS sequences (Cho and Kim 2012). We were unable to compare the pedicel length between the A. rigidulus isotype and two specimens (not type) of A. schoberioides owing to the absence of flowers (https://type.kahaku. go.jp/TypeDB/vascular/1817, http://specimens.kew. org/herbarium/K000901236, http://specimens.kew.org/ herbarium/K000901235). In the future, the synonymy of A. rigidulus should be reconsidered through detailed morphological and molecular phylogenetic analyses.

#### Asparagus kiusianus Makino in Bot. Mag. (Tokyo) 21: 161 (1907)

#### Hama-Tamaboki (in Japanese)

This asparagus species is a wild relative endemic to Japan, listed as Endangered IB (EN) in the Red List 2020 published by the MOE. This species has drawn attention as a breeding material for asparagus because it is a cross-compatible with it (Ito *et al.* 2011), has resistance to stem blight caused by *Phomopsis asparagi* (Sacc.) Grove—a major disease of asparagus (Abdelrahman *et al.* 2017)— and is tolerant to salinity, allowing it to inhabit beaches. Its molecular mechanisms for stem blight resistance and sex determination are being elucidated with the whole genome sequence (Shirasawa *et al.* 2022).

FK01 was collected from eight female plants growing naturally at the top of a sand dune (approximately 30 m<sup>2</sup>), several tens of meters away from the edge of the surf in Itoshima City, Fukuoka Prefecture. We surveyed a radius of approximately 2 km of sandy beach from the point where FK01 was found, but no other populations were discovered. During the survey in May, the male plants flowered, while the fruits of the female plants were immature, appearing green (Figs. 2-5). Therefore, we surveyed the same site again in October to collect red fruits with mature seeds from the female plants (Figs. 6-8). While this species has been reported in Fukuoka Prefecture as well as in Saga and Yamaguchi

Prefectures (Kyushu University, https://www.tropic-agrkyushu-u.jp/category/recent activity/page/2/), both their habitat and population numbers are decreasing. A portion of the sand dune where this population was found in May was eroded during the October survey, and part of the population had disappeared (Fig. 9). Sea level along the Japanese coast in 2022 was 63 mm higher than the average for the 1991-2020 period; this represented the second highest level recorded since 1906, when statistics began (Japan Meteorological Agency, https://www.data. jma.go.jp/gmd/kaiyou/shindan/a 1/sl trend/sl trend. html), after the 71 mm measured in 2021. Additionally, five typhoons were observed approaching the northern Kyushu region and Yamaguchi Prefecture between July and September 2022 (Japan Meteorological Agency, https://www.data.jma.go.jp/fcd/yoho/typhoon/statistics/ accession/index.html). The high tides caused by these phenomena probably eroded the sand dunes conforming the habitat of this species. Although A. kiusianus has significant physical seed dormancy, after heat treatment at 30 °C for 2 days, 6 out of 10 sown seeds germinated and are growing well as of August 2023 (Fig. 10). As the habitat of FK01 is in danger, this study represented an opportunity for its ex-situ conservation.

#### Asparagus oligoclonos Maxim. in Mém. Acad. Imp. Sci. St.-Pétersbourg Divers Savans 9: 286 (1859) Tamaboki (in Japanese)

Asparagus oligoclonos, an asparagus wild relative inhabiting East Asia, has only been found in Japan in the region around the Kuju mountains in Oita Prefecture and Aso mountain in Kumamoto Prefecture. It has been designated as a specified class I nationally rare species of wild fauna and flora under the Act. This species is recognized as "one of the endemic halophytes used in folk medicine" in Korea, and its biochemical activity is being studied (Lee *et al.* 2018ab). Furthermore, a phylogenetic tree based on partial chloroplast DNA sequences has placed this specie in the same clade as *A. kiusianus* (Kubota *et al.* 2012).

Under the guidance of Mr. Atsushi Kamiyoshi, a chairman of the "Kuju-Kogen Michikusa Annainin Kurabu" non-profit organization (NPO), we were able to find this species at an altitude of 832 m in Taketa City, Oita Prefecture. The collection site was a south-facing slope with dense bamboo grasses approximately 1 m tall and scattered shrubs that hindered accessibility (Fig. 11). During the May survey, the plants amounted to less than 20 without flowers or fruits, but we found some fruits from the previous year (Figs. 12 and 13). Completely dead plants were observed having disease symptoms similar to those of asparagus stem blight caused by P. asparagi (Fig. 14). There are expansive grasslands around the Aso and Kuju mountains that have seemingly been used as grazing land for more than 1,000 years, during which locals have continuously burned dead grass, grazed livestock, and cut the grass (Government of Japan, https://www.gov-online.go.jp/eng/publicity/ book/hlj/html/202108/202108 12 en.html). However, maintaining the grasslands has become more difficult owing to a shortage of people taking on farming and livestock work, which has led to growing concerns regarding their deterioration and reduction. Currently, volunteers from the NPO are mowing bamboo grasses at this point, but the vegetation has continued to gradually change, becoming unsuitable for this species. The location is near an asparagus production area (Fig. 15), which may have been invaded by stem blight. Burning dead grass not only resets the vegetation transition, but also reduces stem blight and provides potassium and lime content in grass and tree ash. In modern asparagus production, unnecessary plants are burned to control stem blight (Ogiso et al. 2012). Thus, the habitats of crop wild relatives are in danger because modernization has eliminated the use of traditional practices. This has increased the need for ex-situ conservation of crop wild relatives or gene banks.

FK05.5 is a cultivated plant derived from a wild population around Aso mountain in Kumamoto Prefecture. Mr. Sumio Sei from the "Aso flower field association" provided us with two plants collected from a wild habitat before the promulgation of the Act in 1992. He has been cultivating these plants in a private land ever since and informed us that "the shoots of this plant are as thick as the commercially available asparagus" (Fig. 16). So far, the provided plants have been growing well in the NARO Genebank, Tsukuba City, Ibaraki Prefecture (Fig. 17). As this species is used as a wild vegetable in China, it could be directly used as a new crop as well as breeding material for asparagus. This could in turn potentially contribute to the revitalization of the region as a source of specialized products.

#### Asparagus cochinchinensis (Lour.) Merr. in Philipp. J. Sci. 15: 230 (1919)

#### Kusasugi-Kazura (in Japanese)

Asparagus cochinchinensis is a perennial plant distributed along the coasts of East Asia and Southeast Asia. Its spindle-shaped tubers that occur in large numbers on short rhizomes are processed to produce the Chinese medicine "Tian Men Dong." Unlike other species of the genus Asparagus distributed in Japan, its fruits are white when fully ripe.

FK24 was found growing naturally behind a cemetery near a fishing port in Miyazaki Prefecture. Mr. Masato Watanabe, from Kumamoto University, collected its seeds on June 25, 2011, and has cultivated them in the Medicinal Plant Garden of the university since then (https://www.pharm.kumamoto-u.ac.jp/yakusodb/detail/003365.php). The cultivated plants were over 2 m tall and producing plenty of fruits as of May 2022 (Figs. 18 and 19). Although seeds that had fallen in the field the previous year were collected with permission (Fig. 20), the passport data records the location where Mr. Watanabe originally collected these plants (Table 2).

#### *Vitis kiusiana* Momiy. in J. Jap. Bot. 11: 525 (1935) Kumagawa-Budo (in Japanese)

*Vitis kiusiana* is a grape wild relative distributed in Kumamoto, Miyazaki, and Kagoshima Prefectures, listed as an Endangered Species IA (CR) in the Red List 2020 of the MOE. This species grows on forest edges and easily disappears due to urban development, such as road improvements. This species may be potentially used as a liquor resource; in addition, it possesses edible fruit such as those of other wild species of the genus *Vitis* (Mochioka 1996).

FK29 was found distributed in the forest canopy along a forest road (Figs. 21 and 22). We obtained 17 seeds from 5 fruits (Figs. 23 and 24). The authors (S.Y. and H.I.) believe that FK29 is derived from the same individual as JP251040, collected in 2014 (Iketani *et al.* 2014). Plants that appeared to belong to this species were found in the forest canopy at several sites, but fruit was only available at this site. As this species has difficulty generating adventitious roots (Mochioka *et al.* 2002), we introduced these seeds into the NARO Genebank, while an author (T.S.) is attempting to propagate the genetic resources by grafting with scions onto rootstocks of JP251040, which is now a mature individual (Fig. 25).

We believe that some scientific names in this taxon should be reconsidered. Although some authors have equated V. kiusiana and Vitis heyneana Roemer et Schult with Vitis quinqueangularis Rehder (Chen et al. 2007; Li 1996), we determined that V. quinqueangularis was distinct from both these species. Our claim is based on the specimen examined as V. quinqueangularis by its nomenclator, Rehder, being hairless and having significantly smaller leaves than those of the other two species (https://kiki.huh.harvard.edu/databases/ specimen\_search.php?mode=details&id=128235). While a molecular phylogenetic analysis based on 18 proteincoding gene sequences has been conducted including closely related species, it did not include the Japanese samples (Qin *et al.* 2020). Although the WFO lists as *V. kiusiana* is a synonym of *V. heyneana* (http://www. worldfloraonline.org/taxon/wfo-0001145753), detailed morphological and molecular phylogenetic analyses including the Japanese samples may provide new insights into the taxonomy of this species.

#### The others

Some crop wild relatives and crop landraces were collected during the survey in October. FK30 is a kiwifruit wild relative, "Shima-Sarunashi" (*Actinidia rufa* (Siebold et Zucc.) Planch. ex Miq.), that was found in the immediate vicinity of the habitat occupied by FK29 (*V. kiusiana*). Fruits of this species resembled small kiwifruit (Fig. 26).

FK31 and FK32 are both the citrus landrace "Kunebu" (Citrus nobilis Lour. var. kunep Tanaka), which is a local specialty used to produce various processed products in Itsuki Village (Fig. 27). We collected the fruits at two different farmers' fields in Itsuki Village (Figs. 28-30). "Kunebu" is a possible synonym for the variety "Kunenbo," which may be the pollen parent of satsuma mandarins ("Unshumikan" in Japanese), the predominant cultivated citrus variety in Japan (Fujii et al. 2016). However, further investigation is necessary, as some characteristics such as the spines and leaf-like petiole of FK31 and FK32 are different from those observed in a "Kunenbo" accession (JP117387 in the NARO Genebank). We collected seeds of FK31 and FK32 to compare their resulting plants with JP117387 in the juvenile stage. The NARO Genebank will distribute their vegetative organs after they become adult trees, similarly to other citrus plants.

FK33 and FK34 are a yam wild relative, "Yamanoimo" (*Dioscorea japonica* Thunb.), representing a wild food plant species that is sometimes cultivated in the field. We collected their bulbils at two different sites in Itsuki Village (Figs. 31-33). This species is also called "Jinenjo" in Japan, including this area. Finally, FK35 is a Konnyaku landrace (*Amorphophallus rivieri* Durieu var. *konjac* (K. Koch) Engl.), which was collected at the farmers' field in Minamiaso Village (Fig. 34).

#### **Future prospects**

A notable highlight of this report is the successful *ex-situ* conservation of endangered asparagus wild relatives. Environmental changes have reduced the habitat of coastal plants, including "Hama-Tamaboki (*A. kiusianus*). Moreover, modernization and the loss of traditional practices in the region around the Aso

and Kuju mountains have reduced the habitat of wild plants adapted to grasslands, including "Tamaboki" (*A. oligoclonos*). Thus, as the decline in crop wild relatives is expected to worsen, we will continue *ex-situ* conservation of endangered crop wild relatives as plant genetic resources for food and agriculture. The NARO Genebank will distribute these genetic resources after multiplication for research and educational purposes (https://www.gene.affrc.go.jp/index\_en.php).

#### Author contributions

YT and SY planned the study with input from HA. YT, SY, and HA surveyed the field. YT and SY wrote the manuscript with input from HA (general), TS (wild grape), and HI (taxonomy).

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## 福岡県, 熊本県, 大分県における

# アスパラガス属,ブドウ属,ミカン属,ヤマノイモ属, マタタビ属,コンニャク属の作物近縁野生種および在来品種の 生息域外保全

## 高橋 有<sup>1)</sup>·有賀 裕剛<sup>1)</sup>·山本 伸一<sup>1)</sup>·清水 健雄<sup>2)\*</sup>·池谷 祐幸<sup>3)</sup>

1) 国立研究開発法人 農業・食品産業技術総合研究機構 (農研機構) 遺伝資源研究センター

2) 農研機構 果樹茶業研究部門 果樹品種育成研究領域

3) 岡山理科大学 生物地球学部 生物地球学科

\* 現所属: 農研機構 植物防疫研究部門 果樹茶病害虫防除研究領域

#### 和文摘要

本報告は福岡県, 熊本県, 大分県における作物近縁野生種および在来品種の保全に関する報告書である. 我々 は, 熊本県, 福岡県, 大分県の自然環境または人為攪乱環境を調査し, アスパラガス近縁野生種 (*Asparagus kiusianus* Makino, *Asparagus oligoclonos* Maxim, *Asparagus cochinchinensis* (Lour.) Merr.), ブドウ近縁野生種 (*Vitis kiusiana* Momiy.), キウイフルーツ近縁野生種 (*Actinidia rufa* (Siebold et Zucc.) Planch. ex Miq.), ヤマノイモ近 縁野生種 (*Dioscorea japonica* Thunb.) に加え, ミカン在来品種 (*Citrus nobilis* Lour. var. *kunep* Tanaka) とコン ニャク在来品種 (*Amorphophallus ribieri* Durieu var. *konjac* (K. Koch) Engl.) を収集した. 本報告の特筆事項は, 絶滅危惧種であるハマタマボウキとタマボウキの生息域外保全に成功したことにある. 農研機構のジーンバ ンクは, これらの種子を十分に増殖後, その一部を研究または教育を目的とする利用のために配布する予定 である (https://www.gene.affrc.go.jp/index\_en.php).

	assport data of the collections	1	r	1		r
ID, Accession number	Scientific name, "Japanese name"	Statas, Collected tissues, Sampling method	Collection date in 2022	Latitude, Longitude, Altitude (m)*	Collection site	Topography Soil type
FK01, JP286623	Asparagus kiusianus Makino, "Hama-Tamaboki"	Wild, Seeds, Population	17-Oct	Confidential,	Itoshima City, Fukuoka	Plain, Sand
FK05.5, JP286625	Asparagus oligoclonos Maxim., "Tamaboki"	Wild, Plants, Population	19-Oct	Confidential, Confidential, 350	Minamiaso Village, Kumamoto	Mountains, Organic soil
FK24, JP286626	Asparagus cochinchinensis (Lour.) Merr., "Kusasugi-Kazura"	Wild, Seeds, Population	30-May	32.24745, 131.5748, 10	Tsuno Twon, Miyazaki	Plain, Sand
FK29, JP288575	Vitis kiusiana Momiy., "Kumagawa-Budo"	Wild, Seeds, Individual	18-Oct	Confidential, Confidential, 504	Sagara Village, Kumamoto	Mountains, Organic soil
FK30, JP288576	Actinidia rufa (Siebold et Zucc.) Planch. ex Miq., "Shima-Sarunashi"	Wild, Seeds, Population	18-Oct	Confidential, Confidential, 510	Sagara Village, Kumamoto	Mountains, Organic soil
FK31, JP288577	<i>Citrus nobilis</i> Lour. var. kunep Tanaka "Kunebu"	Cultivated, Seeds, Individual	18-Oct	32.35092, 130.8514, 519	Itsuki Village, Kumamoto	Mountains, Organic soil
FK32, JP288578	<i>Citrus nobilis</i> Lour. var. kunep Tanaka "Kunebu"	Cultivated, Seeds, Individual	18-Oct	32.35756, 130.8535, 614	Itsuki Village, Kumamoto	Mountains, Organic soil
FK33, JP288579	<i>Dioscorea japonica</i> Thunb. "Yamanoimo"	Cultivated, Bulbils, Individual	18-Oct	32.35375, 130.8528, 504	Itsuki Village, Kumamoto	Mountains, Organic soil
FK34, JP288580	<i>Dioscorea japonica</i> Thunb. "Yamanoimo"	Wild, Bulbils, Individual	18-Oct	32.40067, 130.8251, 269	Itsuki Village, Kumamoto	Mountains, Organic soil
FK35, JP288581	Amorphophallus rivieri Durieu var. konjac (K. Koch) Engl., "Konnyaku"	Cultivated, Tubers, Individual	18-Oct	Confidential, Confidential, 350	Minamiaso Village, Kumamoto	Mountains, Organic soil

Table 2. Passport data of the collections

\*The latitude and longitude on the habitats of endangered species is confidential in this paper.



1. Plant of Asparagus schoberioides. Fig.



Fig. 3. Plant of Asparagus kiusianus (FK01).



Fig. 5. Plants with immature fruits of Asparagus kiusianus (FK01).



Fig. 7. Mature fruits of Asparagus kiusianus (FK01).



Fig. 2. Habitat of Asparagus kiusianus (FK01).



Fig. 4. Staminate flower of *Asparagus kiusianus* (FK01).



Fig. 6. Plants with mature fruits of Asparagus kiusianus (FK01).





Fig. 9. Habitat of *Aaparagus kiusianus* eroded by high tides.



Fig. 11. Habitat of Aaparagus oligoclonos.



Fig. 13. Fruits and seeds of *Aaparagus oligoclonos*.



Fig. 15. Cultivation of asparagus in Minamiaso Village.



Fig. 10. Cultivated plant of *Aaparagus kiusianus* in the NARO Genebank.



Fig. 12. Plant of Aaparagus oligoclonos.



Fig. 14. Stems of *Asparagus oligoclonos* showing disease symptoms.



Fig. 16. Shoot of Asparagus oligoclonos.



Fig. 17. Cultivated plant of *Aaparagus oligoclonos* in the NARO Genebank.



Fig. 19. Cultivated plants with immature fruits of *Aaparagus cochinchinensis* (FK24).



Fig. 21. Habitat of Vitis kiusiana (FK29).



Fig. 23. Plant with mature fruits of *Vitis kiusiana* (FK29).



Fig. 18. Cultivated plant of *Aaparagus* cochinchinensis (FK24).



Fig. 20. Seeds of Aaparagus cochinchinensis (FK24).



Fig. 22. Leaves of Vitis kiusiana (FK29).



Fig. 24. Fruits of Vitis kiusiana (FK29).



Fig. 25. Cultivated plant of Vitis kiusiana (JP251040).



Fig. 27. Examples of "Kunebu" processed products.



Fig. 29. Fruits of Citrus nobilis var. kunep (FK31).



Fig. 31. Plant of Dioscorea japonica (FK33).



Fig. 26. Fruits of Actinidia rufa (FK30).



Fig. 28. Tree of Citrus nobilis var. kunep (FK31).



Fig. 30. Fruits of Citrus nobilis var. kunep (FK32).



Fig. 32. Bulbil of Dioscorea japonica (FK33).



Fig. 33. Plant and bulbils of *Dioscorea japonica* (FK34).



Fig. 34. Tubers of Amorphophallus rivieri var. konjac (FK35).