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石垣島におけるマメ類および共生微生物遺伝資源多様性の現地調査と保全

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Ecological Survey and Conservation of Legumes - symbiotic Rhizobia Genetic Diversity from Ishigaki Island, Okinawa, Japan, 2004

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Summary

A field survey was conducted for the conservation of legumes and symbiotic rhizobia genetic diversity on Ishigaki island, Okinawa prefecture, Japan from September 28 to 30, 2004. A total of 23 nodule samples from 16 legume species were collected. Nine nodule samples were collected mainly from the cultigens (pigeon pea, lima bean, cowpea, hyacinth bean, creole bean and winged bean) cultivated in an experimental field of Okinawa Subtropical station, JIRCAS. Fourteen nodule samples were collected from wild legumes (*Vigna marina*, *V. riukiuensis*, *Macroptilium atropurpureim*, *Canavalia lineata*, *Glycine koidzumii*, *V. luteola* and *V. reflexo-pilosa*) growing in natural habitats.

Introduction

Most legume species form root nodules which can fix atmospheric nitrogen. Root nodules

are formed by soil bacteria called rhizobium. Among nodule forming bacteria, those in the genus *Bradyrhizobium* form nodules on soybean roots and is the target bacteria taxa in the present survey. The genus *Bradyrhizobium* currently consists of six species, *B. japonicum*, *B. elkanii*, *B. liaoningense*, *B. betae, B.yuanmingense* and *B. canariense*. Recently many strains of root nodulating bacteria have been collected from wild *Vigna* plants in Thailand. These bacteria belong to the genus *Bradyrhizobium* and have novel and diverse *nod* gene RFLP types (Yokoyama *et al*., 2005). These *Vigna Bradyrhizobium* strains produce diverse *Nod* factor (signal compound produced by bacteria to initiate nodule formation) and can form nodules on soybean. Wild *Vigna* plants are adapted to various environmental conditions such as in dry sandy soils, marsh land or lime stone areas in Thailand (Tomooka *et al*., 2002). Therefore, it is expected that these *Bradyrhizobium* strains are also adapted to various environmental conditions and can be gene resources for improving the soybean symbiotic system. The objective of the present survey was to collect and conserve legumes (especially wild *Vigna*) and nodule forming bacteria from varied environments on the subtropical island of Ishigaki, Japan.

Methods

The field survey was conducted on Ishigaki island, Okinawa, Japan from September 28 to 30, 2004. After the collection in the experimental field at JIRCAS, we conducted a survey across Ishigaki island and collected root nodules and legume seeds if available.

Results

A total of 23 root nodule samples were collected from 16 legume species (Table 1). The locality of sampling sites are shown (Fig. 1).

Sampling at JIRCAS experimental field

Nine nodule samples (I - 1 ～ I - 9) were collected in the experimental field of Okinawa Subtropical Station, JIRCAS. The soil was sandy. Nodules collected from cultigens include those formed on pigeon pea (*Cajanus cajan*), lima bean (*Phaseolus lunatus*), cowpea (*Vigna unguiculata*), hyacinth bean (*Lablab purpureus*), creole bean (*Vigna reflexo-pilosa* var. *glabra*) and winged bean (*Psophocarpus tetragonolobus*). Sword bean (*Canavalia gladiata*) was also cultivated but no nodules was found on it. Nodules formed on wild legumes, *Vigna trilobata*, *Vigna tenuicaulis* and wild rice bean (*Vigna umbellata*) were also collected.

Sampling in natural habitats

Vigna marina grows on sandy beaches. Nodules were collected at Ohama (I-10), Fusaki (I-12) and Ibaruma (I-16) (Fig. 1). *V. marina* can form large populations. Plants have long lateral branches and roots are developed from lateral branches. Many large nodules were formed along spreading branches on the roots(Fig. 2: picture).

Nodule samples were collected from *Vigna riukiuensis* at four locations, Tojin baka (I-11), Uganzaki (I-15), Hirakubozaki (I-19) and Banna park (I-22). At Tojin baka and Banna park, *V. riukiuensis* is growing in the grass that is frequently cut. Uganzaki and Hirakubozaki are headlands with lighthouses on the cliff. Both places are sightseeing spots. At Uganzaki, a large population of *V. riukiuensis* grows in the grass that is periodically cut by workers keeping the place clean. At Hirakubozaki, several smaller-sized populations were growing on cliff top

and surrounding areas used for pasture. The habitat is sometimes affected by strong winds containing sea salt water particularly during typhoons. A population of *Glycine koidzumii* (I-19) was found at Hirakubozaki. *Glycine koidzumii* plants grow in pasture grass. The plants show a perennial growth habit with a thick tap root system. It was very difficult to find nodules from

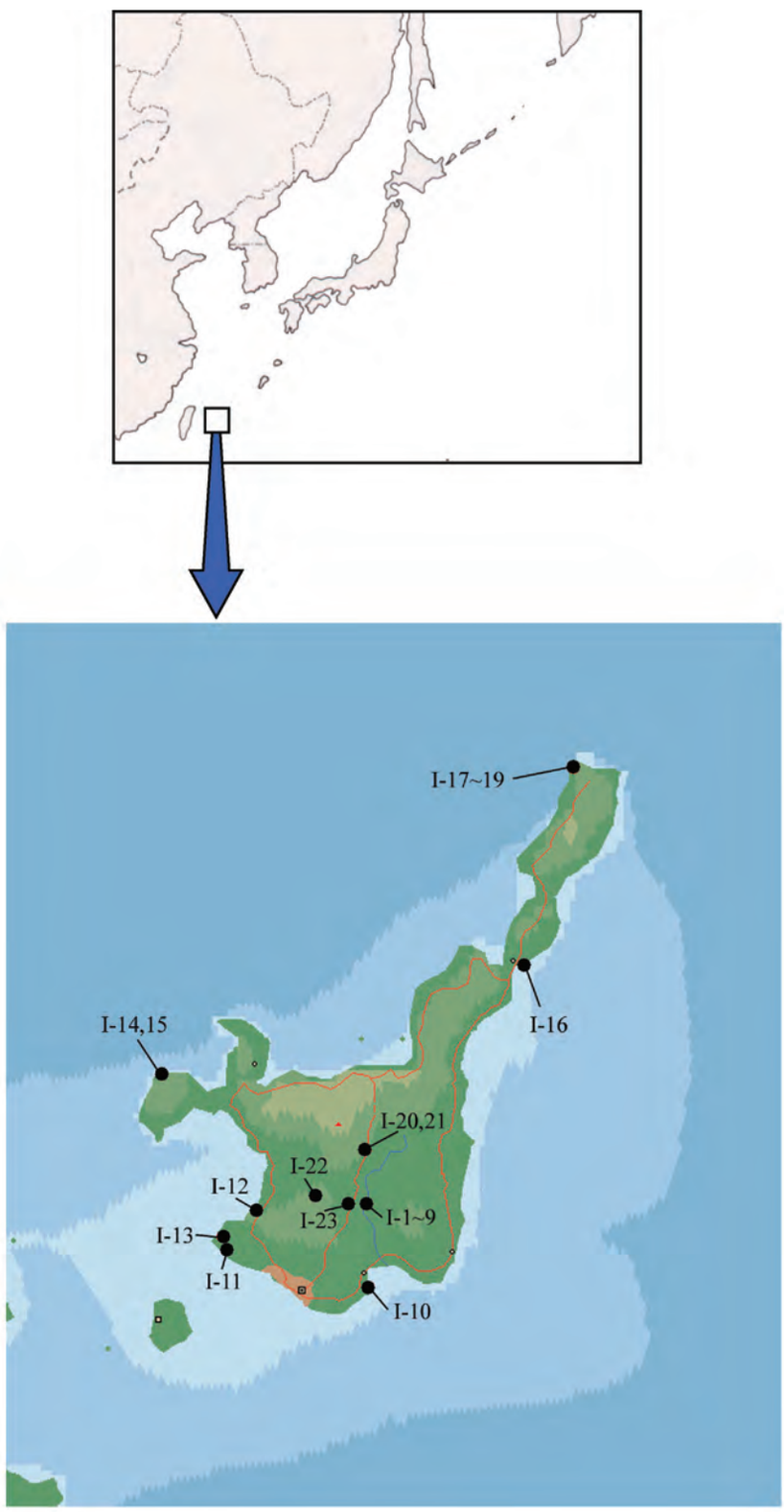

Fig.1. A map of collection sites on Ishigaki island, Okinawa prefecture, Japan.

this species.

Vigna reflexo-pilosa grows in inland sites. The collection site at Omoto (I-21) was wet and soil was muddy. At this site *Vigna luteola* also formed a large population (I-21). *Vigna reflexo-pilosa* also grew in a ruderal habitat, for example beside upland fields (I-23).

Discussion

Improvement of the soybean symbiotic system using elite rhizobium strains with higher nitrogen fixing ability has been conducted primarily based on the genetic variation of *Bradyrhizobium japonicum* genetic resources collected from cultivated soybean grown in temperate regions. Improvement of nitrogen fixing efficiency of soybean using inoculants produced with elite *Bradyrhizobium japonicum* strains frequently fail in tropical countries. In many cases, unexpected endemic rhizobium strains forms nodules instead of inoculated elite bacteria. This suggests that nodulation ability is dependent on specific soil ecological conditions.

Nodulation is a process of complex interaction between legumes and rhizobia. Roots of legume species secrete specific kinds of flavonoid compounds in the soil. Some rhizobia respond to this chemical signal and the *nod* genes are activated. *Nod* factors are chemical products of the *nod* genes and recognized by receptors present on the surface of legume roots if they are compatible. *Nod* genes in rhizobium and *Nod factors receptor* (*NFR*) genes in legume plants are the first key genes for the symbiotic nodulation process.

In the present survey, root nodules from various legume species in different ecological locations were collected. There were variations in size, shape and number of nodules. It may be significant that *Vigna marina* grows in highly salt affected beach habitat and forms many root nodules. *Vigna riukiuensis* growing at a cliff with strong salty wind also forms many root nodules. We plan to study the genetic diversity in nod gene region of newly collected strains of rhizobium. The taxonomic treatment will be studied by the sequence of 16S-rRNA gene. Physiological characterization of bacteria especially the response to salt concentration will also be studied.

Acknowledgements

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References

1)Tomooka N., D.A. Vaughan, H. Moss and M. Maxted (2002) The Asian *Vigna*: genus *Vigna* subgenus *Ceratotropis* genetic resources. Kluwer Academic Publishers. 270 pages.

2)Yokoyama T., N. Tomooka, M. Okabayashi, A. Kaga, N. Boonkerd and D.A. Vaughan (2005) Variation in the *nod* gene RFLPs, nucleotide sequence of 16S-rRNA gene, Nod factors and nodulation abilityes of *Bradyrhizobium* strains isolated from Thai *Vigna* plants. Canadian J Microbiology (accepted).

和文摘要

石垣島におけるマメ類および共生微生物遺伝資源多様性の現地調査と保全

作物栽培環境（農耕地）には見られないような多様な自生地（限界環境）に適応し生息する作物近縁野生種に着生する根粒菌が，農耕地の栽培種に着生する菌とどの程度異なるのかを共生関連遺伝子（根粒形成遺伝子）や種分類マーカーに着目して明らかにすることを目的に，多様な限界環境に自生する作物近縁野生種が豊富に分布している沖縄県石垣島を対象として現地調査を行った。

今回の調査によって，合計 23 サンプルの根粒を 16 種のマメ科植物から採集した。農耕地環境からのサンプルとしては，国際農林水産業研究センター沖縄支所の圃場に栽培されていた 9 種のマメ科植物から 9 サンプルの根粒採集を行った。自生地（限界環境）からのサンプルとしては，7 種のマメ科野生植物から 14 サンプルの根粒採集を行った。

根粒の形態や着生の程度には，宿主による大きな変異が見られた。野生植物には，栽培種が生育できない限界環境に自生している種がある。本調査で特に興味深かったのは，海水を頻繁にかぶるような砂浜に自生しているハマアズキや海崖の塩風が強く吹き抜けるような風衝草原に自生するヒナアズキに多数の根粒が着生していたことである。今後このような自生地に生息する根粒菌の遺伝的，生理的特性を中心に研究を進めていきたい。

Table 1. A list of nodule samples and their host legume species

No.	Collection Site	Environment	Host legume species
Sampling at the JIRCAS experimental field			
I-1	Maesato	sandy soil	*Cajanus cajan*
I-2	〃	〃	*Phaseolus lunatus*
I-3	〃	〃	*Vigna unguiculata*
I-4	〃	〃	*Vigna umbellata*
I-5	〃	〃	*Lablab purpureus*
I-6	〃	〃	*Vigna trilobata*
I-7	〃	〃	*Vigna reflexo-pilosa* var. *glabra*
I-8	〃	〃	*Vigna tenuicaulis* or *V. hirtella*
I-9	〃	〃	*Psophocarpus tetragonolobus*
Sampling in natural habitats			
I-10	Ohama	sandy beach	*Vigna marina*
I-11	Tojin baka	grass with frequent cutting	*Vigna riukiuensis*
I-12	Husaki	sandy beach	*Vigna marina*
I-13	〃	ruderal area beside road	*Macroptilium atropurpureum*
I-14	Uganzaki	grass on cliff	*Canavalia lineata*
I-15	〃	〃	*Vigna riukiuensis*
I-16	Ibaruma	sandy beach	*Vigna marina*
I-17	Hirakubozaki	pasture near cliff	*Glycine koidzumii*
I-18	〃	〃	*Desmodium sp.*
I-19	〃	〃	*Vigna riukiuensis*
I-20	Omoto	wet land	*Vigna luteola*
I-21	〃	〃	*Vigna reflexo-pilosa*
I-22	Banna	grass with frequent cutting	*Vigna riukiuensis*
I-23	Maesato	ruderal area beside road	*Vigna reflexo-pilosa*

Sampling at JIRCAS experimental field

Nine nodule samples were collected from 9 legume species here

Sampling in natural habitats

A beach habitat of *V. marina* (Fusaki) and nodules formed

Natural habitats of *V. riukiuensis* : windy cliffs at Uganzaki (left) and Hirakubosaki (right)