（第３号様式）(Form No. 3)

学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Symbiotic nitrogen fixation regulatory mechanism in *Lotus japonicus* and *Clitoria ternatea*
（マメ科植物ミヤコグサおよびチョウマメにおける共生窒素固定制御機構）

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Leguminous plants have ability to form symbiotic relation with soil bacteria, rhizobia which able to fix atmospheric nitrogen into ammonia. Therefore, legume is considered to be agricultural and environmental important as soil quality improvement.

Lotus japonicus has been considered as one of the model leguminous plant which from small genome size, diploidgenome and a short life cycle (about 2 to 3 months), providing essential information for nodulation and nitrogen fixation. To fix nitrogen, many irons are for legume-rhizobium symbiosis. During nodule senescence, free iron is released from proteins such as nitrogenase, leghemoglobin, which lead to cell damage. Microarray data indicated that ferritin accumulates during the nodule aging because ferritin is considered to be an iron storage protein. Free iron concentration has to be strictly controlled to avoid toxicity. We have studied on ferritin gene expression of *Ljfer1*, which was induced in *Lotus japonicus* during nodule senescence. The *Ljfer1* promoter contains a conserved Iron-Dependent Regulatory Sequence (IDRS). The *Ljfer1* expression was induced after application of iron or sodium nitroprusside, which is a nitric oxide (NO) donor. The iron application to the medium leads to NO accumulation in the nodule. These data indicate that free iron- induced *Ljfer1* expression through the NO signal in the nodule. To know the function of ferritin in the nodule, we produced two transgenic plants, overexpression (FerOx) and suppression (FerRNAi). Acetylene reduction activity (ARA) in FerOX showed increased whereas ARA in FerRNAi showed

increased until 2 wpi, and slightly decreased until nodule senescence (8wpi). These data suggest that ferritin might maintain the ARA activity at the senescence nodule.

Next we focused on *Clitoria ternatea,* which is a local legume in Thailand. This has been called as blue pea because of the color of flower. The blue colored-flower has been used in many foods and drinks. It is recently found to be important in drug because of its alternative production of cyclotides, which have anti-HIV or uterotonic activity. However, there is no report for the bacteria isolation from nodule. To know the rhizobia of *C. ternatea,* we have isolated bacteria and identified. The phylogeny of the isolates was investigated using 16S rDNA and the internal transcribed spacer (ITS) region between 16S to 23S rDNA. The phylogenetic tree of the 16S rDNA reveals that most isolates belonged to *Bradyrhizobium elkanii,* and one belonged to *Bradyrhizobium japonicum*. The topology of the ITS tree was similar to that of 16S rDNA. The acetylene reduction activity of nodules inoculated with the isolated *B. elkanii* strains was higher than those inoculated with *B. japonicum*. From these data we propose that effective rhizobia inoculant were identified for *C. ternatea* cultivation.

In conclusion, this study is beneficial in understanding mechanism of symbiotic nitrogen fixation in *Lotus japonicus* and *Clitoria ternatea,* which will be useful for further study on extend nitrogen fixing period and develop crop agriculture quality.