（第３号様式）(Form No. 3)

学 位 論 文 要 旨
Dissertation Abstract

氏名： Sirinapa Chungopast
Name

学位論文題目：
Title of Dissertation

Functional analysis of nodule senescence in *Lotus japonicus* inoculated with *Mesorhizobium loti*.
ミヤコグサ根粒菌感染による根粒老化の機能解析

学位論文要旨：
Dissertation Abstract

The senescence of plant is part of the growth and development of plants that need to happen in their life cycle. Genetic changes control a plant or plant tissue caused by senescence condition influencing physiological and morphological changes. The senescence is motivated by a mechanism that is not yet completely understood in *Lotus japonicus* nodule including complexity of the signs of hormonal and environmental factors that involved. Here, we used a signature-tagged mutant of *Mesorhizobium loti* (STM30) with a transposon inserted into the glutamine synthetase I (mll0343) gene which associated with the induction of early nodule senescence. Transcriptomic study was perfomed to explore senescence associated genes of *L. japonicus* nodule by the agilent 4×44 microarray chip comparing 2, 4 and 8 weeks post inoculation (wpi) of wild type (WT). Dehydrate nodules were used analysis of metabolome. In addition, we focused ferritin gene which may involve iron homeostasis in the senescent nodule.

In the deficiency of GSI mutant, STM30, inoculation, senescence symptoms, such as, increasing of green nodules and dead bacteria, and decreasing of nitrogen fixing activity in the nodules and chlorophyll content in the leaves, were found. The concentration of glutamine and glutamic acid in the STM30 inoculated nodules reduced 43.9 and 54% respectively along with nitrogen content lower. However, there is no difference between carbon content of the wild-type-and STM30–infected nodules. Notably, nitric oxide accumulation increased around 40% in the STM30-infected nodule. A cystein protease gene expression in the STM30-infected nodule was high at 4wpi. The deficiency of GSI in bacteroid had influence indirectly nitrogen fixation decrease by 40% and led to an early

nodule senescence.

To comprehensively elucidate the senescence-induced gens in the nodules, microarray analysis was performed. Overlying expression data showed 641 up-regulated genes and 416 down-regulated genes out of 20,165 genes during nodule senescence. Profiling of global gene expression revealed four clusters. Prediction of gene function in the euKaryotic orthologous groups (KOG) database point out that 4.45% of up-regulated genes correlated to cell wall/membrane/ envelope biogenesis or 6.02% of that related to extracellular structures and the notably 2.77% of down-regulated genes responsible for defense mechanisms. Metabolome data revealed level metabolic compounds during early nodule senescence of the mutant inoculation.

From micraoarray data, ferritin gene was induced during nodule senescence. Multi element analysis by ICP-MS showed that iron and free iron concentration of the residues and cell sap in the senescent nodules were temporary increased then decreased at 8 wpi because this legume accumulated ferritin. Then the ferritin transfer to young or reproductive tissue such as shoot and seed. Ferritin gene of *L. japonicus* conserved iron-dependent regulatory sequence (IDRS) in a promoter region at position from -237 to -224 bp. Moreover, nitric oxide (NO) was increased in the senescent nodules by iron-citrate treatment and expression of ferritin was increased by NO donor, sodium nitroprusside treatment. These data suggest that accumulation of iron motivated NO for the expression of ferritin. Ferritin accumulation in *L. japonicus* nodule plays a role for iron homeostasis.

To conclude, this study is valuable in understanding the biology of nodule senescence in legume plants. These data is useful in the further study of genes that help keep nitrogen fixation longer in order to the quality of crop agriculture.