ABSTRACTS (MASTER THESIS)

Analysis of two transporters, LjSWEET4 and LjALMT, in nodules of *Lotus japonicus*

**(Graduate School of Agriculture,
Laboratory of Plant Gene Expression, RISH, Kyoto University)**

Yuka Saida

Symbiotic nitrogen fixation in legume plants takes place in nodules, specialized organs formed in roots. In infected cells of nodules, *Rhizobium* exists in forms of bacteroids that are capable of reducing atmospheric N_2 to NH_3, thereby supplying the fixed nitrogen to the host plant. In turn, host legume plant cells provide photosynthetic metabolites mainly in forms of dicarboxylates. Inorganic compounds are also required for the function of bacteroid and are transported across the membrane in nodule. In this process, various types of transporters should be involved at different membrane systems in nodules; however, at the molecular level there remain little insights on the flow of carbon source from the plant cells to the symbiotic bacteria. In this study, I analyzed a putative sugar transporter expressed in nodules of *Lotus japonicus* in order to characterize the molecular mechanism of carbon source transport to bacteroids. To identify the genes possibly involved in sugar transporter in nodules, we focused on the gene homologs of a recently identified sugar transporter family (AtSWEET) in *Arabidopsis* and an aluminum-activated malate transporter (ALMT) family member. In this abstract, results on LjSWEET is described.

AtSWEET1 was shown to be an efflux transporter of monosaccharides such as glucose. In BLAST search on genomic database of *L. japonicus*, at least 13 homologs of *AtSWEET* exist in the genome of *L. japonicas*. We performed semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) with cDNA samples of the roots, nodules and leaves. It was found that only *LjSWEET4* is highly expressed in the nodule.

Real-time quantitative PCR analysis revealed that the *LjSWEET4* expression level in the nodule was approximately 10 and 3 times higher than those of the leaves and the root tissue, respectively. We also performed time course expression analysis of *LjSWEET4* in the underground plants with real-time PCR. It was revealed that the expression of *LjSWEET4* slowly increased after infection of *Mesorhizobium loti* up to 3 weeks of infection.

We also investigated the cell-type specificity of the *LjSWEET4* expression using *L. japonicus* nodules transformed with β-glucuronidase (GUS) reporter gene under the control of *LjSWEET4* promoter, i.e., 2 kb upstream genomic region of *LjSWEET4*. Whole mount analysis of the transgenic plants showed strong and specific activity of GUS in the vascular systems both in roots and nodules. LjSWEET4-GFP fusion protein expressed in *Coptis japonica* protoplasts showed that LjSWEET4 was located at the plasma membrane. These results suggest that LjSWEET4 functions as a transporter in the movement of metabolites from the root to the infected zone of nodules.