
ABSTRACTS (MASTER THESIS)

**Analysis of two MATE-type transporters expressing in *Lotus japonicus*
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Nitrogen is an essential nutrient which circulates in the ecosystem by changing its chemical form. Rhizobia are soil-born bacteria that form nodules on the roots of leguminous plants such as soybean and *Lotus japonicus*. These symbiotic bacteria are involved in symbiotic nitrogen fixation which fixes atmospheric N₂. Upon the infection of rhizobia into legume roots, legume-*Rhizobium* symbioses is established, and in mature nodules many metabolites such as carbon source and amino acids and inorganic ions are transported between rhizobia and host plants; however, most of transporters involved in this process remain to be clarified.

In order to analyze transporter genes expressed in nodule comprehensively, LM (laser micro-dissection) coupled with microarray analysis was performed, where we found that yet uncharacterized Multidrug and toxic compound extrusion (MATE) transporter, LjMATE2 (chr2.LjT36E17.20.r2.d) and LjMATE3 (chr2.CM0008.1100.r2.d) belonging to were preferentially expressed in outer parts of nodule (uninfected cells).

MATE type transporter was first identified in bacteria as a transporter exporting broad range of xenobiotics. MATE type transporters have in general 9-12 putative transmembrane regions, and use electrochemical gradient to transport substrates in plant cells. Many plant MATE transporters identified to date show high substrate specificity for particular endogenous compounds such as alkaloids, flavonoids and citric acids. In this study, I have analyzed the expression profiles of both *LjMATE2* and *LjMATE3* to elucidate their functions in the mature nodules.

In order to analyze the tissue specific expression of *LjMATE2* and *LjMATE3*, reverse transcription polymerase chain reaction (RT-PCR) analysis was performed using cDNA from flowers, leaves, anthocyanin-accumulating stems, uninfected roots, infected roots and nodules of wild type *Lotus japonicus* (Gifu), as well as the stems of wild type *L. japonicus* (Miyakojima) for comparison between anthocyanin accumulation in stems. The expression of *LjMATE2* was high in anthocyanin-accumulating stems, whereas *LjMATE3* expression was detected throughout the plant including nodules. *LjMATE2* was also shown to be localized at the vacuolar membrane in plant cells, using the analysis of GFP-tagged protein.

To analyze the expression of *LjMATE3* in nodule more in detail, quantitative real-time PCR was also performed. The real-time PCR analysis revealed that *LjMATE3* expression was increased during the nodulation, and the expression level of *LjMATE3* in 26 day-old nodules was approximately five times higher than that of roots before infection. The expression of *LjMATE3* was also induced by glutathione treatment in the similar pattern as genes involved in flavonoid synthesis. Promoter: GUS transformants of *Lotus japonicus* showed that the expression of *LjMATE3* in mature nodules was restricted to fibro vascular bundle of nodules.

From these analyses, it was suggested that *LjMATE2* functions as anthocyanin transporter mainly in the stem, while *LjMATE3* function could be a transporter involved in flavonoid accumulation in nodules.