
 ABSTRACTS (MASTER THESIS)

Biochemical analysis of flavonoid secretion in soybean
(Graduate School of Agriculture,
Laboratory of Plant Gene Expression, RISH, Kyoto University)

Kazuaki Yamashita

Legume plants such as soybean (*Glycine max*), pea (*Pisum sativum*) and kidney bean (*Phaseolus vulgaris*) are cultivated as important grains in 12-15% agricultural land in the world. Legume plants establish symbiosis with soil bacteria collectively called rhizobium to fix the atmospheric nitrogen. The first event of this symbiotic process is the secretion of signaling molecules (e.g., flavonoids) from roots, which activate the transcription factor, nodD, of rhizobium leading to the nodule formation. Genistein, daidzein, and coumestrol have been so far identified as signaling molecules in soybean. The transport activity of isoflavonoid aglycon (e.g. genistein) has been analyzed using membrane vesicles of soybean roots, and ATP-dependent isoflavone-specific transport has been reported (Sugiyama et al., 2007).

Root exudates of soybean also contain flavonoid glycosides such as malonyldaidzin and malonylgenistin, whereas β -glucosidase for flavonoid malonyl glycoside occurs at apoplast, suggesting the existence of efflux mechanisms for flavonoid malonyl glycoside at plasma membrane of soybean roots. However, no transporter genes mediating the secretion of flavonoids, regardless of aglycons or glycosides, have been identified thus far. The aim of this study is the identification of flavonoid transporters in soybean roots in order to gain the molecular mechanism of signal molecule movements on the onset of symbiosis.

Analysis of root exudates during soybean growth was carried out, in which hydroponic medium was subjected to high performance liquid chromatography (HPLC) analysis. A large amount of flavonoid derivatives are highly secreted to the medium under the -N condition, in particular daidzein and 6''-O-malonyldaidzin were detected as the main flavonoids, in which daidzein decreased and 6''-O-malonyldaidzin increased during the growth of soybean.

To understand the transport mechanisms of 6''-O-malonyldaidzin in soybean roots, transport activity measurement using plasma membrane vesicle and vacuole membrane vesicle from soybean root were performed, but no activities were found. These studies suggest that yet uncharacterized transport mechanisms may function in the secretion of malonylated flavonoids.

References

- [1] Sugiyama, A., Shitan, N. and Yazaki, K., "Involvement of a soybean ATP-binding cassette-type transporter in the secretion of genistein, a signal flavonoid in legume-Rhizobium symbiosis.", *Plant Physiology*, 144., 2000-2008, 2007.