ABSTRACTS (MASTER THESIS)

Analysis of shikonins in the rhizosphere of *Lithospermum erythrorhizon*

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

Ko Sato

The rhizosphere is a small region around root defined as "region affected by plant roots", and plant metabolites are one of the major effects from plants to the rhizosphere soil. To define the rhizosphere area in soil, the dynamics of these plant metabolites are important, but it is difficult to analyze the dynamics of these plant metabolites in soil. Thus, defining the spatiotemporal distribution of plant metabolites in the rhizosphere is a crucial step to understand the rhizosphere. In order to analyze the metabolites in the rhizosphere, visualization is a good indicator for the detailed information on the dynamics of metabolites. Visualization is expected to lead to the elucidation of the mechanisms of various rhizosphere interactions in soil. So far, methods to visualize the molecules have been established to clarify the localization of minerals and metabolites in the rhizosphere using gels containing adsorbents, radioisotopes, fluorescent proteins, and computer tomography scans. On the other hand, there remain difficult for the application of these methods to analyze the dynamics of the metabolites in long period, mostly due to the limitation of observation time, soil condition, and scale of observation system. In this study, we analyzed the dynamics of plant metabolites in the rhizosphere using shikonins, which are colored purple. Shikonin is produced in *Lithospermum erythrorhizon*, and have high biological activities and are involved in interactions between organisms in the rhizosphere.

In order to observe the secretion of shikonins in *L. erythrorhizon*, plants grown in a pot were transferred to the root box containing Toyoura sand. These plants were cultivated in a dark room to prevent the inhibition of the shikonin biosynthesis, and the root growth was observed by time-lapse imaging. In order to analyze the distribution of shikonins in the soil, roots and soils in pots were fractionated into root, root surface soil, rhizosphere soil, and root zone soil by the water fractionation method using phosphate buffer, following the established protocol. The amount of shikonins in these samples was analyzed using high pressure liquid chromatography. In order to analyze the transfer of shikonins to the water phase in the soil fraction, the shikonins transferred to the water phase were quantified as well. It was shown that an average of 5.9% was transferred to the aquatic fraction when the rhizosphere soil was fractionated using phosphate buffer, and an average of 2.6% was transferred to the root surface soil when the rhizosphere soil was fractionated. It was shown that the phosphate buffer fractionation method is applicable to soil fractionation in the rhizosphere of *L. erythrorhizon*. It was shown that most of the shikonins in pots were localized in the root extract and root surface.