ABSTRACTS (MASTER THESIS)

Establishment of virus-induced gene silencing method in *Lithospermum erythrorhizon*, a model plant for plant specialized metabolism

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Plants produce a large variety of specialized metabolites, which appear non-essential for plant life, while humans utilize many of those natural organic compounds for human life, such as dye, flavor, spice, functional food, and medicines. Recent years, productions of valuable plant metabolites in microorganisms using plant genes are actively studied as being designated synthetic biology. For achievement of synthetic biology, understanding the complete biosynthetic pathway of interest and genes involved in each biosynthetic reaction step is necessary, while there are only a few examples for such valuable metabolites. This is because the elucidation of biosynthetic pathway of a particular compound is still laborious works, and thus there are many targets to study specialized metabolisms in plants.

An effective approach to identify biosynthetic genes in plants is gene silencing via T-DNA tagging, RNAi, genome editing, etc., which all require the stable transformation method of a target plant. However, there is no ubiquitous method for plant stable transformation, rather researchers should pay large efforts to establish the particular protocol to introduce genes to the plant of interest. It is also known that plant species producing a large amount of specialized metabolites are often difficult to be transformed due to the strong biological activities for their own metabolites. Hence, virus-induced gene silencing technology has a strong merit for the application to analyze the gene function. This is a transient system to shut down the expression of target genes and thus the evaluation of gene functions is achieved in much shorter period than stable transformation-mediated methods.

Lithospermum erythrorhizon is a medicinal plant utilized in many Asian countries as a crude drug and natural dye, as well. The biological active compound produced by this plant is a red naphthoquinone pigment, shikonin, which exists as several ester derivatives in the root bark of this plant. We have an appropriate model system of this plant, i.e., shikonin-producing cell cultures and axenic shoot cultures, in which shikonin production is induced. I this study, we have applied a couple of plant viruses including domestic apple latent spherical virus, which shows relative broad infection spectrum for various plant species and does not exhibit pathogenic symptoms, to *L.erythrorhizon* using LeDI2 gene as a trial. It was already reported that the expression of LeDI2 has strong positive correlation with shikonin production, and the suppression of LeDI2 expression by conventional antisense RNA caused decrease in shikonin production [1]. There are some more experimental steps to improve, but basic methodology has nearly been established.

Reference

[1] Yazaki, K., Matsuoka, H., Shimomura, K., Bechthold, A., and Sato, F. (2001). A novel dark-inducible protein LeDI-2 and its involvement in root-specific secondary metabolism in *Lithospermum erythrorhizon*. *Plant Physiol.*, 125, 1831-1841.