

ABSTRACTS (MASTER THESIS)

***In silico* search of candidate genes involved in shikonin production
in *Lithospermum erythrorhizon*****(Graduate School of Agriculture,
Laboratory of Plant Gene Expression, RISH, Kyoto University)****Hao Li**

Shikonin is a red naphthoquinone pigment produced by some boraginaceae plants. *Lithospermum erythrorhizon* is a representative of those plants, and other limited species belonging to *Arnebia*, *Anchusa*, *Alkanna*, *Echium*, and *Onosma* also produce the red pigment. Shikonin derivatives exist as ester derivatives with low molecular weight fatty acids in the root bark of those plants. The dried roots of *L. erythrorhizon* and other shikonin-containing plants are widely used as crude drugs in the area from Europe to Asian countries, taking the advantage that these naphthoquinone derivatives exhibit a variety of biological activities, such as anti-inflammatory, anti-bacterial, hemostatic activities [1]. Recently, an inhibitory effect on main protease of COVID-19 causing serious pandemic is also reported in shikonin [2]. Shikonin derivatives have also been used as a natural dye to stain cloths for over 2 thousand years to have bright purple color with aluminum-containing mordants. *L. erythrorhizon* is, however, recognized as an endangered species despite the high market needs in the last decades. By those backgrounds, cell suspension culture systems were established, which were applied for the industrial production of shikonin derivatives in 1980s. This success brought an outbreak of plant cell/tissue culture studies in scientific communities, and also in bio-industry sectors at the time.

Biosynthetic rout of shikonin were actively studied by biochemical methods using enzymatic analysis, and also by feeding experiments with radio-labeled compounds from 1970s, as summarized in a review article [1]. The biosynthetic pathway of shikonin has been, however, remained to be clarified yet for over 50 years, in particular the latter half of the pathway, in particular the naphthalene ring formation reaction.

The genome sequence read of *L. erythrorhizon* is deposited in public databases without assembling in 2018. Thus, a draft genome sequence was constructed by use of public softwares, which enabled us to search genes of interest at the genome level. By analyzing the data, it was figured out that some secondary metabolic genes exist in the genome as single exon genes. Taking the shikonin biosynthesis as a model, it was shown that, at least some transferase families, several genes are present in the genome as single exon genes, which are potentially involved in the biosynthesis of shikonin [3].

References

- [1] Yazaki, K., *Lithospermum erythrorhizon* cell cultures: Present and future aspects, *Plant Biotech.*, 34: 131-142 (2017).
- [2] Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, X., You, T., Liu, X., Yang, X., Bai, F., Liu, H., Liu, X., Guddat, L. W., Xu, W., Xiao, G., Qin, C., Shi, Z., Jiang, H., Rao, Z. and Yang, H. (2020) Structure of Mpro from COVID-19 virus and discovery of its inhibitors. *Nature*, in press.
- [3] Kusano, H., Li, H., Minami, H., Kato, Y., Tabata, H., Yazaki, K., Evolutionary developments in plant specialized metabolism, exemplified by two transferase families, *Frontiers Plant Sci.*, 10, Article 794, 2019.