## LABORATORY OF PLANT DEVELOPMENT AND PHYSIOLOGY



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There has been growing evidence that metabolic regulation has specific impacts on plant development. The picture emerging depicts the metabolism as a dynamic system that controls and/or supports developmental progression. Despite these advances, the metabolic regulation behind developmental process remains largely unclear. We aim to uncover as-yet-unknown relationships between developmental and metabolic processes in plants and their biological meaning by elucidating molecular mechanisms for the system. To address this, we primary use molecular genetics and metabolomics approaches using *Arabidopsis thaliana* as a model, in conjunction with standard molecular biology and biochemistry techniques.

## I. Exploring as-yet-unknown relationships between development and metabolism

To explore as-yet-unknown relationships between developmental and metabolic processes, we carried out phenome screening of Arabidopsis thaliana enzyme mutants. We examined more than 11 traits including leaf size, primary root length, seed color, etc. A large number of mutants grew normally compared to wild type, probably due to gene functional redundancy. However, we found that one mutant had a shorter primary root than wild type. Further detailed morphological analysis revealed that this could be attributed to a delay of germination and seedling establishment. Irregular patterning of the cotyledon is also observed in the mutant (Figure 1) although the penetrance was low. Based on these observations, together with the expression profile, we now hypothesize that the enzyme works during seed development to control seed quality, and presumably embryo patterning. To know the in vivo substrate of this enzyme and which metabolic pathway is involved in this developmental process, metabolomics analysis will be conducted.



Figure 1. Irregular patterning of cotyledons in the enzyme mutant. Wild type has two symmetrically-arranged cotyledons (A). In contrast, our enzyme mutant shows asymmetrically-arranged (B and C), fused (D-E) and cup-shaped cotyledons (G). Asterisks indicate cotyledons. Bar = 2 mm.

## II. Characterizing an interaction between development and metabolism

In addition to the phenome screening using enzyme mutants, as another topic, we are now examining coordinated regulation of plant growth and amino acid metabolism. We previously found that one of key factor for plant development regulates the expression of amino acid metabolismrelated genes. Moreover, we uncovered that the mutant for this gene shows hypersensitivity specifically to leucine. Growth arrest is observed when this mutant is grown on culture media containing higher concentrations of leucine. Other amino acids such as histidine did not inhibit the mutant's growth, indicating that there is a specific interaction between the responsible gene and leucine metabolism. To directly know whether the gene indeed regulates leucine metabolism, we have established an amino acid profiling system with high sensitivity and accuracy using liquid chromatography-mass spectrometry (LC-MS) in collaboration with the Functional Genomics Facility in NIBB, and are now examining the amino acid prolife in the mutant. Although the gene is well studied as a key component for plant development, our data shed light on a novel aspect of the gene's function, which could be a key to deciphering how amino acid metabolism is interwired with vigorous plant growth.

## **Publication List:**

[Original paper]

 Nakayama, H., Kawade, K., Tsukaya, H., and Kimura, S. (2015). Detection of the cell proliferation zone in leaves by using EdU. Bioprotocol 5, 18.

[Review articles]

- Hisanaga, T., Kawade, K., and Tsukaya, H. (2015). Compensation: a key to clarifying the organ-level regulation of lateral organ size in plants. J. Exp. Botany 66, 1055-1063.
- Kawade, K., and Tanimoto, H. (2015). Mobility of signaling molecules: The key to deciphering plant organogenesis. J. Plant Res. 128, 17-25.