# Studies on the strigolactone signaling pathway mediated by an α/β-fold hydrolase in *Arabidopsis thaliana*

(シロイヌナズナにおける加水分解酵素を介したストリゴラクトン信号伝達経路に関する研究)

活性分子動態分野
曹 萌萌

Strigolactones (SLs) are plant hormones that play an important role in shoot branching regulation. They also function as root-derived signals for parasitic and symbiotic interactions. The rice DWARF14 (D14; AtD14 in Arabidopsis), an α/β-fold hydrolase family protein, functions as a possible SL receptor. D53 was characterized from a rice SL-insensitive mutant as a repressor of the SL signaling pathway. D53 interacted with D14 in an SL-dependent manner, and was degraded through the 26S proteasomal pathway in an $SCF^{D3}$-dependent manner. The SL signaling repressors in Arabidopsis have not been identified. Therefore, we performed functional analysis of Arabidopsis D53 homologs. This work is presented in Chapter 1. An AtD14 paralog in Arabidopsis called HYPOSENSITIVE TO LIGHT (HTL)/KARRIKIN-INSENSITIVE2 (KAI2) has been identified, but the KAI2 signaling mechanism has not been elucidated. To examine the KAI2 signaling mechanism, I prepared KAI2 active-site mutants and analyzed their functions. These results are presented in Chapter 2.

**Chapter 1: Studies on the SL signaling pathway mediated by an α/β-fold hydrolase in Arabidopsis**

Arabidopsis possesses three *D53* homologous genes called *SMXL6*, *SMXL7*, and *SMXL8*. I tested the interaction between a potential SL receptor, AtD14, and these SMXL proteins by yeast two-hybrid (Y2H) analyses. Yeast cells carrying AtD14-AD and SMXL7-BD had favorable growth in the presence of the SL synthetic analog GR24, suggesting that AtD14 physically interacted with SMXL7 in a GR24-dependent manner (Fig. 1). I found that SMXL6 weakly interacted with AtD14 under mildly selective conditions. To analyze SMXLs function, we generated Arabidopsis *atd14 smxl6*, *atd14 smxl7*, and *atd14 smxl8* double-knockout mutants. Only the *atd14 smxl7* mutant showed partial suppression of the *atd14* shoot branching phenotype. I also generated *smxl6 smxl7* and *smxl6 smxl8* double-knockout mutants, and found that only *smxl6 smxl7* showed a constitutive SL response phenotype. These results demonstrate that SMXL7, and to a lesser extent SMXL6, function as repressors in the Arabidopsis SL signaling pathway.

The α/β-hydrolase family proteins contain a conserved catalytic triad (Ser-His-Asp), and these amino acids are necessary for enzymatic catalysis. To analyze the importance of the AtD14 catalytic triad for interaction with SMXL7, I performed Y2H experiments using point mutant proteins (AtD14:S97A, AtD14:H247A, and AtD14:D218A). Our previous studies showed that these three point mutants drastically reduced the hydrolase activity in *vitro*. AtD14:D218A, but not AtD14:S97A and AtD14:H247A, complemented the *atd14* mutant phenotype. In agreement with the complementation results, Y2H experiments showed that only AtD14:D218A interacted with SMXL7 in the presence of GR24. These results suggest that AtD14 hydrolase activity is not necessary for formation of the AtD14-SMXL7 complex.

I also examined the SL structural requirements for the interaction between AtD14 and SMXL7 using Y2H methods. Previous work showed that the SL (2'*R*) configuration and the enol ether bridge are crucial for shoot branching inhibition in rice and Arabidopsis. In agreement, I found that only the (2'*R*) SL stereoisomers induced the AtD14-SMXL7 and D14-D53 interactions (Fig. 2). The derivative 3,6'-dihydroGR24, in which the enol ether double bond is replaced by a single bond, did not induce the AtD14-SMXL7 and D14-D53 interactions. These results reveal good correlation between the biological activities of tested compounds and the important stereostructural characteristics of SLs for inducing formation of the potential receptor complex.

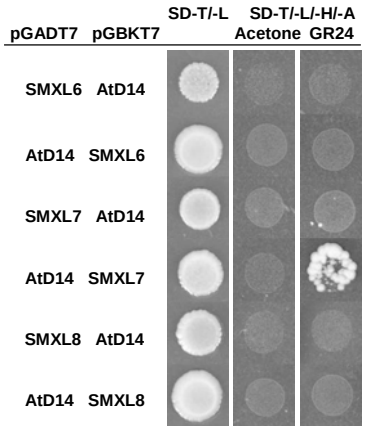


**Figure 1.** Interaction between AtD14 and SMXL6, SMXL7, or SMXL8 in Y2H.

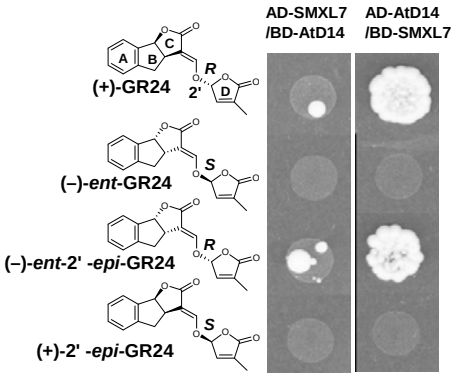


**Figure 2.** Effects of four GR24 stereoisomers on the interaction between SMXL7 and AtD14.

**Chapter 2: Functional analysis of active-site point mutations in KAI2**

KAI2 is an α/β-hydrolase family protein and a paralog of AtD14 in Arabidopsis. The structures of AtD14 and KAI2 are quite similar, and these two pathways share a common F-box protein, MAX2, as a downstream signaling component. KAI2 functions in the perception of a smoke-derived germination stimulant called karrikin (KAR). The endogenous ligand (substrate) of KAI2 has not been identified, and the Arabidopsis KAI2 signaling pathway has not been fully elucidated. To understand the importance of the catalytic triad amino acid residues for KAI2 biological function, we performed complementation tests using three KAI2 mutant proteins (KAI2:S95A, KAI2:H246A, and KAI2:D217A) in the Arabidopsis *kai2* mutant. The *kai2* mutant exhibits an elongated hypocotyl phenotype and delayed seed germination compared with those of WT plants. None of the point mutants complemented the *kai2* elongated hypocotyl phenotype. However, KAI2:S95A could partly complement the delayed germination phenotype of *kai2* mutant seeds. These results suggest that the KAI2 hydrolase activity is not necessary, at least for Arabidopsis seed germination. Further experiments are required to fully elucidate this system.

**Conclusion**

Our results conclusively demonstrate that SMXL6 and SMXL7 function as repressors in the SL signaling pathway in Arabidopsis. We used Y2H experiments to clarify important parameters for the AtD14-SMXL7 interaction, and identified the AtD14 catalytic triad and the SL stereochemistry. Although additional experiments are needed to understand KAI2 function in Arabidopsis, I provided data supporting the important role of the catalytic triad for KAI2 function.

**Publication list**

Mikihisa Umehara*, Mengmeng Cao* et al. Structural Requirements of Strigolactones for Shoot Branching Inhibition in Rice and Arabidopsis. Plant & Cell Physiology, 2015. (*equal contribution)