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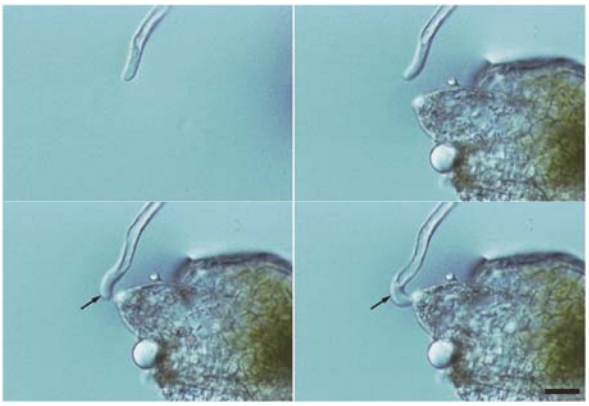
**Grant Title: Action Mechanism of Bio-Active Sugar Chain AMOR**

AMOR is bio-active, female-derived arabinogalactan sugar chain, which activates male pollen tubes in plant fertilization. In a flowering plant *Torenia*, pollen tubes become competent to be attracted by female-derived LURE attractant peptides. The figure below indicates an activated pollen tube of *Torenia* that are attracted to a micromanipulated female tissue secreting LURE peptides (arrows; scale bar = 20 μm). Structure-activity relationship study by synthetic chemistry revealed that a very specific terminal disaccharide structure, 4-Me-GlcA-β-(1→6)-Gal, was responsible for AMOR activity. However, action mechanism of AMOR remains largely unknown. AMOR function in *Arabidopsis*, a model plant species, remains also unknown. The purpose of this study was to tackle with these issues by combination of synthetic chemistry and physiological/genetic studies.

In this study, we at first examined phenotypes of mutants defective in AMOR (methyl-glucuronosyl galactose) synthesis pathway in *Arabidopsis* and *Torenia*. We also tried to find new function of AMOR and its derivatives in plant growth and development. Finally, we tried to develop derivatives of AMOR by adding a crosslinker to investigate the action mechanism of AMOR in plants.

Arabinogalactan sugar chain is bio-synthesized as a part of arabinogalactan proteins (AGPs). *Arabidopsis* has 151 AGP-encoding genes, many of which are expressed and function in reproductive organs (Su and Higashiyama, 2018). In *Arabidopsis*, mutants defective in glucuronic acid transferases were analyzed. Reduced pollen tube growth, abnormal morphology of pollen tubes, and impaired pollen tube guidance were detected, although phenotypes were not severe in these double and triple mutants. We found 8 more closely related genes were expressed in the female organs, therefore further knockout of genes with redundant function would be critical in the future. Reduced gluconic acid modification was likely to be detected easily by western blotting with a monoclonal antibody. In *Torenia*, we could perform knockout of candidate genes of AMOR synthesis pathway genes, which must be an important step for functional analysis of AMOR in vegetative and reproductive phases in *Torenia*. We also found AMOR derivatives showed some bioactivity including growth-promoting activity for *Arabidopsis* seedling. Adding a crosslinker to AMOR was also possible by collaboration with synthetic chemists for visualization, antigenization, and partner identification of AMOR. These results suggest power of combination of synthetic chemistry and genetic analysis, which would provide breakthrough in plant sugar-chain-signaling field.



**Reference:**

Su S., Higashiyama T. (2018) Arabinogalactan proteins and their sugar chains: functions in plant reproduction, research methods, and biosynthesis. ***Plant Reprod.*** 31, 67-75.