RECENT RESEARCH ACTIVITIES

Characterization of cell wall phenylpropanoids of grass bioenergy plants and characterization of ubiquitin ligase involved in secondary cell wall formation in *Arabidopsis*

(Laboratory of Metabolic Science of Forest Plants and Microorganisms, RISH, Kyoto University)

Masaomi Yamamura, Shiro Suzuki, and Toshiaki Umezawa

1. Characterization of cell wall phenylpropanoids of grass bioenergy plants

Lignocelluloses are produced mainly by trees and large gramineous plants such as *Erianthus* spp., switchgrass, nepier grass, miscanthus etc. Because large gramineous plants produce large amounts of biomass, do not compete with food, and can grow under a wide range of conditions, they are drawing attention as potential materials for biofuel and industrial feedstock production. Generally, both lignin and ferulate (or diferulate) cross-linking in the cell wall are known as obstructive factors in the enzymatic saccharification process for the production of biofuel. This study established a system to quantitate diferulates in cell walls of various grass energy plants using stable isotope dilution method.

In this study, various deuterium-labeled and unlabeled diferulates were synthesized, and it was shown that some diferulates are rather unstable; the unstability was partially eliminated by the introduction of protecting groups: acethylation of the phenolic hydroxyls and ethyl ester formation from the carboxyls. In addition, it was shown for the first time that the amounts of diferulates in cell wall materials of *Erianthus arundinaceus* were very small. The present result suggested that the role of the diferulate residues as the obstacles of enzymatic saccharification of *E. arundinaceus* internodes may be insignificant [1].

2. Characterization of ubiquitin ligase involved in secondary cell wall formation in *Arabidopsis*

Recently, many transcription factors which coordinately regulate biosynthesis of the secondary wall components have been uncovered in a model dicotyledonous plant *Arabidopsis thaliana*. However, little was known about other regulatory systems of secondary wall formation. The present study indicated that an E3 ubiquitin ligase, which plays an important role in the selective protein degradation via the ubiquitin-proteasome pathway, was involved in secondary wall formation.

Using a gene co-expression network analysis, we found that *Arabidopsis Tóxicos en Levadura54* (*ATL54*, At1g72220) encoding a putative ubiquitin ligase was co-expressed with some genes involved in secondary wall formation. The recombinant ATL54 protein catalyzed E1- and E2-dependent auto-ubiquitination. Expression of some biosynthetic genes of secondary wall components was up-regulated in apical stem portions of the *ATL54*-knock-out mutants, while expression of a gene involved in programmed cell death of tracheary elements was significantly repressed in both *ATL54*-knock-out and *ATL54*-overexpressed mutants. Moreover, we found that the *β-glucuronidase* (*GUS*) reporter gene driven by the *ATL54* promoter was significantly expressed in interfascicular fibers, xylary fibers, and vessels in inflorescence stems. The dual luciferase transient transfection assay demonstrated that *ATL54* was transactivated by MYB46, a master regulator of secondary wall biosynthetic genes. An electrophoretic mobility shift assay showed that MYB46 directly bound to *ATL54* promoter fragments. These results suggested that ATL54 was an E3 ubiquitin ligase involved in secondary wall biosynthesis and programmed cell death during xylogenesis [2], and indicated that *ATL54* expression was directly regulated by MYB46 [3] .

References

[1] Yasui A, Yamamura M, Suzuki S, Umezawa T (2013) “Establishment of the system to measure diferulates in the cell wall of gramineous plants”, The 63rd Annual Meeting of the Japan Wood Research Society (in Matsuyama)

[2] Noda S, Takahashi Y, Tsurumaki Y, Yamamura M, Nishikubo N, Yamaguchi M, Sakurai N, Hattori T, Suzuki H, Demura T, Shibata D, Suzuki S, Umezawa T (2013) ATL54, a RING-H2 domain protein selected by a gene co-expression network analysis, is associated with secondary cell wall formation in *Arabidopsis*. *Plant Biotechnol* 30: 169–177

[3] Noda S, Yamaguchi M, Tsurumaki Y, Takahashi Y, Nishikubo N, Hattori T, Demura T, Suzuki S, Umezawa T (2013) ATL54, a ubiquitin ligase gene related to secondary cell wall formation, is transcriptionally regulated by MYB46. *Plant Biotechnol* 30: 503–509