ABSTRACTS (PH D THESIS)

Functional characterization of a RING-type ubiquitin ligase and MYB transcription factors involved in secondary cell wall formation

**(Graduate School of Agriculture,
Laboratory of Metabolic Science of Forest Plants and Microorganisms,
RISH, Kyoto University)**

Soichiro Noda

Lignified secondary cell walls are formed in sclerenchymatous cells such as vessel elements, tracheids, and fibers. In lignified secondary walls, regularly arranged and rigid cellulose microfibrils are coated with hemicelluloses, and the gap is filled with lignin. Deposition of lignified secondary walls gives high mechanical strength to sclerenchymatous cells and enables them to support plant bodies and to resist negative pressure caused by water transportation. 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. In addition, the transcriptional regulation of secondary wall formation in Gramineae plants, which have cell wall compositions different from those of dicotyledonous plants, is not known well. In the present study, the author 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. Furthermore, novel rice MYB transcription factors involved in secondary wall formation were characterized.

First, the author conducted a gene co-expression network analysis and 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. These results suggested that ATL54 was an E3 ubiquitin ligase involved in secondary wall biosynthesis and programmed cell death during xylogenesis [1].

Second, the author showed 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. In addition, an electrophoretic mobility shift assay showed that MYB46 directly bound to *ATL54* promoter fragments. These results indicated that *ATL54* expression was directly regulated by MYB46 [2].

Third, using a gene co-expression analysis, six genes of MYB transcription factors were found to be co-expressed with cellulose, xylan, and lignin biosynthetic genes in rice (*Oryza sativa* cv. Nipponbare). A phylogenetic analysis of *Arabidopsis* and rice MYB transcription factors showed that the six MYB transcription factors shared similarities with *Arabidopsis* MYB transcription factors regulating secondary wall biosynthesis [3]. A quantitative real-time PCR analysis revealed that four *MYB* genes were highly expressed in culm internodes, culm nodes, hulls, or leaf sheaths of rice, where secondary wall develops well. The transcriptional activation activity of the MYB transcription factors showed that all the four MYB transcription factors can activate gene expression in yeast.

To identify downstream target genes of the MYB transcription factors, a dual luciferase transactivation assay was performed. A cellulose synthase gene required for secondary wall biosynthesis [4], *OsCesA7*, was transactivated by two MYB transcription factors. In addition, an electrophoretic mobility shift assay showed that one of the MYB transcription factors caused a band shift of oligonucleotides containing *cis*-elements within the *OsCesA7* promoter. These results indicated that *OsCesA7* was a direct downstream target of the MYB transcription factor.

Taken together, this study suggested that selective protein degradation via the ATL54-mediated ubiquitin-proteasome pathway may be involved in secondary wall formation. In addition, it also provided a deeper insight into transcriptional regulation of secondary wall biosynthesis in Gramineae.

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