論文要約

Functional characterization of a RING-type ubiquitin ligase and MYB transcription factors involved in secondary cell wall formation
（二次細胞壁形成に関与するRING型ユビキチンリガーゼおよびMYB転写因子の機能解析）

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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 is known about other regulatory systems of secondary wall formation. In addition, the transcriptional regulation of secondary wall formation in Gramineae plants, which have the cell wall compositions different from those of dicotyledonous plants, is not known well. In the present study, the author strongly suggested 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.

The author first conducted a gene co-expression network analysis and found *Arabidopsis Tóxicos en Levadura54* (*ATL54*, At1g72220) encoding a putative ubiquitin ligase co-expressed with genes involved in secondary wall formation. The recombinant ATL54 protein catalyzed E1- and E2-dependent auto-ubiquitination. Expression of 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.

Next, 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 AtMYB46, a master regulator of secondary wall biosynthetic genes. In addition, an electrophoretic mobility shift assay showed that AtMYB46 directly bound to *ATL54* promoter fragments. These results indicated that *ATL54* expression was directly regulated by AtMYB46. Taken together, it was suggested that selective protein degradation via the ATL54-mediated ubiquitin-proteasome pathway may be involved in secondary wall formation.

Third, the author found that six rice *MYB* transcription factor genes were co-expressed with secondary wall biosynthetic genes. Among the *MYB*s, the expression of *OsMYB63* (Os04g0594100), *OsMYB61* (Os01g0285300), *OsMYB61-L* (Os05g0140100), and *OsMYB85* (Os09g0532900) was relatively high in internodes and nodes of culms. OsMYB63 and OsMYB61-L were shown to transactivate *OsCesA7*, a secondary wall-specific cellulose synthase gene. In addition, OsMYB63 directly bound to two *cis*-elements existing in the *OsCesA7* promoter. These results indicated that OsMYB63 directly activated a cellulose biosynthetic gene. Considering that OsMYB63 is a homolog of *Arabidopsis* AtMYB63 and AtMYB58, which specifically activate lignin biosynthetic genes, the results suggested that the transcriptional regulation of secondary wall biosynthesis by MYB transcription factors in rice may be different from that in *Arabidopsis*.