論文要約

Generation of transgenic rice with altered lignin composition and comparative characterization of their biomass utilization properties

（リグニン組成を改変した形質転換イネの作出とそのバイオマス利用特性の評価）

Metabolic Science of Forest Plants and Microorganisms

Yuri Takeda

Lignocellulose, a complex bio-composite composed of lignin, cellulose, and hemicelluloses, constitutes the secondary cell walls of vascular plants and accounts for the highest proportion of terrestrial biomass on Earth. Hence, sustainable production and utilization of lignocellulosic biomass are critical for achieving the sustainable development goals (SDGs). Trees and grass biomass crops represent major lignocellulose feedstocks. While the annual biomass production of typical tree species is estimated to be less than 20 t ha^{-1} $year^{-1}$, large-sized grass biomass crops, such as *Sorghum*, sugarcane, and *Erianthus*, produce several-fold higher biomass yields compared with those of typical trees. In addition, delignification of grass lignocellulose is generally easier than that of wood lignocellulose. These characteristics render grass biomass crops potential materials for biochemical and thermochemical platforms and/or combustion use, although trees are indispensable for wood-based materials and for pulp and paper production.

Lignin is mainly composed of three different types of aromatic component, guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) units. Lignin aromatic composition, i.e., G/S/H lignin unit ratio, has been considered to be an important structural trait that affects the physico-chemical properties of lignocellulosic biomass. However, our knowledge regarding the relationship between the lignin aromatic composition and biomass utilization characteristics is yet considerably limited especially in monocotyledonous grass species. The main objectives of this PhD research were therefore to generate novel transgenic rice plants that produce lignocellulose with distinct lignin aromatic composition (Chapters 1-4) and then, through comparative characterization of their lignocellulose properties, to elucidate the effect of lignin composition on the utilization properties of grass lignocellulose as a source of biofuels and biomaterials (Chapter 5).

In Chapter 1, the author successfully obtained transgenic rice lines augmented with G and S lignin units through manipulating coniferaldehyde 5-hydroxylase (*CAld5H*) gene expression. Among three *CAld5H* genes identified in rice, *OsCAld5H1* appeared to be predominantly expressed in lignin-producing rice vegetative tissues. RNAi-mediated down-regulation of *OsCAld5H1* resulted in altered lignins largely enriched in G units, whereas up-regulation of *OsCAld5H1* under the control of a modified ubiquitin promoter resulted in lignins substantially enriched in S units, as demonstrated by a series of wet-chemical and NMR structural analyses. The results collectively demonstrate that *OsCAld5H1* expression is a key factor controlling the S/G lignin composition in rice cell walls.

In Chapter 2, to further investigate the effect of *OsCAld5H1*-suppression on rice lignin structure, the author characterized loss-of-function mutants of *OsCAld5H1* generated by the CRISPR/Cas9 genome editing system. A series of cell wall analysis demonstrated that, although lignins in the mutant were predictably enriched in G units, all tested mutant lines produced considerable amounts of S units. Intriguingly, lignin *γ-p*-coumaroylation analysis by the DFRC method revealed that the enrichment of G lignin units in the *OsCAld5H1*-knockout mutants was limited to the non-γ-*p*-coumaroylated lignin units, whereas grass-specific γ-*p*-coumaroylated lignin units were almost unaffected. These data suggested that CAld5H is mainly involved in the production of non-γ-*p*-coumaroylated S lignin units, common in both eudicots and grasses, but not in the production of grass-specific γ-*p*-coumaroylated S units in rice.

In Chapter 3, the author successfully obtained transgenic rice lines augmented with H lignin units through manipulating p-*coumaroyl ester 3-hydroxylase* (*C3'H*) gene expression. *C3'H*-knockdown lines generated via RNAi-

mediated gene silencing, with about 0.5% of the residual expression levels, reached maturity and set seeds, whereas *C3'H*-knockout rice mutants generated via the CRISPR/Cas9-mediated targeted mutagenesis were severely dwarfed and sterile. Cell wall analysis of the mature *C3'H*-knockdown RNAi lines revealed that their lignins were largely enriched in H units while being substantially reduced in the normally dominant G and S units. Interestingly, however, lignin *γ-p*-coumaroylation analysis by the DFRC method revealed that the enrichment of H units was limited to the non-acylated lignin units, with grass-specific γ-*p*-coumaroylated lignin units remaining apparently unchanged. These data suggested that, similar to what we have showed for CAld5H (Chapter 2), C3′H is mainly involved in the production of non-γ-*p*-coumaroylated lignin units, but not in the production of grass-specific γ-*p*-coumaroylated lignin units in rice. Suppression of *C3'H* also resulted in a substantial reduction in wall cross-linking ferulates. These results demonstrate that *C3'H* expression is an important determinant not only of lignin content and composition but also of the degree of cell wall cross-linking in grasses.

In Chapter 4, the author further characterized the CRISPR/Cas9-derived *C3'H*-knockout mutants generated in Chapter 3. In line with their severely impaired growth phenotype, the *C3'H*-knockout lines appeared to show irregular vasculature and ectopic lignification in culm and root. Transcriptomic analysis data suggested that, along with altered transcript levels of phenylpropanoid biosynthetic genes, various biological processes were perturbed in the *C3'H*-knockout lines. Lignin analysis of the mutant cell walls demonstrated that, as observed in the *C3'H*-knockdown lines in Chapter 3, H lignin units were substantially increased but the augmentation of H lignin units in the mutants were limited to non *γ-p*-coumaroylated lignin units, with grass-specific *γ-p*-coumaroylated lignin units being mostly unaffected, further supporting the author's notion that there is C3′H-independent metabolic pathway(s) responsible for the production of grass-specific *γ-p*-coumaroylated lignin units.

In Chapter 5, the author used the transgenic rice lines with distinct lignin aromatic compositions, which were obtained in the previous chapters, to study the impact of altered lignin composition on the chemical reactivity, enzymatic saccharification efficiency and calorific value of rice lignocellulose. It was suggested that H and G lignins are more susceptible to acid-induced condensation reactions than S lignins. The H-lignin-enriched transgenic rice was shown to display significantly enhanced biomass saccharification efficiency without any pretreatment or with alkali and acid pretreatments, whereas the S-lignin-enriched transgenic rice showed further enhanced saccharification efficiency after liquid hot water pretreatment. While no significant differences in the biomass heating values were observed between the transgenic rice materials tested, analysis of synthetic lignins comprising only G, S or H units suggested that increasing H or G units may be beneficial to increase the heating value of biomass.