ABSTRACTS (MASTER THESIS)

Structural modification of rice lignin by regulating aromatic hydroxylase gene expressions

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

Yuri Takeda

Lignocellulose, a promising resource for productions of biofuels and bio-based materials, is majorly composed of three different polymers, i.e., cellulose, hemicelluloses, and lignin. While numerous technologies that transform biomass polysaccharides (cellulose and hemicelluloses) to bioethanol and various materials are becoming mature, cost-effective utilization of lignin has been substantially more challenging, representing one of the major issues that are to be overcome for establishing an economically feasible biorefinery system. In this context, our laboratory has been studying metabolic engineering of lignin to upgrade biomass feedstocks for biorefinery. Our study focuses on lignin bioengineering using a grass model plant, rice (*Oryza sativa*), in particular to obtain fundamental knowledge for improving the properties of grass biomass crops, e.g., swichgrass, *Erianthus*, and *Miscanthus*, that are expected as a potent biomass resource especially because of their high biomass productivity [1-3].

Lignin is mainly composed of three different types of aromatic component, guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) units. Lignin composition, i.e., G/S/H lignin unit ratio, has been identified as an important structural trait that impacts usability of lignin and also lignocellulose as a whole. In the lignin biosynthetic pathway in angiosperms, ferulate 5-hydroxylase (F5H) and *p*-coumaroyl ester 3-hydroxylase (C3´H) have been identified as the major enzymes that modulate aromatic ring structures of lignin monomer units. In this study, we identified major genes encoding F5H and C3´H functioning in the deposition of lignin in rice, and demonstrated that modulation of their expressions led to substantial alterations in G/S/H lignin unit ratio of rice cell walls.

Based on our analyses on protein sequence and gene expression, we determined major rice F5H (*OsF5H1*) and C3´H (*OsC3H1*) genes that are homologous to the previously-characterized F5H and C3´H enzymes in dicots, and they are predominantly expressed in lignin-producing rice organs. We then generated transgenic rice plants in which *OsF5H1* or *OsC3H1* is up- or down-regulated, and characterized their cell wall chemotypes using wet-chemical and 2D NMR approaches. As expected, down- and up-regulation of *OsF5H1* produced altered lignins largely enriched in G and S units, respectively, and down-regulation of *OsC3H1* resulted in lignins considerably enriched in H units. In addition, down-regulation of *OsC3H1* also led a substantial decrease in wall-bound ferulates, cross-linkers between lignin and polysaccharide and one of the unique structural traits of grass lignocellulose. Our data collectively demonstrate that *OsF5H1* and *OsC3H1* are primary genes controlling G/S/H lignin composition in rice cell walls. Overall, we contemplate that manipulation of F5H and C3´H gene expressions could be a promising strategy to upgrade grass lignocellulose for future biorefinery.

References

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