ABSTRACTS (PH D THESIS)

Studies on lignocellulose supramolecular structures and deconstruction properties in lignin-altered rice mutants

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Lignocellulosic biomass represents abundant and renewable carbon sources that can be exploited for the sustainable production of bio-based energy and chemicals. Lignocellulose is majorly produced in plant cell walls and mainly composed of three structural polymers, i.e., cellulose, hemicelluloses and lignin, which intricately interact with each other through both non-covalent and covalent linkages. Lignin, a phenylpropanoid polymer typically accounting for 15%-30% of raw lignocellulose feedstocks, has long been recognized as a key recalcitrant factor limiting the efficiency of lignocellulose deconstruction and downstream processing in polysaccharide-oriented biomass utilization processes, for example, those used in the production of pulp and paper and the generation of fermentable sugars for biomaterials and biofuels. To mitigate such lignin-associated biomass recalcitrance, numerous mutants and transgenic plants that produce lignocellulose with reduced lignin contents and/or lignins with altered chemical structures have been produced and characterized. However, largely because of technical challenges in characterizing the highly complex and heterogeneous structure of lignocellulose, it is not fully understood how altered lignin chemistry affects the supramolecular structure of lignocellulose, and consequently, its utilization properties. This study aimed to dissect the impacts of genetic modifications of lignin on the supramolecular structure and deconstruction properties of lignocellulose. Particular focus was directed to the characterization of rice mutants deficient in *CINNAMYL ALCOHOL DEHYDROGENASE* (*CAD*) and *5-HYDROXYCONIFERALDEHYDE* O-*METHYL-TRANSFERASE* (*CAldOMT*), both of which encode key enzymes in lignin biosynthesis and represent major gene targets in lignin bioengineering research.

Altered lignocellulose chemical structure and molecular assembly in *CAD*-deficient rice

In the first part of this study, the author conducted comprehensive chemical and supramolecular structural analyses of lignocellulose produced by a *CAD*-deficient mutant rice, which harbors a *Tos17* retrotransposon insertion in *OsCAD2*, a major CAD gene involved in lignin biosynthesis of rice. The solution-state two-dimensional NMR and complementary wet-chemical methods elucidated the structural details of the altered lignins enriched with unusual hydroxycinnamaldehyde-derived subunits produced by the *CAD*-deficient mutant rice. In parallel, lignocellulose supramolecular structure was investigated by solid-state NMR, X-ray diffraction and Simon's staining approaches. The obtained data indicated that cellulose assembly and mobility were notably disrupted in the *CAD*-deficient mutant lignocellulose. In particular, both solid-state NMR and X-ray diffraction data suggested that *CAD*-deficient lignocellulose has less well-defined cellulose alignment compared to that in the wild-type control lignocellulose, which may account for the improved saccharification performance of lignocellulose produced by the *CAD*-deficient mutant rice [1].

Insights into lignocellulose molecular assembly and its deconstruction from lignin-altered rice mutants deficient in *CAldOMT* and *CAD*

In the second part of this study, impacts of lignin-modifications induced by deficiencies of *CAldOMT* and *CAD* were comparatively investigated. Rice mutants deficient in either or both *OsCAldOMT1*, a major OMT gene involved in lignin biosynthesis of rice, and *OsCAD2* genes investigated in the earlier part of this study were generated in part by using the Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated 9 (CRISPR/Cas9) system. Isolated homozygous mutant lines and wild-type

control rice plants were grown side-by-side and subjected to in-depth analyses of lignocellulose structures and enzymatic saccharification efficiency. In line with the proposed functions of CAldOMT and CAD in the lignin biosynthetic pathways, *CAldOMT*-deficient mutant lines produced altered lignins largely depleted in syringyl and tricin subunits and partially incorporated with atypical 5-hydroxyguaiacyl units, whereas *CAD*-deficient mutant lines produced lignins incorporated with unusual hydroxycinnamaldehyde-derived subunits. Solid-state NMR and X-ray diffraction analyses suggested that, whereas disruptions of *CAldOMT* and *CAD* both prominently affects the lignocellulose supramolecular structure, the disruption of *CAldOMT* more prominently affects lignocellulose supramolecular structures than does the disruption of *CAD*, resulting in higher cellulose mobility as primarily gauged by nuclear magnetic relaxation. Partly in line with this observation, *CAldOMT*-deficient mutant lignocellulose showed significantly greater glucose release upon enzymatic saccharification compared with those of the wild-type control and *CAD*-deficient mutant lignocellulose [2].

Comparative analysis of lignocellulose chemical degradability and enzymatic saccharification performance of *CAD*- and *CAldOMT*-deficient rice mutants

In the last, third part of this study, the author investigated the deconstruction properties of *CAD*- and *CAldOMT*-deficient rice mutant lignocellulose in terms of their chemical reactivities in typical biomass processing reactions. 2D NMR and chemical structural analyses on the rice lignocellulose samples before and after dilute alkaline, dilute acid and liquid hot water treatments revealed different reactivities of lignin and polysaccharide components comprising *CAD*- and *CAldOMT*-deficient mutant cell walls. Saccharification efficiency of the *CAD*- and *CAldOMT*-deficient mutant lignocellulose was differently improved by using the three chemical reactions as pretreatments to facilitate dissociations of lignocellulose prior to enzymatic polysaccharide hydrolysis. In particular, dilute alkaline treatment was effective to promote saccharification of both *CAD*- and *CAldOMT*-deficient mutant lignocellulose, whereas dilute acid and liquid hot water treatments were effective for *CAldOMT*-deficient mutants but apparently not for *CAD*-deficient mutants. Overall, the use of biomass processing reactions in combination with genetic lignin alterations based on manipulations of *CAD* and *CAldOMT* can be strategic to boost lignocellulose deconstructions [3].

References

[1]Martin AF et al. (2019) Altered lignocellulose chemical structure and molecular assembly in CINNAMYL ALCOHOL DEHYDROGENASE-deficient rice. *Scientific Reports* 9: 17153.

[2]Martin AF et al. manuscript submitted.

[3]Martin AF et al. manuscript to be submitted.