

(続紙 1)

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論文題目	Studies on lignocellulose supramolecular structures and deconstruction properties in lignin-altered rice mutants (リグニンを改変したイネ変異体におけるリグノセルロースの超分子構造と分解特性に関する研究)		
(論文内容の要旨)			
<p>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 <i>CINNAMYL ALCOHOL DEHYDROGENASE (CAD)</i> and <i>5-HYDROXYCONIFERALDEHYDE O-METHYL-TRANSFERASE (CaldOMT)</i>, both of which encode key enzymes in lignin biosynthesis and represent major gene targets in lignin bioengineering research.</p> <p>Altered lignocellulose chemical structure and molecular assembly in <i>CAD</i>-deficient rice</p> <p>In the first part of this study, the author conducted comprehensive chemical and supramolecular structural analyses of lignocellulose produced by a <i>CAD</i>-deficient mutant rice, which harbors a <i>Tos17</i> retrotransposon insertion in <i>OsCAD2</i>, a major <i>CAD</i> 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 <i>CAD</i>-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 <i>CAD</i>-deficient mutant lignocellulose. In particular, both solid-state NMR and X-ray diffraction data suggested that <i>CAD</i>-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</p>			

lignocellulose produced by the *CAD*-deficient mutant rice.

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 triclin 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.

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.

注) 論文内容の要旨と論文審査の結果の要旨は1頁を38字×36行で作成し、合わせて、3,000字を標準とすること。

論文内容の要旨を英語で記入する場合は、400～1,100 wordsで作成し
審査結果の要旨は日本語500～2,000字程度で作成すること。

(続紙 2)

(論文審査の結果の要旨)

リグノセルロースの分解利用性を高める目的で、リグニンの量や化学構造を改変した様々な遺伝子組換え植物が報告されている。しかし、これらのリグニンの改変が、リグノセルロース超分子構造の変動に及ぼす影響、ならびにリグノセルロースの分解反応性向上に及ぼす効果に関する分子レベルでの知見は未だ乏しい。本論文では、リグニン生合成酵素遺伝子*CAD*及び*CaldOMT*の機能欠損によりリグニンの量及び化学構造が改変されたイネ変異株を作出した。次いで、これらの変異株のリグノセルロースについて、固体高次構造、ならびに酵素糖化及び各種化学処理における分解特性の比較解析を行い、リグニンの化学的性状の変化に伴うリグノセルロースの超分子構造及び化学反応性の変化と、それらに基づくリグノセルロースの分解特性向上機構の一端を明らかにした。評価すべき点は以下の3点である。

1. ゲノム編集により、リグノセルロース酵素糖化特性が野生株より向上したイネの*CaldOMT*欠損一重変異株と*CAD*及び*CaldOMT*欠損多重変異株を新たに作出し、既存の*CAD*欠損一重変異株と合わせて、改変されたリグニンの化学構造を各種化学分析法と2D NMR法を用いて詳細に明らかにした。
2. 固体NMR法及びX線回析法等によるリグノセルロースの固体高次構造解析に基づき、上記の*CAD*及び*CaldOMT*欠損イネ変異株のリグノセルロースにおいて、リグニンの化学的性状のみならず、多糖の分子配向や運動性に顕著な変化が生じていることを明らかにし、特に多糖の分子集合状態に生じる緩みが各変異株のリグノセルロース酵素糖化特性の向上に寄与している可能性を示した。
3. *CAD*及び*CaldOMT*欠損イネ変異株のリグノセルロースについて、アルカリ、酸、加圧熱水処理に対する反応特性の相違を明らかにした。また、各イネ変異株のリグノセルロースの反応特性に適した化学前処理法を用いることで、リグノセルロース糖化効率をさらに大きく向上させることにも成功した。

以上のように、本論文は、*CAD*及び*CaldOMT*欠損イネ変異株におけるリグニン改変に伴うリグノセルロース超分子構造の変動の詳細を明らかにし、特に多糖の分子集合状態に生じる緩みが各イネ変異株のリグノセルロース糖化特性の向上に寄与している可能性を示した。また、各種化学前処理法を用いることで、各イネ変異株のリグノセルロース糖化効率を大きく向上させることにも成功した。これらの結果は、植物代謝工学、植物二次代謝化学、木質科学及び植物生理学の基礎及び応用研究の発展に寄与するところが大きい。

よって、本論文は博士(農学)の学位論文として価値あるものと認める。

なお、令和2年2月18日、論文並びにそれに関連した分野にわたり試問した結果、博士(農学)の学位を授与される学力が十分あるものと認めた。

また、本論文は、京都大学学位規程第14条第2項に該当するものと判断し、公表に際しては、当該論文の全文に代えてその内容を要約したものとすることを認める。

注) 論文内容の要旨、審査の結果の要旨及び学位論文は、本学学術情報リポジトリに掲載し、公表とする。

ただし、特許申請、雑誌掲載等の関係により、要旨を学位授与後即日公表することに支障がある場合は、以下に公表可能とする日付を記入すること。

要旨公開可能日： 年 月 日以降 (学位授与日から3ヶ月以内)