

(続紙 1)

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論文題目	Studies on Metabolic Engineering Approaches to Manipulate Lignin Aromatic Composition in Grasses（イネ科植物におけるリグニンの芳香核組成を制御するための代謝工学的アプローチに関する研究）		
（論文内容の要旨） Lignin, a complex aromatic biopolymer produced in the secondary cell walls of vascular plants, is a key recalcitrant component of lignocellulosic biomass. In general, lignins produced by gymnosperms and eudicots are derived from the polymerization of monolignols, mainly coniferyl and sinapyl alcohol, which give rise to guaiacyl (G) and syringyl (S) units, respectively, in the final lignin polymer. On the other hand, lignins produced by monocotyledonous grasses additionally utilize monolignol <i>p</i>-coumarate conjugates, i.e., coniferyl and sinapyl <i>p</i>-coumarate, as canonical lignin monomers, which give rise to G and S units appended with grass-specific <i>p</i>-coumarate (P) decoration units. The complex aromatic composition of lignin, primarily determined by the types of lignin monomers used for lignification, significantly impacts the utility of lignocellulosic biomass. Accordingly, numerous efforts have been dedicated to genetically manipulating lignin aromatic composition in both eudicot and grass model plants. In eudicots, transgenic and mutant lines highly enriched in G or S lignin units have been successfully generated by manipulating the expression level of <i>CONIFERALDEHYDE 5-HYDROXYLASE</i> (<i>Cal</i>d5<i>H</i>, or <i>FERULATE 5-HYDROXYLASE</i>, <i>F5H</i>). This demonstrated the key role of <i>Cal</i>d5<i>H</i> in channeling the biosynthesis of G- and S-type monolignols, the conserved lignin monomers. However, the same bioengineering approach proved less effective in grass rice likely due to the presence of a <i>Cal</i>d5<i>H</i>-independent pathway leading to the G- and S-type monolignol <i>p</i>-coumarate conjugates, the grass-specific lignin monomers. In this study, the author further explored bioengineering approaches to manipulate lignin aromatic composition in rice by simultaneously targeting key enzyme genes involved in both conserved and grass-specific lignin monomer biosynthesis. Newly developed rice mutant and transgenic lines, highly enriched in G and S lignin units, were subjected to in-depth lignin structural analyses and evaluations of lignocellulose deconstruction properties. In Chapter 1, the author aimed to develop rice mutants highly enriched in G lignin via stacked mutations blocking both conserved and grass-specific S lignin biosynthesis. Based on the hypothesis that grasses possess a <i>Cal</i>d5<i>H</i>-independent pathway leading to the monolignol <i>p</i>-coumarate conjugates, the author generated genome-edited rice mutants harboring stacked mutations in a rice <i>Cal</i>d5<i>H</i> gene along with genes encoding <i>p</i>-COUMAROYL-COENZYME A:MONOLIGNOL ACYLTRANSFERASE (PMT) which is required for the generation of monolignol <i>p</i>-coumarate conjugates. Chemical and two-dimensional (2D) nuclear magnetic resonance (NMR) analyses of the cell walls from the developed mutants revealed that, as anticipated, they produced altered lignin highly enriched in G units by eliminating S units along with P decoration units, further strongly supporting the existence of a <i>Cal</i>d5<i>H</i>-independent S lignin pathway in rice. Further in-depth structural analyses of G-dominated lignins produced in the developed rice mutants along with other G-dominated lignins from <i>Cal</i>d5<i>H</i>-deficient eudicot <i>Arabidopsis</i>, natural			

gymnosperm pine, and *in vitro* polymerization, revealed unique features of G lignins produced in different plant lineages. These findings highlight the influence of the lineage-specific cell wall environment on the polymerization of the G-type lignin monomer (coniferyl alcohol) *in planta*.

In Chapter 2, given the potential of more easily processable S lignin to enhance lignocellulosic biomass valorization, the author aimed to develop rice transgenic lines enriched in S lignin by boosting its biosynthesis in the *PMT*-deficient mutant. As anticipated, the overexpression of a rice *Cal5H* gene in the *PMT*-deficient mutant background produced rice lines highly enriched in S lignin units by eliminating G units along with P decoration units. Comparative structural analysis of S-dominated lignins from the S-lignin-enriched rice transgenic lines along with those from *Cal5H*-overexpressing Arabidopsis and natural hardwoods (eucalyptus and acacia woods), as well as previously generated G-lignin-enriched rice and Arabidopsis lignins, revealed distinct features of S- and G-lignins produced in different plant species, reflecting how S- and G-type monolignols (sinapyl and coniferyl alcohol) undergo polymerization in different cell wall environments *in planta*.

In Chapter 3, the author conducted a comparative assessment of lignocellulose deconstruction properties in the rice mutant and transgenic lines developed in Chapter 1 and Chapter 2. It was revealed that the S-lignin-dominated lines exhibited particularly high glucose production yields in enzymatic saccharification, especially when combined with liquid hot water pretreatment. On the other hand, G-lignin-dominated lines showed significant improvement with alkaline pretreatment. Furthermore, enrichment of G and S lignins proved beneficial in increasing the respective yields of G- and S-type phenolic monomers in the complex product mixtures released by the oxidative depolymerization of lignin. Notably, the S-lignin-enriched lines displayed greater total yields of aromatic monomers from oxidative lignin degradation than both wild-type and G-lignin-enriched lines. Overall, these data demonstrate that precise manipulation of G and S lignin units, achieved through the simultaneous engineering of conserved and grass-specific lignin biosynthetic pathways, offers a promising approach to enhance grass lignocellulose valorization.

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)

単子葉類イネ科植物は、樹木と共に、持続型社会構築を担うリグノセルロース供給源として重要な植物グループである。しかし、イネ科植物のリグニンは、他の維管束植物のリグニンと比較して、より多様なリグニンモノマーから生じる複雑かつ多様な芳香核構造を有しており、この特性がイネ科リグノセルロースを有用化成品へと変換する際の大きな阻害要因となっている。本論文は、リグニンモノマー生合成遺伝子群の多重制御によって、リグニン芳香核組成が大幅に単純化された新規な形質転換イネ株を作出するとともに、改変されたリグニンの性状とそのリグノセルロース分解特性への影響を明らかにした一連の研究成果をまとめたものである。評価すべき点は以下の通りである。

1. イネ科特異的なリグニンモノマー生合成経路を遮断した*PMT*遺伝子ノックアウトイネをバックグラウンドとした*Cald5H*遺伝子のノックアウト及び過剰発現によって、グアイアシル (G) 型及びシリングル (S) 型芳香核が大幅に増強された形質転換イネ株の作出に成功した。
2. 上記の形質転換イネ株を含む異なる植物種から調製されたG型及びS型リグニンの詳細な化学構造比較解析に基づき、植物種に依存したG型及びS型リグニンモノマーの重合挙動の違いを明らかにした。
3. 上記の形質転換イネ株のリグノセルロース糖化性及びリグニン酸化分解特性の評価に基づき、G型及びS型芳香核の増強がイネ科リグノセルロースの分解利用特性の向上に有効であることを実証した。

以上のように、本論文は、*PMT*及び*Cald5H*遺伝子の同時制御が、イネ科植物のリグニン芳香核組成の単純化とそれによるイネ科リグノセルロースの分解利用特性の向上に有効であることを示すとともに、改変されたリグニンの精密構造解析に基づき、植物種に依存したリグニンモノマーの重合挙動の違いを明らかにしたものである。本論文の成果は、植物生化学、植物代謝工学、木質科学、作物育種学の基礎及び応用利用研究の発展に寄与するところが多い。

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7年2月12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)