

(続紙 1)

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論文題目	Studies on the Roles of Parallel Lignin Biosynthetic Pathways Derived from Phenylalanine and Tyrosine in Rice（イネにおけるフェニルアラニン及びチロシンから生じる並行リグニン生合成経路の役割に関する研究）		
（論文内容の要旨）			
Monocotyledonous grasses, an important plant lineage from both ecological and economic perspectives, utilize diverse lignin monomers derived from the cinnamate/monolignol pathway to form a complex lignin polymer in their cell walls. Due to the significant interest in manipulating lignin biosynthesis for agro-industrial applications through molecular breeding and bioengineering, the regulatory mechanisms of the cinnamate/monolignol pathway in grasses have been the focus of the related research. Notably, while all vascular plants utilize the aromatic amino acid L-phenylalanine (Phe) as the common entry substrate of the cinnamate/monolignol pathway for lignin biosynthesis, grasses have evolved a parallel pathway that allows them to utilize L-tyrosine (Tyr) as an additional entry point. However, the contributions of these dual pathways in coordinating the production of diverse grass lignin monomers remain unclear. In this study, the author investigated the roles of the Phe- and Tyr-derived parallel lignin biosynthetic pathways in the grass model rice (<i>Oryza sativa</i>) using genetic and biochemical approaches.			
In Chapter1, the author conducted functional characterizations of rice genes encoding CINNAMATE 4-HYDROXYLASE (C4H), which, alongside PHENYLALANINE AMMONIA-LYASE (PAL), initiates the entry of Phe into the cinnamate/monolignol pathway for lignin biosynthesis. Despite extensive research on C4H in eudicots, genetic studies of C4H in grasses remain considerably limited. Notably, the role of C4H in the presence of PHENYLALANINE/TYROSINE AMMONIA-LYASE (PTAL), a grass-specific ammonia-lyase that can bypass the conserved Phe-derived PAL-C4H pathway by recruiting Tyr into the cinnamate/monolignol pathway, remains unclear. To address this gap, a set of genome-edited rice mutants harboring knockout mutations in rice C4H genes were generated and subjected to the analysis of growth phenotype and cell wall chemotype, alongside isotopic feeding and chemical inhibitor assays to test the contributions of the PAL-C4H and PTAL pathways. The phenotype and chemotype characterizations of <i>C4H</i>-knockout rice mutants demonstrated that class I (OsC4H1) and class II (OsC4H2a and OsC4H2b) C4Hs cooperatively contribute to lignin biosynthesis in different rice organs. Nevertheless, the effects of <i>C4H</i> deficiency on plant development and lignin formation in rice appeared to be markedly less prominent compared to those reported in eudicots. The ¹³C-labeled Phe and Tyr feeding experiments and chemical inhibitor assays using C4H and PAL/PTAL inhibitors demonstrated that even with the Phe-derived PAL-C4H pathway completely blocked, <i>C4H</i>-knockout rice could still produce substantial levels of lignin and maintain sound cell walls by utilizing the Tyr-			

derived PTAL pathway. Taken together, the study findings demonstrate the essential but dispensable role of C4H in grass cell wall lignification.

In Chapter 2, the author further delved into the contributions of the parallel Phe- and Tyr-derived lignin pathways in rice using isotopic feeding approaches. Firstly, the author established methods to track the incorporation of ^{13}C -labeled Phe and Tyr into major lignin polymer units, namely those derived from three canonical lignin monomers, i.e., monolignols, as well as grass-specific lignin monomers, i.e., tricin, *p*-coumarate, and ferulate, in rice cell walls. Using this approach, the author demonstrated that Tyr supplied via the PTAL pathway can contribute up to 54% of the major lignin and associated cell wall polymer units alongside Phe supplied via the canonical PAL-C4H pathway. Notably, the Phe-to-Tyr incorporation ratio varies between different types of lignin polymer units and also among the examined organs. These data indicate that Phe- and Tyr-derived metabolite pools are not entirely shared in the biosynthesis of different lignin monomers and may also differ substantially depending on tissue and cell types. In addition, the author examined Phe/Tyr incorporation profiles in various rice mutants and transgenic lines with disrupted lignin biosynthesis. Despite the ability to compensate with Tyr, rice often prioritizes Phe incorporation when adjusting to changes in phenylpropanoid levels in rice mutants and transgenic lines. This trend was especially pronounced in mutants and transgenic lines where genes encoding enzymes that channel the production of different types of lignin monomers were altered.

Overall, the present study demonstrates that, alongside the canonical Phe-derived PAL-C4H pathway, rice can effectively leverage the grass-specific Tyr-derived PTAL pathway for the production of diverse lignin monomers to develop its cell walls. This flexibility in the entry point of the lignin biosynthetic pathway allows rice to adapt to changes in the downstream pathway branches. These data support the view that grasses evolved the Tyr-derived PTAL pathway to supplement the canonical Phe-derived PAL-C4H pathway, meeting the higher carbon demands of their diverse phenylpropanoid metabolism. Unraveling the underlying mechanisms may provide valuable genetic tools for engineering grass cell walls and aromatic metabolisms. This, in turn, can facilitate the production and utilization of grass biomass and biochemicals for various applications.

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、食糧および木質バイオマスの生産を担う重要な植物グループであり、その農業形質や木質バイオマスの利用特性と密接に関係するリグニン生合成機構の理解は重要な研究課題である。本論文は、イネ科植物に特徴的なフェニルアラニン及びチロシンから生じる並行した2つの代謝経路(PAL-C4H経路及びPTAL経路)の存在に着目して、イネにおけるリグニン生合成代謝経路の解析を行っ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. 各種バイオインフォマティクス解析およびゲノム編集技術による変異株の作出と性状解析を通じて、イネのリグニン生合成に寄与する3つのC4H遺伝子を同定し、それらの器官ごとの役割を明らかにした。
2. 野生株とC4Hノックアウト株の細胞壁の性状解析およびフィーディング実験から、イネのリグニン生合成におけるPAL-C4H経路およびPTAL経路の寄与を明らかにした。特に、PAL-C4H経路が完全に遮断されても、PTAL経路による補完によって、イネが細胞壁中のリグニン量の顕著な低下とそれに伴う極度の成長阻害を免れることを明らかにした。
3. 種々のリグニン生合成遺伝子の発現を制御した一連の形質転換イネを用いたフィーディング実験から、リグニン生合成代謝経路の攪乱に伴うPAL-C4H経路およびPTAL経路の寄与の変化を明らかにした。

以上のように、本論文は、イネのリグニン生合成に関与するC4H遺伝子を同定するとともに、イネ科植物が特徴的に有するフェニルアラニンおよびチロシンから生じる並行したリグニン生合成経路の役割と相互作用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6年7月26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)