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学 位 論 文 要 旨
(Summary of the Doctoral Dissertation)

学位論文題目 (Dissertation Title)	The Role of Glycosylation in Modulating the Storage and Function of Volatile Organic Compounds: Case Studies of 1-Octen-3-ol and Linalool in Soybeans (配糖体化がダイズ 1-オクテン-3-オールとリナロールの貯蔵と機能を調節する)
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Glycosylation involves the enzymatic addition of sugar moieties to hydrophobic molecules, altering their physical and chemical properties. In plants, glycosyltransferases (GTs) catalyze this process, using activated sugar donors such as UDP-glucose to conjugate volatiles compounds. The glycosylation of 1-octen-3-ol and linalool results in glycosides like 1-octen-3-yl primeveroside (Pri) and linalyl vicianoside (Vic), respectively. 1-Octen-3-ol, often referred to as "mushroom alcohol," is a volatile organic compound (VOC) associated with fungal metabolism but is also synthesized in plants. It is a product of lipid oxidation mediated by a dioxygenase, which acts on linoleic acid to generate this compound in mushrooms. Known for its distinct fungal aroma, 1-octen-3-ol plays a role in plant-pathogen interactions and herbivore deterrence. In soybeans, it is implicated in signaling pathways that activate defense genes, making the plant less palatable to herbivores. Linalool, on the other hand, is a terpene alcohol emitted by plants under herbivory or stress. It serves dual functions: directly repelling herbivores and indirectly attracting parasitoids or predators of herbivores. Linalool biosynthesis involves the methylerythritol phosphate pathways, culminating in its production via linalool synthase enzymes. Its multifunctional nature makes it a cornerstone of plant defense strategies. Glycosylation of VOCs extends their ecological utility by modulating their roles in plant-herbivore and plant-environment interactions. Glycosides like 1-octen-3-yl Pri and linalyl Vic function as slow-release systems, ensuring a sustained emission of VOCs when needed. This strategy seems useful not only to deter herbivores but also to recruit beneficial organisms. Additionally, glycosylation may reduce autotoxicity, preventing the VOCs from damaging the plant itself while retaining their defensive efficacy against herbivores and pathogens. This balance between activity and safety underscores the evolutionary advantage of glycosylation as a regulatory mechanism.

Linalool is anticipated to have significant ecological roles. In this study, linalyl 6-*O*-α-arabinopyranosyl-β-D-glucopyranoside (linalyl β-vicianoside: LinVic) purified from soybean leaves was identified as LinVic by using liquid chromatography-mass spectrometry by using an authentic compound. High levels of LinVic were detected in leaves and sepals during soybean plant growth. The LinVic content did not significantly increase following methyl jasmonate treatment of the leaves, indicating that its synthesis is independent of the jasmonic acid signaling pathway. In addition to LinVic, soybean also contains 1-octen-3-yl primeveroside. I treated soybean leaves with vaporized

linalool and 1-octen-3-ol to determine whether the glycosylation system discriminates between these two volatile alcohols. Linalool treatment resulted in the accumulation of LinVic, while 1-octen-3-ol treatment caused little change in the amount of 1-octen-3-yl primeveroside, suggesting discrimination between these compounds. Linalool-treated soybean leaves exhibited increased resistance against common cutworms, indicating that LinVic may contribute to herbivore resistance.

It was previously found that soybean seeds contain 1-octen-3-yl β-primeveroside. To elucidate the physiological significance and the biosynthesis of 1-octen-3-ol in plants, changes in the amount of 1-octen-3-yl Pri during development of soybean plants was examined. A high 1-octen-3-yl Pri level was found in young developing green organs, such as young leaves and sepals. Treatment of soybean leaves with methyl jasmonates resulted in a significant increase in the amount of 1-octen-3-yl Pri; suggesting its involvement in defense responses. Although 1-octen-3-ol was below the detection limit in intact soybean leaves, mechanical damage to the leaves caused rapid hydrolysis of almost all 1-octen-3-yl Pri to liberate volatile 1-octen-3-ol. Under the same conditions, the other glycosides, including isoflavone glycoside and linalool diglycoside, were hardly hydrolyzed. Therefore, the enzyme system to liberate aglycone from glycosides in soybean leaves should have strict substrate specificity. 1-Octen-3-yl Pri might function as a storage form of volatile 1-octen-3-ol for immediate response against stresses accompanying tissue wounding.

The glycosylation of 1-octen-3-ol and linalool in soybeans exemplifies the sophistication of plant metabolic networks in modulating VOC functionality. By stabilizing, storing, and controlling the release of these compounds, glycosylation not only enhances their ecological roles but also ensures the plant's safety and efficiency in deploying chemical defenses. Exploring these pathways further could unlock new strategies for sustainable agriculture, leveraging the power of plant biochemistry to address modern challenges in pest management and crop protection. Through glycosylation, soybeans have evolved a remarkable mechanism to amplify the utility of volatile compounds, ensuring their survival and resilience in dynamic ecosystems. Understanding and harnessing this mechanism holds promise for both scientific discovery and practical applications in crop science.

(様式９号)

学位論文審査の結果及び最終試験の結果報告書

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論文題目	The Role of Glycosylation in Modulating the Storage and Function of Volatile Organic Compounds: Case Studies of 1-Octen-3-ol and Linalool in Soybeans (配糖体化がダイズ 1-オクテン-3-オールとリナロールの貯蔵と機能を調節する)

【論文審査の結果及び最終試験の結果】

本論文では、ダイズから 1-オクテン-3-オール、並びにリナロールの配糖体を精製し、共同研究者の協力を仰いでその構造をそれぞれ 1-オクテン-3-イルプリメベロシド、リナリルビシアノシドと決定した。その上で、それぞれの定量的分析系を確立し、ダイズの成長過程に伴うそれぞれの配糖体の蓄積量を調査し、ジャスモン酸処理によって 1-オクテン-3-イルプリメベロシドの蓄積が増加するのに対してリナリルビシアノシド量が増加しないことを確認した。1-オクテン-3-イルプリメベロシドは特に成熟種子の子葉に高濃度で蓄積しており、種子を水で膨潤させて破砕すると速やかに加水分解が進み、1-オクテン-3-オールが生成することを確認した。こうして生成した 1-オクテン-3-オールはダイズ加工食品のフレーバー特性に影響を与えるため、ダイズ種子の加工方法を工夫することでダイズ加工食品のフレーバー特性をコントロールできる可能性を示した。また、ダイズをリナロールの蒸気に曝すことでリナリルビシアノシドが増加することを発見した。こうした発見を通じて、1-オクテン-3-オールとリナロールの配糖体化が異なる機構によって進むことを示した。また、リナロール蒸気処理でリナリルビシアノシド蓄積量を増加させたダイズは植食者のハスモンヨトウ幼虫に対する抵抗性が増加することも示し、リナロールのような植物由来揮発性化合物を有効に使うことで環境負荷が少ない形で植物の抵抗性を高める技術への応用可能性を示した。こうした成果は本学の創成科学研究科博士論文としてふさわしいものであり、博士（生命科学）を授与するにふさわしい内容であることを確認した。

また、2025 年 2 月 4 日に公開の口頭試験を実施した。その中で JULIANO MWENDA NTORURU 君は自らの論文に記載の成果を丁寧に説明し、審査委員や他の参加者の質問に適切に答えたことから、本学創成科学研究科博士号を授与するに値すると判断した。