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授与した学位	博　士
専攻分野の名称	学　術
学位授与番号	博甲第　　７４０５　　号
学位授与の日付	２０２５年　９月　２５日
学位授与の要件	環境生命科学研究科　　農生命科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Involvement of myrosinases in stomatal closure in Arabidopsis （シロイヌナズナの気孔閉口へのミロシナーゼの関与）
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学位論文内容の要旨

This thesis comprises four chapters. Chapter 1 provides background information and outlines the research objectives. Stomatal pores, surrounded by pairs of specialized guard cells, serve as crucial gateways for regulating gas exchange and transpirational water loss. Guard cells respond to various biotic and abiotic stimuli, including plant hormones, light, drought, and pathogen attacks. Myrosinases are known to catalyze the hydrolysis of glucosinolates into bioactive compounds such as isothiocyanates (ITCs), which deter herbivores and pathogens. The two myrosinase isoforms, TGG1 and TGG2, are highly expressed in guard cells and have previously been found to function redundantly in guard cell signaling in response to abscisic acid (ABA) and methyl jasmonate (MeJA). Allyl isothiocyanate (AITC), a myrosinase product; chitosan (CHT), an elicitor; and salicylic acid (SA), a defense molecule, have all been shown to promote stomatal closure in *Arabidopsis*, a process accompanied by reactive oxygen species (ROS) production mediated by SHAM-sensitive peroxidases. In Chapter 1, AITC significantly induced stomatal closure in the wild type (WT) and in the single mutants *tgg1* and *tgg2*, but not in the *tgg1 tgg2* double mutant, indicating that TGG1 and TGG2 function redundantly in AITC-induced stomatal closure. However, AITC-induced ROS production was not impaired in the double mutant, consistent with observations for ABA- and MeJA-induced ROS responses in the same mutant. Similarly, AITC-induced NO accumulation occurred normally in all lines. AITC also triggered cytosolic alkalization in both WT and mutant lines. Additionally, AITC, ABA, and MeJA all induced $[Ca^{2+}]_{cyt}$ oscillations in both WT and *tgg1 tgg2* mutants. These findings indicate that while TGG1 and TGG2 are positive regulators of AITC-induced stomatal closure, they do not mediate early signaling events such as ROS and NO production, cytosolic alkalization, or calcium oscillations in guard cells. Chapter 3 **e**xplores the effects of CHT and SA. CHT and SA significantly induced stomatal closure in WT and in the single mutants *tgg1* and *tgg2*, but not in the *tgg1 tgg2* double mutant, indicating that TGG1 and TGG2 also function redundantly in CHT- and SA-induced stomatal closure. Application of reactive carbonyl species (RCS) scavengers inhibited stomatal closure in all genotypes in response to both CHT and SA. In contrast, treatment with acrolein, a representative RCS, induced stomatal closure in WT, *tgg1*, and *tgg2*, but not in the *tgg1 tgg2* mutant, suggesting that TGG1 and TGG2 are required for RCS-mediated stomatal signaling. Additionally, treatment with CHT induced ROS accumulation in guard cells, and this ROS production was unaffected by the application of RCS scavengers, indicating that ROS generation occurs independently of or upstream of RCS production in CHT signaling pathway. TGG1 and TGG2 are thus required for stomatal closure in response to both CHT and SA. Chapter 4 presents a summary of the entire thesis.

論文審査結果の要旨

高等植物において、気孔閉口は、ストレス応答の一つとして極めて重要である。シロイヌナズナのアブシシン酸誘導気孔閉口とジャスモン酸メチル誘導気孔閉口において、ミロシナーゼが関与していることが報告されているが、その機能は明らかにされていない。

本論文では、シロイヌナズナを材料として、ミロシナーゼの酵素反応の生成物であるイソチオシアネート誘導気孔閉口におけるミロシナーゼの関与について調べ、さらに、サリチル酸やキトサンが誘導する気孔閉口への関与について調査した。

イソチオシアネート誘導気孔閉口にもミロシナーゼが関与し、ミロシナーゼが酵素としてではなく、他の機能を有することを明らかにした。また、サリチル酸やキトサン誘導気孔閉口にも関与し、セカンドメッセンジャーである活性酸素種の生成酵素の違いによらず、その下流で孔辺細胞信号伝達経路に関与していることを明らかにした。

本研究内容は、学術的な価値のみならず、気孔運動に着目した植物（作物）生産制御のための技術の基礎となるものである。従って、本審査委員会は本論文が博士（学術）の学位論文に値すると判断した。