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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４７７９号 |
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| 学位授与の要件 | 自然科学研究科　バイオサイエンス専攻<br>（学位規則第５条第１項該当） |
| 学位論文の題目 | Roles of glucosinolate-myrosinase system in stomatal closure in Arabidopsis<br>（シロイヌナズナの気孔閉口におけるグルコシノレートーミロシナーゼ系の役割） |
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## 学位論文内容の要旨

**Introduction:**

Glucosinolates are plant secondary metabolites present in crucifer plants. Myrosinases (*β*-thioglucoside glucohydrolases, TGGs) are responsible for the degradation of glucosinolates, resulting in the formation of variety of products such as isothiocyanates (ITCs), nitriles, and thiocyanates. The myrosinase-glucosinolate system is involved in a range of biological activities because ITCs have repellent effect on herbivores and insects. However, nitriles and thiocyanates are not as bioactive as isothiocyanates and consequently evidences of their physiological roles in plants are limited. ITC including allyl isothiocyanate (AITC) induced stomatal closure in Arabidopsis. Whether other degradation products, 3-butenenitrile (3BN), and ethyl thiocyanate (ESCN) induce stomatal closure remain to be clarified. Moreover, myrosinases are involved in abscisic acid (ABA)- and methyl jasmonate (MeJA)-induced stomatal closure in Arabidopsis. But, it remains unknown whether myrosinases (TGG1 and TGG2) function in AITC-induced stomatal closure.

**Objectives and methods:**

I investigated the effects of the degradation products, AITC, 3BN, and ESCN on stomatal movement, ROS production, and $[Ca^{2+}]_{cyt}$ oscillation in Arabidopsis in order to clarify the roles of degradation products of glucosinolate in Arabidopsis. I also investigated function of myrosinases in guard cell signaling and also investigated whether one of myrosinase products, AITC, induces stomatal closure in the *tgg* mutants. Arabidopsis wild type, ecotype Columbia (Col-0), transgenic Col-0 plants expressing $Ca^{2+}$ reporter yellow cameleon3.6 (YC3.6), and *tgg1 tgg2* double mutant plants expressing YC3.6 were used in this study. Stomatal apertures in the epidermal tissues were observed under a microscope. Production of ROS and cytosolic alkalization in guard cells were measured under a fluorescence microscope using 2',7'-dichlorodihydrofluorescein diacetate ($H_2$DCF-DA) and 2′,7′-bis-(2-carboxyethyl)-5,(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM), respectively. $[Ca^{2+}]_{cyt}$ oscillations in guard cells was measured using a $Ca^{2+}$-sensing fluorescent protein, YC3.6.

**Results and discussion:**

The degradation products induced stomatal closure in a dose dependent manner in wild type and *atrbohD atrbohF* mutant. The degradation product-induced stomatal closure and ROS production were completely inhibited by catalase (CAT), and SHAM but not by diphenyleneiodonium chloride (DPI) or the *atrbohD atrbohF* mutation, suggesting that the degradation products induce stomatal closure *via* ROS production mediated by peroxidases but not by NADPH oxidases. Moreover, the degradation products induced $[Ca^{2+}]_{cyt}$ oscillation in guard cells, which were inhibited by SHAM. These results suggest that the degradation products induce stomatal closure accompanied by extracellular ROS production mediated by cell wall peroxidases, intracellular ROS accumulation, and $[Ca^{2+}]_{cyt}$ oscillation in Arabidopsis. AITC induced stomatal closure in the *tgg1* and the *tgg2* single mutants but not in the *tgg1 tgg2* double mutant, suggesting that TGG1 and TGG2 cooperatively function in stomatal closure in response to AITC. AITC also induced ROS production, cytosolic alkalization, and $[Ca^{2+}]_{cyt}$ oscillation in guard cells of the *tgg1*, *tgg2*, *tgg1 tgg2* mutants as well as wild-type plants. These results suggest that TGG1 and TGG2 positively function in AITC-induced stomatal closure but are not involved in AITC-induced ROS production, cytosolic alkalization, and $[Ca^{2+}]_{cyt}$ oscillation in guard cells.

## 論文審査結果の要旨

本論文は，陸上植物にとって重要な生理的現象である気孔閉口において，グルコシノレートーミロシナーゼ系がどのように関与しているかを，シロイヌナズナを用いて明らかにしようとしたものである。

初めに，傷害を受けた際に，ミロシナーゼが触媒して生成する種々のグルコシノレート分解産物が気孔閉口を誘導することを見つけ，傷害による水損損失の増加を気孔閉口によって抑制していることを明らかにした。

次に，アブシジン酸やジャスモン酸メチル誘導気孔閉口が観察されないミロシナーゼ二重変異体（*tgg1 tgg2*）において，グルコシノレート分解物誘導気孔閉口が観察されないことを明らかにし，気孔閉口において，ミロシナーゼがグルコシノレートの分解以外にも機能を持っていることを明らかにした。

以上の結果から，グルコシノレートーミロシナーゼ系が気孔閉口にも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。