氏　　名	MD. MOSHIUL ISLAM
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５０５４号
学位授与の日付	平成２６年　９月３０日
学位授与の要件	自然科学研究科　バイオサイエンス専攻 （学位規則第５条第１項該当）
学位論文の題目	Involvement of reactive carbonyl species in stomatal movements （気孔運動への活性カルボニル種の関与）
論文審査委員	教授　村田　芳行　　准教授　中村　宜督　　教授　木村　吉伸

学位論文内容の要旨

Introduction

Abscisic acid (ABA)- and methyl jasmonate (MeJA)-induced stomatal closure is accompanied by production of reactive oxygen species (ROS) mediated by plasma membrane NAD(P)H oxidases while salicylic acid (SA)-induced stomatal closure is accompanied by peroxidase-mediated extracellular ROS production and intracellular ROS accumulation. However, in spite of the importance of ROS in guard cell signaling, the mechanism of how the generating ROS is transduced into downstream reactions in guard cells is unknown. A variety of stresses induce overproduction of ROS in plants and the accumulated ROS oxidizes lipids, especially polyunsaturated fatty acids (PUFA), resulting in production of reactive compounds including aldehydes, ketones, and hydroxyl acids. Aldehydes and ketones containing *α,β*-unsaturated carbonyl structure are termed as reactive carbonyl species (RCS). Accumulated RCS impairs growth of plants because RCS is toxic to plants. Alkenal reductase (AER), aldehyde dehydrogenase (ALDH), aldo-keto reductase (AKR), and aldehyde reductase (ALR) detoxify RCS and overexpressing AER, ALDH, AKR, and ALR improves stress tolerance in plants. However, information about signal transduction mediated by RCS is limited.

Objectives and methods

I investigated the involvement of RCS in ABA-, MeJA-, and SA-induced stomatal closure, and acrolein inhibition of light-induced stomatal opening using wild-type tobacco (*Nicotiana tabacum*) (WT), and transgenic tobacco overexpressing Arabidopsis (*Arabidopsis thaliana*) alkenal reductase (AER-OE), *A. thaliana* ecotype Columbia-0 and scavengers of RCS. RCS was extracted from the epidermal tissues and derivatized with 2,4-dinitrophenylhydrazine (DNPH) and then identified and quantified by reverse-phase HPLC. Stomatal apertures in the epidermal tissues were observed under a microscope. Production of ROS in guard cells were measured using 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA). Arabidopsis expressing YC3.6 were used for the measurement of cytosolic free-calcium concentrations ($[Ca^{2+}]_{cyt}$) elevation in guard cell. Plasma membrane Ca^{2+}-permeable channels (I_{Ca}) and plasma membrane inward-rectifying K^+ (K^+_{in}) channels were measured using patch-clamp technique.

Results and discussion

Both overexpression of the RCS scavenging enzyme 2-alkenal reductase (AER) and application of RCS scavengers, carnosine and pyridoxamine, inhibited ABA- and hydrogen peroxide (H_2O_2)-induced stomatal closure and RCS accumulation but not ABA-induced H_2O_2 production. Acrolein- and HNE-induced stomatal closure was inhibited in AER-OE plants and also by the application of carnosine and pyridoxamine. Moreover, acrolein significantly activated I_{Ca} channels and more effectively initiated $[Ca^{2+}]_{cyt}$ elevation and induced stomatal closure than H_2O_2 did. Furthermore, ABA-suppressed K^+_{in} currents was inhibited in AER-OE. These results indicate that production of RCS following ROS production and regulation of $[Ca^{2+}]_{cyt}$ elevation by RCS are important mechanisms for ABA-induced stomatal closure. MeJA induced stomatal closure in the WT but not in the AER-OE. MeJA-induced stomatal closure in WT was inhibited by RCS scavengers, carnosine and pyridoxamine. MeJA induced H_2O_2 production in guard cells of both WT and AER-OE, and H_2O_2 production was not suppressed by the application of RCS scavengers. These results suggest that RCS is involved in MeJA-induced stomatal closure and function downstream of ROS production. SA induced stomatal closure in the WT but not in the AER-OE. SA-induced stomatal closure in WT was inhibited by RCS scavengers, carnosine and pyridoxamine. Moreover, SA induced H_2O_2 accumulation in guard cells of both WT and AER-OE. These results suggest that RCS mediates SA-induced stomatal closure and function downstream of ROS accumulation. Acrolein inhibited light-induced stomatal opening in a dose-dependent manner. Acrolein at 100 µM inhibited plasma membrane inward-rectifying potassium (K_{in}) channels in guard cells. Acrolein at 100 µM inhibited K_{in} channel KAT1 expressed in a heterologous system using *Xenopus leaves* oocytes. These results suggest that acrolein inhibits light-induced stomatal opening through inhibition of K_{in} channels in guard cells.

論文審査結果の要旨

気孔開閉は、陸上植物にとって重要な生理的現象である。本論文は、植物ホルモンであるアブシジン酸やジャスモン酸メチル、サリチル酸が誘導する気孔閉口における信号伝達経路の新たな因子を明らかにしようとしたものである。

初めに、アブシジン酸が誘導する気孔閉口において、活性酸素種産生の下流で活性カルボニル種が産生し、この活性カルボニル種がさらに下流の細胞質内カルシウム上昇を誘導することを明らかにした。

次に、ジャスモン酸メチルやサリチル酸が誘導する気孔閉口においても、活性カルボニル種が関与することを明らかにし、気孔閉口誘導信号伝達経路において、活性カルボニル種の関与を明らかにした。

さらに、活性カルボニル種が内向き整流性カリウムチャネルの阻害を介して、光誘導気孔開口を阻害することを明らかにした。

以上の結果から、活性カルボニル種が気孔運動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。