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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４４３１号
学位授与の日付	平成２３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	Regulation of methyl jasmonate signaling in *Arabidopsis* guard cells （シロイヌナズナ孔辺細胞のジャスモン酸メチルシグナリングの制御）
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学位論文内容の要旨

Introduction:

Guard cells respond to a variety of external and internal stimuli such as light, drought, CO_2 concentration, external Ca^{2+}, pathogen attack, and phytohormones, abscisic acid (ABA), methyl jasmonate (MeJA), and salicylic acid (SA), to control transpirational water loss and innate immunity *via* stomatal movement. The volatile phytohormone MeJA induces stomatal closure similar to ABA, and MeJA signaling shares several signal components with ABA signaling in guard cells. MeJA as well as ABA induce the elevation and oscillation of cytosolic free-calcium concentrations ($[Ca^{2+}]_{cyt}$) in guard cells, resulting in stomatal closure. So far, ABA signal transduction has been extensively studied, but little is known about MeJA signal transduction.

Objectives and methods:

I investigated involvement of cyclic adenosine 5'-diphosphoribose (cADPR) and cyclic guanosine 3',5'-monophosphate (cGMP) in MeJA-induced stomatal closure in *Arabidopsis thaliana* (Col-0) using an inhibitor of cADPR synthesis, nicotinamide (NA), and an inhibitor of cGMP synthesis, LY83583 (LY, 6-anilino-5,8-quinolinedione). Mechanism of MeJA-induced $[Ca^{2+}]_{cyt}$ oscillation in guard cells was examined using K252a (a broad-range protein kinase inhibitor) and okadaic acid (OA, a protein phosphatase 1 and 2A inhibitor). I also investigated involvement of endogenous ABA in MeJA-induced stomatal closure using an inhibitor of ABA biosynthesis, fluridon (FLU), and an ABA-deficient *Arabidopsis* mutant, *aba2-2*. *Arabidopsis* wild type, ecotype Columbia (Col-0), transgenic Col-0 plants expressing Ca^{2+} reporter Yellow Cameleon 3.6 (YC3.6), and *aba2-2* plants expressing YC3.6 were used in this study. Stomatal apertures in the epidermal tissues were observed under a microscope. Production of reactive oxygen species (ROS) and nitric oxide (NO) production in guard cells were measured using 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) and 4,5-diaminofluorescein-2 diacetate (DAF-2DA), respectively. $[Ca^{2+}]_{cyt}$ in guard cells was analyzed using YC3.6 technique. Gene expression analysis was performed by reverse transcription-PCR.

Results and discussion:

Treatment with NA and LY inhibited MeJA-induced stomatal closure. NA inhibited MeJA-induced production of ROS and NO production and NA and LY inhibited MeJA-elicited $[Ca^{2+}]_{cyt}$ oscillation in guard cells and expression of an MeJA-inducible gene, *VEGETATIVE STORAGE PROTEIN1 (VSP1)*, in leaves. These results suggest that cADPR and cGMP are involved in MeJA signal transduction in *Arabidopsis* guard cells. K252a abolished MeJA-induced stomatal closure and reduced MeJA-elicited $[Ca^{2+}]_{cyt}$ oscillation. The protein phosphatase inhibitor OA, on the other hand, did not inhibit these processes. These results suggest that MeJA signaling involves activation of K252a-sensitive protein kinases upstream of $[Ca^{2+}]_{cyt}$ oscillation but not activation of an OA-sensitive protein phosphatase in guard cells of *A. thaliana* ecotype Columbia. Pretreatment with FLU, inhibited MeJA-induced stomatal closure but not ABA-induced stomatal closure in wild-type plants. The *aba2-2* mutation impaired MeJA-induced stomatal closure but not ABA-induced stomatal closure. In wild-type guard cells, FLU inhibited MeJA-induced $[Ca^{2+}]_{cyt}$ elevation but not ABA-induced $[Ca^{2+}]_{cyt}$ elevation. The *aba2-2* mutation did not affect ABA-elicited $[Ca^{2+}]_{cyt}$ elevation but suppressed MeJA-induced $[Ca^{2+}]_{cyt}$ elevation. In the *aba2-2* mutant, MeJA did not induce *VSP1* expression. In wild-type leaves, FLU inhibited MeJA-induced *VSP1* expression. Pretreatment with ABA at 0.1 μM, which is not enough concentration to evoke ABA responses in wild type, rescued the observed phenotypes of the *aba2-2* mutant. Finally, I found that in wild-type leaves, MeJA stimulates expression of *9-CIS-EPOXYCAROTENOID DIOXYGENASE3*, which encodes a crucial enzyme in ABA biosynthesis. These results suggest that endogenous ABA could be involved in MeJA signal transduction and lead to stomatal closure in *Arabidopsis* guard cells.

論文審査結果の要旨

本論文は、植物ホルモンの一つであるジャスモン酸メチルが誘導する気孔閉口が、どのようなシグナル伝達経路を介して行われているかを明らかにしようとしたものである。

初めに、ジャスモン酸メチルシグナリングにおいて、サイクリック ADP リボースやサイクリック GMP が、ジャスモン酸メチルが誘導する細胞内カルシウムオシレーションの上流で、関与していることを明らかにした。

次に、ジャスモン酸メチルシグナリングにおいて、タンパク質キナーゼやタンパク質ホスファターゼが関与していることを明らかにし、また、野生株の種類によって、タンパク質ホスファターゼの関与に違いがあることを見出した。

さらに、ジャスモン酸メチルが誘導する気孔閉口には、アブシジン酸が必要であることを明らかにした。

以上の結果から、ジャスモン酸メチルが誘導する気孔閉口におけるジャスモン酸メチルシグナリングの制御機構に明らかにした。

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。