氏　　名	MOHAMMAD ISSAK
授与した学位	博　士
専攻分野の名称	学　術
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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 （学位規則第５条第１項該当）
学位論文の題目	Regulation of salicylic acid, yeast elicitor, and chitosan signaling in Arabidopsis guard cells （シロイヌナズナ孔辺細胞におけるサリチル酸、酵母エリシター、キトサンシグナリングの制御）
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) induce reactive oxygen speices (ROS) production, cytosolic alkalization, the elevation and oscillation of cytosolic free-calcium concentrations ($[Ca^{2+}]_{cyt}$), and activation of S-type anion channels in guard cells. Salicylic acid (SA) induces stomatal closure accompanied by peroxidase-mediated extracellular ROS production and intracellular ROS accumulation. Both yeast elicitor (YEL) and chitosan (CHT) induce stomatal closure accompanied by peroxidase-mediated intracellular ROS accumulation, cytosolic alkalization, $[Ca^{2+}]_{cyt}$ oscillation, and activation of S-type anion channel in guard cells. ABA, MeJA, and SA are major phytohormones of cellular signal transduction pathways mediating various biotic and abiotic stress responses. It has been reported that endogenous ABA is involved MeJA-induced stomatal closure and MeJA priming is required for AITC-induced stomatal closure. However, it has not yet been elucidated whether these phytohormones involved in SA, YEL, and CHT signaling in Arabidopsis guard cells.

Objectives and Methods

I investigated SA-, YEL-, and CHT-induced stomatal closure using Arabidopsis wild-type, ecotype Columbia-0 (Col-0), *aba2-2*, *aos*, *nahG*, and the ABA inhibitor fluridon (FLU). Stomatal apertures in the epidermal tissues were observed under a microscope. Production of intracellular ROS and extracellular ROS in guard cells were measured using 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA), 3,3´-diaminobenzidine tetrahydrochloride hydrate (DAB), and nitro blue tetrazolium (NBT), respectively. Cytosolic alkalization in guard cell was measured using 2´,7´-bis-(2-carboxyethyl)-5,(6)-carboxyfluoresceinacetoxymethyl (BCECF-AM). The wild-type and *nahG* plants expressing yellow cameleon 3.6 were used for the measurement of guard cell $[Ca^{2+}]_{cyt}$ oscillation.

Results and Discussion

Salicylic acid (SA), yeast elicitor (YEL), and chitosan (CHT) induced stomatal closure in Arabidopsis wild-type and *aba2-2* plants, induced stomatal closure in fluridon-treated wild-type plants, and induced stomatal closure in *aos* mutants. These results suggest that neither endogenous abscisic acid nor endogenous jasmonic acid is involved in SA-, YEL-, or CHT-induced stomatal closure. YEL and CHT induced stomatal closures in the Arabidopsis wild-type plants but not in the *nahG* transgenic lines. On the other hand, SA at 50 µM after the YEL or CHT application induced stomatal closure in the *nahG* transgenic lines. YEL and CHT induced extracellular reactive oxygen species (ROS) production in the whole leaves and intracellular ROS accumulation in the guard cells of wild-type plants but not in the *nahG* plants. However, application of SA at 50 µM in the presence of YEL or CHT induced extracellular ROS production in the whole leaves and intracellular ROS accumulation in the guard cells of *nahG* plants. SA, YEL, and CHT induced cytosolic alkalization in the wild-type guard cells but not in the *nahG* guard cells. In contrast, SA at 50 µM just after YEL or CHT application induced cytosolic alkalization in the *nahG* guard cells. YEL and CHT induced $[Ca^{2+}]_{cyt}$ elevation and oscillations in the wild-type guard cells. On the other hand, YEL- and CHT-induced $[Ca^{2+}]_{cyt}$ elevations and oscillations were impaired in the *nahG* guard cells. However, SA at 50 µM just after YEL or CHT application induced and $[Ca^{2+}]_{cyt}$ elevations in the *nahG* guard cells. SA at 50 µM, which is not enough concentration to evoke SA responses in the wild-type plants, had no effect on stomatal closure, ROS production, and cytosolic alkalization in the *nahG* guard cells. Taken together, my results suggest that endogenous SA could be synthesized by the YEL and CHT and is involved in YEL and CHT signal transduction leading to stomatal closure in Arabidopsis.

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，サリチル酸，酵母エリシター，キトサンが誘導する気孔閉口における信号伝達経路を明らかにしようとしたものである。

初めに，サリチル酸，酵母エリシター，キトサンが誘導する気孔閉口における信号伝達経路が内因性のアブシジン酸やジャスモン酸に依存しないことを変異体や生合成阻害剤を用いて明らかにした。

次に，酵母エリシター，キトサンが誘導する気孔閉口に内因性のサリチル酸が必要であることをサリチル酸欠損変異体を用いて明らかにした。

以上の結果から，サリチル酸，酵母エリシター，キトサンの信号伝達経路を相互作用が存在す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。