学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Roles of reactive carbonyl species in stomatal closure of *Arabidopsis thaliana*

シロイヌナズナの気孔閉口における活性カルボニル種の役割

学位論文の要旨 Abstract of Thesis

The epidermis of leaves is characterized by specialized guard cells, which form stomatal aperture to regulate gas exchange and water loss. Guard cells modulate stomatal aperture in response to numerous biotic and abiotic signaling stimuli. Phytohormone abscisic acid (ABA) is synthesized in plant cells under water deficit and is known to induce stomatal closure to reduce water loss. ABA-induced stomatal closure is accompanied by production of reactive oxygen species (ROS) and that ROS production is catalyzed by the plasma membrane NADPH oxidases AtrbohD and AtrbohF in Arabidopsis. 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, and ketones. Aldehydes and ketones containing α,β-unsaturated carbonyls such as acrolein and 4-hydroxy-*E*-2-nonenal (HNE) are termed as reactive carbonyl species (RCS) because of their high reactivity and high cytotoxicity. Due to this reactivity, RCS at low concentration may function as signaling molecules. In plants, there is a positive correlation between RCS accumulation and damage under stress conditions. The RCS functions downstream of ROS production in ABA signal pathway in guard cells. However, signal transduction mechanisms downstream of ROS in ABA signal pathway in guard cells still unclear.

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In Chapter 2, In order to clarify the ABA signal pathway, I investigated cytosolic alkalization and cytosolic free calcium concentration $[Ca^{2+}]_{cyt}$ elevation in Arabidopsis guard cells during RCS-induced stomatal closure of wild type (WT), *abi1-1*, *abi2-1*, and *ost1-3* mutants. I found that RCS induced stomatal closure and triggered cytosolic alkalization in the WT but not in the *abi1-1*, the *abi2-1*, and the *ost1-3* mutants. Acrolein induced $[Ca^{2+}]_{cyt}$ elevation in guard cells of the WT plant but not in the *abi1-1*, *abi2-1*, and *ost1-3* mutants. Exogenous Ca^{2+} induced stomatal closure, cytosolic alkalization, and $[Ca^{2+}]_{cyt}$ elevation in all mutants as well as in the WT. An intracellular acidifying agent, butyrate inhibited acrolein-induced stomatal closure, cytosolic alkalization, and $[Ca^{2+}]_{cyt}$ elevation in guard cells of the WT plant. These results suggest that cytosolic alkalization and $[Ca^{2+}]_{cyt}$ elevation are the signal components of RCS signaling and cytosolic alkalization along with $[Ca^{2+}]_{cyt}$ elevation function downstream RCS signaling in Arabidopsis guard cells.

In Chapter 3, I investigated the involvement of myrosinase in RCS signaling in Arabidopsis guard cells. Myrosinase (thioglucoside glucohydrolase, EC 3.2.1.147, TGG) is a highly abundant protein in Arabidopsis guard cells and TGG1 and TGG2 redundantly function in abscisic acid (ABA)- and methyl jasmonate (MeJA)-induced stomatal closure. I found that acrolein induced stomatal closure and triggered cytosolic alkalization in the wild type (WT), the *tgg1-3* single mutant, and the *tgg2-1* single mutant, but not in the *tgg1-3 tgg2-1* double mutant. Acrolein induced $[Ca^{2+}]_{cyt}$ elevation in guard cells of the WT plant but not in the *tgg1-3 tgg2-1* double mutant. Exogenous Ca^{2+} induced stomatal closure and cytosolic alkalization not only in WT but also in all the mutants. I also found that exogenous Ca^{2+} elicited $[Ca^{2+}]_{cyt}$ elevation in guard cells of the WT and the *tgg1-3 tgg2-1*. Acrolein- and Ca^{2+}-induced stomatal closure were inhibited by an intracellular acidifying agent, butyrate, a Ca^{2+} chelator, EGTA, and a Ca^{2+} channel blocker, $LaCl_3$. These results suggest that TGG1 and TGG2 redundantly function not between ROS production and RCS production but downstream of RCS production in ABA signal pathway in Arabidopsis guard cells.