学位論文の要旨

Abstract of Thesis

研究科 School	Graduate School of Environmental and Life Science
専　攻 Division	Agricultural and Life Science
学生番号 Student No.	77D21656
氏　名 Name	Israt Jahan

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）

Involvement of reactive carbonyl species and glutathione in chitosan-induced stomatal closure

（キトサン誘導気孔閉口への活性カルボニル種とグルタチオンの関与）

学位論文の要旨 Abstract of Thesis

The epidermis of leaves is characterized by specialized guard cells, which form stomatal apertures to regulate gas exchange and transpiration. Guard cells regulate stomatal pore apertures via integration of both endogenous hormonal stimuli and environmental signals. An elicitor, chitosan (CHT), is a deacylated derivative of chitin, which is a major component of fungal cell walls, arthropods exoskeletons, and crustacean shells. Chitosan improves growth and yield performance and induces the expression of a variety of genes involved in plant defense responses. Chitosan also induces stomatal closure in *Solanum lycopersicum*, *Commelina communis*, *Pisum sativum*, and *Arabidopsis thaliana*. Abscisic acid (ABA)- and methyl jasmonate (MeJA)-induced stomatal closure is accompanied by plasma membrane NAD(P)H oxidases-mediated reactive oxygen species (ROS) production, while salicylic acid (SA)- and CHT-induced ROS production is mediated by salicylhydroxamic acid (SHAM)-sensitive peroxidases. A variety of stresses induce overproduction of ROS in plants. The accumulated ROS oxidize membrane lipids, especially polyunsaturated fatty acids (PUFA) to form lipid peroxides. Decomposition of lipid peroxides via enzymatic and non-enzymatic radical-catalyzed reactions generate reactive compounds including aldehydes, ketones and hydroxyl acids. Aldehydes and ketones comprising α,β-unsaturated bonds are termed as reactive carbonyl species (RCS) because of their high electrophilicity and reactivity. Reactive carbonyl species function as intermediate downstream signaling components of ROS production in ABA and MeJA signaling in guard cells. However, the involvement of RCS in CHT-induced stomatal closure is still unknown. Glutathione (GSH) is the most abundant non-protein thiol compound in plants and regulates many physiological functions via the redox state of GSH pools. It has been reported that GSH negatively regulates ABA-, MeJA-, and SA-induced stomatal closure. However, the involvement of GSH in CHT-induced stomatal closure is still unknown.

氏名 Name	Israt Jahan

In chapter 2, I investigated the involvement of RCS in CHT-induced stomatal closure using *A. thaliana* ecotype Columbia-0, wild-type tobacco (*Nicotiana tabacum*) (WT), and transgenic tobacco overexpressing *A. thaliana* 2-alkenal reductase (AER-OE), and scavengers of RCS, carnosine and pyridoxamine. I found that CHT-induced stomatal closure was impaired in the tobacco AER-OE plants. Application of RCS scavengers, carnosine and pyridoxamine, significantly inhibited CHT-induced stomatal closure in the Arabidopsis and tobacco WT plants. Chitosan significantly increased RCS accumulation in WT tobacco and Arabidopsis plants but not in the AER-OE plants. I also found that overexpression of AER or the application of RCS scavengers did not suppress chitosan-induced ROS production. Moreover, treatment with the peroxidase inhibitor SHAM significantly inhibited CHT-induced RCS accumulation in Arabidospsis and tobacco WT plants. These results suggest that RCS is involved in CHT-induced stomatal closure and functions downstream of ROS in CHT signaling in guard cells.

In chapter 3, I investigated the involvement of GSH in CHT-induced stomatal closure using GSH-deficient mutants, *cad2-1 and ch1-1*, a GSH-decreasing chemical, 1-chloro-2,4-dinitrobenzene (CDNB), and a GSH-increasing agent, glutathione monoethyl ester (GSHmee). Chitosan significantly induced stomatal closure and decreased GSH in guard cells of the *A. thaliana* wild-type plants. Depletion of GSH by the *cad2-1* and *ch1-1* mutations and by CDNB enhanced CHT-induced stomatal closure. Treatment with GSHmee restored the GSH level in the guard cells of the *cad2-1* and *ch1-1* and complemented the stomatal phenotype of the mutants. These results suggest that GSH negatively regulates CHT-induced stomatal closure in *A. thaliana*. Taken together, it can be concluded that RCS is involved in CHT-induced stomatal closure, which is negatively regulated by glutathione.