論文の内容の要旨

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氏名　鹿内 勇佑
指導教員　藤原 徹

論文題目

Molecular mechanisms of adaptation to low calcium conditions in *Arabidopsis thaliana*
(シロイヌナズナにおける低カルシウム条件に対する適応の分子機構)

Plants are basis of existence of humanity. In a historical view, improvement of agriculture has preceded economic growth. Understanding of plant physiology and genetics is essential to make the agriculture further efficient in the future, thus, I believe that accumulation of knowledge on plant contribute to promotion of human welfare.

Plant growth depends on absorption of essential elements from soils and photosynthesis in leaves. Insufficient supply of essential elements causes deficiency symptoms negatively affecting agriculture. Sixteen essential elements including calcium (Ca) are described in plants. In plants, Ca is mainly transported from roots to shoots through xylem along transpiration stream. Transpiration rate per leaf is higher in old leaves and lower in new and small leaves, and also in organs where no or little stomata is, such as fruits. In addition, Ca is difficult to be translocated through phloem because of strong binding to pectin in cell wall. Due to these characteristics, even when Ca in soil is sufficient, Ca deficient symptoms often appear in new tissues accompanying necrosis: blossom-end rot in tomato; tip burn in Chinese cabbage. These deficiency symptoms reduce their commercial values. Breeding of low-Ca tolerant crops is a possible and promising solution for this problem, however, knowledge on low-Ca adaptation mechanisms in plants required for efficient molecular breeding is still limited.

In my thesis, I clarified a molecular mechanism of low-Ca adaptation in plants through genetical and physiological analysis of low-Ca sensitive mutants in *Arabidopsis thaliana*. In Chapter 1, I revealed that synthesis of callose, one of the cell wall polysaccharide, prevented cell death under low-Ca in *A. thaliana*. I found that the wild-type plants accumulated callose in response to low-Ca condition, depending on *Glucan synthase like* (*GSL*) *10*. I also demonstrated that the inhibition of callose synthesis enhanced cell death lesion. From these results, I established that callose synthesis is indispensable process to prevent cell death for low-Ca adaptation. In Chapter 2, I clarified that redundant contributions of several *GSL* genes were required for the suppression of cell death and adaptation to low-Ca. I found that *gsl1-5* and *gsl8-11* mutants also exhibited the reduced callose accumulation under low-Ca. Further, I demonstrated that the *GSL1* and *GSL10* additively contributed to suppression of cell death under low-Ca. These results established that several *GSL* genes contribute to the ectopic callose accumulation, suppression of cell death, and low-Ca adaptation in *A. thaliana*. In Chapter 3, I demonstrated a potential involvement of defense responses in the development of cell death under low Ca.

Chapter 1: Callose synthesis suppresses cell death induced by low calcium condition in Arabidopsis

Necrosis in new leaves is one of the characteristics of Ca deficiency symptoms in plants. Mechanisms of necrosis induced by low-Ca condition or adaptation remains unclear.

A low-Ca sensitive mutant, *lcs3*, cannot develop true leaf under low-Ca. Before my study in Ph.D., it had been demonstrated in the laboratory of Plant Nutrition and Fertilizers at the University of Tokyo that the causal gene of *lcs3* (hereafter referred to as *gsl10-5*) is *GSL10*. In my master thesis, I have demonstrated that *A. thaliana GSL10* complemented a yeast β-1,3 glucan (callose) synthase mutant, establishing that *GSL10* encodes callose synthase.

In this Ph.D. study, I performed detailed physiological characterization and transcriptome analysis of *gsl10-5*. I characterized the cell death pattern in new leaves, observation of callose, and RNA sequence analysis. Observation of cell death by trypan blue staining revealed that *gsl10-5* developed cell death in new leaves under a low-Ca condition and the introduction of *GSL10* alleviated cell death phenotype in *gsl10-5*, showing that *GSL10* has a function of prevention of cell death under low-Ca conditions. Callose staining with aniline blue revealed that the wild-type plants accumulated ectopic callose in response to low Ca and that *gsl10-5* accumulated less callose than the wild-type plants. Callose accumulation in *gsl10-5* was recovered by introduction of the wild-type gene, showing that *GSL10* is a responsible gene of the ectopic callose accumulation induced by low Ca. These results suggest that callose synthesis is required for the adaptation to low Ca and prevention of cell death. To test this, the wild-type plants were grown under low-Ca condition in the presence of callose synthase inhibitors, 2-deoxy-D-glucose (DDG) or caspofungin. Shoot growth of the wild-type plants under low Ca with DDG

or caspofungin were inhibited comparable to that of *gsl10-5* under low-Ca condition. Application of DDG enhanced the cell death and reduced callose accumulation in the wild type under low-Ca condition. These results showed that callose synthesis is required for the prevention of cell death under low-Ca. To get insight into the transcriptome change(s) in GSL10-mediated low-Ca adaptation, RNA-sequencing analysis was performed. The results showed similar expression profiles between wild-type plants treated with low Ca and *gsl10-5* grown under normal condition. Especially, the expressions of genes related to cell wall and defense responses are commonly altered, suggesting the requirement of *GSL10* for the alleviation of both cell wall damage and defense responses caused by low Ca. These results demonstrate that callose synthesis is essential for the prevention of cell death and is a key part of plant strategy to survive under low-Ca condition.

Chapter 2: Contributions of *GSL* family to low-Ca adaptation in *A. thaliana*

Low-Ca sensitive mutants, *lcs4*, *lcs5* and *lcs6* cannot develop true leaves under low Ca as observed in *gsl10-5*. Before my study in Ph.D., in the laboratory, it had been revealed that the causal gene of *lcs4* and *lcs5* is *GSL1* (*lcs4* and *lcs5* are hereafter referred to as *gsl1-5* and *gsl1-6*, respectively) and that of *lcs6* is *GSL8* (*lcs6* is hereafter referred to as *gsl8-11*). It had also been demonstrated that *GSL1* and *GSL8* complemented a yeast β-1,3 glucan synthase mutant, suggesting *GSL1* and *GSL8* encodes a functional callose synthase.

In my Ph.D. study, I performed detailed physiological characterization of the *gsl* mutants. I analyzed that the contributions of *GSL* genes to the low-Ca adaptation, the ectopic callose accumulation, and the suppression of cell death under low-Ca condition. To test whether *GSL1* and *GSL8* contribute to the callose accumulation under low Ca, I performed callose staining. *gsl1-5* and *gsl8-11* mutants showed the reduced ectopic callose accumulation compared to the wild-type plants, showing that callose accumulation is positively correlated with the adaptation to low-Ca condition. Under the low-Ca condition, double mutant of *gsl1-5* and *gsl10-5* showed enhanced growth inhibition and cell death compared to its parental single mutant lines. In the *A. thaliana* genome, 12 genes are annotated as putative callose synthase. I checked the low-Ca sensitivity of T-DNA insertion lines of other 9 members of *GSL* genes and *GSL11* T-DNA line showed sensitivity to low Ca. These results suggest that the callose synthesized by GSL proteins additively contributes to the tolerance and the prevention of cell death under low-Ca in *A. thaliana*.

Chapter 3: Analysis of the involvement of callose in a low-Ca sensitive mutant *lcs7*

Before my study in Ph.D., in the laboratory, it had been revealed that a low-Ca sensitive mutant *lcs7* exhibits growth inhibition under low-Ca condition similar to that of *gsl* mutants, and the low-Ca

sensitivity of *lcs7* is caused by a gain-of-function mutation in a transcription regulation factor. From microarray analysis, it had been shown that callose degrading enzyme genes was up-regulated in *lcs7*, implying the possibility that the reduction of callose is the cause of low-Ca sensitivity of *lcs7*. In addition, it had been shown that double mutants between *lcs7* and *gsl10-5* or *gsl8-11* showed severe growth inhibition compared to single mutants.

In this Ph.D. study, I performed callose staining to test whether less callose accumulation is related with low-Ca tolerance in *lcs7* as is observed in *gsl* mutants. There was no significant difference between the wild-type plants and *lcs7*, suggesting that the low-Ca sensitivity of *lcs7* is not caused by the reduction of callose and the enhanced low-Ca sensitivity of double mutant between *lcs7* and *gsl* mutants is caused by additive effects of different low-Ca adaptation mechanisms. To speculate the reason for low-Ca sensitivity of *lcs7*, I re-analyzed the microarray data and performed gene ontology analysis. The results showed that the genes related to defense response and cell wall are enriched in differentially expressed genes in *lcs7*. These results suggest that the proper transcription regulation of defense responses and cell wall is crucial for the prevention of cell death under low Ca.

In this Ph.D. thesis, I concluded that *A. thaliana* adapts to low-Ca conditions by synthesizing callose, and that redundant contributions of *GSL* family is required for the suppression of the cell death. In addition, I suggested the involvement of defense response in cell death under low Ca. I believe this knowledge will contribute in the future to the breeding of Ca deficiency tolerant crops and the improvement of agricultural productivity.