論文の内容の要旨

応用生命化学専攻
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論文題目　　Synthesis of boron transport systems and analysis of boron toxicity mechanisms in roots of *Arabidopsis thaliana*
（シロイヌナズナの根におけるホウ素輸送の統合的理解と、過剰ホウ素による生育阻害機構の解明）

Boron is an essential element for plants. Boric acid is required for crosslinking of pectin side chains in cell walls, and boron deficiency hampers plant growth. Boron is toxic in excess for organisms including plants. Excess boron stress increase accumulation of DNA damage and causes developmental defects including cell death in root tips. Because of these properties, both deficiency and toxicity of boron have caused agricultural problem through the world. Approaches for this problem from plant biology can be at two different levels. One is at the level of transport, the other is at the usage or effect of boron in planta.　It has been known that plants have developed boron transport system consists of multiple boron transporters with distinct functions for efficient boron uptake and maintenance of its homeostasis. Improvement of the transport system has been shown to provide plants tolerance to undesirable boron conditions. The other level, the effect of boron in planta, is to improve tolerance to deficient/excessive boron after uptake in planta. Understanding of molecular mechanisms of growth defects caused by inadequate boron dose would make it possible to propose strategy for breeding of tolerant plants.

This thesis is composed of five chapters and overall conclusions, with the central theme of “boron as a plant nutrient”. In Chapter 1, I established a 2-dimentional mathematical model to simulate boron transport through roots, which suggested distinct functions of root tips and other parts of roots in boron uptake. In Chapter 2, I demonstrated behavior of boron transport regulation system to propose significance of the swift regulation in preventing instability. In Chapter 3, I obtained experimiental evidences which suggest NADPH oxidase *RBOHC* is responsible for root growth inhibition under excess boron stress. In Chapter 4, I identified a transcription factor *NAC103* is involved in excess boron stress. Chapter5 is a subject developed from the course of this study. A

previously uncharacterized gene *TPR5* was revealed to be crucial for root meristem maintenance and cell division.

Chapter1. Mathematical modelling and experimental validation of spatial distribution of boron in the root of *Arabidopsis thaliana* identify high boron accumulation in the tip and predict a distinct root tip uptake function

Boron is transported in roots of *Arabidopsis thaliana* mainly by two different types of transporters, BORs and NIPs. Both are plasma membrane-localized, but have distinct transport properties and patterns of cell-type specific accumulation with different polar localizations, which are likely to affect boron distribution. Here, I used mathematical modelling and an experimental determination to address boron distributions in the root. A computational model of the root is created at the cellular level, describing the boron transporters as observed experimentally. Boron is allowed to diffuse into roots, in cells and cell walls, and to be transported over plasma membranes, reflecting the properties of the different transporters. The model predicts that a region around the quiescent centre has a higher concentration of soluble boron than other portions. To experimentally evaluate this prediction, with collaborators, we determined boron distribution in roots using laser ablation-inductivity coupled plasma-mass spectrometry. The analysis indicated that boron concentration is highest near the tip and is lower in the more proximal region of the meristem zone, similar to the pattern of soluble boron distribution predicted by the model. The mathematical model also predicts that upward boron flux does not continuously increase from the root tip toward the mature region, indicating that boron taken up in the root tip is not efficiently transported to shoots. This suggests that root-tip absorbed boron is likely used for local root growth, and that instead it is the more mature root regions which bear a greater role in transporting boron toward the shoots.

Chapter2. Rapid transporter regulation prevents substrate flow traffic jams — a case study of boron transport

Nutrient uptake by roots often involves substrate-dependent regulated nutrient transporters. For robust uptake, the system requires a regulatory circuit within cells and a collective regulatory behaviour across the tissue. A paradigm for such systems is boron uptake, known for its directional transport and homeostasis, as boron is essential but also toxic at high concentrations. Boron uptake occurs via diffusion facilitators (NIPs) and exporters (BORs), each presenting distinct polarity. Intriguingly, although boron soil distributions are dynamically stable, both transporters manifest strikingly swift boron-dependent regulation. Through mathematical modelling, I demonstrated that

slowing down regulation drives the root tissue to unstable and physiologically detrimental oscillatory behaviour. Cytosolic boron concentrations peaked to high, potentially cytotoxic, levels, whereas nutrient throughput to the xylem was hampered. I conclude that, while maintaining homeostasis, swift regulation of the transporters within a polarized tissue context is critical to prevent intrinsic traffic-jam like behaviour of nutrient flow.

Chapter3. NADPH oxidase *RBOHC* is responsible for root growth inhibition caused by excess B stress in *Arabidopsis thaliana*

Excess boron toxicity for plants has been a significant problem in agriculture and its prevention contributes better production. There have been a number of studies on boron toxicity, and several mechanisms are proposed, but the understanding of the molecular process of toxicity occurrence remains in many parts unclear. It has been reported that excess boron stress causes oxidative stress in plants. In this chapter, I screened ROS production genes for excess boron stress inducibility in *A. thaliana* roots by microarray and studied their roles in root growth inhibition caused by excess boron stress. NADPH oxidase *RBOHC* is expressed mainly in the root elongation zone and induced by excess boron stress. A knockout mutant of *RBOHC* exhibited better root elongation under excess boron conditions while under the normal condition the growth difference was not evident. In the wild type, root meristem shrinks under high boron condition, but the extent of the meristem size reduction was significantly smaller in the *rbohc* mutant. These results suggest importance of *RBOHC* under high B condition. RBOHC is known to produce superoxide, an oxidation source, and I examined the extent of lipid peroxidation in roots, the tissue displays RBOHC dependent high boron response. Levels of lipid peroxidation under excess boron stress in roots were less in *rbohc* compared to that in the wild type, suggesting that *rbohc* mutant undergoes less oxidative stress under excess boron conditions. Taken together, I conclude that *RBOHC* is responsible for induction of oxidative stress and growth inhibition in roots under excess boron stress

Chapter4. A mutation in *ANAC103* alleviates DNA damage in *Arabidopsis thaliana* mutant sensitive to excess boron.

Excess boron (B) is toxic to plants, causing DNA damage accumulation and cell death in root meristems. However, the underling mechanisms which link boron and DNA damage remains unclear. It has been reported that *rpt5a-6*, a mutant of 26S proteasome, is sensitive to excess boron, exhibiting more frequent cell death in its root meristem and reduced root elongation. In this chapter, I revealed that reduction in root growth under high boron caused by the *rpt5a-6* mutation is suppressed by a mutation in a NAC domain containing transcription factor *NAC103*, a substrate of

proteasome, which functions in the unfolded protein response (UPR) pathway. The mutation in NAC103 alleviates excess-B-induced DNA damage accumulation and cell death in root meristems. Superoxide (O_2^-) staining with nitroblue tetrazolium (NBT) revealed that excess boron stress causes O_2^- accumulation in root tips and accumulation is higher in *rpt5a-6*, whereas the accumulation was reduced in *rpt5a-6 nac103-1* double mutant. Through the chapter, I demonstrate that regulation of *NAC103* through proteasome pathway is essential for root meristem maintenance under excess boron stress, and the involvement of *NAC103* in novel cellular processes.

Chapter5. TPR5 is involved in directional cell division and is essential for the maintenance of meristem cell organisation in *Arabidopsis thaliana*

Root growth in plants is achieved through the coordination of cell division and expansion. In higher plants, the radial structure of roots is formed during embryogenesis and maintained thereafter throughout development. Here I show that the tetratricopeptide repeat domain protein *TPR5* is necessary for maintaining radial structure and growth rates in *Arabidopsis thaliana* root. An *A. thaliana* mutant with reduced root growth was isolated and I determined that *TPR5* was the gene responsible for the phenotype. The *tpr5-1* mutant root growth rate was reduced to ~60% of that in wild-type plants. The radial structure was disturbed by the occurrence of occasional extra periclinal cell divisions. While the number of meristematic cells was reduced in the *tpr5* mutants, the cell length in the mature portion of the root did not differ from that of the wild type, suggesting that TPR5 is required for proper cell division but dispensable for cell elongation. Expression of the TPR5-GFP fusion protein driven by the *TPR5* promoter displayed fluorescence in the cytoplasm of root meristems, but not in mature root regions. DNA staining revealed that frequencies of micronuclei were increased in root meristems of *tpr5* mutants. Through this study, I concluded that *TPR5* is involved in preventing formation of micronuclei, and is necessary for both the activity and directionality of cell division in root meristems.

In my Ph.D study, I revealed important aspects of boron transport and response to boron conditions in plants from two different approaches and their combinations. In the transport modeling, I established modeling frameworks for boron transport and transporter regulations to capture the behavior of the system. In the experimental analysis of excess boron stress response, I revealed the involvement of two novel genes and illustrated a model for the mechanisms of growth inhibition caused by excess boron stress. Taken together I believe that my thesis represent a big heap in the understanding of boron as a plant nutrient.