論文の内容の要旨

応用生命工学専攻

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論文題目　**Analysis of genes that respond to boron deficiency in model and crop plants**

(ホウ素欠乏に応答する遺伝子のモデル植物や穀物を用いた解析)

Boron (B) has been recognized as an essential microelement for higher plants since 1923. B deficiency is a major agricultural problem that affects vegetative and reproductive growth of plants. Thus, it is important to advance the knowledge of molecular basis of B deficiency and tolerance in plants. So far, two types of boron transporters are found as effective B transporters under low B conditions in *Arabidopsis thaliana,* nodulin 26 (NOD26)-like intrinsic protein 5;1 (NIP5;1), a boric acid channel, involved in B uptake from soils to root cells, and BOR1, a B efflux-type transporter, involved in xylem loading of B in roots. These two types of boron transporters play important roles for normal growth under low B conditions.

In this thesis, I focused on genes that respond to B deficient condition in crop plants, wheat, maize and rice, and also in a model plant, *A. thaliana*. This research consists of three parts as follows. First part is the characterization of the *BOR1*-like genes responsible for B transport under B deficiency in wheat. In the second part, the accumulation of *BOR1*-like gene transcript was observed in several tissues in response to B stress between two genotypes with different B efficiencies of Thai wheat, Thai maize and Thai rice. I found the tendency that the *BOR1*-like genes are more expressed in the B efficient cultivars than in inefficient cultivars. Finally, I identified a novel gene, *GDSL LIPASE-LIKE 1* (*GLIP1*), that response to B deficiency in *A. thaliana,* and expression and function of *GLIP1* were investigated in the third part.

1. Differential expression of three *BOR1* genes corresponding to different genomes in response to B conditions in hexaploid wheat (*Triticum aestivum* L.)

To overcome the problem of B deficiency in wheat, it is of fundamental importance to understand the molecular mechanisms of B transport through identification of transporters. Before I initiate this study, three *BOR1*-like genes (Ta *BOR1.1*, Ta *BOR1.2*, and Ta *BOR1.3*) were identified in wheat by Dr. Fujibe. He demonstrated that Ta BOR1s are capable of reducing the B concentrations in BY-2 cells expressing Ta BOR1s. Then Mr. Wang demonstrated Ta BOR1s-GFP expressed in *A. thaliana* leaf cells showed plasma membrane localization. Taken together, these results suggest that Ta BOR1s are efflux-type B transporters in plant cells.

I confirmed the nucleotide sequence of the *BOR1*-like genes and performed phylogenetic analysis. I found that the three *BOR1*-like genes (Ta *BOR1.1*, Ta *BOR1.2*, and Ta *BOR1.3*) in the wheat genome were the most similar to Os *BOR1* (91% in amino acid identity).

Then I demonstrated that three Ta *BOR1* genes were expressed with a different tissue-specificity and different response patterns to B conditions. Ta *BOR1.1* and Ta *BOR1.3* transcripts were up-regulated under low B conditions, while Ta *BOR1.2* transcript was up-regulated under excess B conditions. Taken together with functional analysis using BY-2 and plasma membrane localization, these results demonstrated that Ta *BOR1s* are the B efflux transporters in wheat. Interestingly, the genes on the A, B and D genomes have different expression patterns.

2. Comparison of *BOR1*-like gene expression in two genotypes with different B efficiencies in wheat (*Triticum aestivum*L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) in Thailand

Genotypic variation in the response to low B supply has been reported in wheat, maize and rice. Here, I presented a comparative investigation of the differential transcript levels of *BOR1*-like genes that expressed in roots, leaves and reproductive organs of two genotypes with different B efficiencies in wheat (*Triticum aestivum L.*), maize (*Zea mays*) and rice (*Oryza sativa*) which were grown under B deficiency in Thailand.

The mRNA expression levels of Ta *BOR1*-like genes between B-efficient (Fang60) and B-inefficient (Bonza) wheat genotypes were compared. Ta *BOR1s* genes were expressed at relatively high levels in Fang60 compared to Bonza in most of the tissues I observed. These results suggest that Ta *BOR1s* may be required for wheat growth and development, especially in the reproductive stage under B limitation.

Zm *BOR1* mRNA expression levels were compared between B efficient variety (Sweet corn) and B inefficient variety (NS72) of maize. The expression of Zm *BOR1* in Sweet corn was higher than that in NS72 in ear at the reproductive stage and roots at both the vegetative and reproductive stages. There was no significant difference in Zm *BOR1* expression between two genotypes in leaves at the vegetative and reproductive stages, and baby corn. These results showed that the expression of Zm *BOR1* in roots is correlated with the B deficiency tolerance.

The expression patterns of Os *BOR1* gene were compared between B-efficient variety (SPR1) and B-inefficient variety (CNT1). Os *BOR1* gene was expressed at higher level in SPR1 compared to CNT1 in roots at the vegetative and reproductive stages, and leaves at the vegetative stage. The expression levels of Os *BOR1* in leaves at the reproductive stage and panicle were detected only in SPR1, not in CNT1. These results showed that Os *BOR1* expression levels correlated with the B deficiency tolerance in rice.

Taken altogether, I found the tendency that *BOR1*-like gene transcripts are accumulated to higher levels in a low boron tolerant cultivar than the sensitive ones. By selecting appropriate tissues and growth stages, the difference in transcript accumulation becomes more evident.

3. Analysis of *GLIP1* gene that is required for boron and calcium deficiency tolerance

To identify the B deficiency responsive genes, I searched for genes up-regulated under B deficient conditions in *A. thaliana* roots using a combination of DNA microarray and quantitative real-time PCR analysis. In this study, the *GLIP1* gene (At5g40990) was identified as a gene whose expression is increased significantly under B deficient condition.

To characterize the function of *GLIP1* gene in *A. thaliana*, two independent T-DNA insertion mutant lines, *glip1-2* and *glip1-3*, were used. While both *glip1-2* and *glip1-3* grew normally under normal B condition, both *glip1* mutant plants showed reduced growth rate under B limitation compared to wild type. The B concentrations in shoots of both *glip1* mutants were reduced under normal B condition compared to wild type.

To confirm whether the mutant phenotype is specific to B deficiency, mutant plants were grown under various nutrient deficient medium. The results showed that the shoot weight of both *GLIP1* mutant lines was significantly reduced in calcium (Ca) deficient condition compared with wild type plants. Ca concentrations showed significant reductions in *glip1-2* and *glip1-3* plants under low Ca conditions compared to wild type plants, suggesting that *GLIP1* is required for Ca deficient condition.

Conclusion

In this thesis, I have identified Ta *BOR1*s as efflux-type B transporters that localize to the plasma membrane in wheat. In addition, this thesis also demonstrated that the expression levels of *BOR1*-like genes correlate with the B deficiency tolerance in wheat, maize and rice. I have also identified *GLIP1* as low-B up-regulated gene in *A. thaliana* roots. *GLIP1* is required for *A. thaliana* roots and shoots growth under B and Ca deficiency, respectively and plays a role in the B and Ca transport from roots to shoots.

Publication

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