学 位 論 文 内 容 の 要 旨

Dissertation Abstract

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学 位 論 文 題 名

Title of dissertation

The effects of type II Golgi-localized proton pyrophosphatase *AVP2;1*/*VHP2;1* mutations on cell wall and root growth under low boron condition in *Arabidopsis thaliana*
（シロイヌナズナのタイプ II ゴルジ体局在プロトンピロホスファターゼ *AVP2;1*/*VHP2;1* 遺伝子変異の低濃度ホウ素条件における細胞壁と根の成長に対する影響）

Plants generally require mineral nutrients for growth and development. One of the mineral nutrients required by plants is boron. Boron exists in the soil as an uncharged molecule and requires in addition to passive diffusion, transport mechanisms to get it into the plant cells. Boron, though a trace nutrient because it is required in a little amount, is essential for plant growth. Its deficiency symptoms have been observed in many plants and it is widely spread across the globe. Some approaches to combat low boron stress such as fertilizer application and upregulation of boron transporters have been exploited. The approach of fertilizer application is limited by cost, potential environmental pollution when washed out of the soil, narrow window between boron deficiency and toxicity and hence inappropriate application of borate fertilizer may lead to toxicity and most importantly the difficulty to ascertain the optimum required fertilizer amount because of the wide range of boron requirement among plant species. The approach of upregulation of boron transporters, although useful for the reduction of boron fertilizer application, does not provide a lasting solution to boron deficiency. The implication of the upregulation of boron transporters is an increase in boron uptake which will eventually lead to a depletion of the limited soil boron in long term. Knowing the down sides of the approaches to boron deficiency thus far, there is the need for a better system. The major role of boron known so far is the stability of the plant cell wall. The heterogenous plant cell wall is made of cellulose, hemicellulose, pectin, and soluble proteins. The pectin component, which makes up a higher percentage of the plant cell wall compared to the other cell wall components, is composed of three major polysaccharides homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). RG-II exists in the cell wall as a dimer and this dimerization/crosslinking of the RG-II monomers is by boron. The crosslinking of RG-II by boron adds to the overall stability of the plant cell wall. The synthesis of the pectic polysaccharides takes place in the Golgi apparatus, acidified by proton pumps. AVP2;1/VHP2;1 is a type II proton pyrophosphatase localized in the Golgi apparatus, which possesses proton pumping activity coupled with pyrophosphate hydrolysis. Its activity and expression patterns have been previously revealed but its role in plants remains unknown. The aim of the present thesis therefore was to explore the physiological role of AVP2;1 in *Arabidopsis thaliana*. In the screening of mutants under low boron, a mutant carrying a missense mutation in *AVP2;1* was isolated. This mutant showed increased primary root growth under low boron conditions but no significant difference under normal boron condition compared to wild type plants. T-DNA insertion mutants showed a similar phenotype of enhanced root growth under low boron. The quantification of mRNA level of *AVP2;1* in *avp2;1*

mutants and wildtype confirmed that T-DNA insertion mutants were knock down mutants suggesting that reduced function of AVP2;1 was responsible for the improved root growth. The mutants also showed an improved root growth under low boron condition in hydroponic culture system (a more natural system), consistent with the growth tests previously conducted in solid media. To check root phenotype in the mutants under different stress conditions, growth tests of the plant lines under low pH and low phosphorus were conducted. Result obtained from this analysis revealed that mutants showed no obvious difference in growth under both low pH and low phosphorus conditions compared to the wildtype. This suggested that increased roots by *AVP2;1* mutation were specific to low boron. For a better understanding of what could possibly have impacted the improved root growth of mutants under low boron, root cells of the plant lines were observed. The root cell observation revealed an increase in meristematic zone length, cell number in meristem and length of matured cell in *avp2;1* mutants compared to wildtype under low boron. To explore the possible mechanism behind the increased root growth and root cell under low boron in *avp2;1* mutants compared to wildtype Col-0, firstly the possibility of enhanced boron uptake was considered. However, results obtained from total boron measurement in both root and shoot of plant lines revealed no obvious changes in boron concentration between the wildtype and mutant lines. mRNA quantification of boron transporter genes (*BOR1*, *BOR2* and *NIP5;1*) was also measured and it was found that expression of the transporters especially *BOR1* and *BOR2* were unchanged and *NIP5;1* expression was rather reduced in the mutants under low boron compared to the wildtype. These results suggested that an enhanced boron uptake is not the probable mechanism behind the improved root growth of mutants under low boron condition. Another possible mechanism considered was changes in cell wall components as boron function in cell wall stability. Analysis of the root cell wall revealed that calcium concentration was reduced in mutant root cell wall under low boron condition and RG-II specific sugars also tended to be decreased in mutant root cell wall under low and normal boron conditions. For a wholistic understanding of the mechanism behind the phenotypes, the question of which component of AVP2;1 (hydrolytic component, or proton pumping component) contributes to the observed phenotypes was discussed. Based on previous research and the analysis of *AVP1* mutants under low boron condition, it was suggested that the proton pumping component of AVP2;1 may possibly be the driving force behind the observed phenotypes. With the assumption of the proton pumping activity as the key for improved root growth, it was also considered that reduced proton pumping due to the reduced function of AVP2;1 in *avp2;1* mutants affect the activity of pectin (RG-II) synthesis enzymes such as glycosyltransferase in the Golgi apparatus. This effect of *AVP2;1* mutation on glycosyltransferase then could lead to reduced amount of pectin (RG-II) been synthesized. The implication of the reduction of RG-II in mutants is that the amount of boron required for crosslinking of RG-II would be reduced and hence the improved root growth of mutants under low boron supply. To support this, previous research has shown that there is a positive correlation between cell wall boron concentration and RG-II amount among different plant species. This implies pectin amount or RG-II amount determines sensitivity to boron deficiency hence the reduced sensitivity to low boron in *avp2;1* mutants. Taken together, the results obtained from this study suggest that changes in cell wall component by mutations in *AVP2;1* may possibly explain the increased root length of *avp2;1* mutants under low boron by the mechanism of reduced boron requirement. This study supports the idea that AVP2;1 plays a role in proton pumping and acidification of Golgi apparatus for maintenance of pectin synthesis by examination of *avp2;1* mutants. It also proposes the idea of a reduction of boron requirement by molecular approach as a sustainable strategy against low boron stress rather than fertilizer application and upregulation of boron transporters.