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Article Addendum

Boron deficiency

How does the defect in cell wall damage the cells?

Taichi Koshiba, Masaru Kobayashi* and Toru Matoh

Laboratory of Plant Nutrition; Graduate School of Agriculture; Kyoto University; Kyoto, Japan

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Boron (B) is an essential micronutrient for vascular plants. Boron plays a structural role in cell walls through binding to pectic polysaccharides. It still remains unclear how B deficiency, and hence probably alterations in cell wall structure, leads to various metabolic disorders and cell death. To understand the process, we analyzed the physiological changes in suspension-cultured tobacco (*Nicotiana tabacum*) BY-2 cells under B deficiency. The results indicated that the cells deprived of B did not undergo a typical programmed cell death process. Oxidative damage was proven to be the direct and major cause of cell death. We discuss possible mechanisms for the generation and accumulation of reactive oxygen species under B deprivation.

Boron (B) deficiency is the most widespread micronutrient deficiency around the world and causes large losses in crop production both quantitatively and qualitatively.[1] Boron deficiency affects vegetative and reproductive growth of plants resulting in inhibition of cell expansion, death of meristem and reduced fertility.[2]

Plants contain B both in a water-soluble and insoluble form. In intact plants, the amount of water-soluble B fluctuates with the quantity of B supplied, while insoluble B does not.[3] The appearance of B deficiency symptoms coincides with the decrease of water-insoluble B, from which it is concluded that the insoluble B is the functional form while the soluble B represents the surplus. We found at least 98% of the insoluble B in tobacco cells bound to the cell wall,[4] and identified their molecular entity as the borate diester with rhamnogalacturonan II (RG-II) regions of pectic polysaccharides.[5] The diester crosslinks pectic polysaccharides to form a network and thereby contributes to construction of a supramolecular cell wall structure.[6] Mutant plants with altered RG-II structures are dwarf and sterile, indicating that the B-RG-II complex is essential for normal plant growth and development.[7] Increasing evidence indicates that B is also essential for animals.[8] The requirement for B in organisms lacking cell walls implies that B may also have additional roles in plants. To date, however, no molecule other than apiosyl residues in pectic polysaccharides has been demonstrated to form a borate ester which could be stable enough under physiological conditions. Thus it is reasonable to consider that B functions primarily, if not exclusively, as a structural component of the cell wall, and B deficiency symptoms arise from disturbance of the cell wall structure. How, then, does the disturbed cell wall structure lead to the damage and cell death that are observed under B deficiency? To understand the linkage, we have analyzed physiological changes of suspension-cultured tobacco (*Nicotiana tabacum*) BY-2 cells under B deficiency.

When cells at the log phase of growth were transferred to B-free media, cell death was detectable as early as 12 h after the treatment. As cell walls play pivotal roles in plant development and growth, we assumed that the B deprivation, which probably causes aberrant cell wall structure, might induce programmed cell death (PCD) as an active response to eliminate damaged cells. Then we examined if the known biochemical hallmark of PCD could be observed in cells deprived of B (hereafter referred to as -B cells). However, internucleosomal DNA fragmentation, decrease in antioxidant content and antioxidant enzyme expression,[9] or protection from death by cycloheximide, were not detected in these cells, suggesting that the cell death is necrosis. We found oxidative damage to be the direct and major cause of cell death, because -B cells contained more reactive oxygen species (ROS) than control cells, and because cell death was effectively suppressed by supplementing the media with lipophilic antioxidants. The deprivation treatment did not induce an oxidative burst, as the extracellular H_2O_2 concentration was not significantly different between -B and control cells at all time points examined. Resupply of B immediately suppressed cell death. Collectively, these results suggest that low but persistent ROS production occurred under the -B condition.

In the study described above, we demonstrated that B deprivation, and hence probably a defective cell wall structure, leads to oxidative damage. How and why B deprivation induces ROS

*Correspondence to: Masaru Kobayashi; Laboratory of Plant Nutrition; Graduate School of Agriculture; Kyoto University; Kyoto 606-8502 Japan; Tel.: +81.75.753.6407; Fax: +81.75.753.6107; Email: masaru@kais.kyoto-u.ac.jp

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overproduction remains to be clarified. We hypothesize that ROS are originally produced as a signal for disturbance of the cell wall structure, and build up to a toxic level unless B is resupplied and the cell wall structure is restored. It has been reported that the mechanical strength of the squash root cell wall decreases within minutes after B deprivation.[10,11] The mechanical change could be brought about by insufficient crosslinking of pectic polysaccharides at RG-II regions, as the B-RG-II complex significantly contributes to the wall tensile strength.[12] If the cell wall becomes weaker and less resistant to turgor, then the plasma membrane would stretch. The change may lead to opening of mechanosensitive channels[13] and generation of signals for the altered cell wall structure. To test this hypothesis, we are now analyzing the immediate and early responses of tobacco BY-2 cells to B deprivation, and preliminary results do indicate the involvement of Ca^{2+} influx in the responses. Identification of the mechanism by which cells sense the external B status will greatly contribute to our understanding of the cell wall-symplast interaction in plants.[14]

References

1. Shorrocks VM. The occurrence and correction of boron deficiency. Plant Soil 1997; 193:121-48.
2. Marschner H. Boron. In: Mineral Nutrition of Higher Plants, 2nd edition. pp. San Diego: Academic Press 1995; 379-96.
3. Matoh T, Matsuda A, Akaike R, Hara Y, Kobayashi M. Increased supply of boron to swiss chard plants did not affect the level of boron in a cell wall-bound form: A possible application to the diagnosis of B nutrition of plants. Soil Sci Plant Nutr 2001; 47:779-84.
4. Matoh T, Ishigaki K, Mizutani M, Matsunaga W, Takabe K. Boron nutrition of cultured tobacco BY-2 cells I. Requirement for and intracellular localization of boron and selection of cells that tolerate low levels of boron. Plant Cell Physiol 1992; 33:1135-41.
5. Kobayashi M, Matoh T, Azuma J. Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plants cell walls. Plant Physiol 1996; 110:1017-20.
6. Kobayashi M, Nakagawa H, Asaka T, Matoh T. Borate-rhamnogalacturonan II bonding reinforced by Ca^{2+} retains pectic polysaccharides in higher-plant cell walls. Plant Physiol 1999; 119:199-204.
7. O'Neill MA, Eberhard S, Albersheim P, Darvill AG. Requirement of borate cross-linking of cell wall rhamnogalacturonan II for Arabidopsis growth. Science 2001; 294:846-9.
8. Hunt CD. Dietary boron: An overview of the evidence for its roles in immune function. Trace Elem Exp Med 2003; 16:291-306.
9. de Pinto MC, Paradiso A, Leonetti P, De Gara L. Hydrogen peroxide, nitric oxide and cytosolic ascorbate peroxidase at the crossroad between defence and cell death. Plant J 2006; 48:784-95.
10. Findeklee P, Wimmer M, Goldbach HE. Early effects of boron deficiency on physical cell wall parameters, hydraulic conductivity and plasmalemma-bound reductase activities in young *C. pepo* and *V. faba* roots. In: Bell RW, Rerkasem B, eds. Boron in Soils and Plants. Netherland: Kluwer Academic Publishers 1997; 221-7.
11. Goldbach HE, Yu Q, Wingender R, Schulz M, Wimmer M, Findeklee P, et al. Rapid response reactions of roots to boron deprivation. J Plant Nutr Soil Sci 2001; 164:173-81.
12. Ryden P, Sugimoto-Shirasu K, Smith AC, Findlay K, Reiter WD, McCann MC. Tensile properties of Arabidopsis cell walls depend on both a xyloglucan cross-linked microfibrillar network and rhamnogalacturonan II-borate complexes. Plant Physiol 2003; 132:1033-40.
13. Nakagawa Y, Katagiri T, Shinozaki K, Qi Z, Tatsumi H, Furuichi T, et al. Arabidopsis plasma membrane protein crucial for Ca^{2+} influx and touch sensing in roots. Proc Natl Acad Sci USA 2006; 104:3639-44.
14. Baluška F, Šamaj J, Wojtaszek P, Volkmann D, Menzel D. Cytoskeleton-plasma membrane-cell wall continuum in plants. Emerging links revisited. Plant Physiol 2003; 133:482-91.