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Boron transport in plants: co-ordinated regulation of transporters

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INTRODUCTION

Boron (B) is an essential micronutrient for plants, and its deficiency generally causes growth defects mainly in young and growing portions of plants. Boron deficiency is one of the most widespread deficiencies among plant micronutrients in agriculture (Loomis and Durst, 1992). Boron deficiency causes a wide range of symptoms including the cessation of root elongation, reduced leaf expansion and the loss of fertility, depending on the plant species and developmental stage (Gupta, 1979; Dell and Huang, 1997; Goldbach et al., 2001). Boron deficiency reduces not only yield but also the quality of crops (Shorrocks, 1997; Brown et al., 2002). Boron-deficient soils are mostly found in regions with high rainfall such as southeast Asia and southeast China. Boron in soil is relatively soluble and easily leached by rainfall.

High concentrations of B are toxic to living organisms including plants (Nable et al., 1997). Boron toxicity reduces growth, particularly of shoots, and causes chlorosis starting at the leaf tip and margins of mature leaves (Nable et al., 1997; Reid et al., 2004; Reid and Fitzpatrick, 2009). This presumably results from the accumulation of B transported through the transpiration stream. As retranslocation of B is negligible in many plant species, B tends to accumulate in old leaves (Marschner, 1995). Boron toxicity is a significant problem in semi-arid areas in the world including South Australia, Turkey, Mediterranean countries, California and Chile.

Boron belongs to Group 13 in the periodic table and its properties lie in between those of metals and non-metals. Although it belongs to Group 13, its chemical properties are very different from those of the other elements in this Group, such as Al or Ga, and B is referred to as an orphan group, such as Al or Ga, and B is referred to as an orphan element in organic chemistry. A wide range of B-containing chemicals including boronic acids and borohydrides are commonly used in the synthesis of organic compounds.

Boron in soil solution is mainly present as boric acid or boric acids. The pH of boric acid is 9.25 and, at neutral pH, the equilibrium is shifted greatly toward boric acid. Boric acid, a charge-neutral molecule, is the major chemical form of B...
taken up by plants (Marschner, 1995). Among the elements required by plants that are taken up from the soil, B is the only element that is taken up by plants not as an ion, but as an uncharged molecule. When the Casparian strip, a hydrophobic layer in the cell wall between endodermal cells, develops, it blocks apoplastic flow of solutes into the stele. In the root tip where the Casparian strip is not matured, solutes are accessible to the xylem through apoplastic flow, and apoplastic flux is shown to be an important pathway for transport to shoots in the case of Ca\(^{2+}\) (White, 2001). For the majority of the root length, for most mineral nutrients to be transported across the root from the soil to the xylem, they need to move across plasma membranes at least twice because of the Casparian strip: first uptake into cells and subsequently export out of the cells into the xylem. For solutes that do not permeate lipid membranes readily, two types of transport protein must be present in the plasma membrane of root cells, an influx transporter and an efflux transporter for symplastic flux. In the case of B, however, only passive diffusion (i.e. transport that is not catalysed by proteins) across the plasma membrane was thought to occur and, until the 1990s, this was considered capable of fulfilling the B requirements of plants. This hypothesis was based on the high permeability of lipid bilayers to boric acid (Raven, 1980). Furthermore, the idea of B transport by passive diffusion appeared to coincide well with the patterns of symptoms of B deficiency and toxicity in young growing and mature tissues, respectively (Marschner, 1995). This hypothesis is now known not to be true. The first B transporter in biological systems was identified in Arabidopsis thaliana and this protein is required for the transport of boric acid against its concentration gradients under low B conditions. The identification of this transport protein revealed the physiological functions of transporters for B homeostasis and expanded our views of B in biology.

**PHYSIOLOGICAL FUNCTION OF B: BORATE CROSS-LINKING OF PECTIC POLYSACCHARIDE RHAMNOGALACTURONAN II**

Although the essential nature of B was established >85 years ago, the molecular basis of the B requirement of plants has been understood for <10 years. Boric acid forms esters with alcohols in a pH-dependent manner (Power and Woods, 1997). The most stable borate esters are formed with cis-diols on a furanoid ring, such as ribose and apiose.

Biochemical and genetic studies have uncovered that one of the physiological functions of B in plants is dimerization of the pectic polysaccharide rhamnogalacturonan II (RG II) by borate for binding to cis-diols, caused the disruption of cytoplasmic strands and cell–cell wall detachment in cultured tobacco cells. Wimmer et al. (2009) reported isolation of possible B-binding proteins from microsomes by phenylboronate affinity chromatography. Molecular mechanisms underlying these functions of B remain to be uncovered.

Of the 17 elements essential for plant growth and development, B has long been considered to be required only for plants but not for animals (Marschner, 1995). A specific requirement for B in plant cell walls supports this idea. However, increasing evidence suggests essential roles for B in other organisms including animals and bacteria. The functions of B are obviously not cell wall related in these organisms. Boron-containing compounds have been identified in micro-organisms. Borophycin and boromyacin are B-containing antibiotics produced by the cyanobacterium Nostoc spingioformer var. tenue and by Streptomyces griseus, respectively (Dembitsky et al., 2002). Autoinducer-2, a molecule for quorum sensing in the marine bacterium Vibrio harveyi, contains B (Chen et al., 2002), and vibrioferrin, a B-containing siderophore produced by particular marine bacteria, is required for the growth of the toxic, bloom-forming dinoflagellate Gymnodinium catenatum (Amin et al., 2007).

**ROOT UPTAKE AND XYLEM LOADING OF B UNDER LIMITED B AVAILABILITY**

Boron occurs in the soil solution predominantly as undissociated, electroneutral boric acid, B(OH)\(_3\). The uptake of B by plant roots from the soil solution was long considered to be a passive process that was not facilitated by transport proteins. However, recent physiological studies have revealed the presence of channel-mediated facilitated diffusion and energy-dependent active transport against concentration gradients in B transport systems (Dannell et al., 2000, 2001; Stangoulis et al., 2001). The molecular identity of the transport systems then became a major focus. Subsequently, molecular genetic studies of A. thaliana successfully identified two types of B transporters, NIP5;1 and BOR1, both of which are important for efficient transport of B across the plasma membrane under B limitation.

In the initial uptake process, NIP5;1, a boric acid channel, facilitates B influx to root cells. NIP5;1 was identified from microarray experiments as a gene upregulated by low B supply in roots of A. thaliana. NIP5;1 mRNA accumulation in roots increased by about 15 fold 24 h after the initiation of a low B treatment (Takano et al., 2006). Expression of NIP5;1 facilitated the influx of boric acid to Xenopus laevis oocytes. T-DNA insertion mutants of A. thaliana exhibited reduced uptake of boric acid by roots and growth defects.
NIP5;1 is a member of the major intrinsic protein (MIP) superfamily, which includes the aquaporins. The MIP superfamily can be divided into four sub-families including the small basic intrinsic proteins (SIPs), tonoplast intrinsic proteins (TIPs), nodulin-26-like intrinsic proteins (NIPs) and the plasma membrane intrinsic proteins (PIPs). Involvement of other members of the MIP superfamily in B transport is also suggested. In addition to NIP6;1, as discussed below, it has been demonstrated that expression of maize Zm-PIP1 in *Xenopus laevis* oocytes resulted in a 30% increase in B permeability (Dordas and Brown, 2000, 2001) and expression of Hv-PIP1;3 and Hv-PIP1;4 from *Hordeum vulgare* increased the sensitivity of yeast cells to B (Fitzpatrick and Reid, 2009). However, the functions of these transporters in plant cells remain to be clarified.

BOR1, a B exporter, plays a key role in xylem loading. The *A. thaliana* bor1-1 mutant was isolated as being more susceptible to B deficiency than the wild type. In bor1-1, leaf expansion in upper leaves was severely inhibited under low B conditions (Noguchi et al., 1997). When B concentrations of root cell saps and xylem saps were determined in plants exposed to various concentration of B, the B concentrations in root cell saps were almost the same as the B concentrations in the media, both in wild-type plants and in the bor1-1 mutant (Takano et al., 2002). In contrast, B concentrations in xylem saps were always higher in the wild type than in the mutant. In the wild type, B concentrations in xylem saps were higher than those in the media with low B supply, but those of the bor1-1 mutant were not (Takano et al., 2002), suggesting that BOR1 was required for B transport to the xylem against its concentration gradient. BOR1 was identified by positional cloning. BOR1 showed a high similarity to anion exchanger proteins, including the well-characterized Band3 protein of animal erythrocytes (Takano et al., 2002). BOR1 is expressed predominantly in the root pericycle cells surrounding the xylem. The BOR1–green fluorescent protein (GFP) fusion protein was localized to the plasma membrane. When *A. thaliana* BOR1 is expressed in a *Saccharomyces cerevisiae* strain that lacks the yeast BOR1 gene, B concentrations in yeast cells were reduced by 3-fold, suggesting that BOR1 is an exporter of B. BOR1 was the first B transporter identified in biological systems. It has been demonstrated that *Oryza sativa* BOR1, an orthologue of *A. thaliana* BOR1, plays a critical role in B acquisition by roots and translocation of B into shoots (Nakagawa et al., 2007).

Expression of *A. thaliana* BOR1 is regulated post-transcriptionally (Takano et al., 2005). Accumulation of BOR1 protein decreases in response to a high B concentration in the medium. Under low B supply, BOR1 is accumulated mainly in the plasma membrane. Upon exposure to high B, BOR1 is incorporated into endosomes and transported to the vacuole for degradation (Takano et al., 2005). This B-dependent degradation of BOR1 might be beneficial for plants as it allows them to avoid overaccumulation of B in shoots when B concentrations in soils are high.

Under B-limited conditions, the combination of NIP5;1 for uptake and BOR1 for efflux into xylem is likely to be key to the efficient transcellular transport of B from the soil solution to the xylem (Fig. 1). BOR1 is likely to generate a concentration gradient between root cells and the medium. This concentration gradient is probably essential for NIP5;1 to facilitate B uptake into root cells because NIP5;1 is likely to be a boric acid channel.

**B DISTRIBUTION IN SHOOTS OF PLANTS WITH LIMITED B SUPPLY**

BOR1 is involved not only in B transport into the xylem but also in B distribution within shoots. Takano *et al.* (2001) demonstrated that the wild-type *A. thaliana* distributed B preferentially to young leaves, while such preferential transport was not evident in the bor1-1 mutant. BOR1 is expressed in shoots, but it is not known how BOR1, an efflux transporter of B, regulates B distribution among leaves. It is possible that BOR1 may be involved in directing B from the xylem to phloem for preferential delivery to young leaves.

Another transporter has also been shown to be involved in B distribution in shoots. *NIP6;1* is the gene most similar to *NIP5;1* among the nine *NIP* genes present in the *A. thaliana* genome. *NIP6;1* is also localized to the plasma membrane and facilitates boric acid uptake into oocytes, but does not show water channel activity (Tanaka *et al.*, 2008). This is in contrast to the case of *NIP5;1*, which is permeable to both boric acid and water. T-DNA insertion mutant lines of *NIP6;1* exhibited defects in the expansion of young leaves. In the mutant, B concentrations in young leaves under B limitation were lower than in wild-type plants. Strong *NIP6;1* promoter activity was observed in the phloem region at nodes of shoots.

**Fig. 1.** A schematic model of B transport in *A. thaliana* roots under B limitation. Cell layers of a cross-section of *A. thaliana* roots where the Casparian strip is developed in the endodermis (State I; White, 2001) are illustrated. It is likely that endodermal cells subsequently become suberized and unable to take up solutes directly from the root apoplast (Moore *et al.*, 2002). Under B limitation, NIP5;1 increases the permeability of boric acid to cell membranes, and facilitates influx of B into root cells from the soil. BOR1 exports B out of the cells toward the xylem against the concentration gradient. It is likely that the co-ordinated expression patterns of BOR1 and NIP5;1 are essential for efficient transcellular transport of B as NIP5;1 possibly facilitates B influx, following the B concentration gradient that BOR1 generates. Under high levels of B supply, expression of both NIP5;1 and BOR1 is decreased by transcriptional and post-transcriptional regulation, respectively. The downregulation of NIP5;1 and BOR1 might be beneficial for avoidance of overloading of high concentrations of B to shoots.
B EFFLUX AT TOXIC LEVELS OF B IN ROOTS AND SHOOTS

One of the physiological functions of the A. thaliana BOR1 homologue in a single cell was shown to be the efflux of B back to the environment to reduce the B concentration in cells. YNL275w (Sc-BOR1) is the sole At-BOR1 homologue in S. cerevisiae. It was previously characterized as an anion transporter localized to plasma membrane, and potential substrates include HCO$_3^{-}$, I$^{-}$, Br$^{-}$, NO$_3^{-}$ and Cl$^{-}$ (Zhaob and Reithmeier, 2001). Bor1p, the product of Sc-BOR1, is a B exporter (Takano et al., 2002). Disruption of Sc-BOR1 increased sensitivity to toxic levels of B, presumably resulting from elevated concentrations of B in cells (Takano et al., 2002, 2007). These findings suggested that one physiological function of Bor1p was to export B from the cell. Nozawa et al. (2007) demonstrated the possible involvement of FPS1 (a glycerol channel) and DUR3 (a urea transporter) in tolerance of high B in yeast. Kaya et al. (2009) recently found that ATR1 is responsible for most of the tolerance of high B in S. cerevisiae. ATR1 encodes a multidrug resistance transport protein of the major facilitator superfamily. Disruption of ATR1 increased sensitivity to high B in the environment, and expression of ATR1 conferred a greater tolerance. ATR1–GFP was localized to the cell membrane and it was suggested that ATR1 was a B exporter. Genes similar to ATR1 are widely present in bacteria and fungi, but not in plants.

It has also been demonstrated that homologues of A. thaliana BOR1 are involved in tolerance of high B in other plant species. Hayes and Reid (2004) observed that the high B-tolerant barley cultivar, Sahara, maintained low B concentrations in roots by active B efflux, and this resulted in decreased accumulation of B in shoots and elevated tolerance of high B. Sutton et al. (2007) performed a quantitative trait locus (QTL) analysis of two barley cultivars differing in their tolerance of high B conditions, and found that the mapped region contained a BOR1 homologue. They named this gene Bot1. The same gene was cloned by Reid (2007) and was named Hv-BOR2, as it is similar to rice BOR2. The terminology for Bot1 may be correct in terms of the effect of the gene, but obviously it potentially causes confusion as similar transporters are now named differently. Sutton et al. (2007) demonstrated that expression of Bot1 conferred tolerance of high B in S. cerevisiae, as was reported for At-BOR1. mRNA from Bot1 has been detected in both roots and shoots, but more is present in roots. The tolerant cultivar possesses multiple copies of Bot1 and has greater amounts of Bot1 mRNA than the susceptible cultivar. Sutton et al. (2007) suggested that increased expression of the B exporter in roots leads to reduction in B concentrations in roots. At the same time, Reid (2007) cloned Hv-BOR2 and Ta-BOR2 from barley and wheat, respectively. Both are similar to At-BOR1. Positive correlations between mRNA levels of BOR2 genes and tolerance of high B were described among different cultivars in both barley and wheat, supporting roles for BOR2 in tolerance of high B. Furthermore, Reid and Fitzpatrick (2009) noticed that necrosis in leaves was observed at higher leaf B concentrations in the tolerant cultivars in barley and wheat. It was shown that B concentrations in leaf protoplasts were lower in the tolerant cultivar, suggesting different partitioning of B within the shoot. It is likely that B transporters export toxic B out of cells into the apoplast and alleviate B toxicity symptoms in the cytoplasm of leaf cells. Boron exporters encoded by BOR1 homologues not only reduce B uptake by roots as a primary B tolerance mechanism, but also redistribute toxic B in leaves to confer further tolerance of high B.

In addition to B exclusion, the presence of another mechanism affecting tolerance of high B in plants has been suggested. Ochiai et al. (2008) found that ‘japonica’ rice cultivars were more tolerant than ‘indica’ cultivars to high B supply. However, B concentrations in roots and shoots were not significantly different. The molecular basis of this phenomenon has not been reported yet, but a major QTL has been identified that accounts for the phenotypic variation.

PLANTS TOLERANT TO B DEFICIENCY AND B TOXICITY GENERATED BY ENHANCED EXPRESSION OF TRANSPORTERS

When A. thaliana BOR1 was over-expressed under the control of the cauliflower mosaic virus 35S RNA promoter in A. thaliana plants, shoot growth and fertility were increased compared with wild-type plants when grown under low B conditions (Miwa and Takano, 2006). Elevated levels of BOR1 presumably increased translocation of B from roots to shoots. This elevated B transport to shoots is likely to be beneficial for plants with low B supply. This study represented the first example of altering the expression of genes encoding transport proteins for essential nutrients to generate plants tolerant to a low availability of the essential nutrient. The advantage of this method is the lack of negative effects of over-expression of BOR1 on growth under normal or high levels of B supply. This is probably due to the degradation of BOR1 in plants grown in high B environments.

Over-expression of BOR1 enhanced shoot growth under limited B supply, but not root growth. Kato et al. (2009) demonstrated that enhanced expression of NIP5;1 improved root elongation in low B environments. The combination of enhanced expression of BOR1 and NIP5;1 enabled A. thaliana plants to become remarkably tolerant to B limitation in terms of the growth of both shoots and roots. From these experiments, it could be speculated that a limiting process for root and shoot growth under low B conditions is B uptake by NIP5;1 and xylem loading by BOR1 in A. thaliana wild-type plants.

To increase tolerance of high B, over-expression of BOR4, a BOR1 parologue, in A. thaliana was very effective (Miwa et al., 2007). The transgenic plants tolerated 10 mM boric acid in the medium, while the untransformed wild-type plants barely extended roots. In contrast to BOR1, BOR4 is not degraded even under high levels of B supply. In one BOR4-expressing A. thaliana line, B concentrations in shoots and roots were decreased by about 3-fold, and plant growth was greatly improved under high levels of B supply. This result further supports the idea that endogenous

the stem. It is proposed that NIP6;1 functions in xylem-phloem transfer for preferential distribution of B into young growing tissues (Tanaka et al., 2008).
Hv-BOR2/Bot1 and Ta-BOR2 function in tolerance of high B in barely and wheat.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Molecular, genetic and physiological studies have revealed that B transport in plants not only occurs by passive diffusion across membranes but is also catalysed by regulated transport proteins. These transporters function to support normal growth under both low and high B conditions. Our understanding of B acquisition from soils by roots has greatly advanced; however, the processes of B distribution within the aerial portions of plants are still poorly understood. In particular, more emphasis should be placed on the molecular study of B transport to reproductive organs during the reproductive stages under limited B conditions, because B limitation causes sterility, which is the major cause of yield reduction of crops under low B conditions. At the cellular level, mechanisms for preferential partitioning of B into cell walls remain to be uncovered.

It is established that the expression of some B transporters is regulated in response to B conditions; NIP5;1 at the mRNA level and BOR1 at the protein level. This observation supports the idea that plants sense their B status and control B transport activities to maintain B homeostasis. It is very important to know where plants sense (local or systemic), what plants sense (boron acid or metabolites) and how plants sense (binding protein) their B status.

Distinct roles and transport properties of the gene products have been found among the BOR genes present in plants, although all of them so far tested encode efflux-type B transporters. At-BOR1 is required for B export into xylem for efficient translocation of B from beneath B supply, and is degraded under high B supply. In contrast, Hv-BOR2/Bot1, Ta-BOR2 and At-BOR4 are likely to remove B from plant cells to confer tolerance to high B. Since a high requirement for B and a requirement to load B into the xylem probably emerged during the evolution of vascular plants, it is of great interest to elucidate how BOR genes have evolved from the ancestral gene common to the animal exchangers in humans, which do not seem to transport boric acid, to fulfill their distinct roles in B transport.

From the view of application to agriculture, modulation of the expression of genes encoding B transporters has been shown to have great potential to improve tolerance of B stresses in A. thaliana plants. In addition to the transgenic approaches, these genes can be useful as genetic makers for crop breeding since genes homologous to BOR1 and NIP5;1 are widely present in plant species. It would be satisfying if the identification of the key genes and processes involved in tolerating B stresses in model plants led to the improvement of crop plants.

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