Boron in plants: deficiency and toxicity

Running title: Boron deficiency and toxicity

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Abstract

Boron (B) is an essential nutrient for normal growth of higher plants, and B availability in soil and irrigation water is an important determinant of agricultural production. To date, a primordial function of B is undoubtedly its structural role in the cell wall; however, there is increasing evidence for a possible role of B in other processes such as the maintenance of plasma membrane function and several metabolic pathways. In the last years, the knowledge of the molecular basis of B deficiency and toxicity responses in plants has advanced greatly. The aim of this review is to provide an update on recent findings related to these topics, which can contribute to a better understanding of the role of B in plants.

Key words: Boron-binding molecules; Boron transporter; Cell wall-related genes; Gene expression; Nitrogen metabolism.

Introduction

Boron (B) is a member of the subgroup III of metalloids and has intermediate properties between metals and nonmetals (Marschner 1995). Despite its low abundance in the nature, B is widely distributed in both lithosphere and hydrosphere, B concentration ranging from 5-10 mg kg^{-1} in rocks (Shorrocks 1997), 3-30 μg kg^{-1} in rivers (Power and Woods, 1997) and ~4.5 mg L^{-1} in ocean (Lemarchand et al. 2000).

B is essential for plants (Warington 1923**)**, and B availability in soil and irrigation water is an important determinant of agricultural production (Tanaka and Fujiwara 2007). In soil solution B exists primarily as boric acid [$B(OH)_3$], which can be easily leached under high rainfall conditions (Shorrocks 1997; Yan et al. 2006) leading to deficiencies in plants that grow there (e. g., many regions in Japan, China, USA, and Brazil). On the contrary, under low rainfall conditions, B can not be sufficiently leached and therefore may accumulate to levels that become toxic to plant growth (Reid 2007b). This is very often in arid and semiarid regions with high-boron groundwater, where the accumulation of B in topsoil due to the evaporation of groundwater reaches toxic levels that reduce crop yields (Tanaka and Fujiwara 2007).

Both boric acid and borate are capable to form complexes with a wide variety of biological compounds having two hydroxyl groups in *cis*-configuration. To date, one of the primary functions of B in higher plants has been reported to be derived of its capacity to form borate esters with apiose residues of rhamnogalacturonan II (RG-II) (Kobayashi et al. 1996). The formation of this complex is essential for cell wall structure and function (O'Neill et al. 2004) since contributes significantly to the control of cell wall porosity (Fleischer et al. 1999) and tensile strength (Ryden et al. 2003). For instance, abnormally swollen cell walls and a decreased RG-II dimer formation have been shown to result from B deficiency (Matoh 1997; Ishii et al. 2001). In addition, the essentiality of the RGII-borate complex for normal plant growth has been shown in the *Arabidopsis thaliana* mutant *mur1-1* and *mur1-2* plants with a reduced amount of this complex (O'Neill et al. 2001). Noguchi et al. (2003) have also reported a lower degree of cross-linking in the cell walls of Arabidopsis *bor1-1* mutant compared to wild-type plants under limited B supply, which seemed to be a consequence of its lower shoot B levels.

Boron uptake and translocation

As above mentioned, agricultural regions that contain insufficient or toxic levels of B in soil have problems with yield and quality of many crops. Hence, understanding the mechanisms that are involved in B uptake and distribution in plants can be critical to improve agricultural production. On this matter, important advances in the knowledge of the molecular aspects that control these processes have been reported in the last years.

B uptake by root plants

B is present in soil solution in several forms but, at common soil pH values (5.5-7.5), the most plentiful form is the soluble undissociated boric acid [$B(OH)_3$]. It is accepted that plants take up B from soil in form of boric acid. Depending on B availability, boric acid uptake by roots can be performed by three different molecular mechanisms: (1) passive diffusion across lipid bilayer, (2) facilitated transport by major intrinsic protein (MIP) channel, and (3) an energy dependent high-affinity transport system induced in response to low B supply, which is mediated via BOR transporters (Tanaka and Fujiwara 2007).

Under conditions of adequate or excessive B availability, boric acid absorption by roots is mediated through a passive process that involves mostly B diffusion across lipid bilayer (Brown et al. 2002; Tanaka and Fujiwara 2007). In fact, the lipid permeability coefficient for boric acid, calculated both theoretically (Raven 1980) and experimentally (Dordas and Brown 2000; Dordas et al. 2000; Stangoulis et al. 2001b), supports the idea that B can cross membranes by a passive process to satisfy plant B requirements (Brown et al. 2002).

Several data suggest that B uptake may be mediated by MIPs channels, which can transport small neutral molecules (Dannel et al. 2002). The first experimental evidence suggesting the involvement of channel proteins in B transport were provided by Dordas et al. (2000), who described that B permeation across plasma-membrane vesicles obtained from squash roots was partially inhibited by channel blockers such as mercuric chloride and phloretin. These results were subsequently verified in *in vivo* assays performed with intact squash roots (Dordas and Brown 2001). In addition, Dordas et al. (2000) showed that expression of the maize PIP1 (a member of MIP

family) in *Xenopus laevis* oocytes resulted in an increase of B absorption. Recently, it has been identified a novel boric acid channel in *Arabidopsis* (AtNIP5;1) that belongs to nodulin 26-like intrinsic proteins (NIP) subfamily of MIPs family (Takano et al. 2006). AtNIP5;1 is localized and expressed in the plasma membrane of root epidermal, cortical, and endodermal cells. The expression of AtNIP5;1 is up-regulated in B-deficient roots, which suggests that this channel is crucial for the uptake of the B required for plant development under B limitation (Takano et al. 2006). In rice, OsNIP3;1 shows a close homolog sequence to AtNIP5;1 and it has been also identified as a boric acid channel required for efficient growth under B-deficient conditions (Hanaoka and Fujiwara 2007).

Physiological studies also have shown the occurrence of an active B uptake by roots under low B conditions (Dannel et al. 2000 and 2002; Stangoulis et al. 2001b). This active absorption of B is supported by the fact that B uptake was inhibited by both metabolic inhibitors and cold treatment in roots (Pfeffer et al. 1999; Dannel et al. 2000). However, to date, only one BOR transporter (OsBOR1 in rice) has been suggested to be involved in the efficient B uptake into root cells under B deficiency (Nakagawa et al. 2007; Tanaka and Fujiwara 2007).

B allocation in plants

Once B has been absorbed by root cells this micronutrient must be loaded into xylem. In well B supplied plants this process is mediated by a passive mechanism that involves both B diffusion across lipid bilayer and facilitated permeation of boric acid via MIPs channel (MIPs) (Dannel et al. 2002). However, an energy dependent high-affinity transport system mediated via BOR transporters is induced in response to low B supply. The first B transporter involved in the process of xylem loading was identified as BOR1 in *A. thaliana*, which is accumulated in plasma membrane of pericycle cells under low B conditions (Takano et al. 2002). In addition, *bor1-1* mutant plants showed a reduced transport of B to the shoot under B deficiency when compared to wild type Arabidopsis plants (Takano et al. 2002). All these findings demonstrate that BOR1 is an efflux-type B transporter for xylem loading under low B limitation (Takano et al. 2002). Afterwards, other *BOR1*-like genes have been identified in *Eucalyptus* (Domingues et al. 2005) and rice (Nakagawa et al. 2007).

After being loaded into xylem, B is transported through this vascular system to shoot in a process mediated by transpiration stream (Raven 1980; Shelp et al. 1995).

However, B can be also transported via phloem to both reproductive and vegetative tissues (Shelp et al. 1995; Matoh and Ochiai 2005), although this capacity varies among species (Brown and Shelp 1997). One mechanism that has been suggested to mediate phloem transport of B involves the formation of boron-diol complexes as transport molecules (Brown and Hu 1996; Hu et al. 1997). In fact, B can readily bind to *cis*-hydroxyl groups of sugar alcohols (mannitol and sorbitol), which allow B to be transported through phloem. For instance, B-polyol complexes have been isolated and characterized from the phloem sap in *Apium graveolens* (Hu et al. 1997). In addition, it has been observed that tobacco transgenic plants with an enhanced sorbitol levels had higher capacity to transport B by phloem and increased tolerance to B deficiency (Bellaloui et al. 1999; Brown et al. 1999). However, B transport via phloem, especially to young tissues, also occurs in plants that are not able to produce these types of carbohydrates (Stangoulis et al. 2001a; Takano et al. 2001; Matoh and Ochiai 2005). Very recently it has been demonstrated that B is transported from mature leaves into actively growing reproductive organs via phloem in white lupin (Huang et al. 2008). Nevertheless, the molecular mechanism involved in this B-phloem transport is still unknown.

Despite in the last years our knowledge about molecular mechanisms that mediate B uptake and distribution in plants has advanced notably, further investigations will be needed to understand better both processes. Newly, it has been identified several *BOR1*-like genes in Arabidopsis and rice (Nakagawa et al. 2007), and some of them could be involved in B uptake and distribution in plants.

Boron deficiency

It is well known that B deficiency causes different effects on very diverse processes in vascular plants such as root elongation, IAA oxidase activity, sugar translocation, carbohydrate metabolism, nucleic acid synthesis, and pollen tube growth (Blevins and Lukaszewski 1998; Goldbach and Wimmer 2007). As if these were not enough, membrane potential, plasmalemma-bound enzymes and ion fluxes across membranes (Blaser-Grill et al. 1989; Goldbach et al. 2001), cytoskeletal proteins (Yu et al. 2001, 2003), accumulation of phenolics and polyamines (Camacho-Cristóbal et al.

2002, 2004, 2005), and nitrogen metabolism (Camacho-Cristóbal and González-Fontes 1999, 2007), among others, are processes in which B can be also involved.

Cell wall, cytoskeleton, and membranes

It is widely known that B deficiency results in the formation of abnormal cell wall with altered physical properties (Fleischer et al. 1999; Ryden et al. 2003), these effects being a consequence of the role of B in cross-linking of cell wall RG-II and pectin assembly. Other works have investigated the short-term effect of B deprivation on structural changes in cell walls. Thus, in maize root apices it has been shown that B-cross-linked RG-II pectins are internalized in brefeldin A-induced compartments (Baluska et al. 2002), and their accumulation within compartments of the endocytic pathway is inhibited by B deficiency resulting in an enhanced build-up of pectins in the cell wall (Yu et al. 2002). Moreover, it has reported that the expression of several cell wall-modifying enzymes is down-regulated after 6 and 24 h of B deprivation (Camacho-Cristóbal et al. 2008), which could alter the cell wall loosening that results in cell elongation (Cosgrove 1999). For instance, the decrease of several xyloglucan endotransglycosylase/hydrolases transcript levels observed under B deficiency (Camacho-Cristóbal et al. 2008) might affect the rearrangement of the xyloglucan cross-linked microfibrillard network with the consequent alteration in the tensile properties of cell walls (Ryden et al. 2003).

Several studies have shown a possible role for B in cytoskeleton structure and associated processes (Yu et al. 2001, 2003; Bassil et al. 2004). Thus, B deprivation increased the levels of actin and tubulin proteins in Arabidopsis roots (Yu et al. 2001) and changed the cytoskeletal polymerization patterns in cells of maize root apices (Yu et al. 2003). This accumulation of cytoskeletal proteins has been proposed to be an adaptative response for contributing to mechanical reinforcement of cells of root periphery under B deficiency (Yu et al. 2003).

There is increasing evidence that B is required for the maintenance of the structure and functions of membranes and, especially, plasma membrane (Shelp 1993; Cakmak and Römheld 1997; Goldbach et al. 2001; Brown et al. 2002). For example, B deficiency altered the membrane potential and reduced the activity of proton-pumping ATPase in *Helianthus annuus* (Ferrol and Donaire 1992) and *Daucus carota* (Blaser-Grill et al. 1989) roots. Furthemore, it has been also described that B deficiency alters plasma membrane permeability for ions and other solutes (Cakmak et al. 1995; Wang et

al. 1999). Despite the clear and rapid effects of B deprivation, the underlying mechanisms by which B deficiency affects the structure and function of plasma membrane are still unknown (Blevins and Lukaszewski 1998; Brown et al. 2002; Goldbach and Wimmer 2007). Therefore, it has been suggested that some membrane molecules containing hydroxylated ligands such as glycoproteins and glycolipids are good candidates for a possible B function in membranes (Goldbach and Wimmer 2007). However, to date, the occurrence of these B complexes has not been proved yet. A recent study showed that at least three potentially B-binding membrane glycoproteins were neither detected in B-deficient pea nodules nor in other B-deficient plant tissues, which could indicate that B and certain membrane glycoproteins are involved in membrane processes associated with general cell growth (Redondo-Nieto et al. 2007). In addition, surface proteins attached to the membrane via a glycosyl-phosphatidyl-inositol anchor such as arabidogalactan proteins (AGP) have been suggested to be putative B-binding structures (Goldbach and Wimmer 2007). Interestingly, a similar alteration in cell wall pectins has been demonstrated in pollen tubes suffering from B deficiency (Yang et al. 1999) or exposed to Yariv reagent (Roy et al. 1998), a compound that cross-links plasma membrane-associated AGPs. Moreover, recently it has been shown that B deficiency causes a rapid decrease in the expression of several AGP genes in Arabidopsis roots (Camacho-Cristóbal et al. 2008). Therefore, it is important to point out that B might exert its action in membranes not only by stabilizing of membrane-molecules with *cis*-diol groups (Bolaños et al. 2004a), but also by regulating the expression of genes involved in membrane structure and function.

Nitrogen fixation and nitrate assimilation

Several studies have pointed out the essentiality of B for N_2 fixation in the heterocyst of the cyanobacterium *Anabaena* PCC 7119 (Mateo et al. 1986; García-González et al. 1990) and in the vesicles of actinomycetes of the genus *Frankia* (Bolaños et al. 2002). Both types of microorganisms require B for the stability of the envelopes that protect nitrogenase from inactivation by oxygen when grown under N_2-fixing conditions. Moreover, it has been described a lower number of developed nodules and capacity to fix N_2 in legumes under B deficiency (Bolaños et al. 1994; Yamagishi and Yamamoto 1994), which could be attributable to the possible role of B in *Rhizobium*-legume cell surface interaction (Bolaños et al. 1996). Specifically, B is needed for the targeting of nodule-specific plant derived glycoproteins (Bolaños et al.

2001) that are crucial as signals for bacteroid differentiation into a N_2-fixing form (Bolaños et al. 2004b). In addition, the cell walls of B deficient nodules have low levels of hydroxyproline-/proline-rich proteins such as ENOD2, which results in a higher oxygen diffusion into the nodules and the consequent inactivation of nitrogenase (Bonilla et al. 1997).

There are several reports on the possible involvement of B in nitrogen assimilation. For instance, a reduced nitrate reductase (NR) activity and enhanced accumulation of nitrate have been described in B deficient plants (Kastori and Petrovic 1989; Ramón et al. 1989; Shen et al. 1993), these effects being attributable to the possible role of B in the *de novo* synthesis of the NR protein or facilitation of nitrate absorption (Ruiz et al. (1998a). However, tobacco plants subjected to a severe B deficiency (B-deprived plants for 6 weeks) had a significant decrease in leaf NR activity, as well as in magnesium, calcium, potassium and, especially, nitrate concentrations in comparison to control plants (Camacho-Cristóbal and González-Fontes 1999). More recently it has been shown that short-term B deficiency led to a decline in root and, especially, leaf nitrate contents without affecting NR activity (Camacho-Cristóbal and González-Fontes 2007) or the concentrations of other macronutrients such as magnesium, calcium, potassium or phosphate (Camacho-Cristóbal et al. 2005). This decreased nitrate content was attributable to the lower net nitrate uptake rate found in B-deficient plants, probably as a consequence of the drop in the levels of root plasma membrane H^+-ATPase (*PMA2)* transcript during the B deficient treatment (Camacho-Cristóbal and González-Fontes 2007). In addition, B deficiency may also promote ammonium assimilation via asparagine synthetase in tobacco roots (Camacho-Cristóbal and González-Fontes 2007).

Secondary metabolism and oxidative stress

There is evidence that B is one of the nutrients responsible for the changes in concentration and metabolism of phenolic compounds in vascular plants. In fact, it is well known that B deficiency causes an accumulation of phenolics through the stimulation of the enzyme phenylalanine-ammonium lyase (PAL) (Cakmak et al. 1995; Ruiz et al. 1998b; Camacho-Cristóbal et al. 2002). Other reports have shown that B deficiency not only induced quantitative changes but also qualitative changes in the phenolic pool of plants (Camacho-Cristóbal et al. 2002; 2004; Karioti et al. 2006). Thus, B deficiency caused an accumulation of two polyamine-phenolic conjugates that were

not detected in B-sufficient conditions. This is consistent with the increased accumulation of polyamines reported in B-deprived tobacco plants (Camacho-Cristóbal et al. 2005).

B deprivation also increased the activity of polyphenoloxidase activity (PPO) (Pfeffer et al. 1998; Camacho-Cristóbal et al. 2002), enzyme that catalyses the oxidation of phenolic compounds into quinones. Although it has been proposed that the loss of membrane integrity under B deficiency may be due to accumulated phenolics and their oxidation products (Cakmak and Römheld 1997), it has been demonstrated that resupply of B to deficient leaves does not recovery plasma-membrane integrity throughout complexing phenols or inhibiting PPO activity (Pfeffer et al. 1998; Ruiz et al. 1999; Cara et al. 2002).

The ascorbate/glutathione cycle plays an essential role in the oxygen toxic species detoxification mechanisms in cells, and different researchers have shown that B deficiency has an effect on this cycle. In fact, both ascorbate and glutathione levels have been shown to decrease in root and leaves under B-deficient conditions (Lukaszewski and Blevins 1996; Cakmak and Römheld 1997). For example, Lukaszewski and Blevins (1996) observed a decrease in ascorbate concentration in root tips of squash suffering from B deficiency that was not related to ascorbate oxidation. Interestingly, the ascorbate concentration declined in proportion to growth rate under low B supply, and the external addition of ascorbate to the low B medium improved root growth. Thus, these authors proposed that root growth inhibition in B-deficient squash may be a result of impaired ascorbate metabolism. More recently it has been reported an induction in the expression of glutathione S-transferase and glucosyltransferase in tobacco BY-2 cells, which might constitute a rescue system against oxidative damage under B deficiency (Kobayashi et al. 2004). Furthermore, B induced an enhancement of glutathione levels in sunflower and maize plants subjected to aluminium stress (Ruiz et al. 2006; Corrales et al. 2008), which supports the view that adequate B supply stimulates antioxidant responses in aluminium stressed plants.

Boron toxicity

B toxicity is a worldwide problem that limits significantly crop yield in agricultural areas of Australia, North Africa, and West Asia characterized by alkaline

and saline soils together with a low rainfall and very scarce leaching. In addition, B-rich soils also occur as a consequence of over-fertilization and/or irrigation with water containing high levels of B (Nable et al. 1997).

B toxicity exerts different effects on very diverse processes in vascular plants, such as altered metabolism, reduced root cell division, lower leaf chlorophyll contents and photosynthetic rates, and decreased lignin and suberin levels, among others (Nable et. al. 1997; Reid 2007b). Accordingly a reduced growth of shoots and roots is typical of plants exposed to high B levels (Nable et al. 1990).

B accumulation follows a pattern from leaf base to tip in many plants and this leads to typical toxicity symptoms on older leaves which appear as marginal or tip chlorosis or both and necrosis (Marschner 1995; Roessner et al. 2006). Although the physiological basis for B toxicity is not clear enough, three main causes have been proposed taking into account our knowledge on B chemistry, that is, the ability of B to bind compounds with two hydroxyl groups in the *cis*-configuration: (1) alteration of cell wall structure, (2) metabolic disruption by binding to the ribose moieties of molecules such as ATP, NADH or NADPH, and (3) disruption of cell division and development by binding to ribose, either as the free sugar or within RNA (Reid et al. 2004).

There is no evidence to support the hypothesis that toxicity in leaves is due to osmotic stress induced by the accumulation of B (Reid et al. 2004). Although growth was rapidly inhibited by internal B concentrations in the range 1-5 mM, this inhibition was not attributable to effects of B on either energy supply or inhibition of protein synthesis, but the toxicity to mature tissues was rather due to the accumulated retardation of many cellular processes, enhanced in light by photooxidative stress (Reid et al. 2004).

Boron toxicity and salt stress

Simultaneous stress by B toxicity and salinity can occur when either plants are irrigated with water containing high levels of B and salts (Nable et al. 1997), or plants are grown in soils with natural presence of high concentrations of salts and B, usually in semiarid and arid regions characterized by low rainfall and poor drainage (Marschner 1995; Nable et al. 1997). Very recently it has been reported that combined B toxicity and salinity caused less severe toxic effects on growth than what would be expected if effects of the separate factors were additive, suggesting as possible explanations reduced uptake of B in the presence of chloride and reduced uptake of chloride in the

presence of B (Yermiyahu et al. 2008). Also it has been proposed that, under simultaneous presence of B and salt stress, boric acid could affect the activity of specific membrane components regulating the functions of certain aquaporin isoforms and ATPase as possible components of the salinity tolerance mechanism (Martínez-Ballesta et al. 2008). In fact, at high external B levels, significant B transport occurs through the plasma membrane aquaporins (Dordas et al. 2000; Dordas and Brown 2001).

Interestingly, it has been reported that increased B and calcium supplies enhance crop salt tolerance and improve yield production in saline soils (El-Hamdaoui et al. 2003a,b; Bonilla et al. 2004), which could be useful for agriculture.

Boron-toxicity tolerance

B-tolerant varieties are characterized by a decreased B concentration in their leaf tissues in comparison to non-tolerant varieties (Nable et al. 1990), probably due to a reduced uptake of B into both roots and shoots. In this sense, the basis for B-tolerance in the landrace cv Sahara has been explained by its high ability to efflux B, and it has been reported two models for this mechanism of active efflux of B, namely, anion (borate) exchange or an anion channel (Hayes and Reid 2004).

As above set out, BOR1 is an efflux-type borate transporter required for the transport of B from roots to shoots under low B supply (Takano et al. 2002). However, in the presence of B toxic levels BOR1 is degraded via endocytosis (Takano et al. 2005), and overexpression of *BOR1* gene does not result in a better plant growth (Miwa et al. 2006). These results suggest that BOR1 is not involved in B tolerance. Nevertheless, an independent transgenic *A. thaliana* line has been generated showing that BOR4, another efflux-type borate transporter, is not degraded at posttranslational level as occurs with BOR1 (Miwa et al. 2007). Accumulation of BOR4-GFP and tolerance of B were positively correlated and the overproduction of BOR4-GFP improved plant growth under high B levels through B efflux. Moreover, GFP fluorescence derived from BOR4-GFP was strongly detected in the plasma membrane of epidermal cells in the root elongation zone of *A. thaliana* (Miwa et al. 2007).

Also in the barley landrace Sahara 3771 has been identified *Bot1*, a *BOR1* ortholog, as the gene responsible for the B-toxicity tolerance. This cultivar contains 3.8 times more *Bot1* gene copies than the B-intolerant malting variety Clipper, and *Bot1* transcript levels in Sahara are about 160-fold and 18-fold higher in roots and leaf

blades, respectively, when compared with Clipper (Sutton et al. 2007). Furthermore, Sahara Bot1 protein has a higher capacity to provide B tolerance in yeast than Clipper Bot1 or Arabidopsis BOR1. *Bot1* transcript levels are consistent with a lower net entry of B into the barley root and in a higher B disposal from leaf guttation through hydathodes (Sutton et al. 2007). Therefore, the ability of Sahara cultivar to maintain lower shoot B concentration is at least due to a mechanism of active B efflux from the root, that is, *Bot1* encodes a functional B efflux transporter responsible for B tolerance. Also it has been suggested that BOR2 gene encodes an efflux-type borate transporter responsible for tolerance to B toxicity in wheat and barley (Reid 2007a).

It has been also described that B inhibits one step of *in vitro* pre-mRNA splicing reaction (Shomron and Ast 2003), which could suggest that B toxicity is primarily due to disruption of RNA splicing (Reid 2007b). Interestingly, several B-tolerance genes from lupin and Arabidopsis that encode transcription factor or ribosomal proteins conferred tolerance to high B in yeast (Nozawa et al. 2006; Reid 2007b). These proteins could act by protecting the splicing sites from attack by B, which would indicate the occurrence of a mechanism to confer B tolerance other than the ability to efflux B from the cells (Reid 2007b).

Boron and gene expression

In the last years, several reports have described that B deficiency affects the expression level of genes related to nitrogen metabolism (Redondo-Nieto et al. 2001; Camacho-Cristóbal and González-Fontes 2007), oxidative stress (Kobayashi et al. 2004), B uptake (Takano et al. 2006; Kasajima and Fujiwara 2007), and cell wall (Camacho-Cristóbal et al. 2008). However, there is no direct evidence to explain how the signal from B deficiency is transferred to nuclei. Kobayashi et al. (2004) proposed that a quick signal transfer from the cell wall to the cytoplasm could be involved for gene induction after the cellular redox imbalance imposes by B deficiency. Goldbach and Wimmer (2007) suggested that changes in B concentrations may lead to a mechanical cascade of signals extending into the cytoplasm via the cell wall-plasma membrane-cytoskeleton continuum, with the possible involvement of AGPs. This hypothesis is supported by the fact that B deprivation led to an altered polymerization pattern of cytoskeletal proteins (actin and tubulin) assemblies (Yu et al. 2001, 2003) and

to an inhibition of the endocytic pathway for internalization of B-cross-linked RG-II pectins in brefeldin A-induced compartments (Yu et al. 2002). Finally, González-Fontes et al. (2008) have also suggested a putative role of B as a cellular signal capable of interacting with transcription factors, which could explain why the expression of several genes involved in different physiological processes are rapidly affected when vascular plants are subjected to B deficiency. Furthermore, there is also the possibility that B is affecting calcium-mediated signaling (see Bolaños et al. 2004a).

In addition, it has been reported that high B induces the expression of several genes in both roots and rosette leaves of Arabidopsis (Kasajima and Fujiwara 2007). One of these genes has been identified as a zinc finger family transcription factor (At1g03770), which could regulate the expression of downstream genes involved in the physiological response pathways to B toxicity in plants (Kasajima and Fujiwara 2007). This result might support the putative role of B as a cellular signal capable of interacting with transcription factors (González-Fontes et al. 2008).

Concluding remarks

Boron is an essential element for the growth and development of vascular plants, and adequate B nutrition is crucial for crop production. This fact highlights the importance of understanding the role of B in plants as well as the molecular mechanisms in response to B deficiency and toxicity, which will allow to improve the tolerance of crops to B stresses.

It has been established that a primary role of B is the cross-linking of cell wall RG-II and pectin assembly (Kobayashi et al., 1996). Nevertheless, Bolaños et al. (2004a) hypothesize that the primary role of B in biological systems is stabilization of molecules with *cis*-diol groups, independently of their function. Thus, it has been suggested that B could be involved in membrane function by complex formation with glycoproteins (Goldbach and Wimmer 2007). More recently, it has been proposed the possible role of B as a cellular signal through the interaction with transcription factors (González-Fontes et al. 2008). Future research should be focused to identify the B-binding ligands as well as their functions in plant physiology.

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