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Role of Boron in Vascular Plants and Response Mechanisms to Boron Stresses

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ABSTRACT

To date, the primordial function of boron is its structural role in the cell wall through stabilization of molecules containing *cis*-diol groups (borate esters with apiose residues of rhamnogalacturonan II). Nonetheless, boron is a micronutrient also involved in a great variety of physiological processes in vascular plants. However the mechanisms underlying the various metabolic disorders caused by boron deficiency are indeed unknown. Recently it has been reported that boron deficiency and toxicity induce stress-responsive genes. In this contribution we review the mechanisms involved in boron uptake and distribution, the role of boron in vascular plants, the effects of boron deficiency and toxicity on them, as well as the interaction boron toxicity and salt stress. In addition, we discuss the most recent hypotheses proposed to explain how boron could exert its function in vascular plants from a mechanistic point of view. The importance of understanding the role of boron in plants as well as the response mechanisms to its deficiency and toxicity will allow us to improve the tolerance of crops to boron stresses.

Keywords: boron deficiency, boron toxicity, boron transport, cell signalling, gene expression

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INTRODUCTION

Boron is the most electronegative element in the Group III of the periodic table, with properties intermediate between metals and electronegative non-metals. Boron has one less valence electron than the number of valence orbital, which causes an ‘electron deficiency’ that has a dominant effect on the behaviour of boron in chemical processes (Kot 2009).

The requirement of boron for plant growth was first established in the beginning of the 20th century, and nowadays it is widely known that boron is an essential element for all vascular plants whose deficiency or toxicity causes impairments in several metabolic and physiological processes (Nable *et al*. 1997; Blevins and Lukaszewski 1998; Bolaños *et al*. 2004; Reid 2007a; Camacho-Cristóbal *et al*. 2008b).

To date, one of the primary functions of boron in vascular plants has been related with the cell wall structure and function. There is direct evidence for a role of boron in cross-linking of cell wall rhamnogalacturonan II (RGII) and pectin assembly showing that boron is essential for cell wall structure and function (O’Neill *et al*. 2004). As a result, boron has a critical role in growing tissues. Actually boron deficiency decreases or inhibits the growth of both vegetative and reproductive plant parts (Dell and Huang 1997). Structural function of boron, together with its limited mobility in most cultivated plant species (Brown and Shelp 1997), makes that boron must be supplied continually

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throughout the life of the plant. Therefore, controlling the boron availability in soils for plant growth and production is of considerable agronomic significance.

BORON IN SOILS AND PLANTS

Boron in soils

The abundance of boron in the universe is extremely low: about 10^9-fold less than hydrogen and about 10^6-fold less than carbon, nitrogen, or oxygen (Kot 2009). In spite of this scarcity, boron is broadly distributed in both lithosphere and hydrosphere, boron concentration ranging from 5 to 10 mg kg^{-1} in rocks (Shorrocks 1997), 3-30 µg kg^{-1} in rivers (Power and Woods 1997) and about 4-5 mg L^{-1} in oceans (Lemarchand *et al.* 2000).

Boron is never found in its elemental form in nature, but is found in rocks and concentrated in deposits as borates, i.e. bound to oxygen together with sodium, calcium, silicon, or magnesium, for example, and it will usually be hydrated (Argust 1998). At least 200 minerals are known to contain the element boron; however, only four boron-containing minerals, borax (hydrated sodium borate), kernite (another form of hydrated sodium borate), colemite (hydrated calcium borate hydroxide), and ulexite (hydrated sodium calcium borate hydroxide), are of commercial importance (Parks and Edwards 2005).

Most soils have less than 10 mg boron kg^{-1} and, hence, are considered to be poor in boron (Woods 1994). Moreover, the majority of this boron is immobilized in rocks and not readily available for plants. Aluminium and ion oxides, magnesium hydroxide, calcium carbonate, organic matter, or clays can act as soil adsorbing surfaces for boron (Goldberg 1997). During rock weathering, boron goes easily into soil solution mainly as boric acid (Nable *et al.* 1997) and is readily available for plant uptake, but this pool constitutes about 10 % of total soil boron (Power and Woods 1997). In soils, boron movement follows the water flux; hence, in cool humid climates soil boron is leached downward in soil profiles, whereas in soils of warm humid, or arid and semi-arid regions, boron is likely to concentrate in surface horizons (Kot 2009).

Boron availability can be affected by several soil factors such as pH, texture, temperature, and organic matter, among others (Goldberg 1997), soil pH being one of the most important parameters. In fact, boric acid is a very weak acid and when the pH is below 7 appears in its undissociated form; at alkaline pH, boric acid dissociates to form the borate anion:

$$B(OH)_3 + H_2O \rightleftarrows B(OH)_4^- + H^+ \ (pK_a\ 9.25)$$

Therefore, in neutral or slightly acid soils boron occurs mainly as undissociated boric acid, and in this form is absorbed by plant roots (Hu and Brown 1997; Power and Woods 1997), which is mobile and easily lost by leaching under high rainfall conditions leading to boron deficiency in plants that grow there. On the contrary, under low rainfall conditions boron cannot be sufficiently leached and therefore may reach levels that become toxic to plant growth (Reid 2007a). Furthermore, various anthropogenic sources of excess boron may increase soil boron to levels that are toxic for plants. The most important boron source is irrigation water (Nable *et al.* 1997), but others include waste from surface mining, fly ash, and industrial chemicals (Kot 2009).

Boron uptake by roots and xylem loading of boron

Boron deficiency and toxicity cause major disorder that can limit plant growth on soils with high rainfall, and of arid and semiarid environments, respectively (Nable *et al.* 1997). Hence, awareness should be taken to understand the mechanisms that take part in boron transport and distribution in plants in order to improve agricultural production. During the last ten years, important advances in elucidation of the molecular mechanisms involved in boron uptake by root cells and boron allocation in plants have been reported.

As above-mentioned, in soil solution boron is found mainly as the soluble uncharged boric acid [$(B(OH)_3$]. It is acknowledged that plants take up boron from soil in this chemical form. It has long been believed that this nutrient is passively absorbed by root cell through simple diffusion mechanism, which can meet the plant requirement of this element under condition of adequate or excessive boron availability. This affirmation was supported by experimental data from lipid permeability coefficients calculated in both the membrane vesicles isolated from *Cucurbita pepo* (Dordas *et al.* 2000) and giant internodal cells of the *Chara corallina* (Klein ex Will. Esk. R. D. Wood) (Stangoulis *et al.* 2001b).

Physiological studies have also suggested the occurrence of an active boron transport in roots (Dannel *et al.* 2000; Stangoulis *et al.* 2001b; Dannel *et al.* 2002). This hypothesis is supported by experimental data showing that both metabolic inhibitors and cold treatments inhibit boron absorption by roots (Dannel *et al.* 2000). Moreover, high boron concentrations in sunflower root symplasm have been measured when plant culture was supplied with low boron (Dannel *et al.* 2002).

However, recent studies have demonstrated that channel proteins also perform boric acid transport into root cells. Thus, Takano *et al.* (2006) characterized a boric acid channel in *Arabidopsis* (AtNIP5;1) that is localized in both plasmalemma of root elongation cells and root hair cells. This protein is crucial for boron absorption into root cells when boron concentration in soil is low. In addition, in rice, OsNIP3;1 – which shows a close homolog sequence to AtNIP5;1 – has been proposed as a boric acid channel required for efficient boron uptake into the root cells under boron-limiting conditions but, unlike AtNIP5;1, it is also detected in shoots (Hanaoka and Fujiwara 2007).

As root cells absorb boron, this micronutrient must be exported from endodermal, pericycle or xylem parenchyma cells into the stelar apoplasm (xylem loading). When plants grow in media with enough boron availability, xylem loading of boron is performed both by boron diffusion across lipid bilayer and facilitated permeation via protein channel (Dannel *et al.* 2002). Nevertheless, an active transport system mediated by BOR transporters is mainly responsible for export boron towards xylem under boron-deficient conditions. BOR1 was identified as the first efflux-type boron transporter for xylem loading in *Arabidopsis* under boron-limiting conditions (Takano *et al.* 2002). In *Eucalyptus*, based on amino acid similarity and *in silico* expression patterns, a BOR1-like sequence has been proposed as a putative candidate BOR1 homolog (Domingues *et al.* 2005). Afterwards, another *BOR1*-like gene has been identified in rice (*OsBOR1*), which is involved in both xylem loading of boron and its absorption into the root cells under boron limitation (Nakagawa *et al.* 2007).

In summary, three different molecular mechanisms have been described to transport boric acid from soil solution towards root cells and xylem loading of boron. Thus, depending on boron availability, boron transport can be performed by: (1) passive transport across plasmalemma mediated by simple diffusion. This system operates mainly when adequate or excessive boron is available in the soil; (2) energy dependent high-affinity transport that is induced in response to low boron supply, and it is mediated via BOR transporters; and (3) facilitated transport carried out by NIP channel proteins (Tanaka and Fujiwara 2008).

Boron distribution in plants

Once boron is into xylem, it is transported towards shoot in a process mediated by transpiration stream (Shelp *et al.* 1995). In addition, boron can also be transported via phloem to both reproductive and vegetative tissues (Shelp *et al.* 1995; Matoh and Ochiai 2005), although the mobility of boron through this vascular system drastically changes among species (Brown and Hu 1996; Brown and Shelp 1997). In plants that transport sugar alcohols via phloem, quick and significant movement of boron has been described (Brown and Hu 1996). For instance, mannitol-boron-mannitol complex has been isolated from the phloem sap of *Apium graveolens* L. (Hu *et al.* 1997). Importantly, it has been observed that transgenic tobacco and rice plants with an enhanced sorbitol levels had higher capacity to transport boron through phloem towards young tissues (Brown *et al.* 1999; Bellaloui *et al.* 2003). All these results point out the possibility that phloem transport of boron involves the formation of boron-diol complexes with sugar alcohols as transport molecules (Brown and Hu 1996; Hu *et al.* 1997). However, boron transport via phloem, especially to young tissues, also occurs in plant species (such as broccoli, lupin, *Arabidopsis*, wheat, canola, sunflower) that are not able to produce these types of carbohydrates, although this translocation is not as efficient as in those plants that produce sugar alcohols (Shelp *et al.* 1998; Huang *et al.* 2001; Stangoulis *et al.* 2001a; Takano *et al.* 2001; Matoh and Ochiai 2005; Huang *et al.* 2008). Nevertheless, the molecular basis that supports this boron-phloem transport is still unknown. Very recently a boric acid channel (NIP6;1) involved in boron transfer from xylem towards phloem in young shoot sink tissues under low boron supply has been identified in *Arabidopsis thaliana* (Tanaka *et al.* 2008).

BORON DEFICIENCY AND PLANT DEVELOPMENT

The essentiality of boron has been shown not only in vascular plants, but also in cyanobacteria, algae, fungi and animals (Brown *et al.* 2002; Goldbach and Wimmer 2007). Many physiological processes are affected by boron deficiency (Blevins and Lukaszewski 1998; Brown *et al.* 2002; Camacho-Cristóbal *et al.* 2008b). However, bearing in mind that it is a micronutrient and between 80 and 90 % of the boron is located in the cell wall of vascular plants (Hu and Brown 1994) – i.e. outside the protoplast –, is really surprising that boron deficiency can lead to negative effects in such a wide variety of processes (González-Fontes *et al.* 2008).

Physiological effects of boron deficiency during the vegetative stage

Boron deprivation affects the functional structure of the cell wall as a consequence of the role of boron in dimerization of rhamnogalacturonan II (RGII) and pectin assembly by borate cross-linking (O'Neill *et al.* 2004). Yu *et al.* (2002) showed that RGII pectins increased under boron deficiency and their internalization was inhibited. These authors suggested that cell wall boron might regulate internalization of RGII pectins by interference with receptor-mediated endocytosis. Also, as an early consequence of boron deprivation, increased levels of actin and tubulin proteins and altered polymerization pattern of their cytoskeletal assemblies were observed in maize root apices (Yu *et al.* 2003). It was tentatively proposed that this response to boron deficiency is an adaptative mechanism in order to strengthen the cell periphery complex of their maize root cells (Yu *et al.* 2003). Furthermore, a short-term starvation of boron down-regulates the expression of several cell wall-modifying enzymes (Camacho-Cristóbal *et al.* 2008a). Thus, boron deficiency decreases transcript contents of several xyloglucan endotransglycosylase/hydrolase (Camacho-Cristóbal *et al.* 2008a), which might influence the rearrangement of the xyloglucan cross-linked microfibrillard network and, consequently, the tensile properties of cell wall (Ryden *et al.* 2003).

Several genes involved in the rescue system for oxidative damage showed up-regulated expression when tobacco BY-2 (*Nicotiana tabacum* L. cv. 'Bright Yellow 2') cells were cultured under low boron conditions (Kobayashi *et al.* 2004). As boron deficiency alters cell wall structure and also causes oxidative damage, both processes were connected each other (Kobayashi *et al.* 2004). These authors suggested that boron deficiency might impose cellular redox imbalance because of the impaired cell wall structure. However, Cakmak and Römheld (1997) supported a possible protective role of boron to shield plasma membrane from oxidative damage caused by toxic oxygen species. In addition, boron was also involved in a protective role of thylakoid membrane through decreasing the production of free oxygen radicals (El-Shintinawy 1999).

Lower levels of root ZmPIP1 aquaporins have been reported under boron deficiency (Goldbach *et al.* 2001). Therefore, the decreased plasmalemma hydraulic conductivity found under chilling conditions may be more severe under boron deficiency, which would explain why this mineral deficiency aggravates chilling damages in leaves (Huang *et al.* 2005).

It has long been known that boron deficiency inhibits root elongation (Dugger 1983; Marschner 1995) and that root growth is more sensitive to boron deficiency than shoot growth (Dell and Huang 1997). This primary effect of boron seems to be a direct consequence of loosing the cell wall plasticity (Hu and Brown 1994), whereas the inhibition of cell division of the root meristematic region is a secondary effect of boron deficiency (Dell and Huang 1997).

With regard to shoots, boron deficiency first affects the shoot apex and the actively growing leaves. The latter become small, dark green, deformed in shape, with brown purple pigmentation and necrotic spots if this nutritional deprivation is prolonged (Dell and Huang 1997). Shoots subjected to boron deficiency appear with shorter internodes, and petioles and stems with bigger diameter (Marschner 1995).

Two boron pools of different water solubility in the roots and shoots have been distinguished: boron in the water-insoluble residue (bound to cell wall) and boron in cell sap (soluble in the symplasm) (Dannel *et al.* 1998). Boron deprivation reduced significantly the concentration of this anion in root cell sap of pea plants, and also diminished cytokinin level as well as the indoleacetic acid export out of the shoot apex (Li *et al.* 2001). According to these authors, hormonal effects could be responsible for the decline in elongation growth and apical dominance.

Further studies were performed in order to revert the hormonal changes caused by boron deficiency. Boron application to the shoot apex restored the endogenous levels of Z/ZR-type cytokinins and indoleacetic acid, increased the export of the latter from the shoot apex, and apical dominance was partly restored (Wang *et al.* 2006).

Physiological effects of boron deficiency during the reproductive stage

Reproductive growth is often more sensitive to boron deprivation than vegetative growth (Dell and Huang 1997). These differences could be consequence of the limitation of boron delivery to reproductive tissues (Dell and Huang 1997; Brown *et al.* 2002). Typical symptoms of boron deficiency at reproductive stage are drop of buds, flowers and developing fruits, alterations in fruit quality and lower viability of seeds (Marschner 1995).

Boron requirement for flowering depends on the sensitivity of pollen development to low boron (Dell and Huang 1997). Under boron deficiency pollen become empty, deformed, shrivelled and without storage material as starch (Dell and Huang 1997). Pollen sensitivity to boron deprivation depends on the variety of flowering species, being more sensitive in compact-inflorescences species than in species with axillary inflorescences (Dell and Huang 1997). Furthermore it has been described that pollen tube growth and anther development are also sensitive to boron deprivation (Rawson 1996). In the absence of boron, pollen tubes may burst possibly due to the primary boron functions in the cell wall structure of the pollen tubes (Brown *et al.* 2002). Experimental investigations were focussed on the identification of the critical period of microsporogenesis of anther and floret fertility to try to establish a protocol to correct in time boron deficiency effects. The most sensitive stage of microspororogenesis to boron deficiency was the phase from premeiotic interphase through meiosis to late tetrad (Huang *et al*. 2000).

In addition to male sterility, pistil sterility is another alteration of reproductive system due to boron deficiency (Agarwala *et al*. 1981), although boron requirement is minor than in the male organs (Rerkasem *et al*. 1997).

It was also described that boron concentration controls suspensor development in embryogenic cultures of the conifer *Larix decidua* Mill. and no suspension formation was observed in boron-free conditions (Behrendt and Zoglauer 1996).

It has been reported that apparently normal seeds from boron-deficient plants may have altered physiological and growth properties (Dell and Huang 1997). Plants without any symptom of boron deficiency in the vegetative phase had a decreased seed production (Nyomora *et al*. 1997). Recently it was established that boron requirement is higher for seed than for forage production in alfalfa plants (Dordas 2006).

Metabolic effects of boron deficiency

Boron deficiency affects photosynthesis reducing the photosynthetic oxygen evolution rate and efficiency of photosystem II (Kastori *et al*. 1995; El-Shintinawy 1999). Moreover, in citrus seedlings, growth and photosynthesis decreased in boron deficiency. Leaves from sweet orange seedlings subject to boron deprivation had lower photosynthetic enzymes activities by excess hexoses, which led to a decrease in growth (Han *et al*. 2008). Other authors also suggested that accumulation of soluble sugars in boron-deficient leaves could inhibit net photosynthesis (Dugger 1983; Dell and Huang 1997). However, Goldbach and Wimmer (2007) stated that the mechanism for a primary role of boron in photosynthesis is unknown and, therefore, the effects of boron deficiency on photosynthesis are secondary in nature. Increased sensitivity to boron deprivation observed under high light intensity (Cakmak *et al*. 1995) could implicate photooxidative damage (Cakmak and Römheld 1997).

Boron seems to play a role in phenolic metabolism since accumulation of phenolics takes place under boron deprivation (Dugger 1983; Cakmak and Römheld 1997; Blevins and Lukaszewski 1998). It was shown that the concentration of phenylpropanoid and the polyphenol oxidase and phenylalanine ammonia-lyase increase mainly in tobacco leaves during short-term boron deficiency (Camacho-Cristóbal *et al*. 2002). Analysis by HPLC-mass spectrometry revealed that the levels of leaf chlorogenic acid increase significantly under boron deficiency, and also accumulate two caffeic acid amides, namely *N*-caffeoylputrescine and dicaffeoylspermidine (Camacho-Cristóbal *et al*. 2004).

It has been suggested that under boron deprivation an altered cell wall synthesis takes place and, consequently, an increase in soluble polyamine, as the linkage between these compounds and cell wall pectins would be modified (Camacho-Cristóbal *et al*. 2005). This is consistent with structural alterations of cell wall pectins and cytoskeleton found under boron deficiency (Yu *et al*. 2002, 2003).

Boron deficiency also affects nitrogen metabolism in vascular plants. Thus, a smaller number of developed nodules capable of nitrogen fixation have been reported in legumes grown under boron deprivation (Bolaños *et al*. 1994). Furthermore, a decreased nitrate uptake, probably due to a lower expression of plasma membrane H^+-ATPase, was observed in boron-deficient tobacco plants. In addition, boron deficiency caused a dramatic increase in the levels of asparagine (Camacho-Cristóbal and González-Fontes 2007).

Mechanisms for tolerance to boron deficiency

Data indicate that boron is supplied to sink tissues through the phloem (Shelp *et al*. 1998), even though it has been demonstrated that differences in boron mobility depend on the species variety (Brown and Hu 1996; Brown and Shelp 1997). For instance, it has been suggested that boron cannot mobilize in tobacco plants from mature tissues to reproductive organs and that the exclusive boron source in reproductive structures should provide by root uptake (Brown *et al*. 1999). Nevertheless, boron remobilization from mature tissues to flag leaves in rice plants has also been reported (Bellaloui *et al*. 2003). Recently boron retranslocation from the shoot to the root has been shown in white lupin, as well as boron re-translocation out of mature leaves into rapidly growing reproductive organs via phloem and xylem (Huang *et al*. 2008).

From data highlighting the importance of sugar alcohols in phloem boron mobility (Hu *et al*. 1997), Brown *et al*. (1999) used transgenic tobacco plants with enhanced sorbitol synthesis to show an increased tolerance to boron deficiency possibly mediated by the boron-sorbitol complex.

Arabidopsis BOR1 is an efflux transporter required for normal growth under low boron supply that is related to boron xylem loading and its distribution to young rosette leaves (Takano *et al*. 2001). As explained before, *Arabidopsis* NIP5;1 and NIP6;1 are plasma membrane boric acid transporters that belong to NIPs proteins and their expression is up-regulated by boron limitation (Tanaka *et al*. 2008). Under boron deficiency NIP6;1 is required for boron transport to growing shoot tissues via xylem-phloem transfer (Tanaka *et al*. 2008). New approaches to generate tolerant plants to boron deficiency are now being carried out by overexpression of boron transporters (BOR1 and NIP5;1) using the genetic manipulation of these proteins (Miwa *et al*. 2006; Kato *et al*. 2009).

BORON TOXICITY AND PLANT DEVELOPMENT

Boron toxicity has been recognized as an important problem limiting crop production in low-rainfall, and highly alkaline and saline soils in regions of Australia, West Asia, and North Africa (Roessner *et al*. 2006). For crop production, boron toxicity is more difficult to manage than its deficiency, which can be prevented by fertilization. However, fertilization with boron to avoid deficiency can result in toxicity (Takano *et al*. 2008), since the concentration range between boron deficiency and toxicity is narrower than for any other plant essential nutrient (Goldberg 1997).

Physiological effects of boron toxicity during the vegetative stage

Toxic boron concentration leads to different physiological effects during the life cycle of vascular plants. Specifically, Bañuelos *et al*. (1999) observed an inhibition in the percentage germination of seeds for both the Chilean and domestic (U.S.) germplasm, including corn (*Zea mays* L., PI No. 9068919 from Chile and Golden Jubilee, USA), carrots (*Daucus carota* L., PI No. 9068933 from Chile and Imperator, USA), tomato (*Lycopersicum esculentum* L., PI No. 9068924 from Chile and Cal Ace, USA), and alfalfa (*Medicago sativa* L., No. 9068931 from Chile and Southern Special, USA). Furthermore, the excess boron decreased the percentage germination and polyphenol oxidase activity in embryos and endosperm of maize seeds (*Zea mays* L. cv. 'Arifiye') during germination (Ölçer and Kocaçaliskan 2007).

The vegetative plant growth is also affected by toxic boron concentrations. The physiological effects of boron toxicity include reduced root cell division (Liu *et al*. 2000), inhibition of cell wall expansion and shoot and root growth, lower leaf chlorophyll contents and photosynthetic rates, and decreased lignin and suberin contents (Nable *et al*. 1997; Reid 2007a).

In contrast to the deficiency symptoms, the initial symptoms of boron toxicity in plant occur in the older leaves tip, and this portion become chlorotic or necrotic, progressing along the leaf margin and into the blade. Toxicity effects appear to be loosely correlated with the accumulation of high concentrations of boron in old leaves, especially at the margin of leaves. This is because boron introduced into the transpiration stream accumulates at the end of this stream (Tanaka and Fujiwara 2008).

Physiological effects of boron toxicity during the reproductive stage

At this stage of the life cycle of plants, boron toxicity especially affects the flowering and the development of both fruits and seeds. High boron concentrations caused various deleterious effects on melon growth and development. It was accompanied by a large accumulation of boron concentration in fruits. A significant delay in the number of days to first flowering was also observed when the melon plants were treated with high boron concentrations. Additionally, fruit set was completely inhibited at the highest boron treatment (Goldberg *et al.* 2003). This effect on fruits and seeds is likely to be indirect, being mediated by the longer-term accumulation of boron. For example, inhibition of root growth by excess boron will limit the ability of the plant to take up essential nutrients and water from soil solution, while necrosis in leaves will limit photosynthesis and, consecutively, the ability to supply photosynthate to developing fruits, seeds, and storage organs (Reid 2007a).

Metabolic effects of boron toxicity

Additionally, accumulation of high leaf concentrations of boron might lead to osmotic imbalances, and might reduce the capacity of plant cells to resist photooxidative damage (Reid 2007a). Excess boron also resulted in increased membrane leakiness, peroxidation of lipids and proline accumulation (Eraslan *et al.* 2007). Recently, in apple and grapevine, it has been reported that boron toxicity induces oxidative damage by lipid peroxidation and hydrogen peroxide accumulation (Gunes *et al.* 2006). Furthermore, Cervilla *et al.* (2007) described oxidative damage in tomato leaves and a general increase in antioxidant enzyme activity provoked by boron toxicity. These authors have also reported increased ascorbate pool size and higher enzyme activities both of L-galactose dehydrogenase, a key enzyme involved in the ascorbate biosynthesis, and enzymes from the Halliwell-Asada pathway.

Key enzymes related with the nitrogen assimilation in plants (i.e. nitrate reductase and asparagine synthetase) were shown significantly affected by environmental stresses such as boron deficiency, heavy metals (e.g. iron), drought and salinity (Camacho-Cristóbal and González-Fontes 1999; Herrera-Rodríguez *et al.* 2007). Boron toxicity also affects enzymes related to the nitrogen assimilation. Thus, Eraslan *et al.* (2007) reported a higher nitrate reductase activity with increasing levels of boron in tomato plants.

High irradiance appears to increase the harmful effects of boron toxicity, probably because elevated boron concentrations may impair plant mechanisms to cope with photooxidation stress (Reid *et al.* 2004).

In summary, boron toxicity causes significant changes in the physiology and activity of numerous enzymes and, consequently, the metabolism during the life cycle of plants. However, the physiological basis for boron toxicity is not clear, although three main candidate sites have been suggested in view of the ability of boron to bind compounds with two hydroxyl groups in the *cis*-configuration: (i) alteration of cell wall structure; (ii) metabolic disruption by binding to the ribose moieties of molecules such as adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (reduced form, NADH), or nicotinamide adenine dinucleotide phosphate (reduced form, NADPH); and (iii) disruption of cell division and development by binding to ribose, either as the free sugar or within RNA (Reid *et al.* 2004).

Mechanism for tolerance to boron toxicity

It has long been known that boron tolerance is related to the ability to restrict boron accumulation in both roots and shoots (Reid 2007a). In this sense, to save problems with boron toxicity, the activity of boric acid transporters must be tightly regulated to maintain the rate of radial transport of boron within an acceptable range, independently of the soil boron concentration (Takano *et al.* 2008). Recent studies have indicated a role for boron efflux transporter in tolerance to boron toxicity (Reid 2007b). For example, in yeast, knock-out and overexpression studies have demonstrated that ScBor1p is a boric acid/borate efflux transporter that confers tolerance to boron toxicity (Nozawa *et al.* 2006; Takano *et al.* 2007). This boric acid/borate efflux transporter shows significant homology to previously reported boron efflux transporters from *Arabidopsis*, BOR1 (Takano *et al.* 2002). Moreover, in barley, physiological comparisons between the highly boron-tolerant cultivar Sahara and the susceptible cultivar Schooner revealed in the tolerant cultivar a much lower root boron concentration than in the external medium, whereas no similar results were observed in sensitive cultivar (Hayes and Reid 2004). Decreased root boron concentrations were translated into lower concentrations in the xylem and much lower concentrations in the shoots. Since the apparent membrane permeability to boron was similar in both cultivars, the authors concluded that the reduced boron concentration in roots resulted not from an exclusion mechanism, but from active efflux pumping in the root cells of the tolerant cultivar (Hayes and Reid 2004). Similar results were obtained in tolerant cultivars of wheat (Reid 2007b). Molecular marker techniques have identified loci in cereals that are correlated with tolerance to high boron concentration. In barley, loci on chromosomes 4 and 6 are related to lower leaf boron accumulation and the gene(s) at these loci might encode boron efflux transporters. The loci on these chromosomes also may contain regulatory genes to control the activity or expression of transporter genes located on the other chromosomes (Reid 2007a). Currently, the identification and cloning of genes conferring tolerance to boron toxicity in cereals is potentially a major advance in the development of varieties capable of growing in high soil boron levels (Reid 2007b). In this sense, genes from roots of boron-tolerant cultivars of wheat and barley have been cloned and they present high similarity to previously reported boron efflux transporters from *Arabidopsis* (At*BOR1*) and rice (Os*BOR1*) (Reid 2007b). In a boron-tolerant cultivar of barley (Sahara 3771), Sutton *et al.* (2007) characterized a *BOR1*-like gene, *Bot1*, which provides tolerance to high boron concentrations and its gene expression was localized in roots and youngest leaf blades. In addition, based on its similarity to rice genes, Reid (2007b) has found other genes conferring tolerance to boron toxicity in cultivars of wheat (cv. 'India') and barley (cv. 'Sahara'), namely Ta*BOR2* and Hv*BOR2*, respectively.

However, in *Arabidopsis* plants, the activity of the BOR1 transporter, which catalyses the loading of boron from xylem parenchyma cells into the xylem – as discussed previously – was rapidly downregulated because BOR1 protein is degraded via endocytosis at high levels of boron (Takano *et al.* 2005, 2008). The overexpression of the *BOR1* gene does not result in a better plant growth under toxic levels of boron (Miwa *et al.* 2006). These results suggest that BOR1 transporter is not involved in boron tolerance in *Arabidopsis* plants. That is why Miwa *et al.* (2007) focused on *BOR4*, one of the six *BOR1* paralogs present in the *Arabidopsis* genome. This gene was accumulated in the presence of a high boron supply, suggesting that *BOR4* is exempt from the posttranslational *BOR1* degradation system (Miwa *et al.* 2007). By using of GFP fluorescence, BOR4 protein was detected in the plasma membranes of the distal sides of epidermal cells in the root elongation zone of

Arabidopsis transgenic lines. Distal localization is important for the directional export of boron content to the soil and, in this way, to avoid high boron concentrations could be achieved in the xylem and growing cells (Miwa *et al.* 2007). Recently, Takano *et al.* (2008) have showed that *Bot1* gene cloning in the boron-tolerant barley cultivar Sahara (Sutton *et al.* 2007), as discussed previously, it is not a *BOR1*-like gene but its function suggests that it is a *BOR4* ortholog. In summary, boric acid/borate efflux transporters appear to be key determinants of plant boron tolerance, and provide a molecular basis for the generation of highly boron-tolerant crops (Takano *et al.* 2008).

It has been suggested that excess boron could provoke disruption of RNA splicing, this effect being a major reason for explaining boron toxicity (Shomron and Ast 2003; Reid 2007a). Several genes from *Arabidopsis* and *Lupinus* encoding transcription factors or ribosomal proteins provide tolerance to boron toxicity in yeast (Nozawa *et al.* 2006; Reid 2007a). Therefore, these proteins might prevent the attack from boron at splice sites of mRNA, which would be another mechanism to confer tolerance to high boron concentrations (Reid 2007a).

Furthermore, the induction of proline accumulation in response to boron toxicity and other stresses commonly found in natural environments (e.g. heavy metal toxicity, nutrient deficiency, photooxidative stress) suggests that genetic engineering of proline overproduction in agriculturally-important crops might increase their overall environmental tolerance and thereby enhancing productivity (Eraslan *et al.* 2007).

Boron toxicity and salinity

The concentration of boron can reach toxic levels in saline soils and with poor drainage (Nable *et al.* 1997), this being particularly harmful for plants because they suffer from a double stress. Thus, salinity increased leaf injury due to boron toxicity in tomato and, especially, cucumber plants (Alpaslan and Gunes 2001), and these authors observed that membrane permeability of the plants was significantly augmented in the presence of salinity by the rising levels of applied boron. Also salinity intensified boron toxicity effects since salinity together with boron toxicity increased soluble boron concentrations in inter- and intracellular compartments of basal leaf sections in wheat when compared to either stress alone, likely related to salinity-induced changes in water status (Wimmer *et al.* 2003).

However, according to other works, boron concentration in the tissues of spinach plants was decreased by sodium chloride, even though concentrations of sodium and chloride ions increased in the plant tissues (Eraslan *et al.* 2008). In addition, it has been proposed that there would be a lower uptake of chloride in the presence of boron and vice versa, what would explain why toxic effects on growth are less harmful for combined salinity and boron toxicity than what would be predicted if effects of the separate stresses were additive (Yermiyahu *et al.* 2008).

Interestingly, the exogenous silicon addition improved the combined salinity and boron tolerance of spinach by the enhancement of antioxidant mechanisms that diminish oxidative membrane damage (Gunes *et al.* 2007; Eraslan *et al.* 2008). It has also been suggested that silicon moderates salinity and boron toxicity of spinach and tomato plants by preventing translocation of sodium, chloride, and boron from root to shoots and/or soil to plants (Gunes *et al.* 2007).

Therefore, the interplay between boron toxicity and salinity is a topic open to debate because different plant responses to both combined stresses have been reported, which do not allow us to achieve a definitive conclusion until we advance in the understanding of the mechanism by which plants respond simultaneously to both stresses.

BORON AND CELL SIGNALLING

Different hypotheses have arisen to explain how boron may exert a regulatory role in the physiology of plants. Thus, it has been suggested that there is a rapid transfer of the signal from the cell wall to the cytoplasm, which could be involved in gene induction that would be generated as a result of the redox imbalance caused by the deficiency of boron (Kobayashi *et al.* 2004). It is also proposed that changes in the concentrations of boron can lead to a mechanical cascade of signals through cell wall-plasma membrane-cytoskeleton continuum to reach the cytoplasm, in which arabinogalactan proteins might probably be involved (Goldbach and Wimmer 2007). Recently it has been suggested that boron may act as a signal capable of interacting with cellular transcription factors to regulate various plant physiological processes affected by boron deficiency (González-Fontes *et al.* 2008).

CONCLUSIONS

Many agricultural areas of our planet are under stress of boron deficiency or toxicity, which limits their growth, productivity and yield of major crop plants. In the past years our knowledge at molecular level of the mechanisms that mediate boron uptake and distribution in plants has advanced notably. On this knowledge, and others to come, lay the groundwork for obtaining genetically modified plants that allow us to improve agricultural productivity through development of plants that are tolerant to both soils containing insufficient or excess boron.

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