ADVANCES IN PLANT AND ANIMAL BORON NUTRITION

Advances in Plant and Animal Boron Nutrition

Proceedings of the 3rd International Symposium on all Aspects of Plant and Animal Boron Nutrition

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Cover illustration courtesy of Yunhua Wang, unpublished (the 'disorders' of 'multi-apex' in cotton, a typical boron deficiency symptom. In 1970s, the discovery of cotton and oilseed rape (Brassica napus) suffering from B deficiency initiated a new research area for plant B nutrition and B fertilization in China (See this book p.83-91).

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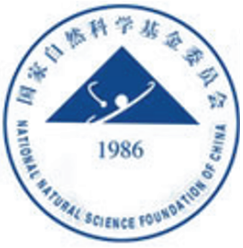

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Preface

Boron has been known as an essential micronutrient for higher plants since 1923 by the work of Katherine Warington, but the physiological role of boron in plants and its molecular basis have not been known for a long time. This lack of knowledge left ample room for conflicting views and hypotheses. Even today, many questions are left open. However, considerable progress has been made in understanding the role of boron with the development of new techniques for boron analysis, as well as those for the examination of pertinent aspects of physiology and cellular and molecular biology. Significant progress has been made not only in understanding the uptake, translocation, and physiological role of boron in plants, but also in establishing boron as an essential element in animals and humans. Thus, the **Third International Symposium on all Aspects of Plant and Animal Boron Nutrition** was held from September 10 to 13 in 2005, Wuhan, P.R. China, and provided a forum for scientists focusing on boron research to exchange their latest achievements and organize and collaborate in planning future research activities.

The Boron 2005 symposium focused on all aspects of B research in soils, plants, animals, and humans, similar to its predecessors held in Chiangmai, Thailand (1997) and in Bonn, Germany (2001), despite being a satellite meeting to the XV International Plant Nutrition Colloquium. So, a total of one hundred and four representatives from eighteen nations of the world gathered at Wuhan, and enjoyed six sessions of reports on recent developments regarding B sorption mechanisms in soils, deficiency and toxicity of B, B fertilizer application and basic research on the physiology and molecular biology of plant B nutrition, and nutritional function of B in animals and humans, and exchanged their views and experiences. Some recent key findings were reported, such as new information about gene expression and control of B transporters, the continuum of B re-translocation and the cold tolerance of low B plants, and many more.

Based on the aim of this symposium and the submission of manuscripts from the participants, the conference book of the symposium Boron 2005, **Advances in Plant and Animal Boron Nutrition,** consists of four parts: Plenary Review, Boron in Plants, Boron in Animals and Humans, and Boron in Soils. The second part "Boron in Plants" is again divided into three sections: Physiology and Metabolism of Boron in Plants, Boron Nutrition and Boron Application in Crops, Genotypic Differences of Boron Nutrition in Plants. In this book, readers will find thorough coverage of all recent developments in boron nutrition research as well as suggestions for future research focus.

We would like to thank all participants for their contributions to the symposium Boron 2005 and conference book. We also sincerely thank our sponsors on behalf of the International Scientific Committee and the Local Organizing Committee. Without their generous funding support, it would have been impossible to hold the symposium and publish this volume. Meanwhile, we would also like to express our sincere thanks to the members of the Local Organizing Committee and the large number of Ph.D. and Masters students for their dedicated efforts that made the symposium an outstanding success. We would especially like to thank Professor Yunhua Wang, well known for his 30 years of research on plant boron nutrition and boron fertilizer application in China. He enthusiastically engaged himself with many aspects of symposium planning including design of the scientific program, the logistical needs of participants, and the important task of raising funds.

Finally, we would like to thank Springer for its willingness to again publish the conference book in the series of international symposia on boron research. Hopefully, this summary of recent progress in all aspects of boron nutrition research will prove valuable during future implementation of relevant improvements in agricultural and nutritional practices, policy development, and planning of boron nutrition research around the globe. Due to the time constraints, some errors in manuscript presentation might have escaped the attention of the editorial staff. We therefore apologize in advance to authors and readers for any such possible errors. We are confident, though, that the readers of this volume will find the information found herein to be interesting and a motivation for making further advances in boron nutrition research.

Fangsen Xu
In the name of the organizers and
the members of the editorial board

Plenary Review

Boron Functions in Plants and Animals: Recent Advances in Boron Research and Open Questions

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Introduction

Boron deficiency is a widespread problem for field crop production where large losses of yield occur annually both quantitatively (e.g. in southeast China over 40% yield reductions may occur in oilseed rape: Wei et al. 1998), as well as qualitatively (Stephenson and Gallagher 1987; Ram et al. 1989; Bell et al. 1990; Nyomora et al. 1997). Significant losses of yield or quality resulting from boron deficiency may occur as well in vegetable crops (e.g. Kotur 1991). Even eucalyptus trees in large areas of southern China (Dell and Malajczuk 1994), and pine trees in southeast Australia (Hopmans and Flinn 1984) may be severely affected by boron deficiency in both growth and quality.

Although reports from agricultural practice have well established that adequate boron supply is imperative for obtaining high yields and good quality, and increasing evidence suggests a metabolic function or at least beneficial effects of boron in animal metabolism (Park et al. 2002, Hunt, Nielsen et al. and Spears, this volume), knowledge about metabolic functions of boron is yet incomplete. Nevertheless, recent research findings have greatly improved our understanding for boron uptake and transport processes (Brown and Shelp 1997; Hu and Brown 1997; Brown et al. 2002; Frommer and von Wiren 2002; Takano et al. 2002), and roles of boron in cell wall formation (Matoh 1997; O'Neill et al. 2004), cellular membrane functions (Goldbach et al. 2001), and anti-oxidative defence systems have been suggested (Cakmak and Römheld 1997). A beneficial or even essential role of boron in animal metabolism is supported by the findings that low boron concentrations induce the MAPK pathway in cultured animal cells and that cell lines with a knockout of the boron transporter NaBC1, the mammalian homolog of AtBor1, stop to develop and proliferate (Park et al. 2004). The finding of boron being an essential part of a signal molecule (AI2) in bacteria (see below) highlights the possibility that, besides being an indispensable factor for RGII cross-linking (see review by O'Neill et al. 2004), boron might play further roles in both, animal and plant metabolism.

This paper summarizes recent advances and major achievements in boron research, mostly since the last boron meeting in 2001, and highlights open questions for

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further elucidating the role(s) of boron in plant and animal metabolism.

Boron binding bio-molecules and stability of boron complexes

There are already well known boron containing biomolecules such as the boron containing macrolides (aplasmomycine, boromycine, and tartrolon B: Moore and Hertweck 2002).

The first boron-containing compound identified in the plant kingdom, which is stable under physiological conditions, is the pectic polysaccharide rhamnogalacturonan II (RGII), where boron cross-links two RGII monomers and thus provides stability to the cell wall matrix (O'Neill et al. 2004). As can be seen in RGII, the steric arrangement of molecules can make a large difference in the stability of boron complexes: although the pectic polysaccharide contains two apioses on side-chains A and B, only the one in chain A is responsible for forming the stable borate bridge, whereas apiose from chain B does not participate in the formation of the RGII dimer (Reuhs et al. 2004). Furthermore, replacing L-fucose by L-galactose in the GDP-D-mannose-4,6-dehydratase deficient *Arabidopsis mur1* mutant, significantly reduces growth and leads to malformation, which can be compensated by higher boron concentrations (Reuhs et al. 2004) or by supply of fucose (O'Neill et al. 2001). The stability of the altered RGII complex is thus lowered through a comparatively small change, and for obtaining a regular degree of cross-linking, the ratio of boron to RG II has to be increased. The glycosyl sequence and the three-dimensional conformation of RG-II are therefore important in regulating the interaction of this pectic polysaccharide with borate (Reuhs et al. 2004).

Other putative boron binding biomolecules have also been identified. Using capillary electrophoresis, Ralston and Hunt (2001) compared adenosine and molecules with adenosine moieties including S-adenosylmethionine (SAM) and diadenosine polyphosphates (Ap_nA). S-adenosylmethionine (SAM) proved to be the compound forming the most stable borate complexes next to apiose. The stability of boron complexes decreases in the order: SAM ≅ Ap_6A ≅ Ap_5A > Ap_4A > Ap_3A ≅ NAD^+ > Ap_2A > NADH ≅ 5'ATP > 5'ADP > 5'AMP > adenosine > 3'AMP ≅ 2'AMP ≅ cAMP ≅ adenine. Species with vicinal *cis*-diols bind boron, species without those moieties do not. Boron binding affinity increases when proximal cationic moieties are present, and anionic moieties remote from the *cis*-hydroxyl binding site also seem to positively influence boron binding affinity. In the Ap_nA species, cooperative complexing of boron between the terminal ribose moieties apparently occurs, as boron affinity is greater than expected for two monocomplexes and binding affinities increase as more phosphate groups (beyond three) are present separating the terminal moieties, probably by reducing the strain on the bonds (Hunt 2002). At physiological pH, the adenine moieties of Ap_nA are driven together by hydrophobic forces and stack interfacially (Kolodny and Collins 1986, Hunt this volume). The work of these authors thus clearly showed the ability of different

biomolecules to form borate complexes. It has to be emphasised, though, that the capillary electrophoresis used by Hunt and co-workers to show the complex stability of these borate esters has been carried out at pH 8.4, which is higher than the pH usually found in living cells. This favours complex formation due to a higher ratio of borate to undissociated boric acid (about 12% *vs*. less than 2% in natural systems). Thus, complex formation might be overestimated. This should not, however, question the results cited before as the observations made by Hunt's group show the *relative* complex stability of a number of relevant biomolecules. It is, however, extremely difficult to assess the stability of the respective borate-complexes *in vivo*, as the cellular fluids are a complex multi-solute system where water activity may be lower than in simplified *in vitro* systems. Furthermore, the complex stability of the borate complexes depends highly on steric conditions, charge distribution and the molecules environment (e.g. hydrophobic interactions). Borate complexes could be stabilized in a way similar to the inhibition of bacterial enoyl-reductase by diazaborines (Baldock et al. 1996).

Considering compartmentation within cell organelles and differences in the steric arrangement of potentially B-binding molecules, it is yet hard to decide which of those ligands besides RGII monomers do play a major role *in vivo*.

An interesting hypothesis has been launched by Ricardo et al. (2004), suggesting that the formation of di-pentose-borate complexes might have stabilized ribose/ribulose (besides other cyclic pentoses such as arabinose, xylose, and lyxose) in pre-biotic phases in interstellar dust or early during earth history. When formed from glycolaldehyde or formaldehyde, borate prevents the hydrolytic decay of pentoses under alkaline conditions. In a reaction mixture at pH values around 12 and in the presence of borate, ribose was the main reaction product, whereas in the absence of borate, pentoses rapidly degraded to ill-defined brown polymers. Formation of borate complexes might thus have enabled the accumulation of ribose as a pre-condition for early development of life on earth. The fact that boric acid resp. borate react with hydroxyl groups is considered as the key for understanding boron functions (Bolaños et al. 2004). The authors stated that "the primary role of boron in biological systems is stabilization of molecules with *cis*-diol groups, independently of their function", and "boron chemistry makes it a perfect candidate for atomic diester bridging". Whether the formation of one- or two-sided complexes is related to boron's function(s) is still under debate. According to Hu et al. (personal communication), the chemical conditions in living systems do not favour monoester formation, and only di-esters could achieve stabilities high enough to be of physiological relevance.

Using phenylboronic acids as a probe for boron binding ligands and the fact that these are forming exclusively one-sided esters makes it thus possible to search for those functions where boron is required for (cross-) linking ligands with *cis*-hydroxyl groups as in RGII (Bassil et al. 2004).

Boron in the animal metabolism

In the past years, a wealth of new evidence has been gathered for boron being an essential or beneficial element in animals/humans (Hunt 2003, see as well contributions by Hunt, Nielsen et al. and Spears, this volume).

Earlier reports showed that boron is essential for embryo development, at least for vertebrates. Boron deprivation disrupted embryonic development resulting in a high percentage of necrotic eggs and abnormal development of the gut in *Xenopus laevis* (Fort et al. 1999). It seems as if at least the early stage of development is especially sensitive to boron deficiency such as described for mated zebrafish (*Danio rerio*) (Rowe and Eckhert 1999). Although the target molecules are likely to be different, there is a coincidence in animal and plant metabolism for boron to be especially required at initial phases of differentiation, as Behrendt and Zoglauer (1996) have shown for *Larix decidua*.

At least beneficial effects of boron were suggested in a number of nutritional studies, with many of the effects related to bone metabolism. For example, boron supplementation of a low-boron diet reduced gross bone abnormalities in the vitamin D-deficient chick (Hunt and Nielsen 1981; Bai and Hunt 1996). In vitamin D-deficient rats fed a low-boron diet, supplemental dietary boron enhanced the apparent absorption and retention of calcium and phosphorus and increased femur magnesium concentrations (Hegsted et al. 1991). In male pigs, bone lipid was lower and the bending moment higher when boron was supplemented to a low-boron diet (Armstrong et al. 2000). It might even be envisaged that the effect of boron on bone metabolism could be at least one of the essential functions of boron in humans. It has been found as well that physiologic concentrations of boron reduce the amount of insulin required to maintain plasma glucose (Bakken and Hunt 2003).

Recently, Park et al. (2004) identified the mammalian homologue NaBC1 of the AtBOR1 transporter (see below), which suggests that there is possibly a need for a relatively close control of boron levels in animal cells, too. The finding that low concentrations of borate activate the MAPK pathway and that the knockdown of NaBC1 halted cell growth and proliferation, point to a possibly essential functional role of boron in animal metabolism.

There is evidence from several laboratories that dietary boron plays a role in immune function in a variety of organisms. The boron-containing antibiotic boromycin from *Streptomyces sp*. strain A-3376 was recently found to be a potent anti-human immunodeficiency virus (HIV) antibiotic (Kohno et al. 1996), acting by (in part) still unknown mechanisms. Generally, boron-containing antibiotics such as boromycin, tartrolon B and aplasmomycin are borodiesters and act as ionophores. When boron is removed from at least one of these antibiotics (aplasmomycin) by mild acid hydrolysis (pH 3), the resulting desboroaplasmomycin loses its functionality as K^+ ionophore (Sato et al. 1978), which can be re-constituted by treatment with boric acid at pH 6 and 8 (Chen et al. 1980).

Interleukin-6 (IL-6) is a systemic "proinflammatory" and as such an important regulator of the immune system by inducing several processes (Lowik 1992). When using rhamnogalacturonan IIs isolated from *Panax ginseng* leaves, monomerization of the RG-II dimer significantly decreased its IL-6 production-enhancing activity. Boron may affect production of TNF, a proinflammatory cytokine, in chicks and humans, since TNF concentrations were elevated in the culture medium of pelvic cartilage isolated from chick embryos after they were incubated with boron as a 3% boric acid solution (Benderdour et al. 1997). Further examples are presented by Hunt (2003); and Nielsen et al. and Hunt, this volume). The treatments, though, were carried out with elevated, non-physiological amounts of boron, and there is a need to determine whether the effects of boron on TNF production are of physiological importance.

As mentioned above, diadenosine phosphates (Ap_nA), which function as signal nucleotides associated with platelet aggregation and neuronal response and which are putative "alarmones" reportedly regulating cell proliferation, stress response, and DNA repair (McLennan 1992), have higher affinities for boron than any other currently recognized boron ligand present in animal tissues including NAD^+ (Ralston and Hunt 2001). A close interaction between boron and serine proteases has also been suggested (Hunt 2003). Serine proteases are major proteolytic enzymes and have, in addition to degrading structural proteins, regulatory roles in normal inflammation processes (Kettner et al. 1988). Boron is able to form covalent bonds with the nitrogen atom of amine groups, e.g. in hemerythrin (a nonheme iron-containing oxygen transport protein of *Golfingia gouldii*), where it binds near the coordination iron site (Garbett et al. 1971). Whether the stability of this type of boron complex is high enough and comparable to polyols to be of biological relevance remains to be shown. In serine proteases, the boron atom is thought to inhibit the formation of a tetrahedral boron adduct (the transition-state analogue) that mimics the tetrahedral adduct formed during normal substrate hydrolysis (Berry et al. 1988). The adduct includes a covalent bond between boron and a specific nitrogen at the active site of these enzymes. Nanomolar concentrations of certain synthetic peptide boronic acids effectively inhibit chymotrypsin, cathepsin G, and both leukocyte and pancreatic elastase *in vitro* (Kettner and Shenvi 1984). The serine protease thermitase (E.C. 3.4.21.66) was partially inactivated by hydrogen peroxide in the presence of 50 mM sodium borate (Hausdorf et al. 1987). This concentration, however, is rather high and well beyond concentrations usually found in organisms *in vivo*. To favour the formation of such a complex, compartmentation and/or hydrophobic interactions would be needed, if boron would not reach similar concentrations in biological systems. Whether this effect is part of the anti-inflammatory pharmaceutical properties of elevated doses of boric acid (e.g. Newnham 2002) still remains to be elucidated.

Boron uptake and translocation

Boron uptake has earlier been considered as a merely passive process following mass flow of water (Hu and Brown 1997). These authors pointed out, though, that the wide variation of boron uptake under field conditions indicates that there are more processes involved in boron uptake and distribution. Since then, there has been a tremendous growth of information in this special field, ranging from the discovery of complex formation with polyols as a mechanism for boron phloem mobility (Brown and Shelp 1997), the finding of several transport mechanisms (Dordas et al. 2000), to the recent identification of boron transporters (Takano et al. 2002). Below, we will only briefly address the development in this area during the past years.

For agricultural purposes, it is important to find cultivars which thrive well under boron–limited conditions. In rape, Xu et al. (2001) found by QTL analysis, that there are one major and three minor gene loci for boron efficiency. In this species, boron efficiency was also more closely related to the (free) sugar contents than to tightly bound or "free" boron. Boron use efficiency is further related to phenology: the boron efficient cultivar shows earlier bolting and flowering (Du et al. 2002), thus probably reducing the amount of boron needed for vegetative biomass development.

In wheat, boron efficiency was likely conferred by two major genes *Bo*d*1* and *Bo*d (Jamjod et al. 2004). Enhanced boron transport into the ear was identified as the mechanism for boron efficiency rather than re-translocation of boron (Huang et al. 2001). Breeding for boron efficiency may boost cereal productivity in SE Asia (Jamjod et al. 2004; Nachiangmai et al. 2004).

Boron and water channels

Water channels (or aquaporins) in the plasma membrane of root cells play an important role in root hydraulic conductivity and plant water relations. In plants, 75-95% of the water moving across the plasma membranes pass through water channels (Steudle 2000). The contribution of aquaporins to water uptake increases in response to changing environmental factors (e.g. drought, nutrient deficiency, low temperature: Tyerman et al. 1999; 2002). Water channels also seem to play an important role in the process of boron uptake across the plasma membrane (Dordas and Brown 2000; Dordas et al. 2000). This was demonstrated in purified plasma membrane vesicles from squash roots, where the boron permeability coefficient (3×10^{-7} cm s^{-1}) was six times higher than that of microsomal vesicles, and where boron permeation across the plasma membrane vesicles was reduced by 30-39% by the addition of the non-specific channel-blocking agent $HgCl_2$ (Dordas et al. 2000). The contribution of channel-mediated boron uptake to total root boron uptake may be particularly significant when external boron concentration is more than adequate, whereas active transporter-mediated uptake seems to be necessary to account for rates of boron uptake required to avoid boron deficiency when boron concentration at the root surface is low.

Severe boron deficiency (e.g. interruption of boron supply to the root tips for one hour) caused a rapid decrease in the amount of the plasma membrane water channel proteins (ZmPIP1 aquaporins) in transgenic tobacco (Goldbach et al. 2001).

Boron transporters

Boron loading into the xylem after uptake into root cells requires the function of boron transporters located in the plasma membrane of the root pericycle, particularly when external boron levels are low (Takano et al. 2002). The recently suggested mechanism of active boron uptake and transport in roots at low boron supply (Dannel et al. 2000; 2002) may be related to the functions of the BOR1 (Takano et al. 2002), and possibly further boron transporters. Wild type *Arabidopsis* plants show an enhanced transport of boron to the shoot under low boron supply, which can be suppressed upon resupply of high levels of boron within a few hours (Takano et al. 2005b). This enhanced transport was not observed in the *bor1-1 Arabidopsis* mutant. Takano et al. (2002; 2005b) were able to show the accumulation in the plasmalemma of a constitutively expressed BOR1-GFP fusion protein under conditions of limited boron supply. Upon resupply of high levels of boron, BOR1-GFP was degraded within several hours. Posttranscriptional mechanisms seem to play a major role in the regulation of BOR1 accumulation. Endocytosis and degradation of BOR1 seem to be regulated by boron availability, possibly to avoid accumulation of toxic levels of boron in shoots under high boron supply, while protecting the shoot from boron deficiency under boron limitation.

Recent reports of a boron transporter as mammalian homologue of the AtBOR1 borate transporter, occurring ubiquitously in animal cell membranes (Park et al. 2004), raises questions about whether related transporters are generally found in plant and animal cell membranes. This NaBC1 transporter conducts Na^+ and OH^- (H^+) ions in the absence of borate, whereas in the presence of borate, NaBC1 behaves as an electrogenic, voltage-regulated, Na^+-coupled $B(OH)_4^-$ transporter (Park et al. 2004). Knockdown of NaBC1 halted cell growth and proliferation.

Boron's function(s) in plants

Structural role(s) in cell walls: RG II

The structural role of boron in the cell wall of higher plants has been reviewed extensively (Brown et al. 2002). It seems as if enhanced formation of RGII (and cross-linking by borate) is closely related to the conquering of land during evolution. Avascular bryophytes contain only about 1% of the amount of RGII of vascular plant species, and the amount of RGII in cell walls increased during the evolution of vascular plants (Matsunaga et al. 2004). Development of boron-dependency during evolution may thus correlate as well with upright growth and lignified secondary walls. The highly conserved structure of RGII and the fact that its genes appeared early during land plant evolution, point to RGII as a fundamental molecule for wall structure (Matsunaga et al. 2004).

The dimerisation of RGII by a borate cross-link has already been addressed above as well as the influence of small molecular changes on its structure (see above). Partial replacement of L-fucosyl by L-galactosyl residues in xyloglucans and in RGII of the dwarf mutant *Arabidopsis thaliana mur1* resulted in reduced growth and malformation of the plants (Reuhs et al. 2004). It was also shown that tensile strength was reduced in the *mur1* mutant compared to the wild type (Ryden et al. 2003). It could, however, be completely rescued with higher boron levels in the hypocotyl and the stem, demonstrating that the lack of fucose in RG II rather than in xyloglucan is important for the mechanical phenotype. Experiments with the *nolac-H18* (non-organogenic-loosely attached cells) tobacco callus mutant also demonstrate the structural importance of dB-RGII for normal growth because the mutant lacks glucuronyltransferase 1, needed for glucuronic acid addition to RGII, causing reduced formation of dB-RGII and consequently reduced intercellular attachment in meristems and tissues as well as the inability to form shoots (Iwai et al. 2002). This highlights the importance of pectin as an adhesion molecule (Lord and Mollet, 2002). This agrees with our earlier observations of a reduced cell wall elasticity modulus under boron deprivation (Findeklee and Goldbach 1996) and observations by Fleischer et al. (1999), showing that RGII is a key component of structural integrity of cell walls. The observations reported by Ryden et al. (2003) also suggest that B–RGII complexation plays a role in the expanded primary wall and is important for secondary wall structure or assembly. Tensile properties of the cell wall depend both on a xyloglucan cross-linked microfibrillar network and RGII-borate complexes (Ryden et al. 2003). Rapid loosening of the cell wall under boron deficiency could also affect the aperture of specific aquaporins, which are responsive to short pressure pulses in a dose dependent manner (Lee et al. 2005; see also below).

The cell walls of the *Arabidopsis* bor1-1 mutant show a lower degree of cross-linking under limited boron supply, which is likely a consequence of lower boron shoot levels in the mutant compared to the WT (Noguchi et al. 2003), highlighting again the importance of boron for maintaining cell wall integrity.

Boron and membranes

Several earlier studies have demonstrated roles of boron in the functioning of enzymes and other proteins of the plasma membrane, transport processes across the membrane and membrane integrity (Cakmak and Römheld 1997; Goldbach et al. 2001; Brown et al. 2002). For example, boron deficiency altered the membrane potential (Blaser-Grill et al. 1989; Schon et al. 1990; Goldbach et al. 1991), and reduced the activity of proton-pumping ATPase and thus the proton gradient across the plasma membrane (Heyes et al. 1991; Ferrol and Donaire 1992; Lawrence et al. 1995; Obermeyer et al. 1996), and of Fe-reductase (Goldbach et al. 1991; Ferrol and Donaire 1992). Some of these changes are observed within minutes after changing the boron supply, which is in line with the assumption of a direct interaction between boron and membranes. Although the results are sometimes contradictory and not

always attributable to an early effect of boron deprivation, the inhibition of PL-bound oxidoreductase activity within minutes of boron deprivation has been confirmed (Barr et al. 1983; Wimmer 2000).

Effects of boron deprivation may exert multiple direct and indirect effects on membrane-bound processes. A direct role of boron in maintaining membrane structure is likely through *cis-diol* complexation with glycoproteins, which are structural constituents of the plasma membrane (see below) (Goldbach et al. 2001; Brown et al. 2002). Effects of boron deficiency pointing to a structural role of boron in membrane stabilization are an altered permeability for K and sugars (Pollard et al. 1977; Parr and Loughman 1983; Goldbach 1985; Cakmak et al. 1995; Wang et al. 1999), a damage of the peribacteroid membrane in nodules (Bolanos et al. 1994; 2001) or a change in membrane-bound Ca levels (Mühling et al. 1998; Wimmer and Goldbach 1999). Also, boron deficiency reactions can be compensated by an enhanced Ca supply in cyanobacteria (Bolanos et al. 1993; 2002) Boron seems to be essential for the full functioning of N_2 fixing rhizobial as well as actinomycal symbioses and for heterocyst formation of free living *Cyanophyceae* (see review by Bolanos et al. 2004). The authors hypothesize that the primary role of boron in biological systems is the stabilization of molecules with *cis*-diol groups, independently of their function.

Verstraeten et al. (2005) suggested that boron preferentially interacts with negatively charged phospholipids, or with those containing sugar moieties in their headgroup. Their work showed that boron, at concentrations as low as 0.5 μM, interacts with the lipid bi-layer affecting membrane rheology. Lipid composition determined the magnitude and direction of the boron effects. Boron may thus play a role in the maintenance of membrane rheology by modulating hydration and fluidity of lipid bilayers. This could be a modulating function equally distributed in animal and plant kingdom. More evidence, though, is still required for a special mode of action, and whether the respective function is essential or just “beneficial”.

The fact that PL-bound enzymatic activities respond remarkably fast to changes in boron supply (within minutes to one hour) point to at least in part post-transcriptional and post-translational control by the boron level. Another evidence for a post-translational control of plasmalemma-bound proteins comes from the observation that the boron transporter AtBor1-1 is regulated by boron levels (see above, (Takano et al. 2005a).

Membrane function could also be affected by the accumulation of oxidative free radicals (including $^{*}OH$) in cells, which is one of the consequences of boron deficiency in root and leaf cells (Cakmak and Römheld 1997). Even though this might be rather one of the later secondary responses, water channels in the plasma membrane are reversibly closed by hydroxyl radicals ($^{*}OH$) (Henzler et al. 2004), which is in line with our findings that GFP-transformed ZMPiP water channel activity almost disappeared in tobacco root tips within one hour of boron deficiency (Yu et al. 2002).

Boron deficiency has been shown to affect leaf photosynthesis, although the existing evidence has been mostly obtained from *in vivo* experiments with far too long (10 days or longer) treatments of plants with deficient boron supply (Kastori et al. 1995; Plesnicar et al. 1997; El-Shintinawy 1999). The primary mechanisms of boron's roles in photosynthesis are unknown, but boron could affect the functions of chloroplastic membranes by disrupting thylakoid electron transport, resulting in photoinhibition. In own preliminary experiments (unpublished), we obtained only weak effects, if at all, of boron deficiency with isolated spinach chloroplasts. It is quite feasible that the effects observed in chloroplasts are secondary and caused by growth inhibition in root and shoot tips, i.e. a reduced sink activity, finally leading to an over-saturation of the electron acceptors of PSII or PSI. These possible effects may increase the rate of photo-oxidative damage in response to further stresses.

Boron and stress responses with special emphasis to chilling

It is challenging to follow more closely possible interactions between boron supply and further stresses such as chilling (Ye et al. 2000; 2003) and salinity (Wimmer et al. 2005). Below, we will focus especially on interactions between boron supply and chilling tolerance as this is discussed since long as a special interaction (Cooling and Jones 1970; Hanson and Breen 1985) and there seems to be an additive or even multiplicatory effect of both stresses (Ye et al. 2000; 2003). So far, however, underlying processes and reactions still remain largely obscure.

The primary interaction site between boron and low temperature may lie with plant cellular membranes, including plasmalemma and chloroplastic membranes. The interaction between boron and low temperature in warm season species has been recently reviewed, particularly in relation to root functions, shoot water use and boron uptake/utilisation in plants (Huang et al. 2005).

Biochemical and physical properties seem to affect both low temperature tolerance and boron permeation at the cellular level. In chilling sensitive species, cellular membrane alterations precede other cellular changes and adverse effects on different cellular organelles are dependent on the duration of chilling and associated growth conditions (e.g. light intensity and relative humidity: Lyons et al. 1979). Dordas and Brown (2000) demonstrated that different proportions of sterols and longer chain fatty acids in the plasma membrane of root cells significantly changed boron uptake in *Arabidopsis thaliana* mutants, and related these changes to different permeability coefficients for boric acid across plasma membranes containing different groups of lipids and fatty acids. The decrease in sterol contents in the plasma membrane may increase membrane fluidity and permeability to water and ions (Lyons et al. 1979), which is correlated with plant chilling tolerance (Hugly et al. 1990). Increased membrane rigidity is a common response to chilling temperature in chilling sensitive species such as *Coffea arabica* L. (Queiroz et al. 1998). As a result, chilling-induced reduction in membrane fluidity and permeability of root cells may

have also contributed to the inhibition of boron uptake in chilling sensitive species (Ye et al. 2000; 2003).

These interactions at the membrane level may be translated into root functions at organ levels, particularly in relation to boron uptake and external boron requirements of plants. Chilling stresses in roots may result in an increased external boron requirement, leading to a higher risk of boron deficiency during the period of seasonal transition from cold to warm climate when air warms up much faster than the soil, such as late winter and early spring. On the other hand, the pre-existence of boron deficiency in young roots may enhance the sensitivity of roots to chilling. Recent work has shown that low root temperature has a consistent set of effects on temperate and tropical species, but at different threshold temperatures (oilseed rape: 5-10°C, sunflower: 12-17°C; e.g. Ye et al. 2000; 2003).

Boron internal requirements in leaf cells may also be altered at low temperatures in warm climate species, which is largely related to the roles of boron in anti-oxidative systems and possibly in the photoinhibition responses of the photosystems after chilling stress. Boron deficiency can decrease the levels of antioxidants in leaves (Cakmak and Römheld 1997). In leaves of a subtropical *Eucalyptus grandis* × *E. urophylla* hybrid, the production rate of superoxide (O_2^-) and polyphenol oxidase activity increased significantly under boron deficiency treatments (0 and 5 μM boron for up to 96 hours at 5°C), but not in plants with adequate (15μM) boron supply at the same temperature (Lu and Huang 2003). In addition, the activities of anti-oxidative enzymes (superoxide dismutase, peroxidase, catalase and ascorbate peroxidase) in leaves were decreased at 5°C with boron deficiency (< 10 μM B), but not at 15 μM B. As a result, boron deficiency may increase the sensitivity of leaf cells to chilling, through enhanced generation of oxidative free radicals and weakened anti-oxidative capacity.

At the whole plant level, root hydraulic conductance is important in both boron uptake and post-chilling recovery of shoots. In species sensitive to root chilling such as cucumber and sunflower, chill-induced water loss is one of the most significant physiological consequences, resulting from decreased root hydraulic conductance and excessive transpiration due to loss of stomatal control (delayed closure or closure failure) (Allen and Ort 2001). At adequate boron levels, root boron uptake is mostly a passive process of permeation of undissociated boric acid across the plasma membrane, which is largely determined by the rate of water uptake through the plasma membrane of root cells, in addition to boron concentration around the root surface (Hu and Brown 1997). The decrease in root hydraulic conductance caused by root chilling would thus have a negative impact on boron supply to new shoot growth due to limited boron uptake and transport from root to shoot. On the other side, boron deficiency rapidly decreased the amount of ZmPIP1 aquaporins in tobacco (Goldbach et al. 2001) and enhanced the accumulation of oxidative free radicals (e.g. *OH) (Cakmak and Römheld 1997), which are also known to (reversibly) close certain aquaporins (Henzler et al. 2004). Boron deficiency-induced

reduced water flow through aquaporins may then lower the ability of roots to maintain hydraulic conductivity in response to chilling stress, even in chilling-tolerant species or genotypes. Experimental evidence is required to test this hypothesis, especially at low boron concentrations, which may be maintained by using the boron-buffered solution culture with realistically low solution boron concentrations (Huang et al. 1999).

Boron and quorum sensing

One of the most exciting findings in the past years was the identification of the bacterial quorum sensor autoinducer 2 (AI2) as a boron containing stabile complex (Chen et al. 2002). This molecule is synthesized by bacteria from S-adenosylmethionine (which by itself shows a rather high complex stability with borate at higher pH values; see Hunt 2002) and activates luminescence when it has accumulated to high enough levels. Bacteria thus sense their cell density *via* the autoinducer concentration (Miller and Bassler 2001; Schauder and Bassler 2001). AI2 is recognized by a large number of different bacteria. In a concerted way, two autoinducers (AI1, a non boron bearing member of the family of AHL signals, and the boron-containing AI2) are required to control a signal chain (Cao and Meighen 1989; Bassler et al. 1993; 1994). Detection of AI-2 requires a periplasmic protein that resembles the ribose binding protein (LuxP) and a second two-component kinase (LuxQ) (Bassler et al. 1994). Both signals are needed for bioluminescence, because in the absence of one signal the cognate receptor acts as a potent kinase to block *lux* gene expression (Mok et al. 2003).

Outlook: how could boron's functional roles be described and assessed?

Until quite recently, the role of boron has only been attributed to an apoplastic function (Kobayashi et al. 1996; O'Neill et al. 1996). Although cross-linking rhamno-galacturonan II (RGII) and hence stabilizing the cell wall is one main and meanwhile well documented structural function (see above), there is increasing evidence for an essentiality in organisms without cell walls such as yeasts (Bennett et al. 1999) and animals (Eckhert 1998; Fort et al. 1998; Rowe and Eckhert 1999; Lanoue et al. 2000). In the latter, boron seems to play a major role in membrane-bound processes or where large amounts of membrane material are required (e.g. dysplasia in zebra fish during embryogenesis: Eckhert and Rowe 1999). In humans, boron shortage resulted in decreased activities of several membrane-bound hormones (Nielsen 2000). In plants, growth (O'Neill et al. 2001) as well as cell differentiation (e.g. xylem differentiation: Lovatt 1985, embryogenesis in *Larix*: Behrendt and Zoglauer 1996) are affected.

Functions of boron at the plasma membrane have been postulated on the basis of quite a number of observations, even though the underlying mechanisms are still a

matter of speculation (see reviews by Goldbach 1997; Blevins and Lukaszewski 1998; Brown et al. 2002). The presence of specific acceptor molecules, which bear ligands able to form complexes with boric acid/borate, is indispensable for any boron function to occur. Sugar moieties (esp. in their furanose form) such as mannose, apiose or galactose, but as well other hydroxylated ligands such as serine or threonine, may form ester-like complexes with boron (Ralston and Hunt 2000). In membranes, glycoproteins and glycolipids are good candidates for a possible boron function. A number of membrane-bound proteins and membrane structures are specifically interesting, as they seem to be related to still not well understood processes in cell growth, differentiation and perception (Kohorn 2000), which are also reported to be boron dependent. Most prominent among these putative B-binding membrane structures are surface proteins attached to the membrane *via* a glycosyl-phosphatidyl-inositol anchor (GPI) (Ferguson and Williams 1988; Thompson and Okuyama 2000). They typically contain three mannose-residues as well as phosphatidylinositol, which are all possible ligands for boron with a strong binding capacity (van Duin et al. 1984; Ralston and Hunt 2000). A modification of the GPI by complexing with boron may alter the access of phospholipases, which loosen this anchor. Thus the ratio of free and bound membrane proteins may be changed under boron deficiency with consequences such as lower contents of hydroxyproline-rich proteins in cell walls of *Phaseolus vulgaris* (Bonilla et al. 1997) or reduced incorporation of proteins into *Petunia* pollen tube walls (Jackson 1989). In both cases, the control was exerted at the post-transcriptional or post-translational level, which is in line with the assumption that boron exerts posttranslational control (Goldbach et al. 2001). A subgroup of GPI proteins is represented by classical arabinogalactanproteins (AGP) (Sherrier et al. 1999; Majewska-Sawka and Nothnagel 2000). There is a striking coincidence between many AGP-dependent processes and their dependency on boron supply (e.g. xylem differentiation: Stacey et al. 1995, pollen tube growth: Jackson 1989; Cheng and Rerkasem 1993; Majewska-Sawka and Nothnagel 2000). GPI-anchored proteins are components of "membrane rafts", micro-domains of membranes with specific functions (Brown and Rose 1992), rich in sphingolipid and cholesterol and insoluble in non-ionic detergents (Brown and London 2000). A specific function of boron in the formation and stability of membrane rafts *via* the formation of two-sided borate-complexes with e.g. mannose residues, seems to be plausible (Brown et al. 2002). Finally, changes in boron concentrations may lead to a mechanical cascade of signals starting by an altered conformation of membrane-bound proteins (Watson 1991; Morris and Homann 2001) and extending into the cytoplasm (Ligterink and Hirt 2001) *via* the cell wall-PL-cytoskeleton-continuum. An altered membrane tension may as well directly influence exo- and endocytosis (Fricker et al. 2000). The accumulation of vesicles at the cytosolic side of the plasma membrane is in line with this assumption (Kouchi and Kumazawa 1976; Hirsch and Torrey 1980), pointing to a possibly inhibited exocytosis. We have shown that actin and tubulin levels increased within 20-40 min of withholding boron in *Arabidopsis* and maize (Yu et al. 2001), but not in zucchini roots. The polimerization rate of these proteins was altered as well (Yu et

al. 2003), likely at the translational or post-translational level. Boron deficiency led to an increased level of JIM5-reactive pectins with low (<40%) methyl esterification and dimeric B-RGII-complexes in the cell walls, whereas their internalization in BrefeldinA induced compartments was reduced or even completely inhibited (Yu et al. 2002).

One or more functions of boron at the plasma membrane are thus highly likely. The challenge is now to identify the relevant components, boron-binding ligands as well as their function. Promising tools for this attempt may include the use of mutants either being deprived off or over-expressing possible target molecules. Also the availability of GFP-fusion proteins or fluorescing markers should greatly improve our understanding of boron's functions. It will be especially challenging to determine the stability of potential boron complexes *in vivo* where the complex stability could be highly influenced by molecules directly surrounding the boron-binding moiety. A very useful tool became available recently with phenylboronic acids such as 3-naphtyl-boronic acid (Bassil et al. 2004), as they bind strongly to *cis*-diols but prevent the formation of cross-links. Boronic acids could be used to test whether the essential function of boron is restricted to the formation of cross-links, i.e. 1:2 boron complexes, or whether it may also occur *via* formation of 1:1 complexes. The latter would suffice for e.g. preventing the oxidation of phenolic acids and alcohols (although this is less likely to occur under physiological conditions).

In any case, it has to be seen, that boron possibly acts only *via* post-translational control. If a boron–containing compound such as AI2 is involved in a signal chain in bacteria, boron might be needed to form or modify signalling complexes in a way similar to AI2 in higher organisms, too. The stability of such complexes can be predicted to vary according to the physicochemical environment (see above). It could be possible that complex formation with boron reduces or modifies the polarity of the complex surface, altering its binding behaviour to apolar sites (from enzymes such as MAPK). To exert a significant effect, concentrations needed could be rather low. It is envisaged that significant progresses will be made in boron research in the next years, especially when considering that evidence accumulates for a more general importance of boron in a wide array of organisms, ranging from bacteria to higher plants, animals and humans.

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