Rapid response reactions of roots to boron deprivation

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Dedicated to Prof. Dr. Dr. h.c. *G. Michael* on occasion of his 90th anniversary

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Summary – Zusammenfassung

Upon B removal from the nutrient solution, several response reactions of root cells can be measured within minutes. These include: reduction of cell wall elasticity modulus ε, increase of hydraulic conductivity, reduced activity of plasmalemma-bound inducible (NADH) reductase, (smaller) changes of the membrane potential, and liberation of Ca^{2+} (apoplastic and membrane-bound). The B most demanding (root) tissues are epidermal and outer cortical cells of the extension zone, xylem vessels, and root hair tips. Deprivation of B leads to morphological changes which can be noticed within hours to days, including browning of tissues, growth inhibition, death of apical meristems, and lack of root hairs. How the primary response reaction(s) lead to the expression of visible symptoms, however, is not yet clear. The present review summarizes rapid responses to B deprivation and shows several possibilities how primary might be linked to secondary reactions, including cytoskeleton-mediated responses.

Key words: Boron / rootgrowth / cytoskeleton / primary responses / secondary responses

Schnelle Reaktionen von Wurzeln auf Bor-Mangel

Nach Umsetzen auf ein B-Mangelmedium können verschiedene Reaktionen von Wurzelzellen innerhalb von Minuten beobachtet werden, wie z.B. Verringerung des Zellwandelastizitätsmoduls ε, Zunahme der hydraulischen Leitfähigkeit, verringerte Plasmalemma-gebundene induzierbare (NADH) Reduktaseaktivität, (kleinere) Änderungen des Membranpotenzials und eine Freisetzung von apoplastischem und membrangebundenem Ca^{2+}. Die Wurzelgewebe mit dem höchsten B-Bedarf scheinen die epidermalen und äußeren Rindenzellen der Streckungszone, Xylem und Wurzelhaarspitzen zu sein. B-Entzug führt zu morphologischen Änderungen, die innerhalb von Stunden und Tagen sichtbar werden, wie Gewebeverbräunung, Wachstumshemmung und Absterben apikaler Meristeme, sowie Fehlen von Wurzelhaaren. Wie die raschen Reaktionen zur (sekundären) Ausprägung von Mangelsymptomen führen, ist jedoch noch nicht klar. In dieser Übersicht werden rasche Reaktionen auf B-Entzug zusammengefasst und Möglichkeiten zur Verknüpfung primärer und sekundärer Reaktionen diskutiert.

1 Introduction

In the older and recent literature, a large number of very different reactions upon B deficiency is described. The observed changes concern the metabolism of nucleic acids, protein synthesis, metabolism and transport of carbohydrates, chemistry, and physics of cell walls, synthesis and transport of plant hormones (especially IAA), regulation of plasma membrane-bound ATPase and oxido-reductase activities, as well as synthesis and metabolism of phenolic compounds, (for a review see *Goldbach*, 1997). Considering that many observations only started several hours or even days and weeks after B deprivation, and taking into account that recent reports point to very rapid reactions within minutes (*Findeklee* and *Goldbach*, 1996; *Findeklee* et al., 1997; *Mühling* et al., 1998; *Wimmer* and *Goldbach*, 1999), it is intriguing to explore the sequence of responses to B deprivation. Below, we summarize effects which have been observed within minutes and discuss how these primary responses may be linked to the expression of secondary reactions or symptoms in the light of recent observations.

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2 Physical properties of cell walls

Early reactions of cells of higher plants to the removal of B include a rapid, but transient decrease of the cell wall elasticity modulus ε accompanied by a simultaneous but also transient increase in the hydraulic conductivity (*Findeklee* and *Goldbach*, 1996; *Findeklee* et al., 1997). 15 to 20 minutes after rinsing plant roots in the B-free nutrient solution, re-hardening of the cell walls commenced. This continued to an extent that it became virtually impossible to impale the cells with the cell pressure probe without rupturing them after 10 hours. Re-addition of boric acid after three hours could not stop this process (Fig. 1). It is thus most likely that *after* deprivation of B a secondary process is induced which is not primarily controlled by B.

Although rhamnogalacturonan II (RGII) has been identified as the major B-binding fraction in plant cell walls (*Kobayashi* et al., 1996; *Matoh*, 1997; *Fleischer*, et al., 1999), it is still unclear how the rapid alterations of the cell

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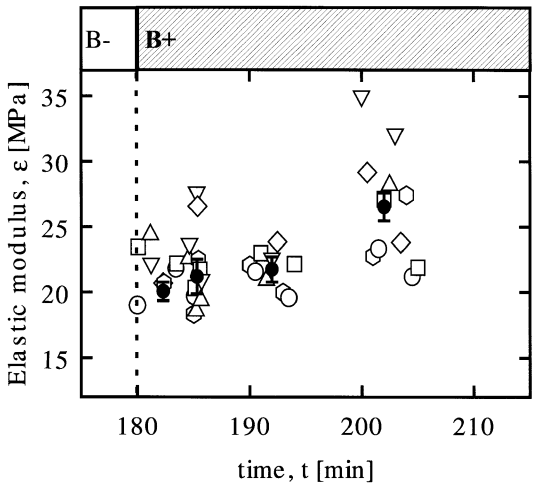

Figure 1: Changes of the cell wall elastic modulus ε after re-supply of B to *Cucurbita maschata* L. roots after 180 minutes of B deprivation; different symbols are values from individual measurements; solid points indicate means ± standard deviation; the cell pressure probe was impaled into the outer or second cortical cell layer.
Abbildung 1: Änderungen des Zellwandelatizitätsmoduls ε nach Zugabe von B zu *Cucurbita moschata* L.-Wurzeln, die für 180 min ohne B-Zugabe gehalten wurden; unterschiedliche Symbole repräsentieren Werte aus unterschiedlichen Messungen; die gefüllten Symbole zeigen den Mittelwert ± Standardabweichung an; die Zelldrucksonde wurde in die äußere oder die darunter liegende Rindenzellschicht eingeführt.

wall properties mentioned above are related to the RGII-borate complex and which processes may be responsible for the re-hardening of the cell walls. Interestingly, the cell wall borate-RG-II complex is mostly located proximal to the plasma membrane, suggesting that B cross-links, especially with pectins can be localized at the plasma membrane cell wall interface (*Matoh* et al., 1998).

Considering the high *in vitro* stability of the borate-RGII complex (*Kobayashi* et al., 1997; *Matoh*, 1997) one would not expect any major exchange of B from these bonds and consequently no significant alteration of ε. As shown in our experiments (*Findeklee* and *Goldbach*, 1996; *Findeklee* et al., 1997), however, a reduction of ε by a factor of almost three was found. Thus, the bonding strength must be lower *in vivo* than *in vitro* (at least in the relatively young cells of our experiments). The discrepancy might be explained by the much higher strain on the RGII-borate bonds in turgid cells.

Fleischer (2000), *Fleischer* et al. (1998) reported from his experimental system (frequently sub-cultured *Chenopodium* cells) that cells can survive without B if kept always in a juvenile state (mostly primary cell walls). However, the cell wall porosity was significantly higher than in the cells with normal B supply (see also *Fleischer* et al., 1999) which points to an altered arrangement of the cell wall matrix. This is in accordance with our observations of the decreased cell wall elasticity modulus ε (*Findeklee* and *Goldbach*, 1996). When allowed longer subculture periods, the *Chenopodium* cells frequently burst (*Fleischer* et al., 1998; *Fleischer*, 2000). These findings are also in line with our observations of an enhanced brittleness of root tissues after 10 h of B deprivation (*Goldbach* et al., 2000).

As the re-hardening of cell walls in root tissues (outer cortical layer) takes place *after* the transitory reduction of the elasticity modulus ε, and as it is not reversible, a signal has to trigger this process. It is hardly conceivable that this reaction is induced by the mere change in cell wall extensibility, otherwise acid induced growth should yield effects comparable to B deficiency.

Based on the effects obtained in *Chenopodium* cell cultures (*Fleischer* et al., 1998; *Fleischer*, 2000), it seems likely that the deleterious effect of B deprivation is related to a disturbance of one or several processes during the formation of secondary cell walls. In cell suspensions, the damage caused by B deficiency might be explained solely by a loss of cell wall stability and subsequent bursting of the cells (*Fleischer*, 2000). The damages to intact tissues (for a review, see e.g. *Bussler*, 1964, and *Al-Badrawy* and *Bussler*, 1977) and all numerous secondary reactions observed thereafter, however, are difficult to explain merely on the basis of differences in elastic cell wall properties. Possible mechanisms will be discussed below.

3 Boron-calcium interactions

An interaction between B and Ca has been reported by several authors (*Yamanouchi*, 1973; *Clarkson* and *Hanson*, 1980; *Yamauchi* et al., 1986; *Teasdale* and *Richards*, 1990; for a review of older literature see also *Goldbach*, 1997). *Wimmer* and *Goldbach* (1999) detected a short-term release of cell wall calcium in apical root segments when B was removed from an equilibrium solution (molar ratio approximately 1:3, B/Ca). All reactions were significant within 5 minutes upon removal of B. Stabilization of RGII-borate-complexes by co-ordinative binding of calcium was shown by Matoh's group (*Kobayashi* et al., 1997), which is in line with our observations on the mutual influence of B and calcium release from incubated apical root segments (*Wimmer* and *Goldbach*, 1999). *O'Neill* et al. (1996), however, did not observe a stabilization of RGII-borate-complexes by Ca, but by Pb, Sr , and Ba. This is in contradiction to the results of *Kobayashi* et al. (1999), but differences in the experimental conditions might be responsible for this apparent discrepancy.

Besides an effect on the cell wall and cell wall-bound calcium, membrane-bound calcium might be involved in early deficiency reactions as well (*Mühling* et al., 1998). Here, deprivation of B led to an increase in free apoplastic calcium whereas re-addition of B decreased it, though the latter effect was less pronounced (*Mühling* et al., 1998). Whether this increase in calcium concentration is a trigger for further reactions and whether B deficiency alters the cytosolic calcium level, has not been shown yet. The enhanced callose formation under B deficiency (*Rajaratnam* and *Lowry*, 1974) indicates that an increase of cytosolic calcium levels might also be possible, but further research is still needed in this respect.

Besides the structural effects of borate-RG II complexes, auxins are affected by B deprivation, too. *Tang* and *de la Fuente* (1986a, b) observed synergistic effects of B and calcium on the polar transport of IAA (see chapter 5).

It is also interesting that concentrations of B which are higher than those needed for an optimum supply are able to

reduce aluminum toxicity in plants (*Lenoble* et al., 1996a, b), at least in non-graminaceous species (see chapter 7).

4 Boron – plasmalemma interactions

For several years, an inhibition of ATPase activities by B deficiency has received much attention (for a review of older literature see *Goldbach*, 1997). *Cakmak* et al. (1995) and *Cakmak* and *Römheld* (1997) attribute the observed higher solute leaching of excised leaves as a membrane damage due to free radical attack. He assumed that phenol oxidation and an enhanced formation of phenolics would be a primary effect of B deficiency. In the light of the rapid effects of B deprivation on cell wall properties as stated above, however, the leaching he found in his experiments, might also be a consequence of cell rupture by incubation in distilled water. Furthermore, the merely *transitory* increase in the hydraulic conductivity of the cortical root cells (*Findeklee* et al., 1997) which reached thereafter again the original level, as well as a slight hyperpolarization of the membrane potential during 24 hours in B-deficient roots of *Cucurbita moschata* (*Blaser-Grill*, 1992; *Goldbach* et al., 1991) are in conflict with *Cakmak*'s notion. There is, however, no doubt that alterations of the phenol metabolism and oxidation of phenols are part of the syndrome which develops after B deprivation (*Shkolnik*, 1984; *Cakmak* and *Römheld*, 1997) and lead to the browning of B deficient plant tissue, especially the apical meristems and vascular bundles. This would also be in line with the observations of *Lukaszewski* and *Blevins* (1996) who found that the levels of ascorbic acid rapidly decline under B deficiency as well as Al toxicity which points to oxidative stress as one consequence of B deficiency. Re-hardening of the cell walls might to a part also be the consequence of an enhanced formation of phenolic crosslinks by esters or ethers as discussed earlier (*Goldbach*, 1997).

Our group (*Goldbach* et al., 1991; *Findeklee* et al., 1997; *Goldbach* et al., 2000), as well as *Barr* et al. (1993) have shown that inducible oxido-reductase activity is rapidly affected by B deficiency and differences are significant even within 5 minutes after B deprivation. Whether this is a primary reaction involving borate-NADH-complexes, or a first reaction to the decrease in cell wall elastic properties, or a still unknown trigger, can not be clarified yet.

The formation of borate esters with ATP and NADH (*Pfeffer* et al., 1999) might be involved in the regulation of ATPase and reductase activities, although it is difficult to imagine how the relatively high stability of these complexes and given the permeability of undissociated boric acid would lead to pronounced differences of ATPase and reductase activities between *Poales* and non-graminaceous species. There are differences in the presence of inducible PL-bound oxido-reductases and to a certain extent also ATPase activities but the underlying mechanism for a control via B is still unknown. *Obermaier* et al. (1996) showed that the plasmamembrane H^+-ATPase of ungerminated lily pollen grains was directly stimulated by boric acid although the increase was only 18 %. Whether this could trigger subsequent processes to such an extent that secondary processes of B deprivation will be initiated or enhanced, remains to be elucidated.

An influence of B deficiency on K-channels as a further mechanism regulated by B has been discussed by *Schon* et al. (1990), *Cakmak* et al. (1995), *Cakmak* and *Römheld* (1997). Upon the addition of boric acid, *Schon* et al. (1990) observed a hyperpolarization of the membrane potential within three minutes. It still needs to be shown whether this is an unspecific "weak acid effect" or a specific reaction to B.

Recently, *Dordas* et al. (2000) provided evidence that at least a part of the B uptake occurs via proteinaceous channels. Some of the plant aquaporins and the glycerol transporter GlpF (from *E. coli*) increased the B permeability of *Xenopus laevis* oocytes by 30 %. This is in line with the partial inhibition (30–39 %) of boric acid permeation through plasmamembrane vesicles by channel blockers. Thus, beside B's high mobility in lipid phases, certain channels which transport small neutral solutes may also be a path through which B enters plants cells and where it may influence processes, especially in the plasmalemma-near cytosol.

5 Boron and IAA-translocation

The basipetal IAA-transport from growing tissues is related to an acropetal calcium transport (*Bañuelos* et al., 1987; 1988; *Guzman* and *de la Fuente*, 1984). *Tang* and *de la Fuente* (1986 a, b) showed that basipetal IAA-translocation in sunflower hypocotyls is highly reduced under B deficiency. Although the initiation and formation of tracheid elements is inhibited under B deficiency (*Pissarek*, 1980), the inhibition of IAA-translocation is likely to be affected by another mechanism. Recently, *Li* et al. (2000) noticed a significant reduction of IAA-translocation by B deprivation. The conflicting data on IAA synthesis and catabolism (for a review see *Goldbach*, 1997) may be due to the different experimental conditions. The possibility that IAA translocation is more directly affected by B deficiency than IAA synthesis is appealing. The possible underlying mechanism will be discussed below.

6 Distribution of boron-binding sites as visualized by the FITC-boronic acid adduct

By using boronic-acid coupled fluorescein-isothiocyanate (FITC) we were able to show the heterogeneous distribution of boric acid/borate binding sites in the tissues (*Glüsenkamp* et al., 1997; *Edelmann* et al., 2000). Especially the walls of the outer cortical/rhizodermal and the vascular bundle cell walls are preferentially binding the dye and thus B (Fig. 2a–c). The distribution of the dye is largely similar to the distribution of RG II (*Matoh*, 1997).

In the context of root hairs (*Michael*, 2001), it is especially interesting that the apical parts of the root hairs stain most intensely with the dye (Fig. 3). As root hairs as well as pollen tubes show tip growth (*Baluška* et al., 2000 b; *Shaw* et al., 2000), there seems to be a locally high demand for boric acid, probably for the secretion of cell wall material (for a hypothesis see *Goldbach*, 1997). The increased demand for the secretion of cell wall material in the growing zone, and the accumulation of vesicles near the plasma membrane in root cortex cells as shown by *Kouchi* and *Kumazawa* (1975a)

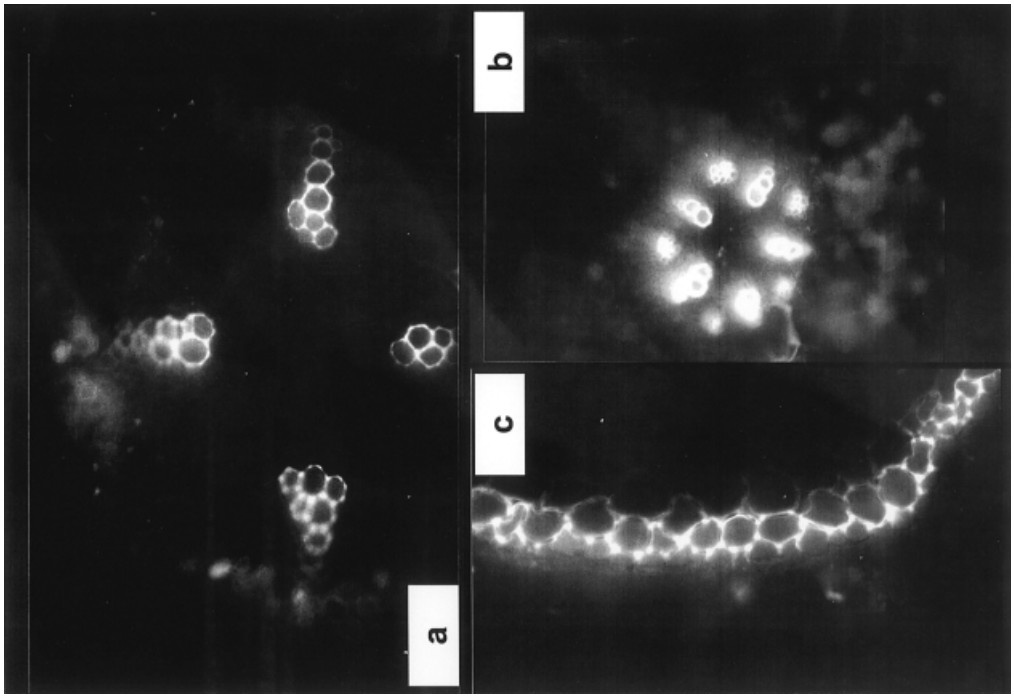

Figure 2: Binding of FITC-boronic acid as a marker for the distribution of B-binding ligands; cryo-sections were made through *Vicia faba* L. roots, stained subsequently with the dye and the surplus of dye removed by repeated rinsing; autofluorescence was low and could hardly be noticed at the same exposure time (16 seconds); a) cross section approx. 20 mm basal from the root tip; b) cross section approx. 50 mm basal from the root tip; c) strong fluorescence in cell walls at the rhizodermis and outer cortex at the beginning of the root hair zone.
Abbildung 2: Bindung von FITC Boronsäure als Indikator für die Verteilung B-bindender Liganden; Gefrierschnitte durch *Vicia faba* L.-Wurzeln wurden mit dem Fluoreszenzfarbstoff angefärbt und danach mehrfach gespült zur Entfernung überschüssigen Farbstoffs; die Autofluoreszenz war sehr niedrig und bei gleicher Belichtungsdauer (16 sec) kaum wahrzunehmen; a) Querschnitt ca. 20 mm basal der Wurzelspitze; b) Querschnitt ca. 50 mm basal der Wurzelspitze; c) starke Fluoreszenz in den Zellwänden der Rhizodermis und äußeren Rindenzellschicht im Bereich der beginnenden Wurzelhaarbildung.

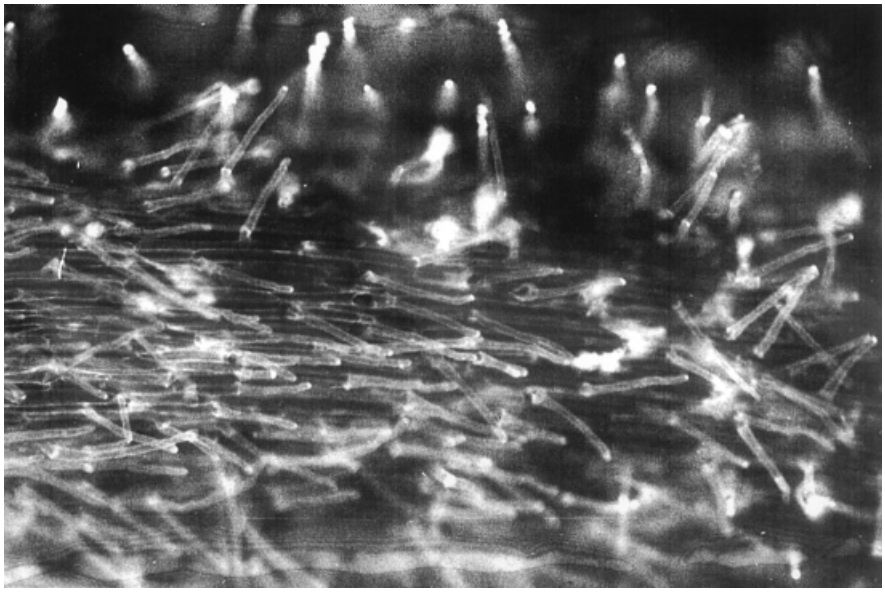

Figure 3: Preferential binding of FITC-boronic acid at the apex of root hairs (*Vicia faba* L.).
Abbildung 3: Bindung von FITC-Boronsäure bevorzugt am Apex von Wurzelhaaren (*Vicia faba* L.).

could be related to the higher B-sensitivity of this tissue. These authors (*Kouchi* and *Kumazawa*, 1975 b; *Kouchi* and *Kumazawa*, 1976) also observed that B deficiency affects the development of root tips.

7 Effects of boron deprivation on the cytoskeleton[1]

The cytoskeleton has been implicated in a variety of cellular functions including cell division, elongation, cell wall synthesis, intracellular signalling and tip growth (for reviews see *Seagull*, 1989; *Volkmann* and *Baluška*, 1999; *Barlow* and *Baluška*, 2000; *Baluška* et al., 2000 a, b). The cytoskeleton – plasma membrane – extracellular matrix continuum was hypothesized by several authors to represent an essential structural assembly directing the growth and morphogenesis of higher plants (*Wyatt* and *Carpita*, 1993; *Miller* et al., 1997). Ionic factors, such as levels of cytosolic Ca^{2+} (*Jones* et al., 1998), Mg^{2+} and calmodulin (*Cyr*, 1991; *Grabski* et al., 1998), are well known to influence the organization and stability of the cytoskeleton. An early response to aluminum toxicity is a disorganization and

[1] all data by *Yu* et al., submitted

destabilization of the cytoskeleton (*Blancaflor* et al., 1998; *Sivaguru* et al., 1999). The cytoskeleton – plasma membrane – extracellular matrix continuum is assumed to be a primary target of aluminum toxicity (*Horst* et al., 1999).

As the early effects of both, aluminum toxicity and B deficiency are located in the same region of young roots, and as aluminum toxicity can be alleviated by the addition of a surplus of B (*Lenoble* et al., 1996 a, b), structural alterations of the pectic matrix after removal of B might interfere directly or indirectly with the cell wall – plasma membrane – cytoskeleton continuum. Thus, we followed early alterations of the cytoskeleton in *Arabidopsis* upon removal of B by using monoclonal antibodies to actin and tubulin. An increasing amount of actin (Fig. 4a) and tubulin (Fig. 4b) proteins were repeatedly observed following 20 min (actin) or 40 min (tubulin) of B deprivation and the increase became more prominent at 120 and 150 min.

SDS-PAGE patterns and western blot of ubiquitinated protein revealed that there was no intensive protein degradation in B deficient samples over the experimental period and the synthesis of house-keeping proteins was constant over 150 min of B deprivation. Thus, we can exclude a rapid effect of B deficiency on overall protein synthesis and turnover. Likewise, the appearance of heat shock proteins as a possible first quick response to the mechanical stress induced by the altered cell wall properties (*Findeklee* and *Goldbach*, 1996) can be excluded, too.

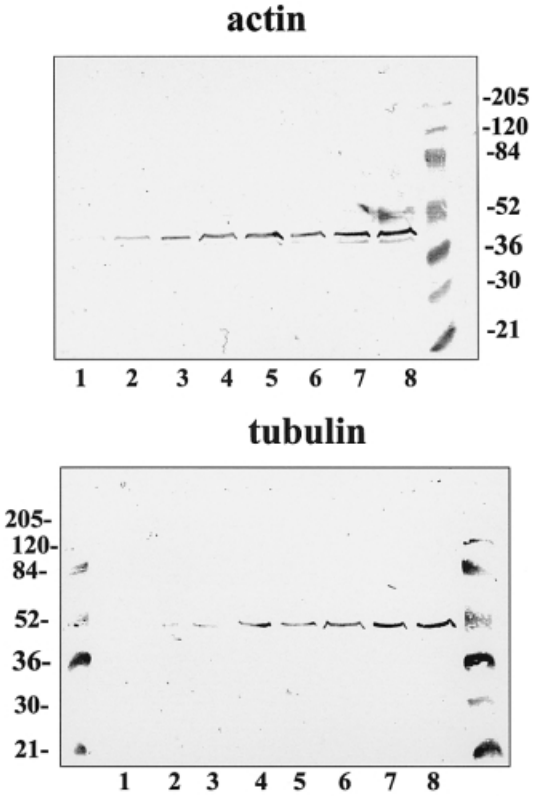

Figure 4: Actin and tubulin immunoblots of root tissue extract from 3-week-old hydroponically grown *Arabidopsis thaliana*. Lane 1 corresponds to control at t = 0, and 10, 20, 40, 60, 90, 120, 150 min, respectively, of B deprivation treatment; at the right are the markers for molecular weight.
Abbildung 4: Actin- und Tubulin-Immunoblots für Wurzelextrakte von *Arabidopsis thaliana* L. in Wasserkultur nach kurzfristiger Unterbrechung der B-Versorgung (Spur 1 = control zu t = 0, 2 = 10 min., 3 = 20 min., 4 = 30 min., 5 = 60 min., 6 = 90 min., 7 = 120 min., 8 = 150 min. Unterbrechung der B-Versorgung).

In addition, preliminary microscopic work with maize root apices also revealed both, increased actin and tubulin fluorescence and more dense cytoskeletal arrays in response to short term (10 to 60 min) B deprivation in the transition and elongation zones of root apices (Fig. 5 a–d). Further work is going on to examine whether increased actin and tubulin fluorescence or denser cytoskeletal arrays can also be detected in *Arabidopsis* root apices subjected to short-term B deprivation as well.

The response of actin and tubulin synthesis to B removal was surprisingly fast and prominent. The increase might be attributed to the up-regulation of specific actin and tubulin isoforms and/or to an inhibited protein degradation. Both, expression of different genes and/or post-translational modification, lead to expression of multiple isoforms. We thus hypothesize that B deprivation could act in a similar way as e.g. symbiotic infection, cold acclimation, hormone treatment, light regime, and wounding (*Kerr* and *Carter*, 1990; *Perez* et al., 1994; *McDowell* et al., 1996).

As can be seen in Figs. 5 a–d and 6 a–d, B deprivation led to an enhanced level of cytoskeleton fluorescence, but in contrast to aluminum toxicity (*Sivaguru* et al., 1999), the array of the cytoskeleton is not altered. We thus noticed both after B deprivation: an increased level of the monomeric forms (as seen from the immunoblots) as well as a higher degree of polymerization as visualized by fluorescence microscopy. Consequently the rigidification of the cytoskeleton could be a response counteracting the weakening of the cell periphery complex (*Baluška* et al., 2000a) in cell walls of B deprived cells. The trigger for this response is still unknown.

8 Conclusions: How could early reactions of boron deprivation lead to secondary events?

As shown above, several rapid reactions can be recognized as short term responses to B deprivation. They can be summarized as follows:

i) altered cell wall physics with a transitory decrease of the elasticity modulus ε and a secondary re-hardening
ii) increase in apoplastic as well as plasmamembrane-bound Ca^{2+} levels
iii) a reduction of plasma membrane-bound inducible reductase activity
iv) higher levels of cytoskeletal proteins (actin and tubulin), and differences in their polymerization pattern

All these reactions take place within less than 5–15 minutes after starting the B deficiency treatment. How they are linked and related to the one definitely known stabile borate-RG II complex, needs to be elucidated.

One possibility is that secondary B deficiency reactions are due to an increased apoplastic/plasmamembrane Ca^{2+} level. Taking into consideration that apoplastic Ca/calmodulin has been identified and that it is involved in reactions to aluminum toxicity (*Ma* et al., 2000), one can imagine that

this triggers a signal chain which leads to the other observed rapid reactions, eventually to the cytosolic Ca/calmodulin and Ca/phosphoinositide signalling pathways, too. Altered cytoskeletal dynamics could thus be one possible consequence and lead to further physiological and morphological alterations. *Pierson* et al. (1993), however, did not find Ca^{2+} fluxes to be influenced in pollen tubes until bursting at the tip. Whether these findings can be generalized, needs to be investigated further. Considering that

i) B deficient roots frequently do not possess root hairs,

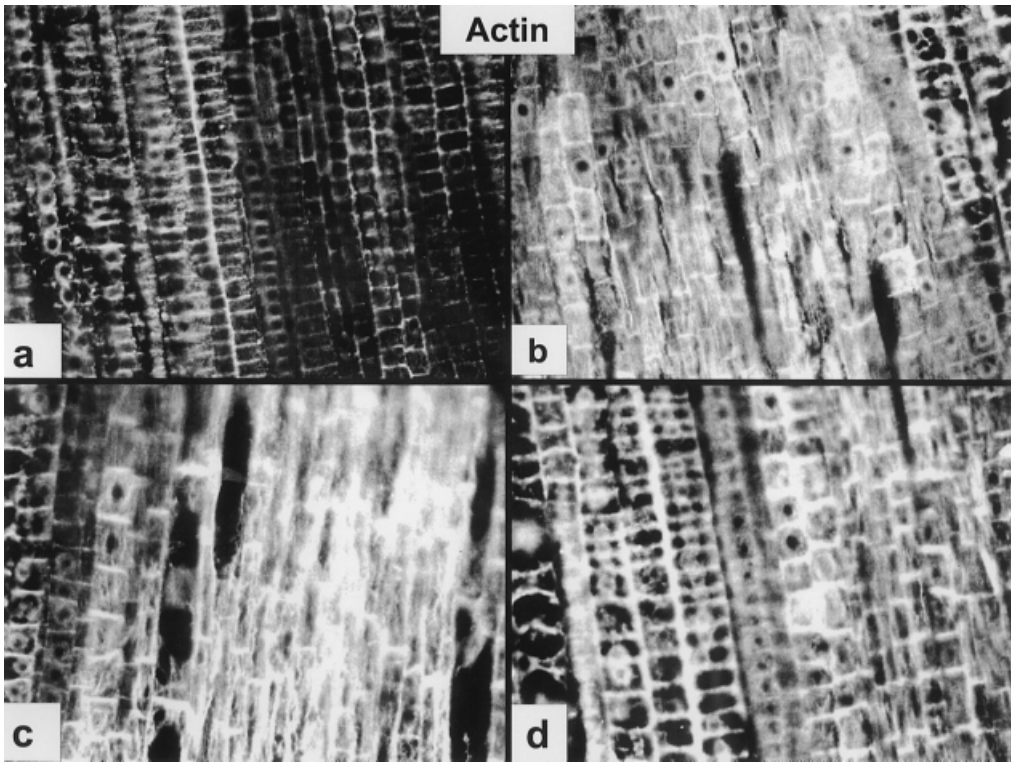

Figure 5: Micrograph of maize root tissue from hydroponic culture after treatment with fluorescent actin antibodies as related to the duration of B deprivation; a) control at t = 0; b) 10 minutes after starting B deprivation; c) 30 minutes after starting B deprivation; d) 60 minutes after starting B deprivation (from Yu and Baluška, unpublished).

Abbildung 5: Mikrophotographie von Maiswurzeln aus Wasserkultur nach Behandlung mit fluoreszierenden Antikörpern gegen Actin nach unterschiedlicher Dauer des B-Entzugs; a) Kontrolle zu t = 0; b) 10 Minuten nach Beginn des B-Entzugs; c) 30 Minuten nach Beginn des B-Entzugs; d) 60 Minuten nach Beginn des B-Entzugs; (Yu und Baluška, unveröffentlicht).

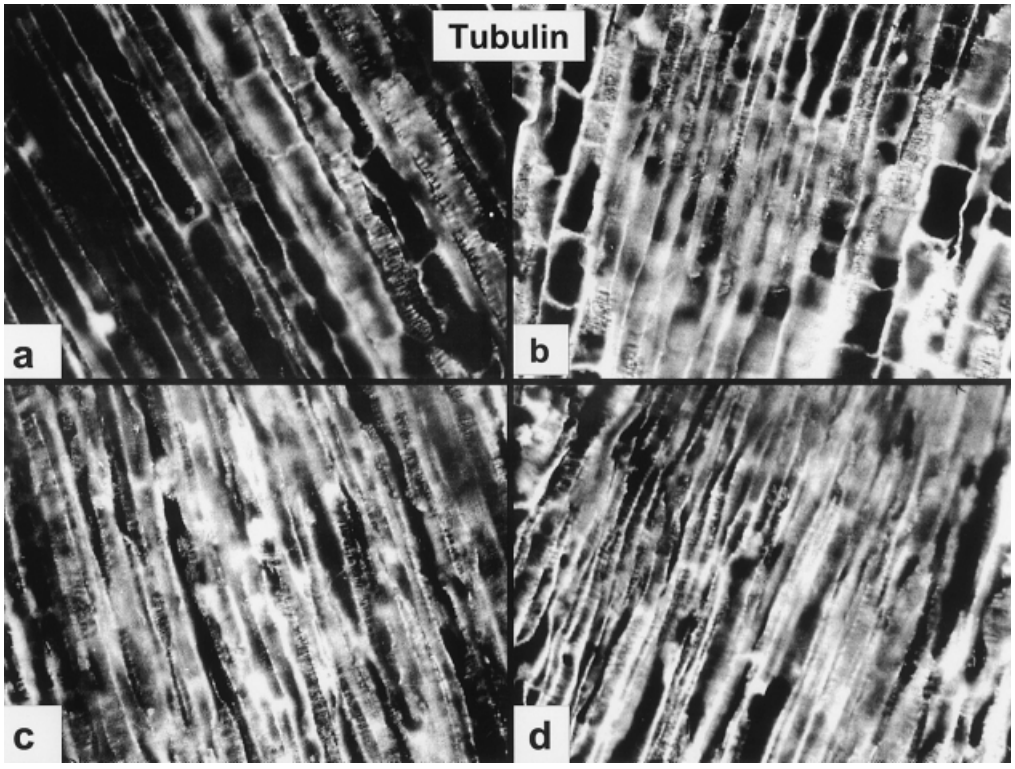

Figure 6: Micrograph of maize root tissue from hydroponic culture after treatment with fluorescent tubulin antibodies as related to the duration of B deprivation; a) control at t = 0; b) 10 minutes after starting B deprivation; c) 30 minutes after starting B deprivation; d) 60 minutes after starting B deprivation (from Yu and Baluška, unpublished).

Abbildung 6: Mikrophotographie von Maiswurzeln aus Wasserkultur nach Behandlung mit fluoreszierenden Antikörpern gegen Tubulin nach unterschiedlicher Dauer des B-Entzugs; a) Kontrolle zu t = 0; b) 10 Minuten nach Beginn des B-Entzugs; c) 30 Minuten nach Beginn des B-Entzugs; d) 60 Minuten nach Beginn des B-Entzugs; (Yu und Baluška, unveröffentlicht).

ii) that growth of pollen tubes (tip growth as in root hairs!) is halted in a B free medium if they do not burst due to the weakening of the tube walls,

one can imagine that B deprivation interferes with several processes which lead to a localized response preceding root hair growth. These can be:

i) *cell wall acidification*: prolonged B deprivation might inhibit plasmalemma-bound reactions which are responsible for the acidification preceding the weakening of the cell wall prior to the initiation of root hair growth
ii) *cell wall weakening*: the re-hardening found as a secondary response to B deprivation could prevent the necessary weakening and the subsequent deposition of (pectic) material to the new root hair tip region.

B deficiency primarily alters cell wall physics (*Kobayashi* et al., 1996; *Findeklee* and *Goldbach*, 1996; *Fleischer* et al., 1999) or plasma membrane associated activities (*Findeklee* et al., 1997) and, consequently, may exert its impact on the cytoskeletal dynamic equilibrium via the extracellular matrix – plasma membrane – cytoskeleton continuum.

Sivaguru et al., (2000) recently observed that aluminum toxicity may lead to an inhibited plasmodesmatal cell-to-cell trafficking of molecules by 1→3-β-D-glucan-formation. Callose-formation is a known rapid effect of Al toxicity in the sensitive distal transition zone (DTZ) as observed by *Sivaguru* and *Horst* (1998) and *Sivaguru* et al. (1999). Considering that

i) callose formation in the sieve tubes has also been observed under B deficiency by *Van de Venter* and *Currier* (1977) and *Rajaratnam* and *Lowry* (1974),
ii) that B deficiency induces accumulation of callose in the tips of B deficient pollen tubes (*Yang* et al., 1999)
iii) as well as the rapid changes in free calcium levels (*Mühling* et al., 1998; *Wimmer* and *Goldbach*, 1999), which according to the distribution of RGII should be effective especially close to the plasmalemma (*Kobayashi* et al., 1999),

the reactions to B deficiency are likely to involve alterations in free Ca concentrations in a signal chain leading to rapid secondary responses to B deficiency. This would also be in line with the increased expression of actin and tubulin proteins at early stages of B deprivation.

This, together with other reported early reactions to B deficiency, points to a probably more complex action of B than just cross linking RGII. Either free boric acid, or other more labile compounds, are involved in the regulation of these rapid responses. Further studies will be aimed at the identification of actin and tubulin isoform identities in response to B deficiency as well as at elucidating its impact on the distribution of microtubules and actin filaments in diverse root cell types.

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References

Al-Badrawy, R. and *Bussler, W.* (1977): Symptome von Bormangel und Borüberschuss bei *Hibiscus esculentus*. Z. Pflanzenernähr. Bodenkd. 140, 505–513.

Baluška, F., Volkmann, D., and *Barlow, P.W.* (2000a): Actin-based domains of the "cell periphery complex" and their associations with polarized "cell bodies" in higher plants. Plant Biol. 2, 253–267.

Baluška, F., Salaj, J., Mathur, J., Braun, M., Jasper, F., Šamaj, J., Chua, N.-H., Barlow, P. W., and *Volkmann, D.* (2000b): Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-enriched F-actin meshworks accumulated within outgrowing bulges. Dev. Biol. 227, 618–632.

Bañuelos, G.S., Bangerth, F., and *Marschner, H.* (1987): Relationship between polar basipetal auxin transport and acropetal Ca^{2+} transport into tomato fruits. Physiol. Plant. 71, 321–327.

Bañuelos, G.S., Bangerth, F., and *Marschner, H.* (1988): Basipetal auxin transport in lettuce and its possible involvement in acropetal Ca^{2+} transport and incidence of tipburn. J. Plant Nutr. 11, 525–533.

Barlow, P.W. and *Baluška, F.* (2000): Cytoskeletal perspectives on root growth and morphogenesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 51, 289–322.

Barr, R., Boettger, M., and *Crane, F.L.* (1993): The effect of boron on plasma membrane electron transport and associated proton secretion by cultured carrot cells. Biochem. Mol. Biol. Int. 31, 31–39.

Blancaflor, E.B., Jones, D.L., and *Gilroy, S.* (1998): Alterations in the cytoskeleton accompany aluminum induced growth inhibition and morphological changes in primary roots of maize. Plant Physiol. 118, 159–172.

Blaser-Grill, J. (1992): Einfluss von Bor auf Membranpotential und Membranwiderstand von *Elodea*-Blattzellen und *Cucurbita*-Wurzelzellen. (Influence of B on membrane potential and membrane resistance of *Elodea* leaf cells and *Cucurbita* root cells) Ph.D. thesis, Technical University of Munich, Germany.

Bussler W. (1964): Die Bormangelsymptome und ihre Entwicklung. Z. Pflanzenern. Düng. Bodenkde. 91, 1–14.

Cakmak, I. and *Römheld, V.* (1997): Boron deficiency-induced impairments of cellular functions in plants. In B. Dell, P. Brown and R. Bell: Boron in Soils and Plants: Reviews; Dordrecht, Netherlands, Kluwer Academic Publishers, p. 71–84.

Cakmak, I., Kurz, H., and *Marschner, H.* (1995): Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. Physiol. Plant. 95, 11–18.

Clarkson, D.T. and *Hanson, J.B.* (1980): The mineral nutrition of higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 31, 239–298.

Cyr, R. (1991): Calcium/calmodulin affects microtubule stability in lysed protoplasts. J. Cell Sci. 100, 311–317.

Dordas, C., Chrispeels, M.J., and *Brown, P.H.* (2000): Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. Plant Physiol. 124, 1349–1361.

Edelmann, H.G., Wimmer, M.A., and *Goldbach, H.E.* (2000): Immunogold labelling of boric acid binding sites by immunoreaction to the FITC-moiety of FITC-boronic acid: first results. J. Trace Microprobe Techniques 18, 451–459.

Findeklee, P. and *Goldbach, H.E.* (1996): Rapid effects of boron deficiency on cell wall elasticity modulus in *Cucurbita pepo* roots. Bot. Acta 109, 463–465.

Findeklee, P., Wimmer, M., and *Goldbach, H.E.* (1997): Early effects of boron deficiency on physical cell wall parameters, hydraulic conductivity and plasmalemma-bound reductase activities in young *C. pepo* and *V.*

faba roots. In R. Bell and B. Rerkasem: Boron in Soil and Plants, Proceedings; Kluwer Academic Publishers Dordrecht, The Netherlands, p. 221–227.

Fleischer, A. (2000): Borwirkungen an primären Zellwänden pflanzlicher Zellen (Effects of boron on primary plant cell walls), Ph.D. thesis, Humboldt-University Berlin, Germany.

Fleischer, A., Titel, C., and *Ehwald, R.* (1998): The boron requirement and cell wall properties of growing and stationary suspension-cultured *Chenopodium album* L. cells. Plant Physiol. 117, 1401–1410.

Fleischer, A., O'Neill, M.A., and *Ehwald, R.* (1999): The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. Plant Physiol. 121, 829–838.

Glüsenkamp, K.H., Kosegarten, H., Mengel, K., Grolig, F., Esch, A., and *Goldbach, H.E.* (1997): A fluorescein boronic acid conjugate as a marker for borate binding sites in the apoplast of growing roots of *Zea mays* L. and *Helianthus annuus* L. In R.W. Bell and B. Rerkasem: Boron in Soils and Plants, Proceedings. Kluwer Academic Publishers, Dordrecht, The Netherlands, p. 229–235.

Goldbach, H.E., Blaser-Grill, J., Lindemann, N., Porzelt, M., Hörrmann, C., Lupp, B., and *Gessner, B.* (1991): Influence of boron on net proton release and its relation to other metabolic processes. In D. D. Randall and D. G. Blevins: Current Topics in Plant Biochemistry and Physiology 10, University of Missouri, USA, p. 195–220.

Goldbach, H.E. (1997): A critical review on current hypotheses concerning the role of boron in higher plants: suggestions for further research and methodological requirements. J. Trace Micr. Techn. 15, 51–91.

Goldbach, H.E., Wimmer, M., and *Findeklee, P.* (2000): Discussion Paper: Boron – how can the critical level be defined? J. Plant Nutr. Soil Sci. 163, 115–121.

Grabski, S., Arnoys, E., Busch, B., and *Schindler, M.* (1998): Regulation of actin tension in plant cells by kinases and phosphatases. Plant Physiol. 116, 279–290.

Guzman, C.C. and *De la Fuente, R.K.* (1984): Polar calcium flux in sunflower hypocotyl segments. I. The effect of auxin. Plant Physiol. 76, 347–352.

Horst, W.J., Schmohl, N., Kollmeier, M., Baluška, F., and *Sivaguru, M.* (1999): Does aluminum affect root growth of maize through interaction with the cell wall – plasma membrane – cytoskeleton continuum? Plant and Soil 215, 163–174.

Jones, D.L., Kochian, L.V., and *Gilroy, S.* (1998): Aluminum induces a decrease in cytosolic calcium concentration in BY-2 tobacco cell cultures. Plant Physiol. 116, 81–89.

Kerr, P.G. and *Carter, J.V.* (1990): Tubulin isotypes in rye roots are altered during cold acclimation. Plant Physiol. 93, 83–88.

Kobayashi, M., Matoh, T., and *Azuma, J.I.* (1996): Two chains of rhamnogalacturonan II are cross-linked by borate diol ester bonds in higher plant cell walls. Plant Physiol. 110, 1017–1020.

Kobayashi, M., Ohno, K., and *Matoh, T.* (1997): boron nutrition of cultured tobacco BY-2 cells. II Characterization of the boron-polysaccharide complex. Plant Cell Physiol. 38, 676–683.

Kobayashi, M., Nakagawa, H., Asaka, T., and *Matoh, T.* (1999): Borate-rhamnogalacturonan II bonding reinforced by Ca^{2+} retains pectic polysaccharides in higher-plant cell walls. Plant Physiol. 119, 199–203.

Kouchi, H. and *Kumazawa, K.* (1975a): Anatomical responses of root tips to boron deficiency. I: Effects of boron deficiency on the elongation of root tips and their morphological characteristics. Soil Sci. Plant Nutr. 21, 218–228.

Kouchi, H. and *Kumazawa, K.* (1975b): Anatomical responses of root tips to boron deficiency. II: Effect of boron deficiency on sub-cellular structure of root tips. Soil Sci. Plant Nutr. 21, 137–150.

Kouchi, H. and *Kumazawa, K.* (1976): Anatomical responses of root tips to boron deficiency. III: Effect of boron deficiency on the cellular growth and development of root tips. Soil Sci. Plant Nutr. 22, 53–71.

Lenoble, M.E., Blevins, D.G., Sharp R.E., and *Cumbie, B.G.* (1996a): Prevention of aluminum toxicity with supplemental boron. 1. Maintenance of root elongation and cellular structure. Plant Cell Environ. 19, 1132–1142.

Lenoble, M.E., Blevins, D.G., and *Miles, J.R.* (1996b): Prevention of aluminum toxicity with supplemental boron. 2. Stimulation of root growth in an acidic, high-aluminum subsoil. Plant Cell Environ. 19, 1143–1148.

Li, C., Pfeffer, H., Dannel, F., Römheld, V., and *Bangerth, F.* (2000): Effects of boron starvation on boron compartmentation, and possibly hormone-mediated elongation growth and apical dominance of pea (*Pisum sativum*) plants. Physiol. Plant. 111, 212–219.

Lukaszewski, K.M. and *Blevins, D.G.* (1996): Root growth inhibition in boron-deficient or aluminum-stressed squash may be a result of impaired ascorbate metabolism. Plant Physiol. 112, 1135–1140.

Ma, L.G., Fan, Q.S., Yu, Z.Q., Zhou, H.L., Zhang, F.S., and *Sun, D.Y.* (2000): Does aluminum inhibit pollen germination via extracellular calmodulin? Plant Cell Physiol. 41, 372–376

Matoh, T. (1997): Boron in plant cell walls. Plant and Soil 193, 59–70.

Matoh, T., Takasaki, M., Takabe, K., and *Kobayashi, M.* (1998): Immunocytochemistry of rhamnogalacturonan II in cell walls of higher plants. Plant Cell Biol. 39, 483–491.

McDowell, J.M., An, Y.Q., Huang, S., McKinney, E.C., and *Meagher, R.B.* (1996): The *Arabidopsis* ACT7 actin gene is expressed in rapidly developing tissues and responds to several external stimuli. Plant Physiol. 111, 699–711.

Michael, G. (2001): The control of root hair formation – suggested mechanisms. J. Plant Nutr. Soil Sci. 164, 111–120.

Miller, D., Hable, W., Gottwald, J., Ellard-Ivey, M., Demura, T., Lomax, T., and *Carpita, N.* (1997): Connections: the hard wiring of the plant cell for perception, signalling, and response. Plant Cell 9, 2105–2117.

Mühling, K.H., Wimmer, M., and *Goldbach, H.E.* (1998): Apoplastic and membrane-associated Ca^{2+} in leaves and roots as affected by boron deficiency. Physiol. Plant. 102, 179–184.

O'Neill, M.A., Warrenfeltz, D., Kates, K., Pellerin, P., Doco, T., Darvill, A.G., and *Albersheim, P.* (1996): Rhamnogalacturonan-II, a pectic polysaccaraide in the walls of growing plant cells, forms a dimer that is covalently cross linked by a borate ester. J. Biol. Chem. 271, 22923–22930 and correction: J. Biol. Chem. 272 (1997), 3869.

Obermaier, G., Kriechbaumer, R., Strasser, D., Maschessnig, A., and *Bentrup, F.-W.* (1996): Boric acid stimulates the plasma membrane H^+-ATPase of ungerminated lily pollen grains. Physiol. Plant. 98, 281–290.

Pérez, H.E., Sánchez, N., Vidali, L., Hernández L.M., Lara, M., and *Sánchez, F.* (1994): Actin isoforms in non-infected roots and symbiotic root nodules of *Phaseolus vulgaris* L. Planta 193, 51–56.

Pfeffer, H., Dannel, F., and *Römheld, V.* (1999): Isolation of soluble boron complexes and their determination together with free boric acid in higher plants. J. Plant Physiol. 154, 283–288.

Pierson, E.S., Smith, P.J.S., Shipley, A.M., Jaffe, L.F., Cresti, M., and *Hepler, P.K.* (1993): Ca^{2+} fluxes around pollen grains and pollen tubes of lily; normal development and effects of thermal shock, BAPTA-type buffer microinjection and depletion of boric acid from the medium. Biol. Bull. 185, 302–303.

Pissarek, H.P. (1980): Makro- und Mikrosymptome des Bormangels bei Sonnenblumen, Chinakohl und Mais. Z. Pflanzenernähr. Bodenkd. 143, 150–160.

Rajaratnam, J.A. and *Lowry, J.B.* (1974): The role of boron in the oil palm. Ann. Bot. 38, 193–200.

Schon, M. K., Novacky, A., and *Blevins, D. G.* (1990): Boron induces hyperpolarization of sunflower root cell membranes and increases membrane permeability to K^+. Plant Physiol. 93, 566–571.

Seagull, R.W. (1989): The plant cytoskeleton. Crit. Rev. Plant Sci. 8, 131–167.

Shaw, S.L., Dumais, J., and *Long, S.R.* (2000): Cell surface expansion in polarly growing root hairs of *Medicago trunculata*. Plant Physiol. 124, 959–969.

Shkolnik, M. Y. (1984): Trace Elements in Plants. Elsevier, Amsterdam, Oxford, New York, Tokyo.

Sivaguru, M. and *Horst, W.J.* (1998): The distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. Plant Physiol. 116, 155–163.

Sivaguru, M., Baluška, F., Volkmann, D., Felle, H.H., and *Horst, W.J.* (1999): Impacts of aluminum on the cytoskeleton of the maize root apex. Short-term effects on the distal part of the transition zone. Plant Physiol. 119, 1073–1082.

Sivaguru, M., Fujiwara, T., Šamaj, J., Baluška, F., Yang, Z., Osawa, H., Maeda, T., Mori, T., Volkmann, D., and *Matsumoto, H.* (2000): Aluminum-induced 1→3-β-D-glucan inhibits cell-to-cell trafficking of molecules through plasmodesmata. A new mechanism of aluminum toxicity in plants. Plant Physiol. 124, 991–1005.

Tang, P.M. and *De la Fuente, R.K.* (1986a): The transport of indole-3-acetic acid in boron- and calcium-deficient sunflower hypocotyl segments. Plant Physiol. 81, 646–650.

Tang, P.M. and *De la Fuente, R.K.* (1986b): Boron and calcium sites involved in indole-3-acetic acid transport in sunflower hypocotyl segments. Plant Physiol. 81, 651–655.

Teasdale, R.D. and *Richards, D.K.* (1990): Boron deficiency in cultured pine cells. Plant Physiol., 1071–1077.

Van de Venter, H.A. and *Currier, H.B.* (1977): The effect of boron deficiency on callose formation and ^{14}C translocation in bean (*Phaseolus vulgaris* L.) and cotton (*Gossypium hirsutum* L.). Amer. J. Bot. 64, 861–865.

Volkmann, D. and *Baluška, F.* (1999): The actin cytoskeleton in plants: from transport networks to signalling networks. Microsc. Res. Tech. 47, 135–154.

Wimmer, M.A. and *Goldbach, H.E.* (1999): Influence of Ca^{2+} and pH on the stability of different boron fractions in intact roots of *Vicia faba* L. Plant Biol. 1, 632–637.

Wyatt, S.E. and *Carpita, N.C.* (1993): The plant cytoskeleton – cell wall continuum. Trends Cell Biol. 3, 413–417.

Yamanouchi, M. (1973): The role of boron in higher plants (Part 2). The influence of boron on the formation of pectic substances. Bull. Fac. Agri. Tottori Univ. XXV, 21–27.

Yamauchi, T., Hara, T., and *Sonoda, Y.* (1986): Distribution of calcium and boron in the pectin fraction of tomato leaf cell wall. Plant Cell Physiol. 27, 729–732.

Yang, X.-D., Sun, S.-Q., and *Li, Y.-Q.* (1999): Boron deficiency causes changes in the distribution of major polysaccharides of pollen tube wall. Acta Botanica Sinica 41, 1169–1176.

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