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Short-term boron deprivation enhances levels of cytoskeletal proteins in maize, but not zucchini, root apices

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By using indirect immunofluorescence microscopy and Western blot analysis, we have demonstrated increased levels of actin and tubulin proteins as well as an altered polymerization pattern of their cytoskeletal assemblies in maize, but not zucchini root apices, as a response to early boron deprivation. Northern blot analysis, however, did not show significant increases in the amount of steady-state mRNAs of actin and tubulin. This finding indicates that these rapid cytoskeletal responses to boron removal are very likely regulated at translational/post-translational levels. Interestingly, these increased levels of cytoskeletal proteins coincided well with a reduction in the water-extractable, but not with the cell wall-bound, fraction of boron. This implicates that free boric acid, or other more labile boron complexes, might be involved in the activation of cytoskeletal responses in maize root apices. In fact, our experimental approach revealed that maize was suffering from boron deprivation as early as zucchini. This was evidenced by its slightly reduced root elongation rate recorded within 3–5 h of boron deprivation. Importantly, however, maize roots can recover from this early inhibition indicating an effective adaptation mechanism. In contrast, zucchini roots apparently lack this boron-deprivation response pathway and suffer extensively when exposed to boron-free environment. This leads to the tentative working hypothesis for an adaptive mechanism of maize roots to boron deprivation by enhancing its cytoskeletal protein levels and altering their polymerization patterns in order to mechanically reinforce the cell periphery complex of their cells. This testable hypothesis requires further experimental verification.

Introduction

In recent years, many functions of boron in plant cell biology and physiology have been postulated, including cell wall synthesis and structure, membrane integrity and function, as well as metabolic activities (for reviews see Loomis and Durst 1991, Marschner 1995, Goldbach 1997, Blevins and Lukaszewski 1998, Goldbach et al. 2001, Brown et al. 2002). Nevertheless, the function of boron remains the least understood among all microelements, and with the exception of the direct evidence for a role for B in cross-linking of cell wall rhamnogalacturonan II (RGII) and pectin assembly, no additional role for B is known (Kobayashi et al. 1996, 1999, Matoh 1996, Fleischer et al. 1999, Ishii et al. 1999, Ishii and Matsunaga 2001). This pectate complex can be further reinforced with calcium (Kobayashi et al. 1999, Ehwald et al. 2002) and plays an important role in the structural organization of plant cell walls (Matoh 1997). In plants, boron deficiency induces a number of diverse anatomical, cytological, and biochemical alterations such as cell wall abnormalities, accumulation of vesicles near the plasma membrane, and reduced plasma membrane H^+-ATPase activity (reviewed by Goldbach 1997). However, it is not yet clear if these alterations are of primary or secondary nature. Current research revealed several rapid reactions of higher plants to boron removal (within 5–20 min), including altered cell wall physics with a transitory decrease of the elasticity modulus ε followed by a secondary re-hardening, and a reduction of inducible plasma membrane-bound reductase activity (reviewed by Goldbach et al. 2001). Although RGII molecules were identified as the major boron-binding fraction in plant cell walls, it is still unclear how the above-mentioned rapid alterations in the cell wall/plasma membrane interface are related to the RGII-borate complex.

Roles of the cytoskeleton in cell division, elongation, cell wall synthesis, intracellular signalling and tip growth have been well established (for reviews see Seagull 1989, Volkmann and Baluška 1999, Baluška et al. 2000, Barlow and Baluška 2000, Staiger 2000). Increasing evidence supports the hypothesis that a putative cytoskeleton–plasma membrane–extracellular matrix continuum (Wyatt and Carpita 1993, Miller et al. 1997, Kohorn 2000) represents an essential structural framework directing the growth and morphogenesis of higher plants. Structural alterations of the pectic matrix, due to the removal of boron, can be expected to interfere directly or/indirectly with the cell wall–plasma membrane–cytoskeleton continuum. As summarized recently by Matoh and Kobayashi 2002), cell wall pore size (Fleischer et al. 1998, 1999) and cell wall swelling (Matoh et al. 2000) are determined by the degree of borate-linked RGII-dimer formation. Having such an impact on cell wall structural properties, considerable influence of boron on the cell wall–plasma membrane–cytoskeleton continuum can be expected. In fact, continuous supply of boron is essential for the actin cytoskeleton-mediated endocytosis of cell wall pectins in cells of maize and wheat root apices (Baluška et al. 2002, Yu et al. 2002). Moreover, the apoplastic side of this structural continuum has been suggested to represent the primary target of aluminium toxicity (Horst et al. 1999). Using actin and tubulin antibodies, we have revealed an increase in levels of actin and tubulin proteins upon 20–40 min of boron deprivation in roots of hydroponically grown *Arabidopsis thaliana* (Yu et al. 2001).

The present study was aimed at investigating the rapid response of the cytoskeleton to short-term boron deprivation as well as its regulation at protein and mRNA transcript levels using root apices of maize and zucchini, two contrasting species with regard to boron sensitivity. Maize, like other graminaceous species, is considered to be boron insensitive while zucchini is well known for its high sensitivity to boron deprivation (Blevins and Lukaszewski 1998). Our ultimate goal is to elucidate how the primary boron deficiency reactions are linked to already extensively described secondary symptoms. The dynamic root cytoskeleton may obviously play a direct adaptive role in early responses to boron deprivation.

Materials and methods

Plant growth

Maize (*Zea mays* L. cv. Careca S230) and zucchini (*Cucurbita pepo* L. cv. Diamant) seeds were soaked in distilled water for 8 h with aeration and germinated in moist rolls of filter paper for 2 days in dark at 24°C (maize) or 28°C (zucchini). Uniform seedlings with straight primary roots were transferred to solution culture which was maintained in a phytotron at 24°C under 14 h light (400 $\mu mol\ m^{-2}\ s^{-1}$) and 70% humidity. The nutrient solution contained (in μM): $Ca(NO_3)_2$, 2000; KNO_3, 3000; KH_2PO_3, 1000; $Mg(NO_3)_2$, 500; NaCl, 100; $MgSO_4$, 1000; FeEDDHA, 44.8; $MnSO_4$, 18.2; $CuCl_2$, 3,1; $ZnSO_4$, 6.1; $(NH_4)_6Mo_7O_{24}$, 0.2; $CoCl_2$, 0.016; $NiCl_2$, 0.017; H_3BO_3, 40. All the nutrient stock solutions were prepared with Milli-Q ultra pure water which was deprived of boron with B-specific ion exchange resin Amberlite IRA-743 (Sigma, Munich, Germany). The pH of the nutrient solution was adjusted to 6.0 by addition of 1 *N* NaOH. Seedlings were allowed to grow for 2 days in a one-tenth strength nutrient solution with 15 min aeration every 45 min, then in a full strength nutrition solution for a further 2 days.

Boron deprivation treatment

Six days after germination, seedlings were transferred to a fresh nutrient solution with (+B) or without boron (–B). In the –B treatment, B-exchange resin was also placed within the nutrient container and the nutrient solution was constantly stirred to adsorb all potential B contaminations. The roots were rinsed in a 5-l –B nutrient solution for a few seconds and then allowed to grow in –B solution for 10, 30 and 60 min, respectively. A detailed description of the method for solution culture and boron deprivation treatment of *Arabidopsis* seedlings was given in our previous study (Yu et al. 2001).

Boron fractionation

Analysis of boron fractions in 3-cm sections of root apex was performed following the procedure of Wimmer and Goldbach (1999).

Indirect immunofluorescence microscopy

Indirect fluorescence microscopy was performed essentially according to Sivaguru et al. (1999) with few modifications. Apical segments of primary root from +B and –B treated seedlings were vacuum infiltrated for 10 min with 3.7% formaldehyde made up in stabilizing buffer (SB: 50 m*M* 1,4 piperazinediethanesulphonic acid (Pipes), 5 m*M* EGTA and 5 m*M* $MgSO_4$, pH 6.9) and then fixed at room temperature for 1 h. After a brief rinse in SB, they were dehydrated in a graded ethanol series diluted with PBS (pH, 7.3) and embedded in Steedman's wax (for details see Baluška et al. 1992). Eight-µ*M*-thick longitudinal sections were prepared from the embedded materials and the most median sections were allowed to expand on a drop of distilled water onto slides coated with glycerol-albumen (Serva, Heidelberg, Germany).

After drying at room temperature overnight, the mounted sections were dewaxed in ethanol and rehydrated in PBS diluted ethanol series, and then left in SB for 30 min before being rinsed with methanol at −20°C for 20 min. The sections were transferred to SB for 30 min and incubated with the following primary antibodies for 1 h at room temperature: the monoclonal antiactin antibody raised against chicken gizzard actin (ICN Biomedicals, Eschwege, Germany); the monoclonal antitubulin antibody raised against chicken brain

α-tubulin (Amersham, Freiburg, Germany). All the primary antibodies were diluted in PBS supplemented with 0.1% BSA. After a rinse in SB, the sections were incubated with FITC-conjugated antimouse IgG (Sigma) and diluted 1:100 in PBS containing 0.1% BSA. The labelled sections, after being rinsed with PBS and further stained with 0.01% toluidine blue to diminish autofluorescence from root tissue, were mounted using an antifade mountant. Fluorescence was detected with an Axiovert 405M inverted microscope (Zeiss, Oberkochen, Germany) equipped with epifluorescence and standard FITC exciter and barrier filters (BP 450-490, LP520). Photos were taken on Kodak T-Max films rated at 400 ASA.

Western blot analysis of cytoskeletal proteins

The roots of maize and zucchini seedlings were frozen in liquid nitrogen and homogenized (5 min with a mortar and pestle) in an ice-cold buffer solution containing 125 m*M* Tris-HCl (pH 7.4), 2 m*M* EDTA, 20% (v/v) glycerol, 10 m*M* dithiothreitol (DTT), 5 m*M* ascorbic acid, and a cocktail of protease inhibitors (500 *M* Pefabloc SC (4-(2-aminoethyl)-benzenesulphonyl fluoride Pefabloc), 100 *M* leupeptin, 100 *M* phenylmethysulphonyl fluoride (PMSF), and 50 $\mu g\ ml^{-1}$ aprotinin). The homogenate was then centrifuged at 15 000 *g* for 15 min at 4°C. The resulting supernatant was used immediately or frozen in aliquots at −20°C. Protein concentration of the samples was determined by the Bradford method and protein separation was achieved by SDS electrophoresis (15% polyacrylamide gels) using the samples which were diluted in sample buffer (4% SDS, 20% glycerol, 125 m*M* Tris/HCl (pH 6.8), 100 m*M* DTT and 0.001% bromophenol blue), heated to 90°C and centrifuged. Gels were electrophoretically transferred to nitrocellulose (0.45 μ*M*) with a Bio-Rad transblot cell. Successful electrotransfer of separated proteins was indicated by Ponceau staining. Nitrocellulose membrane was blocked in PBS buffer containing 5% (w/v) non-fat milk powder for 1 h at room temperature, and incubated for 1 h with the primary antibodies (antiactin and tubulin as used in indirect fluorescence microscopy) diluted with PBS containing 1% (w/v) BSA and 0.025% Tween-20. After washing in PBS containing 0.1% Triton X-100 (15 min, 3 times), the membrane was incubated for 1 h with the secondary antibody (antimouse IgG conjugated to alkaline phosphatase). The membrane was washed again (15 min, 3 times) before immunoreactive spots were visualized by colour development with BCIP and NBT.

RNA extraction and Northern blot analysis

Total RNA was isolated from 7-day-old maize and 3-week-old *Arabidopsis* roots by the guanidine-HCl method according to Logemann et al. (1987). Five-μg of total RNA was resolved on a 1% agarose-formaldehyde gel and transferred onto positively charged nylon membranes. The RNA loading was normalized by photometric measurement and checked by visualization of total RNA in the gel after ethidium bromide staining. The blots with *Arabidopsis* RNA were hybridized with probes representing *Arabidopsis* ACT2 (An et al. 1996) and TUA3 coding sequences (a gift from Peter Snustad at the University of Minnesota, USA, Ludwig et al. 1987), respectively, and blots from maize RNA were probed with *Arabidopsis* ACT2 and maize TUA4 coding sequences (a gift from Silvana Dolfini at the University of Milano, Italy, Dolfini et al. 1993). Probes were labelled by PCR in the presence of DIG-labelled dNTPs using the expand high fidelity PCR system (Roche Diagnostics, Germany) according to the instructions of the supplier. The 34-mer oligonucleotide 5′-GC-GGATCCATGGCTGAGGCTGATGATATTCAACC-3′ (*Bam*MI and *Nco*I restriction sites underlined) and 36-mer oligonucleotide 5′-CGTCTAGACCATGGAA-CATTTTCTGTGAACGATTCC-3′ (*Xba*I and *Nco*I restrition sites underlined) were used as primers in order to amplify the open reading frame of *Arabidopsis* ACT2. The 30-mer 5′-GCAGATCTATGCGAGAAAT-CATAAGCATTC-3′ (*Bgl*II restriction site underlined) and 27-mer 5′-CGACTAGTAGTCTTCACCTTCAT-CTTC-3′ (*Spe*I restriction site underlined) were designed for the amplification of the *Arabidopsis* TUA3 coding sequence. The 29-mer 5′-CAGAATTCGAGCACACT-GATGTTGCTGTC-3′ (*Eco*RI restriction site underlined) and 29-mer 5′-CATCTAGACTAGTACTCGTC-ACCCTCGTC-3′ (*Xba*I restriction site underlined) were used to amplify the 3′ half of maize TUA4 coding region. The maize actin transcripts were hybridized to heterologous *Arabidopsis* ACT2 probe. The other mRNAs were hybridized to homologous probes. Hybridizations were carried out overnight at 50°C using DIG Easy Hyb (Roche Diagnostics, Germany), except that the blot with the heterologous *Arabidopsis* ACT2 was conducted at 42°C. Hybridized blots were then washed at high stringency (0.1 × SSC, 0.1% SDS, at 50°C) except that blots hybridized to heterologous probes were washed at low stringency (2 × SSC, 0.1% SDS, at 42°C). The relative mRNA abundances on the blots were detected by NBT/BCIP colour development according to the supplier's instruction. Hybridization experiments were repeated twice with independent RNA preparations.

The whole experiment was repeated twice with identical results.

Results

Immunofluorescence microscopy studies revealed that there was no major destruction or reorientation of actin filaments (AFs) and arrays of microtubules (MTs) in cells of maize and zucchini root apices in relation to short-term boron deprivation (within 60 min, data not shown). However, increased F-actin and MT fluorescence from both meristematic and elongating zones of root apices were reproducibly registered (Figs 1 and 2 are representive images). The increase in F-actin and MT fluorescence in maize root apex was recorded as early as 10 min following removal of boron (see also Goldbach

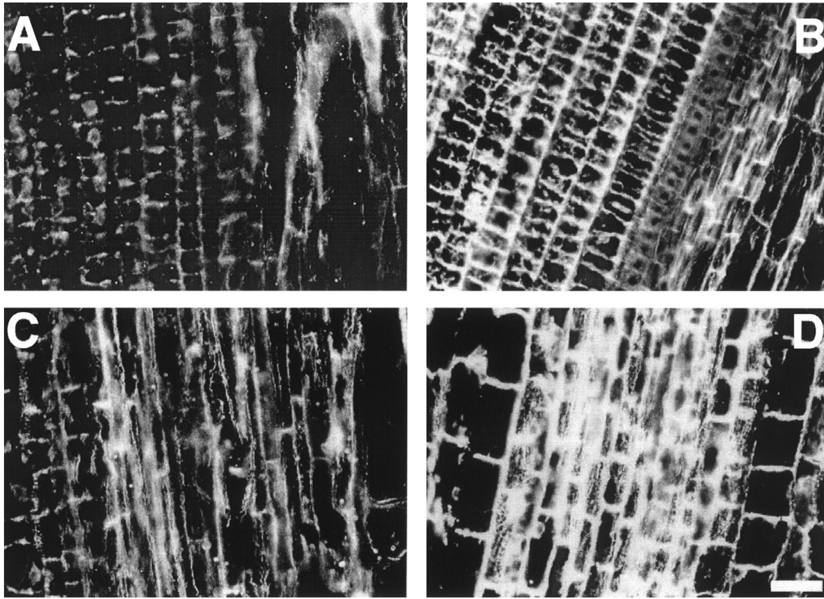

Fig. 1. F-actin immunofluorescence of maize root apices of 7-day-old seedlings grown hydroponically in response to short-term boron deprivation (1 h). Images were taken from the basal part of the meristem (A-B) and the apical part of the elongation region (C-D) under the same exposure time comparing +B (A,C) and −B (B,D) samples.

et al. 2001). Interestingly, this phenomenon was never observed in −B zucchini root apices (data not shown).

Western blot with monoclonal antibodies detected actin protein at about 45 kDa and tubulin at about 50 kDa (Fig. 3), which were in line with values expected for plant species (Yu et al. 2001). Marginally higher amounts of actin and tubulin proteins were repeatedly observed in −B maize, but not in −B zucchini, root extracts as early as 10 min upon boron removal (Fig. 3).

Northern blots from maize RNA, which was hybridized with heterologous *Arabidopsis* actin or homologous maize tubulin cDNA probe, identified a 1.5–1.6 kb band (Fig. 4). Blots from *Arabidopsis* RNA, which was hybridized with homologous *Arabidopsis* actin or tubulin cDNA probe also revealed a 1.5–1.6 kb band (Fig. 5). These bands corresponded well in size to those reported earlier (Kopczak et al. 1992; Dolfini et al. 1993, An et al. 1996). Northern blot analysis showed no significant increase in the amount of steady-state mRNAs of actin and tubulin in roots of maize (Fig. 4) or *Arabidopsis* (Fig. 5) that would correspond to the increase observed in Western blot at their protein levels (for *Arabidopsis* Western blot see Yu et al. 2001). Nevertheless, there seemed to be a slight increment of RNA levels after longer treatments (60 min for maize and 2.5 h for *Arabidopsis*).

Boron fraction analysis showed that both water- and $BaCl_2$-soluble boron was reduced in maize and zucchini root tips within 60 min, when the most prominent

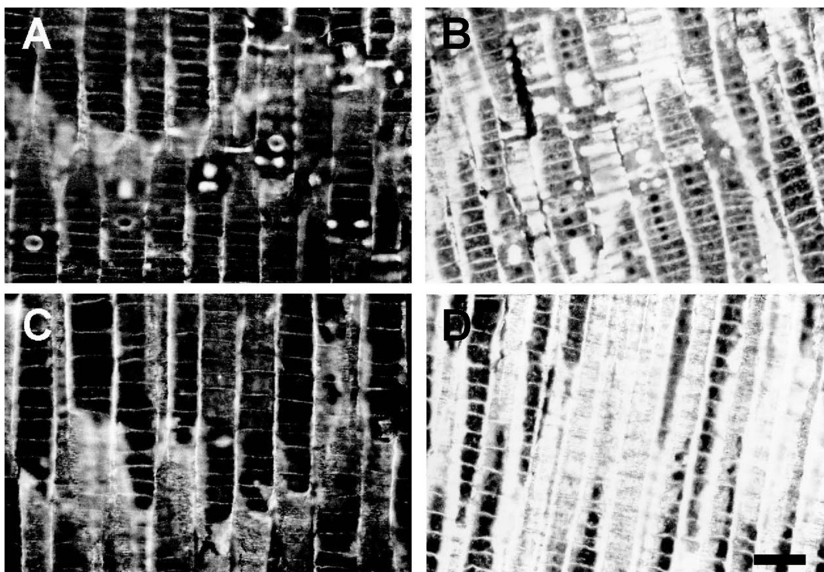

Fig. 2. Microtubule immunofluorescence of maize root apices of 7-day-old seedlings grown hydroponically responding to short-term boron deprivation (1 h). Images were taken from the apical part of the meristem (A-B) and the transition region (C-D) under the same exposure time comparing +B (A,C) and −B (B,D) samples.

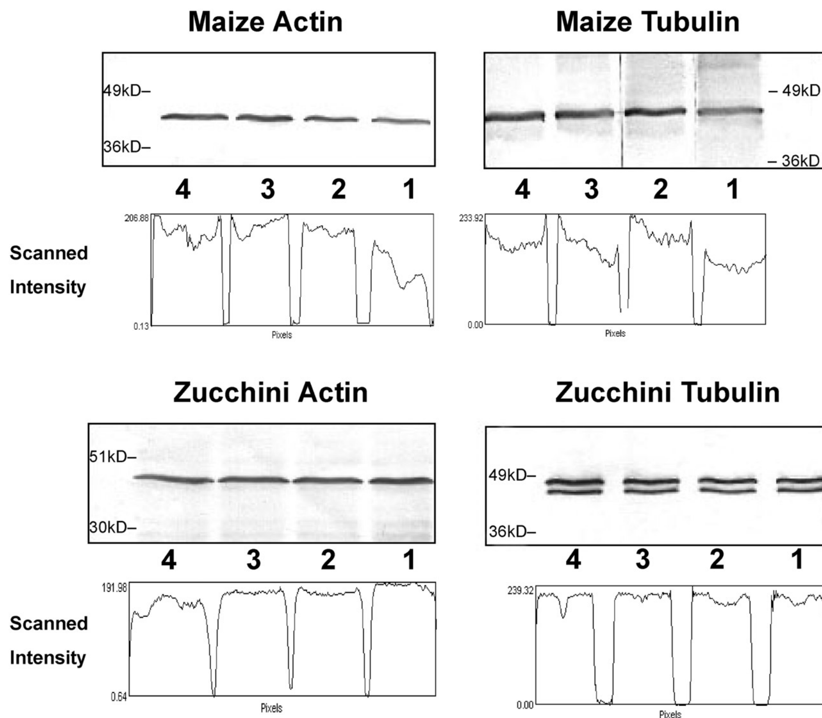

Fig. 3. Western blots of actin and tubulin proteins in the root tissue extracts of 7-day-old maize and zucchini seedlings grown hydroponically as related to short-term boron deprivation. Lanes 1–4 correspond to +B, 10, 30 and 60 min of –B treatments.

response was recorded 10 min after the commencement of boron deprivation (Fig. 6). On the other hand, the cell wall-bound fraction was not affected (not shown). Since RGII (cross-linked by borate) is not likely to be exchangeable by $BaCl_2$, $BaCl_2$-soluble boron may represent a fraction in which boron is more loosely bound.

Growth measurement demonstrated that root elongation rates of both maize and zucchini were affected immediately after the onset of boron deprivation, though to a much lesser extent for maize roots (Fig. 7). The maximum inhibition of root elongation rate in maize occurred after around 5 h of boron deprivation. Importantly, maize, but not zucchini, roots were able to restore the normal elongation rate within 10 h of boron deprivation.

Discussion

There are several parallels between the aluminium toxicity and boron deficiency. The early effects of both aluminium toxicity and boron deficiency are located in the same region of young roots and cell wall pectin appears to be critically involved in these responses. Moreover, the aluminium toxicity can be alleviated by the addition of a surplus of boron (Lenoble et al. 1996). Therefore, it is reasonable to assume that aluminium toxicity might encompass similar responses as boron deficiency; or at least that aluminium toxicity reactions may be in part parallel to boron deficiency responses. Research from the Horst's group have shown both disintegration of microtubules (MTs) and altered

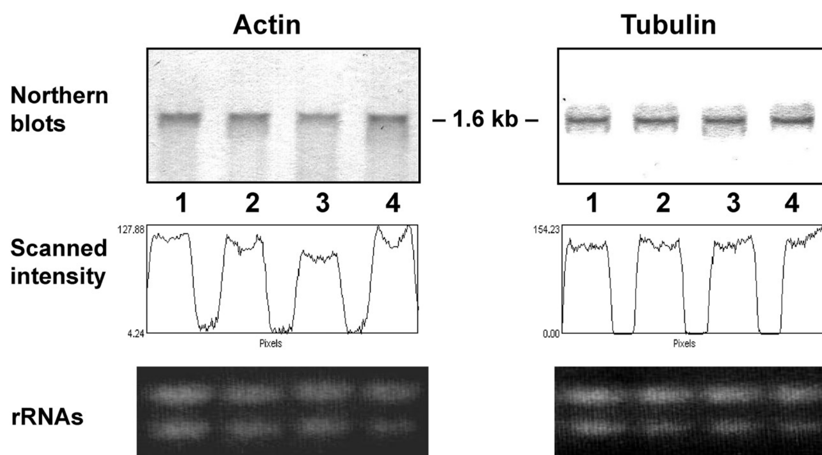

Fig. 4. Northern analysis of actin and tubulin transcripts in root tissue of 7-day-old maize seedlings grown hydroponically in relation to short-term boron deprivation. Lanes 1–4 correspond to +B, 10, 30 and 60 min of –B treatments. Each lane contains 5 μg of total RNA. The blots were hybridized with actin cDNA (ACT2) from *Arabidopsis* or tubulin cDNA (TUA4) from maize.

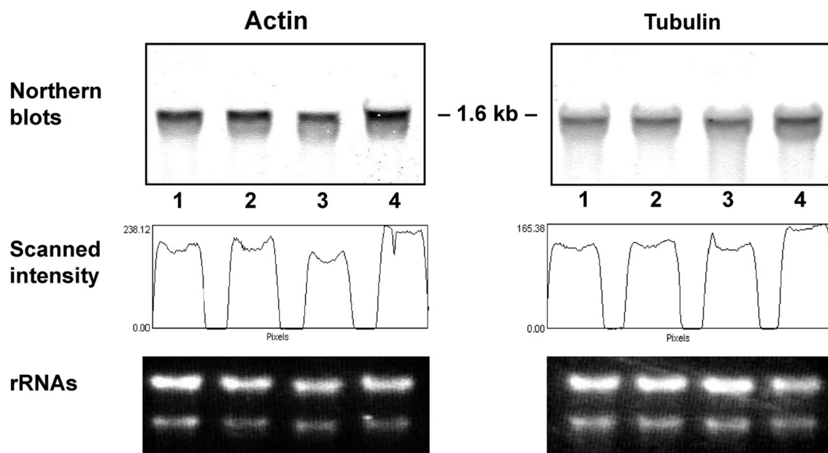

Fig. 5. Northern analysis of actin and tubulin transcripts in root tissue of 3-week-old *Arabidopsis* seedlings grown hydroponically in relation to short-term boron deprivation. Lanes 1–4 correspond to +B, 30, 60 min and 2.5 h of –B treatments. Each lane contains 5 µg of total RNA. The blots were hybridized with *Arabidopsis* actin cDNA (ACT2) and tubulin cDNA (TUA3). For its corresponding Western blot analysis, refer to Yu et al. (2001).

polymerization pattern of actin filaments (AFs) following 60 min of aluminium toxicity in maize root apices (Horst et al. 1999, Blancaflor et al. 1998). However, in our present experimental approach no prominent disorganization of either MTs or AFs were observed in either maize or zucchini root apices within 60 min of boron deprivation. This suggests that aluminium toxicity and boron deficiency exert different influences on the plant cytoskeleton and that the cytoskeletal reaction to boron deprivation is more specific.

Increased fluorescence labelling of cytoskeleton from maize root apex of –B treatment may be due to either altered polymerization pattern or/and increased levels of the actin monomers and tubulin dimers. Western blots probed with monoclonal antiactin and tubulin antibodies confirmed a mild increase in levels of cytoskeletal proteins, in parallel to a dramatic increment in fluorescence. Since the increase in fluorescence signal is much more prominent than the increase in protein levels, we consider that both the enhancement of cytoskeletal protein production and altered polymerization pattern contribute to the strong increase of fluorescence.

The observed increase of cytoskeletal proteins responding to short-term boron deprivation might be attributed to the upregulation of specific actin and tubulin isoforms or to the inhibited targeting of proteins for degradation. Previous work with *Arabidopsis* demonstrated that the amount of ubiquitin which is essential for selecting proteins for degradation, was not different between +B and –B samples (Yu et al. 2001). It seems therefore that inhibited protein degradation is not a likely factor contributing to increased protein levels. However, it can not be excluded that degradation machinery is operating less effectively for actin and tubulin proteins under boron deprivation.

Considering that the response of actin and tubulin synthesis to boron removal was fast and prominent, the upregulation could very likely be fulfilled at translational and post-translational levels. Data from mammalian cell studies with drug treatment are in support of an autoregulatory control of actin and tubulin synthesis at the mRNA level on the basis of the monomeric (dimeric)–polymeric subunit ratio (Cleveland and Sullivan 1985, Bershadsky et al. 1995, Lyubimova et al. 1997). In fact, actual levels of actin monomers are obviously monitored and can have relevant signalling outputs, for instance via regulation of DNA transcription (Sotiropoulos et al. 1999). Whether such mechanisms also exist in plant cells is not yet known.

Our Northern blot analysis clearly demonstrates that no appreciable increases in the amount of steady-state transcripts of actin and tubulin can be detected until 60 min of –B treatment. This is inconsistent with the remarkable increase in immunofluorescence as well as a mild increase in protein levels, as shown in Western blots. The data presented favour a quick stimulation of cytoskeletal response to the short-term boron removal at translational and/or post-translational levels. The results from *Arabidopsis* Northern blot analysis gave further support for this conclusion.

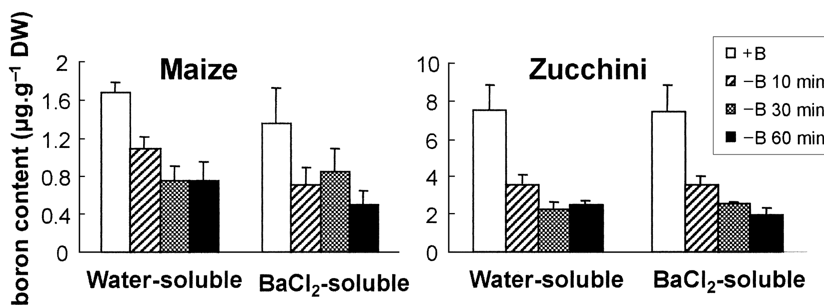

Fig. 6. Analysis of boron fractions in 3-cm long root apices of 7-day-old maize and zucchini seedlings grown in solution culture in response to short-term boron deprivation.

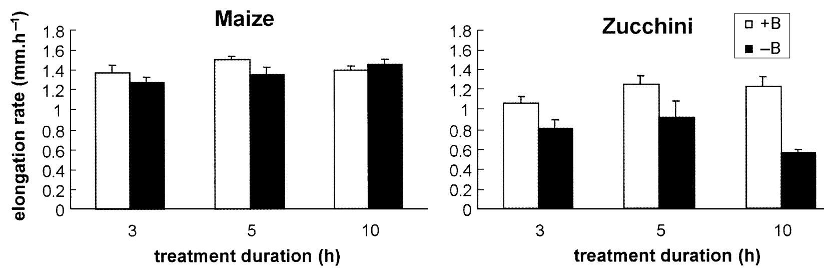

Fig. 7. Root elongating rate of 7-day-old maize and zucchini seedlings grown hydroponically as related to the duration of boron deprivation treatment; vertical bars represent SD.

It is well known that maize (monocot) is less susceptible to boron deficiency than zucchini (dicot). In this respect it is interesting that our root growth analysis indicated that maize was suffering from boron deprivation as early as zucchini, as evidenced by its reduced root elongation rate during the first 3–5 h of –B treatment. Importantly, however, maize roots can recover from the inhibition very soon, indicating an effective adaptation mechanism. Maize cytoskeleton responds to early boron removal by increasing actin and tubulin expression and altering polymerization pattern in order to reinforce the mechanical strength of the cell periphery complex (Baluška et al. 2000). This phenomenon, among other factors, may contribute to the rapid adaptation of maize roots to the boron-free environment via the putative boron-deprivation response pathway. The lack of reponse of the cytoskeleton in zucchini roots might be associated with differences in its susceptability to boron deprivation and differences in the amount, distribution, and eventually chemical nature of boron binding compounds in their cell walls.

The observed increase in fluorescence and levels of cytoskeletal proteins coincided well with the reduction in water- and $BaCl_2$-soluble boron, indicating that free boric acid or other more labile compounds might be involved in the induction of plant cytoskeleton responses to boron deprivation in maize root apices. As boron is cross-linking rhamnogalacturonan II pectins forming rhamnogalacturonan II-borate ester cross-linked dimers, which are essential for the cell wall integrity (O'Neill et al. 2001), it might be that there is a feedback mechanism stopping pectin endocytosis if the newly formed rhamnogalacturonan II pectins are not cross-linked with boron (Yu et al. 2002). This scenario suggests that altered actin cytoskeleton responds rapidly and very sensitively to boron deprivation (this study, Yu et al. 2001) in order to regulate endocytosis/recycling of cell wall pectins (Baluška et al. 2002, Yu et al. 2002).

It is not clear from the present study how these rapid cytoskeletal responses are triggered in root cells. But from other systems it is well known that cellular mechanoprotective adaptations involve co-ordinated actions performed by neighbouring structural systems (Ko and McCulloch 2000). Altered membrane tension (Chanturiya et al. 2000, Morris and Homann 2001), resulting in different conformation of transmembrane proteins (e.g. Watson 1991), is one possible candidate for a sensing mechanism in this mechano-signalling cascade which is typically conveyed further down into the cytoplasm via mitogen-activated protein kinases (for yeast cells see Kamada et al. 1995, Verna et al. 1997, for a review of plant mitogen-activated protein kinases see Ligterink and Hirt 2001). Cell wall weakness in plants due to the reduced availability or absence of RGII-boron complexes in newly synthesized cell wall portions, could increase the plasma membrane tension resulting in direct effects on both exocytosis and endocytosis (Kell and Glaser 1993, Sheetz and Dai 1996). Such rather global weakness of the cell periphery complex (Baluška et al. 2000) can be expected to be immediately challenged by the high internal turgor pressure (Fricker et al. 2000). Another aspect to be considered is the alteration of cell wall pore sizes under boron deprivation (Fleischer et al. 1998, 1999). As dimeric RGII is required for turgor driven growth (O'Neill et al. 2001), and the compartmentation of larger molecules (proteins) enables the ordered deposition of wall material, it is conceivable that maize and zucchini roots differ in cell wall properties under boron deprivation, which lead to an enhanced formation and polymerization of actin and tubulin proteins in maize but not zucchini. This important aspect merits attention in further research.

Note added in proof: Borate cross-linking of RG-II was shown to correlate with growth oscillations of actin dependent tip growth of *Lilium formosanum* pollen tubes (Holdaway-Clarke et al., 2003). These authors suggest that borate is possibly a major player in the phenomenon of oscillatory tip growth.

Conclusions

Immunofluorescence and biochemical data reveal that boron deprivation induces rapid enhancement of actin and tubulin proteins, as well as their altered polymerization patterns, in cells of maize, but not of zucchini roots. The regulation of this rapid response is not accomplished at the transcriptional level, indicating a translational and post-translational enhancement of cytoskeletal proteins. The plant cytoskeleton responds to boron removal by rapidly increasing subunit expression and by altering its polymerization pattern in order to reinforce the mechanical strength of the cellular periphery complex. Given the rapid effect of boron deprivation on cell wall physical properties, the different responses of maize and zucchini

are intriguing. Among other factors, increased levels of cytoskeletal proteins and altered polymerization patterns may contribute to the adaptation of maize roots to the boron deprivation via altered pectin trafficking. Additionally, enhanced levels of cytoskeletal proteins coincided well with the reduction in water- and $BaCl_2$-soluble boron. The chemical nature of these boron ligands is not known yet, but they could represent RGII precursors. This suggests that free boric acid, or other more labile B-containing compounds, might be related to the induction of rapid responses of plant cytoskeleton to the boron deprivation in cells of maize root apices.

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