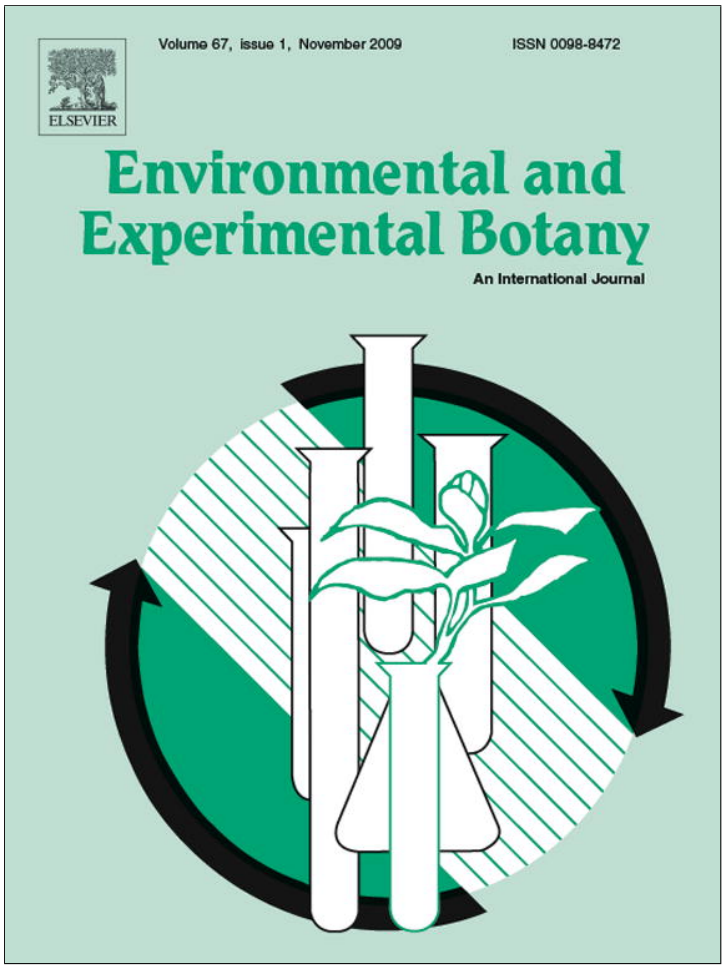

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Boron-regulated hypocotyl elongation is affected in *Arabidopsis* mutants with defects in light signalling pathways

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ABSTRACT

We studied the effect of elevated boron (B) concentrations on the growth and development of *Arabidopsis thaliana in vitro* with respect to different light conditions. Two basic responses were observed. At high concentrations (above 5 mM) a clear toxicity effect of B on plant growth was apparent. Seedlings were short, stunted and pale. However at concentrations between 1 and 3 mM H_3BO_3, hypocotyl elongation was stimulated in all *Arabidopsis* ecotypes tested relative to plants grown at 0.1 mM H_3BO_3. The stimulation of hypocotyl elongation by elevated B was proportionally greater with increasing irradiance. We also showed that blue light (BL) and red light (RL) did not alter the sensitivity of *Arabidopsis* hypocotyls to boron, but, dependent on genotype, BL and RL increased or reduced capacity of boron-induced hypocotyl elongation. Analysis of photomorphogenic mutants indicated the existence of an interaction between boron and light signalling pathways during plant growth and development. This interaction was supported by the observation that the expression of the *BOR1* gene in *Arabidopsis* hypocotyls was stimulated by BL and RL. Our results suggest that in etiolated or light-grown seedlings the stimulation of hypocotyl growth by boron can be mediated by cryptochromes and phytochromes.

1. Introduction

Boron (B) is an essential micronutrient required for plant growth and development, which affects not only yield but also the quality of crops (Brown et al., 2002). Shortly after B was identified as an essential element for higher plants, structural damage was attributed to B deficiency (for review see Blevins and Lukaszewski, 1998). Worldwide, B deficiency is more extensive than deficiencies of any other plant micronutrient (Loomis and Durst, 1992). Boron-deficient plants may exhibit a wide variety of symptoms, depending on the species and the age of the plant (Gupta, 1979; Shelp, 1993; Goldbach, 2001). In general, there is a small concentration range between deficiency and toxicity. The mechanisms of B toxicity are not entirely clear. Symptoms of B toxicity are reduced growth, particularly of the shoot, and chlorosis, then necrosis of older leaves, starting at the leaf tip and margins (Bennett, 1993; Reid et al., 2004).

To date many roles for B in plants have been proposed, including functions in sugar transport, cell wall synthesis and lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid metabolism, phenol metabolism and membrane transport (Blevins and Lukaszewski, 1998; Brown et al., 2002; Bolaños et al., 2004). However, the mechanism of B involvement in many cases remains unclear. Up to 90% of the B in plants is in the cell walls, where it forms an important crosslink between rhamnogalacturonan (RG-II) dimers (O'Neill et al., 1996). The physiological role of B in plants is also depicted as that of a transducer in several processes initiated by light, gravity, and some plant hormones (Tanada, 1995).

In vascular plants, B available in the soil moves from the roots with the transpiration stream and accumulates in the growing points of leaves and stems (Hu et al., 1997). Boron enters plant roots as uncharged boric acid (Woods, 1996). Initially, it was hypothesized that passive diffusion of boric acid across the lipid bilayer represented the major and possibly only mechanism of membrane transport of B. However, recent experiments (Dordas and Brown, 2000; Stangoulis et al., 2001) have revealed that the permeability of lipid membranes for boric acid is much lower than calculated earlier (Raven, 1980). These observations require the presence of

Abbreviations: B, boron; BL, blue light; BM, basal medium; CLSM, confocal laser scanning microscope(y); MES, 2-[N-Morpholino]ethanesulfonic acid; MS, Murashige–Skoog medium; RL, red light; wt, wild-type; WL, white light.

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B transporting membrane proteins to satisfy a plant's demand for B, especially under conditions of B limitation. In *Arabidopsis*, the movement of B from the root surface to the shoot includes at least two transmembrane transport events. In the first step, B is imported into epidermal, cortical, or endodermal cells (i.e. uptake) and then exported from pericycle or xylem parenchyma cells into the stellar apoplasm (i.e. efflux or xylem loading). A high affinity transport system BOR1 involved in the xylem loading has been identified by analysis of the *bor1-1* mutant, that is highly sensitive to B deficiency (Noguchi et al., 1997, 2000). BOR1 was identified as a membrane protein (Takano et al., 2002, 2005). Besides BOR1, NIP5;1 (NOD26-like intrinsic protein), a member of major intrinsic protein family has been identified (Takano et al., 2006). It was demonstrated to function as a boric acid channel for B uptake and, like BOR1, was found to be crucial for plant growth under B limitation in *Arabidopsis* (Takano et al., 2006). Recently, it was reported that BOR4, one of the six BOR1 paralogs, accumulates in the presence of a high B supply and it correlates with plant tolerance of B (Miwa et al., 2007).

In this paper, we present the results of our studies on boron-induced changes in hypocotyl elongation in *Arabidopsis* seedlings *in vitro* and the interaction between this micronutrient and light quality and irradiance. In our experiments we wished to determine if the responsiveness of *Arabidopsis* hypocotyls to the stimulatory as well as inhibitory effect of B could be modulated by light quality and irradiance. For the analyses, we used various well-characterized photomorphogenic mutants to determine which photoreceptors or other elements of light signalling pathways are involved in mediating responses to elevated B.

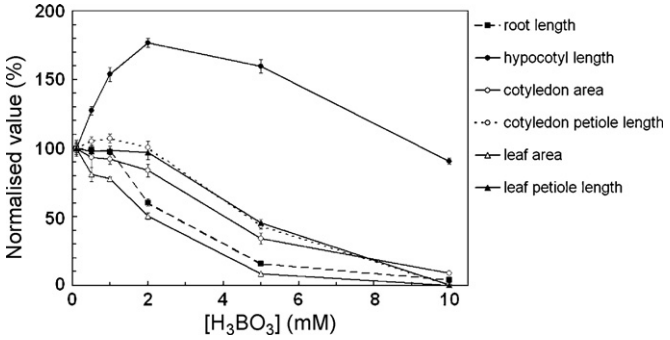

Fig. 1. Effect of B on growth of different organs in wild-type seedlings of *Arabidopsis thaliana* (Col-0) grown at irradiance 250 μmol m^{-2} s^{-1}. The results are mean values ± S.E. obtained in four independent experiments (n = 20 per each experiment). Results have been normalised to values obtained from plants grown at 0.1 mM B.

2. Materials and methods

2.1. Plant material

Experiments were conducted with *Arabidopsis thaliana* (L.) Heynh. ecotypes Columbia (Col-0; NASC stock code N1092), Landsberg *erecta* (L*er*; NW20), and Wassilewskija (Ws; N915). The following photomorphogenic mutants were also used in the experiments: *hy4-1* (*cry1*) (N70; generated by fast neutrons in L*er* ecotype; Ahmad and Cashmore, 1993), *cry2-1* (N3732; fast neutrons, Col-0; Koornneef et al., 1980; Lin et al., 1996), *hy1-1* (N67;

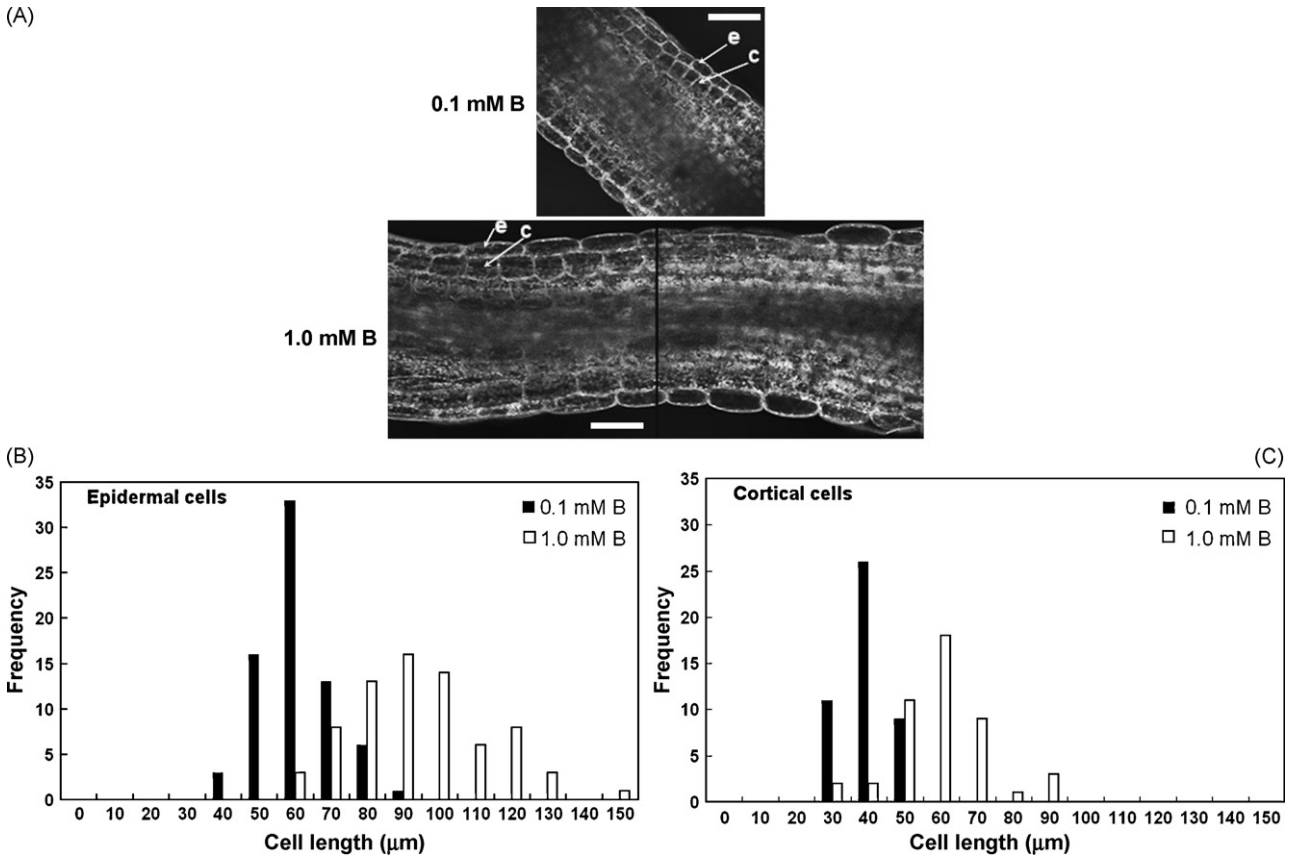

Fig. 2. Epidermal and cortical cells in *A. thaliana* hypocotyls, ecotype Col-0. (A) Longitudinal CLSM optical section (25 μm depth) of hypocotyls of 10-day-old seedlings grown on 0.1 and 1.0 mM B. The cortical (c) end epidermal (e) cell layers are indicated by arrows (scale bar for both sections 100 μm). Frequency distribution of cell lengths of the *Arabidopsis* seedlings grown at irradiance of 250 μmol m^{-2} s^{-1} on medium containing 0.1 mM (solid bars) and 1.0 mM (open bars) H_3BO_3. (B) Average length of epidermal cells was 55.0 ± 1.2 μm for hypocotyls grown on 0.1 mM B and 89.2 ± 2.2 μm on 1.0 mM B. (C) Average length of cortical cells was 34.9 ± 0.9 μm for hypocotyls grown on 0.1 mM B and 54.7 ± 1.8 μm on 1.0 mM B.

L*er*, mutagen not known; Koornneef et al., 1980), *hy2-1* (N68; EMS, L*er*; Koornneef et al., 1980), *phyA-205* (N6222; EMS, L*er*; Boylan and Quail, 1991), *phyB-1* (formerly *hy3-1*; N69; EMS, L*er*, Koornneef et al., 1980), *hy5-1* (N71; EMS, L*er*; Koornneef et al., 1980), *phot1* (formerly *nph1*; Liscum and Briggs, 1995), *phot2* (formerly *npl1*; Jarillo et al., 2001), double mutant *phot1phot2*. All *phot* mutants were generated in the genetic background *glabra-1* (*gl-1*; background Col-0). Seeds of accessions Col-0, L*er*, Ws, and of mutants *hy4-1*, *cry2-1*, *hy1-1*, *hy2-1*, *phyA-205*, *phyB-1* and *hy5-1* were ordered via TAIR (The *Arabidopsis* Information Resource, http://www.arabidopsis.org) and supplied by NASC (The Nottingham Arabidopsis Stock Centre; http://arabidopsis.info). Seeds of the *gl-1*, *phot1*, *phot2*, and *phot1phot2* were kindly provided by W.R. Briggs, Carnegie Institution of Washington, Stanford, CA, USA). Mutant *bor1-1* plants generated by T-DNA insertion in Col-0 accession (Takano et al., 2002) were kindly provided by J. Takano and T. Fujiwara from University of Tokyo.

2.2. Culture conditions

In vitro experiments were performed independently in two separate laboratories in the United Kingdom (further designated as UK lab) and in the Czech Republic (CZ lab) (see authors affiliations), in which slightly different methods for seedling growth were used. Results of experiments on white light presented in Figs. 1–3 were obtained mainly by the methods used in UK lab, while results presented in the other figures were obtained in CZ laboratory.

For sterile (*in vitro*) cultures, seeds were surface-sterilized with commercial bleach containing 1.6% (v/v) sodium hypochlorite and one drop of Tween 20 for 15 min, and rinsed five times with sterile distilled water. Sterilized seeds were arranged on culture medium containing MS salts (Murashige and Skoog, 1962), 1.5 or 3.0% (w/v) sucrose (also sugar-free MS medium was used to test possible effect of sucrose on the response to elevated B), 0.5 g l^{-1} MES and 1% Phytagar (CZ lab, Fluka, Buchs, Switzerland; UK lab, Sigma–Aldrich, Gillingham, UK). The pH was adjusted to 5.8 by 1 M KOH before autoclaving. Additional B (as boric acid) from a 0.5 M stock was added to produce final concentrations of 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 mM B. Seeds were stored at 5 °C for 3–5 days in distilled water before sowing to promote even germination. The seeds were then cultured and plants were grown in a temperature-controlled growth chamber (Microclima 1000, Snijders Scientific B.V., The Netherlands) at a temperature of 22–23 °C. The day length was 16 h. In some experiments (CZ lab), more uniform growth of the seedlings of specific mutants was achieved by allowing seeds to germinate on medium without supplemental B and immediately after germination, transferring to new MS medium with the required boric acid content. Basal MS medium (BM) normally contains 0.1 mM H_3BO_3, which is considered basic sufficient for plant growth as it corresponds to approximately 10 mg kg^{-1} B typically found in many soils. Seedlings were dispersed on square plastic Petri dishes (100 mm × 100 mm × 21 mm) in a 5 mm grid pattern to ensure even spacing. Each plate contained 4 or 5 rows with approximately 20 plants.

Petri dishes were vertically aligned (75° angle) and placed under white light (WL), blue light (BL), red light (RL), or kept in the dark. The position of the plates within the cabinet was randomised by rotation every 1–2 days. Irradiance was controlled by adjustment of the distance of the plants from the light source (CZ lab) or by using neutral density filter (UK lab).

In the experiments with plants grown under WL, 300 W ELH tungsten halogen lamps were used providing an irradiance up to 250 μmol m^{-2} s^{-1} and a high red (RL)/far-red (FR) ratio (2.9). For growth under BL or RL, white fluorescent tubes Philips TL-D 36W/54 were wrapped in filters. For BL treatments tubes were wrapped in one layer of blue Roscolux filter (#83; Roscolux, Hollywood Light Inc., USA) providing a Photon Flux Density (PFD) of 9 μmol m^{-2} s^{-1} (maximum irradiance at 420 nm). RL was provided by filtering light through one layer of red Roscolux (#27;) and provided a PFD of 4 μmol m^{-2} s^{-1} (max. irradiance at 660 nm). The PFD of the lights was measured with a portable spectroradiometer (model LI-1800; Li-Cor, Lincoln, NE, USA) calibrated by the Department of Biophysics at Palacky University in Olomouc at the start of the experiment.

2.3. Measurement of seedling growth

Ten days after sowing, the seedlings were straightened with forceps and the plates were placed in an overhead projector and projected. The junction between the hypocotyl and the root and the top of the seedlings was marked. Magnified images of petioles, hypocotyls, and roots were then measured to the nearest 0.1 mm with a ruler. Accurate measurements of cotyledon and leaf areas were performed by taking images of the seedlings by CCD camera. Pictures were than analysed by a computational image analysis system (CZ lab, Lucia 4.81 software, Laboratory Imaging™, Czech Republic; UK lab, Image Pro Plus, Media Cybernetics, Workingham, UK). Since the seedlings grown under BL, RL or in darkness were relatively long in the comparison with plants grown under WL, the hypocotyl length was measured in a simpler way. The seedlings were spread on a foil with a millimetre screen and length of hypocotyl and root was measured to the nearest millimetre.

2.4. Measurement of cell number and dimensions

The size and number of hypocotyl cells in epidermal and cortical layers were measured by confocal microscopy. Hypocotyls were excised from 10-day-old seedlings grown at 0.1 and 1 mM B and placed between two circles of glass fibre filter paper (Whatman GF/A, UK), moistened with distilled water. The filter paper was placed on top of several layers of tissue paper. Approximately 2 ml of 1% (w/v) periodic acid, 0.2% (w/v) Tween 20 solution was added to the upper filter paper disc and drawn through the sample by capillary action. Schiff's reagent (0.4% (w/v) Basic fuchsin, 13% (v/v) 1.0 M HCl, and 1% (w/v) $K_2S_2O_5$) was then applied to the samples in the same way. The stain was infiltrated into the sample by applying a gentle vacuum for 5 min. The sample was then washed five times with distilled water. Optical sections through the stained hypocotyls were obtained using a Zeiss laser scanning confocal microscope (LSCM Meta 510). Images were acquired using a 20× objective (Plan-Nefluor) with an excitation wavelength of 543 nm provided by an argon laser producing image stacks of dimension 461 μm × 461 μm × 50 μm at a resolution of 0.45 μm.

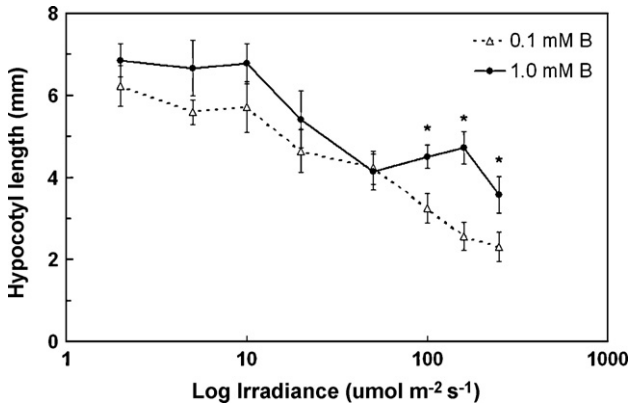

Fig. 3. The effect of B on the hypocotyl length of *Arabidopsis* (Col-0) seedlings grown under different irradiances of WL with 0.1 mM B (open symbols) or 1 mM B (filled symbols). The results are mean values ± S.E. ($n = 80$). *Denotes significant statistical difference ($P \leq 0.05$) from hypocotyls grown on 0.1 mM B.

Image stacks were inspected to identify epidermal and cortical cells and the length of individual cells measured using Image Pro Plus package (Media Cybernetics). For each individual hypocotyl a frequency distribution histogram of epidermal and cortical cell length was constructed. In total 20 hypocotyls were measured for each treatment.

2.5. Statistical analysis

The measurements were performed on at least 15 plants per experiment. Each experiment was repeated at least three times and the presented data are mean values ± S.E. Statistical significance of the treatment differences was assessed using Student's *t*-test. Analysis of measurements of leaf area, leaf petiole length and cotyledon petiole length was carried out on log-transformed data. All data collected were analysed using Microsoft Excel and Minitab version 13 software.

2.6. Analysis of gene expression

The expression of the *BOR1* gene (Takano et al., 2002, 2005) was determined by reverse transcription PCR (RT-PCR). Total RNA was extracted from the hypocotyls of 10-day-old seedlings grown in darkness, under BL or RL using RNeasy Plant Mini RNA kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. After washing RNA samples in RNeasy column by RW1 buffer during the purification procedure, the samples were treated by RNase-free DNase set (Qiagene Inc., Valencia, CA, USA) for 10 min. First-strand cDNA was synthesized from total RNA (1 μg) using Oligo(dT)20 with Thermoscript Reverse Transcriptase (InVitrogen Co., Carlsbad, CA, USA). The cDNA product was amplified by PCR. A PCR reaction of 30 cycles was performed by denaturing the template cDNAs at 95 °C for 2 min followed by cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min. A *BOR1* fragment (Arabidopsis *BOR1* gene accession no. AB073713) was amplified using oligonucleotides 5′-AATCTCGCAGCGGAAACG-3′ and 5′-TGGAGTCGAACTTGAACTTGTC-3′. Fragments of the housekeeping gene EF1α (accession no. NM100667) was amplified using primers 5′-CCTTGGTGTCAAGCAGATGA-3′ and 5′-TGAAGACACCTCCTTGATGATTT-3′. Fragments of the housekeeping gene UBQ10 (accession no. NM202787) was amplified using oligonucleotides 5′-GGAGGTGGAGAGTTCTGACA-3′ and 5′-AGACAAAGTGAAGTGTGGAC-3′. PCR products were size fractionated by electrophoresis in a 1% (w/v) agarose gel stained with ethidium bromide.

3. Results

3.1. Responses of Arabidopsis seedlings to excess of boric acid under white light

To examine the impact of elevated B on the growth and development of *Arabidopsis*, Col-0 seedlings were grown for 10 days under white light on differing concentrations of B and the development of different organs quantified. Fig. 1 shows the growth of each organ normalised to growth on 0.1 mM B. Different organs of the seedlings were differentially affected by elevated concentrations of B. Fig. 2 shows that true leaf and root growth were the most sensitive parts of seedlings to increasing concentrations of H_3BO_3 present in the culture medium. A significant reduction in true leaf area was evident at B concentrations of 0.5 mM and above. At concentrations above 2.0 mM H_3BO_3 the true leaves were not apparent anymore. Cotyledons (area and petiole length) and leaf petioles were less sensitive than true leaves to elevated B since they exhibited reduced growth only at concentrations above 2–5 mM B. Overt toxicity symptoms were apparent for all the organs at 10 mM H_3BO_3. Roots were feeble, practically not developing, and chlorophyll content decreased in cotyledons noticeably at elevated B. In contrast to the other organs of the seedlings, the growth of the hypocotyl was stimulated by elevated B over the range 0.5–5 mM B with inhibition only apparent at 10 mM B where overt toxicity symptoms became apparent (Fig. 1).

As hypocotyl elongation could result from an increase in cell division and/or cell expansion, CLSM of *Arabidopsis* hypocotyls were used to measure cell number and cell size in the epidermal and cortical layers of the hypocotyl. Fig. 2a shows optical longitudinal sections of hypocotyls of plants grown at 0.1 and 1 mM B and Fig. 2b shows the frequency distribution of epidermal and cortical cell lengths within these individual hypocotyls. Both the epidermal and cortical cells in the hypocotyl of plants grown at 1 mM H_3BO_3 were approximately 60% longer than those of plants grown at 0.1 mM H_3BO_3 (Fig. 2b). The increase in individual cell lengths was the same as that observed in the overall hypocotyl length (Fig. 1), thus cell expansion was sufficient to account for the increase in hypocotyl length without any cell division. No significant differences in total cell number per cell file (not shown) were found between seedlings grown on 0.1 and 1.0 mM B.

3.2. Response to elevated boron under different light conditions

Hypocotyls are extremely responsive to environmental signals, particularly light. To determine if the responsiveness of *Arabidopsis* hypocotyls to the stimulatory as well as inhibitory effect of B could be modulated by light quality and irradiance the interaction between B and light on hypocotyls elongation was investigated.

3.2.1. Hypocotyl growth responses to elevated boron under different irradiances

Arabidopsis seedlings (Col-0) were grown for 10 days under white light at irradiances ranging from 2 to 250 $\mu mol\ m^{-2}\ s^{-1}$ and the length of the hypocotyl measured. For seedlings grown with 0.1 mM B, increasing irradiance led to an inhibition of hypocotyl elongation (Fig. 3). The hypocotyls of seedlings grown at 1 mM B were longer than those grown at 0.1 mM B although the differences were only statistically significant at irradiances above 50 $\mu mol\ m^{-2}\ s^{-1}$ ($P \leq 0.05$).

3.2.2. Involvement of blue light receptors in B-induced stimulation of hypocotyl elongation

To determine which regions of the light spectrum are required for the boron-induced promotion of hypocotyl growth observed in white light, the hypocotyl elongation responses to elevated B were investigated for seedlings grown under blue light (BL) and red light (RL). To find out whether the promotion of hypocotyl elongation by B was a light-specific response, the growth of etiolated hypocotyls as a function of B concentration was also studied.

As shown in Fig. 4A, H_3BO_3 at concentrations from 1 to 3 mM promoted hypocotyl growth in L*er* (most of photomorphogenic mutant plants used in our experiments are of L*er* background) seedlings grown under BL, RL or in darkness. The maximum stimulation of hypocotyl elongation in BL and RL was induced by B at concentrations of 2 and 3 mM, respectively ($P \leq 0.05$) and hypocotyls were 26 and 24% longer than those of seedlings grown under the same illumination conditions on medium containing 0.1 mM B. B also promoted hypocotyl growth in etiolated seedlings but the responses were less pronounced. The hypocotyls grown on 3 mM B were approximately 15% longer than those grown with 0.1 mM B. Hypocotyl elongation was inhibited under all illumination conditions at B concentrations greater than 5 mM B. The hypocotyls of dark- and RL-grown seedlings supplied with 10 mM B were 20% shorter than those supplied with 0.1 mM B, and 35% shorter in BL-grown seedlings (Fig. 4A).

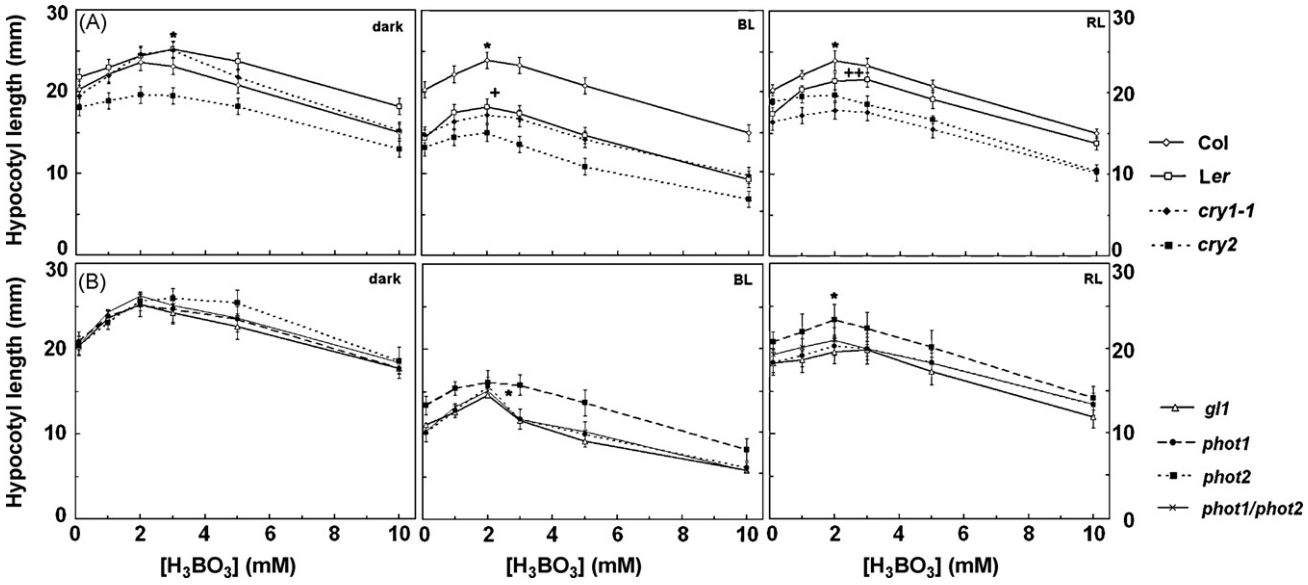

Fig. 4. (A) Response of *Arabidopsis* ecotypes Col-0, and derived mutant *cry2*; L*er* and derived mutant *cry1-1* to elevated B under blue light (BL), red light (RL) and in darkness. (B) Response of *Arabidopsis* single and double *phot* mutants and corresponding control genotype *gl1-1* to elevated B under blue light (BL), red light (RL) and in dark. In L*er* and Col-0 the results are mean values ± S.E. obtained in 16 independent experiments. The results further represent mean values ± S.E. obtained in 3 (*gl1* and *phot* mutants) or 6 (*cry1-1* and *cry2-1*) independent experiments (10 ten-day-old seedlings were measured in each experiment). $n = 15$ per each experiment. (A) *, +, and ++, significantly different ($P \leq 0.05$) from seedlings grown on 0.1 mM B. (B) *Significantly different ($P \leq 0.05$) from seedlings grown on 0.1 mM B.

To investigate the involvement of BL and RL photoreceptors in boron-induced hypocotyl elongation, mutants in genes coding for cryptochromes CRY1 and CRY2, phototropins PHOT1 and PHOT2, and phytochromes (or chromophore biosynthesis) were tested for responsiveness to elevated boron.

The *hy4-1* mutation (generated in L*er* background) results in defective photoreceptor CRY1 (Ahmad and Cashmore, 1993). As shown in Fig. 4A, the mutation in *CRY1* led to the elimination of the stimulatory effect of elevated B on hypocotyl growth in BL-, RL- and dark-grown seedlings (Fig. 4A). The mutant *cry2-1* was generated in the Col-0 ecotype. Like in L*er*, H_3BO_3 promoted hypocotyl growth in Col-0. The maximum stimulation was induced by B at concentrations between 1 and 2 mM. In etiolated hypocotyls, the maximum growth promotion was 18% at 2 mM H_3BO_3. Under BL and RL, the maximum degree of the hypocotyl growth promotion was 29% (at 1 mM). In RL, boron stimulated hypocotyl growth in Col-0 very weakly, i.e. in maximum by 10% at 2 mM H_3BO_3. From the statistical point of view the B effect was at the limit of significance. At the highest concentration tested (10 mM H_3BO_3), B inhibited hypocotyl growth. In dark-grown plants, the inhibition reached 26%, whereas in BL- and RL-grown plants it was 42 and 46%, respectively (Fig. 4A).

Like in Col-0, B stimulated hypocotyl elongation in dark-grown *cry2-1* mutant seedlings with a maximum stimulation of 29% at a concentration of 3 mM B. However, in contrast to wild-type Col-0, there was no significant B-induced hypocotyl elongation in the *cry2-1* mutant seedlings grown under BL. Like in BL, B did not promote hypocotyl growth in *cry2-1* mutants grown in RL (Fig. 4A).

Single mutants *phot1* and *phot2*, and double mutant *phot1phot2* were generated in the genetic background *glabra-1* (*gl-1*) mutant, which is a mutation in the Col-0 accession (W.R. Briggs, personal communication). We therefore used the *gl-1* mutation as a corresponding control for the *phot* mutants. In dark, H_3BO_3 at concentrations from 2 to 3 mM promoted hypocotyl growth in *gl-1* seedlings. A similar stimulation of hypocotyl elongation by elevated B was observed in BL-grown *gl-1* seedlings with the maximum stimulation at 2 mM H_3BO_3 but similarly like in Col-0 there was little stimulation observed in RL-grown plants. Under all illumination conditions tested, single mutants in the PHOT1 and PHOT2 photoreceptors, and the double mutant *phot1phot2* showed a similar responsiveness to elevated B to that observed in control *gl-1* genotype (Fig. 4B).

3.2.3. Role of phytochromes in B-induced stimulation of hypocotyl growth

We further studied the involvement of phytochrome and other elements of RL signalling pathways in boron-stimulated hypocotyl growth. All mutations *hy1-1*, *hy2-1*, *hy5-1*, *phyA-205* and *phyB-1* used in our experiments were generated in L*er* background.

The *hy1-1* mutant, defective in phytochrome chromophore biosynthesis (coding for heme oxygenase), showed a significant B-induced stimulation of hypocotyl elongation in dark-, BL- and RL-grown plants, similar to that observed in L*er* (Fig. 5A). In dark and RL, the maximum of growth promotion of *hy1-1* hypocotyls was observed at a concentration range between 3 and 5 mM H_3BO_3. Differently from L*er*, B-induced growth stimulation of hypocotyl growth in *hy1-1* under BL was observed only at narrow concentration frame, namely at a single concentration, 3 mM. Like in L*er*, inhibition of hypocotyl growth in mutant *hy1-1* at 10 mM H_3BO_3 reached similar extent in dark, BL and RL. The gene *HY2* codes for phytochromobilin synthase, and the *hy2-1* mutation results in seedlings with defects in chromophore biosynthesis. In our experimental conditions, B at concentration between 1 and 3 mM did not significantly promote hypocotyl elongation in the mutant seedlings grown in dark, BL or RL (Fig. 5A). However, whatever the light conditions, the *hy2-1* mutation resulted in a greater inhibition of hypocotyl growth at the highest B concentration (10 mM). In dark-, BL-, and RL-grown plants the inhibition reached approx. 30, 42 and 35%. To determine whether a downstream element of light signalling pathways may play a role in hypocotyl growth induced by boric acid, we tested growth responses to B in *Arabidopsis* seedlings with a mutation in the gene coding the transcription factor HY5. In darkness, hypocotyl growth in *hy5-1* mutant seedlings was not significantly stimulated by B in comparison with the corresponding control genotype L*er*. B-induced hypocotyl elongation in BL- and RL-grown plants with a maximum growth induction of 22 and 16%, respectively was observed at concentration 3 mM B. Elevated B, over 5 mM, had an inhibitory effect on hypocotyl growth similar to that

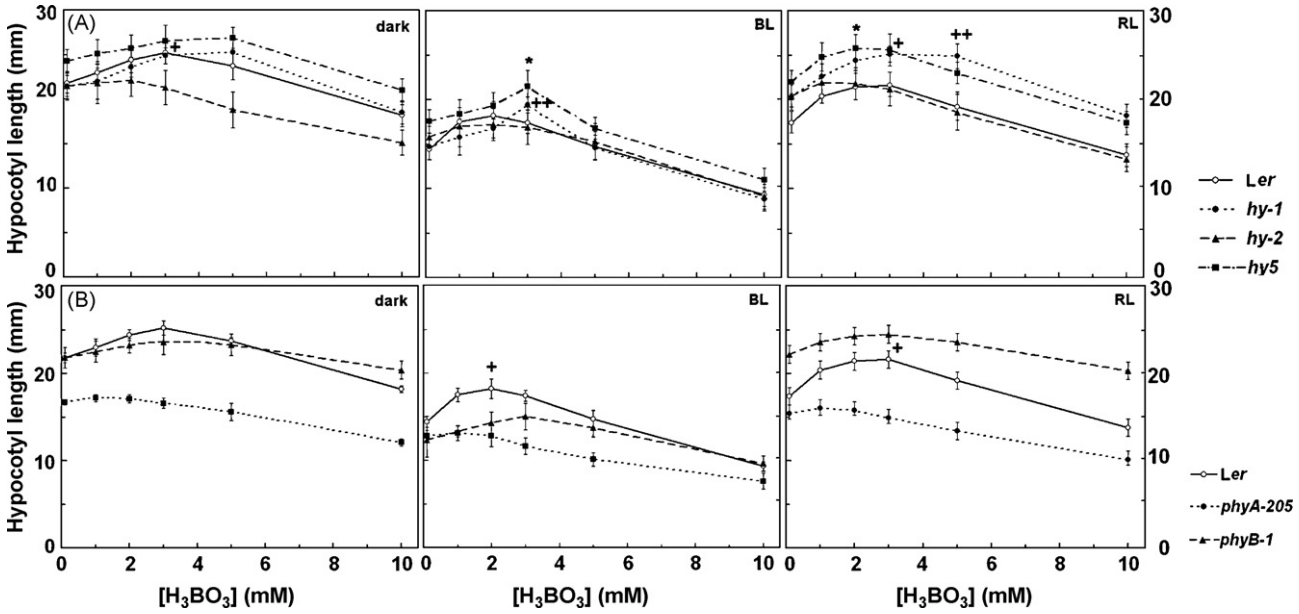

Fig. 5. Response of *Arabidopsis hy1-1*, *hy2-1* and *hy5-1* (A) and *phyA-205*, *phyB-1* (B) mutants and corresponding wild-type (*Ler*) to elevated B under blue light (BL), red light (RL) and in dark. The results are mean values ± S.E. obtained in 3 (*phyA-205*), 5 (*hy1-1*, *hy5-1*, *phyB-1*) or 7 (*hy2-1*); independent experiments ($n = 15$ per each experiment). (A) *, +, and ++, significantly different ($P \leq 0.05$) from seedlings grown on 0.1 mM B. (B) +, significantly different ($P \leq 0.01$) from mutant seedlings grown on 1 mM B.

observed in L*er*. Mutant hypocotyls were the least responsive to the inhibitory effect of 10 mM boric acid in the dark (inhibition approx. 14%), more in RL (21%), and they showed the highest responsiveness to H_3BO_3 in BL (38%) (Fig. 5A).

To further investigate the involvement of phytochrome in boron-induced hypocotyl elongation, we analysed hypocotyl growth responses in the mutants *phyA-205* and *phyB-1*. *PhyA* and *PhyB* mutants are deficient in *phyA*/*B* mRNA and apoprotein. The product of the phyA gene is light labile and predominates in etiolated seedlings, where it accumulates to relatively high levels. phyB is more light stable, predominating in light-grown tissues (Quail, 1994). In etiolated *phyB-1* seedlings, B at all concentrations tested did not stimulate hypocotyl elongation. In BL and RL, H_3BO_3 at concentration from 2 to 5 mM had a weak, but not significant, stimulatory effect on hypocotyl growth with a maximum at 3 mM. The *phyB-1* mutation further resulted in a reduced responsiveness of hypocotyl growth to inhibitory effects of B at the highest concentrations. In etiolated and RL-grown mutant plants boric acid at 10 mM had little or no effect on hypocotyl elongation. Inhibition of hypocotyl growth by boric acid at 10 mM was observed only in seedlings grown in BL (22%), which was however still distinctly lower than that observed in L*er* plants (35%) (Fig. 5B). The response of *phyA-205* mutation did not result in significant hypocotyl growth stimulation on B concentrations find to be positive for control L*er* plants under all light conditions tested. In dark-, BL-, and RL-grown plants the inhibition reached approx. 28, 41 and 35%.

The hypocotyl growth responses to boron in dark, BL and RL observed in all photomorphogenic mutants tested and corresponding ecotypes are summarized in Table 1.

3.3. Growth responses of bor1-1 mutant to elevated boron

Mutant *bor1-1* was generated by T-DNA mutagenesis in Col-0 background (Noguchi et al., 1997). In the greenhouse, plants of mutant *bor1-1* require treatment by boric acid to grow normally and to develop seeds. In our experimental conditions, i.e. on the basal MS medium containing 0.1 mM H_3BO_3 mutant seedlings elongated similarly like wild-type plants in darkness as well as in BL or RL (Fig. 6). *bor1-1* seedlings did not show any significant B-induced hypocotyl elongation when grown under B or RL and a very limited stimulation in etiolated seedlings grown at 2 mM B. The degree of inhibition observed at higher B concentrations was similar between WT and *bor1-1* plants under all growth conditions.

3.4. Expression of BOR1 in etiolated and light-developed hypocotyls

In other experiments we found that among *Arabidopsis* ecotypes, Wassilevskaja (Ws) shows the greatest differences between dark and light in responsiveness to the stimulatory as well as to the inhibitory effects of boron on hypocotyl growth (Fellner, unpublished results). We therefore investigated the effect of light and boron on the expression of gene *BOR1* in *Arabidopsis* hypocotyl. We found that in the hypocotyl of *Arabidopsis* ecotype Ws grown on basal MS medium (0.1 mM H_3BO_3) light upregulated the expression of the *BOR1* gene. In etiolated seedlings abundance of the *BOR1*

Table 1
Summary of hypocotyl growth responses to the effect of boron in all genotypes tested.

Genotype[a]	Dark	Blue	Red
Ler	+	+	+
hy4-1 (*cry1-1*)	–	–	–
hy1-1	+	+	+
hy2-1	–	–	–
phyB1	–	–	–
hy5-1	–	+	+
Col-0	+	+	±[b]
cry2-1	+	–	–
gl-1	+	+	–
phot1	+	+	–
phot2	+	+	–
phot1phot2	+	+	–

+: indicates presence of B-induced hypocotyl elongation; –: indicates absence of B-induced hypocotyl growth.

[a] Genotypes in bold indicate background of the mutants listed below.

[b] In Col-0, stimulation of hypocotyl elongation by B under RL was at the limit of the significance.

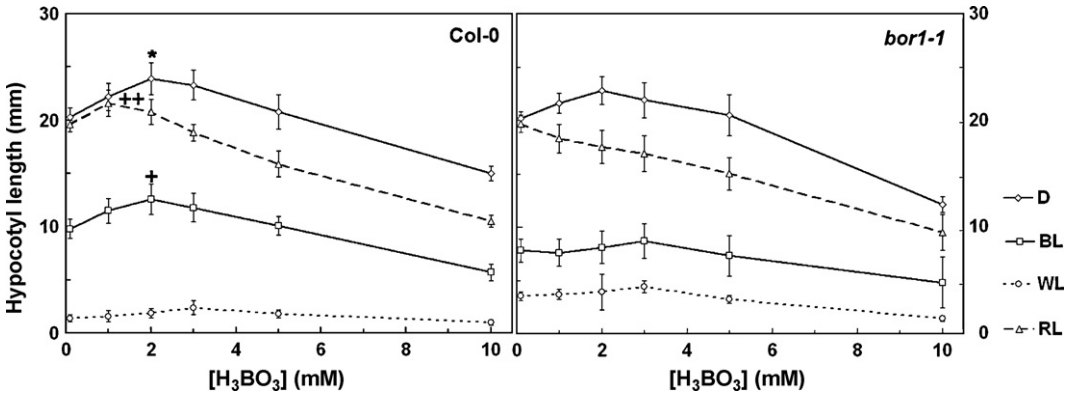

Fig. 6. The effect of B on the hypocotyl length of *bor1-1* mutant (A) and corresponding wild-type (Col-0) (B) grown in dark or under BL, RL. The results are mean values ± S.E. obtained in 4 (*bor1-1*) and 16 (Col) independent experiments (15 ten-day-old seedlings were measured in each experiment). *, +, and ++, significantly different ($P \leq 0.05$) from 0.1 mM boron in dark, BL and RL, respectively.

transcript was relatively low compared to that observed in BL- or particularly RL-grown plants (Fig. 7). A similar expression pattern was observed in hypocotyls of L*er* (Fig. 7B) but in Col-0 BL did not appear to induce *BOR1* expression. Interestingly, a marked upregulation of *BOR1* expression by BL and RL was observed in mutants *cry1-1* and *hy2-1* (Fig. 7B), but not in *cry2-1*. Interestingly, low level of *BOR1* transcript was still detected in *bor1-1* mutant, but the level was not altered by light (Fig. 7).

As mentioned above (Figs. 4 and 5) we found that in the dark the inhibition of hypocotyl growth by boron at the highest concentration tested (10 mM) is less than in BL- and RL-grown plants. To determine whether the effect of BL and RL on B-induced inhibition of hypocotyl correlated with the expression of *BOR1*, we investigated effect of 10 mM B boron on *BOR1* gene expression as a function of illumination conditions. Whatever the light conditions, incubation of L*er* seedlings in the presence of 10 mM H_3BO_3 led to marked increase in expression of *BOR1* in hypocotyls (Fig. 7B). However, in the ecotype Col-0 a marked B-induced expression of *BOR1* was observed in BL, whereas in dark or RL boron reduced gene expression (Fig. 7). Boron also distinctly reduced the expression of *BOR1* BL-grown hypocotyls of the *cry1-1* mutant, in etiolated hypocotyls of mutant *cry2-1*, and in RL-grown *hy2-1* seedlings. Interestingly, in the presence of 10 mM H_3BO_3 the expression of *BOR1* was barely detected (Fig. 7B).

4. Discussion

4.1. Responses of Arabidopsis plants to elevated B

Most essential micronutrient elements have a fairly narrow window for concentrations between optimal and toxic. Boron is no exception (Bennett, 1993; Goldberg, 1997). In fact, within the plant kingdom there is a range of sensitivity to B, depending on the concentration as well as the time and method of application (Nable et al., 1997). Most of the studies were done on barley (Nable et al., 1990; Mahboobi et al., 2000; Sutton et al., 2007) or other crop plants (Li et al., 2001; Stangoulis et al., 2001; Yu et al., 2002) focusing on B deficiency. Working with B deficient plants is technically quite difficult because plants require relatively little amount of B (Loomis and Durst, 1992). Therefore, we originally decided to investigate the effect of elevated B on model plant *A. thaliana* to contribute to elucidating the mechanism of B action on molecular level.

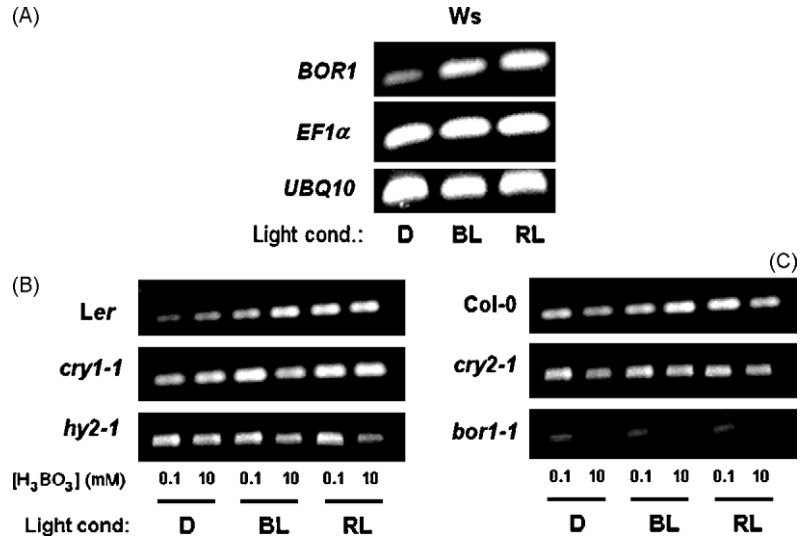

Fig. 7. Reverse transcription-PCR analysis of inducible expression of *BOR1* in *A. thaliana* hypocotyls in ecotype Ws (Wassilevskaja) (A), ecotype L*er* and derived mutants *cry1-1* and *hy2-1* (B), and ecotype Col-0 and derived mutants *cry2-1* and *bor1-1* (C). Seedlings were grown *in vitro* on the basal MS medium containing 0.1 mM or 10 mM H_3BO_3. 10-day-old seedlings were used to isolate RNA. One microgram of total RNA was used to synthesize cDNA. The synthesized cDNA was used for amplification of *Arabidopsis BOR1* (141 bp).

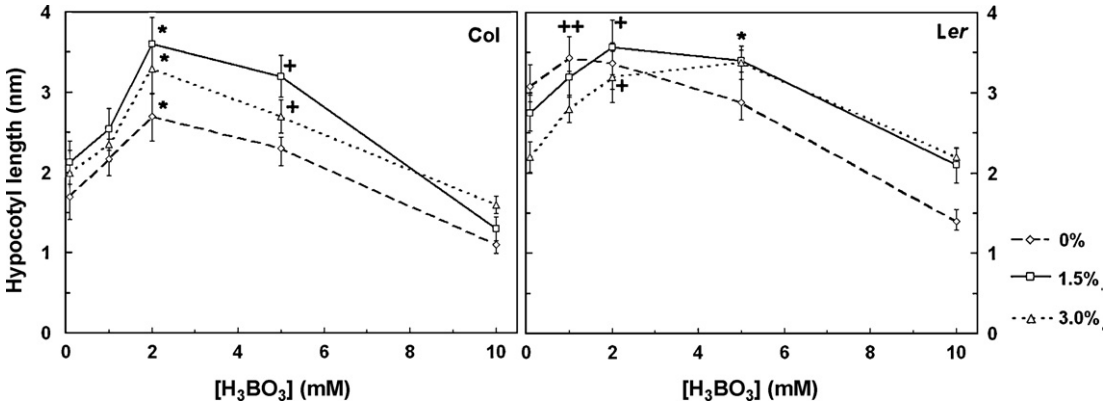

Fig. 8. The effect of sucrose concentration on the response of Col and Ler wt seedlings to elevated B under white-light (WL) at irradiance 180 μmol m^{-2} s^{-1}. The results are mean values ± S.E. obtained in 3 independent experiments (15 ten-day-old seedlings were measured in each experiment). *, +, and ++, significantly different ($P \leq 0.05$) from 0.1 mM B.

For plants grown in the white light, two responses of intact hypocotyl were observed. Addition of B to plant medium up to 5.0 mM stimulated growth of hypocotyls and also slightly cotyledon petioles whereas growth of other plant organs tested, i.e. leaf petioles, leaves and cotyledons, and roots, was inhibited (Fig. 2). The stimulation of hypocotyl growth was due to increased elongation of hypocotyl cells, and likely not increased cell division. *Arabidopsis* hypocotyl is relatively simple plant organ, which contains approximately 20 epidermal cells from top to base. Most of the cells are formed in the embryo and therefore hypocotyl grows mainly due to longitudinal expansion, while cell division occurs only exceptionally (Gendreau et al., 1997; Vandenbussche et al., 2005).

Fleischer et al. (1998) found that B functions as a stabilizer of the cell wall pectic network. From the opposite side, significantly reduced cell wall expansion was observed in B deficient plants (Dell and Huang, 1997). In our experiment, visible symptoms of B toxicity were apparent at concentrations above 2 mM B (except hypocotyls), similar to observations in other plant species (Karabal et al., 2003; Papadakis et al., 2004; Sutton et al., 2007).

Because growth of hypocotyl can be influenced by many endogenous and environmental signals and B is involved in sugar transport (Blevins and Lukaszewski, 1994) we have tested B response on media with different sucrose content (0; 1.5 and 3.0%, w/v) to find out if the sucrose can influence the response of the seedlings to elevated B concentrations. 3% of sucrose is considered as normal level for full MS medium. As seen in Fig. 8, the length of *Arabidopsis* hypocotyl was reduced on the sugar-free medium but the differences between hypocotyls grown on 1.5 and 3% sucrose did not differ significantly. The response to B-stimulation on 1–3 mM B was observed on all sucrose concentrations for each ecotype (59% on 0%; 71% on 1.5% and 55% on 3.0% sucrose in the medium).

4.2. Role of light in the responses to elevated B

Because we decided to study effect of B on plant growth as hypocotyl length changes, we have to calculate also with other factors affecting hypocotyl growth. These include temperature, humidity, nutrient availability, mechanical stress, seed size and phytochrome activity. Several of these factors interact with light signals (Casal et al., 2003). Reduction of elongation by light is one of the main photomorphogenic responses, which has been extensively studied on *Arabidopsis* hypocotyl (Vandenbussche et al., 2005). The fact that plant growth is dramatically promoted by B treatment in mutants highly sensitive to B deficiency (Noguchi et al., 1997; Takano et al., 2006) raises legitimate question of possible cross-talk between light and boron signalling pathways in the elongation process in *Arabidopsis* hypocotyl.

We have observed that the response to elevated B in white light becomes more marked as the irradiance increases (Fig. 3). The experiment performed at lower irradiances (below 50 μmol m^{-2} s^{-1}) showed that the response was lost or weakened at least. Higher irradiance increased stimulation effect of higher B concentration find to be optimal for hypocotyl growth. Following absorption of light, photoreceptors transfer the signal to other transduction elements that eventually lead to many molecular and morphological responses (Quail, 2002). While a complete signal transduction cascades are not known yet, molecular genetic studies on *Arabidopsis* have led to substantial progress in dissecting the signal transduction network (Fankhauser and Chory, 1997).

To elucidate how various parts of light spectrum are responsible for the dual boron-induced hypocotyl growth responses, and whether or not the hypocotyl responses to boron are light-specific, we investigated hypocotyl elongation as a function of boron concentration in dark and under blue light (BL) and red light (RL). Also, it is known that there is a wide variability in the tolerance of plants to high B, not only inter- but also intra-specifically (Carpena et al., 2000). We therefore decided to test several commonly used *Arabidopsis* accessions (ecotypes) to the responsiveness to B. This was reasonable since many mutants from collections available through the stock centres and other sources used in our analyses have different background.

We showed that the pattern of growth responses to elevated B was similar in all tested genotypes, i.e. stimulation of hypocotyl growth by boron at lower concentrations (usually from 1 to 3 mM B), and inhibition of elongation at the highest B concentration tested (above 5 mM). These data suggest that *Arabidopsis* ecotypes did not differ essentially in sensitivity to B. However, the ecotypes differed markedly in the extent of B-induced stimulation or inhibition of hypocotyl growth, relative to the growth on the basal MS medium containing 0.1 mM B. We further showed that B is able to promote essentially hypocotyl growth even in etiolated seedlings. Relative capacity of hypocotyl growth stimulation by boron in dark did not differ distinctly over the genotypes. However, the ability of boron to promote hypocotyl elongation in *Arabidopsis* accessions tested was significantly altered by BL and RL. Herein, it is necessary to point out that the BL and RL sources used in experiments described in this paper contain FR portion of the spectrum. If we think that PHYA mediated interaction with CRY1 occurs then the FR light in the spectra may be a issue. However, at present we use in our lab light sources free of FR portion, i.e. Philips TLD-36W/18-Blue and Philips TLD-36W/15-Red. We therefore checked B-induced growth responses in all experimental genotypes under the new BL and RL sources. We did not find any differences between plant responses

to boron under BL and RL containing FR portion and under BL and RL lacking FR part of the spectra (data not shown).

Although, it was not a goal of this paper to compare in details ability of *Arabidopsis* accessions to respond to B, it is interesting to mention that *Arabidopsis* ecotypes tested differed essentially in the sensitivity to light with respect to the capacity of boron-induced hypocotyl growth. In some ecotypes, BL and RL strongly amplified boron-induced stimulation of hypocotyl growth (L*er*). In other accessions, BL or RL interfere with the boron-induced hypocotyl growth. The inhibitory effect of B on hypocotyl growth was even intensified by BL and RL in all ecotypes at the highest concentration (10 mM). Interestingly, in the ecotypes where light intensified most the stimulatory effect of B on hypocotyl elongation, it intensified least the inhibitory effect of B at the highest concentration. In opposite, in the ecotypes showing weak stimulation of hypocotyl responses to boron, BL and RL intensified most the inhibitory effect of boron on hypocotyl growth. These data confirm revealing of natural variations between *Arabidopsis* ecotypes in many aspects of growth and development (Eichenberg et al., 2000; Maloof et al., 2001; Pepper et al., 2002; van Leeuwen et al., 2007; Filiault et al., 2008).

4.3. Responses of various photomorphogenic Arabidopsis mutants to elevated B

Blue light rapidly and strongly inhibits hypocotyl elongation during the photomorphogenic response known as de-etiolation (Cosgrove, 1981). A genetic approach consisting in isolation and characterization of photomorphogenetic mutants altered in their capacity to respond specifically to BL was used to define the role of blue light in boron-induced stimulation of hypocotyl growth. In *Arabidopsis*, the *hy4* mutant was isolated showing specific insensitivity to BL-induced inhibition of hypocotyl elongation (Koornneef et al., 1980). The analysis of the mutant revealed that gene *HY4* encodes a blue light receptor CRY1 controlling hypocotyl elongation in longer-term growth response to blue light in *Arabidopsis* (Ahmad and Cashmore, 1993; Lin et al., 1995). Unlike in L*er*, hypocotyl in mutant *hy4-1* in our experiments was not significantly stimulated by B in dark, BL or RL. The results suggest that functional photoreceptor CRY1 is involved in B-induced stimulation of hypocotyl growth. The results further indicate that the defect in CRY1 affects signalling pathway of RL involved in boron-induced stimulation of hypocotyl growth. It is not quite surprising since it was shown that CRY1 interacts with phytochrome A (Ahmad et al., 1998). Finally, the fact that mutation *hy4-1* impairs hypocotyl responses to boron in etiolated plants indicates that functional photoreceptor CRY1 is required for B-induced hypocotyl growth even in etiolated plants. In summary, our results suggest that functional protein CRY1 maintains the capacity of boron to stimulate growth in *Arabidopsis* hypocotyls. Interestingly, results of our experiments on *cry2* mutant indicate that CRY2 is involved in B-induced stimulation of hypocotyl elongation under blue, but not in dark and likely not in RL.

It was thought that CRY1 and CRY2 are the unique BL receptors involved in the control of hypocotyl elongation (Cashmore et al., 1999; Lin, 2000). However, it was demonstrated that BL-mediated growth inhibition in *Arabidopsis* is also mediated by phototropins (Parks et al., 1998; Folta and Spalding, 2001). Our analysis showed that single mutants *phot1* and *phot2*, and double mutant *phot1phot2* and corresponding control plants *gl-1* responded similarly to the elevated boron, i.e. by essential stimulation of hypocotyl elongation at 2 mM B in dark and BL, but not in RL. Our data indicate that in BL and in dark photoreceptors *PHOT1* and *PHOT2* do not participate in boron-induced hypocotyl growth.

Light perception by the plant photoreceptor phytochrome requires the tetrapyrrole chromophore phytochromobilin (PΦB), which is covalently attached to a large apoprotein. *Arabidopsis* mutants *hy1* and *hy2*, which are defective in PΦB biosynthesis, display altered responses to light due to a deficiency in photoactive phytochrome. The *Arabidopsis HY1* gene encodes a plastid heme oxygenase necessary for synthesis of biliverdin from heme, and thus consequently for biosynthesis of phytochrome chromophore (Muramoto et al., 1999). The *HY2* gene encodes phytochromobilin synthase, a ferredoxin-dependent biliverdin reductase responsible for the next and final step in biosynthesis of phytochrome chromophore from biliverdin in plastids (Kohchi et al., 2001). Our analysis of *hy1-1* and *hy2-1* responsiveness to elevated B revealed that whatever the light conditions, functional PΦB synthase coded by *HY2*, but surprisingly not heme oxygenase coded by *HY1*, is required for *Arabidopsis* hypocotyl growth induced by elevated B. At present, *hy1-1–hy2-1* discrepancies are very difficult to explain.

Chromophore phytochromobilin PΦB is synthesized in plastid compartment and then released to the cytosol, where apoprotein is synthesized. PΦB is then covalently attached to large apoprotein, and the assembly results in functional holoprotein or phytochrome (Terry et al., 1993). Mutant *phyB-1* in *Arabidopsis* has defect in *PHYB* coding for apoprotein PhyB and shows no de-etiolation under RL. In our experiments, in comparison with corresponding wild-type of ecotype L*er*, *phyA-205* as well as *phyB-1* lack essential boron-induced stimulation and inhibition of hypocotyl elongation in dark and shows just negligible promotion effect of B on hypocotyl growth in RL. Our data indicate that functional PhyA/B is involved in B-induced stimulation of hypocotyl growth, and further suggest that both, apoprotein and chromophore phytochromobilin are required for the response.

Transcription factor HY5 acts downstream of multiple families of the photoreceptors and promotes photomorphogenesis, and it is responsible for regulation of fundamental developmental processes of the plant cell, such as cell elongation and proliferation, and chloroplast development (Oyama et al., 1997). Our results showed that in BL and RL hypocotyl in mutant *hy5-1* responds to elevated B more or less similarly like the hypocotyl in WT seedlings. The data suggest that B-induced stimulation of hypocotyl elongation in BL and RL can be controlled by early elements of light signalling pathways, i.e. photoreceptors, but that functional transcription factor HY5 is not involved in the signalling pathway of B-induced stimulation of hypocotyl elongation. The fact that in dark the defect in transcription factor HY5 results in reduction of B-induced hypocotyl elongation suggests that mutated HY5 is not properly degraded and somehow interferes with B-induced hypocotyl growth. In other words, this suggests that proper degradation of HY5 in dark by ubiquitination process allows hypocotyl to elongate in response to boron.

4.4. Expression studies of BOR1 in Arabidopsis hypocotyls

Our expression analysis showed that in hypocotyls of wild-type seedlings grown on the basal medium (0.1 mM H_3BO_3) *BOR1* is essentially upregulated by BL and RL, whereas mRNA level of *BOR1* was low in dark-grown plants. It could possibly explain greater sensitivity of hypocotyls in BL- and RL-grown plants to the inhibitory effect of elevated boron. The fact that blue and red light induces expression of *BOR1* gene in *cry1* mutant indicates that it could likely happen via phytochromes. It was supported by the fact that the light-induced expression of *BOR1* was not detected in mutant *hy2-1*.

It was reported that under high B conditions, BOR1 protein (but not *BOR1* transcript) is degraded via endocytosis (Takano et al., 2005), and overexpression of BOR1 does not improve plant growth in the presence of toxic levels of B (Miwa et al., 2006, 2007). We found that in BL boron at 10 mM induces expression of *BOR1* in ecotypes L*er* and Col. Interestingly, the upregulation of *BOR1* by high boron was not observed in either photoreceptor mutant. In opposite, in *cry1-1* and *hy2-1* elevated boron essentially reduced

expression of *BOR1* in BL and RL, respectively. These data indicate that defects in cryprochromes and phytochromes alter B-mediated expression of *BOR1* in hypocotyls. Our analysis of *BOR1* expression in high boron requiring mutant *bor1-1* (Noguchi et al., 1997; Takano et al., 2002) showed that *BOR1* transcript can be still detected at very low level, but it could not be regulated by light. Interestingly, the expression of *BOR1* was impaired in seedlings developed at 10 mM H_3BO_3.

However, our data also indicate that expression pattern of *BOR1* does not often correlate with hypocotyl growth responses and cannot explain boron-induced stimulation or inhibition of hypocotyl elongation. For example, hypocotyls of *bor1-1* mutant with very low expression of *BOR1* still show high sensitivity to the inhibitory effect of elevated boron. Our results strongly suggest that boron and light signalling pathways interact during plant growth and development, and that cryptochromes and phytochromes are involved in the B-induced hypocotyl elongation. However, more analysis are needed to understand how boron and light signalling pathways interact in hypocotyl elongation. Since both, light and boron can upregulate expression of *BOR1* in WT plants, and this regulation is altered in cryptochome, phytochrome, and boron transporter mutants, we could speculate that the light and boron regulate expression of *BOR1* via an element common in both signalling pathways.

5. Conclusion

In this paper we showed that boron at elevated concentrations induces dual hypocotyl responses. It stimulates hypocotyl elongation at concentration approx. from 1 to 3 mM, but it has toxic effect on hypocotyl growth at concentrations higher than 5 mM. The stimulation of the elongation by elevated B is due to increased cell expansion and it was proportionally more obvious with increasing irradiance. Blue and red light did not alter the sensitivity of *Arabidopsis* hypocotyls to boron, but the lights can change capacity of boron-induced hypocotyl elongation. Our results suggest that boron and light signalling pathways interact during plant growth and development, and that cryptochromes, phytochromes, and boron transporter BOR1 are somehow involved in the B-induced hypocotyl elongation.

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