Plant Growth Regul (2012) 66:293–301
DOI 10.1007/s10725-011-9646-2

BRIEF COMMUNICATION

Boron and blue light reduce responsiveness of *Arabidopsis* hypocotyls to exogenous auxins

Štěpán Stočes · Marie Karlická · Martin Fellner

Received: 4 August 2011 / Accepted: 20 December 2011 / Published online: 4 January 2012

Abstract We reported earlier that boron stimulates hypocotyl growth in several *Arabidopsis* ecotypes but not in the boron-deficient mutant *bor1-1*. Others have shown that boron influences the metabolism and transport of the plant hormone auxin. We investigated how boron, in interaction with light, influences *Arabidopsis* hypocotyl growth responses to the exogenous auxins 1-NAA, 2,4-D and IAA. In either light condition, 1-NAA similarly inhibited hypocotyl growth in *bor1-1* and the corresponding WT (Col-0), while in both genotypes, boron did not essentially affect the extent of the inhibition. Whatever the light conditions and in the absence of boron, 2,4-D inhibited hypocotyl elongation in WT, while in BL seedlings, high responsiveness to 2,4-D vanished when boron was added to the culture medium. Hypocotyl of *bor1-1* seedlings in all boron concentrations tested and grown in the dark or RL responded to the auxin similar to WT plants. In BL, the mutant hypocotyls retained full sensitivity to 2,4-D at 0.1 mM H_3BO_3 but lost that sensitivity by 2 mM. In both genotypes tested, in the dark or RL, IAA inhibited hypocotyl growth. Conversely, IAA stimulated hypocotyl elongation in both genotypes developed in BL at 0.1 mM H_3BO_3. That stimulation disappeared when the boron supply increased to 2 mM. Our results suggest that specifically in BL, boron reduces hypocotyl responsiveness to auxins 2,4-D or IAA via the functional transporter BOR1. Our results lead to a discussion of how BL and BOR1 influence the mechanisms of auxin transport into and out of the cell.

Keywords *Arabidopsis thaliana* · Auxin · Boron · BOR1 · Hypocotyl · Light

Abbreviations

BL	Blue light
1-NAA	1-Naphthalene acetic acid
IAA	Indole-3-acetic acid
RL	Red light
WL	White light
WT	Wild-type
2,4-D	2,4-Dichlorfenoxyacetic acid

Introduction

Boron is an essential microelement for plant growth and development (Warington 1923). In vascular plants, boron 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). At high concentrations, boron is transported by passive diffusion (Dordas and Brown 2000), whereas under low boron conditions plants need an active transport mechanism. Using the *Arabidopsis* mutant *bor1-1* that normally suffers from boron insufficiency, dwarf growth and female sterility (Noguchi et al. 1997), a high affinity transport membrane protein BOR1 was identified (Takano et al. 2002, 2005). In addition to BOR1, NIP5;1, BOR4, and NIP6;1 protein transporters have been identified (Takano et al. 2006; Miwa et al. 2006; Tanaka et al. 2008; reviewed by Takano et al.

Š. Stočes · M. Karlická · M. Fellner (✉)
Laboratory of Growth Regulators, Group of Molecular Physiology, Palacky University in Olomouc and Institute of Experimental Botany, Academy of Science of the Czech Republic, v.v.i., Šlechtitelů 11, 783 71 Olomouc, Czech Republic
e-mail: martin.fellner@upol.cz

2008 and by Robert and Friml 2009). Along with the role in plant growth and development, boron is considered to be involved in cell wall synthesis and structure (reviewed in Blevins and Lukaszewski 1998; Brown et al. 2002; Bolaños et al. 2004). Most boron in plants is fixed in the cell wall, where it forms an important crosslink between rhamnogalacturonan (RG-II) dimers (O'Neill et al. 1996). It was documented that nutritional signaling interacts with signaling pathways of hormones to coordinate plant growth (reviewed by Krouk et al. 2011). Recently, Martín-Rejano et al. (2011) showed that a low boron supply increases the activity of the auxin reporter DR5-GUS in *Arabidopsis* primary roots and it correlated with an increased root inhibition. The authors proposed a hypothetical model showing that a low boron condition increases auxin synthesis in the primary root meristematic region and it is transported (via AUX1) to the elongation zone where the auxin accumulates and induces local auxin responses that inhibit root cell elongation. The results are in agreement with the observations of others who showed that boron is involved in auxin metabolism and transport (Eaton 1940; Dyar and Webb 1961; Tang and Dela Fuente 1986a, b; Brown et al. 2002; Wang et al. 2006).

Although boron is essential for plant cells, it can be toxic in higher concentrations. The range between boron deficiency and toxicity is very small. We previously showed that at high concentrations (above 5 mM) a clear toxicity effect of boron on hypocotyl growth was apparent, whereas at concentrations between 1 and 3 mM H_3BO_3, hypocotyl elongation was stimulated in all *Arabidopsis* ecotypes tested. The capacity of stimulation was however light-dependent. In addition, we showed that in *Arabidopsis* mutants *bor1-1* and *cry1-1* (*hy4*), boron cannot induce hypocotyl elongation (Kocábek et al. 2009).

The effect of auxin on plant growth is often studied by the application of exogenous auxin to experimental plants. In intact seedlings and on a longer time scale, exogenous auxin (native or synthetic) often reduces the elongation of various plant organs (Boerjan et al. 1995; King et al. 1995; Thomine et al. 1997; Fellner et al. 2003, 2006). In *Arabidopsis*, we similarly observed that as in the case of boron, auxin-induced inhibition of hypocotyl growth is affected by blue light BL (Fellner, unpublished results). Therefore, here we wanted to investigate how the interaction of light with boron influences *Arabidopsis* hypocotyl growth responses to exogenous auxins.

Materials and methods

Experiments were conducted with *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia (Col-0) and the high boron requiring mutant *bor1-1* (generated by T-DNA insertion in Col-0 accession; Noguchi et al. 1997; Takano et al. 2002). Seeds of Col-0 were ordered via TAIR and were kindly provided by NASC (The Nottingham *Arabidopsis* Stock Center—http://nasc.nott.ac.uk). Seeds of the mutant *bor1-1* were kindly provided by J. Takano and T. Fujiwara from University of Tokyo.

For sterile (in vitro) cultures, seeds were stratified for 5 days in distilled water at the temperature 4°C. Afterwards, the seeds were soaked for 15 min in Savo® original solution (~1.5% sodium hypochlorite; Bochemie, s.r.o, Czech Republic) supplemented with a drop of Tween®20 (Calbiochem, USA), and then rinsed extensively with sterile distilled water. Seeds were sown and germinated on 0.7% (w/v) agar basal medium (BM) containing Murashige and Skoog salts (Murashige and Skoog 1962), 1% (w/v) sucrose and 1 mM MES (2-(N-morpholino)-ethanesulfonic acid) (pH adjusted to 6.1 by KOH before autoclaving). The Petri dishes with the seeds were sealed with microporous surgical tape, placed in a temperature-controlled growth chamber (Microclima 1000, Snijders Scientific B.V., The Netherlands) and incubated in the dark at 23°C. The BM normally contains 0.1 mM boric acid (H_3BO_3), which is considered basically sufficient for plant growth as it corresponds to approximately 10 mg kg^{-1} B typically found in many soils. After the beginning of germination (3rd day from sowing), seeds were transferred onto a new BM with the tested auxin concentrations (0, 10^{-7}, 10^{-6}, 5×10^{-6} and 10^{-5} M) and two boron concentrations (0, 0.1 or 2 mM). Three auxins were used in our experiments: 2,4-dichlorfenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA) or 1-Naphthalene acetic acid (1-NAA). Petri dishes with the germinating seeds were placed vertically into growth chambers with blue light (BL), red light (RL) or in the dark and incubated for 10 days at 23°C. In experiments with photo-labile IAA, germinating seeds were transferred onto medium supplemented with IAA and incubated as described above. At the same time seedlings were transferred onto IAA-free medium every other day and incubated in the same manner. BL (maximum irradiance at 460 nm) and RL (maximum irradiance at 660 nm) were provided by blue (Philips TLD-36 W/18-Blue, Phillips, USA) and red (Philips TLD-36 W/15-Red, Phillips, USA) fluorescent tubes, respectively. The total photon fluence rates of BL as well as RL was 10 μmol m^{-2} s^{-1}. 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. After a 10-day-incubation, the hypocotyl length was measured with a ruler to the nearest millimeter. Changes in growth (i.e. inhibition or stimulation) caused by exogenous auxin (2,4-D, IAA or NAA) in the individual genotypes were expressed in percents based on the following formula: $X = 100 \times (A - B)/A$, where

"X" is the change in growth (in %), "A" and "B" stand for growth (in mm) in the absence and presence, respectively, of the exogenous auxin. When necessary, the statistical significance of the treatment differences was assessed using Student's *t* test.

Results

To determine the effects of boron and light on hypocotyl sensitivity to exogenous auxin, seedlings of Col-0 (WT) and the high boron requiring mutant *bor1-1* were transferred onto MS medium supplemented with boric acid (0.1 or 2 mM) or onto MS medium without boric acid supplement. Simultaneously, auxin 2,4-D, IAA or 1-NAA (10^{-7}–10^{-5} M) was added to the medium. Seedlings were incubated for 10 days in the dark or in BL or RL. Hypocotyl growth of the seedlings was measured as described above.

Effect of boron and light on hypocotyl growth responses to 2,4-D

On the basal medium free of boron and auxin, BL or RL inhibited hypocotyl growth in Col-0 as well as the mutant *bor1-1* (Fig. 1a). When the medium was supplemented with auxin 2,4-D but still lacked boric acid, elongation of etiolated or RL-grown hypocotyls in both genotypes was strongly inhibited in a concentration-dependent manner. We further observed that the inhibitory effect of auxin on WT and mutant hypocotyl growth was much reduced in seedlings developed under BL conditions (Fig. 1a). When H_3BO_3 at the concentration of 0.1 mM was present in the medium (Fig. 1b), 2,4-D still markedly inhibited hypocotyl elongation in WT and mutant seedlings developed in the dark or in RL, although the inhibitory effect of auxin was less than in seedlings developed in the absence of boron. The inhibitory effect of 2,4-D on hypocotyl growth

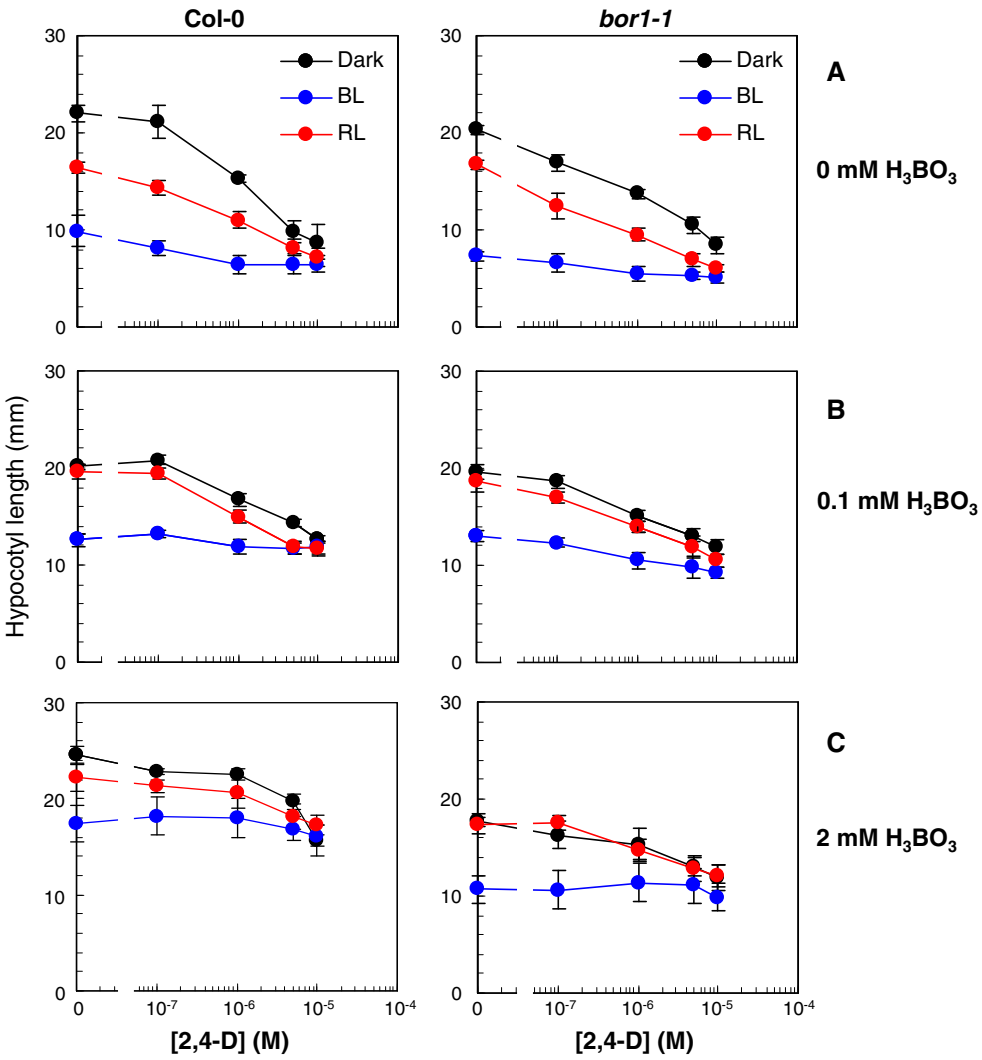

Fig. 1 The effect of auxin 2,4-D on the hypocotyl length of *bor1-1* mutant (*right*) and corresponding WT (Col-0) (*left*) in the dark, BL or RL and in the absence of boron (**a**) or in the presence of 0.1 mM (**b**) or 2 mM boron (**c**). The results are the mean values ± SE obtained in four independent experiments for each genotype, each boron concentration and each light condition (10–15 ten-day-old seedlings were measured in each experiment)

disappeared in WT seedlings grown under BL, whereas hypocotyls of *bor1-1* seedlings grown in BL conditions kept a high sensitivity to the inhibitory effect of 2,4-D (Fig. 1b). Finally, the sensitivity of WT hypocotyls to 2,4-D was strongly reduced when the seedlings developed on the medium supplemented with 2 mM H_3BO_3 (Fig. 1c). The most evident lack of responsiveness to auxin was observed for hypocotyls developed under BL conditions. Interestingly, boron at the concentration of 2 mM suppressed sensitivity to 2,4-D not only in WT, but also in the hypocotyls of *bor1-1* seedlings (Fig. 1c). The inhibitory effect of the selected 2,4-D concentration (5×10^{-6} M) on hypocotyl elongation in both genotypes grown in the absence or presence of H_3BO_3 is illustrated on Fig. 2. The figure particularly and clearly illustrates that in contrast to WT, hypocotyl in *bor1-1* seedlings developed in BL conditions does not change its response to the auxin 2,4-D when 0.1 mM of boric acid is applied, but it does when seedlings developed in the presence of 2 mM of boric acid.

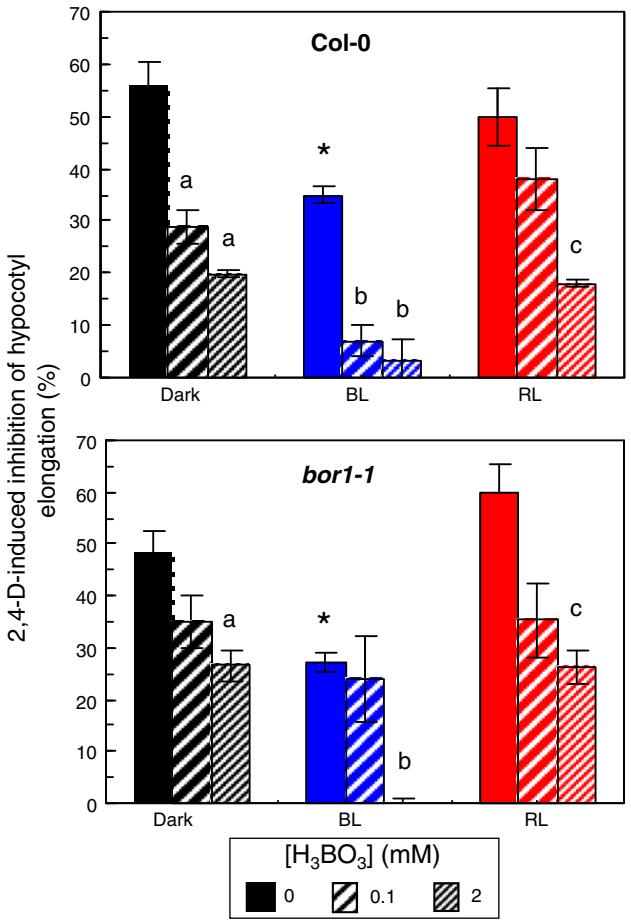

Fig. 2 The inhibition of hypocotyl elongation by 5×10^{-6} M 2,4-D as a function of boron concentrations in the culture medium. The inhibition was calculated as described in the "Materials and methods" section. The results represent the mean inhibition values ± SE obtained in four independent experiments for each genotype, each boron concentration and each light condition. *a*, *b* and *c*, significantly different ($P \leq 0.05$) from the corresponding controls (0 mM H_3BO_3); * significantly different ($P \leq 0.05$) from the controls in the dark and RL

Role of boron and light in hypocotyl growth responses to IAA

Similar to the case of the synthetic auxin 2,4-D, the natural auxin IAA added to the growth medium inhibited hypocotyl growth in WT and the mutant seedlings developed in the dark or RL. Conversely, in BL conditions, IAA distinctly stimulated hypocotyl growth in WT and did not have a significant effect on hypocotyl elongation in *bor1-1* plants (Fig. 3a–c). Different from 2,4-D, the inhibitory effect of IAA on WT and mutant hypocotyl growth was not markedly affected by the tested boron levels when seedlings developed in the dark or RL. However, in BL conditions, increasing boron concentrations caused the disappearance of the IAA-induced stimulation of hypocotyl growth in WT. In fact, this led to an increase of IAA-induced hypocotyl growth in mutant *bor1-1* seedlings (Fig. 3a–c). Figure 4 clearly demonstrates that in BL conditions, boron has the opposite effects on the sensitivity to 5×10^{-6} M IAA of WT and *bor1-1* hypocotyls.

Hypocotyl growth responses to 1-NAA

Like 2,4-D, the exogenous auxin 1-NAA inhibited the elongation of hypocotyls in *Arabidopsis* seedlings in a concentration-dependent manner (data not shown), and in WT as well as in *bor1-1* the inhibitory effect was much less when seedlings developed in BL conditions (Fig. 5). However, unlike 2,4-D and IAA, and in either light condition, hypocotyl sensitivity to 1-NAA was not affected by the level of H_3BO_3 in the culture medium. Figure 5 shows the inhibition of WT and *bor1-1* hypocotyl growth caused by 5×10^{-6} M 1-NAA at the different boron concentrations and light conditions tested.

Discussion

We previously reported that boron at high concentrations (above 5 mM) has a strong toxic effect on the growth of *Arabidopsis* hypocotyl. 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 (Kocábek et al. 2009). We also showed that BL and RL did not alter the sensitivity of *Arabidopsis* hypocotyls to boron, but dependent on the genotype, BL and RL increased or reduced the capacity of boron to induce hypocotyl growth. Our previous analyses of the *bor1-1* mutant indicate that the boron transporter

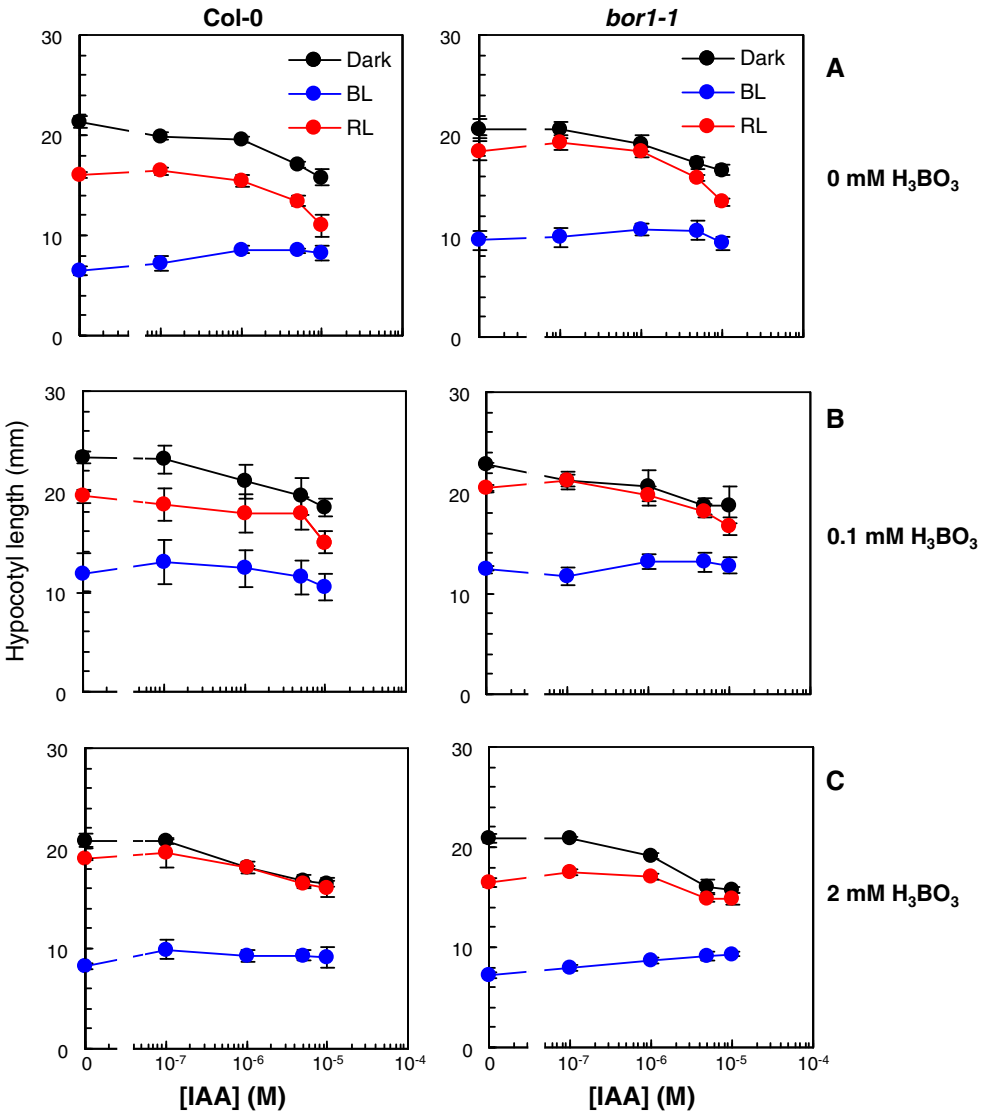

Fig. 3 The effect of auxin IAA on the hypocotyl length of *bor1-1* mutant (*right*) and corresponding WT (Col-0) (*left*) in the dark, BL or RL and in the absence of boron (**a**) or in the presence of 0.1 mM (**b**) or 2 mM boron (**c**). The results are the mean values ± SE obtained in three independent experiments for each genotype, each boron concentration and each light condition (10–15 ten-day-old seedlings were measured in each experiment)

BOR1 mediates the stimulation of hypocotyl growth by boron (Kocábek et al. 2009).

It is well known that plant growth and development is controlled not only by external signals, such as nutrient availability and by light, but also internal factors, such as plant hormones. Thus, it is not surprising, and it has been documented that nutritional and hormonal signaling pathways are coordinated to tune plant growth (reviewed by Krouk et al. 2011). Concerning this, it has been demonstrated that boron could influence metabolism and the transport of the auxin IAA (Eaton 1940; Dyar and Webb 1961; Tang and Dela Fuente 1986a, b; Brown et al. 2002; Wang et al. 2006; Martín-Rejano et al. 2011; reviewed by Blevins and Lukaszewski 1998), while the mechanisms have remained unclear.

Earlier, we found that the auxin-induced inhibition of *Arabidopsis* hypocotyl growth is affected by BL (Fellner, unpublished results), as similarly observed for boron. In the present work, we show that the elongation of wild type *Arabidopsis* hypocotyl is inhibited by the exogenous auxins 2,4-D, 1-NAA or IAA (10^{-7}–10^{-5} M), while the inhibitory effect of auxin was much less in BL than in the dark or RL. Moreover, in BL (10 μmol m^{-2} s^{-1}) IAA did not inhibit but on the contrary stimulated hypocotyl elongation (e.g. 5×10^{-6} M; Figs. 3a, 4). Consistent with our observation, Keuskamp et al. (2011) recently showed that IAA itself (15×10^{-6} M) (or in combination with brassinosteroids) stimulated elongation of Col-0 hypocotyl in low blue light conditions (25 μmol m^{-2} s^{-1}). Similarly in *Lectuca sativa*, IAA with GA_3 added to culture medium were able to eliminate partially the inhibition of hypocotyl elongation by low blue light (7.2 μmol m^{-2} s^{-1}) (Volmaro et al. 1998). Differential seedling responses to auxin in BL conditions could be explained for example by the fact that BL through photoreceptor cry1 may repress sensitivity to, or levels of, auxin in *Arabidopsis* seedlings (Folta et al. 2003).

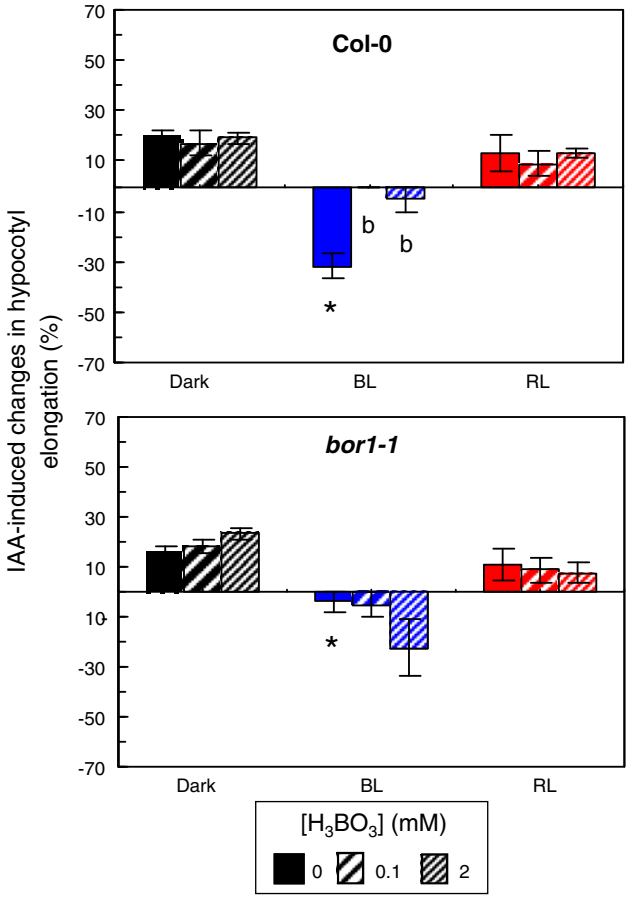

Fig. 4 The inhibition or stimulation of hypocotyl elongation by 5×10^{-6} M IAA as a function of boron concentrations in the culture medium. The inhibition was calculated as described in "Materials and methods" section. The results represent mean inhibition values ± SE obtained in four independent experiments for each genotype, each boron concentration and each light condition. *b* significantly different ($P \leq 0.05$) from the the corresponding control (0 mM H_3BO_3); * significantly different ($P \leq 0.05$) from the controls in the dark and RL

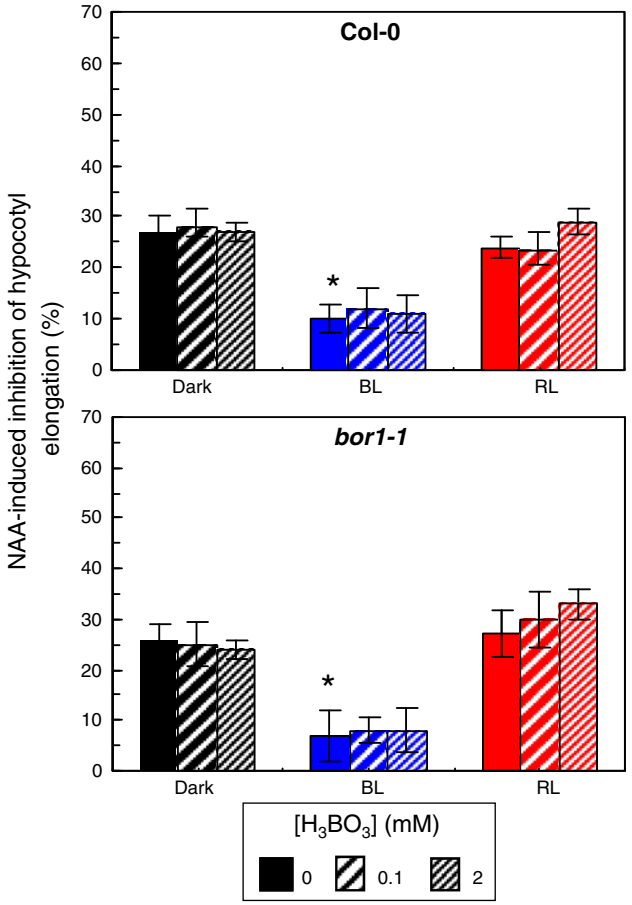

Fig. 5 The inhibition of hypocotyl elongation by 5×10^{-6} M 1-NAA as a function of boron concentrations in the culture medium. The inhibition was calculated as described in "Materials and methods" section. The results represent mean inhibition values ± SE obtained in four independent experiments for each genotype, each boron concentration and each light condition. * significantly different ($P \leq 0.05$) from the controls in the dark and RL

Consistent with this, Aux/IAA proteins intricately involved in regulation of auxin-dependent gene expression could be phosphorylated by phytochromes (Colón-Carmona et al. 2000). It is obvious that this molecular mechanism integrating auxin and light signaling (reviewed by Halliday et al. 2009) will affect, in dependence on light conditions, growth responses to auxin. We also revealed that the inhibitory effect of 1-NAA and the stimulatory effect of IAA in BL were reduced by high concentrations of boron in the culture medium. In addition, boron-induced suppression of hypocotyl responsiveness to 2,4-D or IAA was striking in BL (Figs. 2, 4), whereas boron did not alter the inhibition of hypocotyl growth by 1-NAA (Fig. 5). Finally, analysis of the high boron requiring mutant *bor1-1* strongly suggests that the transporter BOR1 mediates, at least partially, the effect of boron on the hypocotyl responses to auxins.

These interesting observations led us to the hypothesis that the various effects of exogenous auxins on hypocotyl elongation influenced by light and boron have a basis in the different mechanisms controlling the uptake and accumulation of 2,4-D, IAA, and 1-NAA in plant cells as demonstrated by Delbarre et al. (1996). On tobacco suspension cells these authors showed that 2,4-D uptake is mostly ensured by the influx carrier, that this auxin is not secreted by the efflux carrier, and that both auxin carriers contribute to IAA accumulation. By contrast, 1-NAA enters cells by passive diffusion and the efflux carrier controls its accumulation. Subsequent research has revealed that auxin molecules can be actively transported into the cell via the AUX1/LAX family of plasma membrane permeases (Parry et al. 2001; reviewed by Zažímalová et al. 2010). It was further established that members of the PIN (PIN-formed) family are associated with auxin uptake carriers (Galweiler et al. 1998; Luschnig et al. 1998; reviewed by Zažímalová

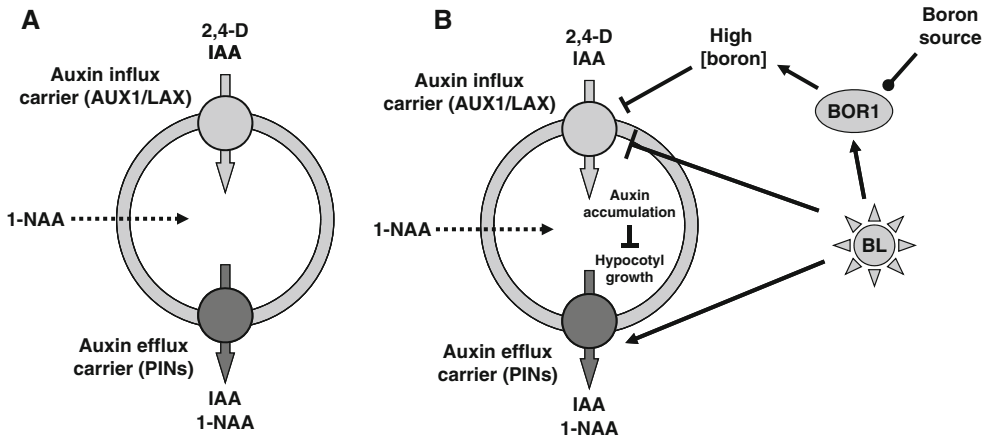

Fig. 6 Simplified scheme of the routes by which auxins enter or leave plant cells (re-drown from Morris 2000) (**a**), and hypothetical working model explaining how boron at high concentrations and light could influence hypocotyl responses to exogenous auxins (**b**). **a** The scheme shows that 2,4-D uptake is mostly ensured by the influx carrier (AUX1/LAX) and auxin is not secreted by the efflux carrier (PINs). Both auxin carriers however contribute to the IAA accumulation in the cells. Finally, 1-NAA enters cells by passive diffusion and the efflux carrier controls its accumulation. **b** In this model, the arrows and T-bars represent positive and negative effects, respectively. The model proposes that the strong inhibitory effect of 2,4-D on hypocotyl elongation could be caused by the relatively high accumulation of the auxin in the cell (over the optimum level). This accumulation is because the auxin is barely able to leave the cell either by diffusion or by an auxin efflux carrier (Delbarre et al. 1996). The observed boron-induced decline of the inhibitory effect of 2,4-D could be caused by a decreased level of 2,4-D inside the cell as a result of the reduced activity of the influx carrier by high boron concentrations. Since the *bor1-1* mutant requires a high boron supply, at the 0.1 mM H_3BO_3 the activity of the auxin influx carrier is not reduced and relative to the WT, the *bor1-1* mutant shows greater inhibition of hypocotyl growth. Only when 2 mM of boron is applied, enough boron is available in the mutant tissue to reduce the activity of the influx carrier and accumulation of 2,4-D inside the cell. It therefore suggests that the functional transporter BOR1 is involved in the boron-induced reduction of hypocotyl sensitivity to 2,4-D. The Boron-induced reduction of hypocotyl sensitivity to the inhibitory effect of 2,4-D was essentially amplified by BL. Earlier we reported that the *BOR1* gene expression is up-regulated in BL (Kocábek et al. 2009). The data therefore indicate that BL could increase accumulation of boron in plant tissues via BOR1 and thus contribute to the reduction of 2,4-D influx. The natural auxin IAA uses both carriers for its transport into and out of the cell. Therefore, this system allows tuning IAA accumulation in the cell, which could result in a low inhibitory effect of IAA on etiolated and RL-grown hypocotyls. Stimulation of WT hypocotyl by IAA in BL and in the absence of boron could be explained by additional fine-tuning of IAA accumulation in the cell by means of BL-induced stimulation of the efflux carrier. This is supported by the results of Laxmi et al. (2008), who showed that BL maintains the steady state PIN2 plasma-membrane-location (a member of PIN family associated with the auxin efflux; reviewed by Zažímalová et al. 2010). Again, at high boron concentrations, the activity of AUX1/LAX influx is reduced, which results in decreasing auxin amounts below an optimum level. Similarly, stimulation of IAA-induced growth of *bor1-1* hypocotyls could be explained by the fine-tuning of the IAA accumulation in the cell by high boron-induced inhibition of the influx carrier and by promotion of the efflux carrier by BL. Finally, the fact that 1-NAA enters cells by passive diffusion and that the efflux carrier controls the auxin accumulation, nicely fits with our results. The absence of the AUX1/LAX in the 1-NAA transport to the cell explains the absence of the boron-induced effect on hypocotyl responsiveness to 1-NAA. In addition, the efflux of 1-NAA stimulated by BL could cause an essential reduction of the auxin accumulation in the cell, which explains the reduced sensitivity of the BL-hypocotyl to the inhibitory effect of 1-NAA

et al. 2010). An illustration of the routes by which auxins enter or leave a plant cell is shown on the simplified model of Morris (2000) (Fig. 6a). Based on our results and reports of others, we have built a model described in detail in Fig. 6b. The model proposes that boron at higher concentrations (at least over 2 mM) somehow reduces the activity of the auxin influx carrier AUX1/LAX. Our model can explain how the observed regulation of *Arabidopsis* hypocotyl growth responded to the auxins 2,4-D, IAA or 1-NAA and were influenced by boron and light. The availability of boron is provided by the transporter BOR1. As we showed earlier, the expression of the *BOR1* gene in *Arabidopsis* hypocotyls could be stimulated by light (Kocábek et al. 2009). We therefore presume that BL could regulate the activity and/or amount of BOR1 protein. However, as far as we know, no information about the regulation of BOR1 (or other boron transporters) and boron transport by light were reported.

Our concept that BL may tune the polar auxin transport in *Arabidopsis* hypocotyl (Fig. 6b) is quite new since literature data about this topic are very limited. In tomato hypocotyls, polar auxin transport was significantly increased in the *cry1* mutant after BL exposure, suggesting a negative role of cry1 in polar auxin transport (Liu et al. 2011). In root system, Laxmi et al. (2008) proposed that light via blue light receptor pathways positively influences the activity of the auxin efflux carrier by controlling the intracellular distribution of PIN2, maintaining its plasma membrane location. Also for roots, Zeng et al. (2010) deduced that *Arabidopsis* cry1 reduces expression of PIN1.

Additionally, the normal polar auxin transport from shoot apex to cotyledons requires a member of the ABC-type auxin transporters (Lewis et al. 2009) called ABCB19 (formerly MDR1 or PGP19) (Vevrier et al. 2008). In *Arabidopsis* hypocotyl, expression of *FLABBY*, which encodes ABCB19, is suppressed by the activation of phytochromes and cryprochromes (Nagashima et al. 2008). Consistent with this, Wu et al. (2010) reported that *cry1* and *phyB* mutations increase level of ABCB19 protein at the plasma membrane. Recent results also indicate that another blue light photoreceptor phototropin 1 phosphorylates ABCB19. It results to the inhibition of its efflux activity, auxin level increases in and above hypocotyl apex, while vertical growth halts (Christie et al. 2011).

The effect of the boron influx carrier system is legitimate. Martín-Rejano et al. (2011) showed in the *Arabidopsis* root system that a low boron supply (0.4 μM) caused inhibition of primary root growth and induced formation of root hairs. It was further associated with the increased activity of auxin reported DR5:GUS induced by 0.4 μM boron. Moreover, elongation of the primary root in the *aux1-22* mutant was less sensitive to a low boron treatment than in WT plants. The authors went on to propose a hypothetical model showing that low boron condition increases auxin synthesis in the primary root meristematic region and its transport (via AUX1) to the elongation zone where auxin accumulates and induces local auxin responses that inhibit root cell elongation (Martín-Rejano et al. 2011). Others also reported the interaction of boron with polar auxin transport. They showed that boron-deficiency causes reduction of auxin distribution in pea shoots and sunflower hypocotyl (Tang and Dela Fuente 1986a, b; Wang et al. 2006). In contrast to the others, we tested three various auxins with various light conditions. Based on our results and based on the information of Delbarre et al. (1996) we hypothesize that a high boron supply, tested from 0.1 to 2 mM, reduces AUX1 transport activity (Fig. 6b). Our model actually fits very well with the observations of the others mentioned above. However, it is important to remember that in our experimental conditions, *Arabidopsis* seeds were first germinated on the basal MS medium containing 0.1 mM H_3BO_3. The reason was that in our experimental conditions *Arabidopsis* seeds germinated very poorly in the absence of boron. After germination, the seeds were transferred onto a boron-free medium. This procedure means that seedlings developing on boron-free medium still had some boron available during their growth. It explains the high hypocotyl sensitivity to auxin effects after the transfer of the seedlings onto the boron-free medium. It seems that the auxin transport system, at least the influx carrier, is very sensitive to boron concentrations. With boron-deficiency (Tang and Dela Fuente 1986a, b; Wang et al. 2006) or a high boron supply (our results here), the activity of the influx carrier seems to be reduced, whereas at a low boron supply (Martín-Rejano et al. 2011) the auxin influx was stimulated.

In conclusion, we have shown that in intact *Arabidopsis* seedlings, boron at high concentrations or BL essentially reduces the growth response of hypocotyl to an exogenously applied auxin, and we hypothesize that this happens via modification of the polar auxin transport. However, since boron and light also affect auxin metabolism and/or synthesis (Dyar and Webb 1961; Martín-Rejano et al. 2011; boron reviewed by Blevins and Lukaszewski 1998; light reviewed by Halliday et al. 2009), these processes cannot be excluded in order to fully understand the complexity of boron- and light-regulated plant responses to auxin.

Acknowledgments We thank Renáta Plotzová and Marcela Foltýnová for their excellent technical assistance. We specially thank J. Takano and T. Fujiwara (University of Tokyo) for providing seeds of the *Arabidopsis* mutant *bor1-1*. We also thank Jan. Nauš (Palacky University in Olomouc) for the PFD measurements of the lights. We also thank Pamela Lewis for her help in revising the English text. This work was supported by the Czech Science Foundation (grant no. 521/09/0445 to MF).

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