Boron Tolerance in Barley Is Mediated by Efflux of Boron from the Roots[1]

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Many plants are known to reduce the toxic effects of high soil boron (B) by reducing uptake of B, but no mechanism for limiting uptake has previously been identified. The B-tolerant cultivar of barley (*Hordeum vulgare* L.), Sahara, was shown to be able to maintain root B concentrations up to 50% lower than in the B-sensitive cultivar, Schooner. This translated into xylem concentrations that were approximately 64% lower and leaf concentrations 73% lower in the tolerant cultivar. In both cultivars, B accumulation was rapid and reached a steady-state concentration in roots within 3 h. In Schooner, this concentration was similar to the external medium, whereas in Sahara, the root concentration was maintained at a lower concentration. For this to occur, B must be actively extruded from the root in Sahara, and this is presumed to be the basis for B tolerance in barley. The extrusion mechanism was inhibited by sodium azide but not by treatment at low temperature. Several anion channel inhibitors were also effective in limiting extrusion, but it was not clear whether they acted directly or via metabolic inhibition. The ability of Sahara to maintain lower root B concentrations was constitutive and occurred across a wide range of B concentrations. This ability was lost at high pH, and both Schooner and Sahara then had similar root B concentrations. A predictive model that is consistent with the empirical results and explains the tolerance mechanism based on the presence of a borate anion efflux transporter in Sahara is presented.

Boron (B) toxicity is a significant problem in agricultural regions across the world (Cartwright et al., 1986; Nable et al., 1997), where high levels of soil B can seriously affect the growth and yield of many crop species. More than 40 years ago, Oertli and Kohl (1961) made the observation that visual symptoms of B toxicity seemed to occur in leaves of a wide variety of plant types at about the same local tissue concentration. From this they inferred that tolerance to B toxicity was unlikely to be due to an ability of shoots to better tolerate high concentrations of B, but to be due to the ability to accumulate less B. Within species such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) there is substantial variation in tolerance to high soil B (e.g. Moody et al., 1988). Nable (1988) and Nable et al. (1990) were able to identify two genotypes of barley with a large difference in tolerance that they related to lower accumulation of B in both shoots and roots. The concentrations that they measured in roots were much lower than in the external medium in both varieties, and so they proposed that the tolerant variety was able to more effectively exclude B. Dordas and Brown (2000) and Dordas et al. (2000) provided a rational basis for such a mechanism by showing that the permeability of membranes to B could be reduced by altering the lipid composition of the membrane, and that two different Arabidopsis mutants with altered membrane lipid composition had either higher or lower uptake of B than the wild type. The viability of such an exclusion mechanism was challenged by the experiments of Stangoulis et al. (2001) who showed that in Chara, the membrane permeability to B was very high, as suggested by Raven (1980) on the basis of ether to water partition coefficients. These observations were consistent with the earlier work of Bingham et al. (1970) who found rapid influx of B into excised barley roots, and Garnett et al. (1993) who had measured both rapid influx and efflux of B in wheat roots. Reexamination of the methods used by Nable et al. (1990) revealed that the roots had been rinsed for 30 min prior to analysis, and if B is as permeable as suggested, then there must have been a substantial loss of root B during the rinse period. These studies left several crucial questions unanswered. Firstly, do roots of the tolerant variety of barley really maintain a lower root concentration, or were the previous results simply an artifact of the rinsing procedure? Secondly, if the differences in root concentration were real, did they arise from an ability of the tolerant variety to prevent B entering the root, a mechanism that conceivably could occur passively simply by maintaining a low permeability to B, or was the lower concentration due to active pumping of B from the roots? There was clearly a need for a careful examination of the permeability of root cell membranes to B, of the kinetics of B fluxes in roots, and an evaluation of the need for energy for altering root B concentrations. Each of these issues has been addressed and resolved in this paper. The clarity of the picture that emerges permits prediction of the mechanism by which tolerant plants are able to maintain lower root concentrations of B and, in so

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Table I. *Relative root and shoot yields (compared to low B-grown seedlings), and boron concentrations in roots, shoots, and xylem of Sahara and Schooner barley varieties, after 16 d of growth in nutrient solution, pH 5.5 containing 5 mM B*

B concentrations were calculated based on root and shoot water content. Data are presented as means ± SE ($n = 4$).

Variety	Relative Yield		B Concentration		
	Roots	Shoots	Roots	Shoots	Xylem[a]
	%		mM		
Sahara	83.7%	94.6%	2.36 ± 0.03	10.1 ± 1.7	0.60 ± 0.09
Schooner	20.9%[b]	48.9%[b]	4.90 ± 0.30	45.8 ± 11.6	1.64 ± 0.21

[a]Xylem concentrations were measured after 13 d of growth ($n = 3$). [b]Yields in 5 mM B treatment were significantly ($P < 0.05$) lower than yields in low B nutrient solution.

doing, to prevent the transfer of toxic amounts of B to the shoots.

RESULTS

Sahara and Schooner barleys show very different tolerance to high B in solution culture experiments. After 16 d growth in nutrient solutions amended with 5 mM B, root and shoot yields of Schooner barley were reduced by 79% and 51%, respectively, relative to low B-grown plants ($P < 0.05$), while growth of Sahara barley was not significantly different from control plants (Table I). Analysis of tissue B concentrations revealed that B in the roots of Schooner barley had equilibrated with B in the growth solution, while B concentrations in the roots of Sahara barley were 53% less than outside. Similarly, xylem and shoot B concentrations were lower in Sahara compared to Schooner, by 2.7- and 4.5-fold, respectively ($P < 0.05$; Table I). These results indicate that the roots of Sahara barley are able to exclude or to efflux B, resulting in lower concentrations of B in the xylem and less accumulation of B in the shoot.

Root and shoot concentrations of B were lower in Sahara compared to Schooner across a wide range of external B concentrations up to 10 mM B (Fig. 1). For Schooner roots, B concentrations equilibrated with concentrations in the external growth solution (Fig. 1A). In contrast, root B concentrations of Sahara barley were maintained below the external solution, by between 25% and 53% at external concentrations above 0.1 mM B. Root B concentrations were higher than the external medium for both barley varieties when treated with 11.7 μM B (22 and 24 μM B for Sahara and Schooner roots, respectively), which most likely reflects incorporation of B into cellular components such as cell walls. The exclusion or efflux of B appeared to be a constitutive trait. Lower root B concentrations were paired with lower shoot B concentrations in Sahara compared to Schooner, with the differences between the two varieties magnified in the shoots (Fig. 1B). It is interesting to note that just as root concentrations of Schooner showed a linear dependence on the B concentrations in the external medium, shoot concentrations were linearly related to root concentrations. The same was not true for Sahara, which showed a more effective exclusion of B from the shoot in the range 1 to 5 mM, compared to 10 mM where the exclusion mechanism appeared to break down (Fig. 1B). To investigate B influx kinetics across Schooner and Sahara barley roots, the time course of accumulation of B following transfer to nutrient solutions containing 5 mM B was measured. There was a rapid influx of B across the roots of both varieties, with half-times for influx of approximately 6 min (Sahara) and 7 min (Schooner; Fig. 2). B in the roots appeared to reach a steady level after 2 h. However, the roots of Sahara reached a concentration of only

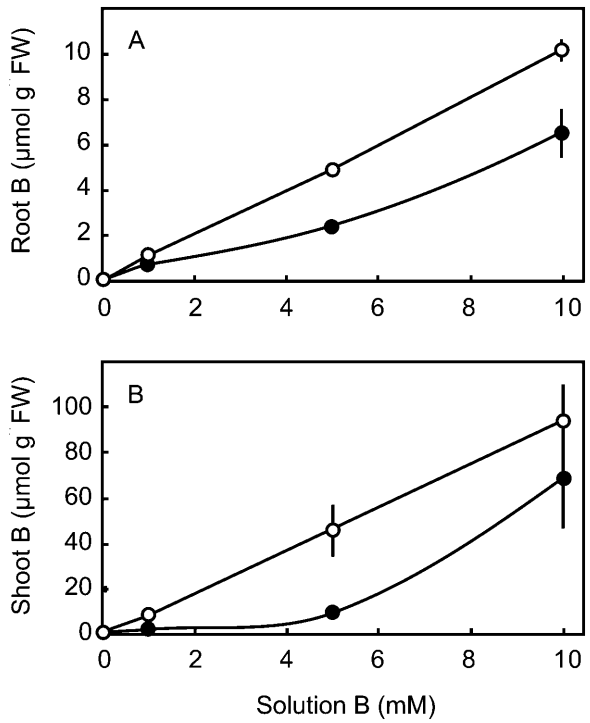

Figure 1. Concentrations of B in roots (A) and shoots (B) of Sahara (black symbols) and Schooner after 16 d growth in nutrient solutions containing different concentrations of B. Each point is the mean ± SE of four replicates.

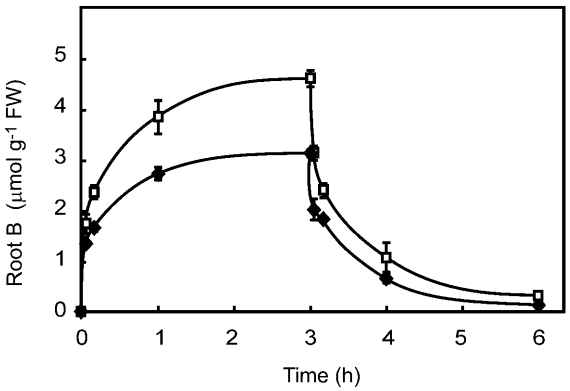

Figure 2. Concentrations of B in the roots of Sahara (black symbols) and Schooner barley varieties during short-term exposure to nutrient solutions, pH 5.5, containing 5 mM B (influx) and after removal of B (efflux). Each point represents a mean ± SE of three replicates.

3.2 μmol g^{-1} root fresh weight (FW), while B concentrations in Schooner increased to almost 5 μmol g^{-1} root FW over the same period, an equivalent concentration to that of B in the treatment solution. The rapid establishment (within 3 h) of a concentration difference between Sahara and Schooner roots was consistent with a mechanism for exclusion or efflux of B acting constitutively.

On removal of B from the treatment solution, B efflux was also rapid, but slower than influx (Fig. 2). Approximate half-times for efflux of 13 and 10 min were determined for Sahara and Schooner, respectively. After 3 h of incubation in B-free nutrient solution, the roots of both varieties contained less than 0.33 μmol B g^{-1} root FW. This was likely to have been exclusively cell wall-bound or complexed B, rather than free, cellular B. The measures of influx and efflux were made under conditions of low transpiration to minimize the influence of transpiration on root concentrations of B. However, separate experiments indicated that transpiration had little effect on root B concentrations in either variety. Plants of both varieties were exposed to high transpiration conditions (high light with fan) or low transpiration conditions (placed on damp filter paper and covered with bell jar in darkness) overnight. The final root concentrations (calculated on the basis of root water content) for high/low transpiration conditions were 3.0 ± 0.5 mM and 3.3 ± 0.1 mM for Sahara, and 6.0 ± 0.3 and 6.0 ± 0.4 mM for Schooner (n = 8 replicates).

Microscopy studies with transverse sections of root revealed no anatomical differences between Sahara and Schooner roots (data not shown), and we were unable to identify a compartment within the roots of Sahara barley with a capacity to exclude sufficient B to account for a difference of up to 50% in B concentrations between the varieties. A possible explanation, however, may be that Sahara is able to actively pump boric acid from the root or has a permeability to the borate anion (e.g. via anion channels), and through efflux of either B species is able to maintain lower root B concentrations than in the external solution. Such mechanisms would involve either direct or indirect input of energy to prevent B equilibrating. Several experiments were designed to investigate this possibility. The addition of metabolic inhibitors to roots that had been preexposed to B gave mixed results (Table II). Treatment with 0.5 mM sodium azide specifically increased the root B concentration of Sahara by 66% ($P < 0.05$) but did not affect B concentrations in Schooner, consistent with an inhibition of energy-driven B efflux activity in Sahara roots. However, low temperature treatment did not affect root B concentrations of either barley variety (Table II).

Ryan et al. (1995) reported inhibition of malate efflux from wheat roots treated with ethacrynic acid, anthracene-9-carboxylic acid (A9C), or niflumate at concentrations between 20 μM and 100 μM for 1 h. These compounds, plus several other inhibitors of anion channel activity in vitro, were tested for their effects on root B concentrations. When applied at 100 μM for 2 h, ethacrynic acid, A9C, diisothiocyanatostilbene-2,2′-disulfonate (DIDS), and α-cyano-4-hydroxycinnamic acid (CHCA) had no effect, while diphenylamine-2-carboxylic acid (DPC) and niflumate increased root B concentrations by 50% and 80%, respectively in Sahara (Fig. 3) so that the concentrations approached that of untreated Schooner roots. Root B concentrations were unaffected by ethanol at a concentration of 0.2% (as in treatments in which the channel blockers added from stock dissolved in ethanol). Root B concentrations in Schooner were not affected by any of the channel blockers (data not shown).

To check for possible nonspecific toxicity of the channel inhibitors that increased B concentrations in Sahara, DPC and niflumate were applied to cells of the giant alga, *Chara corallina*. In these cells, metabolic inhibition is easily detected by a slowing in the rate of protoplasmic streaming, which is approximately linearly dependent on the cytosolic ATP concentration (Reid and Walker, 1983). After 2 h at 100 μM DPC, the streaming rate was reduced to 15% ± 5% of the control, and in 100 μM niflumate to 10% ± 9% of the control. In both treatments a proportion of the cells (three out of eight for DPC, and seven out of eight for niflumate) had ceased to stream and had lost turgor.

Table II. *B concentrations in roots of Sahara and Schooner barley after overnight exposure to 5 mM B in nutrient solution, pH 5.5, followed by a further 2-h exposure to solutions precooled to 4°C (low temperature treatment) or containing 0.5 mM sodium azide*

Data are presented as mean ± SE of three replicates.

Treatment	B Concentrations	
	Sahara	Schooner
	mM	
Control	2.26 ± 0.14	4.58 ± 0.08
Low termperature	2.18 ± 0.02	4.75 ± 0.11
Azide	3.55 ± 0.09	4.53 ± 0.18

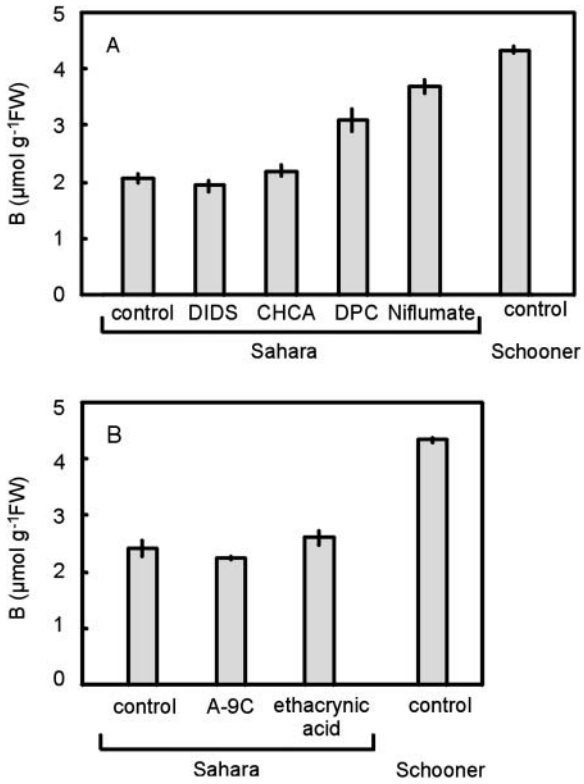

Figure 3. Boron concentrations in roots of Sahara and Schooner barley after 2-h treatment in solutions containing 5 mM B with or without various anion channel inhibitors applied at 100 μM. A and B represent separate experiments. Data are presented as mean ± SE of three replicates (A) and five replicates (B).

At 50 μM, streaming rate was reduced to approximately 35% for both inhibitors. These results clearly demonstrate that in Chara, at least, DPC and niflumate are strongly inhibitory to metabolism.

Relative proportions of borate $[B(OH)_4^-]$ and boric acid $[B(OH)_3]$ present in the external solution were altered, by varying the pH of treatment solutions containing 5 mM B, and the resulting concentrations of B in roots were measured. B concentrations in the roots of Schooner barley decreased with increasing external pH; at pH values below the pK_a for $B(OH)_3$, root B concentrations reflected the proportion of $B(OH)_3$ present in the external solution (Fig. 4). At higher pH, the relationship between Schooner root B concentrations and $B(OH)_3$ in the external solution did not hold, with B concentrations of 2.9 and 2.2 μmol g^{-1} root FW measured at pH 9.5 and 10.0, respectively (for which $B(OH)_3$ concentrations present in the treatment solutions would be 1.8 and 0.8 mM). These discrepancies could be explained if the apoplastic pH was lower than the bulk solution pH by about 0.5 units, which on the basis of other studies seems probable (Amtmann et al., 1999; Kosegarten et al., 1999). In Sahara roots, B concentrations were maintained at around 2 μmol B g^{-1} root FW, independently of external pH and the speciation of B in the treatment solutions (Fig. 4).

DISCUSSION

This study provides strong evidence that active transport of B is related to B tolerance in plants and provides a strong physiological basis for molecular investigations into genes involved either directly in the transport or in the control of B transport.

Tolerance to B toxicity in the two barley varieties studied was correlated with lower B concentrations in the roots and shoots. This appears to rule out internal complexation as a major mechanism for tolerance since this would have the effect of increasing total internal B rather than reducing it. Careful measurements of influx revealed that in the sensitive variety, B equilibrated between the external solution and the root symplasm within several hours, which is explained by a high membrane permeability to B, as predicted by previous studies. The tolerant variety also showed rapid uptake of B but was able to maintain steady-state root concentrations of B that were up to 50% below the concentration in the external medium. This lower accumulation of B by Sahara was unaffected by transpiration and was therefore not simply due to an ability of Sahara to rapidly move B out of the roots via the transpiration stream.

The lower root concentrations in Sahara were matched by reduced xylem and shoot concentrations. In one experiment with 5 mM B in the nutrient solution, the roots of Sahara contained 1.7-fold less B, while xylem and shoot concentrations were 2.7-fold and 4.5-fold lower, respectively, than in Schooner.

On simple thermodynamic grounds, maintenance of a concentration difference of a neutral solute across a membrane must require an input of energy. Addition of the metabolic inhibitor sodium azide to B solutions resulted in a specific increase in the B concentrations of Sahara roots, consistent with efflux being an active

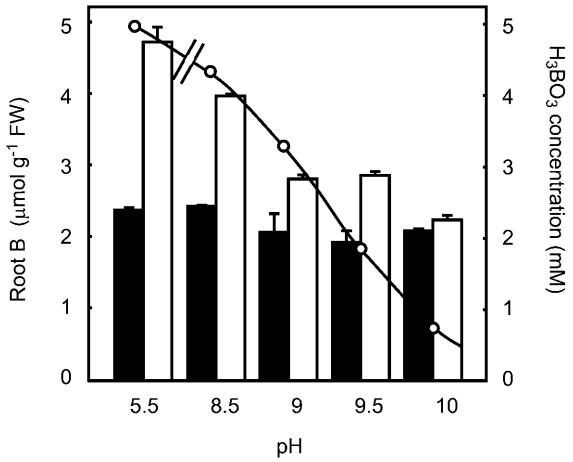

Figure 4. Boron concentrations in roots of Sahara (shaded bars) and Schooner (white bars) barley varieties after overnight exposure to 5 mM B in nutrient solution, pH 5.5, followed by a further 2-h exposure to solutions containing 5 mM B and adjusted to pH 5.5 to 10.0. The line indicates the concentration of $B(OH)_3$ in solution at each pH. Data are presented as means ± SE (n = 4).

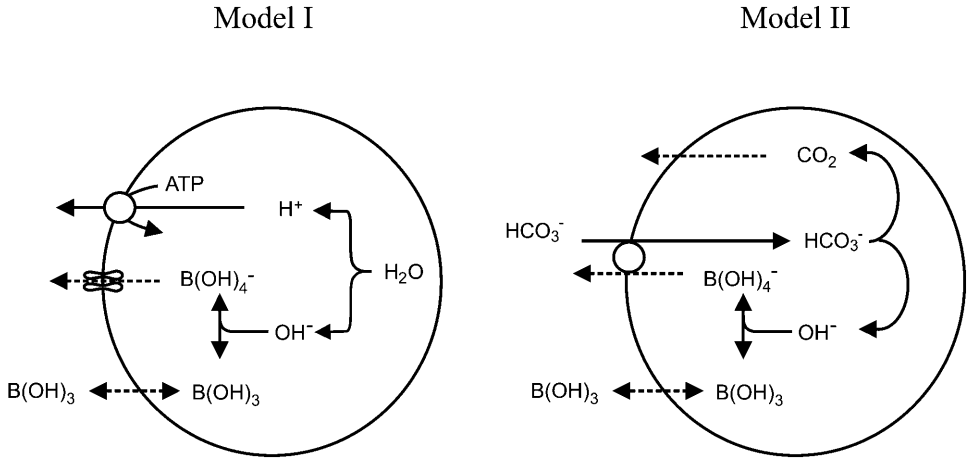

Figure 5. Two models for boron efflux as the basis of B tolerance in barley. It is proposed that B-sensitive genotypes lack a capacity to efflux $B(OH)_4^-$. B enters rapidly from the external medium and accumulates in the cytoplasm as $B(OH)_3$, which undergoes pH-dependent conversion (pKa = 9.25) to $B(OH)_4^-$. In model I, efflux of $B(OH)_4^-$ is driven through an anion-permeable transporter by the negative membrane potential difference and at low external pH by the outwardly directed concentration gradient. Energy input to the H^+-ATPase is required to prevent depolarization of the plasma membrane due to anion efflux and to prevent acidification of the cytoplasm. In model II, $B(OH)_4^-$ efflux occurs by anion-exchange. If the anion is HCO_3^- no charge compensation is needed and pH stability will occur through dissociation into OH^- and CO_2 in the cytoplasm. Dashed lines indicate passive fluxes.

process. A low temperature treatment, while expected to also inhibit metabolism, did not affect B concentrations in the roots of either variety. However, low temperatures would also reduce diffusion rates for B and changes in the concentration of root B may have only become evident after prolonged treatment.

It could be argued that active efflux would be futile in the presence of such high influx, analogous to trying to bale out a sinking boat with gaping holes in the bottom. The influx is indeed high (around 30 μmol g^{-1} FW h^{-1} at 5 mM), but the actual energy gradient is relatively small, 1.7 kJ mol^{-1} for a 50% reduction, compared to other effluxes such as Ca^{2+} of around 40 kJ mol^{-1} (Ca_i = 0.1 μM, Ca_o = 1 mM, electrical potential difference (PD) = −120 mV), and 23 kJ mol^{-1} for H^+ at pH_o of 5 (pH_i = 7.5, PD = −120 mV). In terms of the energetics, efflux of B seems entirely feasible.

The identity of the efflux transporter has not been resolved in this study, but there are a number of possibilities. B is known to form complexes with sugars and sugar alcohols in plants, but membrane transport of such complexes seems unlikely because it would result in the loss of large amounts of fixed carbon from the roots. A more feasible mechanism involves efflux of the borate anion. The concentration of B in roots of Schooner decreased with increasing pH, presumably reflecting the change in the concentration of the permeant species $B(OH)_3$. In Sahara, however, root concentrations did not significantly decrease at higher pH, despite the large change in the proportion of the neutral $B(OH)_3$ species. Such a situation would be expected if the efflux mechanism in the tolerant variety was due to the presence of a higher permeability to $B(OH)_4^-$ for which there is a strong outwardly directed electrochemical gradient at lower external pH. The magnitude of this gradient progressively reduces with increasing pH due to the shift in speciation of B in the external solution from $B(OH)_3$ to $B(OH)_4^-$, thereby reducing the effectiveness of $B(OH)_4^-$ efflux in maintaining low root B.

High fluxes across membranes are often mediated by ion channels, and if the tolerance mechanism was due to efflux of $B(OH)_4^-$ through channels, then efflux might be sensitive to anion channel inhibitors. Efflux

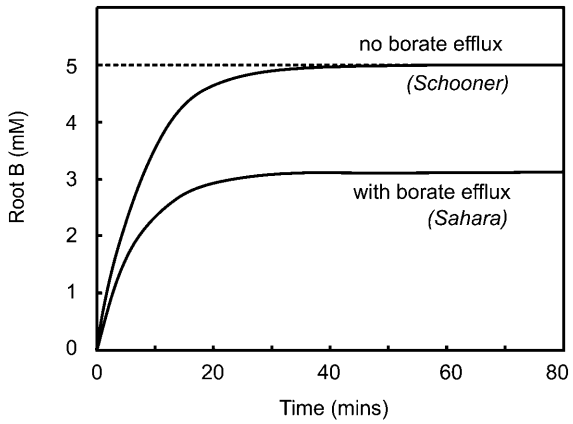

Figure 6. Simulation of B influx into roots of tolerant and sensitive varieties of barley from a solution containing 5 mM B at pH 6. The model is based on a half-time for equilibration of B of 7 min for Schooner (equivalent to an influx of 30 μmol g^{-1} h^{-1}) and a ratio of permeability of $B(OH)_4^-$ to $B(OH)_3$ of 2 (Sahara only). Cytoplasmic pH = 7.67 (from Reid et al., 1985a) and membrane PD = −118 mV (from Reid et al., 1985b). The dashed line represents the external concentration.

of B was inhibited by both DPC and niflumate, but both of these compounds were metabolically inhibitory when applied to Chara. Doubt therefore remains whether the effects of these channel inhibitors were exerted directly or via reduced metabolic activity. The metabolic inhibition in Chara could be due either to the consequences of blocking anion channels or to a direct effect on metabolism. If the latter is the case, then previous studies with these inhibitors on intact cells may need to be reevaluated.

Is the tolerance mechanism induced by high B? There are several lines of evidence that the efflux mechanism is constitutive. Reduced root B concentrations in Sahara compared to Schooner were seen within minutes of addition of B, and this applied to plants not previously exposed to B. The differences could also be seen at concentrations that are not toxic to plant growth (i.e. 0.1 mM B). The mechanism appeared to break down at very high external B concentrations, perhaps because the efflux required exceeded the capacity of the transporter to drive B out.

Models for Borate Efflux

Two models are proposed for the efflux of B from root cells, both based on efflux of the borate anion (Fig. 5). To be credible, each model needs to be energetically feasible and to account for the observed pH dependence and effect of metabolic inhibition. Additionally, given the high fluxes involved, there need to be compensatory mechanisms for charge transfer across the plasma membrane resulting from anion efflux, and for the acid load imposed on the cytoplasm caused by the formation of borate from boric acid.

Model I is based on borate efflux through an anion channel while model II involves anion exchange. The parameters needed to test model I are all reasonably well known and can be used to predict the internal concentration of B under various conditions. The response of cytoplasmic pH to external pH that is required for the estimation of internal and external borate concentrations is given in Reid et al. (1985b), while the membrane PD that is needed for the calculation of the electrochemical gradient for borate is given in Reid et al. (1985a) up to pH 8 (higher pHs were assigned the same PD as pH 8). The permeability of the plasma membrane to B was estimated based on the influx required to achieve equilibration according to the measured half-time of 7 min. Figure 6 shows an example for B accumulation at pH 6 based on the assumption that the tolerant variety possesses a transporter for borate while the sensitive variety does not. The effectiveness of the efflux mechanism is strongly dependent on the borate permeability. In the example shown, the ratio of permeabilities of borate:boric acid that matches the measured concentrations was set at 2:1.

The observed loss of tolerance as the external pH increases can be explained by the greater increase in the concentration of borate in the external medium compared to the cytoplasm, due to the low sensitivity of cytoplasmic pH to changes in external pH. The concentration gradient for borate reverses and eventually exceeds the outward driving force due to the negative membrane PD.

The energy dependence is explained by the need to actively extrude H^+ to maintain the electrical driving force for borate efflux, as well as to prevent cytoplasmic acidification. An argument against this model is that it would be energetically wasteful. H^+ efflux normally occurs via H^+-pumping ATPases and hydrolysis of 1 ATP/H^+ would release around 30 kJ mol^{-1} ATP, which greatly exceeds the overall B gradient of approximately 1.7 kJ mol^{-1}.

Model II was proposed previously by Frommer and von Wirén (2002) as a possible mechanism for BOR1, a putative borate transporter involved in xylem loading of B (Takano et al., 2002). Anion exchange accounts for the need for charge balance but not necessarily for pH regulation. If the exchanger anion were Cl^- then it would still be necessary to pump out protons that would then necessitate the flux of another ion (e.g. K^+ influx) to balance the charge and maintain a stable membrane PD. However, we have found that B concentrations in roots of Sahara do not rise if Cl^- is removed from the external solution (J. Hayes, unpublished data). If the exchanger anion were HCO_3^- then both pH and charge balance could be accommodated (Fig. 5B). While this is a simple and attractive mechanism, it is hard to develop a predictive model because the external and internal concentrations of HCO_3^- are too difficult to estimate. There is also the question of whether there would be sufficient HCO_3^- in the apoplast at pH less than about 6 to drive the exchange reaction. Exchange with OH^- could also occur but would be most effective at high pH, and this was not observed.

These models should serve as the starting point for probing the molecular identity of the transporter, research that we are currently undertaking.

MATERIALS AND METHODS

Plant Culture, Harvest, and B Analysis

Seeds of Schooner and Sahara barley (*Hordeum vulgare*) varieties were surface-sterilized in 0.5% sodium hypochlorite and germinated on damp filter paper in petri dishes. After 2 to 3 d, germinated seedlings were transferred to buckets of nutrient solution with continuous aeration. Between six and eight seedlings were grown in 4 L of nutrient solution. Nutrient solutions, pH 5.5, contained (in mM): 3.75 NO_3-N, 1.5 K, 1.25 Ca, 0.5 Mg, 0.5 S, and 0.25 P; and (in μM): 8.52 Na, 4.6 Cl, 4.5 Fe-EDTA, 2.3 Mn, 0.26 Mo, 0.19 Zn, and 0.08 Cu. Standard nutrient solutions also contained 11.7 μM B as $B(OH)_3$. Treatment solutions were supplemented with additional $B(OH)_3$ of up to 10 mM, while in most experiments, 5 mM additional B was used as a high B treatment because it enabled clear differentiation between the two varieties in their tolerance to B. Solutions were replaced at least weekly and seedlings were grown for between 13 and 25 d.

At harvest, the roots of individual plants were excised and carefully but briefly blotted dry between two layers of blotting paper. FWs were determined and samples were stored at 4°C in 50-mL, B-free plastic tubes until analysis. In some cases, roots were dried for 48 h at 70°C to obtain a fresh-to-dry weight ratio for calculation of root B concentrations in mM. Fresh and oven-dry

weights of shoots, where sampled, were also determined before analysis. All samples were digested in a mixture of nitric and hydrochloric acids and analyzed for B by inductively coupled plasma atomic emission spectrometry.

Measurements of B Efflux

The influx of B across barley roots was investigated by following the time course of accumulation in 25-d-old seedlings after transfer to nutrient solutions containing 5 mM B. The time course of B efflux was investigated by pretreating seedlings in 5 mM B for 3 h, then transferring to solution without B and sampling roots at various intervals. To minimize transfer of B from roots to shoots via transpiration during the experiment, seedlings were maintained in low light, high humidity conditions. Individual plants (three replicates for each variety and time point) were harvested throughout the influx-efflux period and the roots excised, blotted dry, and weighed for analysis of B.

Experiments measuring the effects of solution pH and of metabolic and anion channel inhibitors on B efflux used seedlings grown in low B nutrient solutions for 18 to 22 d, which were then exposed to 5 mM B in nutrient solution, pH 5.5, overnight (approximately 20 h) before treatment. For the metabolism inhibition study, pretreated seedlings were further exposed for 2 h to 5 mM B that was amended with 0.5 mM sodium azide or cooled to 4°C. Roots were sampled immediately following the 2-h period for B analysis by inductively coupled plasma atomic emission spectrometry. In a separate experiment, the effects on B efflux of several anion channel inhibitors were also determined. Seedlings preexposed to high B were treated for a further 2 h in 5 mM B nutrient solutions containing 100 μM DIDS (Sigma, St. Louis), ethacrynic acid (Sigma), DPC (Aldrich), CHCA (Sigma), niflumate (Sigma), or A9C (Aldrich). CHCA, niflumate, and ethacrynic acid were first dissolved in ethanol before dilution into nutrient solution from a stock solution of 0.5 M. The final concentration of ethanol in this treatment was 0.02%. DIDS was dissolved in water and DPC in dimethyl sulfoxide (final concentration 0.02%). A9C was added to treatment solution from a 50 mM stock solution prepared in 1 N NaOH. The treatment solutions were readjusted to pH 5.5 after addition of the inhibitors. To determine the effect of pH on B efflux, seedlings preexposed to 5 mM B were transferred to buffered nutrient solutions containing 5 mM B and 5 mM 3-[cyclohexylamino]-2-hydroxy-1-propanesulfonic acid (Sigma) and adjusted to pH values between 8.5 and 10.0. After 2 h of incubation in the treatment solutions, the roots were harvested and analyzed for B.

Collection of Barley Xylem Fluid

Xylem fluid was collected from seedlings grown for 13 d in nutrient solution containing 5 mM B by slicing off the shoots at approximately 2 cm above the junction between shoot and root. After blotting the roots to remove adherent nutrient solution, a detopped seedling was secured in a Scholander pressure bomb. Pressures of 700 to 1,000 kPa were applied to the compartment containing the roots for 2 to 3 min and the xylem fluid exuded through the cut stem was collected with a pipette. For each replicate, xylem fluid from at least 10 seedlings was collected by this method.

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LITERATURE CITED

Amtmann A, Jelitto TC, Sanders D (1999) K^+-selective inward-rectifying channels and apoplastic pH in barley roots. Plant Physiol **120:** 331–338

Bingham F, Elseewi A, Oertli J (1970) Characteristics of boron absorption by excised barley roots. Soil Sci Soc Am Proc **34:** 613–617

Cartwright B, Zarcinas BA, Spouncer IA (1986) Boron toxicity in South Australian barley crops. Aust J Agric Res **37:** 351–359

Dordas C, Brown PH (2000) Permeability of boric acid across lipid bilayers and factors affecting it. J Membr Biol **175:** 95–105

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

Frommer W, von Wirén N (2002) Ping-pong with boron. Nature **410:** 282–283

Garnett TP, Tester MA, Nable RO (1993) The control of boron accumulation by two genotypes of wheat. Plant Soil **155:** 305–308

Kosegarten H, Grolig F, Esch A, Glusenkamp KH, Mengel K (1999) Effects of NH_4^+, NO_3^- and HCO_3^- on apoplast pH in the outer cortex of root zones of maize, as measured by the fluorescence ratio of fluorescein boronic acid. Planta **209:** 444–452

Moody D, Rathjen A, Cartwright B, Paull J, Lewis J (1988) Genetic diversity and geographical distribution of tolerance to high levels of soil boron. *In* T Miller, R Koebner, eds, 7th International Wheat Genetics Symposium, July 13–19, 1988. IPRS, Cambridge, pp 859–865

Nable RO (1988) Resistance to boron toxicity amongst several barley and wheat cultivars: a preliminary examination of the resistance mechanism. Plant Soil **112:** 45–57

Nable RO, Bañuelos GS, Paull JG (1997) Boron toxicity. Plant Soil **193:** 181–198

Nable RO, Cartwright B, Lance RC (1990) Genotypic differences in boron accumulation in barley: relative susceptibilities to boron deficiency and toxicity. *In* N El Bassam, M Dambroth, B Loughman, eds, Genetic Aspects of Plant Mineral Nutrition. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 243–251

Oertli JJ, Kohl HC (1961) Some considerations about the tolerance of various plant species to excessive supplies of boron. Soil Sci **92:** 243–247

Raven JA (1980) Short- and long-distance transport of boric acid in plants. New Phytol **84:** 231–249

Reid RJ, Dejaegere R, Pitman MG (1985a) Regulation of electrogenic pumping in barley by pH and ATP. J Exp Bot **36:** 535–549

Reid RJ, Field LD, Pitman MG (1985b) Effects of external pH, fusicoccin and butyrate on the cytoplasmic pH in barley root tips measured by ^{31}P-nuclear magnetic resonance spectroscopy. Planta **166:** 341–347

Reid RJ, Walker NA (1983) Adenlyate concentrations in *Chara*: variability, effects of inhibitors and relationship to protoplasmic streaming. Aust J Plant Physiol **10:** 373–383

Ryan PR, Delhaize E, Randall P (1995) Characterisation of Al-stimulated efflux of malate from the apices of Al-tolerant wheat roots. Planta **196:** 103–110

Stangoulis JC, Reid RJ, Brown PH, Graham RD (2001) Kinetic analysis of boron transport in *Chara.* Planta **213:** 142–146

Takano J, Noguchi K, Yasumori M, Kobayashi M, Gajdos Z, Miwa K, Hayashi H, Yoneyama T, Fujiwara T (2002) *Arabidopsis* boron transporter for xylem loading. Nature **420:** 337–340