Boron in Plant Biology

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Abstract: The interest of biologists in boron (B) has largely been focused on its role in plants for which B was established as essential in 1923 (Warington, 1923[153]). Evidence that B has a biological role in other organisms was first indicated by the establishment of essentiality of B for diatoms (Smyth and Dugger, 1981[138]) and cyanobacteria (Bonilla et al., 1990[14]; Garcia-Gonzalez et al., 1991[44]; Bonilla et al., 1997[16]). Recently, B was shown to stimulate growth in yeast (Bennett et al., 1999[8]) and to be essential for zebrafish *(Danio rerio)* (Eckhert and Rowe, 1999[33]; Rowe and Eckhert, 1999[121]) and possibly for trout *(Oncorhynchus mykiss)* (Eckhert, 1998[32]; Rowe et al., 1998[120]), frogs *(Xenopus laevis)* (Fort et al., 1998[41]) and mouse (Lanoue et al., 2000[75]). There is also preliminary evidence to suggest that B has at least a beneficial role in humans (Nielsen, 2000[96]). While research into the role of B in plants has been ongoing for 80 years it has only been in the past 5 years that the first function of B in plants has been defined. Boron is now known to be essential for cell wall structure and function, likely through its role as a stabilizer of the cell wall pectic network and subsequent regulation of cell wall pore size. A role for B in plant cell walls, however, is inadequate to explain all of the effects of B deficiency seen in plants. The suggestion that B plays a broader role in biology is supported by the discovery that B is essential for animals where a cellulose-rich cell wall is not present. Careful consideration of the physical and chemical properties of B in biological systems, and of the experimental data from both plants and animals suggests that B plays a critical role in membrane structure and hence function. Verification of B association with membranes would represent an important advance in modern biology. For several decades there has been uncertainty as to the mechanisms of B uptake and transport within plants. This uncertainty has been driven by a lack of adequate methodology to measure membrane fluxes of B at physiologically relevant concentrations. Recent experimentation provides the first direct measurement of membrane permeability of B and illustrates that passive B permeation contributes sufficient B at adequate levels of B supply, but would be inadequate at conditions of marginal B supply. The hypothesis that an active, carrier mediated process is involved in B uptake at low B supply is supported by research demonstrating that B uptake can be stimulated by B deprivation, that uptake rates follow a Michaelis-Menton kinetics, and can be inhibited by application of metabolic inhibitors. Since the mechanisms of element uptake are generally conserved between species, an understanding of the processes of B uptake is relevant to studies in both plants and animals. The study of B in plant biology has progressed markedly in the last decade and we are clearly on the cusp of additional, significant discoveries. Research in this field will be greatly stimulated by the discovery that B is essential for animals, a discovery that will not only encourage the participation of a wider cadre of scientists but will refocus the efforts of plant biologists toward a determination of roles for B outside the plant cell wall. Determination of the function of B in biology and of the mechanisms of B uptake in biological systems, is essential to our understanding and management of B deficiency and toxicity in plants and animals in both agricultural and natural environments. Through an analysis of existing data and the development of new hypotheses, this review aims to provide a vision of the future of research into the biology of boron.

Key words: Boron, function, structure, essentiality, membrane, uptake, transport, cell wall, reproduction.

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Introduction

Because of the rapidity and the wide variety of symptoms that occur following B deprivation, determining the primary function of B in plants has been one of the greatest challenges in plant nutrition (Blevins and Lukaszewski, 1998[10]). Over the past decade, however, very significant advances have been made in understanding the metabolism of B in plants. Primary among these advances has been the determination of the chemical form and function of B in plant cell walls, the identification of the role of polyhydric alcohols in phloem B transport and the characterization of the processes of B transport across membranes. Each of the major advances in our knowledge of the biology of B has been strongly grounded in an understanding of the physical and chemical characteristics of B. Though B is now known to be essential for many organisms, it is clear that B plays a uniquely important role in plants. Among the essential plant micro-nutrients, B deficiencies occur widely and have a significant agronomic impact throughout the world (Gupta, 1979[49]; Shorrocks, 1997[131]). One significant feature of B deficiency that contributes to its importance

in agricultural production is that a deficiency of B inhibits *growing* tissues, specifically reproductive structures, which represent 80% of the worlds agricultural product. Boron deficiency creates a wide range of anatomical symptoms including the inhibition of apical and extension growth, necrosis of terminal buds, cracking and breaking of stems and petioles, abortion of flower initials, and shedding of fruits (Mozafar, 1993[92]; Goldbach, 1997[47]). Boron deficiency also causes many physiological, and biochemical changes including, altered cell wall structure, altered membrane integrity and function, changes in enzyme activity and altered production of a wide range of plant metabolites (Goldbach, 1997[47]). The profound effects of B on meristems reflects the unique role B plays in cell growth and is a consequence of the processes that control B uptake and transport. It is clear, however, that our knowledge of the biology of B is still limited and that we have an inadequate understanding to optimally manage B in agricultural practice.

While a role for B in cell walls has now been well defined, a wealth of evidence suggests that B plays a role in the structure and function of the plasma membrane of plants. An effect of B on membrane structure could occur as a result of B binding to phosphoinositides, glycoproteins and glycolipids of membranes thereby influencing the stability of membrane domains with unique biological activity (Parr and Loughman, 1983[103]). A role for B in membranes is also supported by findings that B deficiency in animals primarily disrupts processes that are highly membrane specific or that require synthesis of new membranes. In both the African clawed frog *(Xenopus laevis)* (Fort et al., 1998[41]) and the zebrafish (Eckhert and Rowe, 1999[33]) B is essential for the normal reproduction while in mature zebrafish, B deficiency results in dystrophy of photoreceptors in the eye (Eckhert and Rowe, 1999[33]). These processes are characterized by a high requirement for membrane synthesis. In humans, B affects several membrane specific processes resulting in decreased brain electrical activity, impaired cognitive performance, altered concentrations and decreased activity of several membrane active hormones (Nielsen, 2000[96]). A primary role for B in membranes is clearly suggested and is consistent with known physical and chemical properties of B.

The cumulative evidence that B is essential across a number of kingdoms of living organisms represents a major paradigm shift and provided the impetus for this review. In the following, we re-examine significant historical observations and recent advances in the study of B in plants, and interpret these findings in light of the discoveries that B plays a role in the biology of both plants and animals.

Boron Chemistry

The physical and chemical properties of B and its complexes are unique and highly varied. An understanding of these properties is essential if we are to predict and interpret the role of B in biology (for more complete review see Woods [1996[158]]).

Under physiological conditions, and in the absence of interaction with bio-molecules, B exists as boric acid ($B[OH]_3$) or borate anion ($B[OH]_4^-$) (Woods, 1996[158]). Boric acid is a very weak acid, with a pKa of 9.24, and at the pH found in the cytoplasm (pH 7.5), more than 98% of B would exist in the form of free $B(OH)_3$ and less than 2% would exist as $B(OH)_4^-$ (Woods, 1996[158]). At pH values found in the apoplast (pH 5.5), greater than 99.95% of B would be in the form of $B(OH)_3$ and less than 0.05% would be in the form of $B(OH)_4^-$. Boric acid and borate, however, can readily react with many kinds of biological molecules and under normal biological conditions available B binding molecules will typically exceed the concentration of free B. An understanding of B binding reactions is therefore central to an understanding of B physiology.

Boric acid forms esters and complexes with a wide variety of mono, di- and poly-hydroxy compounds (Woods, 1996[158]). These borate esters form and dissociate *spontaneously* in dynamic pH-dependent equilibrium and with *rapid* kinetics (Friedman et al., 1974[43]). A number of factors affect the stability of these B complexes. Increasing pH typically stabilizes B complexes, *cis*-diols are favoured over *trans*-diols (Boeseken, 1949[11]); while sugars with a furanoid ring (five-membered ring) configuration form more stable complexes than those with a pyranoid ring (six-membered ring) (Loomis and Durst, 1992[81]; Goldbach, 1997[47]; Brown P. H. and Hu H., unpublished data). The presence and nature of neighboring groups in B complexes can have a significant stabilizing effect on B complexes. Large substituent groups provide steric protection against hydrolysis, while the presence of an adjacent nitrogen increases stability by inducing additional H-bonding; and the presence of a positively charged substituent group confers electrostatic stabilization (Woods, 1996[158]). Thus, the coenzyme NAD^+ has a 15-fold greater affinity for B than the reduced form NADH since the negative charge of NAD-borate is stabilized by the positive charge in NAD^+ (Smith and Johnson, 1976[136]). The stability of the borate-rhamnogalacturonan-II complex is also greatly enhanced by presence of Ca^{2+} (Kobayashi et al., 1999[71]).

Examples of bio-molecules that react strongly with boric acid include ribose, apiose (e.g., cell wall), sorbitol and other polyols (Loomis and Durst, 1992[81]), as well as phenolics and amino acids, such as serine (Tate and Meister, 1978[143]). Complex bio-molecules with predicted B binding capacity include glycoproteins and glycolipids. This has been demonstrated by Frantzen et al. (1995[42]) using protein-boronic acid (a boric acid analog) affinity column to separate glycohemoglobin from non-glycolated hemoglobin. Given the diversity of functional groups with which B can bind a wide range of B containing bio-molecules are likely present in all biological systems. With the exception of the cell wall rhamnogalacturonan-II B complex identified by Kobayashi et al. (1996[70]) and O'Neill et al. (1996[102]), and the B-polyol complex identified by Hu et al. (1997[59]), the functional significance of these putative biological B complexes has not been determined.

The diversity of factors that influence the stability of B complexes is so great and our knowledge of biological B complexation so poor, that currently it is impossible to predict, with any degree of certainty, the binding status of B in biological systems. The ubiquitous nature of potential B binding sites has often confounded the interpretation of experimental results and has resulted in the many purported functions of B that have been published over the proceeding 70 years. For example, the theoretical capacity of sugars and phenolics to bind B has been frequently interpreted as evidence for a role of B in sugar and phenol metabolism as pointed out by Goldbach (1997[47]).

This conclusion has been widely cited even though there is no definitive evidence that B-sugar or B-phenol complex formation has any significant effect on metabolism. Equally problematic are studies of B uptake and within plant transport that fail to adequately consider the potential effects of B-complexation on transport kinetics.

Uptake of Boron

As a consequence of its essential role in growing tissues and of the inherent phloem immobility of B in most plant species, fluctuations in soil B availability can have a profound effect on plant growth and productivity. Many species are also sensitive to high levels of B in soil, and water, and growth inhibition as a result of excess B uptake is experienced in many agricultural regions. Understanding the biology of B uptake by plants is therefore critical to the management of B in natural and agricultural systems. The past three years have seen a progress in our understanding of the mechanisms of B acquisition by plants and the factors that govern transmembrane B transport.

The subject of B uptake has long been controversial and significant evidence supports both the active and passive uptake of B in higher plants (Brown and Shelp, 1997[19]; Hu and Brown, 1997[57]). Hu and Brown (1997[57]) proposed that B uptake, under conditions of adequate or excessive B supply, is the result of passive absorption of undissociated boric acid ($B[OH]_3$). It was further hypothesized that B uptake occurred by a non-metabolic process primarily determined by the B concentration outside the root, B-complex formation inside (Brown and Hu, 1994[18]) or outside of the root, membrane permeability (Raven, 1980[115]), translocation of B within the plant (Brown and Shelp, 1997[19]) and the transpiration rate. In their analysis Hu and Brown (1997[57]) concluded that there is no substantive evidence that B uptake occurred through an energy dependent process, such as a transport carrier molecule.

Though this conclusion was consistent with the majority of available studies, it was incomplete in that it did not provide a mechanistic explanation of B uptake and was based largely upon the theoretical predictions of membrane permeability proposed by Raven (1980[115]), predictions that had not been verified *in vivo*. Furthermore, the conclusion that B uptake was a passive process seemed to be at odds with observed differences in B uptake among plant species or cultivars, that can be as large as seven-fold when those species are grown under identical conditions (Nable et al., 1997[97]). For example, barley cultivars "Sahara 3763" and "Schooner" accumulated 112 and 710 mg kg^{-1} B dry weight in the youngest expanded leaf blade, respectively. Such differences in B uptake cannot easily be explained through differences in water use, as Passioura (1977[104]) reported for wheat that the water use efficiency of 13 cultivars ranged only from 3.1 to 4 g dry matter kg^{-1} water.

The apparent contradiction, between *in vitro* results that suggest that B uptake is a passive process, and the field results that demonstrate significant differences among species and genotypes, is difficult to reconcile but is of fundamental importance to studies of B nutrition. Mechanisms that have been postulated to explain this apparent paradox include active uptake, exudation of B complexing compounds into the rhizosphere, species differences in B binding compounds, such as pectins in the cell walls, physical barriers in the roots, and species differences in membrane permeability (Huang and Graham, 1990[60]; Hu and Brown, 1997[57]).

Evidence for passive boron uptake

As early as 1980 Raven (1980[115]) postulated that B uptake would occur via passive diffusion since in physiological pH range boric acid would have a theoretical lipid permeability coefficient of 8×10^{-6} cm s^{-1}, which is adequate to satisfy the B need of the plant. Only very recently have these theoretical calculations been verified and the specific mechanisms by which B crosses artificial lipid membranes, as well as plant and animal membranes, have been tested (Dordas and Brown, 2000[29]; Dordas et al., 2000[30]).

Using artificial liposomes made of phosphatidylcholine (PC), Dordas and Brown (2000[29]) measured the permeability coefficients of boric acid (Pf_b), urea, glycerol (non-electrolytes of similar size) and water. They calculated the Pf_b to be 4.9×10^{-6} cm s^{-1} which closely corresponds to the theoretical values estimated from ether-water partitioning coefficients and also from the molecular weight and number of H-bonds of B (Raven, 1980[115]). When vesicle characteristics, such as sterol (STL) composition, type of phospholipids, the presence of head groups and length of fatty acid chains were modified, Pf_b was also affected. With a decreasing PC/STL ratio (from 100% PC to 60% STL), the Pf_b was reduced from 5×10^{-6} to 1×10^{-6} cm s^{-1}. These results are consistent with those shown previously for other non-electrolytes, where the presence of STL in the membrane can substantially reduce membrane fluidity, increase mechanical coherence and hence suppresses passive transmembrane permeability (Mouritsen et al., 1995[91]).

In studies utilizing membranes isolated from squash roots, Dordas et al. (2000[30]) determined Pf_b to vary from 3.9×10^{-7} to 2.4×10^{-8} cm s^{-1} in plasma membrane and plasma membrane-depleted vesicles, respectively. In the charophyte alga, *Chara corallina,* Stangoulis et al. (2001[140]) determined Pf_b of 4.4×10^{-7} cm s^{-1} across the combined plasmalemma and tonoplast membrane, which is in good agreement with values from squash liposomes. These values, however, are 20- to 300-fold slower than the permeability predicted by Raven (1980[115]) or determined experimentally in artificial liposomes (Dordas and Brown, 2000[29]). The lower Pf_b found in this study (Dordas et al., 2000[30]) compared to that calculated from the ether-water partition coefficient can occur because of the effect of lipid composition on the membrane properties and permeability. Sterols are major components of plant membranes, and are known to have a strong effect on the permeability of water and non-electrolytes (Schuler et al., 1991[129]; Lande et al., 1995[74]). Plant sterols reduce water permeability to an even greater extent than cholesterol (Schuler et al., 1991[129]) and it is expected that these sterols will have similar effects in reducing membrane permeability for boric acid or other non-electrolytes.

The hypothesis that changes in membrane composition directly affect permeability of B and hence plant B uptake, is supported by experimentation conducted with mutant lines of *Arabidopsis thaliana* differing in membrane lipid composition (Dordas and Brown, 2000[29]). In these experiments, B uptake

varied significantly in a manner that was consistent with results from *in vitro* studies (Dordas and Brown, 2000[29]).

The results of Brown and coworkers (Dordas and Brown, 2000[29]; Dordas et al., 2000[30]; Stangoulis et al., 2001[140]) verify the principle that substantial movement of B occurs through passive membrane diffusion and are consistent with the theory that B uptake is a passive process. These experiments also provide a biophysical explanation for observed differences in B uptake among several species and cultivars, grown under identical conditions (Hu and Brown, 1997[57]; Nable et al., 1997[95]). The results also demonstrate that membrane composition, membrane origin and cultivar differences all have a profound impact on the rate of B permeability. The presence of substantial membrane permeability does not, however, preclude a role for membrane proteins in the facilitation of transmembrane B movement. Indeed, the permeability of water through plant membranes is known to be substantially faster than the permeability of B and yet numerous membrane intrinsic proteins (Aquaporins) are known to be involved in water uptake (Weig et al., 1997[154]).

The possible involvement of channel proteins in B transport was recently examined by Dordas et al. (2000[30]) and Nuttall (2000[97]) utilizing a variety of methods including the application of channel inhibiting mercury and expression of major intrinsic membrane proteins (MIP) in *Xenopus laevis* oocytes. The inhibition of ion transport following application of $HgCl_2$ is widely interpreted as evidence of the involvement of channel proteins in the uptake (Barone et al., 1997[4]). Dordas et al. (Dordas and Brown, 2000[29]; Dordas et al., 2000[30]) demonstrated that B uptake into squash membrane vesicles was inhibited by $HgCl_2$ and that this effect could be reversed by the application of 2-mercaptoethanol. These results were replicated in *in vivo* studies conducted with squash and *Arabidopsis* where the presence of $HgCl_2$ reduced B uptake by 35 % (Dordas and Brown, 2000[29]).

The hypothesis that channel proteins are involved in B transport was further supported by experiments in which certain MIPs with homology to non-electrolyte transporting channels from other species were expressed in *Xenopus laevis* oocytes and B permeability subsequently determined (Dordas et al., 2000[30]). The expression of one of the MIPs (PIP1), resulted in a 30 % increase in Pf_b and this benefit could be reversed with the addition of $HgCl_2$. The effectiveness of additional MIP's at increasing B transport was verified by Nuttall (2000[97]) who demonstrated that expression of PIP1b, PIP2a and PIP2b proteins in *Xenopus* oocytes significantly increased the permeability of the oocyte membrane to boric acid, from below the detection limit to 1×10^{-8} cm s^{-1}. PIP1c did not mediate boric acid transport, despite the fact that it increased the water permeability of oocytes, which was interpreted as evidence of functional expression of PIP1c proteins in the oocyte plasmalemma.

The evidence that substantial B movement can occur through diffusion and channel mediated transport is compelling and could account for B uptake under conditions of adequate or greater B supply. However, this evidence does not address the conditions that might exist when B is at or below adequate concentrations in the rooting medium, conditions that might induce specialized mechanisms for B uptake.

Evidence for active carrier mediated boron uptake

Prior to 1997 there had been only scattered reports of metabolically dependent B uptake and many of these experiments were technically flawed or could not be reproduced (for review see Hu and Brown [1997[57]]). Few, if any, of these early studies utilized sufficiently low levels of B supply or included pretreatment under B deficient conditions. All experiments were limited by difficulties in B determination in the low concentration range and by the lack of a convenient isotope for determination of short-term uptake kinetics. Recently, a series of studies that address these problems have been conducted by Dannel, Pfeffer, and Römheld. These experiments contribute substantially, to our knowledge, of B uptake and suggest that under conditions of restricted B supply, B is assimilated through a metabolically active, carrier mediated transport process.

The first evidence for the occurrence of an active B transport system was presented by Dannel et al. (1997[27]) who observed B concentrations in the root cell sap (consisting of cytoplasmic, vacuolar and apoplastic solutions) and the xylem exudate of sunflower, that were about 22-fold higher than the concentration of B in the rooting medium (1 μM B). These results were interpreted as evidence of an energy dependent transport mechanism capable of accumulating B against a concentration gradient. Subsequent studies (Pfeffer et al., 1999[107]) demonstrated that the putative concentration mechanism for B is suppressed by 24 h of high B supply (100 μM). The energy dependence of this mechanism was subsequently demonstrated by application of the metabolic inhibitors 2,4-dinitrophenol, or low root zone temperature which resulted in a suppression of the B concentrating mechanism (Pfeffer et al., 1999[107]). This suggests that the establishment of a concentration gradient for B between external solution and the inside of the cell is directly or indirectly dependent on metabolic energy. In a more detailed study, using stable isotopes and various B concentrations, Dannel et al. (2000[26]) characterized B uptake in sunflower in greater detail. The results suggest that low B supply during plant pre-culture stimulates B uptake, which can be eliminated by metabolic inhibition treatments. At higher B supplies, uptake then followed a non-saturable linear kinetics suggesting that B uptake occurs by two processes, a saturable carrier mediated transport at low concentrations and a non-saturable diffusion driven process at higher concentrations.

In a series of short-term uptake studies, Pfeffer et al. (2001[109]) suggested that B uptake at low B supply follows Michaelis-Menten kinetics with an apparent K_m of 15 μM and V_{max} of about 31 nmol g_{root} FW^{-1} h^{-1}. Experiments on B uptake have also been conducted by Stangoulis et al. (2001[140]) using the charophyte alga *Chara corallina*. Following at least one day of B starvation, B uptake into *Chara* cells appeared to be a combination of a saturable and a linear component, and at 0 to 10 μM B supply uptake followed Michaelis-Menten kinetics with an apparent K_m of about 2 μM and V_{max} of about 135 pmol m^{-2} s^{-1}.

These experiments are the first detailed investigations of B uptake at conditions of moderate B supply (< 10 μM) and provide evidence that an active B uptake process may function in plants. This conclusion, however, must be considered with caution since cytoplasmic concentrations of B cannot yet be directly determined. The proposed K_m for the putative high affinity B transporter (15 μM) is also much higher than the

Table 1 Comparison of maximum potential contribution of passive uptake rate vs. actual relative uptake rate of B in canola and tobacco

Species	Boron supply (μM)	Relative uptake rate (nmol · g^{-1} FW d^{-1})	Permeability coefficient (cm · s^{-1})	Root surface area (cm^2)*	Maximum passive permeation rate (nmol · g^{-1} FW d^{-1})
Canola	0.1	5	2.4×10^{-8}**	700	0.15
	0.1	5	3.9×10^{-7}***	700	2.4
	0.97	96	2.4×10^{-8}	700	1.5
	0.97	96	3.9×10^{-7}	700	24
	10	100	2.4×10^{-8}	700	15
	10	100	3.9×10^{-7}	700	240
Tobacco	10	20	2.4×10^{-8}	700	15
	10	20	3.9×10^{-7}	700	240

Permeability coefficients determined in squash from Dordas et al. (2000[30]).
* Root surface area is calculated by assuming root hair surface to be 2 · 5-fold root surface and 1 cm^3/g of root.
** Permeability coefficient of plasma membrane-depleted vesicles.
*** Permeability coefficient of Plasma membrane vesicles.

minimal concentration of B at which plant growth can be maintained (Asad et al., 1997[2]) and is significantly higher than the K_m of other micronutrients required in equivalent concentrations by the plant. For example, K_m was found to be 1.5 to 3 μM for Zn in maize and barley, respectively (Veltrup, 1978[150]; Mullins and Sommers, 1986[94]).

Boron uptake: Conclusions and future directions

One of the primary reasons cited against the occurrence of an active (energy dependent) high affinity uptake of B in plants, was the prediction by Raven that the theoretical permeability of B was such that adequate B uptake would occur by a process of passive diffusion through the membrane (Raven, 1980[115]). The predictions of Raven can now be verified by determination of B permeability coefficients across plant membranes (Dordas et al., 2000[30]) and B uptake in plants grown under carefully controlled experimental systems (Asad et al., 1997[2]; Bellaloui et al., 1999[7]).

In Table **1**, we have estimated potential B permeation rates under a variety of external B concentrations in tobacco and canola, for which data on plant B demand have been experimentally determined. These calculations were derived using the measured Pf_b from the membranes of squash, a species that is relatively sensitive to B deficiency. The calculations also presume a constant supply of external B and a constant internal B concentration of 0 μM, hence this represents the maximal potential permeation rates. These data demonstrate that passive permeation of B would be adequate to provide the observed B requirement for both canola and tobacco under normal conditions of B supply (e.g., 10 μM B). However, if B is reduced to 1 μM or Pf_b is decreased to 2.4×10^{-8} cm s^{-1} (as found in plasma membrane-depleted membranes) then passive B permeation is inadequate to satisfy the B requirements of these species.

These calculations support the contention that at low levels of B an active B uptake mechanism may exist. These results also illustrate the very significant effect that small variations in Pf_b, external B supply, plant growth rate or root characteristics (diameter, length) will have on B uptake. The presence of substantial passive permeability under conditions of adequate B supply (> 10 μM B), however, does not preclude a role of active uptake mechanisms in B acquisition at these concentrations. Indeed in *Chara australis,* which has a plasma membrane permeability coefficient for urea that is significantly higher than that for boric acid (Wilson and Walker, 1988[156]), the uptake of urea is known to be facilitated by a high affinity, electrogenic symport with sodium at low concentrations (20 μM) (Wilson et al., 1988[155]; Wilson and Walker, 1988[156]; Walker et al., 1993[152]).

Evidence to support the occurrence of a saturable, active B uptake mechanism is still preliminary, primarily owing to our inability to directly measure cytoplasmic B concentrations or to directly monitor real time B uptake. These results should, therefore, be interpreted with caution and alternative explanations for the reported results should be considered. In this regard the unique physical and chemical characteristics of B described above may provide some insight. The uptake kinetics of B would be greatly complicated by the abundance of potential B complexing molecules in plant cell wall and cytoplasm. At low B supply and specifically following a period of B deprivation, many of these potential B complexing molecules would not be associated with B. With the addition of B at the commencement of a short-term uptake period, there would be a strong driving force as the unassociated B binding sites become saturated, effectively reducing the concentration of free B and maintaining a driving force for further uptake.

The effect of cytoplasmic B binding molecules on B uptake has been demonstrated experimentally in transgenic tobacco plants, genetically engineered to produce enhanced concentrations of sorbitol, a B complexing molecule usually not found in tobacco (Bellaloui et al., 1999[7]). The production of sorbitol in transgenic tobacco significantly increases B uptake, tissue B concentration and B transport to meristematic tissue, when compared with plants not containing sorbitol (Bellaloui et al., 1999[7]). These results suggest that B complexing molecules present in the cytoplasm may facilitate B uptake by maintaining a favourable gradient for B diffusion into the plant. Such a complexation driven uptake process would result in uptake kinetics that significantly complicate the interpretation of uptake data. A more detailed analysis of the quantity and characteristics of putative B binding molecules would contribute to the resolution of this issue.

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