Proposed Physiologic Functions of Boron in Plants Pertinent to Animal and Human Metabolism

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Boron has been recognized since 1923 as an essential micronutrient element for higher plants. Over the years, many roles for boron in plants have been proposed, including functions in sugar transport, cell wall synthesis and lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid metabolism, phenol metabolism and membrane transport. However, the mechanism of boron involvement in each case remains unclear. Recent work has focused on two major plant-cell components: cell walls and membranes. In both, boron could play a structural role by bridging hydroxyl groups. In membranes, it could also be involved in ion transport and redox reactions by stimulating enzymes like nicotine adenine dinucleotide and reduced (NADH) oxidase. There is a very narrow window between the levels of boron required by and toxic to plants. The mechanisms of boron toxicity are also unknown. In nitrogen-fixing leguminous plants, foliarly applied boron causes up to a 1000% increase in the concentration of allantoic acid in leaves. In vitro studies show that boron inhibits the manganese-dependent allantoate amidohydrolase, and foliar application of manganese prior to application of boron eliminates allantoic acid accumulation in leaves. Interaction between borate and divalent cations like manganese may alter metabolic pathways, which could explain why higher concentrations of boron can be toxic to plants.

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**Boron Requirement for Plants**

Boron was proven an essential micronutrient for higher plants by Warington in 1923 (1). Since that time, boron nutrition has been studied extensively; and although several roles of boron have been proposed, its exact metabolic function has not been determined. The quantity of boron required by plants and found in their tissues shows clear-cut variation within the plant kingdom. Species from the Gramineae require and contain less boron than the other monocots and all dicots. The Gramineae are also unique in several other ways. Graminaceous plants contain large quantities of silicon and much less pectin in their cell walls than other monocots and the dicots (2). Loomis and Durst (3) point out that graminaceous plants are uniquely resistant to 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid, while dicots and most other monocots are sensitive to these auxin-type herbicides. Another unique property of the Gramineae is their strategy for obtaining iron, termed strategy II. Strategy II involves the secretion of phytosiderophores, while other monocots and all dicots use a turbo-redox system to reduce Fe" to Fe^2+ in the rhizosphere prior to uptake (4). Perhaps an examination of these differences could provide some clues for the role of boron in plants.

In a recent review, Loomis and Durst (3) list the roles of boron in plants that have been postulated over the years. These roles involve sugar transport, cell wall synthesis and lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid metabolism, phenol metabolism, membrane functions, and DNA synthesis (Table 1). Unfortunately, the mechanism of boron action in each case remains unclear. At the 1992 North American Workshop on Boron in Plants and Soil (University of Missouri, Columbia), it was concluded that boron might serve two primary roles—one in cell wall structure and one in membrane function. In 1988, Durst demonstrated that 96 to 99% of the total boron in cultured carrot cells was localized in cell walls, with only 0.7 to 4.1% present in the protoplasts (5). There is strong evidence that boron is involved in lignin biosynthesis and in cell wall cross-linking. Both were discussed in detail by Loomis and Durst (3). In addition, there is evidence that, in plants, boron nutrition affects cell wall structure; plants grown on sufficient boron bend easily while plants that are grown on low boron are brittle (3).

A significant amount of boron is present in membranes. Tanada (6) reported that the concentration of boron in a mung bean protoplast membrane fraction compared to that in whole protoplasts was 20.1 and 1.8 μg boron/mg protein, respectively. In plants, boron nutrition affects membrane function. Early evidence of this phenomenon came from Parr and Loughman (7) who found that boron increased membrane transport of chlorine (Cl) and phosphorus. These results were explained as a boron stimulation of the plasmalemma ATPase.

**Table 1. Proposed roles of boron in higher plants.**

| Cell walls | Metabolism |
| --- | --- |
| Carbohydrate | Synthesis |
| RNA | Structure |
| Auxin | Phenolic metabolism |
| Phenolic | Lignification |
| Respiration | Transport |
| Membrane | (long distance) |
| Structure | Membrane |
| Sugars | Function |
| Auxin |

*Based on reviews by Gauch and Dugger (20), Loomis and Durst (3), and Parr and Loughman (3).
Other studies have shown that supplemental boron stimulates proton pumping in plants, causes hyperpolarization of the membrane potential, and increases K⁺ uptake (2,8-12). Along with these findings, there have been reports of a boron-mediated increase in ferricyanide reduction (2,8-11).

**Possible Effects of Boron on Plant Membrane Functions**

Proposed changes in membrane functions induced by boron deficiency may result from primary changes in membrane structure. This concept was suggested by Donaire and colleagues in several papers that described the effects of boron on lipid biosynthesis, lipid oxidation, and proton pumping (10,11,13,14). They observed that treating sunflower seedlings with 10 μM boron caused a decrease in neutral lipids and an increase in linolenic acid content, as compared to zero boron controls (14). If boron caused a change in the type of lipid synthesized, or if the availability of boron influenced the bridging of lipids via hydroxyl groups, then boron could affect membrane structure and function. Very little information is available on the role of boron in lipid biosynthesis. No boron–lipid complexes have been isolated from plant membranes.

Other evidence indicates that the effects of boron on membrane function may be associated more directly with proton pumping or changes in a redox system in the plasmalemma. Both the hyperpolarization of the membrane potential (in sunflower roots) and the stimulation of the ferricyanide reduction (in carrot cells) could be related to the activity of NADH oxidase, an enzyme that could provide protons for export (15,16). In 1991, Barr and Crance (17) reported that the activity of plasmalemma NADH oxidase was stimulated by boron. The biologic role of NADH oxidase in plant metabolism and the natural electron acceptor are unknown, but ascorbate free radical (AFR) has been postulated as a likely substrate. NADH oxidase has been referred to as AFR reductase, and may be a part of a larger membrane-spanning, oxido-reductase system (18,19).

The possible connection between boron stimulation of NADH-oxidase activity and the production of reduced ascorbate is intriguing. It could mean boron involvement in ascorbate metabolism and consequential involvement in an important aspect of the redox reactions that affect cell wall extension and membrane transport. In most plants, the first symptom of boron deficiency is impaired root growth. The meristematic region is affected, especially cellular elongation (20). In 1991, Lin and Varner (21) suggested an involvement of ascorbate and/or dehydroascorbate in cell wall loosening, allowing expansive growth. In addition, Hidalgo et al. (22) demonstrated an AFR-mediated increase in cell size in meristematic and elongation zones in onion roots; and Morré et al. found that inhibition of ascorbate metabolism blocked growth (19).

**Hypothesis**

Our investigation of the possible link between boron and ascorbate metabolism was based on the hypothesis that if boron stimulates AFR reduction to ascorbate, and thereby stimulates growth, then ascorbate supplementation may help maintain root growth in boron-deficient plants. Squash seedlings were grown with low and normal boron concentrations in hydroponic culture, and plants from each group were supplemented with 250 μM ascorbate. We found that root growth in boron-deficient medium in the presence of ascorbate was equal to that of boron-sufficient controls and was double that grown with low boron and without supplemental ascorbate. The effect of ascorbate on root growth was negatively correlated with the concentration of boron in the medium. This indicates that a direct interaction may exist between boron and ascorbate.

Besides its function in plant growth processes, boron plays a definite role in pollen germination and pollen tube growth (23). Defined media for pollen germination usually contain sucrose, boron, and calcium. Recently, Robbertse et al. (24) showed that petunia pollen tubes grew toward an increasing boron concentration, seemingly exhibiting a "boronotropic" response. Again, the mechanism of boron action in pollen germination and pollen tube germination is unknown. Boron is also required for diatoms, some species of marine algal flagellates and for Cyanobacteria with heterocysts capable of nitrogen fixation (3).

**Toxicity**

Most essential micronutrient elements have a fairly narrow window for concentrations between optimal and toxic. Boron is no exception. In fact, within the plant kingdom there is a range of sensitivity to boron, depending on the concentration as well as the time and method of application. Many species that respond favorably to boron have very low tolerance for boron applied with the seed at planting. This problem can be solved by incorporating boron into the soil several weeks prior to planting. Necrotic spots commonly form on leaves following foliar application of boron; however, small quantities of boron (<1/3 kg boron/ha) can be applied safely (25). The mechanisms of boron toxicity are unknown. In our research, foliar application of boron on soybean plants resulted in an alteration of nitrogen metabolism. Allantoic acid, the dominant transport molecule for nitrogen in nitrogen-fixing soybeans, was elevated by up to 1000% in leaves sprayed with boric acid when compared to control leaves sprayed with water (26).
The amide asparagine was also elevated in boron-treated leaves. Because allantoate amidohydrolase, which catalyzes the breakdown of allantoic acid, is a manganese-activated enzyme, we proposed that excess boron acid might interfere with the availability of manganese. We found that both boronic acid and asparagine were effective inhibitors of the allantoate amidohydrolase, and increased manganese concentrations were required to overcome the inhibition in in vitro assays (Figure 1). Furthermore, supplemental manganese added to foliar sprays of boronic acid in the field prevented the accumulation of allantoate and resulted in allantoic acid concentrations in the leaf tissue that were equal to or below those in leaves of controls not treated with boron acid (Figure 2). It is possible that boron may alter metabolism by regulating activation of other manganese enzymes. In fact, boron regulation of metal-activated enzymes is a plausible explanation for many of the postulated functions of boron mentioned above. Boron has been shown to inhibit certain enzymes, e.g., 6-phosphogluconate dehydrogenase and aldolase, by binding to substrates or enzyme substrate complexes.

Conclusions
Despite extensive research, the role of boron in plants has not yet been resolved. Boron, along with other metalloids, like silicon and germanium, has properties intermediate to those of metals and nonmetals. The ongoing work on boron involvement in cell wall structure and in membrane structure and function may be a key to defining the principal function of boron in plant growth processes and physiology. It is likely that at least some of the mechanisms of boron action in plants will be the same or related to the role boron plays in animal and human metabolism.

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