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BORON IN PLANT STRUCTURE AND FUNCTION

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ABSTRACT

New and exciting developments in boron research in the past few years greatly contributed to better understanding of the role of boron in plants. Purification and identification of the first boron-polyol transport molecules resolved much of the controversy about boron phloem mobility. Isolation and characterization of the boron-polysaccharide complex from cell walls provided the first direct evidence for boron crosslinking of pectin polymers. Inhibition and recovery of proton release upon boron withdrawal and restitution in plant culture medium demonstrated boron involvement in membrane processes. Rapid boron-induced changes in membrane function could be attributed to boron-complexing membrane constituents. Boron may affect metabolic pathways by binding apoplastic proteins to *cis*-hydroxyl groups of cell walls and membranes, and by interfering with manganese-dependent enzymatic reactions. In addition, boron has been implicated in counteracting toxic effects of aluminum on root growth of dicotyledonous plants. Molecular investigations of boron nutrition have been initiated by the discovery of a novel mutant of *Arabidopsis thaliana* with an altered requirement for boron.

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Introduction

World-wide, boron deficiency is more extensive than deficiency of any other plant micronutrient (39, 103). It is particularly prevalent in light textured soils, where water-soluble boron readily leaches down the soil profile and becomes unavailable for plants (131). Adequate boron nutrition is critical not only for high yields but also for high quality of crops. Boron deficiency causes many anatomical, physiological, and biochemical changes, most of which represent secondary effects. Because of the rapidity and the wide variety of symptoms that follow boron deprivation, determining the primary function of boron in plants has been one of the greatest challenges in plant nutrition.

There are excellent reviews summarizing boron research from agricultural and physiological perspectives (20a, 21, 22, 31, 32, 39, 67, 70, 73, 77, 96, 98, 100, 115, 121, 122). One comprehensive review of the early work (32) was divided into 15 separate sections, each describing a different possible site of boron action. Some later reviews proposed unifying hypotheses that pulled together many of the effects of boron on plant growth and developmental processes (67, 73). In the absence of conclusive evidence, however, the actual role of boron in plants remained speculative.

In recent years, research has progressed to the point where it is possible to demonstrate boron involvement in three main aspects of plant physiology. Thus, this chapter features an in-depth look at a structural role for boron in cell walls, a role for boron in membrane function, and boron involvement in metabolic activities. Analysis of these aspects of boron nutrition provides explanations for several of the controversial areas in the literature. In addition, following the recent breakthrough in isolation, purification, and identification of boron-containing compounds from plant tissues, this review presents and discusses the first direct evidence for boron-bound molecules in plants.

History

In 1910, Agulhon (1) reported that several diverse plant species contained boron but did not claim that boron was essential (70). Subsequently, Mazé (82, 83) claimed that boron, aluminum, fluoride, and iodine were essential, but his experimental techniques have been questioned. Therefore, Warington (132) is credited with the first definitive proof of boron requirement for a higher plant. A key observation by Warington was that plants required a continuous supply of boron, an important concept for today's understanding of boron function in growth. Following Warington (132), Sommer & Lipman (123) established the boron requirement for six nonleguminous dicots and for one graminaceous plant, barley.

Shortly after boron was introduced as an essential element for higher plants, structural damage described as cracked stem of celery, stalk rot of cauliflower, heart rot and internal black spot of beets, top rot of tobacco, internal cork of apples, and yellows of alfalfa, was attributed to boron deficiency (32). Application of boron fertilizer became a common practice for production of several horticultural crops, sugar beets, and alfalfa. This led to an observation that the boron requirement among species was highly variable, and that the optimum quantity for one species could be either toxic or insufficient for other species. Based on their boron requirement, plants could be divided into three groups: graminaceous plants, which have the lowest demand for boron; the remaining monocots and most dicots with an intermediate requirement; and latex-forming plants, with the highest boron requirement among plant species (84). Another important classification was made by Shkolnik (121), who subdivided dicots and monocots based on stage of growth and localization of boron deficiency symptoms. In some boron-deficient dicots (sunflower, tomato, squash, alfalfa), inhibition of root growth and degeneration of meristematic regions appear quickly and simultaneously; in other dicots (pea, soybean, lupine), degeneration of growing points is delayed. Some monocots (maize, sorghum, millet, spiderwort, onion) are able to maintain normal root growth and vegetative growth in boron-free conditions much longer then dicots. The small grains and grasses (rye, oat, wheat, timothy) have normal vegetative growth and show boron deficiency symptoms only during formation of reproductive organs. High demand for boron during reproductive growth is a common feature among plant species and is discussed in a different section (see "Reproduction, Pollen Tube Growth, and Pollen Germination").

Other than vascular plants, boron is required by diatoms, some species of marine algal flagellates, and *Cyanobacteria* dependent on heterocysts for nitrogen fixation (9, 10, 32, 78). As for humans, Nielsen et al (91, 92) presented evidence that boron may be beneficial, especially for calcium retention by older women; however, a strict requirement has not yet been established.

Boron Phloem Mobility and Transport Molecules

In vascular plants, boron moves from the roots with the transpiration stream and accumulates in growing points of leaves and stems. It has been suggested that these local concentrations in apical tissues led to the evolutionary development of dependency on boron for some aspects of metabolism in plant meristems (72). Once in the leaves, boron retranslocation is restricted and it becomes fixed in the apoplast. Based on this pattern, boron is generally considered phloem immobile. However, tracer studies with stable isotope ^{10}B demonstrated that in some fruit trees, foliar application of boron in the fall temporarily increased boron concentration of leaves, but during late fall and winter boron moved to the

bark. In the spring, the boron moved from the bark into flowers and resulted in increased fruit set. This movement of boron required phloem transport (40–42).

Besides fruit trees, phloem transport of boron was reported for some *Brassicas*, radish, cauliflower, and rutabaga (114, 116–119). Subsequently, Hu & Brown (49) evaluated boron mobility in some species within the genera of *Pyrus*, *Malus*, and *Prunus* and connected their phloem boron transport with the key fact that these species transported carbon as polyols. Since the beginning of the twentieth century, chemists have used polyols, such as glycerol or mannitol, to enhance the acidity of borate solutions. The basis for these reactions is the ability of borate to form cyclic diesters with some diols and polyols. Brown and associates (51) isolated and characterized soluble sorbitol-boron-sorbitol complexes from the floral nectar of peach, and mannitol-boron-mannitol complexes from phloem sap of celery. This was the first isolation and identification of boron transport molecules in plants. Brown's group also obtained evidence for phloem boron movement in species transporting dulcitol (51). These results explain much of the confusion about boron phloem mobility in plants. We can now conclude that phloem movement of boron depends on the sugar or polyol transport molecules used by the particular plant. It could be of interest that a major crop in the United States, soybean, contains large quantities of the polyol pinitol and shows some response to foliar applications of boron (38, 101, 112). In the future, perhaps phloem sap from soybean will be analyzed for pinitol-B-pinitol complexes.

Role for Boron in Cell Wall Structure

The primary cell wall of higher plants is an important factor determining cell size and shape during plant development. The mechanical properties of growing cell walls can be modified by crosslinks between their major components, cellulosic polymers, and matrix polymers such as hemicellulosic and pectic polysaccharides (15). Over the years, researchers have observed a close relationship between the primary cell walls and boron nutrition. Up to 90% of the cellular boron has been localized in the cell wall fraction (70). The first symptoms of boron deprivation include abnormalities in cell wall and middle lamella organization (48, 70, 79). Recently, formation of borate esters with hydroxyl groups of cell wall carbohydrates and/or glycoproteins has been proposed as a mechanism for crosslinking cell wall polymers (70). Borate bridging could explain many of the characteristics of boron-deficient and boron-toxic plants. This type of bonding could account for brittle leaves of boron-deficient plants, while plants grown with supraoptimal levels of boron produce leaves that are plastic or elastic in their response to bending (32, 48, 70). In addition, the slipping and sliding properties of "slime" (17, 109), permitted by the H-bonding of

hydroxyl groups on borate molecules and the hydroxyl groups of the polyvinyl alcohol, could explain the properties of primary cell walls at early stages of development (15).

The specific plant molecules that could participate in borate bridging of cell wall polymers were discussed at length by Loomis & Durst (70). Borate forms the most stable diesters with *cis*-diols on a furanoid ring. The compounds in plants that have this configuration are limited to ribose and apiose. According to Loomis & Durst (70), apiose, found in cell walls of a variety of plant species within both dicots and monocots (16), can be the key sugar moiety for borate-crosslinking cell wall polymers, while ribose, present in abundance in ribonucleotides, is likely involved in the chemistry of boron toxicity (70). Another possible candidate for borate cell wall bridging is fucose. Diatoms and certain algae that require boron contain fucose in walls (5, 70). Higher plants, other than the gramineae, have cell wall xyloglucans and rhamnogalacturonans with terminal fucose moieties (5, 16, 44). In addition, many of the glycoproteins secreted into the apoplast are fucosylated (56). It is noteworthy that mutants of *Arabidopsis thaliana*, which lack fucose, have brittle leaves (104), a condition found in boron-deficient plants (70).

It was an early observation that plant boron content was closely correlated with pectin (32). In 1961, Ginsburg (33) showed that a strong chelator, EDTA, mixed with a weak chelator (e.g. IAA), was effective in causing cell separation by removing the pectin/protein matrix, but borate buffer kept the matrix intact longer than any other buffer. Clarkson & Hanson (19) proposed that by forming crosslinks in pectin, boron protects Ca in the cell wall. Results that supported this idea came from Yamanouchi (135) and Yamauchi et al (136), who found that boron-deficient cell walls of tomato contained less calcium. We could hypothesize that the hydroxyl H-bonding and borate ester formation may pull carboxylate groups of polymers into close proximity and allow calcium or magnesium binding by the polymers. In this theme, based on a sophisticated growth analysis of pine cell cultures supplied with various concentrations of boron, calcium, and magnesium, Teasdale & Richards (127) proposed that plant cell wall acceptor molecules efficiently bind calcium after loosely binding boron. The acceptor molecules also bind magnesium competitively with calcium. Teasdale & Richards' work (127) supports the idea that borate esters, formed with hydroxyls of sugars (like apiose or fucose) on pectin or glycoprotein polymers, provide areas for the chelation of calcium or magnesium in cell walls.

Shortly after Loomis & Durst (70) presented their model for boron bridging cell wall polymers, with apiose being the key sugar moiety binding borate, Matoh et al (80) isolated the first boron-polysaccharide complex from driselase-treated radish root cell walls. The complex had a molecular weight of 7.5 kDa

and contained 0.232% boron, 52.3% uronic acid, 17.4% arabinose, 9.8% rhamnose, 4.9% galactose, and 0.3% xylose. Up to 80% of the cell wall boron was localized in this complex, and ^{11}B-NMR analysis suggested that the boron was present as a two-ligand borate-diol, BL2. Matoh's work was complemented by Brown and his group (48), who reported a correlation between pectin fraction and boron in cell walls of squash and tobacco, and by their survey of 14 plant species (50) showing very close correlation between the uronic fraction, pectin sugars, and boron content of the plant.

Kobayashi et al (59) purified the pectin fraction from radish root cell walls and isolated the first boron-containing pectic polysaccharide complex from plants, boron-rhamnogalacturonan-II (RG-II-B). This group also produced the first direct evidence for borate crosslinking two RG-II monomers, by demonstrating that the removal of boron from the RG-II-B complex reduced the molecular weight of the complex by half. Subsequently, Matoh et al (81) found the RG-II-B complex in cell walls of 22 other plant species, including two of *Brassicaceae*, three *Cucurbitaceae*, four *Leguminosae*, two *Apiaceae*, two *Chenopodiaceae*, two *Solanaceae*, two *Asteraceae*, one *Liliaceae*, one *Araceae*, two *Amaryllidaceae*, and three *Gramineae*. On the basis of this research, Matoh et al (81) proposed that RG-II may be the exclusive polysaccharide-binding boron in cell walls.

Using cultured sycamore cells, etiolated pea stems, and red wine as sources of RG-II-B complex, O'Neill et al (95) found that the borate ester was located on C-2 and C-3 of two of the four 3′-linked apiosyl residues of dimeric RG-II. The authors postulated that the dimeric RG-II-B covalently crosslinks the cell wall pectic matrix in dicots, nongraminaceous monocots, and graminaceous plants, though the pectin content of the grasses is much lower than that of the other species (95). Ishii & Matsunaga (52) and Kaneko et al (58) isolated and characterized RG-II-B complexes from sugar beet (a dicot) pulp and bamboo (a monocot) shoot, respectively.

Recently, research on RG-II-B complexes of the wall has moved into a new phase, addressing the formation of dimers from monomers and borate. According to O'Neill et al (95), in vitro dimer formation was increased by addition of strontium, lead, and cadmium, but calcium and magnesium were ineffective. The authors suggest that a catalyst, for example an enzyme, may be required for a rapid dimer formation by boric acid at physiological pH.

Other candidates for borate crosslinking in primary cell walls are hydroxyproline-rich glycoproteins and proline-rich proteins, e.g. extensin. It has been observed that cell walls of boron-deficient bean root nodules contain very low levels of hydroxyproline-rich proteins, compared with those of boron-sufficient controls (11). Interestingly, the mRNA of one of these proteins (an early nodulin called ENOD2) was present in the nodules, but evidently the proteins were not assembled into the wall structure. This is consistent with the work of

Jackson (53) on *Petunia* pollen tube growth, where without boron, proteins were secreted but not assembled into tube walls and therefore "lost" to the medium.

Membranes and Membrane-Associated Reactions

The evidence provided by recent cell wall studies explains many problems caused by boron deficiency. However, there are some aspects of plant boron nutrition that go beyond cell wall structure. These include rapid changes in membrane function induced by addition of boron to boron-deficient tissues.

Boron was first localized in maize root membranes by Pollard et al (99) and was later found in membrane fractions from protoplasts of mung bean by Tanada (125). Although the quantities of boron in membranes were not large, especially compared with those in cell wall fractions, they were significant for ion uptake. Uptake of rubidium (which is a potassium analog) by roots of *Vicia faba* was inhibited in boron-free solutions, and the problem was localized in the terminal 10-mm section of the root (107). Uptake of phosphorus, which was slow in boron-deficient roots of *Vicia faba*, was restored to normal levels when the roots were pretreated with boron for 1 h before the absorption experiment (107). Phosphorus, chloride, and rubidium uptake by boron-deficient roots of maize and *Vicia faba* was restored to 40% of normal within 20 min after boron was added to the rooting medium (99). Both uptake and efflux of phosphorus were decreased in boron-deficient sunflower roots, and both were restored within 1 hr after addition of boron (35). Goldbach et al (36) demonstrated that boron deficiency in suspension cultures of carrot and tomato caused a 50% reduction in the ferricyanide-induced net proton release. The inhibition was reversed within 60 min after addition of boron. This boron effect occurred only in the presence of auxin, so the authors concluded that boron was required for the auxin stimulation of ferricyanide-induced proton release. Vanadate suppressed the proton release, indicating that the plasmalemma proton pump was key to the process (36). In fact, Ferrol and coworkers (27, 28, 108) showed that boron deficiency inhibited the vanadate-sensitive H^+-ATPase in microsomes isolated from sunflower roots. Although immunoblotting showed that the quantity of the enzyme was not affected by boron deficiency, fluorescence anisotropy showed a difference between the membrane preparations from boron-deficient and boron-sufficient roots (26). This difference was interpreted as an increase in rigidity of boron-deficient membranes. In other studies, Lawrence et al (63) showed lower ATPase activity in plasmalemma-enriched vesicles from boron-deficient chickpea roots than in vesicles from control roots, and Obermeyer et al (94) reported boron stimulation of the plasmalemma ATPase from ungerminated pollen grains of lily.

Barr & Crane (3, 4) reported a boron effect on plasma membrane electron transport reactions and showed that the auxin-sensitive plasma membrane NADH oxidase was inhibited in boron-deficient carrot cell cultures. The authors

demonstrated that proton secretion associated with the H^+-ATPase was also decreased by boron deficiency, but not as severely as the ferricyanide-stimulated proton release. This means that ferricyanide activates transmembrane electron transport that is coupled to proton release only when boron is present. In the same work, Barr & Crane (3, 4) showed that addition of exogenous boric acid (with or without 2,4-D) to low boron cells caused an instantaneous stimulation of the plasma membrane NADH oxidase. This was the fastest boron response reported. An earlier report by Schon et al (113) indicated significant hyperpolarization of sunflower membranes within 3 min following the addition of boron.

In 1986, Morré et al (88) presented evidence that identified the activity of auxin-sensitive plasma membrane NADH oxidase as ascorbate free radical (AFR) oxidoreductase. This finding supported an earlier hypothesis of JC Brown (13) that boron nutrition was important in maintaining the "reducing atmosphere" in the apoplast to support ion uptake. By stimulating NADH oxidase, boron could be involved in keeping ascorbate reduced at the cell wall/membrane interface. It is noteworthy that both NADH oxidase activity and ascorbate have been linked with plant growth processes (37, 45, 68, 87, 88).

In summary, boron treatment of low-boron plants leads to hyperpolarization of root membranes, and stimulation of ferricyanide-dependent H^+ release, ATPase activity, NADH oxidase activity, and ion transport (3, 26, 28, 36, 71, 108, 113). Though these changes are associated with membrane function, several researchers have speculated that boron may be affecting physical properties of membrane proteins. In 1977, Pollard et al (99) suggested that rapid restoration of ATPase activity and potassium uptake by boron-deficient roots following supplementation with boron could be explained by boron complexing with polyhydroxy groups of membrane components. Marschner's group (14) demonstrated leakage of potassium, sucrose, phenolics, and amino acids in boron deficient sunflower leaves and discussed a role for boron in maintaining the integrity of plasma membranes. They proposed that boron stabilized the structure of the plasma membrane by complexing membrane constituents. Either H-bonding or ester formation with glycolipids or glycoproteins could easily keep enzymes or channels in an optimum conformation and anchored in the membrane. In agreement with this hypothesis, less phospholipid and galactolipid were found in membranes of boron deficient plants (121).

In addition, Shkolnik (121) observed that several enzymes, normally bound to membranes or walls in a latent form, become active when released under boron deficient conditions. These enzymes include ribonuclease, glucose-6-phosphate dehydrogenase, phenylalanine ammonia lyase, β-glucosidase and polyphenoloxidase. Release of these enzymes under boron-insufficient conditions could severely alter plant metabolism, deplete RNA, and increase phenolic

synthesis. Many of the phenolics are potent growth inhibitors (66), the same phenolics also inhibit ion uptake and thus retard membrane function (34).

The work of Shkolnik (121) indicates that although this response to boron deficiency is common in dicots, it has not been observed in graminaceous monocots. This raises a question of whether glycosylation of apoplastic proteins is different in graminaceous plants, or whether these proteins are less abundant in membranes and cell walls of grasses.

Reproduction, Pollen Tube Growth, and Pollen Germination

Based on the latest research, cell wall composition may be of primary importance in determining the quantity of boron required for growth. However, it has been observed that in most plant species the boron requirement for reproductive growth is much higher than for vegetative growth (32, 70). This is especially true for gramineaceous plants, which have the lowest cell wall pectin content and the lowest boron requirement to maintain normal vegetative growth, but need as much boron as other species at the reproductive stage. The physiological basis for the high boron demand for plant reproduction is not fully understood.

Boron requirement for reproductive growth in plants has long been recognized. Gauch & Dugger (32) cited over 70 references that reported boron effects on pollen germination, or on flowering and fruiting of plants. Boron deficiency caused sterility in maize and flower malformations in a wide variety of both monocots and dicots (32). Piland et al (97) found that boron treatment increased seed yield of alfalfa by 600%, while hay yield was increased by only 3%. Schmucker (110) found that pollen from a tropical species of *Nymphaea* failed to germinate in 1% glucose but germinated readily in a stigma extract. Later, he determined that the stigma extract contained borate and found that addition of boric acid to 1% glucose made it a very effective germination medium. Schmucker (111) proposed that boric acid was bound to hydroxyl-rich organic molecules, like sugars, and was involved in pollen tube wall formation.

Pollen grains of most species are naturally low in boron, but in the styles, stigma, and ovaries, boron concentrations are generally high (32). Visser (130) showed that a continuous and ample supply of boron was required for pollen tube growth, and speculated that the boron was complexing with cellular materials during the tube elongation process. Along this line, Johri & Vasil (57) demonstrated that boron was more critical for pollen tube elongation than for pollen germination.

Rapid growth of pollen tube depends on constant fusion of vesicles forming the plasmalemma, and continuous secretion of cell wall material. Jackson (54) proposed that the "capture" of secreted pollen proteins for membrane and wall building, proceeds through borate complexes with sugar residues.

Pollen of *Petunia* contains many glycoproteins, and the oligosaccharides of plant glycoproteins contain significant amounts of mannose and fucose, both known to form stable esters with borate. Jackson (54) studied protein secretion during pollen tube growth of *Petunia* at different temperatures. He observed that the phase change patterns of lipids in membranes was completely different when boron was present in the medium. This could be related to the fact that in the presence of boron, a greater proportion of the protein was assembled into the membrane and wall matrices. Jackson (54) also noted that pollen tube germination was completely inhibited at temperatures over 21°C unless boron was present. This could explain the importance of boron in reproductive growth of warm season crops, like maize (129).

Robbertse et al (106) found a boron gradient from the stigma through the style to the ovary and showed that pollen tubes of *Petunia* grew toward higher boron concentrations. Perhaps boron is a chemotactic agent for pollen tube growth through reproductive tissues. This idea is consistent with the high boron concentration generally found in female flower parts (32).

The results of pollen growth studies are consistent with boron-complexing cell wall polymers, while the lipid thermostability results show that boron is important in membrane structure and function. Whatever the mechanism, the role of boron in reproductive growth is particularly striking. The uniformly high boron requirement for reproductive growth across the plant kingdom is intriguing and indicates similarities between reproductive structures, so unlike cell walls, perhaps the composition of the pollen tube wall is similar across plant species. Gauch & Dugger (32) quoted Lohnis (69), who said that "it is quite conceivable it will be the study of pollen which may elucidate the very fundamental part boron plays in the biochemical processes."

Nitrogen Fixation

Boron was found essential for nitrogen fixation by heterocysts of *Anabaena* ACC 7119 (78). Loss of nitrogenase activity under boron-deficient conditions was explained by the destruction of nitrogenase by O_2. Altered O_2 status of the boron-deficient heterocysts was also implied in the increased activity of superoxide dismutase, catalase, and peroxidase in boron-deficient heterocysts (29). Heterocysts are morphologically and functionally distinct from vegetative cells and are capable of nitrogen fixation because they maintain a reducing (low O_2) environment (105). The low O_2 status in heterocysts is possible because of a thick envelope comprised of an inner layer of specific glycolipids (133). These glycolipids are absent from vegetative cells and therefore are predicted to provide the O_2 diffusion barrier in heterocysts (62). Mutants of *Cyanobacteria* deficient in these envelope glycolipids fail to fix nitrogen when assayed aerobically (43). Extraction and quantification of heterocyst envelope glycolipids

showed that within 6 h following boron removal, glycolipid content dropped by 33% and within 24 h was less than 1% of that found in boron-sufficient cells (30). It was suggested that boron stabilizes the inner glycolipid layer of heterocyst envelopes and thus retards O_2 diffusion.

In early work, Brenchley & Thornton (12) showed a major reduction in nodule number and in nitrogen fixation by inoculated boron-deficient fava bean. Vascular connections to the nodule were reduced, and so was the number of bacteria that changed into bacteroid. The authors speculated that under boron-deficient conditions, the symbionts may become parasitic. Results of this early study are consistent with the recent work by Bolaños et al (8). In boron-deficient pea nodules, the number of infected host cells was much lower then in sufficient controls. Host cells in boron-deficient plants developed enlarged and abnormally shaped infection threads, which frequently burst. Binding of the plant matrix glycoprotein to the cell surface of *Rhizobium leguminosarum* was inhibited by the presence of borate in the incubation buffer. The authors proposed that binding of matrix glycoprotein in the absence of boron may block the interaction between bacterial cell surface and the plant membrane glycocalyx. Developing soybean root nodules were more sensitive to low boron nutrition than large fully developed nodules (134). Both development and nitrogen fixation of young nodules were retarded after boron removal, while acetylene reduction rates remained unchanged in large nodules.

In the most recent study on bean root nodules (11), the ratio of hydroxyproline to cell wall dry weight was fivefold lower in boron-deficient nodules than in boron-sufficient controls. The levels of hydroxyproline-rich covalently bound ENOD2 protein were extremely low in walls of boron-deficient nodule parenchyma cells, although the Northern blot analysis showed that the mRNA was present in both boron-sufficient and -deficient nodules. The absence of the ENOD2 protein in the wall correlated with an irregular wall structure. The researchers concluded that a failure to incorporate the ENOD2 protein in the absence of boron could lead to wall abnormalities that prevent proper formation of the O_2 barrier, which protects the dinitrogenase complex and allows symbiotic nitrogen fixation.

Sites of Boron Action in Plant Metabolism

Primary cell wall structure and membrane function are now closely linked to boron nutrition. In contrast, boron role in plant metabolism is still a subject of considerable debate. Focusing on the diversity of early responses to boron deficiency, Lovatt & Dugger (73) postulated that boron can be involved in a number of metabolic pathways and can act in regulation of metabolic processes similarly to plant hormones. However, due to a lack of suitable information, boron function in metabolic events has never been properly evaluated.

There is substantial evidence supporting the association of boron with ascorbate metabolism. Among earlier studies, two reports on vegetable crops are particularly interesting. Chandler & Miller (18) found that rutabaga treated with boron had more ascorbate than untreated controls, and that following dehydration and storage, the boron-treated plants maintained about twice as much ascorbate. Mondy & Munshi (86) found that foliar application of borax on potatoes at 10 and 13 weeks after planting resulted in significantly greater quantities of ascorbate in tubers harvested 18 weeks after planting. Following storage for 6 months, discoloration of tubers harvested from boron-treated plants was decreased, and the decrease was attributed to lower phenolic concentrations and higher ascorbate concentrations in tubers from boron-treated plants.

One way boron could increase ascorbate concentration is through its effect on plasma membrane electron transport reactions. Barr and associates (3, 4) showed that boron instantaneously stimulated the auxin-sensitive plasmalemma NADH oxidase. This enzyme, also called ascorbate free radical oxidoreductase (88), catalyzes the transfer of electrons to ascorbate free radical. Inhibition of this process in the absence of boron could result in deprivation of reduced ascorbate. In our studies (74), inhibition of squash root elongation caused by inadequate boron nutrition was correlated with a decline in ascorbate concentration in root apices. However, no boron- or growth-related variation was observed in the concentration of ascorbate free radical and dehydroascorbate, the oxidized forms of ascorbate. This indicates that the decline in ascorbate concentration induced by boron deficiency cannot be ascribed, at least not in full, to boron interference with the redox cycle, but represents a decrease in the total pool of ascorbate in root meristems. It should be noted that ascorbate added to hydroponic medium promoted root elongation in the absence of boron and under low boron conditions. This shows that supplemental ascorbate can, to some degree, compensate for boron in root growth processes (74).

Another site of boron action that is not connected with a structural role in cell walls or membranes is auxin metabolism. Boron interaction with auxin has long been postulated, and although the issue remains controversial, it may be central to our understanding of the role of boron in plants. In the past, boron deprivation has been reported to cause IAA accumulation and toxicity (20, 55, 76, 85, 89) as well as IAA depletion and deficiency (23, 24, 120). The mechanisms of the responses have not been explained. In 1977, Bohnsack & Albert (7) demonstrated a 20-fold increase in IAA oxidation rate in root apices 24 h after boron was withheld from the nutrient medium. The authors attributed the increase to stimulation of the activity by high levels of IAA accumulated in boron-deficient tissues. However, Hirsch & Torrey (47) demonstrated that the ultrastructural changes caused by boron deficit were different from those resulting from IAA toxicity. Furthermore, boron deficiency symptoms were observed with unchanged or reduced IAA levels in apical tissues (25, 46).

Studies in our laboratory confirmed the sizable increase in IAA oxidation rate in boron-deprived squash root tips and ascribed it to boron interaction with the cofactors of IAA oxidase, manganese, and *p*-coumaric acid. It has been reported that foliar fertilization with boron resulted in lower leaf Mn concentrations in mint (124) and soybean (102). Previously, we observed boron inhibition of a manganese-dependent enzyme in soybean leaves, allantoate amidohydrolase (75). Boric acid, applied foliarly on field-grown nodulated soybeans, caused up to a 10-fold increase in allantoate concentration in treated leaf tissue. Accumulation of allantoate in response to boron was either eliminated or greatly reduced in plants presprayed with manganese. In vitro, the activity of partially purified allantoate amidohydrolase reflected the boron/manganese ratio in the incubation solution.

Recently, we observed that the activity of IAA oxidase in squash root apices depended on boron nutrition in a manner very similar to soybean allantoate amidohydrolase. IAA oxidation rate was low in root tips of boron-sufficient plants and high in plants grown without boron. Supplemental manganese had little effect on IAA oxidase activity in boron-deficient root tips but resulted in a substantial increase in the activity in boron-sufficient plants, showing that in the presence of boron more manganese was required for stimulation of the enzyme. The activity of IAA oxidase in excised root tips was modified by a change in the boron/manganese ratio during incubation (90; KM Lukaszewski & DG Blevins, unpublished observations).

IAA oxidation rate was also negatively correlated with root tip ascorbate content, which has been shown to depend on boron nutrition (74; KM Lukaszewski & DG Blevins, unpublished observations). Ascorbate added to the medium suppressed IAA oxidase activity in boron-deficient squash root meristems. Inhibition of IAA oxidase by ascorbate coincided with a decrease in the tissue concentration of *p*-coumaric acid, the monophenolic cofactor of the activity. Although the regulation of endogenous levels of IAA is not fully understood and the importance of IAA oxidase in IAA catabolism has been challenged by experiments with transgenic plants (2, 61, 93), in our studies we found a close negative correlation between root growth and oxidative degradation of IAA, both greatly affected by boron nutrition. In the future, it may be important to determine IAA levels in specific tissues and in the apoplast of cells in the elongation zone of boron-deficient and sufficient plants.

Amelioration of Aluminum-Induced Root Growth Inhibition

Aluminum is an important factor that impairs plant growth in acid soils by causing structural and functional damage to the roots. The mechanisms of aluminum toxicity are complex and not fully understood (60). The toxic form of aluminum in soil solution or nutrient medium is Al^{3+}, and this form is abundant at pH 4.0–4.5. Once aluminum is inside the plant, it is likely to be

in the form of aluminate $Al(OH)_3$, which is structurally similar to boric acid $B(OH)_3$ (6, 60).

Based on the similarities of the molecules and of the symptoms characteristic for aluminum-stressed and boron-deficient plants, most dealing with cell walls, membrane function, and root growth, it was proposed that aluminum may exert its toxic effect by inducing boron deficiency (6). The results from our laboratory supported this hypothesis. We showed that supplemental boron incorporated into acidic, high-aluminum subsoil promoted root penetration into this soil (64). In hydroponic culture, supraoptimal boron prevented aluminum inhibition of root growth at all criteria examined: root length, cell elongation, cell production rate, tissue organization and cell structure, root morphology, and maturation (65). This reaction may be limited to nongraminaceous plants, since Taylor & MacFie (126) showed that boron did not alleviate aluminum toxicity symptoms in wheat. However, as stated earlier, cell wall structure and boron requirement of graminaceous plants are different from dicots, and boron deficiency does not affect root growth of wheat.

Aluminum added to squash rooting medium caused a decline in root tip ascorbate concentration that was very similar to that caused by boron deprivation. The reduction in ascorbate content in root apices by aluminum was parallel to the inhibition of root elongation (74). Supplemental boron in aluminum-toxic medium produced root apices with higher ascorbate concentrations. IAA oxidase activity in the root tips of aluminum-stressed plants decreased in a manner reversely correlated with boron concentration in nutrient solutions (KM Lukaszewski & DG Blevins, unpublished observations). In summary, our findings support the hypothesis that toxic levels of aluminum induce boron deficiency in plants. The results also indicate that root growth inhibition under both boron-deficient or aluminum-toxic conditions may be a consequence of a disrupted ascorbate metabolism (74).

Molecular Approach

Molecular genetics can be a powerful tool in studying plant nutritional disorders. Recently, a mutant of *Arabidopsis thaliana* (*bor1-1*) with increased requirement for boron was identified and characterized by Noguchi et al (92a). In contrast to the wild type, *bor1-1* plants grown in "normal" concentrations of boron showed severe boron deficiency symptoms (reduced expansion of rosette leaves, reduced fertility, and loss of apical dominance) that were reversed by "excess" boron. Tracer experiments with ^{10}B suggested a defect in boron uptake and/or translocation. Genetic mapping showed a linkage of the *bor1* mutation with the simple sequence length polymorphism (SSLP) markers on chromosome 2. This approach may provide much needed information on the genetic mechanisms controlling boron metabolism in plants.

Concluding Remarks

Boron is essential for plant growth and development, and adequate boron nutrition of cultivated plants can be of great economic importance. Boron affects the yield of fruits, vegetables, nuts, and grains as well as the quality of harvested crops. Increased boron applications may promote root elongation in acidic, high-aluminum soils.

In one of his last articles, Joe Varner listed the boron requirement as one of the important unknowns in plant biology (128). Although recent progress in the isolation and characterization of plant boron-polyol transport molecules and pectin RG-II-B complexes greatly improved our understanding of boron mobility and boron chemistry in plant cell walls, it also highlighted the need to learn more about boron complexes with glycolipids and/or glycoproteins in membranes. The concept of boron-binding apoplastic proteins, as well as the effect of boron on manganese-activated enzymes, may be of importance in many metabolic processes. Molecular investigations of boron requirement in plants open new possibilities for improving boron deficiency/toxicity stress tolerance of crops. Elucidation of these aspects of boron nutrition will be a challenging goal for future research.

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