Does boron play only a structural role in the growing tissues of higher plants?

Patrick H. Brown and Hening Hu
Department of Pomology, University of California, Davis, CA 95616, USA

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Abstract

In species where boron (B) mobility is limited, B deficiency only occurs in growing plant organs. As a consequence of the highly localized patterns of plant growth and the general immobility of B it has been extremely difficult to determine the primary function of B in plants. In species in which B is phloem mobile, the removal of B from the growth medium results in the depletion of B present in mature leaves. Thus, it is possible to develop mature leaves with increasingly severe levels of B depletion, thereby overcoming the complications of experiments based on growing tissues. Utilizing this approach we demonstrate here that B depletion of mature plum (Prunus salicina) leaves did not result in any discernible change in leaf appearance, membrane integrity or photosynthetic capacity even though B concentrations were reduced to 6-8 µg/g dwt, which is less than 30% of the reported tissue B requirement. Boron depletion, however, results in a severe disruption of plant growth and metabolism in young growing tissues. This experimental evidence and theoretical considerations suggest that the primary and possibly sole function of B, is as a structural component of growing tissues.

Introduction

Boron deficiency produces a wide variety of symptoms, so it is not surprising that many hypotheses on the function of B have been advanced, yet the function has remained an enigma (Loomis and Durst, 1997). The effects of B deficiency include, but are not limited to, rapid inhibition of meristematic growth, disruption of normal cell wall formation, membrane leakiness and impaired function, altered phenol metabolism, altered auxin metabolism and altered RNA, DNA metabolism etc. (Dugger, 1983; Loomis and Durst, 1997; Lovatt and Dugger, 1984; Parr and Loughman, 1983; Shelp, 1993).

In the majority of reported experiments on B nutrition, plants have been treated with or without B for several days or at least several hours. Visible growth inhibition in roots, however, may occur as early as 3 to 6 hours after B omission (Heyes et al., 1991; Hirsch and Torrey, 1980; Krueger et al., 1987). Thus, the majority of these experiments have been performed on growing tissues that have been B deficient for some period. As the period of B deprivation increases, it becomes increasingly difficult to compare physiologically matched tissue between +B and -B treatments (Heyes et al., 1991). For example, Robertson and Loughman (1973) reported that Rb uptake by Vicia faba plant was reduced by the onset of B deficiency but that successive sections of the root responded differently, indicating that the reduced ion uptake was probably a secondary effect of the reduced root extension rather than a primary effect of B. Shelp (1993) suggested that evidence favours the involvement of B at the membrane level and that changes in membrane properties may result in many secondary effects, including the function of membrane-bound enzymes, the transport of ions, metabolites and hormones. Though several studies have demonstrated an effect of B resupply on membrane function, no study has simultaneously examined cell wall structure or cell division to eliminate its role in the response. Thus, even the role of B in membrane function is far from conclusive.

To overcome these complications, Parr and Loughman (1983), suggested that it would be valuable to develop “An experimental approach designed to assess the primary role of B during the onset of deficiency to eradicate a major extent the secondary effects”. An ideal experimental system would also utilize mature plant tissue to avoid the confounding effects of plant growth. Recently we demonstrated that B is freely phloem mobile in plant species in which B complexing polyols represent a major translocated photosynthetic (Brown and Hu, 1996). In the species Malus, Pyrus and Prunus, B-complexing subtilis is present in mature photosynthesizing tissues at concentrations 100-500 times greater than tissue B concentrations. In
expressed cell sap and phloem exudate all detectable B was present as the sorbitol-B-sorbitol complex (Hu et al., 1997). In these species the transfer of a plant from B adequate growth medium to B deficient medium results in the gradual depletion of B present in leaves developed prior to transfer and the movement of the sorbitol-B-sorbitol complex to growing tissues. With continued growth under B deficient conditions, there will be an increasingly severe B depletion of mature leaves (Brown and Hu, 1996). If B has a function in mature tissues then these leaves should develop B deficiency symptoms.

The use of species in which B is phloem mobile therefore provides an ideal experimental system for the determination of the effects of B depletion in mature tissues and avoids the confounding effects of plant growth. This approach will be utilized here to help define the function of B in plants.

**Materials and methods**

**Plant material**

Plum seeds (*Prunus salicina* cv ‘Myrobalan’) were stratified at 5°C for 120 days then grown hydroponically in a greenhouse (26/15°C, day/night temperature) and supplied with modified 1/5 Hoagland solution, pH 5.8 (Hoagland and Arnon, 1950). Boron was supplied at 12.5 μM as boric acid and the nutrient solution was changed weekly. After 10 weeks of growth, when the plant had developed 9 to 11 mature fully expanded leaves, roots were washed and half of the plants were transferred to -B nutrient medium while the others were transferred to nutrient medium with 25 μM B. Each treatment consisted of two replicate pots (19 liters) each containing 7 plants.

In order to monitor B depletion in the fully mature leaves before physiological measurements, leaves were sampled as follows: One leaf from each plant, either number 5 or 6 (counting from bottom) was sampled immediately following treatment imposition (day 0). From the results of preliminary experiment, second sampling was made at day 21 from leaf either 5 or 6. Boron concentration in these samples were analyzed by inductively coupled plasma mass spectrometer (ICP MS; Elan 5000, Pekin-Elmer Cetus, USA).

Based on the preliminary results, physiological measurements were not made until we presumed that no further depletion of B from fully mature leaves occurred. This was made after B treatment for 28 days.

*Fig. 1. 'Myrobalan' plum was grown for 4 weeks in the presence of 12.5 μM B then transferred to growth medium with no added B (-B) for an additional 49 days. At the time of transfer to -B conditions, leaves basal to leaf 27 had attained full size while leaves above this point were immature and not visible. All leaves younger than leaf 27 developed varying degrees of B deficiency symptoms. Symptoms include leaf chlorosis, decreased leaf size, wavy leaf margins and 'puckered' interveinal regions (contrast leaves 15 and 16 with leaf 27). Laterals 1 and 8 were formed after -B imposition and are severely B deficient. Leaf 27 and below did not develop any signs of B deficiency over the duration of the experiment.*

**Physiological measurements**

At day 28, and thereafter, K+ leakage from whole leaves was determined according to Cakmak et al. (1995). The leaves used were young expanding leaves (leaf 3-5 from top) and fully mature leaves (leaf 8-11 from bottom, which corresponds to leaf position 28 to 31 in Fig. 1). Basically the measurement involved immersion of the entire leaf (with petiole in the air) into a test tube containing 25 ml deionized water for a period of 120 minutes. Potassium content in the test tube was then determined by atomic absorption spectrophotometry. After leakage measurements, the leaf was dry-ashed directly and B was determined by ICP MS.