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Cultivar differences in boron uptake and distribution in celery (*Apium graveolens*), tomato (*Lycopersicon esculentum*) and wheat (*Triticum aestivum*)

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Abstract

Species and cultivar differences in boron (B) uptake at low B availability and tolerance to high external B are known for many species but mechanisms explaining such differences remain obscure. Here we contrast B uptake and distribution between two cultivars of tomato and celery that differ significantly in their susceptibility to B deficiency. The celery cultivar S48-54-1 and tomato cultivar 'Brittle' are known to be more susceptible to B deficiency (inefficient) than the closely related cultivars 'Emerson Pascal' and 'Rutgers' (efficient), respectively. B uptake and distribution was also compared in two wheat lines differing in tolerance to B excess ('Chinese Spring', sensitive and *Lophopyrum* Amphiploid, tolerant). Results showed that there is no significant difference in either the specific uptake rate (I_M) of ^{10}B or the relative growth rate (RGR) between the efficient cultivar (Emerson Pascal) and less efficient cultivar (S48-54-1) of celery. However, the distribution of ^{10}B among plant organs (leaves, stems and roots) of Emerson Pascal was different from S48-54-1. In Emerson Pascal more than 63% of accumulated B was present in the shoots while in S48-54-1 only 45% of accumulated B was present in shoots. In tomato plants, in addition to differences in B distribution among plant organs between the efficient (Rutgers) and less efficient (Brittle) cultivars, the specific uptake rate of ^{10}B was significantly higher in the efficient cultivar. In wheat, the tolerant line (Amphiploid) took up less B than the less tolerant cultivar (Chinese Spring), and the pattern of B distribution among plant organs was different with a greater percentage of B found in roots of Chinese Spring compared to Amphiploid. Differences in sensitivity to B deficiency and excess amongst cultivars and species were a consequence of either reduced B uptake as in wheat (Amphiploid), a restriction in B translocation from roots to shoot as in celery (S48-54-1) or a combination of both process as in tomato (Brittle).

Abbreviations: ICP-MS – inductively coupled plasma-mass spectrometer; RGR – relative growth rate; I_M – specific uptake rate

Introduction

The mechanism of B uptake and the factors governing B distribution in plants are poorly understood. Evidence suggests that B uptake is passive (see Hu and Brown, 1997 for review). However, species and cultivars differ significantly in B uptake even when grown under identical environmental conditions. For example, Brown and Jones (1971) studied the differential B transport in two tomato cultivars, T3238 (B-inefficient) and Rutgers (B-efficient), and found that Rutgers was 15 times more efficient in utilizing B from the medium than T3238. However, roots of T3238 accumulated more B than those of Rutgers. They concluded that T3238 lacks the ability to translocate B from root to shoot. In addition, when T3238 plants were transferred from adequate to inadequate supply of B, some B deficiency symptoms were observed within 48 h. This was in agreement with the observation of Skok (1957) that B is not redistributed within the plant. Similarly, Wall and Andrus (1962) found that leaves of Rutgers contain more B than those of Brittle (an abnormal sublethal phenotype in tomato) yet roots of Brittle contain

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more B than roots of Rutgers. In celery, Pope and Munger (1953) observed striking differences in B efficiency between S48-54-1 (susceptible line to low B) and Summer Pascal. At low to moderate B availability Summer Pascal produced more dry matter than S48-54-1 and S48-54-1 developed pronounced symptoms of B deficiency. However, above a concentration of 0.25 mg/L in the medium no significant difference in total weight was observed between the groups and no signs of B deficiency were observed.

Nable (1988), working on several barley and wheat genotypes, reported that the most susceptible genotypes to excess B accumulate more B in roots and shoots than tolerant genotypes. Further, the ability of tolerant genotypes to tolerate high B in the medium was not a consequence of an ability to tolerate high B concentrations in the plant tissues (Nable, 1988; Nable et al., 1990). Nable (1991) and Paull et al. (1992) also reported in annual medics and peas that the ability to tolerate high B concentration was due to the capacity of the plant to restrict B uptake by roots and transport to shoots. Although the mechanism that causes the restriction of B uptake is not known (Marschner, 1995), several explanation have been proposed. The high accumulation of B by susceptible genotypes or the lower accumulation of B by tolerant genotypes may be related to differences in membrane permeability associated with the composition of membrane and cell wall (Nable and Paull, 1991). Brown and Hu (1994) and Shelp (1993) suggested that the insoluble-B-complexes in the cell wall influence the uptake of B by roots. For example, graminaceous monocots require low (3–10 μg B g^{-1} dry weight) B compared to dicot which require high (20–30 μg B g^{-1} dry weight) B (Jones et al., 1991). The different requirements for B by monocots and dicots may be determined by the differences in cell wall composition between the two groups. The primary cell wall of dicots showed a higher pectic content compared to the primary cell wall of monocots which showed a low pectic content (Darvill et al., 1980). Hu et al. (1996) and Matoh et al. (1996) recently showed that there is a significant positive correlation between B concentration in the leaf or the cell wall and uronic acid, rhamnose and galactose in the cell wall. They concluded that species with high pectic content have a higher tissue B requirement.

It can be concluded, therefore, that the precise mechanism underlying the differential uptake of B by plant species remains unclear. Here, we utilize species and cultivars known to differ in B uptake to further investigate the mechanisms of B uptake and distribution.

Materials and methods

Plant material

The genotypes used here were selected based on the degree of susceptibility to low B and the degree of tolerance to excess B as determined by the work of Brown and Jones (1971) and Wall and Andrus (1962) on tomato; Pope and Munger (1953) on celery; Shumann (1969) and Paull et al. (1991) on wheat. The cultivars selected were as follows:

- B-efficient celery (*Apium graveolens* cv.'Emerson Pascal') and tomato *Lycopersicon esculentum* cv. 'Rutgers').
- B-inefficient celery S48-54-1 and tomato 'Brittle'.
- Wheat-*Lophopyrum* Amhiploid (*Triticum aestivum*) (tolerant to excess B) and wheat 'Chinese Spring' (less tolerant to excess B).

Growth conditions

Seeds were germinated in petri dishes. Seeds of wheat were sterilized with Na-hypochlorite (50% of the commercial product) for 15 min then soaked in water for 30 min and placed on a filter paper until germination. Tomato and celery seeds were only soaked in water for 30 min. All seeds were chilled at 5 °C for three days after which the seeds were transferred to a controlled environment room for germination. After 7 days all the seedlings were transplanted to a perlite medium supplied with 1/4 strength Hoagland's solution (Hoagland and Arnon, 1950) with a concentration of 0.1 mg/L ^{11}B under green house conditions. After two weeks of growth the plants were transferred to a growth chamber with a temperature of 25 °C /18 °C (day/night); photoperiod 16/8 h (day/night); relative humidity 30/60% (day/night); and light intensity 432 μmol m^{-2} s^{-1}. All plants under growth chamber conditions were supplied with 3/4 strength of Hoagland's solution with a concentration of 0.1 mg/L ^{11}B. After a week of growth in the growth chamber plants were treated with 10 mg/L of 99.43% ^{10}B enriched boric acid. In order to investigate the effect of different concentrations of B in the growth medium on the uptake of B, celery cultivars were also treated with either 0.1 or 1 mg/L of 99.43% ^{10}B enriched boric acid. ^{10}B was used as a tracer for B uptake. The pH of the nutrient solution was adjust-

ed to 5.5–6.5. Plants were irrigated twice a week with the nutrient solution and between each nutrient addition plants received irrigation with double deionized water at least once a day. Plants were irrigated with double deionized water subsequent to the final nutrient application and before harvest. The irrigation with nutrient solution was applied after the growth medium was flushed with double deionized water to avoid salt and B accumulation.

Sampling and plant analysis

Two harvests were taken. The first harvest was taken before ^{10}B treatment and the second was taken three weeks after the ^{10}B treatment. At each harvest, four individual plants (replicates) were taken from each cultivar. Each plant was divided into shoot (leaves, stems and petioles) and root. Roots were removed from the medium without being washed and the adhering perlite was removed by hand. Preliminary experiments demonstrated that, due to our frequent irrigation as described above, washing at harvest removed only 5–20% (5–6 for celery, 11–13 for tomato and 17–20 for barley) of root B and did not significantly alter the results. Roots were blotted dry prior to weighing. All healthy expanded leaves were used for leaf analysis. Each leaf was divided into two halves, one half for isotopic B (^{10}B) and the other half for cell wall B analysis (see below). The ^{10}B was determined in stems and roots of each plant. The plants were monitored for growth and any symptoms of B deficiency over the period of the experiment.

Boron analysis

Plant tissues were dry ashed at 500 °C and analyzed for B by using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Elan 500). The method of cell wall B determination was based on those of Hu and Brown (1994) and Matoh et al. (1993, 1996). The fresh plant tissue samples were homogenized with an ice cold mortar and pestle in cold water. The homogenate was then centrifuged at 1000 g for 10 min. The residue was washed three times with 10 volumes of 80% ethanol and once with 10 volumes of methanol:chloroform mixture (1:1, v/v). Finally, the precipitate was washed with 10 volumes of acetone. The samples were then dried and ashed for cell wall B determination using ICP-MS.

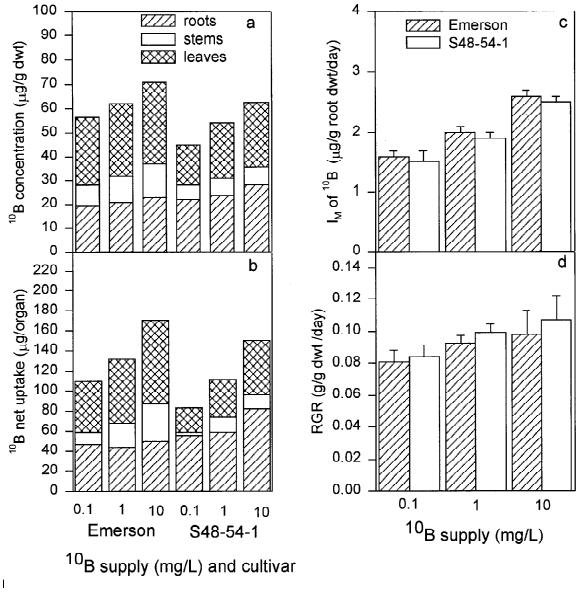

Figure 1. ^{10}B concentration (a), net uptake of ^{10}B per organ in leaves, stems and roots (b), specific uptake rate of ^{10}B (c) and relative growth rate (d) in celery cv. Emerson and S48-54-1 when plants were grown in 0.1, 1 and 10 mg/L ^{10}B for a period of three weeks. Bars represent means of four replicates ± SE.

Specific uptake rate

Specific uptake rate (I_M) of B was calculated according to the equation of Williams (1948):

$$I_M = [(\ln R_2 - \ln R_1)/(t_2 - t_1)] \times [(M_2 - M_1)/(R_2 - R_1)]$$

where R_1 and R_2 are the initial and final root dry weights at t_1 and t_2, respectively, M_1 and M_2 are the initial (t_1) and final (t_2) boron contents.

Experimental design

A randomized complete design was used in this experiment. All values shown in tables and graphs represent means of four individual plants (replicates). Error bars indicate standard error of the means. Statistical analysis were carried out with the SAS package (SAS, 1985).

Results

The plants were healthy throughout the experiment and did not show any symptoms of B deficiency or toxicity.

After three weeks of 10 mg/L treatment, ^{10}B concentration (as μg ^{10}B/g dwt) in S48-54-1, which is B-inefficient, was 21 and 49% lower in the leaves and stems respectively, than in Emerson Pascal (Figure 1a). By contrast, ^{10}B concentration in roots was 18% higher in S48-54-1 compared to Emerson (Figure 1a). Similarly, net uptake of ^{10}B per organ in leaves and stems was lower in S48-54-1 than in Emerson (Figure 1b). Net uptake is defined as B uptake from the commencement of ^{10}B treatment to harvest. No significant difference was observed in either I_M of ^{10}B or RGR (Figure 1c, d). In order to follow the changes of B distribution with different concentrations of B in the growth medium, celery cultivars were treated with 0.1 and 1 mg/L of 99.43% ^{10}B enriched boric acid. The results showed that ^{10}B distribution in different organs of Emerson and S48-54-1 was similar to those of celery cultivars when they were grown in 10 mg/L ^{10}B, i.e., the accumulation of ^{10}B in leaves of Emerson was higher than those of S48-54-1, whereas roots of S48-54-1 accumulated more ^{10}B than those of Emerson (Figure 1a, b). Also, no significant difference was observed in either I_M of ^{10}B or RGR (Figure 1c, d). Leaves represented the major site of B accumulation in Emerson Pascal, while in S48-54-1 roots contained the greatest proportion of plant B (Figure 1a, b).

In leaves, both the concentration of ^{10}B and the net uptake of ^{10}B per organ in Brittle (B-inefficient) were lower than in leaves of Rutgers (B-efficient) (Figure 2a, b). In contrast, RGR and I_M were higher in Rutgers (Figure 2c, d). The contribution of leaves to the total plant ^{10}B was higher in Rutgers (78%) than in Brittle (56%). Whereas, the contribution of roots to the whole plant ^{10}B in Brittle was higher (36%) than the contribution of roots in Rutgers (11%). The percentages shown here, in the case of tomato and wheat, was calculated based on the content (concentration of ^{10}B $\times$ weight) of ^{10}B in each organ and the total content of ^{10}B in the whole plant, and not on the net uptake of ^{10}B in each organ.

The results show that the concentration and the net uptake of ^{10}B per organ in both leaves and roots of Amphiploid, which is considered more tolerant to excess of B, were lower than in leaves and roots of Chinese Spring (Figure 2a, b). I_M of ^{10}B and RGR were significantly higher in Chinese Spring (Figure 2c, d). Roots of Chinese Spring contributed more (59%) to the total plant ^{10}B, compared to (39%) for roots of Amphiploid. On the other hand, the contribution of leaves to the total plant ^{10}B in Amphiploid was higher than those of roots.

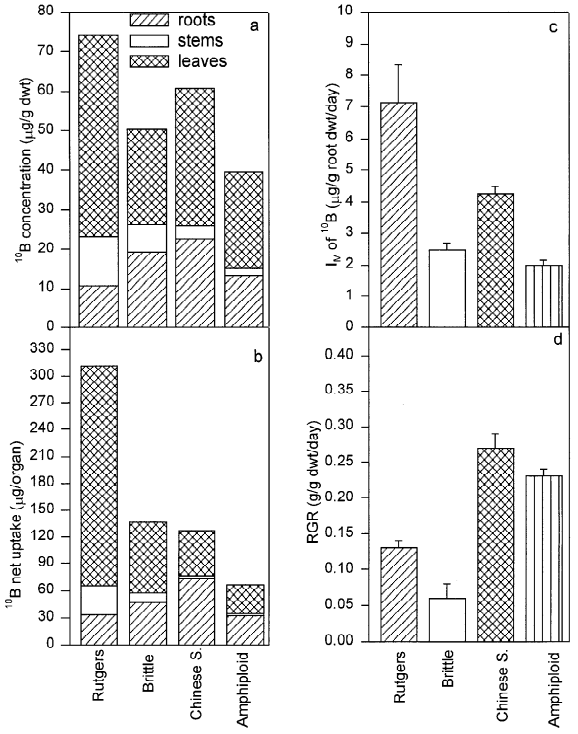

Figure 2. ^{10}B concentration (a), net uptake of ^{10}B per organ in leaves, stems and roots (b), specific uptake rate of ^{10}B (c) and relative growth rate (d) in tomato cv. Rutgers and Brittle, and in wheat cv. Chinese Spring (Chinese S.) and wheat-*Lophopyrum* (Amphiploid) when plants were grown in 10 mg/L ^{10}B for a period of three weeks. Bars represent means of four replicates $\pm$ SE.

The percentage of cellular B present in the cell wall did not differ between cultivars of the same species (Table 1).

Discussion

Frequent irrigation with the blotting of roots apparently removed sufficient apoplastic ^{10}B to account for the 'low' root ^{10}B concentration observed in all species with high ^{10}B application (10 mg/L). The apoplastic B can be washed out very easily as evidenced by rapid desorption in tobacco cell culture (Brown and Hu, 1994).

The concentration and the net uptake of ^{10}B in the shoot of the B-inefficient celery cultivar (S48-54-1) was lower than that in more efficient cultivar (Emerson Pascal). The main site of B accumulation in Emerson was the shoot while in S48-54-1 it was the root. This suggests that the translocation of ^{10}B from root to shoot

Table 1. Contribution of cell wall B to the total leaf B ($^{10}B+^{11}B$) in celery, tomato and wheat. Plants were grown in 10 mg/L ^{10}B for three weeks[a]

Species	Cultivar	Total leaf B (μg/g dwt leaf)	Cell wall B (μg/g dwt leaf)	Cell wall (%)
Celery	Emerson	90 ± 3.5	64 ± 1.2	71
	S48-54-1	69 ± 4.3	45 ± 1.0	65 ns[b]
Tomato	Rutgers	87 ± 12.0	57 ± 1.9	66
	Brittle	76 ± 6.7	45 ± 1.4	59 ns
Wheat	Chinese Spring	63 ± 5.2	38 ± 0.8	60
	Amphiploid	52 ± 5.7	29 ± 0.8	56 ns

[a]Values are the mean of four replicates ± SE.
[b]ns – non-significant. $p < 0.05$ was used as level of significance.

is restricted in S-48-54-1. No significant difference was observed in either I_M or RGR in celery cultivars even when they were grown in 0.1 and 1 mg/L ^{10}B. This indicates that the major difference in B efficiency in these celery cultivars is the pattern of B distribution in the different plant organs rather than the uptake of B.

In tomato, the concentration and the net uptake of ^{10}B into shoots of Brittle was lower than in Rutgers. This is in agreement with Brown and Jones (1971) and Wall and Andrus (1962). This can be explained by an apparent restriction in root to shoot B transport in Brittle. Roots of Brittle have higher ^{10}B concentration and higher net B uptake which results in higher ^{10}B concentrations. In contrast to previous reports (Brown and Jones, 1971; Wall and Andrus, 1962) our study also demonstrates that both distribution of B within the plant and the uptake of B were different between the cultivars of tomato. Here we demonstrate that I_M of ^{10}B uptake was higher in Rutgers. Brown and Jones (1971), working with tomato line, T3238 and Rutgers, found that Rutgers was more efficient in translocating B to the shoot than T3238 plants. On the other hand, the roots of T3238 accumulated more B than was accumulated by Rutgers. It was suggested that T3238 lacked the ability to adequately translocate B from the roots to the leaf tissues. The lack of translocation of B to the leaf tissues in T3238 was reported to be governed by a single recessive gene (Wall and Andrus, 1962).

Comparing both celery and tomato genotypes, it is clear that the major mechanism in B efficiency in these celery cultivars is the rate of transport of B from root to shoot rather than the uptake of B by roots. In tomato, however, two mechanisms of B efficiency contribute, that is B distribution within the plant as well as the uptake of B.

The lower concentration and reduced net uptake of ^{10}B exhibited by Amphiploid wheat could be due to reduced uptake or increased excretion of previously acquired B through an unidentified excretion mechanism. In less tolerant cultivars the capacity to restrict B uptake is lower. Our study showed that the uptake (I_M) of ^{10}B in Chinese Spring was higher than in Amphiploid. Nable (1988) showed that barley and wheat genotypes, B sensitive cultivars, accumulate more B in their tissues than the tolerant genotypes. This was attributed to the fact that genotypes tolerant to high B concentration in the medium have the ability to restrict B uptake by roots (Nable and Paull, 1991; Paull et al., 1992) and was not the result of an ability of tolerant genotypes to tolerate high concentration of B in their tissues (Nable, 1988; Nable et al., 1990). The ability of tolerant genotypes to control B uptake may be due to differences in plasma membrane composition that restricts the passive transport of B (Huang and Graham, 1990; Nable and Paull, 1990).

The results indicate that the mechanism of B efficiency in celery is associated with a restriction in translocation of B from root to shoot. The same mechanism operates in tomato, though tomato cultivars also differ in total B uptake. The differences in B uptake between cultivars of a species were not the result of differences in cell wall composition as the percentage of cell wall B did not differ between cultivars.

It can be concluded that the distribution of B from root to shoot is a major mechanism of B efficiency in celery. In tomato differences in both B uptake and B distribution contribute to B efficiency. While the

mechanism of differential B tolerance in wheat is due solely to differential B uptake into the root.

Tolerance to B excess and susceptibility to B deficiency apparently may involve differential B uptake and/or differences in B distribution within the plant. Much remains to be done before the mechanisms of B uptake are fully understood.

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