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bor1–1, an *Arabidopsis thaliana* Mutant That Requires a High Level of Boron[1]

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bor1–1 **(high *bor*on requiring), an *Arabidopsis thaliana* mutant that requires a high level of B, was isolated. When the B concentration in the medium was reduced to 3 μM, the expansion of rosette leaves was severely affected in *bor1–1* but not in wild-type plants. In a medium containing 30 μM B the mutant grew normally but showed female sterility, whereas the wild type was able to set seeds. These defects of the *bor1–1* mutant were not detected with supplementation of 100 μM B. In vivo concentrations of B in *bor1–1* mutants were lower than those of the wild type, especially in the inflorescence stems. Tracer experiments using ^{10}B suggested that the mutant has defects in uptake and/or translocation of B. The mutation was mapped on the lower arm of chromosome 2.**

Although B was established as an essential element in higher plants more than 70 years ago (for review, see Loomis and Durst, 1992), its mechanism of action is still poorly understood. B-deficient plants exhibit various visible symptoms and disorders. Effects of B deficiency in maize seedlings first appear as cessation of root growth followed by the collapse of meristematic regions, suggesting that B plays a key role in cell division (Kouchi and Kumazawa, 1976). A number of studies have been conducted on the physiological effects of B deficiency. These studies established that B deficiency causes various changes in properties such as membrane integrity and permeability, auxin metabolism, sugar transport, lignification in the cell wall, carbohydrate metabolism and transport, respiration (for review, see Loomis and Durst, 1992), and reduced fertility (Marschner, 1995). However, the primary defects caused by B deficiency in plants are still unclear.

B is predominantly localized in the cell walls in tobacco and squash (Matoh et al., 1992; Hu and Brown, 1994). Recent findings showing that B in cell walls is bound to rhamnogalacturonan II (Ishii and Matsunaga, 1996; Kobayashi et al., 1996; O'Neill et al., 1996) and that this complex is present in a wide range of plant species (Matoh et al., 1996) suggest that it exerts its effects, at least partially, in cell walls. However, for B to be transported from roots into aerial portions of the plant through the xylem, it must cross the plasma membrane at or near the casparian strips. Studies at the cellular level established that the uptake of B occurs mainly by passive diffusion, although other mechanisms may also be involved (Raven, 1980; Brown and Hu, 1994). Translocation of B is also known to occur through transpiration streams, although several observations suggest that B can also be mobilized through phloem (Brown and Hu, 1996). The detailed mechanism of B transport is not yet understood.

One possible approach for understanding the primary action of B in plants is to use genetics. A number of cultivars of major crops are reported to have different sensitivities to B deficiencies and toxicities. In some cases, the differences in sensitivities were attributed to differences in uptake and translocation of B (Nable et al., 1990). Such approaches should help us to understand the molecular components required for the functions of B in plants. However, cloning of the corresponding gene(s) from commercial cultivars is difficult.

A number of *Arabidopsis thaliana* mutants with mutations related to plant nutrition have been isolated (Poirier et al., 1991; Delhaize and Randall, 1995; Delhaize, 1996; Larsen et al., 1996; Wu et al., 1996), although, to our knowledge, mutations related to B requirement have not been described. Here we describe the identification and characterization of a novel mutant of *A. thaliana* that requires high levels of B for completion of its life cycle.

MATERIALS AND METHODS

Arabidopsis thaliana (L.) Heynh. strains Col-0 (ecotype Columbia wild type), Col *gl1–1*, Ler (ecotype Landsberg harboring *erecta* mutation) were from our laboratory stock. The *bor1–1* mutant line was isolated in this study (see below). The *bor1–1 gl1–1* line was constructed by crossing the *bor1–1* mutant and Col *gl1–1*.

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Abbreviations: ICP-MS, inductively coupled plasma MS; SSLP, simple-sequence-length polymorphism.

Growth Conditions

A. thaliana was grown (Hirai et al., 1995) with hydroponic media (Fujiwara et al., 1992) in a greenhouse at 22°C under natural light supplemented with fluorescent lamps. Concentration of B was adjusted by altering the concentration of H_3BO_3 in the medium. Hydroponic solutions containing 150, 30, and 3 μM B are defined as high-B, B-sufficient, and B-deficient media, respectively, in this report. Plants used for the reciprocal crosses were grown with vermiculite and watered with the B-sufficient medium.

Determination of B Concentration

Concentration of B was measured using ICP-MS. Plant materials were harvested and dried in an air incubator at 60°C for more than 60 h. Samples (0.5–3.5 mg) were transferred to 8-mL Teflon tubes (Nalgene, Rochester, NY), and about 0.3 mL of concentrated HNO_3 was added per milligram of sample. The samples were digested at 120 to 130°C to dry completely. The residues were dissolved in 0.08 M HNO_3 and subjected to ICP-MS (model SII SPQ-8000A, Seiko Instruments, Chiba, Japan) analysis.

Tracer Experiments Using ^{10}B

Col-0 and *bor1–1* plants were grown hydroponically in media containing 12 or 30 μM boric acid of natural isotopic abundance (19.9% ^{10}B, based on our measurement) for 4 weeks until inflorescence shoots reached 10 cm in height. The plants were then transferred to a medium containing 30 μM boric acid enriched with ^{10}B (95.9% ^{10}B, ICON, Mt. Marison, NY). After 48 h of incubation plant samples were harvested and dried, and the contents of ^{10}B and ^{11}B were measured by ICP-MS. The following equation was used to determine the fraction of B derived from tracer (B_{dft}; Picchioni et al., 1995):

$$B_{dft} = (\%\ ^{10}B_t - \%\ ^{10}B_u)/(\%\ ^{10}B_s - \%\ ^{10}B_u)$$

The subscripts t, u, and s refer to the atomic percentages of ^{10}B in the treated sample, the untreated sample, and the hydroponic solution used for the tracer experiments, respectively. The percentages of $^{10}B_u$ and $^{10}B_s$ were 19.9 and 95.9%, respectively, in this study.

Linkage Analysis

The *bor1–1* line (female parent) was crossed with Ler. F_2 seeds were obtained by self-pollination. Forty-three F_2 plants were grown with the high-B medium, and the F_3 seeds were collected from individual F_2 lines. The F_3 plants were grown with the B-deficient medium and the Bor1$^-$ phenotype was scored. DNA was isolated from a pool of more than 10 F_3 plants from each F_2 line and the SSLP analysis was performed (Bell and Ecker, 1994). Primers for the SSLP analysis were purchased from Research Genetics (Huntsville, AL). Genetic distances were calculated according to the formulas described by Koornneef and Stam (1992).

RESULTS

Isolation of the *bor1–1* Mutant

During the course of experiments with an *A. thaliana* mutant line PD114 carrying a *tom1* mutation (Ishikawa et al., 1991), in which multiplication of tobacco mosaic virus was reduced to low levels, we noticed that the original isolate segregated lines that showed very poor seed setting when grown with the B-sufficient medium. As reduction of fertility is among the typical symptoms of B deficiency in *Brassica napus*, we suspected that this line might be suffering B deficiency. To test this possibility, we applied tiny crystals of boric acid to pods of these sterile plants. After several days, we found full restoration of their fertility (Fig. 1A). As similar observations were repeated twice with this line, we concluded that the original isolate of PD114 carries a mutation that renders it sensitive to low levels of B for normal seed sets. Therefore, we named the mutation *bor1–1* (high *bor*on requiring).

The PD114 mutant had been isolated by ethylmethane sulfonate mutagenesis of Col-0 and backcrossed twice to Col-0 (Ishikawa et al., 1991). The PD114 mutant was crossed with Col-0 another time, and the phenotypes of reduced tobacco mosaic virus multiplication (the Tom1 phenotype) and high-B requirement (the Bor1 phenotype) were scored in the F_2 and F_3 generations. The Tom1 and Bor1 phenotypes segregated independently (data not shown). Moreover, in the PD378 line, another isolate of *tom1* that is independent from PD114 (Ishikawa et al., 1991), fertility was normal without the addition of boric acid (data not shown). These results indicate that the two phenotypes are caused by different mutations. After another round of backcrossing, a *bor1–1 TOM1*$^+$ line was established and used for subsequent experiments.

Crosses were made between the *bor1–1* mutant and wild-type plants that were grown with the high-B medium, and the F_1 and F_2 plants were tested for fertility with the B-sufficient medium. All of the F_1 plants were fertile, and among the total of 159 F_2 plants, 119 were fertile and the rest were sterile, indicating that the *bor1–1* mutant carries a single recessive mutation ($\chi^2 = 0.95$).

The *bor1–1* Mutation Causes Female Sterility under a Limited Supply of B

When grown under the B-sufficient medium (30 μM B), the *bor1–1* mutant was able to develop apparently normal flowers but failed to set seeds most of the time (Fig. 1B). The expansion of rosette leaves did not seem to be affected by the mutation at 30 μM B.

To determine the nature of sterility of *bor1–1* plants, reciprocal crosses (four each) were performed using plants grown with the B-sufficient media. Pollen from *bor1–1* plants was able to fertilize wild-type female plants, but wild-type pollen failed to fertilize plants carrying the *bor1–1* mutation in all of the crosses tested. The Col *gl1–1* plants used in the study self-pollinated efficiently, whereas *bor1–1* plants formed normal flowers without setting seeds at 30 μM B. These results establish that *bor1–1* plants are female-sterile when grown with 30 μM B.

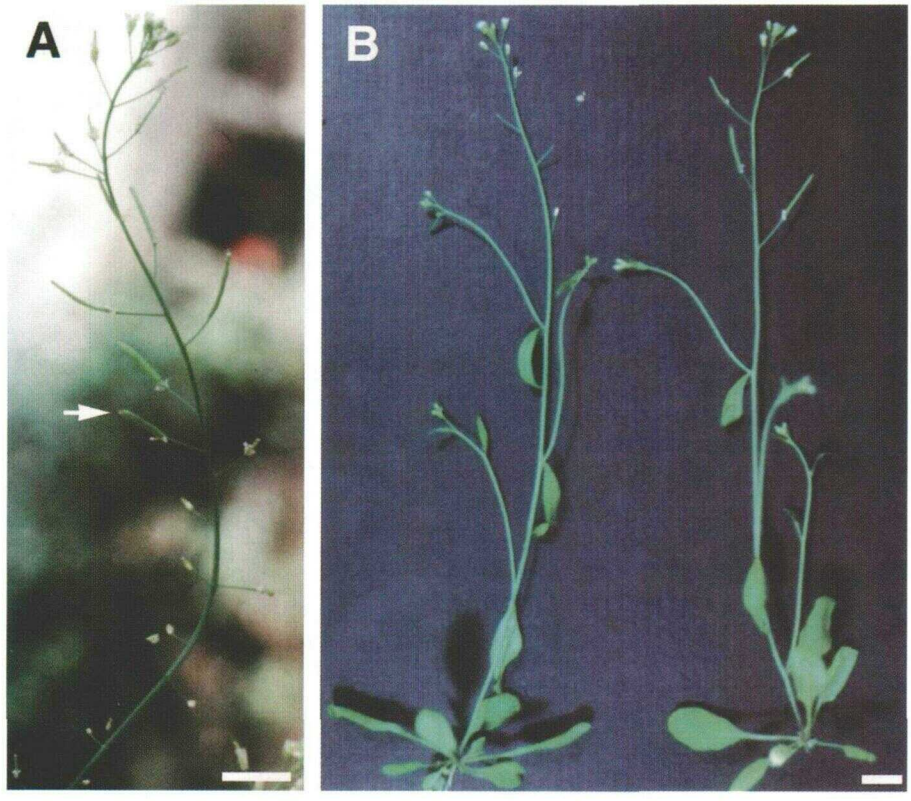

Figure 1. Seed setting in *bor1–1* plants. A, Recovery of fertility by application of 150 μM B to a *bor1–1* plant. The plant was grown with vermiculite supplied with a medium containing 30 μM B until several days after the start of flowering, when it was supplied with a medium containing 150 μM B. Seed setting recovered (indicated by the arrow) within a few days of high-B supply. Bar = 1 cm. B, Pod setting of *bor1–1* (left) and wild-type (right) plants grown hydroponically for 4 weeks with the B-sufficient medium containing 30 μM B. Bar = 1 cm.

Growth under Various Levels of B Supply

The Col-0 wild-type and *bor1–1* plants were grown hydroponically with nutrient solution containing various levels of B for 3 weeks to determine the effects of B supply on growth of the plants (Fig. 2). In wild-type plants dry weights of the aerial portions were relatively constant within a range of 0.3 to 300 μM B, but omission of B from the nutrient solution reduced the growth of aerial portions severely. Wild-type plants failed to set seeds at 0.3 μM B and root growth was reduced markedly (data not shown). Since B is an essential element for higher plants, growth at 0 μM B presumably represents a minor contamination of B in our experiments. Growth of the aerial portion in *bor1–1* plants was diminished when B supply was reduced to 3 μM. Growth of both plant lines was similar when B supply was at or higher than 12 μM. These observations establish that *bor1–1* plants are sensitive to B deficiency, exhibiting decreased vegetative growth and seed setting, as described earlier. Root growth was not significantly altered in *bor1–1* plants until leaf growth became severely retarded (data not shown). These defects were not detected in the *bor1–1* mutant plants grown with 100 μM B.

B-deficiency symptoms of *bor1–1* plants are shown in Figure 3. Moderate reduction in expansion of rosette leaves and severe repression of apical dominance were observed in *bor1–1* plants grown with the B-deficient medium (3 μM B). These symptoms are unique to *bor1–1* plants. That is, when compared with *bor1–1* plants grown with the B-deficient medium, wild-type plants grown with 0 μM B retain apical dominance but suffer more severe effects on rosette leaf expansion.

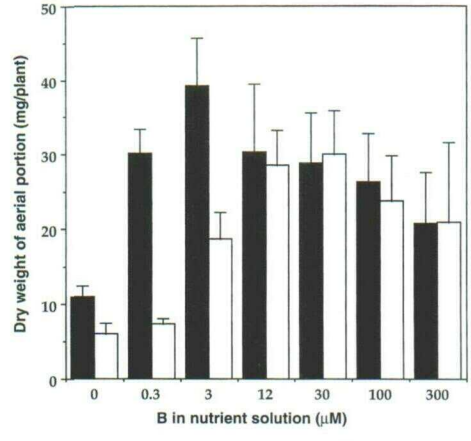

Figure 2. Growth of *bor1–1* (white bars) and Col-0 (black bars) plants under various levels of B supply. Col-0 and *bor1–1* plants were grown hydroponically with various levels of B. Dry weight of aerial portions of plants after 3 weeks of growth was measured. Averages ± SD are shown (n = 8).

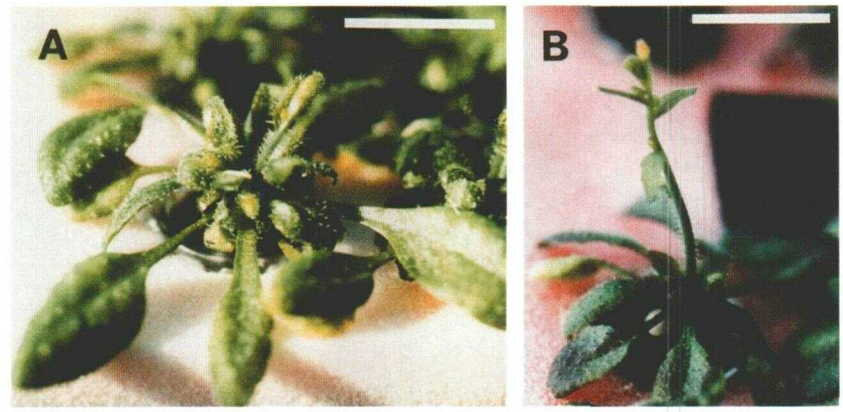

Figure 3. Growth of *bor1–1* and Col-0 plants under a limited supply of B. A, *bor1–1* plant grown with the hydroponic solutions containing 3 μM B. Bar = 1 cm. B, Col-0 plant grown with the hydroponic solutions containing 0 μM B. Bar = 1 cm.

Reduced B Content in *bor1–1* Plants

B contents in leaves and inflorescences were compared with those of wild-type plants. When grown hydroponically with the B-sufficient medium (30 μM B), lower rosette leaves (first to fourth leaves) and the upper portion of the inflorescence (about 3 cm from the top) of *bor1–1* plants had about 66 and 18%, respectively, of the B contained in equivalent portions of wild-type plants (Fig. 4). Similar results were obtained with 12 μM B; B content of *bor1–1* plants was less than 50% of wild-type levels.

Reduced Uptake of B in *bor1–1* Plants

To determine the nature of the reduced B content in *bor1–1* plants, tracer experiments were conducted to compare B uptake and translocation between *bor1–1* and wild-type plants. Plants were grown hydroponically with media containing 30 or 12 μM of natural abundance B (19.9 atom % ^{10}B) for 4 weeks until inflorescence shoots reached 10 cm in height. After 48 h of incubation in medium containing 30 μM boric acid enriched with ^{10}B (95.9 atom % ^{10}B), the lower rosette leaves and the upper portion of inflorescence were harvested and the levels of ^{10}B and ^{11}B were measured by ICP-MS.

Concentrations of B derived from the tracer during the 48-h labeling period were lower in *bor1–1* than in Col-0 plants, especially in the upper portion of the inflorescence (Fig. 5). Together with the results presented in Figure 4, these results suggest that the *bor1–1* mutant plants have defects in the uptake and/or transport, but not the utilization of B.

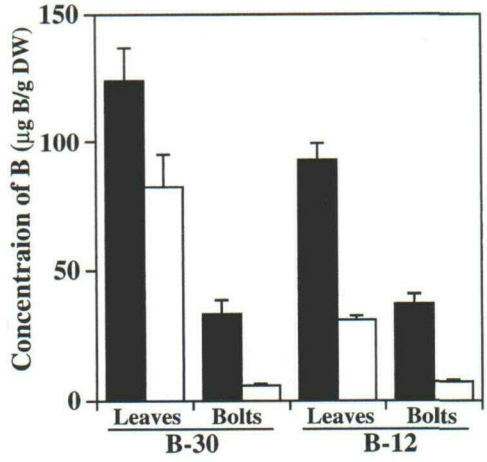

Figure 4. B contents in Col-0 (black bars) and *bor1–1* (white bars) plants. Wild-type and *bor1–1* plants were grown hydroponically with 30 (B-30) or 12 μM (B-12) B supply for 4 weeks. B contents in the lower rosette leaves (first to fourth leaves) and the upper portion of inflorescence (about 3 cm from the top) were determined by ICP-MS. Leaves and Bolts refer to the lower rosette leaves and the upper portion of inflorescence, respectively. Averages ± SD are shown (n = 5). DW, Dry weight.

Genetic Mapping of the *bor1* Mutation

The mutant was crossed with Ler and genetic mapping using SSLP markers was carried out. Markers on the lower

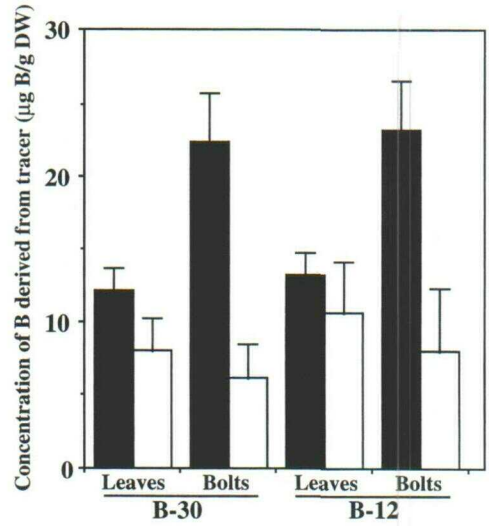

Figure 5. B uptake from the nutrient solution during 48 h of tracer experiments. Col-0 (black bars) and *bor1–1* (white bars) plants were grown hydroponically with 30 (B-30) or 12 μM (B-12) B supply and subjected to the tracer experiments. Based on the ^{10}B and ^{11}B amount in the samples (the lower rosette leaves and the upper portion of inflorescence), contents of B taken up from the nutrient solution during 48-h tracer experiments were calculated (for details, see "Materials and Methods"). Leaves and Bolts refer to the lower rosette leaves and the upper portion of the inflorescence, respectively. Averages ± SD are shown (n = 5). DW, Dry weight.

arm of chromosome 2 showed linkage to the *bor1* mutation. Genetic distances calculated from the data obtained were 21.2 ± 6.8 cM and 18.6 ± 6.8 cM for *bor1*-nga168 and *bor1*-nga361, respectively.

DISCUSSION

Molecular genetics can be a powerful tool for understanding the roles of essential elements in higher plants, especially those elements for which the primary functions are not clearly understood. Here we describe the isolation and characterization of the *bor1–1* mutant of *A. thaliana*. To our knowledge, this is the first report of an *A. thaliana* mutant related to B nutrition. Since all of the visible defects of *bor1–1* plants, i.e. reduced expansion of rosette leaves, reduced fertility, and loss of apical dominance, can be recovered by application of excess B, the defects in *bor1–1* must be specifically related to functions of B in higher plants. Thus, it is likely that the *BOR1* gene is involved, directly or indirectly, in B metabolism in higher plants.

Results of B measurement and uptake experiments suggest that the mutant has a reduced capacity for B uptake and/or translocation, especially to the inflorescence (Figs. 4 and 5). Most of the defects in *bor1–1* can be explained as general B-deficiency symptoms. For example, reduced fertility was observed under B deficiency in various crops including rapeseed (Marschner, 1995). Reduced apical dominance is also reported under B deficiency in various plant species. These symptoms led to the findings that auxin transport was impaired by reduced levels of B supply (Loomis and Durst, 1992). The female sterility evident in *bor1–1* is another typical symptom of B deficiency. It is well established that a high concentration of B is required for pollen germination and pollen tube elongation (Loomis and Durst, 1992). Therefore, the most likely explanation for the female sterility of *bor1–1* plants is that the B concentration in the stigma is reduced to a level that cannot support normal development of pollen tubes.

On the other hand, phenotypes of *bor1–1* plants under moderate levels of B deficiency (at 0.3–3 μM) are somewhat different from symptoms of severe B deficiency (at 0 μM) of wild-type plants. For example, in *bor1–1* plants apical dominance was lost before rosette leaves started to show severe symptoms of deficiency, whereas in the wild type apical dominance was maintained at low levels of B (less than 0.3 μM), which caused a reduction in rosette leaf expansion (Fig. 3). These observations suggest that defects in *bor1–1* plants are not simply caused by the uniform reduction of B levels in all parts of the plant. Levels of reduction in the B content may differ depending on the organs or cell types, which may cause the unusual B-deficiency symptoms of *bor1–1* plants. Our finding that concentrations of B in leaves and inflorescence were different between wild-type and *bor1–1* plants (Fig. 4), presumably resulting from the difference in translocation shown in Figure 5, supports this view.

B contents were lower in *bor1–1* than in wild-type plants. The data from uptake experiments suggested that the ratio of B absorbed during the 48-h labeling period to the total B content was more or less similar between the wild-type and *bor1–1* plants (Figs. 4 and 5). These results suggest that overall efficiency of B uptake and translocation was reduced in *bor1–1* plants. It is generally accepted that B is an immobile element and its distribution within a plant occurs mainly through transpiration streams. Under the conditions of our 48-h uptake experiments, the amount of B derived from the tracer is equivalent to the increase in B in the upper part of the inflorescence (based on the increase in dry weights of the samples in 48 h, data not shown, and Figs. 4 and 5), suggesting that B is also relatively immobile in *bor1–1* plants under our experimental conditions. However, it is also known that remobilization of B occurs under certain conditions such as the presence of excess B. Thus, patterns of B translocation in *bor1–1* plants may differ under different conditions.

Recent reports have shown that B in the cell wall is associated with rhamnogalacturonan II (Ishii and Matsunaga, 1996; Kobayashi et al., 1996; O'Neill et al., 1996) and that the B requirement is correlated with cell wall pectin contents (Hu et al., 1996). It is possible that the reduced levels of B in the *bor1–1* mutant may be caused by an altered composition of the cell wall. B deficiency and toxicity are major nutritional disorders, and cultivars having different sensitivities to B deficiency and toxicity were bred in a number of crop species (Loomis and Durst, 1992). The mutant described here presents a possible route to understanding molecular components that control B requirements in higher plants.

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