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BORON NUTRITION, INTRACELLULAR TRANSPORT, AND KNIFE-CUT DISEASE IN SUNFLOWER.

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ABSTRACT

The present study was conducted with the primary aim to investigate in sunflower the processes of boron uptake, intracellular compartmentation and xylem translocation in response to B supply, ranging from deficiency, incipient toxicity and to short term changes in B supply. The experiments were conducted with two sunflower genotypes, selected on the basis of their susceptibility to knife-cut.

It appears that the roots of the sunflower susceptible genotype (S) were more sensitive to low B contents in the solution media than the shoots. The decrease in root dry weight in high B treatments could also indicate it was more sensitive to B toxicity. Though root dry weight has decreased, the shoot : root dry weight ratio was smaller in the resistant genotype (R), suggesting that this genotype would have a substantially large root volume, capable of supporting the B demand of its shoots.

The B contents in the water insoluble residue (WIR) of roots were similar for all genotypes and treatments. In contrast, the B concentration in WIR of leaves reached values near saturation only when B started to accumulate in the cell sap (CS) of roots to the level as detected in CS of leaves. The critical values of B concentrations in shoot-

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tissues would then be established after the boron requirement for cell walls was satisfied and a proper metabolic B content in CS of roots was reached.

Uptake efficiency (UE) values < 1.00, detected as a result of treatments with high concentration of B in the nutrient solution, suggested the presence of an exclusion mechanism to be working in order to restrict B accumulation. The high UE value obtained with low B treatments indicated that mechanisms, other than mass flow, had played a role in providing the acquired B.

Key words: Plant Nutrition, micronutrients, Boron deficiency, Boron uptake.

Introduction

Boron (B) is an essential element for higher plants and it is found in live tissue in water soluble and water insoluble forms (Matoh, 1997). The water insoluble form is associated with cell wall pectins (Hu and Brown, 1994), where boron is expected to perform an important function related to cell wall structure (Brown et al., 2002). In higher plants, cell walls have been identified to be boron-polysaccharide complexes, formed by two chains of rhamnogalacturonan II, cross-linked by boric acid (Kobayashi et al., 1996; O'Neill et al., 1996). On the other hand, soluble boron is found in the cell sap (CS), formed in simplasmic and apoplasmic aqueous solution (Pfeffer et al., 2001).

The amount of cell wall-bound B, as well as the amount of B in CS, varies with B supply, plant species, and plant organ. For instance, squash plants (*Cucurbita pepo* L.) and cultured tobacco cells (*Nicotiana tabacum*), grown in B deficient nutrient media, showed a greater proportion of the absorbed B to be localized in their cell walls, whereas a much lesser proportion was present in CS (Hu and Brown, 1994). In

sunflower plants (*Helianthus annuus* L.), grown with a B supply varying from 0.1 to 1600 µmol L^{-1}, the proportion of cell wall-bound B ranged from 95 to 15 % for the root and from 66 to 15 % for the shoot (Dannel et al., 1998).

Boron uptake in higher plants has long been studied and evidence has been presented supporting both active and passive uptake of B (Brown et al., 2002). When B supply is high, B uptake by roots is believed to occur by passive diffusion. At low B supply, its accumulation in the symplasm of root cells is considered to depend on two processes working together: (1) an energy dependent process and (2) a passive diffusion process along a gradient, maintained by the formation of B complexes within the cell (Pfeffer et al., 1999). However, Takano et al. (2002) suggested that the concentration mechanism, functioning at low boron supply, was mediating xylem loading.

Globally, boron deficiency is a widespread problem (Shorrocks, 1997), and sunflower is considered a susceptible crop, where B deficiency produces a wide variety of symptoms. A field symptom, related to boron deficiency in sunflower, is the capitulum abscission, commonly known as knife-cut (Furlani et al., 1990). The present study was conducted with the primary aim to investigate in sunflower the processes of boron uptake, intracellular compartmentation and xylem translocation in response to B supply, ranging from deficiency, incipient toxicity and to short term changes in B supply. The experiments were conducted with two sunflower genotypes, selected on the basis of their susceptibility to knife-cut, when grown under similar field conditions. Comparison of the two genotypes for their susceptibility to knife-cut was a secondary aim of this investigation.

Materials and Methods

Experiment 1.

Experimental Design

Two sunflower genotypes, selected on the basis of their degree of susceptibility to suffer from the abscission of the capitulum, were grown in solution cultures, using six levels of B treatments, replicated four times (2 genotypes x 6 B levels x 4 replications = 48 pots). The sunflower genotypes are referred to as S (higher degree of susceptibility to abscission of the capitulum) and R (lesser degree of susceptibility to abscission of the capitulum) throughout the paper. A randomized completed design was used in this experiment.

Growing Conditions

Sunflowers seeds were soaked in aerated tap water for 22 h and germinated on wet paper towels in a growth chamber with a temperature of 20 º C /10º C (day/night). After 4 days, five uniform seedlings were transferred to 300 mL plastic pots, containing tap water. The pots were randomly distributed in a growth chamber, employing a 16 h light and 8 h dark photoperiods. After 5 days, the tap water was replaced by nutrient solutions, containing (μM): 2500 $Ca(NO_3)_2$, 2500 KNO_3, 1000 $MgSO_4$, 500 KH_2PO_4, 9.14 $MnCl_2$, 0.79 $ZnSO_4$, 0.29 $CuSO_4$, 0.015 $(NH4)_6Mo_7O_{24}$, and 10 FeEDTA. Boron was supplied as H_3BO_3 in concentration of 0, 1, 10 ,50, 75, 100 µmol L^{-1}. Distilled water, containing less than 0.015 g B L^{-1}, and analytical grade chemicals were used to make up the nutrient solutions. The nutrient solutions were constantly aerated. During the experiment the solutions were brought back to volume with distilled water every day

and were renewed twice. The consumed water was calculated. The plants were monitored for any symptoms of B deficiency or toxicity over the period of the experiment. Plants of 8 pots (4 pots for each genotype) were harvested just before the B treatment were imposed.

Collection of Xylem Exudate and Harvest

The plants were harvested 10 days after the B treatments were imposed. They were cut just below the cotyledonary leaves and a plastic tube was fixed over the stump to collect xylem exudate. By covering the plants with a polyethylene bag the transpiration was minimized to create conditions, favouring gutation. Xylem exudate was collected over a day period. The roots were then rinsed for 20 seconds with distilled water, blotted dry, and cut at the transition point between the root and hypocotyl. The shoots were divided into leaves (consisting of plant material above the first pair of leaves) and CHS (consisting of cotyledonary leaves, hypocotyl and lower stem). The fresh weight of the plants parts was recorded, and the plant material was then frozen at -18 º C to rupture the cells.

Intracellular Compartmentation

Frozen samples of roots and leaves were thawed, transferred to filter tubes which were placed inside centrifugal tubes and centrifuged for 10 minutes. Filtered solutions were taken as root or leave cell saps. The residue over the filter was homogenized with a mortar and pestle in distilled water, after which the homogenate was then centrifuged for 10 minutes, and the supernatant discarded. The residue was washed 3 times with

distilled water by repeating the homogenization and centrifugation procedures. The water insoluble residue (WIR) contains B, closely bound to cell wall polymers, and the cell sap (CS) is a mixture of intracellular and apoplasmic fluid (Pfeffer et al., 2001). Total B content in cell sap was calculated as follows, assuming that 1 g cell sap equals 1 mL cell sap:

Total B (µg) = (fresh weight - dry weight) (ml) x µg B mL^{-1} cell sap

Boron Analysis

Boron concentrations in cell sap and xylem exudate were determined using an inductively-coupled plasma atomic emission spectrometer (ICPS Shimadzu 1000 III).

The rest of the B concentrations was determined by the Azomethine-H method (Gupta and Stewart, 1975). To prevent volatilization of B at high temperatures, an appropriate amount of saturated $Ca(OH)_2$ solution was added to the samples prior to drying. The dry weight of the plants parts was recorded after drying at 70 º C to constant weight. Plant material was dry ashed at 500 º C for at least 3 h, and the ash dissolved in an appropriate volume of 1 *N* H_2SO_4 prior to B analysis using the Azomethine-H method.

All equipment used in the experiment was washed with diluted HCl and rinsed with distilled water to diminish B contamination.

***Experiment** 2.*

Experimental Design, Grown Conditions, Short Term Treatments and Harvest

Experimental procedures for growing plants were similar to those of experiment 1. Treatments were replicated four times (2 genotypes x 4 B treatments x 4 replications = 32 pots). A randomized completed design was used in this experiment. Plants of 8 pots (4 pots for each genotype) were harvested just before the first period of culture in nutrient solution was imposed. Both sunflower genotypes were grown in a preculture nutrient solution at two levels of boron (4 and 30 µmol L^{-1}). After seven days of preculture, the plants were transferred to nutrient solutions containing 4 or 30 µmol L^{-1}. The resulting four treatments were named 4/4, 4/30, 30/4 and 30/30, whereby the first numbers indicate the B concentration in the nutrient solutions during the first period of culture and the second numbers during the short term treatments. After two hours, xylem exudate was collected over a four hours period as previously described, but the collected volume was not large enough to allow the determination of boron concentration. Afterwards, roots were rinsed for 20 seconds with distilled water, blotted dry, and cut at the transition point between the root and hypocotyl. The shoots were divided into leaves (consisting of plant material above the cotyledonary leaves), hypocotyls and cotyledonary leaves.

Intracellular Compartmentation and Boron Analysis

As previously described, roots, hypocotyls and leaves were subjected to intracellular boron compartmentation analysis. Boron concentrations in cell sap were measured again by inductively-coupled plasma atomic emission spectrometry. The rest of the B concentrations was determined by the Azomethine-H method, as previously described.

Calculation of Uptake Efficiency

Uptake efficiency (UE) defined as total absorbed boron during B treatments divided by the amount of boron that the plant would hypothetically absorb, if the plasma membrane was not offering resistance to boron permeation, was calculate as follows:

UE = (TBP1 – TBP2) (µg B) / µg B mL^{-1} nutrient solution x mL water consumed

where TBP1 is the sum of total B of every compartment in every plant part, TBP2 is the total B content of plants that were harvested before plants started growing in nutrient solution, and µg B mL^{-1} nutrient solution is the boron concentration in the nutrient solution.

Statistical Analyses

The data were subjected to analysis of variance (ANOVA). They were transformed when they did not conformed to homogeneity requirements for the analysis of variance. The original data are shown, and significant treatment effects were separated with the Fisher's protected LSD Test at $p \leq 0.05$.

Results

Experiment 1.

Visual Symptoms of B Deficiency

In both the genotypes, boron deficiency symptoms were similar and became evident only under 0 and 1 B treatments. Under 0 B treatment the visual symptoms occurred: a) by the fourth day, when the leaves were thickened and the tissue was brittle, manifested mainly at the stem above the cotyledonary leaves; b) by the fifth day, when the base of the youngest leaves was whitish in color; c) by the sixth day, when the plants were visibly smaller than those grown under the other B treatments; d) by the eight day, when plants had developed severe symptoms of B deficiency, as noticed by the youngest leaves buckling downward and the leaf base was brown or whitish in color; whereas the older leaves were hard and dark green, with shining or necrotic areas in the margins upward. Splitting of hypocotyls was also observed. The most evident symptoms, as a result of 1 B treatments, were: a) thickening of the leaves by the fourth day; b) whitish coloring by the eight day of the base of the youngest leaves. While some of these leaves were buckling downward; some of the old leaves had shining areas and interveinal crinkling.

At harvest, plants under 0 B treatment had only the first pair of leaves completely expanded. The base of the youngest leaves was brown in color in plants grown under 1 B treatment and B toxicity symptoms (necrosis of the oldest leaf tips) became evident under 100 B treatment.

Dry Weight

Both genotypes suffered a decrease in root dry weight as a result of 0 and 1 B treatments (Table 1), whereas shoot dry weight was affected only by 0 B treatment. Otherwise, when plants of the R genotype were grown in solutions, containing 10, 50,

75 and 100 µmol B L^{-1}, their roots dry weight remained constant. However, roots of plants of the S genotype decreased in dry weight by the 75 and 100 B treatments. The ratio of shoot to root dry weight increased with low B concentrations in solution, but under 0 B treatment, this ratio was higher for plants of the S genotype than that for plants of the R genotype.

Boron Concentration and Boron Content

A linear relationship was obtained (Table 2) between B concentration in nutrient solution and B concentration in root cell sap for each genotype. Boron concentration in root cell sap, at 0 and 1 B treatments, was smaller than that at 0.0µ1 g B mL^{-1}. However, this concentration increased progressively at treatments up to 100 B, where on the average the B content of both genotypes was eight times higher than that at 10 B treatment. In contrast, B concentrations in WIR of roots (Table 2) were similar under all treatments, though on the average the B content in WIR of roots of plants belonging to the S genotype was higher than that of plants in the R genotype.

Boron contents in both WIR and CS of leaves (Table 2) increased progressively with B concentration in nutrient solution. Between 0 and 10 B treatments the B content increased substantially in WIR of leaves. Further increments of B in the nutrient solution, resulted in a slight increase only in B concentration in WIR of leaves (less than twice between 10 and 100 B treatments). In contrast, the increases in B contents in CS of leaves were about five times between 10 and 100 B treatments. However, the B concentration, at 0 and 1 B treatments, was smaller than that at 0.01 µg B mL^{-1}.

It must be underscored that the B concentration in WIR of roots, at 0 and 1 B treatment, was not only higher than that in WIR of leaves (Table 2), but that the

concentration was also close to its greatest value. In contrast, the greatest value of B content in WIR of leaves tended to be reached in a nutrient solution with boron concentrations >5 μ*M*. However, at 0 and 1 B treatments, the B concentration in WIR of leaves in S genotype was higher than that in R genotype.

In general, total B content in WIR of roots and leaves (Table 2) did not differ between genotypes, and it increased when B concentration in nutrient solution was increased from 0 to 10 μ*M*, and the total B content seemed to be unaffected by the latter treatment in both genotypes.

Uptake Efficiency (UE)

The results (Table 3) showed that the uptake efficiency was affected by B supply. As a result of 1 and 10 B treatments, the UE was almost one (0.93 on the average for both genotypes at both B concentrations). It was smaller in value as a result of the other treatments. In this regard, the UE, at 0 B treatment, was on the average 0.47, and remained < 1 (0.26 on the average) at treatments of 50 and 100 B..

Boron Concentration in CS of Roots and Leaves and in Xylem Exudates

In order to make the comparison more meaningful, the B concentrations in CS of roots and leaves and in xylem exudates were expressed in μg B mL^{-1} (Tabla 4). Similarly as in the results, shown in Table 2, a linear relationship was also detected between B concentration in the nutrient solution and B concentration in root cell sap, for each genotype. When plants were grown at 0 and 1 B treatment, the B content in CS and xylem exudate was < 0,01 μg B mL^{-1}. Practically, no differences were noticed between B concentrations in CS of roots and B concentration in nutrient solutions at the 10, 50,

75 and 100 B treatments (Table 4). In contrast, there was a direct relationship between the increase of B concentration in the solutions and the increase in B concentration in CS of leaves. Significant differences were also noticed between B concentration in xylem exudates and B concentration in the nutrient solution. Actually, at the 10 B treatment, the B content in the xylem exudate was three times higher than that in the nutrient solution. In plants, grown in nutrient solutions with B concentrations < 10 μ*M*, the B concentration in the xylem exudate decreased more and more than that in the nutrient solution, and reductions of B contents were as high as 50 % in the nutrient solution.

Experiment 2

Visual Symptoms of B Deficiency and Dry Weight

Under the conditions of experiment 2, no visible symptoms of B deficiencies were observed. Differences between dry weight of roots, hypocotyls and leaves were not found, neither between genotypes nor between B treatments.

Boron Compartmentation in Roots

Boron concentration in WIR of roots (Table 5) was not modified when plants were transferred to nutrient solution of higher B concentration (4/30 B treatment) or from high to lower B concentration (30/4 B treatment) in comparison with plants that were supplied continuously with 4 μ*M* or 30 μ*M*, and no differences between genotypes were also evident.

Preculture conditions indicated that at the 4/4 B treatment, the B concentration in CS of roots was lower than that under the 30/30 B treatment (Table 5). Otherwise, the transfer to nutrient solutions with a different B concentration produced a quite rapid effect on B contents in CS. Six hours after the B supply was changed from 4/30 B to 30/30 B treatments, the B concentration in CS reached similar values than those under the 30/30 B treatment. However, the B concentration in CS decreased to a value similar to that of the 4/4 B treatment, as a result of the change in medium to 30/4 B treatment.

Boron Compartmentation in Hypocotyls

Neither the preculture conditions nor the change in B concentrations in nutrient solutions, into which the plants were transferred for a short period of time, had any effect on B contents in WIR of hypocotyls (Table 5). The values were similar under all treatments and differences between genotypes were not evident.

Boron concentration in CS of hypocotyls (Table 5) increased when plants, precultured with 4 μ*M* B in solution, were transferred to a growth medium containing 30 μ*M* (4/30 B treatment) with respect to plants, grown continuously with 30 μ*M* (30/30 B treatment). As a result of 30/4 B treatment, the B content in CS of hypocotyls attained values of plants, subjected to 4/4 and 30/30 B treatments. There were no evidences of differences between genotypes.

Boron Compartmentation in Leaves

Boron concentration in WIR of leaves (Table 5) was affected by preculture conditions, but not by the change in B contents of nutrient solutions, into which plants

were transferred for a short period of time. Boron concentration in CS was also affected by B supply during preculture, since B content in CS of leaves was lower at the 4/4 B than that at the 30/30 treatments (Table 5). Transferring plants for a short duration in nutrient media of different B concentrations did not produced any effect on B contents CS of leaves at the 4/30 and 30/4 B treatments as compared to those at the 4/4 and 30/30 B treatments.

Uptake Efficiency (UE)

When plants were supplied continuously with 4 μ*M* B, the uptake efficiency for both genotypes was 1.77 on the average, whereas plants supplied continuously with 30 μ*M* B exhibit uptake efficiencies of 0.32 on the average (for both genotypes, Table 3).

Discussion

It appears that the roots of the sunflower S genotype were not only more sensitive to low B contents in the solution media than the shoots, as supported by Blamey et al. (1997), but the decrease in root dry weight by the 75 and 100 B treatments could also indicate the effect of B toxicity, similar to that found by Dannel et al. (1998). Though root dry weight has decreased, the shoot : root dry weight ratio was smaller in the R genotype, suggesting that this genotype would have a substantially large root volume, capable of supporting the B demand of its shoots. For similar genotypes, such an adjustment in shoot : root ratio could be part of an effective strategy, enabling sunflower plants to take up more B in order to satisfy the B demand of shoots, when B supply is restrictive under field conditions.

It should be emphasized that in the S genotype, the decrease in root dry weight due to B deficiency (0 and 1 B treatment) and toxicity (75 and 100 B treatments) corresponded with an increase in B concentration in WIR of the roots, while the total B content did not differ much between genotypes. The observation above suggests that the S genotype exhibits a higher boron requirement threshold value for root cell walls than that of the R genotype.

The B contents in WIR of roots were similar for all genotypes and treatments. In contrast, the B concentration in WIR of leaves reached values near saturation only when B started to accumulate in the CS of roots to the level as detected in CS of leaves. In sunflower plants, it seems apparent that the boron requirement for cell walls of roots must first be met, before the boron requirement for cell walls of shoots can be satisfied. Once these steps have taken place, accumulation of metabolically available boron in CS of roots starts to occur, followed by its accumulation in CS of leaves, where it is concentrated. These findings would confirm that: (a) WIR of roots and leaves behave like a chemical absorber of B, with a limited number of binding sites occupied by B until saturation is attained (Dannel et al., 1998), and (b) the mechanism suggested by Hu and Brown (1997) was effective since with the formation of B complexes in the cell wall a concentration gradient was kept in operation as the key step for translocation of B to the shoots.

The critical values of B concentrations in shoot-tissues would then be established after the boron requirement for cell walls was satisfied and a proper metabolic B content in CS of roots was reached. The above became evident in plants, grown in nutrient solutions with a B concentration between 4 μ*M* (experiment 2) and 10 μ*M* (experiment 1). In addition, the results of experiment 2, with their changes in B supply, showed that: (a) a B concentration of 4 μ*M* in the nutrient solution was sufficient to satisfy the

requirement of root cell walls; and (b) the amount of B absorbed in WIR was stable, since it was not affected by the decrease in B supply, which was in agreement with the results of Pfeffer et al. (1997).

The quick adjustment (six hours) of B concentration in CS of roots that took effect due to changes of B concentration in the nutrient solution, indicated that the changing B concentration in CS of roots or leaves could be used in combination with the more stable B concentration in WIR of roots or leaves, as components of a criterion, relating B status in plant with B availability in the medium, and to predict the likelihood of B deficiency or toxicity to occur. Therefore, B availability in the medium would be sufficient during the vegetative growth of sunflower, when the ratio between B concentration in CS and B concentration in WIR of roots (both expressed in μg B g^{-1} dry weight) was > 0.1 (Table 2). However, in young leaves, the value of the B ratio between both compartments was higher than 0.3.

When the results in the present investigation are compared with those of other authors, it can be seen that the total B concentration in leaves at the 0 and 1 B treatments (12,7 and 24,2 μg B g^{-1} dry weight of WIR, respectively for both genotypes) is in accordance to previously reported findings on B requirement of sunflower (Asad et al., 2001; Asad et al., 2003; Asad et al 2002). However, Blamey et al. (1997) found a marked increase in total dry weight with an increase in B concentration (> 22.0 μg B g^{-1} dry weight) in the youngest mature leaf blade (YMB), and they considered deficiency to occur at a critical value of a B concentration of 190.0 μg B g^{-1} dry weight (90% of maximum yield) in the YMB. The latter value was considerably higher than those reported in other studies, but was close to the total B concentration in leaves (CS and WIR) by the 100 B treatment (255.5 μg B g^{-1} dry weight of WIR on the average for both genotypes) in present investigations, at which B toxicity symptoms were observed.

Uptake efficiency values < 1.00, detected as a result of treatments of 30 to 100μ*M* B in the nutrient solution, suggested the presence of an exclusion mechanism to be working in order to restrict B accumulation. However, this exclusion mechanism was not completely effective at B supplies between 1 and 10 μ*M*, and the high UE value obtained by the 4/4 B treatments indicated that mechanisms, other than mass flow, had played a role in providing the acquired B. The mechanism, causing the restriction in uptake of B, is not exactly known, but an excretion mechanism had been proposed by Bellaloui and Brown (1998), whereas other authors quoted a higher membrane permeability for water as a better explanation with respect to boron absorption (Weig et al., 1997 cited by Brown et al., 2002). The low UE value at the 0 B treatment (0.47 on the average for both genotypes), could be interpreted as a consequence of a severe B deficiency, since B seemed to be of crucial importance for the maintenance of structural integrity of plasma membranes (Cakmak and Römheld, 1997).

The pathways of nutrient transport from root surface to shoot include at least two processes of transmembrane transports: (1) import into epidermal or cortex cells; and, (2) export from pericycle or xylem parenchyma cells into the stelar apoplasm (xylem loading) (Takano et al., 2002). As discussed in the introduction, B uptake and B transport through membranes in higher plants were believed to be facilitated by both active and passive uptake processes (Brown et al, 2002). It is suggested that B uptake by roots would be by passive diffusion when B supply was high, whereas B accumulation in the symplasm of root cells was supposed to be an active process when B supply was low, since such an accumulation would take place against a concentration gradient (Pfeffer et al., 1999). When the values of plant WIRs were near saturation (10 B treatment or higher B supply), the B concentrations in the different liquid compartments indicated that: (a) there was no accumulation of B in CS of roots, since B contents

follow a linear concentration dependence; (b) at the 10 B treatment, the B concentration in xylem exudate was three times higher than the B content in the nutrient solution. At higher B supply, the boron concentrations in the xylem exudates followed a linear concentration dependence; and (c) B accumulation started in leaf CS against a concentration gradient. Furthermore, the results of experiment 2 showed that the great difference, found in B concentration in CS of roots as a result of treatments between 4/4 and 30/30 B, was reduced to a minimum when the B concentration in CS of hypocotyls was included. The data above suggest that a concentration mechanism was perhaps induced and effective at low B supply as a result of treatments of < 4 or 10 μ*M* B. This mechanism was perhaps the key process, controlling B accumulation, which was named as xylem loading by Takano et al. (2002). These authors, working with *Arabidopsis thaliana* mutant bor1-1 (sensitive to boron deficiency) and wild-type plants, showed that the concentration of boron in root cell sap increased in proportion to boron concentration in the medium in both the genotypes, suggesting that B uptake into roots occurred mainly by passive transport. The concentration of boron in xylem exudates of the bor1-1 plants also followed a linear concentration dependence, whereas a combination of saturable and linear concentration dependence was observed in the wild plants. Takano et al (2002) also indicated that xylem loading is the key step for boron accumulation in shoots with a low external boron supply and that BOR1, an efflux-type boron transporter for xylem loading, was an essential component of the process.

The present results also indicated the presence of two factors explaining the differences in susceptibility to knife-cut between the S and R sunflower genotypes, when grown under similar field conditions: (a) a higher capability of adjustment of shoot : root ratio for the R genotype under deficient boron conditions; and (b) a higher B requirement threshold for cell walls for S genotype. Though a clear relationship between

degree of susceptibility and B-efficiency could not be established adequately, the suggestion is made that the higher or lower susceptibility to B deficiency between genotypes is perhaps related to the capability of establishing B concentrations in CS of roots, allowing for proper transport and B accumulation in CS of leaves.

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Table 1. Dry weight of WIR of roots, WIR of leaves and cotyledonary leaves, hypocotyls and lower stems (CHS) in two sunflower genotypes when plants grown for ten days under different B supply. Values represent means of four replications ± SD.

	Genotype	Boron concentratioon in nutrient solution (µM)					
		0	1	10	50	75	100
Dry weight of WIR of roots	S	109.3 ± 14	149.7 ± 11	192.5 ± 19	200.5 ± 22	160.8 ± 14	154.4 ± 16
	R	121.2 ± 12	165.4 ± 22	223.4 ± 50	197.5 ± 36	225.7 ± 18	202.3 ± 35
Dry weight of WIR of leaves	S	213.9 ± 36	324.1 ± 49	325.9 ± 6	301.4 ± 46	272.8 ± 42	259.9 ± 40
	R	201.5 ± 25	344.4 ± 52	374.3 ± 72	348.0 ± 60	364.7 ± 72	300.9 ± 46
Dry weight of CHS	S	489.5 ± 51	526.2 ± 37	591.6 ± 23	516.7 ± 54	564.7 ± 52	567.8 ± 76
	R	441.3 ± 18	545.0 ± 24	573.3 ± 49	521.0 ± 52	689.4 ± 88	590.8 ± 76
Ratio between Dry weight of WIR of leaves + Dry weight of CHS and Dry weight of WIR of roots	S	6.54 ± 1.24	5.68 ± 0.23	4.80 ± 0.51	4.08 ± 0.16	5.20 ± 0.16	5.35 ± 0.29
	R	5.34 ± 0.55	5.41 ± 0.46	4.34 ± 0.70	4.44 ± 0.34	4.65 ± 0.33	4.44 ± 0.35

Table 2. Boron concentration (μg g^{-1} of WIR dry weight) in CS and WIR of roots and leaves and total boron content (μg) in WIR of roots and leaves in two sunflower genotypes when plants grown for ten days under different B supply. Values represent means of three or four replications ± SD. ND not determined. < 0.01 B concentration lesser than 0.01 μg B ml^{-1}.

	Genotype	Boron concentratioon in nutrient solution (μM)					
		0	1	10	50	75	100
Boron concentration in CS of roots	S	< 0.01	< 0.01	4.8 ± 0.5	20.3 ± 2.3	30.0 ± 0.6	37.7 ± 2.8
	R	< 0.01	< 0.01	4.5 ± 0.8	21.7 ± 2.2	29.7 ± 1.9	37.1 ± 3.0
Boron concentration in WIR of roots	S	51.2 ± 4.9	42.1 ± 2.8	42.1 ± 1.9	44.3 ± 0.6	61.6 ± 14.9	62.4 ± 12.3
	R	45.1 ± 2.7	37.5 ± 2.9	40.9 ± 3.4	44.0 ± 2.4	47.6 ± 2.8	49.5 ± 3.4
total boron content in WIR of roots	S	5.6 ± 0.5	6.3 ± 0.7	8.1 ± 0.5	8.9 ± 0.9	10.0 ± 3.0	9.7 ± 2.8
	R	5.4 ± 0.5	6.2 ± 0.5	9.0 ± 1.4	8.6 ± 1.2	10.7 ± 0.7	10.0 ± 1.8
Boron concentration in CS of leaves	S	< 0.01	< 0.01	29.5 ± 1.6	63.8 ± 5.8	ND	140.4 ± 3.4
	R	< 0.01	< 0.01	27.3 ± 2.3	63.0 ± 5.2	92.6 ± 8.3	127.6 ± 15.9
Boron concentration in WIR of leaves	S	13.9 ± 0.9	26.9 ± 6.3	84.8 ± 2.1	100.0 ± 7.3	113.0 ± 4.5	124.5 ± 5.3
	R	11.6 ± 1.3	21.5 ± 1.3	80.0 ± 4.1	97.3 ± 2.9	112.9 ± 4.5	120.2 ± 2.2
total boron content in WIR of leaves	S	3.0 ± 0.6	8.5 ± 1.1	27.6 ± 1.0	30.1 ± 4.6	30.9 ± 5.4	32.2 ± 4.1
	R	2.3 ± 0.2	7.4 ± 0.9	29.7 ± 4.4	33.9 ± 5.9	41.4 ± 9.9	36.2 ± 6.0

Table 3. The effect of B supply on boron uptake efficiency in two sunflower genotypes grown in two independent experiments. In Experiment 1 plants were grown with B concentrations of 0, 1, 10, 50, 75 y 100 µM, and in Experiment 2 under B concentrations of 4 y 30 µM. Values represent means of three or four replications ± SD. ND not determined.

Genotype	Boron concentratioon in nutrient solution (µM)							
	0	1	4	10	30	50	75	100
S	0.67±0.29	1.07±0.15	1.69±0.17	0.89±0.03	0.30±0.04	0.23±0.03	ND	0.26±0.01
R	0.28±0.20	0.90±0.09	1.85±0.12	0.87±0.10	0.35±0.02	0.28±0.02	0.28±0.02	0.26±0.04

Table 4. Boron concentration (µg ml^{-1}) in CS of roots, in xylem exudate and in CS of leaves in two sunflower genotypes when plants grown for ten days under different B supply. Values represent means of three or four replications ± SD. ND not determined. < 0.01 B concentration lesser than 0.01 µg B ml^{-1}.

	Genotype	Boron concentration in nutrient solution (µg ml^{-1})					
		0	0.01	0.11	0.54	0.81	1.08
Boron concentration in CS of roots	S	< 0.01	< 0.01	0.13 ± 0.02	0.54 ± 0.06	0.77 ± 0.02	0.97 ± 0.06
	R	< 0.01	< 0.01	0.12 ± 0.03	0.56 ± 0.04	0.86 ± 0.03	0.98 ± 0.14
Boron concentration in xylem exudate	S	< 0.01	< 0.01	0.32 ± 0.03	0.54 ± 0.02	0.64 ± 0.09	0.78 ± 0.06
	R	< 0.01	< 0.01	0.31 ± 0.01	0.42 ± 0.02	0.57 ± 0.02	0.58 ± 0.09
Boron concentration in CS of leaves	S	< 0.01	< 0.01	1.55 ± 0.12	3.13 ± 0.23	ND	7.63 ± 0.22
	R	< 0.01	< 0.01	1.36 ± 0.08	3.03 ± 0.38	4.72 ± 0.27	6.47 ± 1.14

Table 5. Boron concentration (µg g^{-1} of WIR dry weight) in WIR of roots, hypocotyls and leaves and boron concentration (µg ml^{-1}) in CS of roots, hypocotyls and leaves in two sunflower genotypes. Plants grown for seven days with a B supply of 4 or 30 µM, then were transferred to nutrients solutions containing 4 or 30µmol L^{-1} for six hours, the first numbers indicate the B concentration in the nutrient solution during the first period of culture and the second numbers during the short term treatments. Values represent means of three or four replications ± SD.

	Genotypes	B Treatments			
		4/4	4/30	30/4	30/30
Boron concentration in WIR of roots	S	45.6 ± 3.6	43.1 ± 1.9	44.3 ± 3.3	44.4 ± 2.8
	R	44.7 ± 4.2	47.3 ± 3.4	46.2 ± 4.2	46.3 ± 2.1
Boron concentration in CS of roots	S	0.03 ± 0.01	0.19 ± 0.04	0.05 ± 0.00	0.18 ± 0.03
	R	0.04 ± 0.01	0.20 ± 0.05	0.04 ± 0.01	0.19 ± 0.02
Boron concentration in WIR of hypocotyls	S	66.2 ± 2.4	68.0 ± 1.6	69.6 ± 5.4	73.8 ± 6.2
	R	70.7 ± 9.4	70.8 ± 5.7	76.6 ± 5.5	71.6 ± 11.8
Boron concentration in CS of hypocotyls	S	0.07 ± 0.02	0.11 ± 0.02	0.08 ± 0.01	0.09 ± 0.01
	R	0.07 ± 0.01	0.13 ± 0.04	0.10 ± 0.02	0.11 ± 0.03
Boron concentration in WIR of leaves	S	52.8 ± 0.4	51.1 ± 2.6	61.4 ± 5.6	58.3 ± 2.1
	R	51.2 ± 3.9	51.6 ± 3.5	60.9 ± 1.2	59.8 ± 1.8
Boron concentration in CS of leaves	S	0.70 ± 0.12	0.53 ± 0.10	1.79 ± 0.10	1.76 ± 0.18
	R	0.58 ± 0.05	0.55 ± 0.05	1.52 ± 0.22	1.53 ± 0.08

BORON NUTRITION, INTRACELLULAR TRANSPORT, AND KNIFE-CUT DISEASE IN SUNFLOWER.

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ABSTRACT

The present study was conducted with the primary aim to investigate in sunflower the processes of boron uptake, intracellular compartmentation and xylem translocation in response to B supply, ranging from deficiency, incipient toxicity and to short term changes in B supply. The experiments were conducted with two sunflower genotypes, selected on the basis of their susceptibility to knife-cut.

It appears that the roots of the sunflower susceptible genotype were more sensitive to low B contents in the solution media than the shoots. The decrease in root dry weight in high B treatments could also indicate it was more sensitive to B toxicity. Though root dry weight has decreased, the shoot : root dry weight ratio was smaller in the resistant genotype, suggesting that this genotype would have a substantially large root volume, capable of supporting the B demand of its shoots.

The B contents in the water insoluble residue (WIR) of roots were similar for all genotypes and treatments. In contrast, the B concentration in WIR of leaves reached values near saturation only when B started to accumulate in the cell sap (CS) of roots to the level as detected in CS of leaves. The critical values of B concentrations in shoot-

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tissues would then be established after the boron requirement for cell walls was satisfied and a proper metabolic B content in CS of roots was reached.

Uptake efficiency (UE) values < 1.00, detected as a result of treatments with high concentration of B in the nutrient solution, suggested the presence of an exclusion mechanism to be working in order to restrict B accumulation. The high UE value obtained with low B treatments indicated that mechanisms, other than mass flow, had played a role in providing the acquired B.

Key words: Plant Nutrition, micronutrients, Boron deficiency, Boron uptake.

Introduction

Boron is an essential element for higher plants and it is found in live tissue in water soluble and water insoluble forms (Matoh, 1997). The water insoluble form is associated with cell wall pectins (Hu and Brown, 1994), where boron is expected to perform an important function related to cell wall structure (Brown et al., 2002). In higher plants, cell walls have been identified to be boron-polysaccharide complexes, formed by two chains of rhamnogalacturonan II, cross-linked by boric acid (Kobayashi et al., 1996; O'Neill et al., 1996). On the other hand, soluble boron is found in the cell sap, formed in simplasmic and apoplasmic aqueous solution (Pfeffer et al., 2001).

The amount of cell wall-bound B, as well as the amount of B in CS, varies with B supply, plant species, and plant organ. For instance, squash plants (*Cucurbita pepo* L.) and cultured tobacco cells (*Nicotiana tabacum*), grown in B deficient nutrient media, showed a greater proportion of the absorbed B to be localized in their cell walls, whereas a much lesser proportion was present in CS (Hu and Brown, 1994). In

sunflower plants (*Helianthus annuus* L.), grown with a B supply varying from 0.1 to 1600 µmol L^{-1}, the proportion of cell wall-bound B ranged from 95 to 15 % for the root and from 66 to 15 % for the shoot (Dannel et al., 1998).

Boron uptake in higher plants has long been studied and evidence has been presented supporting both active and passive uptake of B (Brown et al., 2002). When B supply is high, B uptake by roots is believed to occur by passive diffusion. At low B supply, its accumulation in the symplasm of root cells is considered to depend on two processes working together: (1) an energy dependent process and (2) a passive diffusion process along a gradient, maintained by the formation of B complexes within the cell (Pfeffer et al., 1999). However, Takano et al. (2002) suggested that the concentration mechanism, functioning at low boron supply, was mediating xylem loading.

Globally, boron deficiency is a widespread problem (Shorrocks, 1997), and sunflower is considered a susceptible crop, where B deficiency produces a wide variety of symptoms. A field symptom, related to boron deficiency in sunflower, is the capitulum abscission, commonly known as knife-cut (Furlani et al., 1990). The present study was conducted with the primary aim to investigate in sunflower the processes of boron uptake, intracellular compartmentation and xylem translocation in response to B supply, ranging from deficiency, incipient toxicity and to short term changes in B supply. The experiments were conducted with two sunflower genotypes, selected on the basis of their susceptibility to knife-cut, when grown under similar field conditions. Comparison of the two genotypes for their susceptibility to knife-cut was a secondary aim of this investigation.

Materials and Methods

Experiment 1.

Experimental Design

Two sunflower genotypes, selected on the basis of their degree of susceptibility to suffer from the abscission of the capitulum, were grown in solution cultures, using six levels of B treatments, replicated four times (2 genotypes x 6 B levels x 4 replications = 48 pots). The sunflower genotypes are referred to as S (higher degree of susceptibility to abscission of the capitulum) and R (lesser degree of susceptibility to abscission of the capitulum) throughout the paper. A randomized completed design was used in this experiment.

Growing Conditions

Sunflowers seeds were soaked in aerated tap water for 22 h and germinated on wet paper towels in a growth chamber with a temperature of 20 ° C /10° C (day/night). After 4 days, five uniform seedlings were transferred to 300 mL plastic pots, containing tap water. The pots were randomly distributed in a growth chamber, employing a 16 h light and 8 h dark photoperiods. After 5 days, the tap water was replaced by nutrient solutions, containing (µ*M*): 2500 $Ca(NO_3)_2$, 2500 KNO_3, 1000 $MgSO_4$, 500 KH_2PO_4, 9.14 $MnCl_2$, 0.79 $ZnSO_4$, 0.29 $CuSO_4$, 0.015 $(NH4)_6Mo_7O_{24}$, and 10 FeEDTA. Boron was supplied as H_3BO_3 in concentration of 0, 1, 10 ,50, 75, 100 µmol L^{-1}. Distilled water, containing less than 0.015 g B L^{-1}, and analytical grade chemicals were used to make up the nutrient solutions. The nutrient solutions were constantly aerated. During the experiment the solutions were brought back to volume with distilled water every day

and were renewed twice. The consumed water was calculated. The plants were monitored for any symptoms of B deficiency or toxicity over the period of the experiment. Plants of 8 pots (4 pots for each genotype) were harvested just before the B treatment were imposed.

Collection of Xylem Exudate and Harvest

The plants were harvested 10 days after the B treatments were imposed. They were cut just below the cotyledonary leaves and a plastic tube was fixed over the stump to collect xylem exudate. By covering the plants with a polyethylene bag the transpiration was minimized to create conditions, favouring gutation. Xylem exudate was collected over a day period. The roots were then rinsed for 20 seconds with distilled water, blotted dry, and cut at the transition point between the root and hypocotyl. The shoots were divided into leaves (consisting of plant material above the first pair of leaves) and CHS (consisting of cotyledonary leaves, hypocotyl and lower stem). The fresh weight of the plants parts was recorded, and the plant material was then frozen at -18 ° C to rupture the cells.

Intracellular Compartmentation

Frozen samples of roots and leaves were thawed, transferred to filter tubes which were placed inside centrifugal tubes and centrifuged for 10 minutes. Filtered solutions were taken as root or leave cell saps. The residue over the filter was homogenized with a mortar and pestle in distilled water, after which the homogenate was then centrifuged for 10 minutes, and the supernatant discarded. The residue was washed 3 times with

distilled water by repeating the homogenization and centrifugation procedures. The water insoluble residue contains B, closely bound to cell wall polymers, and the cell sap is a mixture of intracellular and apoplasmic fluid (Pfeffer et al., 2001). Total B content in cell sap was calculated as follows, assuming that 1 g cell sap equals 1 mL cell sap:

Total B (µg) = (fresh weight - dry weight) (ml) x µg B mL^{-1} cell sap

Boron Analysis

Boron concentrations in cell sap and xylem exudate were determined using an inductively-coupled plasma atomic emission spectrometer (ICPS Shimadzu 1000 III).

The rest of the B concentrations was determined by the Azomethine-H method (Gupta and Stewart, 1975). To prevent volatilization of B at high temperatures, an appropriate amount of saturated $Ca(OH)_2$ solution was added to the samples prior to drying. The dry weight of the plants parts was recorded after drying at 70 ° C to constant weight. Plant material was dry ashed at 500 ° C for at least 3 h, and the ash dissolved in an appropriate volume of 1 *N* H_2SO_4 prior to B analysis using the Azomethine-H method.

All equipment used in the experiment was washed with diluted HCl and rinsed with distilled water to diminish B contamination.

Experiment 2.

Experimental Design, Grown Conditions, Short Term Treatments and Harvest

Experimental procedures for growing plants were similar to those of experiment 1. Treatments were replicated four times (2 genotypes x 4 B treatments x 4 replications = 32 pots). A randomized completed design was used in this experiment. Plants of 8 pots (4 pots for each genotype) were harvested just before the first period of culture in nutrient solution was imposed. Both sunflower genotypes were grown in a preculture nutrient solution at two levels of boron (4 and 30 µmol L^{-1}). After seven days of preculture, the plants were transferred to nutrient solutions containing 4 or 30 µmol L^{-1}. The resulting four treatments were named 4/4, 4/30, 30/4 and 30/30, whereby the first numbers indicate the B concentration in the nutrient solutions during the first period of culture and the second numbers during the short term treatments. After two hours, xylem exudate was collected over a four hours period as previously described, but the collected volume was not large enough to allow the determination of boron concentration. Afterwards, roots were rinsed for 20 seconds with distilled water, blotted dry, and cut at the transition point between the root and hypocotyl. The shoots were divided into leaves (consisting of plant material above the cotyledonary leaves), hypocotyls and cotyledonary leaves.

Intracellular Compartmentation and Boron Analysis

As previously described, roots, hypocotyls and leaves were subjected to intracellular boron compartmentation analysis. Boron concentrations in cell sap were measured again by inductively-coupled plasma atomic emission spectrometry. The rest of the B concentrations was determined by the Azomethine-H method, as previously described.

Calculation of Uptake Efficiency

Uptake efficiency defined as total absorbed boron during B treatments divided by the amount of boron that the plant would hypothetically absorb, if the plasma membrane was not offering resistance to boron permeation, was calculate as follows:

UE = (TBP1 – TBP2) (µg B) / µg B mL^{-1} nutrient solution x mL water consumed

where TBP1 is the sum of total B of every compartment in every plant part, TBP2 is the total B content of plants that were harvested before plants started growing in nutrient solution, and µg B mL^{-1} nutrient solution is the boron concentration in the nutrient solution.

Statistical Analyses

The data were subjected to analysis of variance (ANOVA). They were transformed when they did not conformed to homogeneity requirements for the analysis of variance. The original data are shown, and significant treatment effects were separated with the Fisher's protected LSD Test at $p \leq 0.05$.

Results

Experiment 1.

Visual Symptoms of B Deficiency

In both the genotypes, boron deficiency symptoms were similar and became evident only under 0 and 1 B treatments. Under 0 B treatment the visual symptoms occurred: a) by the fourth day, when the leaves were thickened and the tissue was brittle, manifested mainly at the stem above the cotyledonary leaves; b) by the fifth day, when the base of the youngest leaves was whitish in color; c) by the sixth day, when the plants were visibly smaller than those grown under the other B treatments; d) by the eight day, when plants had developed severe symptoms of B deficiency, as noticed by the youngest leaves buckling downward and the leaf base was brown or whitish in color; whereas the older leaves were hard and dark green, with shining or necrotic areas in the margins upward. Splitting of hypocotyls was also observed. The most evident symptoms, as a result of 1 B treatments, were: a) thickening of the leaves by the fourth day; b) whitish coloring by the eight day of the base of the youngest leaves. While some of these leaves were buckling downward; some of the old leaves had shining areas and interveinal crinkling.

At harvest, plants under 0 B treatment had only the first pair of leaves completely expanded. The base of the youngest leaves was brown in color in plants grown under 1 B treatment and B toxicity symptoms (necrosis of the oldest leaf tips) became evident under 100 B treatment.

Dry Weight

Both genotypes suffered a decrease in root dry weight as a result of 0 and 1 B treatments (Table 1), whereas shoot dry weight was affected only by 0 B treatment. Otherwise, when plants of the R genotype were grown in solutions, containing 10, 50, 75 and 100 µmol B L^{-1}, their roots dry weight remained constant. However, roots of

plants of the S genotype decreased in dry weight by the 75 and 100 B treatments. The ratio of shoot to root dry weight increased with low B concentrations in solution, but under 0 B treatment, this ratio was higher for plants of the S genotype than that for plants of the R genotype.

Boron Concentration and Boron Content

A linear relationship was obtained (Table 2) between B concentration in nutrient solution and B concentration in root cell sap for each genotype. Boron concentration in root cell sap, at 0 and 1 B treatments, was smaller than that at 0.0µ1 g B mL^{-1}. However, this concentration increased progressively at treatments up to 100 B, where on the average the B content of both genotypes was eight times higher than that at 10 B treatment. In contrast, B concentrations in WIR of roots (Table 2) were similar under all treatments, though on the average the B content in WIR of roots of plants belonging to the S genotype was higher than that of plants in the R genotype.

Boron contents in both WIR and CS of leaves (Table 2) increased progressively with B concentration in nutrient solution. Between 0 and 10 B treatments the B content increased substantially in WIR of leaves. Further increments of B in the nutrient solution, resulted in a slight increase only in B concentration in WIR of leaves (less than twice between 10 and 100 B treatments). In contrast, the increases in B contents in CS of leaves were about five times between 10 and 100 B treatments. However, the B concentration, at 0 and 1 B treatments, was smaller than that at 0.01 µg B mL^{-1}.

It must be underscored that the B concentration in WIR of roots, at 0 and 1 B treatment, was not only higher than that in WIR of leaves (Table 2), but that the concentration was also close to its greatest value. In contrast, the greatest value of B

content in WIR of leaves tended to be reached in a nutrient solution with boron concentrations >5 µ*M*. However, at 0 and 1 B treatments, the B concentration in WIR of leaves in S genotype was higher than that in R genotype.

In general, total B content in WIR of roots and leaves (Table 2) did not differ between genotypes, and it increased when B concentration in nutrient solution was increased from 0 to 10 µ*M*, and the total B content seemed to be unaffected by the latter treatment in both genotypes.

Uptake Efficiency

The results (Table 3) showed that the uptake efficiency was affected by B supply. As a result of 1 and 10 B treatments, the UE was almost one (0.93 on the average for both genotypes at both B concentrations). It was smaller in value as a result of the other treatments. In this regard, the UE, at 0 B treatment, was on the average 0.47, and remained < 1 (0.26 on the average) at treatments of 50 and 100 B..

Boron Concentration in CS of Roots and Leaves and in Xylem Exudates

In order to make the comparison more meaningful, the B concentrations in CS of roots and leaves and in xylem exudates were expressed in µg B mL^{-1} (Tabla 4). Similarly as in the results, shown in Table 2, a linear relationship was also detected between B concentration in the nutrient solution and B concentration in root cell sap, for each genotype. When plants were grown at 0 and 1 B treatment, the B content in CS and xylem exudate was < 0,01 µg B mL^{-1}. Practically, no differences were noticed between B concentrations in CS of roots and B concentration in nutrient solutions at the 10, 50, 75 and 100 B treatments (Table 4). In contrast, there was a direct relationship between

the increase of B concentration in the solutions and the increase in B concentration in CS of leaves. Significant differences were also noticed between B concentration in xylem exudates and B concentration in the nutrient solution. Actually, at the 10 B treatment, the B content in the xylem exudate was three times higher than that in the nutrient solution. In plants, grown in nutrient solutions with B concentrations < 10 μ*M*, the B concentration in the xylem exudate decreased more and more than that in the nutrient solution, and reductions of B contents were as high as 50 % in the nutrient solution.

Experiment 2

Visual Symptoms of B Deficiency and Dry Weight

Under the conditions of experiment 2, no visible symptoms of B deficiencies were observed. Differences between dry weight of roots, hypocotyls and leaves were not found, neither between genotypes nor between B treatments.

Boron Compartmentation in Roots

Boron concentration in WIR of roots (Table 5) was not modified when plants were transferred to nutrient solution of higher B concentration (4/30 B treatment) or from high to lower B concentration (30/4 B treatment) in comparison with plants that were supplied continuously with 4 μ*M* or 30 μ*M*, and no differences between genotypes were also evident.

Preculture conditions indicated that at the 4/4 B treatment, the B concentration in CS of roots was lower than that under the 30/30 B treatment (Table 5). Otherwise, the transfer to nutrient solutions with a different B concentration produced a quite rapid effect on B contents in CS. Six hours after the B supply was changed from 4/30 B to 30/30 B treatments, the B concentration in CS reached similar values than those under the 30/30 B treatment. However, the B concentration in CS decreased to a value similar to that of the 4/4 B treatment, as a result of the change in medium to 30/4 B treatment.

Boron Compartmentation in Hypocotyls

Neither the preculture conditions nor the change in B concentrations in nutrient solutions, into which the plants were transferred for a short period of time, had any effect on B contents in WIR of hypocotyls (Table 5). The values were similar under all treatments and differences between genotypes were not evident.

Boron concentration in CS of hypocotyls (Table 5) increased when plants, precultured with 4 μ*M* B in solution, were transferred to a growth medium containing 30 μ*M* (4/30 B treatment) with respect to plants, grown continuously with 30 μ*M* (30/30 B treatment). As a result of 30/4 B treatment, the B content in CS of hypocotyls attained values of plants, subjected to 4/4 and 30/30 B treatments. There were no evidences of differences between genotypes.

Boron Compartmentation in Leaves

Boron concentration in WIR of leaves (Table 5) was affected by preculture conditions, but not by the change in B contents of nutrient solutions, into which plants

were transferred for a short period of time. Boron concentration in CS was also affected by B supply during preculture, since B content in CS of leaves was lower at the 4/4 B than that at the 30/30 treatments (Table 5). Transferring plants for a short duration in nutrient media of different B concentrations did not produced any effect on B contents CS of leaves at the 4/30 and 30/4 B treatments as compared to those at the 4/4 and 30/30 B treatments.

Uptake Efficiency

When plants were supplied continuously with 4 µ*M* B, the uptake efficiency for both genotypes was 1.77 on the average, whereas plants supplied continuously with 30 µ*M* B exhibit uptake efficiencies of 0.32 on the average (for both genotypes, Table 3).

Discussion

It appears that the roots of the sunflower S genotype were not only more sensitive to low B contents in the solution media than the shoots, as supported by Blamey et al. (1997), but the decrease in root dry weight by the 75 and 100 B treatments could also indicate the effect of B toxicity, similar to that found by Dannel et al. (1998). Though root dry weight has decreased, the shoot : root dry weight ratio was smaller in the R genotype, suggesting that this genotype would have a substantially large root volume, capable of supporting the B demand of its shoots. For similar genotypes, such an adjustment in shoot : root ratio could be part of an effective strategy, enabling sunflower plants to take up more B in order to satisfy the B demand of shoots, when B supply is restrictive under field conditions.

It should be emphasized that in the S genotype, the decrease in root dry weight due to B deficiency (0 and 1 B treatment) and toxicity (75 and 100 B treatments) corresponded with an increase in B concentration in WIR of the roots, while the total B content did not differ much between genotypes. The observation above suggests that the S genotype exhibits a higher boron requirement threshold value for root cell walls than that of the R genotype.

The B contents in WIR of roots were similar for all genotypes and treatments. In contrast, the B concentration in WIR of leaves reached values near saturation only when B started to accumulate in the CS of roots to the level as detected in CS of leaves. In sunflower plants, it seems apparent that the boron requirement for cell walls of roots must first be met, before the boron requirement for cell walls of shoots can be satisfied. Once these steps have taken place, accumulation of metabolically available boron in CS of roots starts to occur, followed by its accumulation in CS of leaves, where it is concentrated. These findings would confirm that: (a) WIR of roots and leaves behave like a chemical absorber of B, with a limited number of binding sites occupied by B until saturation is attained (Dannel et al., 1998), and (b) the mechanism suggested by Hu and Brown (1997) was effective since with the formation of B complexes in the cell wall a concentration gradient was kept in operation as the key step for translocation of B to the shoots.

The critical values of B concentrations in shoot-tissues would then be established after the boron requirement for cell walls was satisfied and a proper metabolic B content in CS of roots was reached. The above became evident in plants, grown in nutrient solutions with a B concentration between 4 μ*M* (experiment 2) and 10 μ*M* (experiment 1). In addition, the results of experiment 2, with their changes in B supply, showed that: (a) a B concentration of 4 μ*M* in the nutrient solution was sufficient to satisfy the

requirement of root cell walls; and (b) the amount of B absorbed in WIR was stable, since it was not affected by the decrease in B supply, which was in agreement with the results of Pfeffer et al. (1997).

The quick adjustment (six hours) of B concentration in CS of roots that took effect due to changes of B concentration in the nutrient solution, indicated that the changing B concentration in CS of roots or leaves could be used in combination with the more stable B concentration in WIR of roots or leaves, as components of a criterion, relating B status in plant with B availability in the medium, and to predict the likelihood of B deficiency or toxicity to occur. Therefore, B availability in the medium would be sufficient during the vegetative growth of sunflower, when the ratio between B concentration in CS and B concentration in WIR of roots (both expressed in µg B g^{-1} dry weight) was > 0.1 (Table 2). However, in young leaves, the value of the B ratio between both compartments was higher than 0.3.

When the results in the present investigation are compared with those of other authors, it can be seen that the total B concentration in leaves at the 0 and 1 B treatments (12,7 and 24,2 µg B g^{-1} dry weight of WIR, respectively for both genotypes) is in accordance to previously reported findings on B requirement of sunflower (Asad et al., 2001; Asad et al., 2003; Asad et al 2002). However, Blamey et al. (1997) found a marked increase in total dry weight with an increase in B concentration (> 22.0 µg B g^{-1} dry weight) in the youngest mature leaf blade (YMB), and they considered deficiency to occur at a critical value of a B concentration of 190.0 µg B g^{-1} dry weight (90% of maximum yield) in the YMB. The latter value was considerably higher than those reported in other studies, but was close to the total B concentration in leaves (CS and WIR) by the 100 B treatment (255.5 µg B g^{-1} dry weight of WIR on the average for both genotypes) in present investigations, at which B toxicity symptoms were observed.

Uptake efficiency values < 1.00, detected as a result of treatments of 30 to 100μ*M* B in the nutrient solution, suggested the presence of an exclusion mechanism to be working in order to restrict B accumulation. However, this exclusion mechanism was not completely effective at B supplies between 1 and 10 μ*M*, and the high UE value obtained by the 4/4 B treatments indicated that mechanisms, other than mass flow, had played a role in providing the acquired B. The mechanism, causing the restriction in uptake of B, is not exactly known, but an excretion mechanism had been proposed by Bellaloui and Brown (1998), whereas other authors quoted a higher membrane permeability for water as a better explanation with respect to boron absorption (Weig et al., 1997 cited by Brown et al., 2002). The low UE value at the 0 B treatment (0.47 on the average for both genotypes), could be interpreted as a consequence of a severe B deficiency, since B seemed to be of crucial importance for the maintenance of structural integrity of plasma membranes (Cakmak and Römheld, 1997).

The pathways of nutrient transport from root surface to shoot include at least two processes of transmembrane transports: (1) import into epidermal or cortex cells; and, (2) export from pericycle or xylem parenchyma cells into the stelar apoplasm (xylem loading) (Takano et al., 2002). As discussed in the introduction, B uptake and B transport through membranes in higher plants were believed to be facilitated by both active and passive uptake processes (Brown et al, 2002). It is suggested that B uptake by roots would be by passive diffusion when B supply was high, whereas B accumulation in the symplasm of root cells was supposed to be an active process when B supply was low, since such an accumulation would take place against a concentration gradient (Pfeffer et al., 1999). When the values of plant WIRs were near saturation (10 B treatment or higher B supply), the B concentrations in the different liquid compartments indicated that: (a) there was no accumulation of B in CS of roots, since B contents

follow a linear concentration dependence; (b) at the 10 B treatment, the B concentration in xylem exudate was three times higher than the B content in the nutrient solution. At higher B supply, the boron concentrations in the xylem exudates followed a linear concentration dependence; and (c) B accumulation started in leaf CS against a concentration gradient. Furthermore, the results of experiment 2 showed that the great difference, found in B concentration in CS of roots as a result of treatments between 4/4 and 30/30 B, was reduced to a minimum when the B concentration in CS of hypocotyls was included. The data above suggest that a concentration mechanism was perhaps induced and effective at low B supply as a result of treatments of < 4 or 10 μ*M* B. This mechanism was perhaps the key process, controlling B accumulation, which was named as xylem loading by Takano et al. (2002). These authors, working with *Arabidopsis thaliana* mutant bor1-1 (sensitive to boron deficiency) and wild-type plants, showed that the concentration of boron in root cell sap increased in proportion to boron concentration in the medium in both the genotypes, suggesting that B uptake into roots occurred mainly by passive transport. The concentration of boron in xylem exudates of the bor1-1 plants also followed a linear concentration dependence, whereas a combination of saturable and linear concentration dependence was observed in the wild plants. Takano et al (2002) also indicated that xylem loading is the key step for boron accumulation in shoots with a low external boron supply and that BOR1, an efflux-type boron transporter for xylem loading, was an essential component of the process.

The present results also indicated the presence of two factors explaining the differences in susceptibility to knife-cut between the S and R sunflower genotypes, when grown under similar field conditions: (a) a higher capability of adjustment of shoot : root ratio for the R genotype under deficient boron conditions; and (b) a higher B requirement threshold for cell walls for S genotype. Though a clear relationship between

degree of susceptibility and B-efficiency could not be established adequately, the suggestion is made that the higher or lower susceptibility to B deficiency between genotypes is perhaps related to the capability of establishing B concentrations in CS of roots, allowing for proper transport and B accumulation in CS of leaves.

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Table 1. Dry weight of WIR of roots, WIR of leaves and cotyledonary leaves, hypocotyls and lower stems (CHS) in two sunflower genotypes when plants grown for ten days under different B supply. Values represent means of four replications ± SD.

	Genotype	Boron concentratioon in nutrient solution (µM)					
		0	1	10	50	75	100
Dry weight of WIR of roots	S	109.3 ± 14	149.7 ± 11	192.5 ± 19	200.5 ± 22	160.8 ± 14	154.4 ± 16
	R	121.2 ± 12	165.4 ± 22	223.4 ± 50	197.5 ± 36	225.7 ± 18	202.3 ± 35
Dry weight of WIR of leaves	S	213.9 ± 36	324.1 ± 49	325.9 ± 6	301.4 ± 46	272.8 ± 42	259.9 ± 40
	R	201.5 ± 25	344.4 ± 52	374.3 ± 72	348.0 ± 60	364.7 ± 72	300.9 ± 46
Dry weight of CHS	S	489.5 ± 51	526.2 ± 37	591.6 ± 23	516.7 ± 54	564.7 ± 52	567.8 ± 76
	R	441.3 ± 18	545.0 ± 24	573.3 ± 49	521.0 ± 52	689.4 ± 88	590.8 ± 76
Ratio between Dry weight of WIR of leaves + Dry weight of CHS and Dry weight of WIR of roots	S	6.54 ± 1.24	5.68 ± 0.23	4.80 ± 0.51	4.08 ± 0.16	5.20 ± 0.16	5.35 ± 0.29
	R	5.34 ± 0.55	5.41 ± 0.46	4.34 ± 0.70	4.44 ± 0.34	4.65 ± 0.33	4.44 ± 0.35

Table 2. Boron concentration (µg g^{-1} of WIR dry weight) in CS and WIR of roots and leaves and total boron content (µg) in WIR of roots and leaves in two sunflower genotypes when plants grown for ten days under different B supply. Values represent means of three or four replications ± SD. ND not determined. < 0.01 B concentration lesser than 0.01 µg B ml^{-1}.

	Genotype	Boron concentratioon in nutrient solution (µM)					
		0	1	10	50	75	100
Boron concentration in CS of roots	S	< 0.01	< 0.01	4.8 ± 0.5	20.3 ± 2.3	30.0 ± 0.6	37.7 ± 2.8
	R	< 0.01	< 0.01	4.5 ± 0.8	21.7 ± 2.2	29.7 ± 1.9	37.1 ± 3.0
Boron concentration in WIR of roots	S	51.2 ± 4.9	42.1 ± 2.8	42.1 ± 1.9	44.3 ± 0.6	61.6 ± 14.9	62.4 ± 12.3
	R	45.1 ± 2.7	37.5 ± 2.9	40.9 ± 3.4	44.0 ± 2.4	47.6 ± 2.8	49.5 ± 3.4
total boron content in WIR of roots	S	5.6 ± 0.5	6.3 ± 0.7	8.1 ± 0.5	8.9 ± 0.9	10.0 ± 3.0	9.7 ± 2.8
	R	5.4 ± 0.5	6.2 ± 0.5	9.0 ± 1.4	8.6 ± 1.2	10.7 ± 0.7	10.0 ± 1.8
Boron concentration in CS of leaves	S	< 0.01	< 0.01	29.5 ± 1.6	63.8 ± 5.8	ND	140.4 ± 3.4
	R	< 0.01	< 0.01	27.3 ± 2.3	63.0 ± 5.2	92.6 ± 8.3	127.6 ± 15.9
Boron concentration in WIR of leaves	S	13.9 ± 0.9	26.9 ± 6.3	84.8 ± 2.1	100.0 ± 7.3	113.0 ± 4.5	124.5 ± 5.3
	R	11.6 ± 1.3	21.5 ± 1.3	80.0 ± 4.1	97.3 ± 2.9	112.9 ± 4.5	120.2 ± 2.2
total boron content in WIR of leaves	S	3.0 ± 0.6	8.5 ± 1.1	27.6 ± 1.0	30.1 ± 4.6	30.9 ± 5.4	32.2 ± 4.1
	R	2.3 ± 0.2	7.4 ± 0.9	29.7 ± 4.4	33.9 ± 5.9	41.4 ± 9.9	36.2 ± 6.0

Table 3. The effect of B supply on boron uptake efficiency in two sunflower genotypes grown in two independent experiments. In Experiment 1 plants were grown with B concentrations of 0, 1, 10, 50, 75 y 100 µM, and in Experiment 2 under B concentrations of 4 y 30 µM. Values represent means of three or four replications ± SD. ND not determined.

Genotype	Boron concentratioon in nutrient solution (µM)							
	0	1	4	10	30	50	75	100
S	0.67±0.29	1.07±0.15	1.69±0.17	0.89±0.03	0.30±0.04	0.23±0.03	ND	0.26±0.01
R	0.28±0.20	0.90±0.09	1.85±0.12	0.87±0.10	0.35±0.02	0.28±0.02	0.28±0.02	0.26±0.04

Table 4. Boron concentration (µg ml^{-1}) in CS of roots, in xylem exudate and in CS of leaves in two sunflower genotypes when plants grown for ten days under different B supply. Values represent means of three or four replications ± SD. ND not determined. < 0.01 B concentration lesser than 0.01 µg B ml^{-1}.

	Genotype	Boron concentration in nutrient solution (µg ml^{-1})					
		0	0.01	0.11	0.54	0.81	1.08
Boron concentration in CS of roots	S	< 0.01	< 0.01	0.13 ± 0.02	0.54 ± 0.06	0.77 ± 0.02	0.97 ± 0.06
	R	< 0.01	< 0.01	0.12 ± 0.03	0.56 ± 0.04	0.86 ± 0.03	0.98 ± 0.14
Boron concentration in xylem exudate	S	< 0.01	< 0.01	0.32 ± 0.03	0.54 ± 0.02	0.64 ± 0.09	0.78 ± 0.06
	R	< 0.01	< 0.01	0.31 ± 0.01	0.42 ± 0.02	0.57 ± 0.02	0.58 ± 0.09
Boron concentration in CS of leaves	S	< 0.01	< 0.01	1.55 ± 0.12	3.13 ± 0.23	ND	7.63 ± 0.22
	R	< 0.01	< 0.01	1.36 ± 0.08	3.03 ± 0.38	4.72 ± 0.27	6.47 ± 1.14

Table 5. Boron concentration (µg g^{-1} of WIR dry weight) in WIR of roots, hypocotyls and leaves and boron concentration (µg ml^{-1}) in CS of roots, hypocotyls and leaves in two sunflower genotypes. Plants grown for seven days with a B supply of 4 or 30 µM, then were transferred to nutrients solutions containing 4 or 30µmol L^{-1} for six hours, the first numbers indicate the B concentration in the nutrient solution during the first period of culture and the second numbers during the short term treatments. Values represent means of three or four replications ± SD.

	Genotypes	B Treatments			
		4/4	4/30	30/4	30/30
Boron concentration in WIR of roots	S	45.6 ± 3.6	43.1 ± 1.9	44.3 ± 3.3	44.4 ± 2.8
	R	44.7 ± 4.2	47.3 ± 3.4	46.2 ± 4.2	46.3 ± 2.1
Boron concentration in CS of roots	S	0.03 ± 0.01	0.19 ± 0.04	0.05 ± 0.00	0.18 ± 0.03
	R	0.04 ± 0.01	0.20 ± 0.05	0.04 ± 0.01	0.19 ± 0.02
Boron concentration in WIR of hypocotyls	S	66.2 ± 2.4	68.0 ± 1.6	69.6 ± 5.4	73.8 ± 6.2
	R	70.7 ± 9.4	70.8 ± 5.7	76.6 ± 5.5	71.6 ± 11.8
Boron concentration in CS of hypocotyls	S	0.07 ± 0.02	0.11 ± 0.02	0.08 ± 0.01	0.09 ± 0.01
	R	0.07 ± 0.01	0.13 ± 0.04	0.10 ± 0.02	0.11 ± 0.03
Boron concentration in WIR of leaves	S	52.8 ± 0.4	51.1 ± 2.6	61.4 ± 5.6	58.3 ± 2.1
	R	51.2 ± 3.9	51.6 ± 3.5	60.9 ± 1.2	59.8 ± 1.8
Boron concentration in CS of leaves	S	0.70 ± 0.12	0.53 ± 0.10	1.79 ± 0.10	1.76 ± 0.18
	R	0.58 ± 0.05	0.55 ± 0.05	1.52 ± 0.22	1.53 ± 0.08