



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

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6 2 **AND KNIFE-CUT DISEASE IN SUNFLOWER.**  
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20 8 **ABSTRACT**  
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23 9 The present study was conducted with the primary aim to investigate in  
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25 10 sunflower the processes of boron uptake, intracellular compartmentation and xylem  
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27 11 translocation in response to B supply, ranging from deficiency, incipient toxicity and to  
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29 12 short term changes in B supply. The experiments were conducted with two sunflower  
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31 13 genotypes, selected on the basis of their susceptibility to knife-cut.  
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35 14 It appears that the roots of the sunflower susceptible genotype (S) were more  
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37 15 sensitive to low B contents in the solution media than the shoots. The decrease in root  
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39 16 dry weight in high B treatments could also indicate it was more sensitive to B toxicity.  
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41 17 Though root dry weight has decreased, the shoot : root dry weight ratio was smaller in  
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43 18 the resistant genotype (R), suggesting that this genotype would have a substantially large  
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45 19 root volume, capable of supporting the B demand of its shoots.  
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48  
49 20 The B contents in the water insoluble residue (WIR) of roots were similar for all  
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51 21 genotypes and treatments. In contrast, the B concentration in WIR of leaves reached  
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53 22 values near saturation only when B started to accumulate in the cell sap (CS) of roots to  
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55 23 the level as detected in CS of leaves. The critical values of B concentrations in shoot-

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3 24 tissues would then be established after the boron requirement for cell walls was satisfied  
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6 25 and a proper metabolic B content in CS of roots was reached.  
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9 26 Uptake efficiency (UE) values  $< 1.00$ , detected as a result of treatments with  
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11 27 high concentration of B in the nutrient solution, suggested the presence of an exclusion  
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13 28 mechanism to be working in order to restrict B accumulation. The high UE value  
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15 29 obtained with low B treatments indicated that mechanisms, other than mass flow, had  
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18 30 played a role in providing the acquired B.  
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20 31  
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22 32 *Key words:* Plant Nutrition, micronutrients, Boron deficiency, Boron uptake.  
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## 25 33 26 27 34 **Introduction** 28

29 35  
30 36 Boron (B) is an essential element for higher plants and it is found in live tissue in  
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32 37 water soluble and water insoluble forms (Matoh, 1997). The water insoluble form is  
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34 38 associated with cell wall pectins (Hu and Brown, 1994), where boron is expected to  
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36 39 perform an important function related to cell wall structure (Brown et al., 2002). In  
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38 40 higher plants, cell walls have been identified to be boron-polysaccharide complexes,  
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40 41 formed by two chains of rhamnogalacturonan II, cross-linked by boric acid (Kobayashi  
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42 42 et al., 1996; O'Neill et al., 1996). On the other hand, soluble boron is found in the cell  
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44 43 sap (CS), formed in simplasmic and apoplasmic aqueous solution (Pfeffer et al., 2001).  
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47 44 The amount of cell wall-bound B, as well as the amount of B in CS, varies with  
48  
49 45 B supply, plant species, and plant organ. For instance, squash plants (*Cucurbita pepo* L.)  
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51 46 and cultured tobacco cells (*Nicotiana tabacum*), grown in B deficient nutrient media,  
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53 47 showed a greater proportion of the absorbed B to be localized in their cell walls,  
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55 48 whereas a much lesser proportion was present in CS (Hu and Brown, 1994). In  
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3 49 sunflower plants (*Helianthus annuus* L.), grown with a B supply varying from 0.1 to  
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5 50 1600  $\mu\text{mol L}^{-1}$ , the proportion of cell wall-bound B ranged from 95 to 15 % for the root  
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7  
8 51 and from 66 to 15 % for the shoot (Dannel et al., 1998).  
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10 52 Boron uptake in higher plants has long been studied and evidence has been  
11  
12 presented supporting both active and passive uptake of B (Brown et al., 2002). When B  
13 53  
14 supply is high, B uptake by roots is believed to occur by passive diffusion. At low B  
15 54  
16 supply, its accumulation in the symplasm of root cells is considered to depend on two  
17 55  
18 processes working together: (1) an energy dependent process and (2) a passive diffusion  
19 56  
20 process along a gradient, maintained by the formation of B complexes within the cell  
21 57  
22 (Pfeffer et al., 1999). However, Takano et al. (2002) suggested that the concentration  
23 58  
24 mechanism, functioning at low boron supply, was mediating xylem loading.  
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30 60 Globally, boron deficiency is a widespread problem (Shorrocks, 1997), and  
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32 sunflower is considered a susceptible crop, where B deficiency produces a wide variety  
33 61  
34 of symptoms. A field symptom, related to boron deficiency in sunflower, is the  
35 62  
36 capitulum abscission, commonly known as knife-cut (Furlani et al., 1990). The present  
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38 study was conducted with the primary aim to investigate in sunflower the processes of  
39 64  
40 boron uptake, intracellular compartmentation and xylem translocation in response to B  
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42 supply, ranging from deficiency, incipient toxicity and to short term changes in B  
43 66  
44 supply. The experiments were conducted with two sunflower genotypes, selected on the  
45 67  
46 basis of their susceptibility to knife-cut, when grown under similar field conditions.  
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48 Comparison of the two genotypes for their susceptibility to knife-cut was a secondary  
49 69  
50 aim of this investigation.  
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## 71 72 **Materials and Methods** 73

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3 74 **Experiment 1.**  
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8 76 *Experimental Design*  
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13 78 Two sunflower genotypes, selected on the basis of their degree of susceptibility  
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15 79 to suffer from the abscission of the capitulum, were grown in solution cultures, using six  
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18 80 levels of B treatments, replicated four times (2 genotypes x 6 B levels x 4 replications =  
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20 81 48 pots). The sunflower genotypes are referred to as S (higher degree of susceptibility to  
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22 82 abscission of the capitulum) and R (lesser degree of susceptibility to abscission of the  
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24  
25 83 capitulum) throughout the paper. A randomized completed design was used in this  
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27 84 experiment.  
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32 86 *Growing Conditions*  
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37 88 Sunflowers seeds were soaked in aerated tap water for 22 h and germinated on  
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39 89 wet paper towels in a growth chamber with a temperature of 20 ° C /10° C (day/night).  
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41  
42 90 After 4 days, five uniform seedlings were transferred to 300 mL plastic pots, containing  
43  
44  
45 91 tap water. The pots were randomly distributed in a growth chamber, employing a 16 h  
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47 92 light and 8 h dark photoperiods. After 5 days, the tap water was replaced by nutrient  
48  
49 93 solutions, containing ( $\mu M$ ): 2500 Ca(NO<sub>3</sub>)<sub>2</sub>, 2500 KNO<sub>3</sub>, 1000 MgSO<sub>4</sub>, 500 KH<sub>2</sub>PO<sub>4</sub>,  
50  
51 94 9.14 MnCl<sub>2</sub>, 0.79 ZnSO<sub>4</sub>, 0.29 CuSO<sub>4</sub>, 0.015 (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, and 10 FeEDTA. Boron  
52  
53 95 was supplied as H<sub>3</sub>BO<sub>3</sub> in concentration of 0, 1, 10 ,50, 75, 100  $\mu mol L^{-1}$ . Distilled  
54  
55 96 water, containing less than 0.015 g B L<sup>-1</sup>, and analytical grade chemicals were used to  
56  
57 97 make up the nutrient solutions. The nutrient solutions were constantly aerated. During  
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59 98 the experiment the solutions were brought back to volume with distilled water every day  
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3 99 and were renewed twice. The consumed water was calculated. The plants were  
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6 100 monitored for any symptoms of B deficiency or toxicity over the period of the  
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8 101 experiment. Plants of 8 pots (4 pots for each genotype) were harvested just before the B  
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10 102 treatment were imposed.

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15 104 *Collection of Xylem Exudate and Harvest*

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20 106 The plants were harvested 10 days after the B treatments were imposed. They  
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22 107 were cut just below the cotyledonary leaves and a plastic tube was fixed over the stump  
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24  
25 108 to collect xylem exudate. By covering the plants with a polyethylene bag the  
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27 109 transpiration was minimized to create conditions, favouring gutation. Xylem exudate  
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29 110 was collected over a day period. The roots were then rinsed for 20 seconds with distilled  
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31 111 water, blotted dry, and cut at the transition point between the root and hypocotyl. The  
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33 112 shoots were divided into leaves (consisting of plant material above the first pair of  
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35 113 leaves) and CHS (consisting of cotyledonary leaves, hypocotyl and lower stem). The  
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37 114 fresh weight of the plants parts was recorded, and the plant material was then frozen at -  
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39 115 18 ° C to rupture the cells.

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47 117 *Intracellular Compartmentation*

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52 119 Frozen samples of roots and leaves were thawed, transferred to filter tubes which  
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54 120 were placed inside centrifugal tubes and centrifuged for 10 minutes. Filtered solutions  
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56 121 were taken as root or leave cell saps. The residue over the filter was homogenized with a  
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58 122 mortar and pestle in distilled water, after which the homogenate was then centrifuged  
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60 123 for 10 minutes, and the supernatant discarded. The residue was washed 3 times with

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3 124 distilled water by repeating the homogenization and centrifugation procedures. The  
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6 125 water insoluble residue (WIR) contains B, closely bound to cell wall polymers, and the  
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8 126 cell sap (CS) is a mixture of intracellular and apoplasmic fluid (Pfeffer et al., 2001).  
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11 127 Total B content in cell sap was calculated as follows, assuming that 1 g cell sap equals 1  
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13 128 mL cell sap:

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$$\text{Total B } (\mu\text{g}) = (\text{fresh weight} - \text{dry weight}) (\text{ml}) \times \mu\text{g B mL}^{-1} \text{ cell sap}$$

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### 21 22 132 *Boron Analysis*

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28 134 Boron concentrations in cell sap and xylem exudate were determined using an  
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30 135 inductively-coupled plasma atomic emission spectrometer (ICPS Shimadzu 1000 III).

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32 136 The rest of the B concentrations was determined by the Azomethine-H method  
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35 137 (Gupta and Stewart, 1975). To prevent volatilization of B at high temperatures, an  
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37 138 appropriate amount of saturated  $\text{Ca}(\text{OH})_2$  solution was added to the samples prior to  
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40 139 drying. The dry weight of the plants parts was recorded after drying at 70 ° C to constant  
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42 140 weight. Plant material was dry ashed at 500 ° C for at least 3 h, and the ash dissolved in  
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44  
45 141 an appropriate volume of 1 N  $\text{H}_2\text{SO}_4$  prior to B analysis using the Azomethine-H  
46  
47 142 method.

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49 143 All equipment used in the experiment was washed with diluted HCl and rinsed  
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51 144 with distilled water to diminish B contamination.

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### 54 55 56 146 *Experiment 2.*

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148 *Experimental Design, Grown Conditions, Short Term Treatments and Harvest*

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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  $\mu\text{mol L}^{-1}$ ). After seven days of preculture, the plants were transferred to nutrient solutions containing 4 or 30  $\mu\text{mol L}^{-1}$ . 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.



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8 176 *Calculation of Uptake Efficiency*  
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13 178 Uptake efficiency (UE) defined as total absorbed boron during B treatments  
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15 179 divided by the amount of boron that the plant would hypothetically absorb, if the plasma  
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17 180 membrane was not offering resistance to boron permeation, was calculate as follows:  
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22 182  $UE = (TBP1 - TBP2) (\mu\text{g B}) / \mu\text{g B mL}^{-1} \text{ nutrient solution} \times \text{mL water consumed}$   
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24 183  
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26  
27 184 where TBP1 is the sum of total B of every compartment in every plant part, TBP2 is the  
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29 185 total B content of plants that were harvested before plants started growing in nutrient  
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31 186 solution, and  $\mu\text{g B mL}^{-1}$  nutrient solution is the boron concentration in the nutrient  
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33 187 solution.  
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45 194 The data were subjected to analysis of variance (ANOVA). They were  
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47 195 transformed when they did not conformed to homogeneity requirements for the analysis  
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49 196 of variance. The original data are shown, and significant treatment effects were  
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51 197 separated with the Fisher's protected LSD Test at  $p \leq 0.05$ .  
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198 *Statistical Analyses*

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260 **Results**

261 **Experiment 1.**

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*Visual Symptoms of B Deficiency*

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

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

219

*Dry Weight*

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

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3 224 75 and 100  $\mu\text{mol B L}^{-1}$ , their roots dry weight remained constant. However, roots of  
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6 225 plants of the S genotype decreased in dry weight by the 75 and 100 B treatments. The  
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8 226 ratio of shoot to root dry weight increased with low B concentrations in solution, but  
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10 227 under 0 B treatment, this ratio was higher for plants of the S genotype than that for  
11  
12 228 plants of the R genotype.

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### 14 15 16 17 18 230 *Boron Concentration and Boron Content*

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22 232 A linear relationship was obtained (Table 2) between B concentration in nutrient  
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24 233 solution and B concentration in root cell sap for each genotype. Boron concentration in  
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26 234 root cell sap, at 0 and 1 B treatments, was smaller than that at  $0.0\mu\text{l g B mL}^{-1}$ . However,  
27  
28 235 this concentration increased progressively at treatments up to 100 B, where on the  
29  
30 236 average the B content of both genotypes was eight times higher than that at 10 B  
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32 237 treatment. In contrast, B concentrations in WIR of roots (Table 2) were similar under all  
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34 238 treatments, though on the average the B content in WIR of roots of plants belonging to  
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36 239 the S genotype was higher than that of plants in the R genotype.

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39 240 Boron contents in both WIR and CS of leaves (Table 2) increased progressively  
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41 241 with B concentration in nutrient solution. Between 0 and 10 B treatments the B content  
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43 242 increased substantially in WIR of leaves. Further increments of B in the nutrient  
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45 243 solution, resulted in a slight increase only in B concentration in WIR of leaves (less than  
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47 244 twice between 10 and 100 B treatments). In contrast, the increases in B contents in CS  
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49 245 of leaves were about five times between 10 and 100 B treatments. However, the B  
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51 246 concentration, at 0 and 1 B treatments, was smaller than that at  $0.01 \mu\text{g B mL}^{-1}$ .

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54 247 It must be underscored that the B concentration in WIR of roots, at 0 and 1 B  
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56 248 treatment, was not only higher than that in WIR of leaves (Table 2), but that the

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3 249 concentration was also close to its greatest value. In contrast, the greatest value of B  
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6 250 content in WIR of leaves tended to be reached in a nutrient solution with boron  
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8 251 concentrations  $>5 \mu\text{M}$ . However, at 0 and 1 B treatments, the B concentration in WIR of  
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10  
11 252 leaves in S genotype was higher than that in R genotype.

12  
13 253 In general, total B content in WIR of roots and leaves (Table 2) did not differ  
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15 254 between genotypes, and it increased when B concentration in nutrient solution was  
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17  
18 255 increased from 0 to 10  $\mu\text{M}$ , and the total B content seemed to be unaffected by the latter  
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20 256 treatment in both genotypes.

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#### 22 258 *Uptake Efficiency (UE)*

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27 259 The results (Table 3) showed that the uptake efficiency was affected by B supply.  
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30 260 As a result of 1 and 10 B treatments, the UE was almost one (0.93 on the average for  
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32 261 both genotypes at both B concentrations). It was smaller in value as a result of the other  
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34  
35 262 treatments. In this regard, the UE, at 0 B treatment, was on the average 0.47, and  
36  
37 263 remained  $< 1$  (0.26 on the average) at treatments of 50 and 100 B..  
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#### 41 265 *Boron Concentration in CS of Roots and Leaves and in Xylem Exudates*

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47 267 In order to make the comparison more meaningful, the B concentrations in CS of  
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49 268 roots and leaves and in xylem exudates were expressed in  $\mu\text{g B mL}^{-1}$  (Table 4).  
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51  
52 269 Similarly as in the results, shown in Table 2, a linear relationship was also detected  
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54 270 between B concentration in the nutrient solution and B concentration in root cell sap, for  
55  
56  
57 271 each genotype. When plants were grown at 0 and 1 B treatment, the B content in CS and  
58  
59 272 xylem exudate was  $< 0,01 \mu\text{g B mL}^{-1}$ . Practically, no differences were noticed between  
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273 B concentrations in CS of roots and B concentration in nutrient solutions at the 10, 50,

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3 274 75 and 100 B treatments (Table 4). In contrast, there was a direct relationship between  
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6 275 the increase of B concentration in the solutions and the increase in B concentration in  
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8 276 CS of leaves. Significant differences were also noticed between B concentration in  
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10  
11 277 xylem exudates and B concentration in the nutrient solution. Actually, at the 10 B  
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13 278 treatment, the B content in the xylem exudate was three times higher than that in the  
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15 279 nutrient solution. In plants, grown in nutrient solutions with B concentrations  $< 10 \mu M$ ,  
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17  
18 280 the B concentration in the xylem exudate decreased more and more than that in the  
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20 281 nutrient solution, and reductions of B contents were as high as 50 % in the nutrient  
21  
22  
23 282 solution.  
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## 25 283 26 27 284 Experiment 2

### 28 285 29 30 286 *Visual Symptoms of B Deficiency and Dry Weight*

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35 288 Under the conditions of experiment 2, no visible symptoms of B deficiencies  
36  
37 289 were observed. Differences between dry weight of roots, hypocotyls and leaves were not  
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39 290 found, neither between genotypes nor between B treatments.  
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### 43 292 *Boron Compartmentation in Roots*

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46 294 Boron concentration in WIR of roots (Table 5) was not modified when plants  
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48 295 were transferred to nutrient solution of higher B concentration (4/30 B treatment) or  
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50 296 from high to lower B concentration (30/4 B treatment) in comparison with plants that  
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52 297 were supplied continuously with  $4 \mu M$  or  $30 \mu M$ , and no differences between genotypes  
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54 298 were also evident.  
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3 299 Preculture conditions indicated that at the 4/4 B treatment, the B concentration in  
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5 300 CS of roots was lower than that under the 30/30 B treatment (Table 5). Otherwise, the  
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8 301 transfer to nutrient solutions with a different B concentration produced a quite rapid  
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10 302 effect on B contents in CS. Six hours after the B supply was changed from 4/30 B to  
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12 303 30/30 B treatments, the B concentration in CS reached similar values than those under  
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15 304 the 30/30 B treatment. However, the B concentration in CS decreased to a value similar  
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17 305 to that of the 4/4 B treatment, as a result of the change in medium to 30/4 B treatment.  
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### 22 307 *Boron Compartmentation in Hypocotyls*

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26  
27 309 Neither the preculture conditions nor the change in B concentrations in nutrient  
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29 310 solutions, into which the plants were transferred for a short period of time, had any  
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31 311 effect on B contents in WIR of hypocotyls (Table 5). The values were similar under all  
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33 312 treatments and differences between genotypes were not evident.  
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36  
37 313 Boron concentration in CS of hypocotyls (Table 5) increased when plants,  
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39 314 precultured with 4  $\mu\text{M}$  B in solution, were transferred to a growth medium containing 30  
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41 315  $\mu\text{M}$  (4/30 B treatment) with respect to plants, grown continuously with 30  $\mu\text{M}$  (30/30 B  
42  
43 316 treatment). As a result of 30/4 B treatment, the B content in CS of hypocotyls attained  
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45 317 values of plants, subjected to 4/4 and 30/30 B treatments. There were no evidences of  
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47 318 differences between genotypes.  
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### 51 319

### 52 320 *Boron Compartmentation in Leaves*

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57 322 Boron concentration in WIR of leaves (Table 5) was affected by preculture  
58  
59 323 conditions, but not by the change in B contents of nutrient solutions, into which plants  
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3 324 were transferred for a short period of time. Boron concentration in CS was also affected  
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6 325 by B supply during preculture, since B content in CS of leaves was lower at the 4/4 B  
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8 326 than that at the 30/30 treatments (Table 5). Transferring plants for a short duration in  
9  
10 327 nutrient media of different B concentrations did not produced any effect on B contents  
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12  
13 328 CS of leaves at the 4/30 and 30/4 B treatments as compared to those at the 4/4 and 30/30  
14  
15 329 B treatments.

330

331 *Uptake Efficiency (UE)*

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333 When plants were supplied continuously with 4  $\mu\text{M}$  B, the uptake efficiency for  
334 both genotypes was 1.77 on the average, whereas plants supplied continuously with 30  
335  $\mu\text{M}$  B exhibit uptake efficiencies of 0.32 on the average (for both genotypes, Table 3).

336

### 337 Discussion

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

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3 349 It should be emphasized that in the S genotype, the decrease in root dry weight  
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6 350 due to B deficiency (0 and 1 B treatment) and toxicity (75 and 100 B treatments)  
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8 351 corresponded with an increase in B concentration in WIR of the roots, while the total B  
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11 352 content did not differ much between genotypes. The observation above suggests that the  
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13 353 S genotype exhibits a higher boron requirement threshold value for root cell walls than  
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15 354 that of the R genotype.

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18 355 The B contents in WIR of roots were similar for all genotypes and treatments. In  
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20 356 contrast, the B concentration in WIR of leaves reached values near saturation only when  
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22 357 B started to accumulate in the CS of roots to the level as detected in CS of leaves. In  
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25 358 sunflower plants, it seems apparent that the boron requirement for cell walls of roots  
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27 359 must first be met, before the boron requirement for cell walls of shoots can be satisfied.  
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30 360 Once these steps have taken place, accumulation of metabolically available boron in CS  
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32 361 of roots starts to occur, followed by its accumulation in CS of leaves, where it is  
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35 362 concentrated. These findings would confirm that: (a) WIR of roots and leaves behave  
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37 363 like a chemical absorber of B, with a limited number of binding sites occupied by B  
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40 364 until saturation is attained (Dannel et al., 1998), and (b) the mechanism suggested by Hu  
41  
42 365 and Brown (1997) was effective since with the formation of B complexes in the cell  
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44 366 wall a concentration gradient was kept in operation as the key step for translocation of B  
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46  
47 367 to the shoots.

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49 368 The critical values of B concentrations in shoot-tissues would then be established  
50  
51 369 after the boron requirement for cell walls was satisfied and a proper metabolic B content  
52  
53 370 in CS of roots was reached. The above became evident in plants, grown in nutrient  
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55 371 solutions with a B concentration between 4  $\mu M$  (experiment 2) and 10  $\mu M$  (experiment  
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57 372 1). In addition, the results of experiment 2, with their changes in B supply, showed that:  
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59 373 (a) a B concentration of 4  $\mu M$  in the nutrient solution was sufficient to satisfy the



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3 374 requirement of root cell walls; and (b) the amount of B absorbed in WIR was stable,  
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6 375 since it was not affected by the decrease in B supply, which was in agreement with the  
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8 376 results of Pfeffer et al. (1997).  
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10  
11 377 The quick adjustment (six hours) of B concentration in CS of roots that took  
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13 378 effect due to changes of B concentration in the nutrient solution, indicated that the  
14  
15 379 changing B concentration in CS of roots or leaves could be used in combination with the  
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18 380 more stable B concentration in WIR of roots or leaves, as components of a criterion,  
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20 381 relating B status in plant with B availability in the medium, and to predict the likelihood  
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22 382 of B deficiency or toxicity to occur. Therefore, B availability in the medium would be  
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25 383 sufficient during the vegetative growth of sunflower, when the ratio between B  
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28 384 concentration in CS and B concentration in WIR of roots (both expressed in  $\mu\text{g B g}^{-1}$  dry  
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30 385 weight) was  $> 0.1$  (Table 2). However, in young leaves, the value of the B ratio between  
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32 386 both compartments was higher than 0.3.  
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34  
35 387 When the results in the present investigation are compared with those of other  
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37 388 authors, it can be seen that the total B concentration in leaves at the 0 and 1 B treatments  
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39 389 (12,7 and 24,2  $\mu\text{g B g}^{-1}$  dry weight of WIR, respectively for both genotypes) is in  
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42 390 accordance to previously reported findings on B requirement of sunflower (Asad et al.,  
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44 391 2001; Asad et al., 2003; Asad et al 2002). However, Blamey et al. (1997) found a  
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46 392 marked increase in total dry weight with an increase in B concentration ( $> 22.0 \mu\text{g B g}^{-1}$   
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49 393 dry weight) in the youngest mature leaf blade (YMB), and they considered deficiency to  
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51 394 occur at a critical value of a B concentration of  $190.0 \mu\text{g B g}^{-1}$  dry weight (90% of  
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54 395 maximum yield) in the YMB. The latter value was considerably higher than those  
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57 396 reported in other studies, but was close to the total B concentration in leaves (CS and  
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59 397 WIR) by the 100 B treatment ( $255.5 \mu\text{g B g}^{-1}$  dry weight of WIR on the average for both  
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398 genotypes) in present investigations, at which B toxicity symptoms were observed.

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3 399 Uptake efficiency values  $< 1.00$ , detected as a result of treatments of 30 to  
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6 400  $100\mu M$  B in the nutrient solution, suggested the presence of an exclusion mechanism to  
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8 401 be working in order to restrict B accumulation. However, this exclusion mechanism was  
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10 402 not completely effective at B supplies between 1 and  $10\mu M$ , and the high UE value  
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12 403 obtained by the 4/4 B treatments indicated that mechanisms, other than mass flow, had  
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14 404 played a role in providing the acquired B. The mechanism, causing the restriction in  
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16 405 uptake of B, is not exactly known, but an excretion mechanism had been proposed by  
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18 406 Bellaloui and Brown (1998), whereas other authors quoted a higher membrane  
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20 407 permeability for water as a better explanation with respect to boron absorption (Weig et  
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22 408 al., 1997 cited by Brown et al., 2002). The low UE value at the 0 B treatment (0.47 on  
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24 409 the average for both genotypes), could be interpreted as a consequence of a severe B  
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26 410 deficiency, since B seemed to be of crucial importance for the maintenance of structural  
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28 411 integrity of plasma membranes (Cakmak and Römheld, 1997).

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34 412 The pathways of nutrient transport from root surface to shoot include at least two  
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36 413 processes of transmembrane transports: (1) import into epidermal or cortex cells; and,  
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38 414 (2) export from pericycle or xylem parenchyma cells into the stelar apoplasm (xylem  
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40 415 loading) (Takano et al., 2002). As discussed in the introduction, B uptake and B  
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42 416 transport through membranes in higher plants were believed to be facilitated by both  
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44 417 active and passive uptake processes (Brown et al, 2002). It is suggested that B uptake by  
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46 418 roots would be by passive diffusion when B supply was high, whereas B accumulation  
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48 419 in the symplasm of root cells was supposed to be an active process when B supply was  
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50 420 low, since such an accumulation would take place against a concentration gradient  
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52 421 (Pfeffer et al., 1999). When the values of plant WIRs were near saturation ( $10\mu M$  B  
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54 422 treatment or higher B supply), the B concentrations in the different liquid compartments  
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56 423 indicated that: (a) there was no accumulation of B in CS of roots, since B contents

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3 424 follow a linear concentration dependence; (b) at the 10 B treatment, the B concentration  
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6 425 in xylem exudate was three times higher than the B content in the nutrient solution. At  
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8 426 higher B supply, the boron concentrations in the xylem exudates followed a linear  
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10 427 concentration dependence; and (c) B accumulation started in leaf CS against a  
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12 428 concentration gradient. Furthermore, the results of experiment 2 showed that the great  
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14 429 difference, found in B concentration in CS of roots as a result of treatments between 4/4  
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16 430 and 30/30 B, was reduced to a minimum when the B concentration in CS of hypocotyls  
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18 431 was included. The data above suggest that a concentration mechanism was perhaps  
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20 432 induced and effective at low B supply as a result of treatments of < 4 or 10  $\mu M$  B. This  
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22 433 mechanism was perhaps the key process, controlling B accumulation, which was named  
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24 434 as xylem loading by Takano et al. (2002). These authors, working with *Arabidopsis*  
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26 435 *thaliana* mutant bor1-1 (sensitive to boron deficiency) and wild-type plants, showed that  
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28 436 the concentration of boron in root cell sap increased in proportion to boron  
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30 437 concentration in the medium in both the genotypes, suggesting that B uptake into roots  
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32 438 occurred mainly by passive transport. The concentration of boron in xylem exudates of  
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34 439 the bor1-1 plants also followed a linear concentration dependence, whereas a  
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36 440 combination of saturable and linear concentration dependence was observed in the wild  
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38 441 plants. Takano et al (2002) also indicated that xylem loading is the key step for boron  
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40 442 accumulation in shoots with a low external boron supply and that BOR1, an efflux-type  
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42 443 boron transporter for xylem loading, was an essential component of the process.

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52 444 The present results also indicated the presence of two factors explaining the  
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54 445 differences in susceptibility to knife-cut between the S and R sunflower genotypes,  
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56 446 when grown under similar field conditions: (a) a higher capability of adjustment of  
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58 447 shoot : root ratio for the R genotype under deficient boron conditions; and (b) a higher B  
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60 448 requirement threshold for cell walls for S genotype. Though a clear relationship between

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3 449 degree of susceptibility and B-efficiency could not be established adequately, the  
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6 450 suggestion is made that the higher or lower susceptibility to B deficiency between  
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8 451 genotypes is perhaps related to the capability of establishing B concentrations in CS of  
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11 452 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  $\pm$  SD.

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

Table 2. Boron concentration ( $\mu\text{g g}^{-1}$  of WIR dry weight) in CS and WIR of roots and leaves and total boron content ( $\mu\text{g}$ ) 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  $\pm$  SD. ND not determined.  $< 0.01$  B concentration lesser than  $0.01 \mu\text{g B ml}^{-1}$ .

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



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  $\mu\text{M}$ , and in Experiment 2 under B concentrations of 4 y 30  $\mu\text{M}$ . Values represent means of three or four replications  $\pm$  SD. ND not determined.

Genotype	Boron concentration in nutrient solution ( $\mu\text{M}$ )							
	0	1	4	10	30	50	75	100
S	0.67 $\pm$ 0.29	1.07 $\pm$ 0.15	1.69 $\pm$ 0.17	0.89 $\pm$ 0.03	0.30 $\pm$ 0.04	0.23 $\pm$ 0.03	ND	0.26 $\pm$ 0.01
R	0.28 $\pm$ 0.20	0.90 $\pm$ 0.09	1.85 $\pm$ 0.12	0.87 $\pm$ 0.10	0.35 $\pm$ 0.02	0.28 $\pm$ 0.02	0.28 $\pm$ 0.02	0.26 $\pm$ 0.04

Table 4. Boron concentration ( $\mu\text{g ml}^{-1}$ ) 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  $\pm$  SD. ND not determined.  $< 0.01$  B concentration lesser than  $0.01 \mu\text{g B ml}^{-1}$ .

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

Table 5. Boron concentration ( $\mu\text{g g}^{-1}$  of WIR dry weight) in WIR of roots, hypocotyls and leaves and boron concentration ( $\mu\text{g ml}^{-1}$ ) in CS of roots, hypocotyls and leaves in two sunflower genotypes. Plants grown for seven days with a B supply of 4 or 30  $\mu\text{M}$ , then were transferred to nutrients solutions containing 4 or 30  $\mu\text{mol L}^{-1}$  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  $\pm$  SD.

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

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3 1 **BORON NUTRITION, INTRACELLULAR TRANSPORT,**  
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6 2 **AND KNIFE-CUT DISEASE IN SUNFLOWER.**  
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15 6 Argentina  
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18 8 **ABSTRACT**  
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20 9 The present study was conducted with the primary aim to investigate in  
21 10 sunflower the processes of boron uptake, intracellular compartmentation and xylem  
22 11 translocation in response to B supply, ranging from deficiency, incipient toxicity and to  
23 12 short term changes in B supply. The experiments were conducted with two sunflower  
24 13 genotypes, selected on the basis of their susceptibility to knife-cut.  
25 14

26 15 It appears that the roots of the sunflower susceptible genotype were more  
27 16 sensitive to low B contents in the solution media than the shoots. The decrease in root  
28 17 dry weight in high B treatments could also indicate it was more sensitive to B toxicity.  
29 18 Though root dry weight has decreased, the shoot : root dry weight ratio was smaller in  
30 19 the resistant genotype, suggesting that this genotype would have a substantially large  
31 20 root volume, capable of supporting the B demand of its shoots.  
32 21

33 22 The B contents in the water insoluble residue (WIR) of roots were similar for all  
34 23 genotypes and treatments. In contrast, the B concentration in WIR of leaves reached  
35 24 values near saturation only when B started to accumulate in the cell sap (CS) of roots to  
36 25 the level as detected in CS of leaves. The critical values of B concentrations in shoot-  
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3 24 tissues would then be established after the boron requirement for cell walls was satisfied  
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6 25 and a proper metabolic B content in CS of roots was reached.  
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11 27 Uptake efficiency (UE) values < 1.00, detected as a result of treatments with  
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13 28 high concentration of B in the nutrient solution, suggested the presence of an exclusion  
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16 29 mechanism to be working in order to restrict B accumulation. The high UE value  
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18 30 obtained with low B treatments indicated that mechanisms, other than mass flow, had  
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21 31 played a role in providing the acquired B.  
22

23 32 *Key words:* Plant Nutrition, micronutrients, Boron deficiency, Boron uptake.  
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### 32 **Introduction**

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35 36 Boron is an essential element for higher plants and it is found in live tissue in  
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37 37 water soluble and water insoluble forms (Matoh, 1997). The water insoluble form is  
38  
39 38 associated with cell wall pectins (Hu and Brown, 1994), where boron is expected to  
40  
41 39 perform an important function related to cell wall structure (Brown et al., 2002). In  
42  
43 40 higher plants, cell walls have been identified to be boron-polysaccharide complexes,  
44  
45 41 formed by two chains of rhamnogalacturonan II, cross-linked by boric acid (Kobayashi  
46  
47 42 et al., 1996; O'Neill et al., 1996). On the other hand, soluble boron is found in the cell  
48  
49 43 sap, formed in simplasmic and apoplasmic aqueous solution (Pfeffer et al., 2001).  
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51  
52 44 The amount of cell wall-bound B, as well as the amount of B in CS, varies with  
53  
54 45 B supply, plant species, and plant organ. For instance, squash plants (*Cucurbita pepo* L.)  
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56 46 and cultured tobacco cells (*Nicotiana tabacum*), grown in B deficient nutrient media,  
57  
58 47 showed a greater proportion of the absorbed B to be localized in their cell walls,  
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60 48 whereas a much lesser proportion was present in CS (Hu and Brown, 1994). In

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3 49 sunflower plants (*Helianthus annuus* L.), grown with a B supply varying from 0.1 to  
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5 50 1600  $\mu\text{mol L}^{-1}$ , the proportion of cell wall-bound B ranged from 95 to 15 % for the root  
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7  
8 51 and from 66 to 15 % for the shoot (Dannel et al., 1998).  
9

10 52 Boron uptake in higher plants has long been studied and evidence has been  
11  
12 presented supporting both active and passive uptake of B (Brown et al., 2002). When B  
13 53  
14 supply is high, B uptake by roots is believed to occur by passive diffusion. At low B  
15 54  
16 supply, its accumulation in the symplasm of root cells is considered to depend on two  
17 55  
18 processes working together: (1) an energy dependent process and (2) a passive diffusion  
19 56  
20 process along a gradient, maintained by the formation of B complexes within the cell  
21 57  
22 (Pfeffer et al., 1999). However, Takano et al. (2002) suggested that the concentration  
23 58  
24 mechanism, functioning at low boron supply, was mediating xylem loading.  
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30 60 Globally, boron deficiency is a widespread problem (Shorrocks, 1997), and  
31  
32 sunflower is considered a susceptible crop, where B deficiency produces a wide variety  
33 61  
34 of symptoms. A field symptom, related to boron deficiency in sunflower, is the  
35 62  
36 capitulum abscission, commonly known as knife-cut (Furlani et al., 1990). The present  
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38 study was conducted with the primary aim to investigate in sunflower the processes of  
39 64  
40 boron uptake, intracellular compartmentation and xylem translocation in response to B  
41 65  
42 supply, ranging from deficiency, incipient toxicity and to short term changes in B  
43 66  
44 supply. The experiments were conducted with two sunflower genotypes, selected on the  
45 67  
46 basis of their susceptibility to knife-cut, when grown under similar field conditions.  
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48 Comparison of the two genotypes for their susceptibility to knife-cut was a secondary  
49 69  
50 aim of this investigation.  
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## 71 72 **Materials and Methods**

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3 74 **Experiment 1.**  
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8 76 *Experimental Design*  
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13 78 Two sunflower genotypes, selected on the basis of their degree of susceptibility  
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15 79 to suffer from the abscission of the capitulum, were grown in solution cultures, using six  
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17  
18 80 levels of B treatments, replicated four times (2 genotypes x 6 B levels x 4 replications =  
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20 81 48 pots). The sunflower genotypes are referred to as S (higher degree of susceptibility to  
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22 82 abscission of the capitulum) and R (lesser degree of susceptibility to abscission of the  
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24  
25 83 capitulum) throughout the paper. A randomized completed design was used in this  
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27 84 experiment.  
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32 86 *Growing Conditions*  
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37 88 Sunflowers seeds were soaked in aerated tap water for 22 h and germinated on  
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39 89 wet paper towels in a growth chamber with a temperature of 20 ° C /10° C (day/night).  
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41  
42 90 After 4 days, five uniform seedlings were transferred to 300 mL plastic pots, containing  
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44  
45 91 tap water. The pots were randomly distributed in a growth chamber, employing a 16 h  
46  
47 92 light and 8 h dark photoperiods. After 5 days, the tap water was replaced by nutrient  
48  
49 93 solutions, containing ( $\mu\text{M}$ ): 2500  $\text{Ca}(\text{NO}_3)_2$ , 2500  $\text{KNO}_3$ , 1000  $\text{MgSO}_4$ , 500  $\text{KH}_2\text{PO}_4$ ,  
50  
51 94 9.14  $\text{MnCl}_2$ , 0.79  $\text{ZnSO}_4$ , 0.29  $\text{CuSO}_4$ , 0.015  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ , and 10  $\text{FeEDTA}$ . Boron  
52  
53 95 was supplied as  $\text{H}_3\text{BO}_3$  in concentration of 0, 1, 10, 50, 75, 100  $\mu\text{mol L}^{-1}$ . Distilled  
54  
55 96 water, containing less than 0.015 g B  $\text{L}^{-1}$ , and analytical grade chemicals were used to  
56  
57 97 make up the nutrient solutions. The nutrient solutions were constantly aerated. During  
58  
59 98 the experiment the solutions were brought back to volume with distilled water every day  
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3 99 and were renewed twice. The consumed water was calculated. The plants were  
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5  
6 100 monitored for any symptoms of B deficiency or toxicity over the period of the  
7  
8 101 experiment. Plants of 8 pots (4 pots for each genotype) were harvested just before the B  
9  
10 102 treatment were imposed.

#### 13 103 14 15 104 *Collection of Xylem Exudate and Harvest*

16 104  
17  
18 105  
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20 106 The plants were harvested 10 days after the B treatments were imposed. They  
21  
22 107 were cut just below the cotyledonary leaves and a plastic tube was fixed over the stump  
23  
24 108 to collect xylem exudate. By covering the plants with a polyethylene bag the  
25  
26 109 transpiration was minimized to create conditions, favouring gutation. Xylem exudate  
27  
28 110 was collected over a day period. The roots were then rinsed for 20 seconds with distilled  
29  
30 111 water, blotted dry, and cut at the transition point between the root and hypocotyl. The  
31  
32 112 shoots were divided into leaves (consisting of plant material above the first pair of  
33  
34 113 leaves) and CHS (consisting of cotyledonary leaves, hypocotyl and lower stem). The  
35  
36 114 fresh weight of the plants parts was recorded, and the plant material was then frozen at -  
37  
38 115 18 ° C to rupture the cells.

#### 39 116 40 41 42 117 *Intracellular Compartmentation*

43 118  
44  
45 119 Frozen samples of roots and leaves were thawed, transferred to filter tubes which  
46  
47 120 were placed inside centrifugal tubes and centrifuged for 10 minutes. Filtered solutions  
48  
49 121 were taken as root or leave cell saps. The residue over the filter was homogenized with a  
50  
51 122 mortar and pestle in distilled water, after which the homogenate was then centrifuged  
52  
53 123 for 10 minutes, and the supernatant discarded. The residue was washed 3 times with



1  
2  
3 124 distilled water by repeating the homogenization and centrifugation procedures. The  
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5  
6 125 water insoluble residue contains B, closely bound to cell wall polymers, and the cell sap  
7  
8 126 is a mixture of intracellular and apoplasmic fluid (Pfeffer et al., 2001). Total B content  
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10 127 in cell sap was calculated as follows, assuming that 1 g cell sap equals 1 mL cell sap:  
11  
12

13 128

15 129 
$$\text{Total B } (\mu\text{g}) = (\text{fresh weight} - \text{dry weight}) (\text{ml}) \times \mu\text{g B mL}^{-1} \text{ cell sap}$$

18 130

### 20 131 *Boron Analysis*

23 132

25 133 Boron concentrations in cell sap and xylem exudate were determined using an  
26  
27 134 inductively-coupled plasma atomic emission spectrometer (ICPS Shimadzu 1000 III).

30 135 The rest of the B concentrations was determined by the Azomethine-H method  
31  
32 136 (Gupta and Stewart, 1975). To prevent volatilization of B at high temperatures, an  
33  
34 137 appropriate amount of saturated  $\text{Ca}(\text{OH})_2$  solution was added to the samples prior to  
35  
36 138 drying. The dry weight of the plants parts was recorded after drying at 70 ° C to constant  
37  
38 139 weight. Plant material was dry ashed at 500 ° C for at least 3 h, and the ash dissolved in  
39  
40 140 an appropriate volume of 1 N  $\text{H}_2\text{SO}_4$  prior to B analysis using the Azomethine-H  
41  
42 141 method.

47 142 All equipment used in the experiment was washed with diluted HCl and rinsed  
48  
49 143 with distilled water to diminish B contamination.

52 144

### 54 145 *Experiment 2.*

57 146

59 147 *Experimental Design, Grown Conditions, Short Term Treatments and Harvest*

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3 149 Experimental procedures for growing plants were similar to those of experiment  
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6 150 1. Treatments were replicated four times (2 genotypes x 4 B treatments x 4 replications  
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8 151 = 32 pots). A randomized completed design was used in this experiment. Plants of 8  
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11 152 pots (4 pots for each genotype) were harvested just before the first period of culture in  
12  
13 153 nutrient solution was imposed. Both sunflower genotypes were grown in a preculture  
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15 154 nutrient solution at two levels of boron (4 and 30  $\mu\text{mol L}^{-1}$ ). After seven days of  
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17  
18 155 preculture, the plants were transferred to nutrient solutions containing 4 or 30  $\mu\text{mol L}^{-1}$ .  
19  
20 156 The resulting four treatments were named 4/4, 4/30, 30/4 and 30/30, whereby the first  
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23 157 numbers indicate the B concentration in the nutrient solutions during the first period of  
24  
25 158 culture and the second numbers during the short term treatments. After two hours,  
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28 159 xylem exudate was collected over a four hours period as previously described, but the  
29  
30 160 collected volume was not large enough to allow the determination of boron  
31  
32 161 concentration. Afterwards, roots were rinsed for 20 seconds with distilled water, blotted  
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34  
35 162 dry, and cut at the transition point between the root and hypocotyl. The shoots were  
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37 163 divided into leaves (consisting of plant material above the cotyledonary leaves),  
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40 164 hypocotyls and cotyledonary leaves.

#### 41 42 165 43 44 166 *Intracellular Compartmentation and Boron Analysis*

45  
46  
47 167  
48  
49 168 As previously described, roots, hypocotyls and leaves were subjected to  
50  
51  
52 169 intracellular boron compartmentation analysis. Boron concentrations in cell sap were  
53  
54 170 measured again by inductively-coupled plasma atomic emission spectrometry. The rest  
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57 171 of the B concentrations was determined by the Azomethine-H method, as previously  
58  
59 172 described.  
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### 174 175 *Calculation of Uptake Efficiency*

176  
177 Uptake efficiency defined as total absorbed boron during B treatments divided by  
178 the amount of boron that the plant would hypothetically absorb, if the plasma membrane  
179 was not offering resistance to boron permeation, was calculate as follows:

$$180$$
$$181 \text{UE} = (\text{TBP1} - \text{TBP2}) (\mu\text{g B}) / \mu\text{g B mL}^{-1} \text{ nutrient solution} \times \text{mL water consumed}$$
$$182$$

183 where TBP1 is the sum of total B of every compartment in every plant part, TBP2 is the  
184 total B content of plants that were harvested before plants started growing in nutrient  
185 solution, and  $\mu\text{g B mL}^{-1}$  nutrient solution is the boron concentration in the nutrient  
186 solution.

### 187 188 *Statistical Analyses*

189  
190 The data were subjected to analysis of variance (ANOVA). They were  
191 transformed when they did not conformed to homogeneity requirements for the analysis  
192 of variance. The original data are shown, and significant treatment effects were  
193 separated with the Fisher's protected LSD Test at  $p \leq 0.05$ .

## 194 195 **Results**

### 196 197 **Experiment 1.**

1  
2  
3 199 *Visual Symptoms of B Deficiency*  
4

5 200 In both the genotypes, boron deficiency symptoms were similar and became  
6  
7 201 evident only under 0 and 1 B treatments. Under 0 B treatment the visual symptoms  
8  
9 202 occurred: a) by the fourth day, when the leaves were thickened and the tissue was brittle,  
10  
11 203 manifested mainly at the stem above the cotyledonary leaves; b) by the fifth day, when  
12  
13 204 the base of the youngest leaves was whitish in color; c) by the sixth day, when the plants  
14  
15 205 were visibly smaller than those grown under the other B treatments; d) by the eighth day,  
16  
17 206 when plants had developed severe symptoms of B deficiency, as noticed by the youngest  
18  
19 207 leaves buckling downward and the leaf base was brown or whitish in color; whereas the  
20  
21 208 older leaves were hard and dark green, with shining or necrotic areas in the margins  
22  
23 209 upward. Splitting of hypocotyls was also observed. The most evident symptoms, as a  
24  
25 210 result of 1 B treatments, were: a) thickening of the leaves by the fourth day; b) whitish  
26  
27 211 coloring by the eighth day of the base of the youngest leaves. While some of these leaves  
28  
29 212 were buckling downward; some of the old leaves had shining areas and interveinal  
30  
31 213 crinkling.  
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40 214 At harvest, plants under 0 B treatment had only the first pair of leaves  
41  
42 215 completely expanded. The base of the youngest leaves was brown in color in plants  
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44 216 grown under 1 B treatment and B toxicity symptoms (necrosis of the oldest leaf tips)  
45  
46 217 became evident under 100 B treatment.  
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51 218  
52 219 *Dry Weight*  
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54 220 Both genotypes suffered a decrease in root dry weight as a result of 0 and 1 B  
55  
56 221 treatments (Table 1), whereas shoot dry weight was affected only by 0 B treatment.  
57  
58 222 Otherwise, when plants of the R genotype were grown in solutions, containing 10, 50,  
59  
60 223 75 and 100  $\mu\text{mol B L}^{-1}$ , their roots dry weight remained constant. However, roots of

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2  
3 224 plants of the S genotype decreased in dry weight by the 75 and 100 B treatments. The  
4  
5 225 ratio of shoot to root dry weight increased with low B concentrations in solution, but  
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7  
8 226 under 0 B treatment, this ratio was higher for plants of the S genotype than that for  
9  
10  
11 227 plants of the R genotype.

12 228

### 15 229 *Boron Concentration and Boron Content*

18 230

20 231 A linear relationship was obtained (Table 2) between B concentration in nutrient  
21  
22 232 solution and B concentration in root cell sap for each genotype. Boron concentration in  
23  
24  
25 233 root cell sap, at 0 and 1 B treatments, was smaller than that at  $0.0\mu 1 \text{ g B mL}^{-1}$ . However,  
26  
27 234 this concentration increased progressively at treatments up to 100 B, where on the  
28  
29  
30 235 average the B content of both genotypes was eight times higher than that at 10 B  
31  
32 236 treatment. In contrast, B concentrations in WIR of roots (Table 2) were similar under all  
33  
34  
35 237 treatments, though on the average the B content in WIR of roots of plants belonging to  
36  
37 238 the S genotype was higher than that of plants in the R genotype.

39 239 Boron contents in both WIR and CS of leaves (Table 2) increased progressively  
40  
41  
42 240 with B concentration in nutrient solution. Between 0 and 10 B treatments the B content  
43  
44 241 increased substantially in WIR of leaves. Further increments of B in the nutrient  
45  
46  
47 242 solution, resulted in a slight increase only in B concentration in WIR of leaves (less than  
48  
49 243 twice between 10 and 100 B treatments). In contrast, the increases in B contents in CS  
50  
51  
52 244 of leaves were about five times between 10 and 100 B treatments. However, the B  
53  
54 245 concentration, at 0 and 1 B treatments, was smaller than that at  $0.01 \mu\text{g B mL}^{-1}$ .

56 246 It must be underscored that the B concentration in WIR of roots, at 0 and 1 B  
57  
58  
59 247 treatment, was not only higher than that in WIR of leaves (Table 2), but that the  
60  
248 concentration was also close to its greatest value. In contrast, the greatest value of B

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3 249 content in WIR of leaves tended to be reached in a nutrient solution with boron  
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5  
6 250 concentrations  $>5 \mu M$ . However, at 0 and 1 B treatments, the B concentration in WIR of  
7  
8 251 leaves in S genotype was higher than that in R genotype.

9  
10 252 In general, total B content in WIR of roots and leaves (Table 2) did not differ  
11  
12 253 between genotypes, and it increased when B concentration in nutrient solution was  
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14 254 increased from 0 to  $10 \mu M$ , and the total B content seemed to be unaffected by the latter  
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16 255 treatment in both genotypes.

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### 21 257 *Uptake Efficiency*

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25 258 The results (Table 3) showed that the uptake efficiency was affected by B supply.  
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27 259 As a result of 1 and 10 B treatments, the UE was almost one (0.93 on the average for  
28  
29 260 both genotypes at both B concentrations). It was smaller in value as a result of the other  
30  
31 261 treatments. In this regard, the UE, at 0 B treatment, was on the average 0.47, and  
32  
33 262 remained  $< 1$  (0.26 on the average) at treatments of 50 and 100 B..

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37 263

### 38 264 *Boron Concentration in CS of Roots and Leaves and in Xylem Exudates*

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43  
44 266 In order to make the comparison more meaningful, the B concentrations in CS of  
45  
46 267 roots and leaves and in xylem exudates were expressed in  $\mu g B mL^{-1}$  (Tabla 4).  
47  
48 268 Similarly as in the results, shown in Table 2, a linear relationship was also detected  
49  
50 269 between B concentration in the nutrient solution and B concentration in root cell sap, for  
51  
52 270 each genotype. When plants were grown at 0 and 1 B treatment, the B content in CS and  
53  
54 271 xylem exudate was  $< 0,01 \mu g B mL^{-1}$ . Practically, no differences were noticed between  
55  
56 272 B concentrations in CS of roots and B concentration in nutrient solutions at the 10, 50,  
57  
58 273 75 and 100 B treatments (Table 4). In contrast, there was a direct relationship between

1  
2  
3 274 the increase of B concentration in the solutions and the increase in B concentration in  
4  
5  
6 275 CS of leaves. Significant differences were also noticed between B concentration in  
7  
8 276 xylem exudates and B concentration in the nutrient solution. Actually, at the 10 B  
9  
10 277 treatment, the B content in the xylem exudate was three times higher than that in the  
11  
12 278 nutrient solution. In plants, grown in nutrient solutions with B concentrations  $< 10 \mu M$ ,  
13  
14 279 the B concentration in the xylem exudate decreased more and more than that in the  
15  
16 280 nutrient solution, and reductions of B contents were as high as 50 % in the nutrient  
17  
18 281 solution.  
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## 25 283 Experiment 2

### 26 27 284 28 29 285 *Visual Symptoms of B Deficiency and Dry Weight*

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32  
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34 287 Under the conditions of experiment 2, no visible symptoms of B deficiencies  
35  
36 288 were observed. Differences between dry weight of roots, hypocotyls and leaves were not  
37  
38 289 found, neither between genotypes nor between B treatments.  
39  
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41

### 42 290 43 44 291 *Boron Compartmentation in Roots*

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48  
49 293 Boron concentration in WIR of roots (Table 5) was not modified when plants  
50  
51 294 were transferred to nutrient solution of higher B concentration (4/30 B treatment) or  
52  
53 295 from high to lower B concentration (30/4 B treatment) in comparison with plants that  
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55 296 were supplied continuously with  $4 \mu M$  or  $30 \mu M$ , and no differences between genotypes  
56  
57 297 were also evident.  
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3 298 Preculture conditions indicated that at the 4/4 B treatment, the B concentration in  
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6 299 CS of roots was lower than that under the 30/30 B treatment (Table 5). Otherwise, the  
7  
8 300 transfer to nutrient solutions with a different B concentration produced a quite rapid  
9  
10 301 effect on B contents in CS. Six hours after the B supply was changed from 4/30 B to  
11  
12 302 30/30 B treatments, the B concentration in CS reached similar values than those under  
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14  
15 303 the 30/30 B treatment. However, the B concentration in CS decreased to a value similar  
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17 304 to that of the 4/4 B treatment, as a result of the change in medium to 30/4 B treatment.  
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#### 21 306 *Boron Compartmentation in Hypocotyls*

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23 307  
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27 308 Neither the preculture conditions nor the change in B concentrations in nutrient  
28  
29 309 solutions, into which the plants were transferred for a short period of time, had any  
30  
31 310 effect on B contents in WIR of hypocotyls (Table 5). The values were similar under all  
32  
33 311 treatments and differences between genotypes were not evident.  
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36  
37 312 Boron concentration in CS of hypocotyls (Table 5) increased when plants,  
38  
39 313 precultured with 4  $\mu\text{M}$  B in solution, were transferred to a growth medium containing 30  
40  
41 314  $\mu\text{M}$  (4/30 B treatment) with respect to plants, grown continuously with 30  $\mu\text{M}$  (30/30 B  
42  
43 315 treatment). As a result of 30/4 B treatment, the B content in CS of hypocotyls attained  
44  
45 316 values of plants, subjected to 4/4 and 30/30 B treatments. There were no evidences of  
46  
47 317 differences between genotypes.  
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#### 51 318 52 319 *Boron Compartmentation in Leaves*

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54 320  
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58 321 Boron concentration in WIR of leaves (Table 5) was affected by preculture  
59  
60 322 conditions, but not by the change in B contents of nutrient solutions, into which plants



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3 323 were transferred for a short period of time. Boron concentration in CS was also affected  
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6 324 by B supply during preculture, since B content in CS of leaves was lower at the 4/4 B  
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8 325 than that at the 30/30 treatments (Table 5). Transferring plants for a short duration in  
9  
10 326 nutrient media of different B concentrations did not produced any effect on B contents  
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12  
13 327 CS of leaves at the 4/30 and 30/4 B treatments as compared to those at the 4/4 and 30/30  
14  
15 328 B treatments.

329

330 *Uptake Efficiency*

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332 When plants were supplied continuously with 4  $\mu\text{M}$  B, the uptake efficiency for  
333 both genotypes was 1.77 on the average, whereas plants supplied continuously with 30  
334  $\mu\text{M}$  B exhibit uptake efficiencies of 0.32 on the average (for both genotypes, Table 3).

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336

### Discussion

337

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

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3 348 It should be emphasized that in the S genotype, the decrease in root dry weight  
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6 349 due to B deficiency (0 and 1 B treatment) and toxicity (75 and 100 B treatments)  
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8 350 corresponded with an increase in B concentration in WIR of the roots, while the total B  
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11 351 content did not differ much between genotypes. The observation above suggests that the  
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13 352 S genotype exhibits a higher boron requirement threshold value for root cell walls than  
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16 353 that of the R genotype.

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18 354 The B contents in WIR of roots were similar for all genotypes and treatments. In  
19  
20 355 contrast, the B concentration in WIR of leaves reached values near saturation only when  
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23 356 B started to accumulate in the CS of roots to the level as detected in CS of leaves. In  
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25 357 sunflower plants, it seems apparent that the boron requirement for cell walls of roots  
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28 358 must first be met, before the boron requirement for cell walls of shoots can be satisfied.  
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30 359 Once these steps have taken place, accumulation of metabolically available boron in CS  
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32 360 of roots starts to occur, followed by its accumulation in CS of leaves, where it is  
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35 361 concentrated. These findings would confirm that: (a) WIR of roots and leaves behave  
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37 362 like a chemical absorber of B, with a limited number of binding sites occupied by B  
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40 363 until saturation is attained (Dannel et al., 1998), and (b) the mechanism suggested by Hu  
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42 364 and Brown (1997) was effective since with the formation of B complexes in the cell  
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45 365 wall a concentration gradient was kept in operation as the key step for translocation of B  
46  
47 366 to the shoots.

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49 367 The critical values of B concentrations in shoot-tissues would then be established  
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52 368 after the boron requirement for cell walls was satisfied and a proper metabolic B content  
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55 369 in CS of roots was reached. The above became evident in plants, grown in nutrient  
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57 370 solutions with a B concentration between 4  $\mu M$  (experiment 2) and 10  $\mu M$  (experiment  
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59 371 1). In addition, the results of experiment 2, with their changes in B supply, showed that:  
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372 (a) a B concentration of 4  $\mu M$  in the nutrient solution was sufficient to satisfy the

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3 373 requirement of root cell walls; and (b) the amount of B absorbed in WIR was stable,  
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6 374 since it was not affected by the decrease in B supply, which was in agreement with the  
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8 375 results of Pfeffer et al. (1997).  
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11 376 The quick adjustment (six hours) of B concentration in CS of roots that took  
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13 377 effect due to changes of B concentration in the nutrient solution, indicated that the  
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15 378 changing B concentration in CS of roots or leaves could be used in combination with the  
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18 379 more stable B concentration in WIR of roots or leaves, as components of a criterion,  
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20 380 relating B status in plant with B availability in the medium, and to predict the likelihood  
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22 381 of B deficiency or toxicity to occur. Therefore, B availability in the medium would be  
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25 382 sufficient during the vegetative growth of sunflower, when the ratio between B  
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27 383 concentration in CS and B concentration in WIR of roots (both expressed in  $\mu\text{g B g}^{-1}$  dry  
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29  
30 384 weight) was  $> 0.1$  (Table 2). However, in young leaves, the value of the B ratio between  
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32 385 both compartments was higher than 0.3.  
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35 386 When the results in the present investigation are compared with those of other  
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37 387 authors, it can be seen that the total B concentration in leaves at the 0 and 1 B treatments  
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39 388 (12,7 and 24,2  $\mu\text{g B g}^{-1}$  dry weight of WIR, respectively for both genotypes) is in  
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42 389 accordance to previously reported findings on B requirement of sunflower (Asad et al.,  
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44 390 2001; Asad et al., 2003; Asad et al 2002). However, Blamey et al. (1997) found a  
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46 391 marked increase in total dry weight with an increase in B concentration ( $> 22.0 \mu\text{g B g}^{-1}$   
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49 392 dry weight) in the youngest mature leaf blade (YMB), and they considered deficiency to  
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51 393 occur at a critical value of a B concentration of  $190.0 \mu\text{g B g}^{-1}$  dry weight (90% of  
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54 394 maximum yield) in the YMB. The latter value was considerably higher than those  
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56 395 reported in other studies, but was close to the total B concentration in leaves (CS and  
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58 396 WIR) by the 100 B treatment ( $255.5 \mu\text{g B g}^{-1}$  dry weight of WIR on the average for both  
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60 397 genotypes) in present investigations, at which B toxicity symptoms were observed.

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3 398 Uptake efficiency values  $< 1.00$ , detected as a result of treatments of 30 to  
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6 399  $100\mu\text{M}$  B in the nutrient solution, suggested the presence of an exclusion mechanism to  
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8 400 be working in order to restrict B accumulation. However, this exclusion mechanism was  
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10 401 not completely effective at B supplies between 1 and  $10\mu\text{M}$ , and the high UE value  
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12 402 obtained by the 4/4 B treatments indicated that mechanisms, other than mass flow, had  
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15 403 played a role in providing the acquired B. The mechanism, causing the restriction in  
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18 404 uptake of B, is not exactly known, but an excretion mechanism had been proposed by  
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20 405 Bellaloui and Brown (1998), whereas other authors quoted a higher membrane  
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23 406 permeability for water as a better explanation with respect to boron absorption (Weig et  
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25 407 al., 1997 cited by Brown et al., 2002). The low UE value at the 0 B treatment (0.47 on  
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28 408 the average for both genotypes), could be interpreted as a consequence of a severe B  
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30 409 deficiency, since B seemed to be of crucial importance for the maintenance of structural  
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32 410 integrity of plasma membranes (Cakmak and Römheld, 1997).

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35 411 The pathways of nutrient transport from root surface to shoot include at least two  
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37 412 processes of transmembrane transports: (1) import into epidermal or cortex cells; and,  
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40 413 (2) export from pericycle or xylem parenchyma cells into the stelar apoplasm (xylem  
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42 414 loading) (Takano et al., 2002). As discussed in the introduction, B uptake and B  
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44 415 transport through membranes in higher plants were believed to be facilitated by both  
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47 416 active and passive uptake processes (Brown et al, 2002). It is suggested that B uptake by  
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50 417 roots would be by passive diffusion when B supply was high, whereas B accumulation  
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52 418 in the symplasm of root cells was supposed to be an active process when B supply was  
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54 419 low, since such an accumulation would take place against a concentration gradient  
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57 420 (Pfeffer et al., 1999). When the values of plant WIRs were near saturation ( $10\text{ B}$   
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59 421 treatment or higher B supply), the B concentrations in the different liquid compartments  
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422 indicated that: (a) there was no accumulation of B in CS of roots, since B contents

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3 423 follow a linear concentration dependence; (b) at the 10 B treatment, the B concentration  
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6 424 in xylem exudate was three times higher than the B content in the nutrient solution. At  
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8 425 higher B supply, the boron concentrations in the xylem exudates followed a linear  
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11 426 concentration dependence; and (c) B accumulation started in leaf CS against a  
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13 427 concentration gradient. Furthermore, the results of experiment 2 showed that the great  
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15 428 difference, found in B concentration in CS of roots as a result of treatments between 4/4  
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18 429 and 30/30 B, was reduced to a minimum when the B concentration in CS of hypocotyls  
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20 430 was included. The data above suggest that a concentration mechanism was perhaps  
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22 431 induced and effective at low B supply as a result of treatments of < 4 or 10  $\mu M$  B. This  
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25 432 mechanism was perhaps the key process, controlling B accumulation, which was named  
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27 433 as xylem loading by Takano et al. (2002). These authors, working with *Arabidopsis*  
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29 434 *thaliana* mutant bor1-1 (sensitive to boron deficiency) and wild-type plants, showed that  
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31 435 the concentration of boron in root cell sap increased in proportion to boron  
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33 436 concentration in the medium in both the genotypes, suggesting that B uptake into roots  
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36 437 occurred mainly by passive transport. The concentration of boron in xylem exudates of  
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38 438 the bor1-1 plants also followed a linear concentration dependence, whereas a  
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40 439 combination of saturable and linear concentration dependence was observed in the wild  
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42 440 plants. Takano et al (2002) also indicated that xylem loading is the key step for boron  
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45 441 accumulation in shoots with a low external boron supply and that BOR1, an efflux-type  
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47 442 boron transporter for xylem loading, was an essential component of the process.  
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52 443 The present results also indicated the presence of two factors explaining the  
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54 444 differences in susceptibility to knife-cut between the S and R sunflower genotypes,  
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56 445 when grown under similar field conditions: (a) a higher capability of adjustment of  
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58 446 shoot : root ratio for the R genotype under deficient boron conditions; and (b) a higher B  
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60 447 requirement threshold for cell walls for S genotype. Though a clear relationship between

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3 448 degree of susceptibility and B-efficiency could not be established adequately, the  
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6 449 suggestion is made that the higher or lower susceptibility to B deficiency between  
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8 450 genotypes is perhaps related to the capability of establishing B concentrations in CS of  
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11 451 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  $\pm$  SD.

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

Table 2. Boron concentration ( $\mu\text{g g}^{-1}$  of WIR dry weight) in CS and WIR of roots and leaves and total boron content ( $\mu\text{g}$ ) 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  $\pm$  SD. ND not determined.  $< 0.01$  B concentration lesser than  $0.01 \mu\text{g B ml}^{-1}$ .

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

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  $\mu\text{M}$ , and in Experiment 2 under B concentrations of 4 y 30  $\mu\text{M}$ . Values represent means of three or four replications  $\pm$  SD. ND not determined.

Genotype	Boron concentration in nutrient solution ( $\mu\text{M}$ )							
	0	1	4	10	30	50	75	100
S	0.67 $\pm$ 0.29	1.07 $\pm$ 0.15	1.69 $\pm$ 0.17	0.89 $\pm$ 0.03	0.30 $\pm$ 0.04	0.23 $\pm$ 0.03	ND	0.26 $\pm$ 0.01
R	0.28 $\pm$ 0.20	0.90 $\pm$ 0.09	1.85 $\pm$ 0.12	0.87 $\pm$ 0.10	0.35 $\pm$ 0.02	0.28 $\pm$ 0.02	0.28 $\pm$ 0.02	0.26 $\pm$ 0.04

Table 4. Boron concentration ( $\mu\text{g ml}^{-1}$ ) 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  $\pm$  SD. ND not determined.  $< 0.01$  B concentration lesser than  $0.01 \mu\text{g B ml}^{-1}$ .

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

Table 5. Boron concentration ( $\mu\text{g g}^{-1}$  of WIR dry weight) in WIR of roots, hypocotyls and leaves and boron concentration ( $\mu\text{g ml}^{-1}$ ) in CS of roots, hypocotyls and leaves in two sunflower genotypes. Plants grown for seven days with a B supply of 4 or 30  $\mu\text{M}$ , then were transferred to nutrients solutions containing 4 or 30  $\mu\text{mol L}^{-1}$  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  $\pm$  SD.

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