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# Effect of Drought on the Mobility of Foliar-Applied Boron in the Plants

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**Abstract:**

*Boron is considered to be phloem immobile or to have only limited phloem mobility in many higher plant species, where it is transported along the transpiration stream and accumulates in the margins of leaves. However, one would expect a phloem transport of boron if the back diffusion into the xylem in some way be prevented. The back diffusion into the xylem may only be possible under reduced transpiration. In this research, the distribution of foliar-applied boron (B) in green gram plants (Vigna radiata L.) under varying transpiration rates was evaluated in the Plant Nutrition greenhouse at the University of Bonn, Germany, between July and September 2009. The top of the second trifoliate leaf was immersed in 100 mM boric acid solution for one hour. The plan samples were digested in pressure digestion and B concentration of digested samples were determined by miniaturized curcumin method. Results revealed that most of the B absorbed from the foliar application was accumulated in the treated leaf. There was no evidence of phloem B movement out of the leaves*

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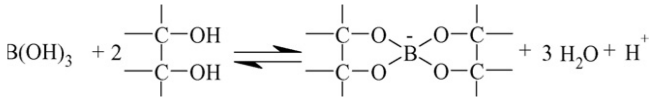
*under reduced transpiration. The transpiration rates affect the foliar-B uptake in plants. However, the reduced transpiration did not support the phloem B transport out of the leaves.*

**Key words:** Boron, foliar-application, mobility, transpiration

## INTRODUCTION

Boron is a unique micronutrient with narrow margin between deficiency and toxicity. It is considered to be phloem immobile or to have only limited phloem mobility in most of the higher plant species (Brown and Hu, 1996, Brown et al, 1999). Evidence suggests that the principal factor that confers phloem B mobility to a plant species is the synthesis of sugar alcohols and the subsequent transport of the B-sugar alcohol complex in the phloem to sink tissues such as vegetative or reproductive meristems (Anonymus, 2006; Brown and Hu, 1998; Brown and Hu 1996; Brown and Shelp, 1997; Hu et al., 1997; Oertli, 2004). In species, where boron (B) is phloem mobile, a polyol-B-polyol complex is formed in the photosynthetic tissues as shown in the following equation:



Adopted from Masrchner (1995)

The polyols are single sugars, as sorbitol, manitol and dulcitol present in many plants (Zimmermann and Ziegler, 1975), but not present in several dicotyledonous plants.

A steep gradient in B concentration has often been found such that B concentration in petioles and midribs is always lower than margins and tips (Oertli and Rechardson, 1970). This pattern of distribution coincides with the appearances of deficiency symptoms in young parts and toxicity symptoms in the margins of developed leaves of plants (Brown and Jones

1971). The occurrence of higher B concentrations in old or matured leaves in comparison to younger leaves is evidence of B immobility (Brown and Shelp, 1997). If B is immobile in a species, then application of foliar B fertilizers will results in enrichment of the treated leaf, but will not result in enhanced B content of leaves formed after treatment or of tissues supplied primarily by the phloem (Brown and Shelp, 1997). It has generally been assumed that boron is relatively immobile in dicotyledonous plants and that a continuous supply of this element in the substrate is required for normal growth (Gauch and Dugger, 1954). Correction of B deficiency is directly affected by B mobility (or immobility) in plants. In those species in which B is immobile, foliar-applied B will not be translocated from the site of application. This B cannot supply the B requirements of tissues not yet formed.

In species that do not produce significant quantities of polyols, boron is transported along the transpiration stream and accumulated in the margins of leaves. There is no evidence of significant retranslocation of B within the plant body (Brown and Hu, 1998; Miwa and Fujiwara, 2009) and thus becoming immobile. Oertli and Richardson (1970) supposed that since the phloem flux will always be low, little or no boron will be removed from the leaf. They also emphasized since that the transport capacity of the xylem normally exceeds the capacity of the phloem, the influx of boron into the leaf should exceed the efflux. Their hypothesis states that B is phloem immobile because it can move out of the phloem easily due to the high membrane permeability of boric acid (small, uncharged and therefore membrane permeable molecules). High membrane permeability of B is thought to induce a rapid efflux of B out of the phloem and its subsequent and immediate retranslocation into the source leaf by the transpiration stream which prevents the long distance phloem transport. The distribution of B is related to the loss of water from shoot organs, suggesting that it is primarily xylem-mobile with limited re-translocation in the phloem (Miwa and Fujiwara, 2009; Shelp *et al.,* 1995).

The consequence of the hypothesis of Oertli and Richardson (1970) would be that, a low transpiration rate would reduce the xylem flux (influx) and thus the re-transport of B into the leaf that ultimately increases the distance which B can be transported out of the leaf. Based on this assumption, the hypothesis of this research is "Under the condition of reduced transpiration boron translocation in the phloem is enhanced".

## MATERIALS AND METHODS

### 1.1 Plant material and growth conditions

A pot experiment was conducted in the Plant Nutrition greenhouse at the University of Bonn, Germany between July and September 2009 to evaluate the mobility of foliar-applied boron in Green gram (*Vigna radiata* L). Green gram seeds (RTS Wholesale Co. LTD Southall, Middlesex, England) were surface sterilized with 1 % sodium hypochlorite (NaOCl) for 10 min followed by thoroughly rinsing with deionised water. The surface sterilized seeds were imbibed in deionised water overnight in the dark at 20$^0$C before sowing. Then pre-germinated (surface sterilized) seeds were sown at 1 cm depth and with a distance of approximately 1 cm between seeds and slightly pressed down. Afterwards de-ionized water was supplied sufficiently to maintain the moisture. Initially, plants were grown without external nutrient supply but with sufficient amounts of deionised water until the first true leaf stage. When plants reached the first true leaf stage nutrients were supplied externally including B. Every second day, nutrient solution was supplied at 1/4 strength during the first week, followed by 1/2 strength during the second week and full strength thereafter. The pH of the solution after dilution was 5.78 at 20$^0$C.

## 1.2 Treatment application

The experiment was laid out in a complete randomized block design (CRBD) comprising three moisture levels and two boron levels. In total, there were three replications for each treatment.

### 1.2.1 Adjustment of moisture levels

When the plants reached the third true leaf stage (40 DAS), three different moisture levels were induced for one week: high moisture level (75% WHC), medium moisture level (50% WHC) and low moisture level (25% WHC) considering low ML creates drought stress to the plants. The moisture levels were maintained accordingly during the treatment application. In order to add equal amounts of nutrients to all pots, the amount of nutrient solution required for the lowest water level was added to all pots. Then, the additional amount of water was added to pots for the medium and high water levels in order to maintain the calculated standard pot weight.

### 1.2.2 Transpiration measurement

Various environmental factors, including those conditions that directly influence the opening and closing of the stomata, affect the plant’s transpiration rate. In the experiment, transpiration rates were measured only under different soil moisture levels. All other factors like light intensity and duration, relative humidity, air temperature and air humidity remained constant. Individual plants were selected randomly and transpiration was measured in the second true leaf of each selected plant by using a Steady State Porometer LI 1600 (LI-CoR, Inc.).

### 1.2.3 Boron Application

After a week of drought stress, foliar B was applied. At the beginning of the B treatment the surface of the pots was covered by aluminum foil to protect the roots from contamination by solutions. Further, silica free water protected paste was applied as a minute ring around the sub-petiole of the treated trifoliate leaf to block the surface movement of treatment solution to the plant system. Thereafter, the top of the second trifoliate leaf was immersed into 100 ml of 100 m*M* boric acid solution for one hour. After one hour, the immersed leaves were removed from the solution and the surface of the treated leaf was blotted carefully with blotting paper before the harvest. The samples were collected one week after boron treatment. During the sample collection, each plant was separated into six different parts comprising the top of the trifoliate treated leaf including the sub-petiole, the rest of the trifoliate treated leaf including the petiole, the parts above the treated leaf including the third true leaf and growing tips, the first true leaf including the petiole, the stems below the treated leaf and the roots (Figure 1). After harvest, all the samples were oven dried at 60$^0$C for three days.

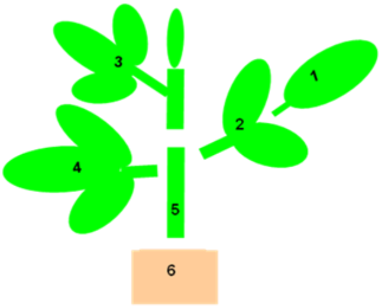


**Figure 1: Illustration of different plant parts harvested in the experiment: top of the trifoliate treated leaf including the sub-petiole (1), rest of trifoliate treated leaf including the petiole (2), parts above the treated leaf including the third true leaf and growing tips (3), first true leaf including the petiole (4), stems below the treated leaf (5) and roots (6)**

## 1.2.4 Tissue Boron Concentration

The plant material was dried at 60$^0$C for 3 days and subjected to a pressure digestion. Tissue B concentration in the digested samples was then determined by miniaturized curcumin method (Wimmer and Goldbach, 1999). Briefly, 100 µl of digest was mixed with 100 µl of 0.1 N HCl and 50 µl 2-Ethyl-1,3-

hexanediol/Chloroform mixture (10% w/w). After phase separation, 20 µl of the lower organic phase were mixed with 200 µl of conc. H2SO4/HAc (1:1, v:v) and 250 µl Curcumin/Methyl-Isobutyl-Ketone (MIBK) (25 mg/50 ml). After one hour, the reaction was stopped by addition of 500 µl of MP water and the colored organic phase was then measured with a Perkin-Elmer Lambda 20 (Serial No: 1) Spectrophotometer (Überlingen, Germany) at a wavelength of 550 nm for the determination of B. The amounts of B originating from foliar uptake were calculated as the difference between the B content in plant parts of foliar treated plants and in the corresponding parts of untreated control plants.

## RESULTS

### 1.3 Transpiration Characteristics and Relationship with B content

The transpiration rates of plants under low ML was significantly lower than higher and medium MLs in both B treated and B controlled plants (Figure 2).

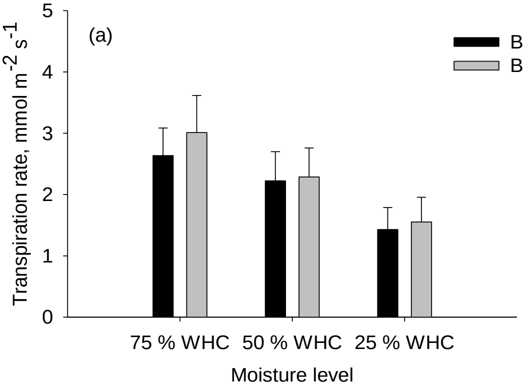


**Figure 21: Transpiration rates (mmolm$^{-2}$s$^{-1}$) of 6 week-old *Vigna radiata* L. plants after treatment application. Black bars and grey bars represents the B treated and untreated controls, respectively. Measurement was taken by using the Steady State Porometer LI 1600 (LI-CoR, Inc.) in the second true leaves of each selected plants.**

There was a significant correlation between transpiration rates and total B uptake of both B treated plants ($r^2$ = 0.70, Figure 3a), and untreated control plants ($r^2$ = 0.99, Figure 3b). The significance of correlation was higher in untreated control plants.

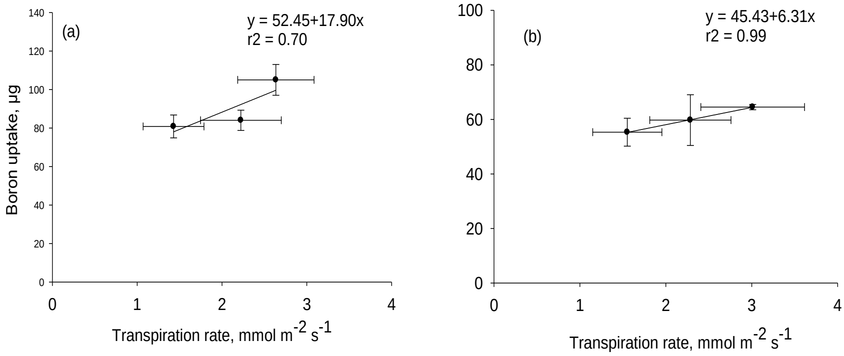


**Figure 3: Relationship between transpiration rate (mmolm-2s-1) and total B uptake (µg) of 6 weeks-old *Vigna radiata* L. plants one week after B treatment; B treated plants (a) and untreated control plants (b).**

## 1.4 Total B Uptake

Comparing the B content of B treated and untreated plants separated after soil moisture level gave a significant difference at high and low moisture levels (Figure 4). The mean B content was 118.7±8.2 µg at high, 96.5±5.7 µg at medium and 92.7±6.1 µg at low moisture level in treated plants, respectively, and it was 75.4±2.2 µg at high, 72.0±11.0 µg at medium and 68.8±5.5 µg at low moisture level, respectively, in untreated control plants. The increment of B in B treated plants compared to untreated control plants was 43.3 µg at high, 24.8 µg at medium and 23.9 µg at low moisture level, respectively, which account for 57, 35 and 35 % of B content of control plants.

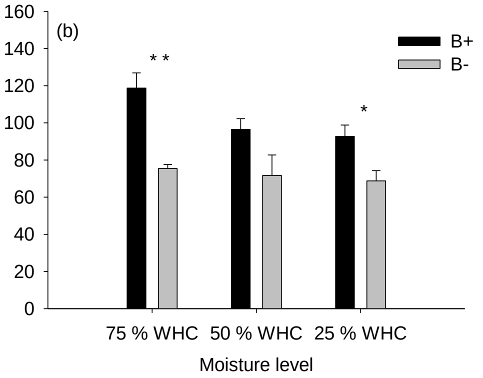


**Figure 4: Total B content (µg) in 6 weeks-old B treated *Vigna radiata* L. plants (black bars) and in untreated control (white bars) one hour after foliar B application (a) and one week after foliar B application (b) as boric acid solution (100 *mM*). Before B application, plants were exposed to different moisture levels for a week. Asterisks indicate significant differences between B-treated and untreated control plants at the respective moisture levels (t-test, n=3, *: p≤0.05, **: p≤0.01, ***: p≤0.001). Vertical bars indicate standard errors of the means.**

The foliar application of B had a highly significant effect on total plant B content. Plants grown under high ML resulted in highest B uptake and low ML resulted in lowest B uptake. It might be due to the fact that plants grown under low ML might have received lower amount of water from the soil compared to plants grown under medium and high MLs. Because of the water scarcity to transpire, there might be the formation of waxes in the cuticle layer of the transpiring leaves of the plants grown under low ML to minimise the water loss from the transpiration which might prevent the foliar-applied B to enter inside the leaf. The significant effect of the B treatment on total plant B content under different soil moisture levels indicates that the plants were able to take up foliar-applied B.

Similarly, high moisture level resulted in the highest B content, followed by the medium and low moisture levels, irrespective of the boron levels. Within the moisture levels, there was a significant difference of B content in B treated plants at medium and low moisture level at high and low moisture level (Figure 4). The significant correlation between transpiration and total B uptake indicates that transpiration rates affected the total B uptake of the plants. The correlation coefficient was higher in B control plants ($r^2 = 0.99$, Figure 3b) than in B treated plants ($r^2 = 0.71$, Figure 3a). The lower the correlation coefficient in the B treated plants compared to control plants (as stated above) might be due to the additional B from foliar application in the B treated plants and not due to transpiration.

Boron level had a highly significant effect on leaf B content of treated leaf. There was a significant difference between the leaf B content from treated leaf of B treated plants and corresponding leaf of untreated control plants. Most of the foliar-applied B accumulated in the treated leaf and did not show the movement towards surrounding leaflets (of treated leaf). Comparatively lower The B content in B treated leaf at low ML indicates that the absorbed B in the source leaf was moved out towards the other plant parts. However, other plant parts including rest of the treated leaf did not received B from the source leaf. It was expected that reduced transpiration could reduce the re-transport of B into the source leaf and thereby increase the distance that B can be transported out of the leaf, resulting in increased amounts of B in untreated plant parts, e.g. the newly growing shoots. But the result was completely opposite. High B concentration at high moisture level might be due to high transpiration. Under the condition of low moisture level, the surface of transpiring leaves become thicker by the formation of was layer in the cuticles to minimise the water loss from transpiration. And, this thick wax layer might prevent to enter the foliar applied boron into the leaves.

The B levels had a significant effect on leaf B content of rest the treated leaf. The significant difference in B content in rest of treated leaf from B treated plants and untreated control plants indicated that part of foliar-applied B, absorbed from the treated leaf moved towards the surrounding leaflets of the treated leaf. Movement of B from treated leaf towards surrounding leaflets (of treated leaf) indicates that the absorbed B in the source leaves was able to move out towards the other plant parts. However, the movement of B was detected only at high moisture level. It might be due to high B concentration in the B treated leaf. It is important to note, however, that even though the data obtained support the view that B moves in the xylem, the complications arising from differential transport mechanisms (influx and efflux) and accumulation in transit (petiole) must be underlined. The lateral leaflets of the treated leaf acquired B not only from the B treated leaf but also from the roots. At the same time, it lost parts of acquired B together with the treated leaf to rest of other (younger) parts. Because of this complication, this research could not quantify the amount of B that received by the particular plant parts and need another research.

## DISCUSSION

The B content of the treated leaf in the B treated plants significantly correlated with transpiration ($r^2 = 0.97$). It might be due to the accumulation of foliar-applied B in the treated leaf and transported B from the roots in the treated leaf. The high transpiration rate increased the amount of B in the treated leaf (highly transpiring organ). This result suggests that B moves with transpiration streams in the non-living xylem tissue and accumulated in the highly transpiring organs (older leaves). The high rate of transpiration might prevent the efflux of B out of the leaf with increasing capacity of xylem flow rate. Once B transported into the leaves in the xylem and as water is lost through the transpiration, it is concentrated in the margin of the leaf (Oertli and Richardson, 1970). The B content of the rest of treated leaf and parts above the treated leaf in the

B treated plants showed a positive correlation with transpiration ($r^2 = 0.58$ and 0.56). The lateral leaflets of the treated leaf probably received B both from the treated leaf via phloem transport and from the roots via transpiration. B might be loaded into the phloem in the margin of treated leaf to equilibrate the concentration and transport towards the basal areas of the leaf with the concentration gradient as suggested by Oertli and Richardson (1970). They hypothesized that part of the phloem loaded B in the leaf is lost into the adjacent tissues in the basal areas of the leaves and petiole where concentration is low. Part of that B might move towards the lateral leaflets of the treated leaf and parts above the treated leaf (growing tips) with the transpiration stream. It was expected that reduced transpiration decrease the xylem flux and reduce the re-transport of B into the source leaf. This ultimately, increase the distance that B can be transported out of the leaf. Shelp et al. (1995) reviewed several experimental evidences and concluded that the distribution of B is related to loss of water from shoot organs, suggesting that it is primarily xylem mobile.

## CONCLUSION

The present study confirms the well-known fact that plants have a capacity to take up foliar-applied B. The total B uptake depended on the moisture levels in the root zone of the plants as well. The uptake (total B mass) and translocation (B in rest of treated leaf) of B in the plant was related to the amount of water consumed and consequently to the transpiration rates of the plants. However, all of the plant parts do not participate to the same extent in B distribution. Movement of B from treated leaf towards surrounding leaflets indicates that the absorbed B in the source leaves was able to move out towards the other plant parts. However, the movement of B was detected only at high moisture level. It might be due to the effect of concentration gradient rather than transpiration. It was expected that reduced transpiration could reduce the re-transport of B into the source leaf and thereby increase the distance that B can be transported out of the leaf, resulting in increased amounts of B in untreated plant parts, e.g. the newly growing shoots. However, the increase in the B content of the parts above the B treated leaf may not only come from the older leaves but could also be due to the increase in dry weight of biomass. Increase in the biomass due to the plant growth also increased the B uptake from the roots which interfered in the present study with B translocation from the treated leaf. B content in the newly growing plant parts cannot be separated whether it come from B treated leaf or from the roots. The results showed no evidence of phloem B movement out of the leaves under reduced transpiration. The suggested conclusion is that the transpiration rates affect the foliar-B uptake and distribution in plants. However, the question of phloem B transport under reduced transpiration is still to be answered. To answer this question it is necessary to analyse the phloem flux too, which is still lacking in this research. Analysis of rubidium (Rb) as a phloem marker may verify the findings of this research. The reduction of transpiration rates by low soil water availability might have not been sufficient to allow the export of the foliar-absorbed B out of the treated leaf. It could thus be better to reduce the transpiration by further lowering the moisture levels below 25% WHC for clearer results. It is necessary to quantify the amount of B that plant received from the soil before and after the B treatment. It would be better to use B isotopes ($^{10}$B and $^{11}$B) to quantify the trace amount of B.

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