Πρόκειται για άρθρο δημοσιευμένο στο περιοδικό Journal of Plant Nutrition & Soil Science 2012, v. 175, pp. 189–195
με τις διορθώσεις της επιστημονική επιτροπής, αλλά χωρίς το λογότυπο του εκδότη

Acclimation of eggplant (*Solanum melongena*) to low boron supply

Georgios Liakopoulos[1]*, Varvara Psaroudi[1], Sotiria Stavrianakou[1], Dimosthenis Nikolopoulos[1], and George Karabourniotis[1]

[1] Laboratory of Plant Physiology, Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, 118 55, Athens, Greece

Abstract

Root active uptake and remobilization of boron (B) have been accepted as mechanisms contributing to nutrient efficiency under low supply of boron. Here, we examined the existence of these mechanisms in eggplant *(Solanum melongena L.)* supplied either with luxury (100 ΙM, B+) or low (7.5 ΙM, B–) B in the growth medium via semihydroponic cultivation. Boron treatment was marginally not limiting growth thus avoiding side-effects and impairment of acclimation mechanisms of plants. The induction of a B-concentrating mechanism was evident in the roots as B con- centration in the xylem sap was only decreased by 23% in B– compared to B+ plants, *i.e.*, B–roots concentrated B by a factor of 2.7 relative to the external solution. Leaf B concentration in the B-treatment decreased by 33% and 40% in young fully expanded and mature leaves, respectively. Larger differences were observed in the soluble B fraction that decreased by 65% in mature leaves. However, both total and soluble B concentrations in developing leaves were almost equal for both treatments exhibiting a pattern commonly observed in B-remobilizing plants. On the other hand, amounts of B export in the phloem sap were small compared to other species in which B is highly mobile. The B export rate from source leaves was slightly increased under low B supply while that of sucrose was not affected. We conclude that the root concentrating mechanism contributes to the alleviation of B deficiency in eggplant under low B supply while B remobilization may also contribute to a lower degree.

Key words: *boron remobilization / boron uptake / phloem sap / xylem sap / eggplant**

1. Introduction

Early studies of the effects of low boron (B) supply in plants have shown that different species or even closely related genotypes may show striking differences in tolerance of low B supply *(Hu and Brown, 1997; Rerkasem and Jamjod, 1997)*. These differences imply that one or more low-B acclimation mechanisms exist in particular genotypes and may operate against the onset of deficiency of this essential micronutrient. We now know that two major mechanisms contribute to B efficiency *(Camacho-Cristóbal et al., 2008; Takano et al., 2008).* One mechanism is a combination of channel-mediated and transporter-mediated absorption of B *(Takano et al., 2008).* Through this mechanism, plants develop sufficient concentrations of the nutrient in the xylem sap, much higher than those in the external solution *(Brown et al., 2002; Dannel et al., 2002; Takano et al., 2002).* The other mechanism is based on B remobilization *(Brown and Shelp, 1997; Bellaloui et al., 2003).*

Active B absorption occurs only under low B supply *(Brown et al., 2002)* and seems to be widespread if not ubiquitous in higher plants *(Stavrianakou et al., 2006, and references there in).* Boron remobilization, the second mechanism, seems to operate regardless of the nutritional regime *(Liakopoulos et al., 2005, 2009)* but it has been reported to occur only in species in which polyols (sugar-alcohols) are among the main photoassimilates translocated via phloem *(Brown and Shelp, 1997).* The significance of B remobilization under adequate B supply is not so obvious. Nevertheless, it contributes to the optimal distribution of the nutrient on a whole- plant scale because growing tissues require considerably higher amounts of B compared to mature ones while in non- B-remobilizing species the distribution is reversed *(Brown and Shelp, 1997)*. However, under limited B supply, B remobilization permits growth of new aerial plant parts which could not be sustained without sufficient B. It has also been proposed that B remobilization in olive (a species that remobilizes B by complexing it with mannitol in the phloem) is maintained under B limitation even if soluble B reserves in mature tissues drop to extremely low concentrations and that this is possibly mediated by increasing phloem mannitol *(Liakopoulos et al., 2009).*

Η λέξη-κλειδί "eggplant**" προστέθηκε κατά την επεξεργασία και καταχώρηση του άρθρου στο Ιδρυματικό Αποθετήριο.*

Despite the fact that B remobilization is more obvious in species that translocate polyols in the phloem, species that do not belong to the above group (i.e., they predominantly translocate sucrose) also show an appreciable level of B mobility *(Shelp, 1988; Liu et al., 1993; Marentes et al., 1997; Stangoulis et al., 2001; Lehto et al., 2004).* Recently *(Stangoulis et al., 2010),* a putative bis-sucrose borate complex has been identified in the phloem sap of canola and wheat. This result extends the possibility of B phloem mobility in plant species that translocate sucrose. Therefore, according to this possibility, virtually all higher plants are inherently capable of showing some extent of boron mobility, at least, in regards to the existence of borate complexes in the phloem.

According to our knowledge, studies on B nutrition of egg- plant are very limited while much more research has been conducted on related solanaceous species. Leaf-tip yellowing has been reported as a result of poor B supply of eggplant *(De Kreij and Bas¸ar, 1997).* Oertli (1993) studied the B distribution in tomato plants after changing to a B-free nutrient solution and concluded that little if any remobilization occurred from old to young aerial parts while a limited amount of B was transported to the roots. Tomato cultivars often differ in B uptake and distribution. Bellaloui and Brown (1998) reported that a low-B–tolerant tomato cultivar was more capable of translocating B from roots to leaf tissues resulting in higher B content in the aerial parts compared to a low-B–sensitive cultivar. A tomato B-efficient cultivar also showed enhanced B uptake by the root cortex cells based on low- B–induced active transport *(Savic et al., 2007).*

Here, we studied the effects of low B supply of eggplant *(Solanum melongena)* plants and the possible existence of the above mentioned acclimation mechanisms, namely, root B-concentrating mechanism and B remobilization. Evidence for the existence of both mechanisms is presented.

2. Materials and methods

2.1 Plant cultivation

Seeds of eggplant *(Solanum. melongena* cv. Tsakoniki) were placed on moist paper laid on Petri dishes and left to germinate in the dark at 24°C for 1 week. Seedlings having a root length of 0.2–0.5 cm were transferred in 2 L plastic containers filled with quartz sand (four seedlings per pot, ten pots per treatment). Plant and substrate preparation was done according to previous reports *(Liakopoulos et al., 2005).* Plants were grown for 38 d in an environmentally controlled growth chamber using a semihydroponic culture system. Environmental conditions were: photoperiod 16/8 h, air temperature 25°C/20°C, and air relative humidity 52%/70% (day/ night). Mean light intensity was 330 lmol $m^{-2} s^{-1}$ (PAR) at plant height, provided by sodium-vapor lamps (VIALOX NAV- T400 4Y, Osram GmbH, Munich, Germany). Plants were supplied with either full composition half-strength Hoagland nutrient solution (B supplied as boric acid at 100 **l**M, control treatment, B+) or low-B solution (7.5 **l**M, low-B treatment, B–). The concentrations of the other essential nutrients are given elsewhere *(Liakopoulos et al., 2005).* Each pot received 15 irrigations per 24 h (every 1–2 h in the light period and every 3 h in the dark period; ca. 67 mL of nutrient solution per irrigation and per pot).

2.2 Sampling and measurements

Forty plants were assigned to each treatment. At harvest, four plants of each pot were pooled for the measurement growth parameters (ten replicates). Leaf dry mass was measured from leaves not used for biochemical analyses. Then, total dry mass was recalculated to account for the missing leaves that were sampled for biochemical analyses and collection of plant fluids. For the above recalculation, leaf area of missing leaves was converted to dry mass using the specific leaf-mass average for each leaf age. For chemical analyses, four to eight plants from different pots were randomly pooled and specific plant parts were subjected to further sample processing (five to ten replicates, depending on the particular measurement). Statistics were performed using Excel v. 12 (Microsoft Corporation, WA, USA). Significant differences between B treatments were determined using Student's t-test after confirming the equality of variances.

2.3 Collection of cell and xylem sap and phloem- sap exudates

Xylem sap (pH 5.9) was collected after removing the upper- ground part from the cut surface using a sharp-end Pasteur pipette. The first 20–30 **l**L of sample were discarded, and a final volume of ca. 400 **l**L was stored at 4°C until analyzed. Phloem sap was collected according to King and Zeevaart (1974) with modifications by Flora and Madore (1993). The submersed petiole was cut in the exudation solution which consisted of 20 mM Na_2EDTA adjusted to pH 7.0 with KOH. Exudation solution was prepared using Merck Ultrapur water (B concentration < 0.2 ng g^{-1}; Merck KGaA, Darmstadt, Germany). Each sample was collected from one leaf immersed in 2 mL exudation solution under low light, and exudation lasted 6 h into the photoperiod. During exudation, water was added to replace evaporation. According to a confirmatory experiment (not shown), losses of exudates through re- uptake into the xylem owing to transpiration of the exudating leaf were negligible. At the end, samples were made up to a final volume and stored at –20°C until analyzed.

Cell sap (400–1000 **l**L) was collected after pressing an appropriate amount of leaves with a mechanical tool. Samples were clarified by centrifugation and stored at –20°C until analyzed.

2.4 Measurements of boron parameters

Total B concentration was determined in dry plant material using the azomethine color reaction *(Banuelos et al., 1992)* as previously reported *(Liakopoulos et al., 2009).* Boron concentration in collected plant fluids was determined using the chromotropic acid reaction after separation of the borate- chromotropic acid complex by HPLC as reported previously *(Matoh et al., 1997; Liakopoulos et al., 2009).*

Insoluble B concentration was calculated by subtracting cell- sap B (soluble B; taken as the average value of six replicates; converted to stathmic units representing leaf soluble B con- tent) from total leaf B (calculated for each one of the ten replicates used for the total leaf B determination; converted to stathmic units representing total leaf B content). Subtracting soluble B content from total boron content yielded insoluble B content which was expressed per unit dry mass of each individual leaf (insoluble B concentration). For the above calculations, it was assumed that the water content of these samples (determined as the difference between fresh and dry mass for each leaf) was equal to the cell-sap mass.

Boron export rate was expressed as moles of B in the phloem-exudate solution per unit of leaf cell sap and per hour of exudation. The ratio of remobilizable to soluble B (also stated as the B relative export rate) was calculated by dividing B export rate by cell-sap B concentration *(Liakopoulos et al.,2009).* The molar ratio of sucrose to B in the phloem was calculated by dividing the concentration of each molecule in the phloem-exudate solution.

2.5 Analysis of sucrose in phloem-sap exudates

Phloem-sap exudates were injected into a Nucleosil Carbohydrate 10 lm, 250 mm × 4 mm column (Macherey Nagel GmbH, Düren, Germany) and chromatographed in a Jasco HPLC (Jasco Corporation, Tokyo, Japan) equipped with a RI930 refractive index detector. Separation was done at 27°C using acetonitrile-to-water-to-phosphoric acid 85 : 14 : 1 as the mobile phase at 2 mL min^{-1} flow rate. Sucrose was identified by reference to pure standard, and quantification was done according to a sucrose reference curve. The presence of sugar-alcohols myo-inositol, mannitol, and sorbitol was also examined during the analyses but none was detected.

2.6 Gas exchange, chlorophyll determination and in vivo chlorophyll-fluorescence measurements

Chlorophyll was measured indirectly using a portable chlorophyll meter (SPAD 502, Konica Minolta Holdings, Inc., Tokyo, Japan). Photosynthetic rate was measured at various light intensities (steady state was

accomplished by applying each intensity for 5 min) to construct light response curves using a portable photosynthesis instrument equipped with an artificial red-blue LED light source (LCPro+, ADC Bioscientific, Hoddesdon, UK). Maximum photosynthetic rate was recorded as the rate at saturating light intensity (reached at 1840 lmol quanta m^{-2} s^{-1} incident on leaf surface for both treatments). In vivo chlorophyll fluorescence was used to determine the intrinsic efficiency of PS II (U_{PSIIo}). A portable chlorophyll fluorometer (Fim 1500, ADC Bioscientific) was used to determine the basal (F_o) and maximal (F_m) fluorescence yield after acclimating leaves in the dark for 30 min. U_{PSIIo} was calculated as the ratio of variable *(Fv = Fm – Fo)* to maximal fluorescence yield.

3. Results

3.1 Plant growth and photosynthesis

A low-B treatment (B–) was chosen to induce acclimation mechanisms of plants without seriously affecting growth and plant homeostasis. Growth parameters of B– plants were not significantly different compared with the control including specific leaf mass which is frequently affected by B deficiency *(Tab. 1).* Photosynthetic rate at light saturation, photochemical efficiency of PS II, and chlorophyll concentration were also not affected by the treatment *(Tab. 2).*

Table 1: Effect of B supply on growth parameters at harvest. Values are means of ten replicates ± standard errors of the mean. Significant differences between means are marked with asterisks (**p < 1%; *p < 5%).

Parameter	B+		B–
Developing leaves			
Leaf dry weight / g $leaf^{-1}$	0.085 ± 0.010		0.068 ± 0.005
Leaf surface area / dm^2 $leaf^{-1}$	0.297 ± 0.039		0.242 ± 0.015
Specific leaf mass / g d.w. dm^{-2}	0.292 ± 0.012		0.283 ± 0.013
Young fully expanded leaves			
Leaf dry weight / g $leaf^{-1}$	0.482 ± 0.066		0.359 ± 0.023
Leaf surface area / dm^2 $leaf^{-1}$	1.725 ± 0.173	*	1.270 ± 0.079
Specific leaf mass / g d.w. dm^{-2}	0.273 ± 0.015		0.287 ± 0.015
Mature leaves			
Leaf dry weight / g $leaf^{-1}$	0.344 ± 0.035		0.353 ± 0.057
Leaf surface area / dm^2 $leaf^{-1}$	1.342 ± 0.132		1.241 ± 0.147
Specific leaf mass / g d.w. dm^{-2}	0.259 ± 0.011		0.275 ± 0.013
Whole-plant parameters			
Total leaf dry weight / g $plant^{-1}$	3.932 ± 0.398		3.901 ± 0.410
Total leaf area / dm^2 $plant^{-1}$	9.021 ± 0.724		8.595 ± 0.545
Total leaf dry weight / g $plant^{-1}$	3.932 0.398		3.901 0.410
Total leaf area / dm^2 $plant^{-1}$	9.021 0.724		8.595 0.545

Table 2: Effect of B supply on photosynthetic parameters and chlorophyll concentration of fully expanded leaves at harvest. Values are means of seven (intrinsic efficiency of PS II photochemistry, U_{PSIIo}), nine (net photosynthetic rate at saturating light intensity, A_n), or ten (concentration of chlorophylls, $Chl_{(a+b)}$) replicates ± standard errors of the mean. Significant differences between means are marked with asterisks (**p < 1%; *p < 5%).

Parameter	B+	B–
$Chl_{(a+b)}$ (SPAD values)	46.88 ± 2.18	42.48 ± 1.44
U_{PSIIo}	0.817 ± 0.005	0.818 ± 0.004
A_n / 1mol CO_2 m^{-2} s^{-1}	20.61 ± 0.86	19.67 ± 1.60

3.2 Boron acquisition and concentration in plants

On the other hand, B treatment caused appreciable reduction in B uptake by the roots indicated by the B concentration in the xylem sap. Nevertheless, B absorption by B– plants was much higher (ca. 2.7-fold) than predicted from the concentration of the external medium, indicating the effect of a concentrating mechanism, while that of B+ plants was ca. 74% lower than that of the external medium *(Fig. 1a).* As a result of reduced B acquisition by the roots of B– plants, B concentration was significantly reduced in mature leaves by 40% and in young fully expanded leaves by 33% but it was equal in developing leaves for the two treatments *(Fig. 1b).*

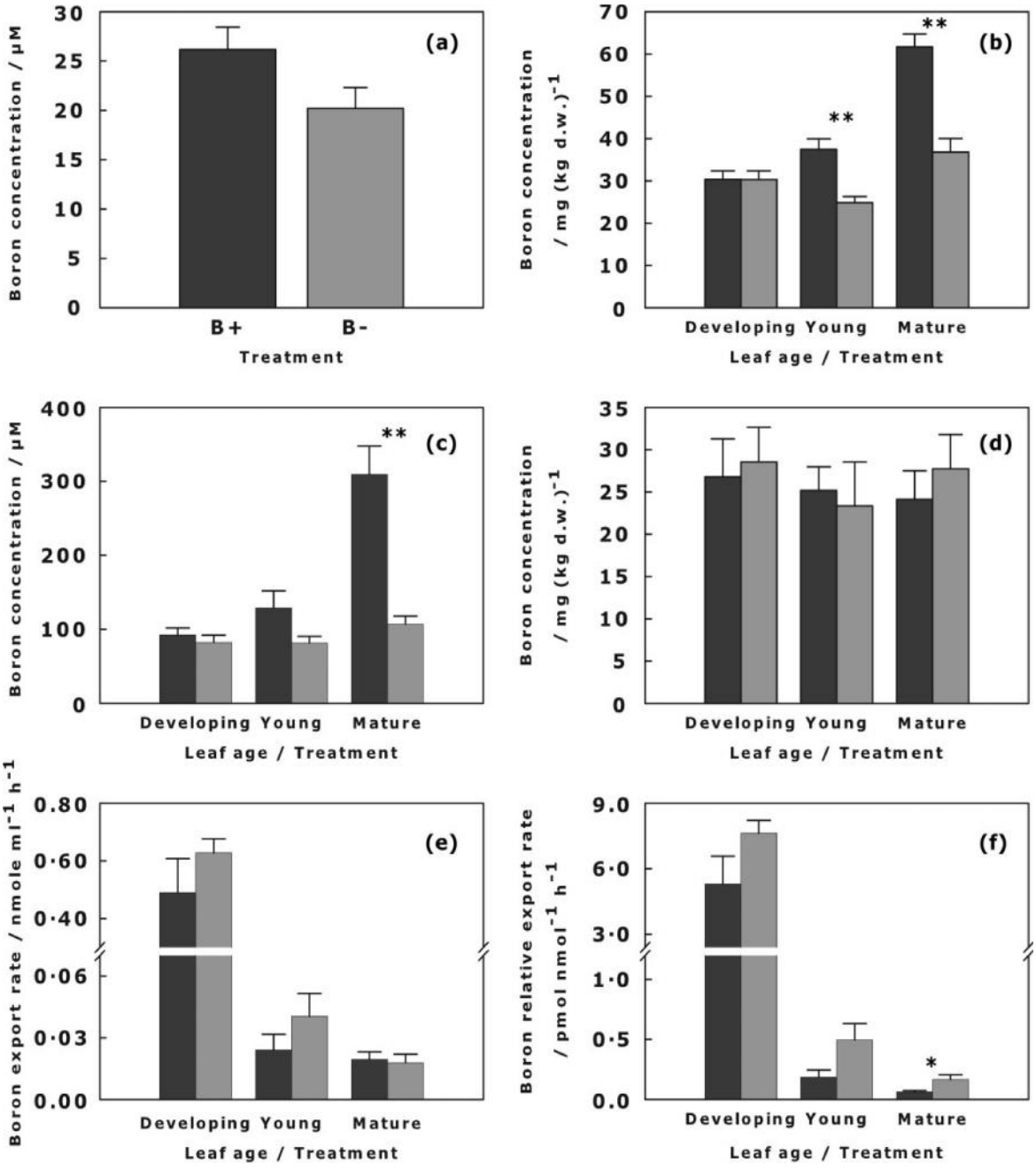

Figure 1: Effect of B supply on B concentrations of plant parts or fluids at harvest. (a) xylem sap (n = 10), (b) total leaf concentration (n = 10), (c) cell sap (n = 6), (d) insoluble B (total leaf B – cell sap B) concentration (n = 10), (e) phloem exudates (n = 5), and (f) relative export rate as the ratio of phloem-sap-exudates B enrichment per hour to cell-sap B. Black bars: B+; gray bars: B–. Values are means ± standard errors of the mean. Significant differences between means are marked with asterisks (**p < 1%; *p < 5%).

The portion of cell-sap B was affected to a greater extent due to low B supply compared to total B. Soluble B concentration in mature leaves was reduced by 65% and in young fully expanded leaves by 37% but again there was no difference in developing leaves between the two treatments *(Fig. 1c)*. The insoluble B pool, measured as the difference between total leaf B and cell-sap B, was not affected by the treatment in all three leaf developmental stages *(Fig. 1d).* Boron concentration in the phloem-sap exudates, expressed per unit of cell sap of the exudating leaf, was essentially similar between treatments indicating that low B supply did not affect B remobilization. Boron export was notably higher in developing leaves compared to young fully expanded and mature leaves (Fig. 1e). Figure 1f shows the remobilizable-to-soluble B ratio also stated as the B relative export rate. This expression has been proposed as an indicator of the intensity of remobilization *(Liakopoulos et al., 2009).* Boron treatment caused an increase of this ratio which, however, was statistically significant only in mature leaves *(Fig. 1f).*

3.3 Phloem sucrose and sucrose to boron ratio

Efflux of sucrose from fully expanded source leaves (ca. 15 nmol cm^{-2} h^{-1}, mature and young, regardless of B treatment) is in close agreement to that measured in potato *(Almon et al., 1997).* In our study, sucrose export was expressed per unit of leaf cell sap and per hour of exudation (Fig. 2) to allow comparison between sucrose and B export rates and calculation of the molar ratio in the phloem *(see also Liakopoulos et al.,2009).* Sucrose export was notably higher in developing leaves compared to young fully expanded and mature leaves while it was unaffected by B treatment *(Fig. 2).* Sugar-alcohols (specifically myo-inositol, mannitol, or sorbitol) were not detected in the phloem samples.

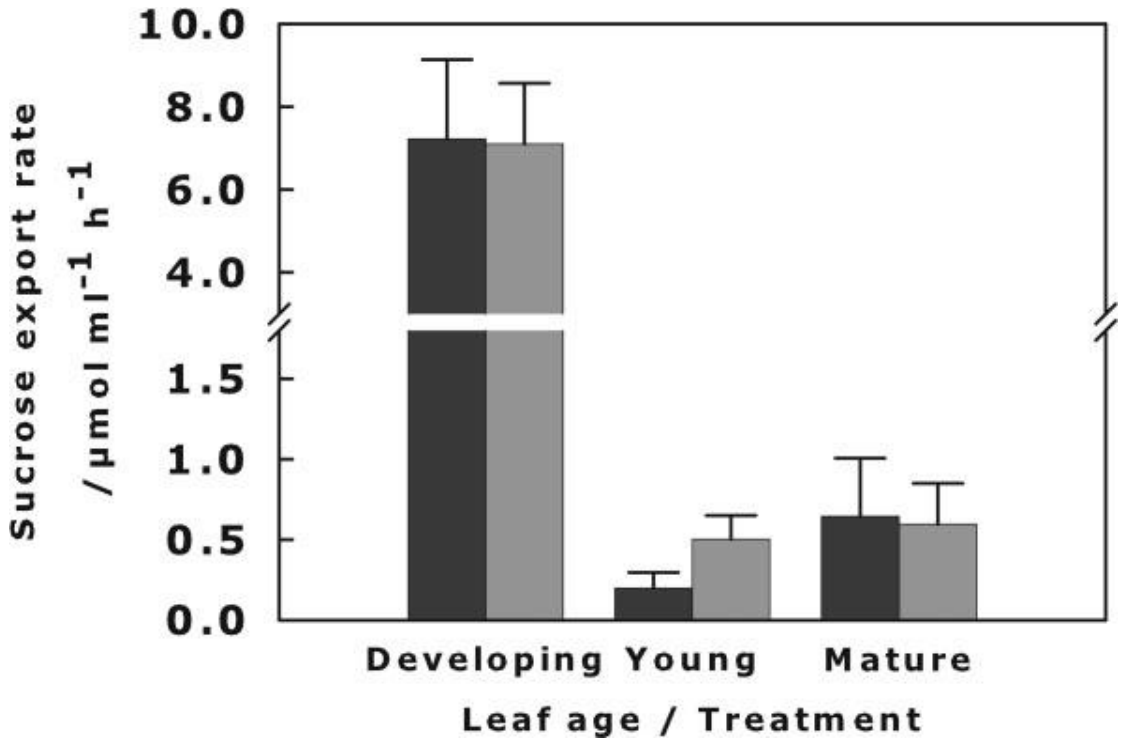

Figure 2: Effect of B supply on sucrose concentration in phloem-sap exudates (n = 5) at harvest. Black bars: B+; gray bars: B–. Values are means ± standard errors of the mean. Significant differences between means are marked with asterisks (**p < %; *p < 5%).

The sucrose-to-B molar ratio in the phloem-sap exudates ranged between 4700 and 22 000 depending on leaf age and treatment *(Fig. 3).* Notably, this ratio was higher in young and mature leaves of B– plants although the differences were statistically non-significant *(Fig. 3).*

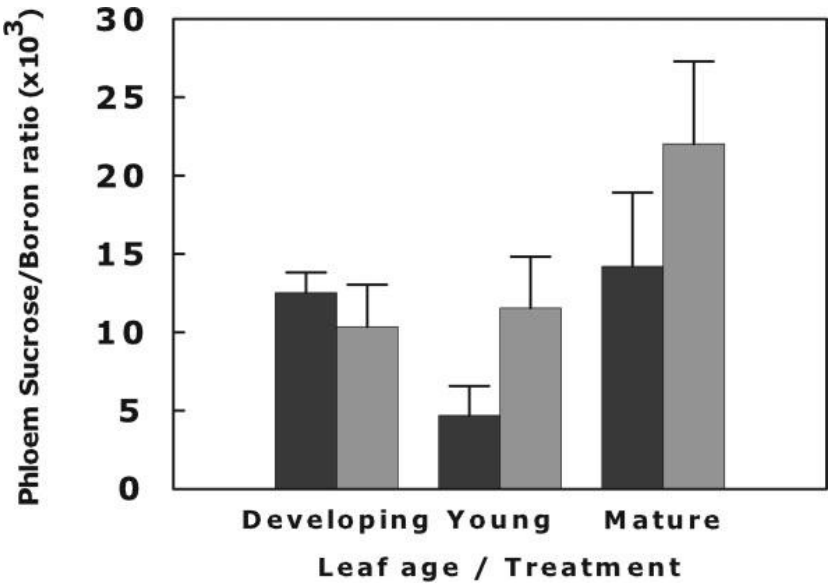

Figure 3: Effect of B supply on the molar ratio of sucrose to boron in phloem-sap exudates. Black bars: B+; gray bars: B–. Values are means of five replicates ± standard errors of the mean. Significant differences between means are marked with asterisks (**p < 1%; *p< 5%).

4. Discussion

With the exception of surface area of young leaves *(Tab. 1),* B– treatment had no apparent effect on plant growth and photosynthetic performance, allowing the study of plant acclimation to low B supply without the interference of side-effects of B deficiency. Chlorophyll-fluorescence measurements also confirmed the absence of stress on the photosynthetic machinery given that the efficiency of excitation energy capture by open PSII reaction centers (U_{PSIIo}) was identical for the two treatments and its values were indicative of a healthy leaf *(Maxwell and Johnson, 2000).*

Plant roots were able to concentrate boron 2.7-fold compared to the external nutrient solution indicating the existence of a B-concentrating mechanism located in root cortex cells, xylem parenchyma cells of the stele, or both, according to previous reports *(Takano et al., 2008).* Active transport of boron, induced by low external boron concentration, has been recently shown for tomato *(Savic et al., 2007)* in which B was concentrated up to 4-fold in the cell sap of the root cortex compared to the external solution (0.5 **I**M). However, the concentrating mechanism showed a saturable uptake pat- tern, similarly to other studies, which resulted in a lower con- centration factor at higher external concentrations (ca. 1.6- fold in the xylem sap at 10 **I**M external B concentration). In the control B+ treatment, primary translocation of B showed a significant reduction of B concentration relative to the extenal solution *(Fig. 1a)* probably owing to efflux from, exclusion by, or retardation of B in the roots. It is notable that while xylem-sap B concentration was 23% lower in the B– plants compared to the control *(Fig. 1a),* large differences were observed in leaf total *(Fig. 1b)* and leaf cell-sap *(Fig. 1c)* B con- centration between the two treatments.

The pattern of B distribution among leaves observed in B– plants is, to some extent, similar to that observed in plant species that are able to remobilize B through the phloem. In particular, under low B supply, young leaves show similar or higher B concentration compared to mature leaves owing to B remobilization from source leaves to growing sinks *(Brown and Shelp, 1997).* In the present study, young and developing leaves of B– plants showed similar B concentrations which may be an indication of B mobility in eggplant. If boron was only supplied through the xylem, mature leaves should always show higher B concentrations. Analysis of phloem exudates

showed the presence of B which, however, was exported at much lower rates (ca. 100–200 times lower, depending on leaf age) compared to olive *(compare Fig. 1f with Fig. 1c in Liakopoulos et al., 2009),* a species in which B mobility in the phloem is facilitated by mannitol translocation *(Perica et al., 2001).*

Chemical analysis of the phloem exudates of eggplant source leaves failed to detect any translocatable sugar other than sucrose. Although we found no reports on the exact composition of phloem sap of eggplant, it seems that polyols are absent or not present at detectable concentrations which, in addition, would facilitate an appreciable increase in B mobility. According to other studies, sucrose is the predominant photoassimilate translocated in *Solanaceae* species such as potato *(Almon et al., 1997),* tomato *(Chengappa et al., 1999), Datura arborea*, and *Solanum giganteum (Zimmermann and Ziegler, 1975)* while, according to the same authors, the last species also translocates some myo-inositol (a sugar-alcohol). However, myo-inositol as well as low concentrations of mannitol and sorbitol present in tomato leaf tissues *(Roessner-Tunali et al., 2003; Schauer et al., 2005)* could facilitate some B mobility, although to a much lesser magnitude compared to species in which B-remobilization is inherently high. Despite that a direct mechanistic association has been established between translocation of polyols and B mobility, a certain degree of B remobilization may be observed in plant species that lack polyols in the phloem or vice-versa *(Lehto et al., 2004).* In addition, sucrose translocation could itself support some B mobility according to recent reports *(Stangoulis et al., 2010).* Reports on B mobility in solanaceaous species are limited. In tomato, B mobility is restricted *(Oertli, 1993, 1994),* which is in accordance with the fact that polyols are absent in the phloem *(Brown et al., 1999).* However, apparent B mobility following foliar application of boron has also been reported for this plant species *(Davis et al., 2003).*

Our results show that B relative export rate from source leaves was slightly increased under low B supply *(Fig. 1f)* which also occurs in olive under low-B supply and it has been proposed as part of the low-B acclimation process. Moreover, increase of relative export rate in olive occurs through an increase of mannitol efflux which in turn results in an increase of the mannitol-to-boron ratio in the phloem *(Liakopoulos et al., 2009)*. In the present study, we did not observe notable changes in sucrose efflux (Fig. 2). On the contrary, sucrose- to-boron ratio in the phloem was increased in B– plants compared to the controls but these values were not significantly different *(Fig. 3).*

5. Conclusion

The present study shows that acclimation of eggplant to low B supply is primarily accomplished by the action of a B-concentrating mechanism which operates in the root. In addition, our results support the possibility of limited B mobility in the phloem which may contribute to B redistribution on a plant scale although more definite evidence is needed to document the existence of such a mechanism.

References

Almon, E., Horowitz, M., Wang, H. L., Lucas, W. J., Zamski, E., Wolf, S. (1997): Phloem-specific expression of the tobacco mosaic virus movement protein alters carbon metabolism and partitioning in transgenic potato plants. Plant Physiol. 115, 1599–1607.

Banuelos, G. S., Cardon, G., Pflaum, T., Akohoue, S. (1992): Comparison of dry ashing and wet acid digestion on the determination of boron in plant tissue. Commun. Soil Sci. Plant Anal. 23, 2383–2397.

Bellaloui, N., Brown, P. H. (1998): Cultivar differences in boron uptake and distribution in celery (Apium graveolens), tomato (Lycopersicon esculentum) and wheat (Triticum aestivum). Plant Soil, 198, 153–158.

Bellaloui, N., Yadavc, R. C., Chern, M.-S., Hu, H., Gillen, A. M., Greve, C., Dandekar, A. M., Ronald, P. C., Brown, P. H. (2003): Transgenically enhanced sorbitol synthesis facilitates phloem- boron mobility in rice. Physiol. Plant. 117, 79–84.

Brown, P. H., Shelp, B. J. (1997): Boron mobility in plants. Plant Soil, 193, 85–101.

Brown, P. H., Hu, H., Roberts, W. G. (1999): Occurrence of sugar alcohols determines boron toxicity symptoms of ornamental species. J. Am. Soc. Hortic. Sci. 124, 347–352.

Brown, P. H., Bellaloui, N., Wimmer, M. A., Bassil, E. S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F., Römheld, V. (2002): Boron in plant biology. Plant Biol. 4, 205–223.

Camacho-Cristóbal, J. J., Rexach, J., González-Fontes, A. (2008): Boron in plants: Deficiency and toxicity. J. Integr. Plant Biol. 50, 1247–1255.

Chengappa, S., Guilleroux, M., Phillips, W., Shields, R. (1999): Transgenic tomato plants with decreased sucrose synthase are unaltered in starch and sugar accumulation in the fruit. Plant Mol. Biol. 40, 213–221.

Dannel, F., Pfeffer, H., Römheld, V. (2002): Update on boron in higher plants – Uptake, primary translocation and compartmentation. Plant Biol. 4, 193–204.

Davis, J. M., Sanders, D. C., Nelson, P. V., Lengnick, L., Sperry, W. J. (2003): Boron improves growth, yield, quality, and nutrient content of tomato. J. Am. Soc. Hortic. Sci. 128, 441–446.

De Kreij, C., Bas¸ar, H. (1997): Leaf tip yellowing in eggplant is caused by boron deficiency. J. Plant Nutr. 20, 47–53.

Flora, L. L., Madore, M. A. (1993): Stachyose and mannitol transport in olive (Olea europaea L.). Planta 189, 484–490.

Hu, H., Brown, P. H. (1997): Absorption of boron by plant roots. Plant Soil 193, 49–58.

King, R. W., Zeevaart, J. A. D. (1974): Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol. 53, 96–103.

Lehto, T., Räisänen, M., Lavola, A., Julkunen-Tiitto, R., Aphalo, P. J. (2004): Boron mobility in deciduous forest trees in relation to their polyols. New Phytol. 163, 333–339.

Liakopoulos, G., Stavrianakou, S., Filippou, M., Fasseas, C., Tsadilas, C., Drossopoulos, I., Karabourniotis, G. (2005): Boron remobilization at low-boron supply in olive (Olea europaea) in relation to leaf and phloem mannitol concentrations. Tree Physiol. 25, 157–165.

Liakopoulos, G., Stavrianakou, S., Nikolopoulos, D., Karvonis, E., Vekkos, K.-A., Psaroudi, V., Karabourniotis, G. (2009): Quantitative relationships between boron and mannitol concentrations in phloem exudates of Olea europaea leaves under contrasting boron supply conditions. Plant Soil 323, 177–186.

Liu, L., Shelp, B. J., Spiers, G. A. (1993): Boron distribution and retranslocation in field-grown broccoli (Brassica oleracea var. italica). Can. J. Plant Sci. 73, 587–600.

Marentes, E., Shelp, B. J., Vanderpool, R. A., Spiers, G. A. (1997): Retranslocation of boron in broccoli and lupin during early reproductive growth. Physiol. Plant. 100, 389–399.

Matoh, T., Akaike, R., Kobayashi, M. (1997): A sensitive and conve- nient assay for boron in plant using chromotropic acid and HPLC. Plant Soil 192, 115–118.

Maxwell, K., Johnson, G. N. (2000): Chlorophyll fluorescence – A practical guide. J. Exp. Bot. 51, 659–668.

Oertli, J. J. (1993): The mobility of boron in plants. Plant Soil 155, 301–304.

Oertli, J. J. (1994): Non-homogeneity of boron distribution in plants and consequence for foliar diagnosis. Commun. Soil Sci. Plant Anal. 25, 1133–1147.

Perica, S., Bellaloui, N., Greve, C., Hu, H., Brown, P. H. (2001): Boron transport and soluble carbohydrate concentrations in olive. J. Am. Soc. Hortic. Sci. 126, 291–296.

Rerkasem, B., Jamjod, S. (1997): Genotypic variation in plant response to low-boron and implications for plant breeding. Plant Soil 193, 169–180.

Roessner-Tunali, U., Hegemann, B., Lytovchenko, A., Carrari, F., Bruedigam, C., Granot, D., Fernie, A. R. (2003): Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development. Plant Physiol. 133, 84–99.

Savic, J., Nikolic, M., Prodanovic, S., Römheld, V. (2007): Boron uptake by the root cortex symplast of tomato and pea plants: Evidence for low-boron-induced active transport. Funct. Plant Biol. 34, 1130–1136.

Schauer, N., Zamir, D., Fernie, A. R. (2005): Metabolic profiling of leaves and fruit of wild species tomato: A survey of the Solanum lycopersicum complex. J. Exp. Bot. 56, 297–307.

Shelp, B. J. (1988): Boron mobility and nutrition in broccoli (Brassica oleracea var. italica). Ann. Bot. 61, 83–91.

Stangoulis, J. C. R., Brown, P. H., Bellaloui, N., Reid, R. J., Graham, R. D. (2001): The efficiency of boron utilization in canola. Aust. J. Plant Physiol. 28, 1109–1114.

Stangoulis, J., Tate, M., Graham, R., Bucknall, M., Palmer, L., Boughton, B., Reid, R. (2010): The mechanism of boron mobility in wheat and canola phloem. Plant Physiol. 153, 876–881.

Stavrianakou, S., Liakopoulos, G., Karvonis, E., Resta, E., Karabour- niotis, G. (2006): Low-boron acclimation induces uptake of boric acid against a concentration gradient in root cells of Olea europaea. Funct. Plant Biol. 33, 189–193.

Takano, J., Noguchi, K., Yasumori, M., Kobayashi, M., Gajdos, Z., Miwa, K., Hayashi, H., Yoneyama, T., Fujiwara, T. (2002): Arabidopsis boron transporter for xylem loading. Nature 420, 337–340.

Takano, J., Miwa, K., Fujiwara, T. (2008): Boron transport mechanisms: collaboration of channels and transporters. Trends Plant Sci. 13, 451–457.

Zimmermann, M. H., Ziegler, H. (1975): List of sugars and sugar alcohols in sieve-tube exudates, in Ziemmermann, M. H., Milburn, J. A.: Encyclopedia of Plant Physiology. New Series, Vol. 1, Transport in Plants. 1. Phloem Transport. Springer-Verlag, Berlin, pp. 480–503.