

**ACCEPTED AUTHOR VERSION OF THE MANUSCRIPT**

**Effect of excess boron on growth, membrane stability, and functional groups of tomato seedlings**

**DOI: 10.37427/botcro-2023-001**

Abeer A. Radi<sup>1</sup>, Hussein Kh. Salam<sup>2</sup>, Afaf M. Hamada<sup>1</sup>, Fatma A. Farghaly<sup>1</sup>

<sup>1</sup> Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt

<sup>2</sup> Biology Department, Faculty of Applied Science, Thamar University, Dhamar, Yemen

**Please cite this article as:** Radi A.A., Salam H.K., Hamada A.M., Farghaly F.A.: Effect of excess boron on growth, membrane stability, and functional groups of tomato seedlings. Acta Bot Croat, DOI: 10.37427/botcro-2023-001.

This is a PDF file of a manuscript that has been accepted for publication. The manuscript will undergo language and technical editing, formatting and author proofing before it is published in its final form.

## Effect of excess boron on growth, membrane stability, and functional groups of tomato seedlings

Abeer A. Radi<sup>1</sup>, Hussein Kh. Salam<sup>2</sup>, Afaf M. Hamada<sup>1\*</sup>, Fatma A. Farghaly<sup>1</sup>

<sup>1</sup> Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt

<sup>2</sup> Biology Department, Faculty of Applied Science, Tamar University, Dhamar, Yemen

\*Corresponding author: e-mail: hamada@aun.edu.eg, afafhamada@yahoo.com

Running title: BORON TOXICITY AND TOMATO SEEDLING GROWTH

**Abstract** - With the scarcity of good quality water, plants like tomatoes will be more susceptible to excess boron (EB) in Mediterranean regions. The effects of EB on the growth, free, semi-bound, and bound boron (B) concentrations, and macromolecules of the *Solanum lycopersicum* L. cultivar Castle Rock, were investigated in this study. Seedlings were exposed to four levels of EB using boric acid. The results manifested that EB inhibited tomato growth, total water content, and photosynthetic pigments. EB harmed the membrane stability, as seen by increased potassium (K) leakage, UV absorbance metabolites, and electrolyte conductivity (EC) in leaf disc solution. EB raised concentrations of free, semi-bound, and bound forms of B in seedlings. Fourier transform infrared spectroscopy (FTIR) data revealed that EB induced uneven wax deposition, altered the shape of cell walls, and lowered cellulose synthesis in seedlings. EB affected the amide I and amide II indicating damage to the protein pools. These results provide new insights into understanding the specific effects of EB on the functional groups of different macromolecules of tomato seedlings.

Keywords: excess boron, FTIR analysis, membrane stability, photosynthetic pigments, plant growth, tomato

### Introduction

Boron (B) serves vital tasks in plant life at ideal levels, whereas excess boron (EB) has negative consequences. The difference in B insufficiency and toxicity levels is minimal (Fang et al. 2016). B-rich soils can be found all over the world and are prevalent in arid and semi-arid areas (Ardic et al. 2009). With reduced precipitation in the Mediterranean area (Cervilla et al. 2012) and irrigation water shortage due to new dams, demand for desalinated water for agriculture is expected to rise, potentially raising the level of B in irrigation water. Moreover, rising sea levels (Mediterranean Sea) can pollute groundwater, resulting in higher B levels in irrigation water (Princi et al. 2016). Since the cultivated area in Egypt is suitable for intensive cultivation, along with anthropological activity, all of this may lead to B contamination (Elbehiry et al. 2017). EB produce different physiological and morphological changes in plants, resulting in decreased plant growth, leaf chlorophyll, membrane stability (El-Shazoly et al. 2019), and ultimately reduced production (Metwally et al. 2018).

Tomatoes are grown in the Mediterranean region, where there is a disturbance with EB in the soil. Tomatoes are among the most important vegetable crops in Egypt throughout the year, with a total production of 6,729,004 tons and a total area of 166,206 hectares (FAO 2017). EB led to alterations in tomatoes, including biomass, membranes, photosynthetic pigments, phenolic compounds, and antioxidant enzymes (Cervilla et al. 2012, Farghaly et al. 2022b), which led to reduced yields (Kaya et al. 2009).

The advantage of Fourier transform infrared spectroscopy (FTIR) is ability to produce spectra on different samples such as powders and liquids with minimal sample preparation, which reduces analysis time (Canteri et al. 2019). FTIR is an appropriate analytical tool for biological macromolecules, assessing the composition of organic components (Wu et al. 2017). Absorption outlines show fixed peaks area that identifies modest modifications of metabolites related to physiological processes after infrared spectra ( $400\text{-}4000\text{ cm}^{-1}$ ) pass through plant samples (Renuka et al. 2016). The peak areas, positions, and bandwidths values are critical to changes in plant macromolecules (Renuka et al. 2016). However, to our knowledge, there are no reports on the use of this technique to assess physiological changes produced by excess boron on tomato seedlings.

In this study, we aimed to focus on how EB treatments alter tomato macromolecules, assessing the composition of organic components using FTIR analysis. Additionally, we measured the growth, photosynthetic pigments, membrane stability, and B forms concentration in tomato seedlings. The findings reveal a fresh understanding of the various structural responses of tomato seedlings when exposed to EB.

## Materials and methods

### Growth conditions

Vegetables Department, Faculty of Agriculture, Assiut University gave seeds of *Solanum lycopersicum* L. (tomato), cultivar Castle Rock. Under a laminar airflow hood, seeds were surface sterilized for 15 minutes with a 5% NaClO solution and rinsed four times with sterile water. We wanted to achieve data without any extraneous influences and repeat the experiment under the same settings, so we did it *in vitro*. Sterilized seeds were grown on sterile, half-strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962). The medium was supplemented with 2.2 g L<sup>-1</sup> MS, 3% sucrose, various concentrations (0, 2, 4, and 6 mM) of H<sub>3</sub>BO<sub>3</sub>, and 0.3% gelatin added after adjusting the pH to 5.7. The medium was sterilized for 15 min at 121 °C, pressed at 105 kPa, and allowed to cool to room temperature. Seedlings were cultured in a growth chamber at 25 ± 1 °C, 65-70% relative humidity, and a photoperiod of 16/8 h with 30 μM m<sup>-2</sup> S<sup>-1</sup> illumination.

After 20 days, some seedlings were separated into shoots and roots, weighed quickly to estimate the fresh weight (FW), and stored at -80 °C. Other seedlings were oven-dried at 60 °C to determine the dry weight (DW). The total water content (TWC) of shoots and roots was determined using the following formula:

$$\text{TWC} = \text{FW} - \text{DW}$$

### Photosynthetic pigments

Using a spectroscopic approach, photosynthetic pigments, including chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*), and carotenoids (carot), were determined (Lichtenthaler 1987). 0.1 g of a fresh leaf was dropped in 5 mL of 95% ethanol at 60 °C until colorless,

and the volume was then finished to 10 mL using 95% ethanol. Using a spectrophotometer (Unico UV-2100), the concentrations of carotenoids and chlorophylls were determined using formulas:

$$\text{Chl } a = (13.36 * A_{663}) - (5.19 * A_{644})$$

$$\text{Chl } b = (27.49 * A_{644}) - (8.12 * A_{663})$$

$$\text{Carot} = \{(1000 * A_{452}) - (2.13 * \text{Chl. } a) - (9.76 * \text{Chl. } b)\} / 209.$$

Results were expressed as  $\text{mg g}^{-1} \text{FW}$ .

### **Cell membrane stability**

Different parameters were assessed, including electrical conductivity (EC%), potassium leakage (K leakage), and UV-absorbing metabolites (metabolite leakage) for determining the cell membrane stability.

The percentage of injury (electrical conductivity; EC) was measured according to the Premachandra et al. (1992) method. Fresh leaf discs (2.1 cm) were soaked in 10 mL of distilled water for 24 h at 10 °C. After measuring the initial electrical conductivity (EC<sub>1</sub>) of all test tubes at 25 °C, the leaf discs were autoclaved for 15 min, cooled to 25 °C, and then the last EC<sub>2</sub> was measured again. The cell membrane stability index was estimated using a percentage of damage:

$$\text{Electrical conductivity (\%)} = (\text{EC}_1 / \text{EC}_2) \times 100$$

Potassium leakage was measured using a flame photometer in the same conductivity solution before and after sterilization (Williams and Twine 1960).

Metabolite leakage was assessed using the Navari-izzo et al. (1989) method in the same solution of conductivity measurements.

### **Boron analysis**

Boron forms were extracted, according to Du et al. (2002) and Li et al. (2017). 5 mL of distilled water was added to 0.2 g of powdered dry seedlings, shaken at 25°C for 24 h, filtered, and the free B was measured. The residue was shaken at 25 °C for 24 h in a plastic tube with 1 M NaCl, filtered, and then semi-bound B was quantified in the filtrate. Finally, the residue was shaken at 25 °C for 24 hours in a plastic tube with HCl (1 M), filtered, and then the bound B was quantified in the filtrate.

According to Mohan and Jones (2018), B concentration was quantified using the curcumin-acetic acid method and detected at 550 nm. The curcumin-acetic acid (1 mL) solution was added to 1 mL of filtrates and 0.25 mL of concentrated H<sub>2</sub>SO<sub>4</sub>, shaken for 30 min, and diluted with 95% ethanol to 5 mL after 30 min read at 550 nm using H<sub>3</sub>BO<sub>3</sub> as a reference.

### **Fourier transform infrared spectroscopy (FTIR) analysis**

To analyze macromolecular alteration, we employed Fourier transform infrared spectroscopy (Nicolet IS 10 FTIR) in Chemistry Department. A translucent, homogeneous tablet was prepared by a tablet-making machine using a little amount of the finely powdered sample (approximately 100 µg) mixed with KBr (1: 100 p/p). The absorbance of spectra was measured (400-4000  $\text{cm}^{-1}$ ) against an ordinary KBr pellet (blank), then the resolution was 4  $\text{cm}^{-1}$ . The functional groups of the sample were determined when comparing the spectroscopic result with a reference.

## Statistical analysis

The studies (25 jars/each treatment) were repeated at least twice, with the findings being an average  $\pm$  standard deviation (SD) of four biological replicates. Charts were generated by Origin 8.6 and Microsoft Excel 2010. Using SPSS Statistical Package 22.0, a one-way analysis of variance test was performed and followed by a Tukey's test for significant differences ( $P \leq 0.05$ ) to compare the means. The correlation between the mean values of different parameters of tomatoes under EB treatments was determined using Pearson's correlation analysis.

## Results

### Growth

EB affected all growth parameters of the tested tomato seedlings, including FW, DW, and TWC of shoots and roots (Figs. 1A-D, On-line Suppl. Fig. 1). Compared to control, tomatoes treated with 2 mM B showed a slight or considerable increase in FW, DW, and TWC of shoots and roots. In contrast, treatments with 4 and 6 mM B lowered FW, DW, and TWC of shoots and roots. After exposure to 6 mM B, the highest DW reductions of 56.90% and 86.52% were recorded in shoots and roots, respectively. Further, treatment with EB showed a significant negative association between shoots and roots, FW, DW, TWC, and an increase in free, semi, and bound B contents. However, the shoot/root ratio was positively and strongly associated with free, semi-bound, and bound B concentrations in the shoots (0.907\*\*, 0.922\*\*, and 0.646\*, respectively, On-line Suppl. Tabs. 1, 2). Although the correlations between bound B and growth parameters were significant, they were the weakest of all the growth criteria associations.

### Photosynthetic pigments

EB had varied effects on the contents of chl *a*, *b*, *a+b*, and carot pigments in leaves (Fig. 2A). Compared to control, EB at a low-level (2 mM) stimulated chl *a* content in leaves by 31.28%, but at a high-level (6 mM), it significantly reduced it by 48.34%. In the shoots, there were strong negative associations between chl *a* and free, semi-bound, and bound B content (-0.768\*\*, -0.822\*\*, and -0.812\*\*, respectively, On-line Suppl. Tab. 1).

Low EB treatments promoted the synthesis of chl *b* in tomato leaves. Compared to control, the rise in chlorophyll *b* content at low EB levels (2 and 4 mM) was considerable (129.45% and 66.23%, respectively), but there was no significant increase at a high EB level (6 mM). Insignificant relationships between chl *b* and free, semi-bound, and bound B levels in shoots confirmed these findings (-0.224, -0.311, and -0.341, respectively, On-line Suppl. Tab. 1).

6 mM B reduced chl *a + b* concentration by 42.69% compared to control. The moderate treatment (4 mM B) showed a lower increase in the chl *a + b* content (0.63%) compared to exposure to 2 mM B, which resulted in a 40.66% rise compared to control. Moreover, results revealed a strong negative association between chl *a + b* and free, semi-bound, and bound B content in shoots (-0.704\*, -0.767\*\*, and -0.675\*\*, respectively, On-line Suppl. Tab. 1).

Regarding carotenoids, EB boosted carot content by 37.18% at a low-level (2 mM) but lowered them by 44.72% at a high level (6 mM) compared to control. Carotenoids and free, semi-bound, and bound B levels had negative associations (-0.655\*, -0.593\*, and -0.686\*, respectively), similar to chlorophylls (On-line Suppl. Tab. 1). Moreover, the data revealed a significant and positive relationship between chl *a*, *a + b*, carot, and

shoot DW (0.669\*, 0.734\*\*, and 0.785\*\*, respectively), except for chl *b*, which was not.

### **Boron concentrations**

The most important for measuring a plant's tolerance to EB is the B concentration in its tissues. Therefore, the B forms in tomatoes grown under various EB treatments were measured (Fig. 2B). Our results indicated that free B content was higher than the content of semi-bound and bound in seedlings. Our results indicated that free B content was higher than the content of semi-bound and bound in seedlings. With increasing EB concentrations, the accumulation of all B forms also increased. Compared with optimal B concentration, EB at the low level (2 mM) increased free, semi-, and bound B by 25.41%, 37.40%, and 88.61%, while the high level (6 mM) increased it by 149.69%, 134.98%, and 367.93%, respectively.

### **Membrane stability**

To quantify the degree of membrane integrity under EB stress, the EC, K leakage, and metabolite leakages in seedlings undergoing various treatments were measured (Figs. 3A-C and On-line Suppl. Tabs. 1 and 2). 6 mM B raised the EC and incidence of K and UV metabolites in leaves by 67.22%, 101.54%, and 91.99%, respectively. Furthermore, EC (0.931\*\*, 890\*\*, and 0.724\*\*, respectively), K (0.943\*\*, 0.915\*\*, and 0.828\*\*, respectively), and metabolite leakages (0.971\*\*, 0.937\*\*, and 0.687\*, respectively) were shown to be strongly connected with free, semi-bound, and bound B levels in shoots.

### **FTIR analysis**

We employed FTIR analysis to assess the effect of EB on seedling ultrastructure (Fig. 4 and Tab. 1). EB did not induce extensive alterations within the four peaks at 3405.17 cm<sup>-1</sup>, 2927.25 cm<sup>-1</sup>, 1384.45 cm<sup>-1</sup>, and 825.42 cm<sup>-1</sup>. Treatment with 4 mM B raised the peak intensity of 3405.17 cm<sup>-1</sup>, but exposure to 2 and 6 mM B lowered it compared to control. Moreover, treatments with 4 and 6 mM B raised the peak intensity at 2927.25 cm<sup>-1</sup> and 1384.45 cm<sup>-1</sup>, respectively, while exposure to 2 mM B lowered them compared to control. However, EB levels raised the peak intensity of 825.45 cm<sup>-1</sup> relative to control.

Regarding the peak at 1653.21 cm<sup>-1</sup> (control), 2 mM B treatment did not significantly alter its transmission, while 4 and 6 mM concentrations lowered it by -6.34 cm<sup>-1</sup> and -12.48 cm<sup>-1</sup>, respectively. Moreover, 4 mM B raised this peak intensity, although other B treatments did not. Meanwhile, the peak at 1540.60 cm<sup>-1</sup> (control) disappeared under B treatments (2, 4, and 6 mM).

The peak recorded at 1058.10 cm<sup>-1</sup> (control) was negatively shifted by -3.20 cm<sup>-1</sup>, -4.27 cm<sup>-1</sup>, and -4.18 cm<sup>-1</sup> upon exposure to 2, 4, and 6 mM B, respectively. Moreover, treatments with 2 and 4 mM B stimulated this peak intensity, but the treatment of 6 mM EB reduced it.

Compared to the peak recorded at 617.04 cm<sup>-1</sup> (control), EB treatments increased from the transmission area by 1.04 cm<sup>-1</sup>, 5.31 cm<sup>-1</sup>, and 3.75 cm<sup>-1</sup> at 2, 4 and 6 mM concentrations, respectively. Furthermore, low levels of EB (2 and 4 mM) increased the peak intensity, while the treatment with 6 mM B decreased it.

The peaks recorded at 540.52 cm<sup>-1</sup>, 536.78 cm<sup>-1</sup>, and 537.63 cm<sup>-1</sup> appeared under treatments with 2, 4, and 6 mM B, respectively. Under the high levels of EB (4 and 6 mM), the 483.52 cm<sup>-1</sup> and 459.94 cm<sup>-1</sup> peaks disappeared, while the exposure to 2 mM B stimulated their values by 0.74 cm<sup>-1</sup> and 4.26 cm<sup>-1</sup>, respectively.

## Discussion

The shoot/root ratio of seedlings was enhanced at the EB lowest concentration, indicating the higher growth (Figs. 1A-D). Moreover, lower growth at high levels of EB was linked to the concentration of B forms within the seedlings, demonstrating that B buildup was limiting growth. As demonstrated by the strong positive relationships between shoot/root ratio and B level within shoots, the negative B effect was more evident in tomato roots than in shoots. EB has a comparable negative effect on tomato growth, according to Kaya et al. (2020). Cell division (Liu and Yang 2000), cell expansion, cell numbers (Choi et al. 2007), water content (Metwally et al. 2018), and cell wall matrix stiffness (Farghaly et al. 2022b) are all linked to decreased seedling growth. Conversely, promoting growth at a low EB level may be associated with active B influx, which lowers intracellular B levels (Reid et al. 2004, Ardic et al. 2009).

The primary organelles damaged by EB are the chloroplasts (Landi et al. 2019). The deficiency of photosynthetic pigments found in this study (Fig. 2A) could be due to a structural damage to thylakoids as a result of abnormal spongy parenchyma growth (Papadakis et al. 2004), oxidation of chlorophyll and chloroplast membranes (Aftab et al. 2012), and a reduction in three types of thylakoid-related proteins (Sang et al. 2015). Our results match the findings of wheat and tomato, which are vulnerable to EB (El-Shazoly et al. 2019, Kaya et al. 2020). Thus, EB has a variety of consequences on photosynthetic processes, including changes in photosynthetic pigment levels, lower CO<sub>2</sub> assimilation, impaired photosystem II performance, and a decreased electron transport rate (Landi et al. 2019).

High content of photosynthetic pigments at a low EB level suggests seedling tolerance. Furthermore, the chloroplasts were less vulnerable to EB since the DW was high at this level of 2 mM EB. Additionally, strong positive relationships between pigment contents and shoot DW revealed that pigment preservation is necessary to stimulate seedling growth. Accordingly, Eraslan et al. (2007) found no significant changes in chl *a* and *b* concentration in carrot plants when exposed to EB.

Boron amount in plants is considered the main physiological feature utilized to examine tolerance to EB in an environment. Our findings revealed that all B forms were significantly increased in EB-stressed seedlings, explaining the symptoms of increased EB (Fig. 2B). Likewise, during exposure to EB, an accumulation of B forms was previously observed in tomato calli (Farghaly et al. 2021, 2022b). Free B demonstrated the ability to cross cell membranes and showed promise as being immediately accessible for potential physiological roles in the cell, according to Dannel et al. (1998). In this study, the content of semi-bound and bound B forms varied from about 6%-76% and 2%-119%, respectively. These data may reveal that a small amount of EB was attached to the cell walls in exchange for increased B availability, but this amount was too low to actively participate in EB detoxification, as indicated by increased free B (Dannel et al. 1998, Farghaly et al. 2022b).

Ionic solutes and cellular metabolites are widely applied to assess membrane integrity (Palta et al. 1977, Navari-Izzo et al. 1993). According to our findings presented in Figs. 3A-C, the membrane damage worsens as EB levels in the medium increased. These findings showed that EB had a significant impact on the permeability of tomato membranes, revealed by the EC and leakage of K and UV metabolites, which were all confirmed in the prior work with wheat (Metwally et al. 2012).

FTIR spectra revealed further information about the influence of EB on seedling macromolecules (Fig. 4 and Table 1). Türker-Kaya and Huck (2017) correlate the first peak, recorded at 3405.17 cm<sup>-1</sup> in control, with O-H and N-H related to alcohol,

carbohydrates, phenols, and proteins. EB did not affect the wavenumber, indicating that no alterations in cell wall components and the reduction in bound-B in seedlings may clarify these findings. Furthermore, EB lowered peak intensity, suggesting that EB may change the pattern in binding between alcohols, carbohydrates, proteins, phenols, and components of walls. Riaz et al. (2021) demonstrated that EB increased lignin and suberin levels in rice plants, perhaps leading to cell wall stiffness. Otherwise, changes in peak intensity can refer to as changes in cell wall shape (Zuverza-Mena et al. 2016).

The peak found around 3000-2800  $\text{cm}^{-1}$  was assigned the C-H stretching area of lipids, wax, and fats (Legner et al. 2018). EB did not affect this peak's value (3000-2800  $\text{cm}^{-1}$ ), but the intensity of the peak increased at 4 and 6 mM EB. These data indicate that no changes were made to wall wax amount, while the shape of wall wax may only change under EB (Morales et al. 2013). Mesquita et al. (2016) found irregular wax deposition on the surface of citrus leaves under EB, which might support our findings.

The peak in the region of 1700-1600  $\text{cm}^{-1}$  is characteristic of the C=O of the amide I (proteins) (Dumas and Miller 2003). Amide II (1480-1580  $\text{cm}^{-1}$ ) is a mixture of N-H and C-N vibrations that aid in ionic reaction response, although it is less well understood (Zhao and Wang 2016). The amide I peak value was reduced by high levels of EB (4 and 6 mM), demonstrating that the protein's structure is changed to chelate EB, and this explanation might be confirmed by the finding of Farghaly et al. (2022a). According to Riaz et al. (2021), EB significantly affected the amide protein, amide II, and amide III, indicating damage to the protein pools. The disappearance of the amide II peak under EB treatments can disclose the binding of B ions to nitrogen amide to chelate the EB, and plants can use this claw to withstand B toxicity. Dunbar et al. (2012) reported the disappearance of amide II (around 1550  $\text{cm}^{-1}$ ; bending of amide N-H) owing to iminol structural coordination between the amide II and magnesium, nickel, and cobalt.

Wei et al. (2015) assigned the peaks between 1500-1000  $\text{cm}^{-1}$  fingerprint regions of the amide III, nucleic acid functional groups, and carbohydrates. Our results revealed that EB treatments induce a change in the intensity of the peak recorded at 1384.45  $\text{cm}^{-1}$ . The increasing peak intensity might reveal that the additional B has changed the fingerprint region's components and linked EB with proteins.

EB reduced the 1058.10  $\text{cm}^{-1}$  peak, which was attributed to cellulose (Wu et al. 2017), indicating a reduction in cellulose production in seedlings under EB. This peak intensity was lowered by EB at its maximum level, indicating a decrease in cellulose synthesis. Similarly, Farghaly et al. (2022b) discovered that EB decreased the cellulose content of tomato calli in their study.

The peak recorded at 825.42  $\text{cm}^{-1}$  in control, which was not affected by EB treatments, was assigned to the trisaccharide (D-(+)-raffinose pentahydrate) with  $\alpha$ -glycosidic bonds (Wiercigroch et al. 2017). The intensity of this peak was increased, indicating B binding to the pentahydrate. EB also boosted the 617.04  $\text{cm}^{-1}$  peak, which was assigned to D-(+)-glucose (Wiercigroch et al. 2017), showing the degradation of cellulose or sucrose into simple monosaccharides. This explanation might confirm a decrease in the cellulose wavelengths. Under EB, the appearance of additional peaks, recorded at 540.52  $\text{cm}^{-1}$ , 536.78  $\text{cm}^{-1}$ , and 537.63  $\text{cm}^{-1}$ , may also demonstrate glucose buildup (Farghaly et al. 2022b). Furthermore, at high EB levels, the disappearance of ribose peaks (484  $\text{cm}^{-1}$  and 460  $\text{cm}^{-1}$ ; Wiercigroch et al. 2017) suggested EB binding to ribose, and this confirmed the ability of EB to stabilize ribose to create a nucleotide of a borate ester (Grew et al. 2011, Scorei 2012).



In conclusion, EB treatments exhibited unfavorable influences on FW, DW, TWC, and photosynthetic pigments of tomato seedlings. EB also caused a reduction in membrane integrity, as seen by higher EC, and K and UV-metabolite leakage. B absorption matched the B content in the nutritional medium, resulting in increased accumulation of various B forms in seedlings. EB inhibited cellulose synthesis in seedlings and altered wax deposition in cell walls. Moreover, EB affected the amide I and amide II indicating damage to the protein pools. Finally, our results reveal that decreased tomato growth under EB might be related to alterations in photosynthetic pigments, membrane stability, and macromolecules.

## Acknowledgments

The authors thank Assiut University for providing the laboratory equipment.

## References

- Aftab, T., Khan, M.M., Naeem, M., Idrees, M., Moinuddin, A.S., Teixeira da Silva, J.A., Ram, M., 2012: Exogenous nitric oxide donor protects *Artemisia annua* from oxidative stress generated by boron and aluminum toxicity. *Ecotoxicology and Environmental Safety* 80, 60-68.
- Ardic, M., Sekmen, A.H., Tokur, S., Ozdemir, F., Turkan, I., 2009: Antioxidant responses of chickpea plants subjected to boron toxicity. *Plant Biology* 11, 328-338.
- Canteri, M.H., Renard, C.M., Le Bourvellec, C., Bureau, S., 2019: ATR-FTIR spectroscopy to determine cell wall composition: Application on a large diversity of fruits and vegetables. *Carbohydrate Polymers* 212, 186-196.
- Cervilla, L.M., Blasco, B., Ríos, J.J., Rosales, M.A., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M.M., Romero, L., Ruiz, J.M., 2012: Parameters symptomatic for boron toxicity in leaves of tomato plants. *Journal of Botany* 2012, 1-17.
- Choi, E.Y., Kolesik, P., Mcneill, A., Collins, H., Zhang, Q., Huynh, B., Graham, R., 2007: The mechanism of boron tolerance for maintenance of root growth in barley (*Hordeum vulgare* L.). *Plant, Cell & Environment* 30, 984-993.
- Dannel, F., Pfeffer, H., Romheld, V., 1998: Compartmentation of B in roots and leaves of sunflower as affected by B supply. *Journal of Plant Physiology* 153, 615-622.
- Du, C.W., Wang, Y.H., Xu, F.S., Yang, Y.H., Wang, H.Y., 2002: Study on the physiological mechanism of boron utilization efficiency in rape cultivars. *Journal of Plant Nutrition* 25, 231-244.
- Dumas, P., Miller, L., 2003: The use of synchrotron infrared microspectroscopy in biological and biomedical investigations. *Vibrational Spectroscopy* 32, 3-21.
- Dunbar, R.C., Steill, J.D., Polfer, N.C., Berden, G., Oomens, J., 2012: Peptide bond tautomerization induced by divalent metal ions: characterization of the iminol configuration. *Angewandte Chemie International Edition in English* 51, 4591-4593.
- Elbehiry, F., Elbasiouny, H., El-Henawy, A., 2017: Boron: Spatial distribution in an area of North Nile Delta, Egypt. *Communications in Soil Science and Plant Analysis* 48, 294-306.
- El-Shazoly, R.M., Metwally, A.A., Hamada, A.M., 2019: Salicylic acid or thiamin increases tolerance to boron toxicity stress in wheat. *Journal of Plant Nutrition* 42, 702-722.

- Eraslan, F., Inal, A., Gunes, A., Alpaslan, M., 2007: Boron toxicity alters nitrate reductase activity, proline accumulation, membrane permeability, and mineral constituents of tomato and pepper plants. *Journal of Plant Nutrition* 30, 981-994.
- Fang, K., Zhang, W., Xing, Y., Zhang, Q., Yang, I., Cao, Q., Qin, L., 2016: Boron toxicity causes multiple effects on *Malus domestica* pollen tube growth. *Frontiers of Plant Science* 7, 208.
- FAO, 2017: Statistical database. Food and Agriculture Organization of the United Nations. <http://www.fao.org/faostat/en/#data/QC>.
- Farghaly, F.A., Hamada, A.M., Radi, A.A., 2022a: Phyto-remedial of excessive copper and evaluation of its impact on the metabolic activity of *Zea mays*. *Cereal Research Communications* <https://doi.org/10.1007/s42976-022-00259-0>.
- Farghaly, F.A., Salam, H.Kh, Hamada, A.M., Radi, A.A., 2021: The role of benzoic acid, gallic acid and salicylic acid in protecting tomato callus cells from excessive boron stress. *Scientia Horticulturae* 278, 109867.
- Farghaly, F.A., Salam, H.Kh, Hamada, A.M., Radi, A.A., 2022b: Alleviating excess boron stress in tomato calli by applying benzoic acid to various biochemical strategies. *Plant Physiology and Biochemistry* 182, 216–226.
- Grew, E.S., Bada, J.L., Hazen, R.M., 2011: Borate minerals and origin of the RNA world. *Origins of Life and Evolution of Biospheres* 41, 307–316.
- Han, S., Tang, N., Jiang, H.X., Yang, L.T., Li, Y., Chen, L.S., 2009: CO<sub>2</sub> assimilation, photosystem II photochemistry, carbohydrate metabolism and antioxidant system of citrus leaves in response to boron stress. *Plant Science* 176, 143–153.
- Kaya, C., Levent, Tuna, A.L., Dikilitas, M., Ashraf, M., Koskeroglu, S., Guneri, M., 2009: Supplementary phosphorus can alleviate boron toxicity in tomato. *Scientia Horticulturae* 121, 284–288.
- Kaya, C., Sarioglu, A., Ashraf, M., Alyemeni, M.N., Parvaiz Ahmad, P., 2020: Gibberellic acid-induced generation of hydrogen sulfide alleviates boron toxicity in tomato (*Solanum lycopersicum* L.) plants. *Plant Physiology and Biochemistry* 153, 53–63.
- Landi, M., Margaritopoulou, T., Papadakis, I.E., Araniti, F., 2019: Boron toxicity in higher plants: an update. *Planta* 250, 1011–1032.
- Legner, N., Meinen, C., Rauber, R., 2018: Root differentiation of agricultural plant cultivars and proveniences using FTIR spectroscopy. *Frontiers of Plant Science* 9, 748.
- Li, M., Zhao, Z., Zhang, Z., Zhang, W., Zhou, J., Xu, F., Liu, X., 2017: Effect of boron deficiency on anatomical structure and chemical composition of petioles and photosynthesis of leaves in cotton (*Gossypium hirsutum* L.). *Scientific Reports* 7, 4420.
- Lichtenthaler, H.K., 1987: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* 148, 183–350.
- Liu, P., Yang, Y.A., 2000: Effects of molybdenum and boron on membrane lipid peroxidation and endogenous protective systems of soybean leaves. *Acta Botanica Sinica* 42, 461–466.
- Mesquita, G.L., Zambrosi, F.C., Tanaka, F.A., Boaretto, R.M., Quaggio, J.A., Ribeiro, R.V., Jr. Mattos, D., 2016: Anatomical and physiological responses of citrus trees to varying boron availability are dependent on rootstock. *Frontiers of Plant Science* 7, 224.
- Metwally, A.M., El-Shazoly, R.M., Hamada, A.M., 2012. Effect of boron on growth criteria of some wheat cultivars. *Journal of Biology and Earth Sciences* 2, B1–9.

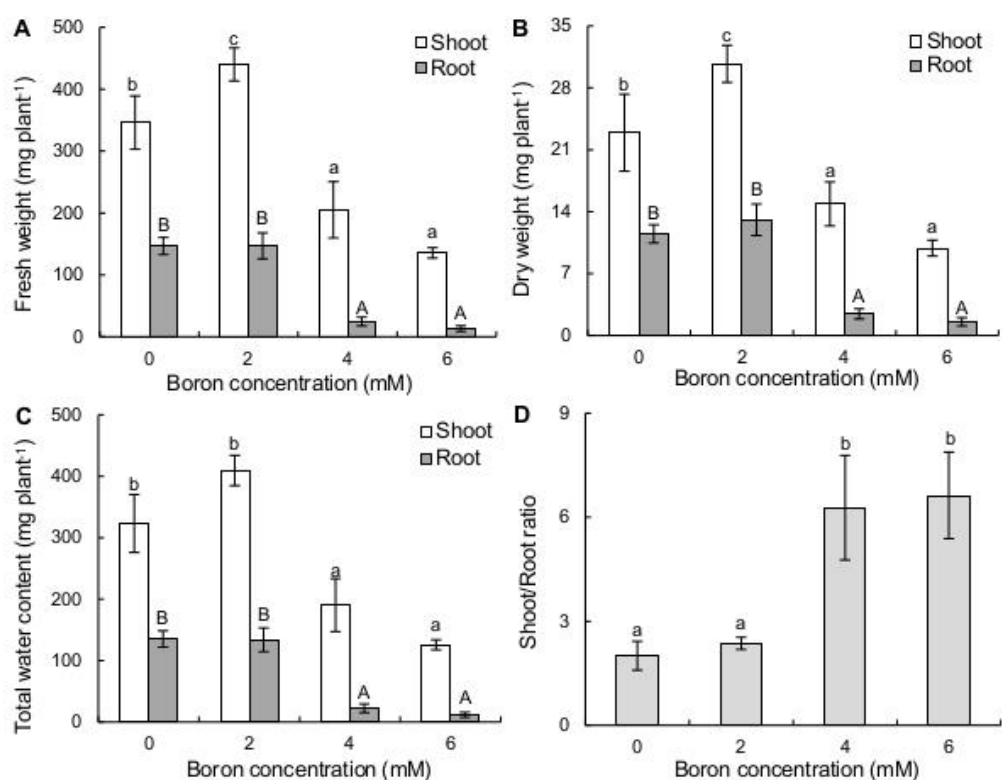
- Metwally, A.M., Radi, A.A., El-Shazoly, R.M., Hamada, A.M., 2018. The role of calcium, silicon and salicylic acid treatment in protection of canola plants against boron toxicity stress. *Journal of Plant Research* 131, 1015-1028.
- Mohan, T.C., Jones, A.M., 2018: Determination of boron content using a simple and rapid miniaturized curcumin assay. *Bio-Protocol* 8, e2703.
- Morales, M.I., Rico, C.M., Hernandez-Viezcas, J.A., Nunez, J.E., Barrios, A.C., Tafoya, A., Flores-Marges, J.P., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013: Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum* L.) plants grown in organic soil. *Journal of Agricultural and Food Chemistry* 61, 6224–6230.
- Murashige, T., Skoog, F., 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15, 473–497.
- Navari-Izzo, F., Quartacci, M.F., Melfi, D., Izzo, R., 1993: Lipid composition of plasma membranes isolated from sunflower seedlings grown under water-stress. *Physiologia Plantarum* 87, 508-514.
- Navari-Izzo, F., Izzo, R., Quartacci, M.F., Lorenzini, G., 1989: Growth and solute leakage in *Hordeum vulgare* exposed to long-term fumigation with low concentration of CO<sub>2</sub>. *Physiologia Plantarum* 76, 445-450.
- Palta, J.P., Levitt, J., Stadelmann, E.J., 1977: Freezing injury in onion bulb cells. I. Evaluation of the conductivity method and analysis of ion and sugar efflux from injured cells. *Plant Physiology* 60, 393-397.
- Papadakis, I., Dimassi, K., Bosabalidis, A., Therios, I., Patakas, A., Giannakoula, A., 2004: Boron toxicity in 'Clementine' mandarin plants grafted on two rootstocks. *Plant Science* 166, 539–547.
- Premachandra, G.S., Saneoka, A.H., Fujita, K., Ogata, S., 1992: Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. *Journal of Experimental Botany* 43, 1569-1576.
- Princi, M.P., Lupini, A., Araniti, F., Longo, C., Mauceri, A., Sunseri, F., Abenavoli, M.R., 2016: Boron toxicity and tolerance in plants: Recent advances and future perspectives. In *Plant Metal Interaction*; Elsevier: Amsterdam, The Netherlands, pp. 115–147.
- Reid, R.J., Hayes, J.E., Post, J.C., Stangoulis, A., Graham, R.D., 2004: A critical analyses of the causes of boron toxicity in plants. *Plant, Cell & Environment* 25, 1405–1414.
- Renuka, B., Sanjeev, B., Ranganathan, D., 2016: Evaluation of phytoconstituents of *Caralluma nilagirina* by FTIR and UV-Vis spectroscopic analysis. *Journal of Pharmacognosy and Phytochemical* 5, 105-108.
- Riaz, M., Kamran, M., El-Esawi, M., Hussain, S., Wang, X., 2021: Boron-toxicity induced changes in cell wall components, boron forms, and antioxidant defense system in rice seedlings. *Ecotoxicology and Environmental Safety* 216, 112192.
- Sang, W., Huang, Z.R., Qi, Y.P., Yang, L.T., Guo, P., Chen, L.S., 2015: An investigation of boron-toxicity in leaves of two citrus species differing in boron-tolerance using comparative proteomics. *Journal of Proteomics* 123, 128–146.
- Scorei, R., 2012: Is boron a prebiotic element? A mini-review of the essentiality of boron for the appearance of life on earth. *Origins of Life and Evolution of Biospheres* 42, 3–17.
- Türker-Kaya, S., Huck, C.W., 2017: A review of mid-infrared and near-infrared imaging: principles, concepts and applications in plant tissue analysis. *Molecules* 22, 168.

- Wei, Z., Jiao, D., Xu, J., 2015: Using Fourier Transform Infrared Spectroscopy to study effects of magnetic field treatment on wheat (*Triticum aestivum* L.) seedlings. *Journal of Spectroscopy* 2015, 1-6.
- Wiercigroch, E., Szafraniec, E., Czamara, K., Pacia, M.Z., Majzner, K., Kochan, K., Kaczor, A., Baranska, M., Malek, K., 2017: Raman and infrared spectroscopy of carbohydrates: A review. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* 185, 317-335.
- Williams, C.H., Twine, J.R., 1960: Flame photometric method for sodium, potassium and calcium. In: Peach K, Tracey MV (eds) *Modern methods of plant analysis*, vol 5. Springer, Berlin, pp 3–5.
- Wu, X.W., Muhammad, R., Yan, L., Du, C.Q., Liu, Y.L., Jiang, C.C., 2017: Boron deficiency in trifoliate orange induces changes in pectin composition and architecture of components in root cell walls. *Frontiers of Plant Science* 8, 1882.
- Zhao, J., Wang, J., 2016: Uncovering the Sensitivity of Amide-II Vibration to Peptide-Ion Interactions. *Journal of Physical Chemistry B* 120, 9590-9608.
- Zuverza-Mena, N., Armendariz, R., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2016: Effects of silver nanoparticles on radish sprouts: root growth reduction and modifications in the nutritional value. *Frontiers of Plant Science* 7, 90.

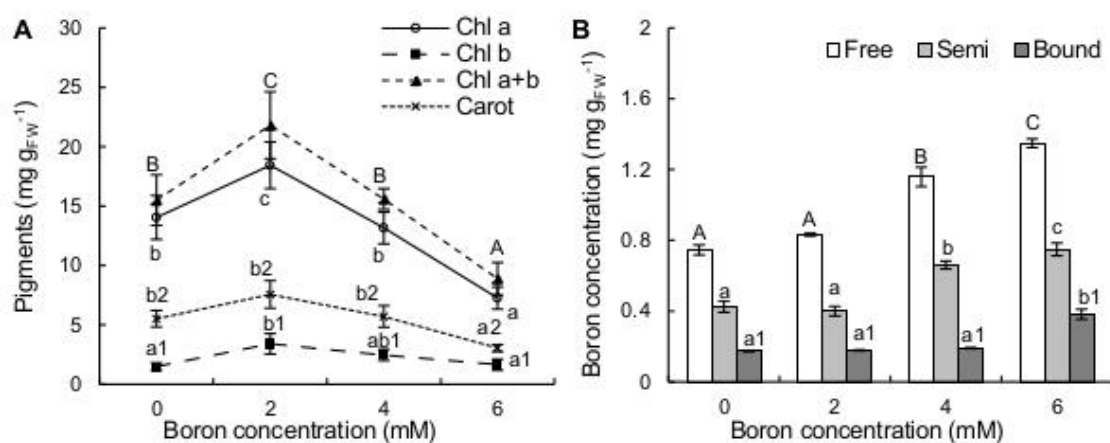
ACCEPTED MANUSCRIPT

**Tab. 1.** FTIR spectra showing observed peaks in tomato seedlings grown under 0, 2, 4, and 6 mM boron for 20 days.

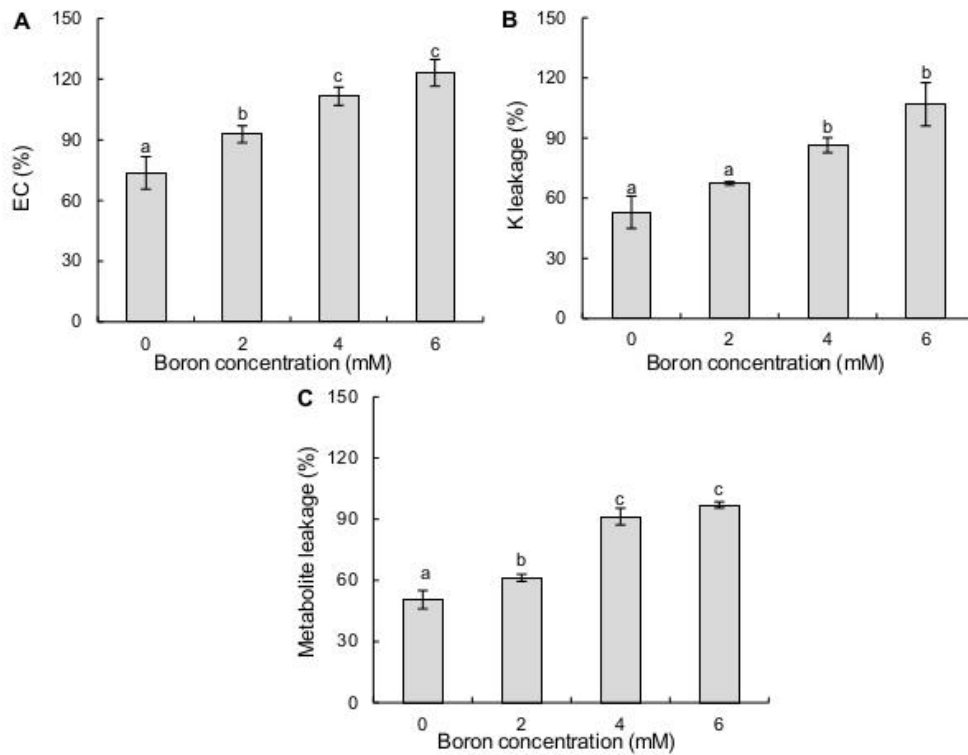
Peak	Frequency (cm <sup>-1</sup> )			
	Excess boron (mM)			
	0	2	4	6
1	3405.17	3404.98	3405.43	3406.01
2	2927.25	2927.25	2926.60	2926.75
3	1653.21	1656.79	1646.87	1640.73
4	1540.60	-	-	-
5	1384.45	1384.28	1384.34	1384.30
6	1058.10	1054.9	1053.83	1053.92
7	825.42	825.55	825.68	825.42
8	617.04	618.08	622.35	620.79
9	-	540.52	536.78	537.63
10	483.52	484.26	-	-
11	459.94	464.20	-	-
12	-	453.29	-	453.74



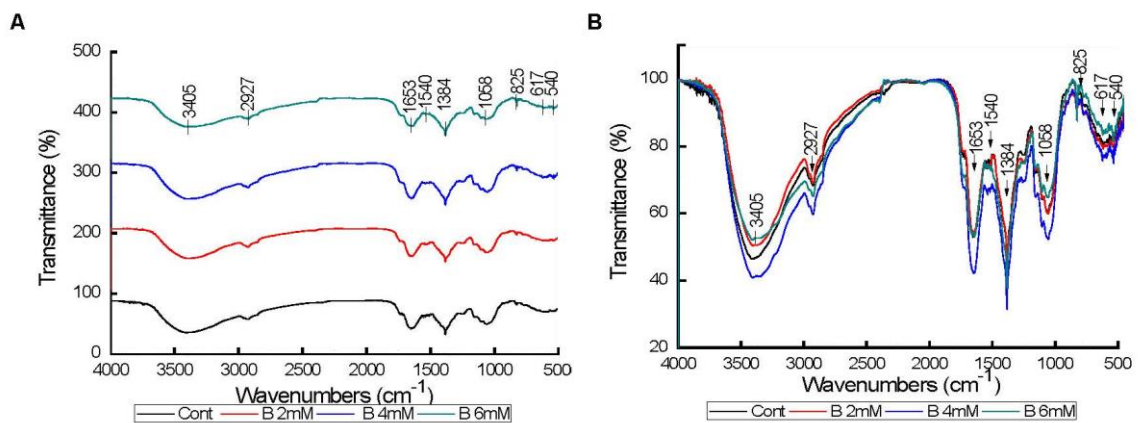
**Fig. 1.** Fresh (A), dry weight (B), total water content (C), and shoot/root ratio (D) in tomato seedlings grown under 0, 2, 4, and 6 mM boron for 20 days. The data are means  $\pm$  SD ( $n = 4$ ). Different letters, capital for roots and small for shoots, indicate statistically significant differences ( $P \leq 0.05$ ).



**Fig. 2.** Photosynthetic pigments (chl *a*; chl *b*; chl *a* + *b*; carot; A) and boron concentration (free, semi-bound; and bound B) in tomato seedlings grown under 0, 2, 4, and 6 mM boron for 20 days. The data are means  $\pm$  SD ( $n = 4$ ). Different letters, capital for chl *a*, free B and small for chl *b*, semi-bound B, small<sup>1</sup> for chl *a* + *b*, bound B, small<sup>2</sup> for carot, indicate statistically significant differences ( $P \leq 0.05$ ).



**Fig. 3.** Electrical conductivity (EC; A), potassium leakage (K leakage; B), and UV absorbing metabolites (metabolite leakage; C) in tomato seedlings grown under 0, 2, 4, and 6 mM boron for 20 days. The data are means  $\pm$  SD ( $n = 4$ ). Different letters indicate statistically significant differences ( $P \leq 0.05$ ).



**Fig. 4.** FTIR spectra (range 4000 - 400  $\text{cm}^{-1}$ ; A, and 0–500  $\text{cm}^{-1}$  region expanded; B) of tomato seedlings grown under 0, 2, 4, and 6 mM boron for 20 days.

## On-line Supplementary material

**On-line Suppl. Tab. 1.** Pearson correlation coefficients obtained for shoots of tomato seedlings under 0, 2, 4, and 6 mM boron for 20 days; bivariate correlation analysis (n = 4). Measured parameters include fresh weight (FW) and dry weight (DW), total water content (TWC), shoot/root ratio (S/R ratio), percent of electrical conductivity (EC%), potassium leakage (K leakage), UV metabolites leakage, chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a+b (Chl a+b), carotenoids (Carot.), free boron (Free B), semi-bound boron (Semi B), and bound boron (Bound B). \*\* Correlation is significant at 0.01 level (two-tailed). \* Correlation is significant at 0.05 (two-tailed) level.

Shoot														
	FW	DW	TWC	S/R ratio	EC%	K leakage	Metabolite leakage	Chl a	Chl b	Chl a+b	Carot.	Free B	Semi B	Bound B
<b>FW</b>	1													
<b>DW</b>	0.932**	1												
<b>TWC</b>	1.000**	0.922**	1											
<b>S/R ratio</b>	-0.837**	-0.798**	-0.835**	1										
<b>EC%</b>	-0.755**	-0.681*	-0.756**	0.827**	1									
<b>K leakage</b>	-0.803**	-0.742**	-0.803**	0.833**	0.977**	1								
<b>Metabolite leakage</b>	-0.865**	-0.800**	-0.866**	0.924**	0.926**	0.915**	1							
<b>Chl a</b>	0.803**	0.862**	0.795**	-0.669*	-0.609*	-0.680*	-0.652*	1						
<b>Chl b</b>	0.458	0.516	0.452	-0.111	-0.005	-0.087	-0.101	0.690*	1					
<b>Chl a+b</b>	0.778**	0.840**	0.770**	-0.599*	-0.527	-0.604*	-0.581*	0.991**	0.780**	1				
<b>Carot.</b>	0.715**	0.783**	0.706*	-0.591*	-0.514	-0.592*	-0.550	0.985**	0.720**	0.984**	1			
<b>Free B</b>	-0.896**	-0.857**	-0.894**	0.907**	0.931**	0.943**	0.971**	-0.768**	-0.224	-0.704*	-0.669*	1		
<b>Semi B</b>	-0.930**	-0.924**	-0.925**	0.922**	0.890**	0.915**	0.937**	-0.822**	-0.311	-0.767**	-0.734**	0.965**	1	
<b>Bound B</b>	-0.719**	-0.713**	-0.715**	0.646*	0.724**	0.828**	0.687*	-0.812**	-0.341	-0.765**	-0.785**	0.799**	0.799**	1



**On-line Suppl. Tab. 2.** Pearson correlation coefficients obtained for roots of tomato seedlings grown under 0, 2, 4, and 6 mM boron for 20 days, bivariate correlation analysis (n = 4). Measured parameters include fresh weight (FW) and dry weight (DW), total water content (TWC), free boron (free B), semi-bound boron (semi B), and bound boron (bound B). \*\* Correlation is significant at 0.01 level (two-tailed). \* Correlation is significant at 0.05 (two-tailed) level.

<b>Root</b>						
	<b>FW</b>	<b>DW</b>	<b>TWC</b>	<b>Free B</b>	<b>Semi B</b>	<b>Bound B</b>
<b>FW</b>	1					
<b>DW</b>	0.988**	1				
<b>TWC</b>	1.000**	0.986**	1			
<b>Free B</b>	-0.953**	-0.930**	-0.954**	1		
<b>Semi B</b>	-0.958**	-0.964**	-0.957**	0.965**	1	
<b>Bound B</b>	-0.665*	-0.661*	-0.655*	0.799**	0.799**	1



**On-line Suppl. Fig.1.** Tomato seedlings grown under 0 (control), 2, 4, and 6 mM boron for 20 days.

ACCEPTED PAPER