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ORIGINAL PAPER

Allocation Pattern, Nutrient Partitioning, Sugar Metabolism, and Pigment Composition in Hydroponically Grown Loquat Seedlings Subjected to Increasing Boron Concentrations

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Abstract

Boron (B) is an essential micronutrient for plant growth and development, but in soil of arid and semiarid environments, B frequently exceeds the plant requirements. B toxicity hampers plant performances and productivity even though there is a lack of information about changes in leaf sugar metabolism and nutrient partitioning provoked by B excess, especially in tree sugar alcohol-producing species where B is highly mobile. In current experiment, hydroponically grown loquat seedlings were subjected to increasing B levels (25, 50, 100, 200, and 400 μM) in the nutrient solution for 69 days. B excess caused visible symptoms in the upper part of loquat shoots (leaves and stem), typical symptoms usually found in species where B is highly phloem mobile. Furthermore, B excess caused significant (i) reduction of plant growth, leaf number, and stem diameter; and (ii) alterations in macro- and micronutrient allocation patterns in different plant organs, e.g., decrease of K, P, Mn, and Mg concentration in roots. Younger fully expanded leaves of B-treated seedlings showed a decline of sucrose paralleled by increments of glucose and fructose concentration in leaves, alteration of leaf pigment composition, and increased peroxidation of lipid bilayers (higher malondialdehyde by-products). Our observations suggest that loquat is very sensitive to B excess and B toxicity can affect dramatically the plant physiology and biochemistry, thus leading to changes in sugar patterns, a reduced growth and, eventually, a reduced productivity of this species.

Keywords Carotenoid · Chlorophyll · *Eriobotrya japonica* L. · Oxidative stress · Proline · Sugars

1 Introduction

Boron is a micronutrient critical to plant growth, with a window between essential and toxic concentrations extremely narrow (Nable et al. 1997). The presence of B in the environment derives primarily from the weathering of B-containing minerals, but also from geothermal steams which significantly contribute to natural enrichment of B in soil and water (Chatzissavvidis et al. 2004; Pennisi et al. 2006). However, the most impactful source of high-concentrated B, with an average of 4.5 mg L^{-1} B dissolved as boric acid, is certainly the seawater which can contaminate fresh water in coastal areas following tidal cycle (Kabay et al. 2010). Differently to other nutrient elements, environmental release of B directly or indirectly attributable to human activities plays a minor role compared to the amplitude of the environmental B enrichment deriving from natural sources, though global change is predicted to increase arid/semiarid environments (Landi and Benelli 2016), therefore exacerbating B accumulation in the soil. In a general way, edaphic concentrations of B in the soil solution ranging from 5 to 100 mg L^{-1} are toxic for many species, though a large degree of tolerance can be observed among species (Maas 1990; Ferreyra et al. 1997) and even genotypes (Papadakis et al. 2003; Papadakis et al. 2004; Cervilla et al. 2007; Landi et al. 2013a, 2013b; Pardossi

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et al. 2015; Meriño-Gergichevich et al. 2017; Sarafi et al. 2018).

In soil, B is found primarily as boric acid $B(OH)_3$, which can either diffuse passively into root hair cells or else is taken up through channels (Tanaka and Fujiwara 2008). Boric acid is a weak acid at cytoplasm pH (about 7.0–7.5) and, under physiological conditions, $B(OH)_3$ can easily bind to molecules with mono-, di-, and polyhydroxyl groups such as ribose, apiose, sorbitol, mannitol, and other polyalcohols (Ralston and Hunt 2001). Under conditions of excessive B supply, B is principally absorbed passively by roots and translocated to leaves via non-living cells of the xylem, driven by the evapotranspiration flux (Brown et al. 2002; Tanaka and Fujiwara 2008). Because xylem provides nutrients to mature leaves, which have the highest transpiration rates, it is expected that the older leaves exhibit more severe symptoms of B toxicity than younger ones (Tanaka and Fujiwara 2008). Until recently, it has generally been accepted that B is an immobile nutrient via the phloem and therefore it tends to accumulate in highly transpiring mature leaves (Brown and Shelp 1997). Differently, in some plant species (e.g., *Pyrus*, *Malus*, *Prunus*, *Allium*, and *Brassica*) where sugar alcohols (i.e., mannitol and sorbitol), in place of sucrose, are predominant for the phloem translocation of photosynthates, B has been found to be uniformly distributed within plants or even at a higher concentration in young tissues than in mature leaves (Brown and Shelp 1997; Camacho-Cristóbal et al. 2008). These results demonstrated the capacity of B to be moved by phloem flux in some plant species due to its ability to bind with *cis*-hydroxyl groups of sorbitol and mannitol giving the origin to diol-B complexes (Reid et al. 2004). Phloem translocation does not follow the evapotranspiration stream and it supplies the major proportion of nutrient requirements for actively growing areas such as young leaves and fruit, organs which do not readily transpire (Brown and Shelp 1997) and in which B symptoms occur in sugar alcohol-translocating species, such as loquat [*Eriobotrya japonica* (Thunb.) Lindl].

Loquat is considered to be very sensitive to high B levels (López-Gómez et al. 2007), given that, according to Keren and Bingham (1985), loquat plants are sensitive to B in irrigation water which ranges from 0.3 to 1.0 mg L^{-1}. Nevertheless, the knowledge on how B excess affects the yield and metabolism of loquat plants is still limited. In particular, there is a lack of information about changes in leaf sugar metabolism and nutrient partitioning provoked by increasing concentrations of B in loquat plants. Previous research highlights that B excess seriously perturbed loquat metabolism by altering several morpho-anatomical, physiological, and biochemical parameters, even before the appearance of typical symptoms of B toxicity over the leaf lamina (Papadakis et al. 2018). In this experiment, increasing levels of B supply (25—as control—50, 100, 200, or 400 μM) were imposed in the nutrient solution during the hydroponic cultivation of loquat seedlings until to the appearance of typical symptoms of B toxicity (yellow-brown necrotic areas over the margin of the leaf lamina). Plant biomass and nutrient allocation patterns, chlorophylls, carotenoids, proline, soluble sugars, and polyols (mannitol and sorbitol) as well as the estimation of lipid peroxidation were assessed to generate knowledge on the physiological/biochemical effects induced by B excess in loquat seedlings.

2 Materials and Methods

2.1 Plant Material and Growing Conditions

Six-month-old seedlings of *E. japonica*, uniform in stem diameter, leaf area, and height (*data not shown*), were grown in a glasshouse located at the arboretum of the Agricultural University of Athens (latitude 37.981907, longitude 23.705639). Initially, the plants were grown in plastic pots containing a mixture of peat/perlite (2:1, *v*/*v*). Afterwards, the plants were singularly transplanted into black plastic bags containing 2 L of a mixture of sand/perlite (1:2, *v*/*v*) for 21 days (acclimation period). During transplanting, the seedling roots were thoroughly washed firstly with tap water and then with distilled water in order to remove residuals of the initial mixture. During the acclimation period, loquat seedlings were irrigated three times per week with tap water. Afterwards, each plant, in each plastic bag, was fertigated with a full-strength Hoagland's nutrient solution, differing in B concentration (25, 50, 100, 200, or 400 μM B). Each fertigation event supplied enough solution (400–600 mL per plant) to fill the pores (field capacity). Furthermore, with this procedure, the B concentrations in the planting media were maintained identical to the original solution (see further details in Papadakis et al. 2003). The control treatment was considered the solution containing 25 μM B, given that in previous experiments, it was a safe and non-limiting concentration (Papadakis et al. 2018). A total of 25 plants was used (five replicates per each B treatment). Climatic parameters were monitored by a weather station located inside the glasshouse. The minimum, maximum, and averaged air temperature during the cultivation period were 18, 28, and 24.8 °C, respectively. Maximum temperature reached up to 30–32 °C in sunny hours. Daily global radiation averaged 11.5 MJ m^{-2}.

Sixty-nine days after the beginning of B treatments, about 10 days after the appearance of the first B toxicity symptoms, the seedlings were harvested and separated into leaves, stems, and roots. After the measurement of the number of leaves per plant as well as the length and mean diameter of the stems, each plant part was weighted (fresh weight), washed initially with tap water, and afterwards twice with distilled water. Finally, all plant parts were oven-dried at 75 °C till constant weight (dry weight). For biochemical analyses, the first and

second fully expanded mature leaves (from the top) were used, where B principally accumulates in polyol-translocating species as loquat.

2.2 Macro- and Micronutrient Determination

Dried plant parts were milled to fine powder. The concentrations of B, P, K, Ca, Mg, Fe, Mn, Zn, and Na were determined in leaves, stems, and roots. B concentration was determined colorimetrically (420 nm; Helios Gamma UV-Vis Spectrophotometer 9423 UVG, Unicam, UK) by the azomethine-H method (Wolf 1971). For measurements of P, K, Ca, Mg, Mn, Zn, Na, and Fe, dried powder (0.5 g) of each sample was also ashed for 5.5 h at 550 °C, dissolved in 3 mL 6 N HCl, and diluted with deionized water up to 50 mL. Concentration of P was determined colorimetrically (470 nm; Helios Gamma UV-Vis Spectrophotometer 9423 UVG, Unicam, UK) by the vanado-molybdo-phosphate yellow color method (Page et al. 1982). Ca, Mg, Mn, Zn, and Fe were quantified using an atomic absorption spectrophotometer (Varian SpectrAA 20, Varian Ltd., Victoria, AU), whereas K and Na using a flame spectrophotometer (PGI 2000, PG Instruments Ltd. Alma Park-Leicestershire, UK) standard method (Lagunen 1992).

2.3 Chlorophyll and Carotenoid Concentration

Fresh leaf samples (75 mg) were grinded in a cold mortar with 10 mL of cold acetone (80%; *v*/*v*), transferred in falcon tubes, kept in the dark for 1 h, and in the meantime vortexed at 15 min intervals. After that, samples were centrifuged at 4400*g* for 5 min at 4 °C. Chlorophyll and carotenoid concentrations were determined spectrophotometrically (Helios Gamma UV-Vis Spectrophotometer 9423 UVG, Unicam, UK) by collecting extract absorbance at 470 nm, 647 nm, and 663 nm and using the equation described by Lichtenthaler and Buschmann (2001):

$$[\text{Chl}a] = 12.25A_{663} - 2.79A_{647}$$
$$[\text{Chl}b] = 21.5A_{647} - 5.1A_{663}$$
$$[\text{Carotenoids}] = \{1000A_{470} - 1.82\,[\text{Chl}a] - 85.02\,[\text{Chl}b]\}/198$$

2.4 Proline Estimation

Fresh leaves (0.1 g) were cut into small pieces and grinded in a cold mortar with 10 mL of 80% (*v*/*v*) ethanol. The homogenate was placed in falcon tubes and then centrifuged at 4400*g* for 5 min at 4 °C. One milliliter of the supernatant was transferred into test tubes containing 2 mL of acid ninhydrin. After vortexing, test tubes were maintained at 95 °C for 25 min in a water bath, then transferred to an ice bath, and finally, they were allowed to cool down at room temperature. After centrifugation (5 min, 4400*g*, 4 °C), the absorption of the supernatant was recorded at 520 nm and proline was quantified as described by Bates et al. (1973).

2.5 Carbohydrate Determination

For soluble carbohydrate determination, 30 mg of freeze-dried leaf tissue was mixed to 2 mL of HPLC-grade water (Carlo Erba Reagents S.A.S, France) and vortexed for 20 s. Then, extraction of the water-soluble carbohydrates was performed in a microwave oven for 2 min at 400 W. After centrifugation (4400*g* for 10 min at 4 °C), the supernatant was removed and the process was repeated twice. The two supernatants were pooled together and filtered by using syringe filters (0.2 μm pore size). HPLC analyses were conducted using a HPLC pump (Waters, model 510) equipped with an HP refractive index, RI (HP 1047A). The mobile phase consisted of HPLC-grade water. A 20-μl aliquot of the extract was injected into an Agilent HI-PLEX $Ca2^{+}$ column. The temperature of the column was 80 °C and the flow was constant at 0.6 mL min^{-1}. The processing of the chromatograms was done by means of a specific program on the computer (Peak Simple Chromatography Data System, SRI Model 302). Sucrose, glucose, fructose, sorbitol, and mannitol quantitative determinations were derived by a specific reference curve made for each sugar (or polyol).

2.6 Determination of Lipid Peroxidation

Leaf tissue (0.5 g FW) was homogenized in 10 mL of 0.1% trichloroacetic acid at 4 °C. A centrifugation at 4400*g* for 15 min at 4 °C was then carried out. Lipid peroxidation was estimated by the quantification of malondialdehyde (MDA) by-products determined by reaction of the extract with 0.5% 2-thiobarbituric acid (TBA) in 20% TCA (*w*/*v*) as described in Heath and Packer (1968).

2.7 Statistical Analysis

Homogeneity of variance was assessed by Bartlett's test and then the data were subjected to one-way analysis of variance (ANOVA) using the "PASW Statistics 18" statistical package (SPSS INC., Chicago, USA) followed by LSD post hoc test ($P \leq 0.05$). Percentage values were arcsine transformed prior analyses. For mean separation, the Duncan multiple range test for $P \leq 0.05$ was applied. Correlations between B concentration in the nutrient solution and studied parameters were examined and the corresponding correlation coefficients (r) were calculated using Pearson's correlation test. The experiments were arranged following a completely randomized experimental design. Experiments were repeated twice with similar results and a representative dataset is reported herein.

3 Results

3.1 Effects of B Treatments on Plant Growth, Symptom Appearance, and B Plant Partitioning

Increasing concentrations of B in the nutrient solution (from 50 to 200 μM) caused a proportionate reduction of loquat organ biomass (Fig. 1a). Conversely, the highest B concentration induced a stronger decline of leaf biomass (− 74%) with respect to that of stem (− 56%) and root (− 46%). Concentration of B and loss of biomass of each plant organ were always significantly positively correlated: r was − 0.733 ($P < 0.01$), − 0.690 ($P < 0.01$), and − 0.459 ($P < 0.05$) for leaves, root, and stem, respectively. Diameter of stem ($r = -0.639$, $P < 0.01$) as well as the number of leaves per plant ($r = -0.594$, $P < 0.01$) were affected negatively by the increment of B concentration in the nutrient solution (Fig. 1b, c, respectively), with a similar extent from 50 to 200 μM (~− 15% on average) whereas the most severe was the effect of 400 μM B (~− 47%). At the end of the experiment, symptoms of B toxicity appeared in top leaves (as wilting and necrosis) and upper portion of plant stem (browning, drying, and necrosis of the apex) even in plants irrigated with 50 μM B (Fig. S1).

The increase of B levels in the nutrient solution resulted in a notable increase of its levels in all studied organs (Table 1). Interestingly, different patterns of fluctuation of B accumulation were observed in roots (non-significant linear correlation coefficient, r), stems ($r = 0.678$, $P < 0.01$), and leaves ($r = 0.853$, $P < 0.01$). In roots, even the moderate concentration of 50 μM of B in the nutrient solution resulted in remarkable increase of B accumulation when compared to the controls (25 μM B) and no significant differences were recorded between the treatments 50–400 μM B. The concentration of B in stems was higher in (but similar between) 50–100 μM and 100–200 μM B, whereas in leaves, B concentration increased in a dose-dependent manner.

3.2 Allocation Pattern of Macro- and Micronutrients in Roots, Stems, and Leaves

The effects of B excess on the concentrations of the most macro- and micronutrients were mainly evident in roots, where the levels of K, P, Mg, and Mn were significantly suppressed by all the treatments 50–400 μM B. Constrained Na accumulation in the root was only found in the range 50–200 μM B (Table 1). The effects of B excess on macro- and micronutrients in stems and in leaves were not uniform as those found in roots. In leaves, B only caused a consistent increment of Mn and Mg (excluded 400 μM B) (Table 1). In loquat stems, in some cases, significant decrease was observed, such as in the levels of Mg (100–400 μM B) and Zn (50–100 μM B), while in some other cases, B toxicity increased the accumulation of minerals such as K and Na (50–400 μM B) (Table 1). The concentrations of Ca and Fe were not affected at all by the B concentration in the nutrient solution nor in leaves or stems and roots (25–400 μM B) (Table 1).

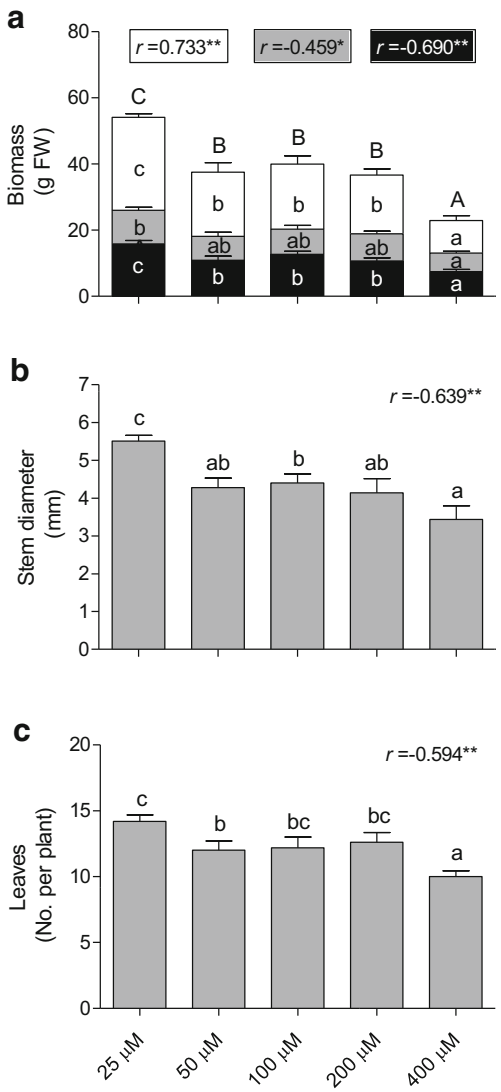

Fig. 1 **a** Fresh weight (g) of root (black), stem (gray), leaves (white), **b** stem diameter (mm), and **c** leaf number of *Eriobotrya japonica* seedlings in relation to increasing B concentrations in the nutrient solution (25–400 μM). Means ($n = 5$; ±SE) keyed with similar lowercase letters are not statistically different among B treatments (Duncan's post hoc; $P \leq 0.05$), while bars keyed with different capital letters indicate that average of total plant biomass is statistically different ($P \leq 0.05$). Linear correlations between **a** each plant portion, **b** stem diameter, and **c** leaf number against B concentration in the nutrient solutions were tested and the correlation coefficients (r) were calculated using Pearson's correlation test (ns = $P \geq 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$)

3.3 Sugar and Sugar Alcohol Patterns, Chlorophyll, Carotenoid, Proline Content, and Lipid Peroxidation in Loquat Leaves Under B Excess

In our experiment, B concentrations higher than 50 μM caused a severe reduction of total sugars *sensu lato* (including

Table 1 Concentrations of various nutrients in loquat organs at increasing B concentration in the nutrient solution

	25 μM B	50 μM B	100 μM B	200 μM B	400 μM B	*r*
B_{leaf} (mg kg^{-1} DW)	69.99 ± 2.61aB	212.66 ± 6.27bA	264.04 ± 17.78cA	372.65 ± 26.57dB	423.72 ± 11.63eB	0.853**
B_{stem}	61.47 ± 4.25aB	347.02 ± 32.51bB	332.95 ± 32.82bB	476.14 ± 40.85cA	484.86 ± 44.55cA	0.678**
B_{root}	40.52 ± 1.96aA	507.11 ± 46.93bC	488.10 ± 66.14bC	442.41 ± 98.77bA	477.92 ± 25.09bA	ns
K_{leaf} (% DW)	2.37 ± 0.07aA	2.49 ± 0.08aB	2.30 ± 0.12aB	2.22 ± 0.19aB	2.06 ± 0.03aB	− 0.501**
K_{stem}	2.16 ± 0.04aA	2.53 ± 0.12bB	2.51 ± 0.21bB	2.86 ± 0.11cC	2.88 ± 0.08cC	0.602**
K_{root}	2.01 ± 0.10bA	1.21 ± 0.09aA	1.23 ± 0.14aA	1.17 ± 0.12aA	1.48 ± 0.04aA	ns
P_{leaf} (% DW)	0.31 ± 0.02aA	0.17 ± 0.04aA	0.34 ± 0.02aA	0.27 ± 0.04aA	0.21 ± 0.01aA	ns
P_{stem}	0.79 ± 0.06aB	0.79 ± 0.02aC	0.71 ± 0.02aC	0.71 ± 0.05aC	0.68 ± 0.05aC	ns
P_{root}	0.82 ± 0.05bB	0.61 ± 0.07aB	0.55 ± 0.06aB	0.54 ± 0.05aB	0.59 ± 0.06aB	ns
Mg_{leaf} (% DW)	0.33 ± 0.02aB	0.44 ± 0.02bB	0.45 ± 0.02bB	0.39 ± 0.04bB	0.33 ± 0.01aB	ns
Mg_{stem}	0.47 ± 0.01bC	0.43 ± 0.03abB	0.39 ± 0.03aB	0.40 ± 0.02aB	0.37 ± 0.01aB	− 0.527**
Mg_{root}	0.25 ± 0.01bA	0.16 ± 0.04aA	0.18 ± 0.02aA	0.19 ± 0.01aA	0.18 ± 0.02aA	ns
Ca_{leaf} (% DW)	2.30 ± 0.10aC	2.43 ± 0.11aB	2.63 ± 0.11aB	2.41 ± 0.19aB	2.32 ± 0.07aB	ns
Ca_{stem}	1.33 ± 0.03aA	1.57 ± 0.09aA	1.55 ± 0.05aA	1.43 ± 0.07aA	1.35 ± 0.10aA	ns
Ca_{root}	1.68 ± 0.14aB	1.37 ± 0.09aA	1.41 ± 0.09aA	1.46 ± 0.08aA	1.28 ± 0.03aA	− 0.422*
Mn_{leaf} (mg kg^{-1}DW)	227.9 ± 10.3aB	304.5 ± 17.2bC	309.6 ± 22.9bC	291.0 ± 24.6bB	282.2 ± 9.4bB	ns
Mn_{stem}	91.58 ± 3.02aA	82.86 ± 1.94aA	85.98 ± 4.12aA	82.46 ± 3.61aA	96.42 ± 6.36aA	ns
Mn_{root}	795.1 ± 84.9bC	248.5 ± 25.2aB	246.7 ± 37.6aB	285.8 ± 46.6aB	358.2 ± 65.9aC	ns
						ns
Zn_{leaf} (mg kg^{-1} DW)	39.56 ± 0.84aB	38.94 ± 1.01aB	39.04 ± 1.26aB	39.02 ± 1.54aB	40.02 ± 0.71aB	ns
Zn_{stem}	25.30 ± 1.22cdA	20.88 ± 0.39aA	21.44 ± 0.91abA	23.72 ± 0.39bcA	27.04 ± 1.20dA	0.516**
Zn_{root}	56.64 ± 2.55cC	41.28 ± 1.94aB	45.12 ± 4.37abB	50.08 ± 3.29abcC	53.78 ± 4.09bcC	ns
Fe_{leaf} (mg kg^{-1} DW)	189.5 ± 12.4aB	170.9 ± 7.2aB	183.9 ± 16.1aB	176.2 ± 8.0aC	191.1 ± 9.4aB	ns
Fe_{stem}	59.94 ± 1.95aA	52.34 ± 1.96aA	58.10 ± 3.93aA	53.56 ± 2.28aA	50.28 ± 2.57aA	− 0.402*
Fe_{root}	424.4 ± 29.4aC	319.3 ± 28.0aC	418.2 ± 54.5aC	418.7 ± 52.0aB	307.9 ± 16.3aC	ns
Na_{leaf} (% DW)	0.13 ± 0.01aB	0.14 ± 0.02aB	0.17 ± 0.02aB	0.16 ± 0.03aB	0.15 ± 0.01aB	ns
Na_{stem}	0.07 ± 0.01aA	0.12 ± 0.01bcB	0.10 ± 0.01bA	0.13 ± 0.01cB	0.12 ± 0.01bcA	0.489*
Na_{root}	0.13 ± 0.01cB	0.09 ± 0.01aA	0.09 ± 0.01aA	0.10 ± 0.01abA	0.12 ± 0.01bcA	ns

In the same row, means (±standard error, SE) followed by different lower case letter(s) as well as means keyed with different capital letter(s) in the same column are not statistically different from each other ($n = 5$, $P < 0.05$). *r* represents the linear correlation coefficient between B concentration in the nutrient solution and the uptake of each element in different plant portions ($n = 25$; ns = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$)

sugar alcohols mannitol and sorbitol), and translocating sugars (sucrose, mannitol, and sorbitol), though in both the cases, the decline was only attributable to the B-induced decrease of foliar sucrose (Table 2). Differently, a build-up of glucose and fructose (and therefore their sum: non-translocating sugars) was observed, again only in the range 100–400 μM B, whereas no changes were found in plants subjected to 50 μM B ($r = 0.635$ for fructose, $P < 0.01$; $r = 0.569$ for glucose, $P < 0.01$; Table 2). Sucrose level was suppressed significantly under the effect of increasing abundance of B especially over 100 μM B ($r = -0.871$, $P < 0.01$). Sorbitol levels remained rather constant in all treatments (25–400 μM B), whereas relatively higher levels of mannitol were found at 100 μM B and a trend to the increment was found also at 50 and 200 μM B.

Incremented concentrations of total chlorophylls (Chl_{TOT}) were found in plant treated with 50–200 μM B (Fig. 2a) and then decreased again at 400 μM B. The increment was principally attributable to higher levels of Chl *b* found in those plants, which induced in turn a reduction of Chl *a*/*b* (Fig. 2b). No changes of carotenoid content were found, irrespectively of treatments (Fig. 2c).

Malondialdehyde by-product and proline levels followed similar patterns in treated plants (Fig. 3a and b, respectively). In particular, a noteworthy increase of MDA by-products was detected in all the plants treated with elevated B levels (50–400 μM B) when compared to controls.

4 Discussion

Toxic effects of B were proportionate among different plant organs and were evident even at 50 μM B. In most cases, the impact of 50 μM B supply on the plant biometric parameters

Table 2 Concentrations of sucrose (Suc), glucose (Glu), fructose (Fru), mannitol, (Man), sorbitol, (Sorb), total sugars (Suc + Glu + Fru + Man + Sorb), translocating sugars (Suc + Man + Sorb), non-translocating sugars (Fru + Glu) and their ratios in loquat leaves as affected by B concentration in the nutrient solution

	25 μM B	50 μM B	100 μM B	200 μM B	400 μM B	*r*
Sucrose (% DW)	3.37 ± 0.12c	2.83 ± 0.10c	1.82 ± 0.37b	1.07 ± 0.10a	1.18 ± 0.20a	− 0.871**
Glucose (% DW)	0.16 ± 0.01a	0.21 ± 0.05ab	0.22 ± 0.08ab	0.39 ± 0.05c	0.33 ± 0.03bc	0.569*
Fructose (% DW)	0.14 ± 0.02a	0.14 ± 0.01a	0.54 ± 0.09b	0.56 ± 0.05b	0.43 ± 0.08b	0.635**
Mannitol (% DW)	0.34 ± 0.10a	0.43 ± 0.12ab	0.64 ± 0.06b	0.52 ± 0.06ab	0.34 ± 0.09a	ns
Sorbitol (% DW)	5.6 ± 0.37a	5.25 ± 0.31a	5.08 ± 0.34a	4.97 ± 0.59a	4.91 ± 0.04a	ns
Total sugars (% DW)	9.62 ± 0.55c	8.86 ± 0.43bc	8.30 ± 0.29abc	7.51 ± 0.58ab	7.19 ± 0.26a	− 0.702**
Translocating sugars (% DW)	9.31 ± 0.53c	8.51 ± 0.42bc	7.54 ± 0.24ab	6.56 ± 0.57a	6.44 ± 0.21a	− 0.781**
Non-translocating sugars (% DW)	0.3 ± 0.03a	0.35 ± 0.05a	0.76 ± 0.17b	0.95 ± 0.06b	0.76 ± 0.10b	0.671**
Translocating/non-translocating	31.46 ± 2.69a	26.4 ± 4.37a	11.35 ± 1.7b	7.01 ± 0.68b	8.9 ± 0.88b	− 0.822**
Translocating/total sugars	0.97 ± 0.003c	0.96 ± 0.01c	0.91 ± 0.02b	0.87 ± 0.01a	0.9 ± 0.010ab	− 0.756**
Non-translocating/total sugars	0.03 ± 0.002a	0.04 ± 0.01a	0.09 ± 0.02b	0.13 ± 0.01bc	0.1 ± 0.010c	0.760**

In the same row, means (±standard error) followed by the same letter(s) were not statistically different from each other ($n = 5$, $P < 0.05$); *r* represents the linear correlation coefficient between B concentration in the nutrient solution and the fluctuation of each one parameter studied ($n = 25$; ns = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$)

was not dissimilar to that induced by 100 and 200 μM B. Differently, the highest level of B (400 μM) constrained more severely the leaf biomass than that of stem and roots of the loquat seedlings. Accordingly, it has been observed in tomato (Kayaa and Ashraf 2015) and citrus (Shah et al. 2017) a similar proportionate reduction of all the plant organs due to excess B.

The appearance of symptoms in younger tissues, even at the first concentration of B excess applied in our experiment (50 μM B), is supportive for the extremely narrow "window" which exists between safe and toxic concentrations of this microelement (Nable et al. 1997). Symptoms of B excess recorded in the present experiment in actively growing loquat organs are typical of plant species characterized by high B mobility in phloem (Brown and Shelp 1997; Chatzissavvidis and Therios 2010) due to the translocation of polyols (sorbitol and mannitol in loquat). From a pomological point of view, the fact that symptoms of B toxicity were present at the apical-growing portions of shoots and that loquat trees produce fruit panicles from apical flower buds suggests that B toxicity can severely affect the productivity of loquat trees.

The severely decremented uptake of K, P, Mn, and Mg induced by B excess in roots of loquat can be attributed to both the toxic effect induced by B at root level, as well as an increased competition for the uptake of these elements caused by excessive presence of borate anions in the nutrient solution. Even though this reduced nutrient uptake (together with the B-triggered oxidative stress) can be on the bases of reduced plant biomass production, it is well evident that loquat seedlings were able to adjust the leaf metabolism in order to maintain the proportions among microelements similar to controls (unchanged levels of most nutrients in leaves). This is in agreement with previous experiment in which the mineral content of leaves was not affected by B excess (Papadakis et al. 2003; Yermiyahu et al. 2008). Other experiments suggest that no pattern exists between B supply and the levels of mineral elements in various plant parts of different plant species, such as broccoli (Shelp 1988), tomato (Gunes et al. 1999), pepper (Eraslan et al. 2007), kiwifruits (Sotiropoulos et al. 1999), olive (Chatzissavvidis and Therios 2010), and apple tree (Sotiropoulos et al. 2006; Paparnakis et al. 2013). Inconsistencies on the effects of B excess on uptake of other nutrients are probably an indication of the different mobility of nutrients in various species, different demands of nutrients, but principally to biochemical/physiological mechanisms adopted by different plant species/cultivars to counteract luxury availability of B in leaves. For this reason, alteration on some physiological parameters, such as pigment profile and composition, sugar metabolism, and reactive oxygen species production, is more consistently observed in plants exposed to toxic B levels (Papadakis et al. 2004; Sotiropoulos et al. 2006; Eraslan et al. 2008; Han et al. 2009; Landi et al. 2013a, 2013c; Landi et al. 2014).

Sugar metabolism is extremely complex and contributes to a plethora of mechanisms that orchestrates the plant defenses against different stressors (Rosa et al. 2009). Boron caused a reduction of total sugar content in loquat leaves, which was principally dependent to severely decremented level of sucrose. This effect was likely dependent to both the decline of photosynthetic rate of top mature leaves, which was compromised by B toxicity and the reduction of phloem integrity, which can cause downregulation of the biosynthesis of sucrose by the mature source leaves (Han et al. 2009; Lemoine et al. 2013). Higher level of glucose and fructose, which are

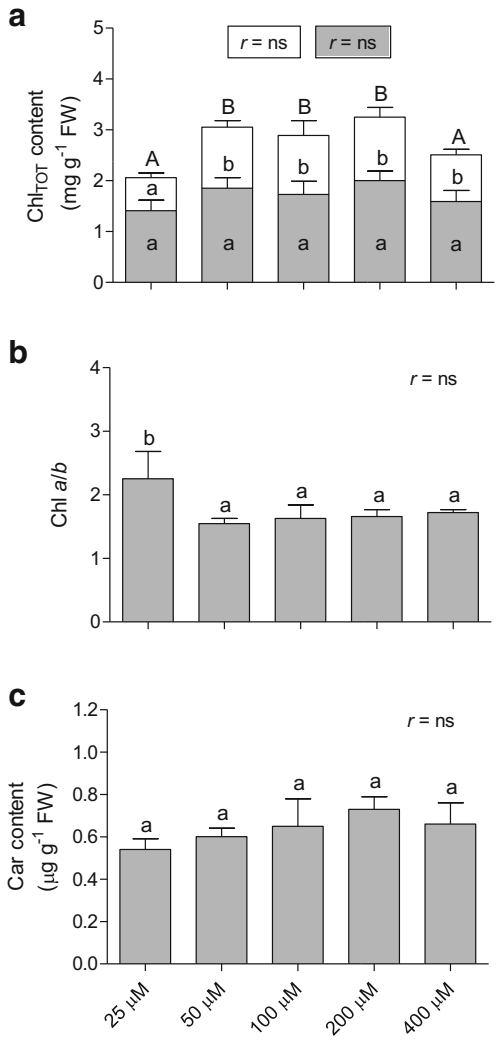

Fig. 2 **a** Content of total chlorophyll (Chl_{TOT}; mg g^{-1} FW) as the sum of Chl *a* (gray bar) and Chl *b* (white bar). **b** Chl *a*/*b* ratio. **c** Total carotenoid content (µg g^{-1} FW) in leaves of *Eriobotrya japonica* seedlings in relation to increasing B concentration in the nutrient solution (25–400 µM). Means ($n = 5$; ±SE) keyed with similar lowercase letters are not statistically different according to Fisher's protected LSD ($P \leq 0.05$). Means keyed with different capital letters indicate that values of Chl_{TOT} are statistically different according to Fisher's protected LSD ($P = 0.05$). Linear correlations between stem diameter (**b**) and leaf number (**c**) and B concentration in the nutrient solutions were tested and correlation coefficients (*r*) were calculated using Pearson's correlation test. (ns = $P \geq 0.05$)

substrates for sucrose biosynthesis, accumulated, in turn, in top mature leaves of plants grown with B excess, according to other works (Cervilla et al. 2007; Han et al. 2009). Fructose is involved in the biosynthesis of several defensive metabolites (Rosa et al. 2009) and this can explain the increment of phenolic compounds (i.e., anthocyanins: Landi et al. 2013a), ascorbic acid, and glutathione (Cervilla et al. 2007; Landi et al. 2013a, 2013c) found in leaves of plant subjected to B stress. In particular, the biosynthesis of powerful antioxidant compounds is useful to counteract B-triggered oxidative stress, which is a response found in our experiments (increment of MDA by-products) as well as by several other authors (reviewed by Landi et al. 2012).

Differently to fructose, compiling evidences show that glucose acts as osmoprotectant under stress conditions (for a review, see Gupta and Kaur 2005), but there is no evidence to support the hypothesis that B toxicity in leaves is due to osmotic stress induced by foliar accumulation of B (Reid et al. 2004). According to other studies that observed increased level of proline under B toxicity (e.g., Eraslan et al. 2008), it is presumable, therefore, that also proline accumulation is not a mean to increase the osmotic potential of the leaf and maintain an active xylem flux, but it seems more realistic that proline biosynthesis represents another additional way to increase the antioxidant pool of the cell (Hayat et al. 2012). Glucose can both represent the substrate for cellular respiration as well as a key signaling molecule in plants under stress (Gupta and Kaur 2005; Sheen 2014). In particular, several genes related to the photosynthetic process are inhibited by glucose-induced phosphorylation (Lemoine et al. 2013; Sheen 2014). Accordingly, Guo et al. (2014) observed that B toxicity in citrus decreased the CO_2 assimilation and the expression of genes involved in photosynthesis and carbohydrate metabolism, such as Rubisco and ADP-glucose pyrophosphorylase, whereas Han et al. (2009) observed a strong increment of fructose and

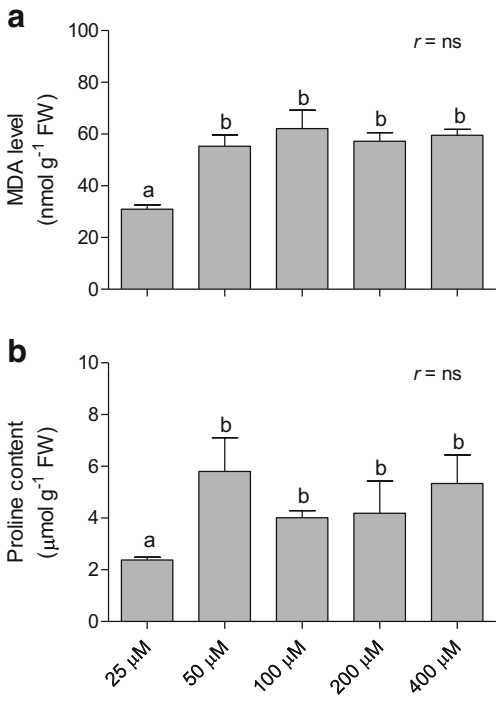

Fig. 3 **a** Malondialdehyde (MDA; nmol g^{-1} FW) by-products. **b** Proline levels (µmol g^{-1} FW) in leaves of *Eriobotrya japonica* seedlings in relation to increasing B concentrations in the nutrient solution (25–400 µM). Means ($n = 5$; ±SE) keyed with similar lowercase letters are not statistically different according to Fisher's protected LSD ($P \leq 0.05$). Linear correlations between each parameter against B concentration in the nutrient solutions were tested and the correlation coefficients (*r*) were calculated using Pearson's correlation test (ns = $P \geq 0.05$)

glucose and a decline of sucrose in citrus leaves. Strong accumulation of hexose can lead to premature leaf senescence (Landi et al. 2015) and this partially explains the lower Chl *a*/*b* ratio found in loquat leaves under B excess. Different papers have reported mixed results about Chl_{TOT} content under B excess (Han et al. 2009; Landi et al. 2013a, 2013c; Shah et al. 2017), but the reduction of Chl *a*/*b* ratio has been consistently found as consequence of B excess. In our experiments, changes of Chl *a*/*b* ratio are mainly dependent to increased biosynthesis of Chl *b* rather than a downregulation of Chl *a* biosynthesis under increasing B concentrations. Higher level of Chl *b*, prevalently connected to photosystem I, could be an adaptation response, transferring "workload" from photosystem II to photosystem I, which is usually less prone to photoinhibition (Tikkanen et al. 2014). Even though the photosynthetic process is usually hampered by B toxicity, in B symptomatic leaves, the maintenance of level of Chl_{TOT} in healthy areas as similar as those recorded in control leaves might be necessary to compensate for the loss of chlorophylls in necrotic areas. Differently, no significant effect on carotenoid content was found in loquat leaves as well as in other species (Keles et al. 2004; Shah et al. 2017), suggesting a minor role of these accessory pigments under B stress.

5 Conclusions

Our dataset offers new evidences about physiological/biochemical adjustments adopted by loquat seedlings subjected to boron excess (summarized in Fig. S2). Loquat plants result highly sensitive to boron excess and in this species, boron-induced symptoms occurred firstly in younger fully expanded leaves, leaf petioles, and top part of the stem, as typically observed in other polyalcohol-translocating species. Roots accumulated high level of boron and offered a first barrier against boron translocation to the shoot, but when the concentration of boron in the medium increased strongly, boron accumulation increased dramatically, even in leaves and stems. B hampered the biosynthesis of sucrose in the leaves which correlates to accumulation of its intermediates (glucose and fructose). This, in turn, can promote early senescence in leaves, as indicated by lower level of chlorophyll *a*/*b* ratio and malondialdehyde by-product accumulation. Conversely, an impairment of translocating sugars can seriously compromise the development of sink organs, such as young leaves and fruits. Overall, these affects pose serious concerns about the possible effect of boron excess to fruit production and further research is necessary to investigate the interplay between nutrient partitioning, sugar metabolism, and progression of leaf senescence in loquat leaves under B excess and to understand whether these processes can significantly compromise the yield of this economically important tree fruit species.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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