

The effects of low boron on growth, gas exchange, boron concentration and distribution of 'Newhall' navel orange (*Citrus sinensis* Osb.) plants grafted on two rootstocks

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ABSTRACT

The effects of low boron (B) on plant growth, photosynthesis, B concentration and distribution of 'Newhall' orange (*Citrus sinensis* Osb.) plants grafted on either Trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) or Carrizo citrange [*C. sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.] rootstocks were investigated. One-year-old plants of the two scion-rootstock combinations were grown for 183 days in sand:perlite (1:1, v/v) medium under greenhouse conditions. The plants were irrigated with half-strength Hoagland's nutrient solutions containing four B concentrations (0.01, 0.05, 0.10 and 0.25 mg l⁻¹). The growth of root, stem of scion and leaves was less affected by low B treatments when 'Newhall' scion was grafted on Carrizo citrange than on Trifoliolate orange. Thus, the growth of scions under low B conditions was mainly depended on the rootstock used, i.e., Carrizo citrange-grafted plants were more tolerant to low B compared to the plants grafted on Trifoliolate orange. Boron concentrations in all plant parts decreased significantly by decreasing the B supply in the nutrient solution. Leaves were the dominant sites of B accumulation and showed the greatest reduction in B concentration compared to the other plant parts, as B concentration in the nutrient solution decreased. Irrespective of the rootstock, B levels in the upper-younger leaves were substantially higher than in basal-older leaves when plants were exposed to low B concentrations (≤ 0.05 mg l⁻¹), suggesting that under such conditions B was preferentially translocated to upper-younger leaves to support their growth. Furthermore, B distribution in different plant parts indicated that there was a restriction in translocation of B from root to scion tissues (stems and leaves of scion) under conditions of limited B availability. In addition, low B treatments decreased leaf photosynthetic rate, stomatal conductance and transpiration rate but increased intercellular CO₂ concentration in the leaves of 'Newhall' plants, irrespective of the rootstock used.

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1. Introduction

Boron (B) is an essential micronutrient for higher plants, and B deficiency is a world-wide nutritional disorder. Although citrus plants are not classified as the most sensitive species to boron deficiency, the occurrence of B deficiency has been reported in the major citrus producing countries of the world, such as Spain, United States, Brazil and China (Shorrocks, 1997). An improved understanding of the responses of citrus plants to low B availability may help the development of more tolerant cultivars and improved management. In eastern and southern China where soil

B levels are low (hot water extraction $B \leq 0.25$ mg kg⁻¹), visible symptoms of B deficiency in leaves of 'Newhall' (*Citrus sinensis* Osb.) orange plants are very common during fruit growth period. The symptoms are deformation in immature leaves and corky split veins in mature leaves, as well as early leaf senescence. Also, the tree vigour declines rapidly after fruit set, a fact that affects fruit yield and quality in coming years (Xiao et al., 2007). Many studies have shown that soil- or foliar-applied B increases fruit yield and improves fruit quality (Wojcik et al., 1999; Wojcik and Lewandowski, 2003). However, apart from the fact that the application of B fertilizers increases the cultivation cost (chemicals and work), it often results in B toxicity since the range between the critical soil B deficiency and toxicity levels is too narrow (Gupta et al., 1985). Another alternative and environmentally friendly way to cope with B deficiency in citrus orchards is the use of rootstocks which can absorb more B from the soil and/or use it efficiently. Indeed, it is

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well known that the success of citrus production depends on the availability of suitable rootstocks which may influence several aspects of citrus growth and development, including yield, fruit quality, and tolerance to stress caused by biotic and abiotic factors (Georgiou and Gregoriou, 1999; Storey and Walker, 1999; Forner-Giner et al., 2003; Smith et al., 2004; Mourão Filho et al., 2007). In fact, rootstock can also greatly affect the scion's tolerance to B toxicity (El-motaium et al., 1994; Edelstein et al., 2005) and deficiency (Wojcik et al., 2003). Although Papadakis et al. (2003, 2004a, b) have investigated the effects of excess B in citrus plants, there is little information about the responses of different citrus scions and rootstocks combinations to low B availability. Trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) has been widely used as a cold-hardy and drafting rootstock for citrus production in China. On the other hand, Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.] is currently one of the most important citrus rootstocks all over the world, mainly due to its vigorous growth and tolerance to tristeza virus (Forner-Giner et al., 2003). Moreover, little is known about the adaptability of these two rootstocks to low B conditions.

The objectives of this work were: (a) to evaluate the effects of low B on plant growth, gas exchange and B concentration and distribution in 'Newhall' navel orange plants grafted on Trifoliolate orange and Carrizo citrange rootstocks, and (b) to compare the differential responses of the two scion-rootstock combinations under low B supply.

2. Materials and methods

2.1. Plant materials and B treatments

'Newhall' (*Citrus sinensis* Osb.) navel orange grafted on two rootstocks, i.e. Trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) and Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.], were used. At budding time, the seedlings of both rootstocks were one-year-old and selected depending on uniform stem diameter, 4–5 mm for Trifoliolate orange and 5–7 mm for Carrizo citrange, respectively. Plant material (seedlings and bud sticks) used in the present experiment was virus-free and harvested from the National Indoor Conservation Center of Virus-free Germplasms of Fruit Crops at Huazhong Agricultural University, Wuhan, China.

According to Papadakis et al. (2004a, b), the roots of the plants were severely pruned (to stimulate new root development following B treatments). Subsequently, all the plants were washed with tap water to remove surface contaminants, followed by transplantation to black pots (one plant per pot) containing 10 l B-free medium composed of quartz sand:perlite (1:1, v/v). The medium had been washed with 3% hydrochloric acid (HCl) overnight and then rinsed thoroughly with deionised water before the transplantation, in order to ensure the B concentration in the medium was less than 0.01 mg l⁻¹ determined by an inductively coupled plasma atomic emission spectrometry (ICP-AES, IRIS-Advan type, Thermo, USA). Thereafter, the plants were put in a greenhouse, and supplied initially with a modified B-free, 1/4 strength Hoagland's No.2 nutrient solution (Hoagland and Arnon, 1950) for 4 weeks, until the sprouting shoots of the scion were about 8–10 cm in length (with 7–9 leaves).

The treatments started at the beginning of May in 2006. The plants were irrigated with a modified Hoagland's No. 2 nutrient solution (prepared with double deionized water), in which the macronutrients were supplied at half strength and micronutrients at full strength, except for B which was supplied at four concentrations (0.01, 0.05, 0.10 and 0.25 mg l⁻¹). Treatment with 0.25 mg l⁻¹ B was considered as the adequate B treatment for normal plant growth (Papadakis et al., 2004a, b; Hoagland and

Arnon, 1950), while the other three B concentrations were considered as treatments of low B. The plants were irrigated with the nutrient solutions every 2 days (approximately 500 ml per plant), allowing some drainage from the growth medium to occur. To avoid salt and B accumulation, the plants were irrigated with 10 l double deionized water once a week, followed by application of 3 l nutrient solution, and the excess solution was flow freely drained from the bottom of the growth medium (Bellaloui and Brown, 1998).

The experiment was terminated after 183 days when visible symptoms of B deficiency appeared. During the B treatments, the plant growth conditions were controlled as follows: 550 to 1200 μmol m⁻² s⁻¹ flux density of natural light, 33 °C/25 °C (day/night) of temperature, and 80% of relative humidity (measured by Li-6400 portable measuring device, Li-COR, Lincoln, USA).

2.2. Leaf gas exchange measurements

At the termination of the experiment, the gas exchange parameters, i.e., the photosynthetic rate (P_n) (μmol CO₂ m⁻² s⁻¹), stomatal conductance (g_s) (mmol m⁻² s⁻¹), intercellular CO₂ concentration (C_i) (μmol mol⁻¹) and transpiration rate (E) (mmol H₂O m⁻² s⁻¹) of all plants were measured using the Li-6400 device. Measurements were performed on basal leaves, located between the middle and the base of the scion's shoots, of each plant (nine replicates per B treatment and scion-rootstock combination). Measurements were carried out between 09:00 and 12:00 a.m. at steady light intensity (1000 μmol m⁻² s⁻¹) under air CO₂ concentration was 385 ± 10 μl l⁻¹ and leaf temperature between 28 and 30 °C.

2.3. Sampling, plant analyses, and B determination

When the plants were harvested, the scion's parts including leaves and stem of scion, and rootstock's parts including root and stem of rootstock, were separately sampled. The leaves were further separated into basal leaves (spring-flush), middle leaves (summer-flush) and upper leaves (autumn-flush) based on the different phases of shoot growth. All the samples were washed initially with tap water and afterwards with double deionized water for three times. The fresh and dry weights of plants were measured. Each dry sample was ground to fine powder and stored in air-tight glass container for subsequent analyses.

For measurement of B concentration, 0.50 g of each sample was dry-ashed in a muffle furnace at 500 °C for 6 h, followed by dissolution in 0.1 N HCl, and B was determined by ICP-AES.

B distribution was expressed as percentage ratios (fraction, %) of B content (concentration × dry weight) in different plant parts to total plant B content (Subedi et al., 1999; Möttönen et al., 2001). The patterns of B distribution in leaves, roots, scion's parts and rootstock's parts are presented to show the changes of fractions of the total amount of B uptaken by plants in these plant parts of the plants upon exposure to low B in relation to rootstock used.

2.4. Experimental design and statistical analyses

The experiment was a completely randomized 2 × 4 factorial design with two rootstocks, Trifoliolate orange and Carrizo citrange, and four B treatments (0.01, 0.05, 0.10 and 0.25 mg l⁻¹). Three replications (3 plants in each) were designed for each treatment. In total, 72 pots (plants) were used in this experiment.

The data were subjected to analysis of variance (ANOVA) using the SAS (SAS Institute Inc., 1996) and the differences were compared by employing the Duncan's test with a significance level of $P < 0.05$.

Table 1

Effects of B concentration (mg l^{-1}) in the nutrient solution on dry weight (g plant^{-1} DW) of different parts of 'Newhall' navel orange plants grafted on Trifoliolate orange and Carrizo citrange. Values are means of three replicates ($n = 3$), with three plants per each replicate. For each rootstock, means within each column followed by the same letter (a–d) do not differ significantly at $P < 0.05$. Values in parenthesis denote percentages (%) of decreases (–) or increases (+) of each measured parameters, for each rootstock and B treatment, in relation to 0.25 mg l^{-1} B treatment of the same rootstock. *F* values for the rootstock (RS), B treatment (B) and the interaction (RS \times B), are also shown.

RS	B (mg l^{-1})	Basal leaves	Middle leaves	Upper leaves	Stem of scion	Stem of rootstock	Root	Scion's parts	RS's part	Total plant
Trifoliolate orange	0.01	1.66 d (–58)	2.79 c (–40)	3.70 c (–39)	4.65 d (–45)	2.53 b (–32)	8.29 d ((52)	12.8 d ((45)	10.8 c ((49)	23.6 d ((47)
	0.05	2.34 c ((41)	3.16 c ((32)	4.04 c ((33)	5.46 c ((35)	2.37 b ((36)	9.70 c ((44)	15.0 c ((35)	12.1 c ((42)	27.1 c ((39)
	0.10	3.10 b ((22)	3.97 b ((14)	5.23 b ((14)	6.04 b ((28)	2.41 b ((35)	12.1 b ((31)	18.3 b ((21)	14.6 b ((31)	32.9 b ((26)
	0.25	3.97 a	4.63 a	6.05 a	8.43 a	3.72 a	17.4 a	23.1 a	21.1 a	44.2 a
Carrizo citrange	0.01	4.14 a ((1.0)	4.27 c ((39)	4.59 c ((51)	9.14 c ((15.4)	8.55 a (+21)	16.5 a ((8.3)	22.1 c ((30)	25.0 a ((0.4)	47.2 c ((16)
	0.05	4.16 a ((0.5)	4.36 c ((38)	8.24 b ((12)	8.47 bc ((22)	7.67 b (+8.5)	17.0 a ((5.6)	25.2 bc ((20)	24.6 a ((2.0)	49.9 bc ((12)
	0.10	4.15 a ((0.7)	6.03 b ((14)	8.04 b ((15)	9.81 b ((9.2)	7.24 b (+2.4)	17.4 a ((3.3)	28.0 ab ((11)	24.6 a ((2.0)	52.8 b ((6.4)
	0.25	4.18 a	6.98 a	9.40 a	10.8 a	7.07 c	18.0 a	31.4 a	25.1 a	56.4 a
<i>F</i> values	RS	284.4***	214.2***	433.1***	636.7***	1382***	343.4***	1101***	768.0***	1643***
	B	37.23***	78.51***	122.9***	88.09***	6.30***	64.87***	219.9***	40.45***	179.2***
	RS \times B	35.21***	4.72*	26.98***	11.60***	18.50***	34.50***	2.12 ^{ns}	37.27***	28.55***

Significance of ANOVA: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: non significant.

3. Results

3.1. Plant growth and symptoms of B deficiency

At the end of the experiment, the symptoms of water-soaked spots, wavy leaf margins and unequal leaf expansion were observed in upper leaves (young leaves) of the plants irrigated with low B ($\leq 0.10 \text{ mg l}^{-1}$) solution. These symptoms became more serious at external B level of 0.01 mg l^{-1} , regardless of rootstock used. While downward curvature of leaf margins occurred in basal leaves (old leaves) at 0.10 mg l^{-1} B, no visual symptoms of B deficiency were found in these plant parts at the other B treatments.

As shown in Table 1, the plants of 'Newhall' scion grafted on Trifoliolate orange exhibited lower dry weights in various plant parts than that grafted on Carrizo citrange, independently of the B supply. The dry weights in various parts of the Trifoliolate orange-grafted plants decreased significantly as external B concentration decreased. When these plants were supplied with 0.01 , 0.05 and 0.10 mg l^{-1} B, the dry weights were reduced by 30, 44 and 52% in roots, and 22, 41 and 58% in basal leaves, respectively, compared with the supply of 0.25 mg l^{-1} B. However, Carrizo citrange-grafted plants showed less reduction in dry weight of various plant parts by irrigation with the nutrient solutions containing B levels from 0.10 to 0.01 mg l^{-1} . Only at 0.01 mg l^{-1} B treatment, the dry weight of middle and upper leaves was reduced by 39 and 51%, respectively. As far as the stem of rootstock of Carrizo citrange-grafted plants is concerned, its dry weight increased from 7.07 to $8.55 \text{ g plant}^{-1}$ by decreasing the B concentration in the nutrient solution from 0.25 to 0.01 mg l^{-1} . In addition, low B treatments significantly decreased dry weights in the scion's parts (leaves and stems of scion) of the plants grafted on both the two rootstocks used, and in the rootstock's parts (roots and stems of rootstock) of the Trifoliolate orange-grafted plants. Although low B treatments did not affect in the dry weight of rootstock's parts of Carrizo citrange-grafted plants, they affected the dry weight of scion's parts (Table 1). At low B growth conditions, 'Newhall' plants had generally higher dry weights and showed less reductions when grafted on Carrizo citrange than on Trifoliolate orange, compared to the condition of relative adequate B supply (0.25 mg l^{-1}).

3.2. Boron concentration and distribution

Boron concentration in all plant parts decreased significantly by decreasing the B supply in the nutrient solution; higher values were recorded in the plants grafted on Trifoliolate orange than in those

grafted on Carrizo citrange (Fig. 1). Leaves contained more B than the other plant parts but they also showed greater reductions compared to the other plant parts as B concentration in the nutrient solution decreased (Fig. 1A). When more than 0.10 mg l^{-1} B was supplied, basal leaves had higher B concentrations than the middle and upper leaves. Instead of this, when less than 0.10 mg l^{-1} B was supplied the variances underlying the B concentrations in basal, middle and upper leaves tended to decrease, regardless of the rootstock used (Fig. 1A). It was also noted that at B level of 0.05 mg l^{-1} , B concentration in upper leaves was significantly higher compared to basal and middle leaves. Root B concentrations decreased promptly as the external B concentration decreased and were reduced by 60% at 0.01 mg l^{-1} B, compared to the 0.25 mg l^{-1} B treatment. For the stems of scion and rootstock, however, B concentrations were comparatively constant across the various B treatments, since decreases of less than 30% at 0.01 mg l^{-1} B were found, compared to the 0.25 mg l^{-1} B treatment (Fig. 1B and C).

The effects of B treatments and rootstocks on B distribution (%) in different parts of 'Newhall' plants are presented in Fig. 2. Leaves represented the dominant sites of B accumulation, regardless of rootstock and B treatment. The fractions of total plant B content in leaves and scion's parts decreased while in roots and rootstock's parts increased, as the B concentration in the solution declined from 0.25 to 0.01 mg l^{-1} . At less than 0.10 mg l^{-1} B treatments, the fractions of B distributed to leaves and scion's parts were less in Carrizo citrange-grafted plants (23–53% and 40–65%, respectively) than in Trifoliolate orange-grafted plants (32–62% and 52–73%, respectively). However, plants grafted on Carrizo citrange rootstock contained more B in roots (38–24%) and in rootstock's parts (60–35%) than those grafted on Trifoliolate orange (34–22% in roots and 48–27% in rootstock's parts, respectively) (Fig. 2).

3.3. Leaf gas exchange parameters

Leaf gas exchange parameters were greatly influenced by B treatments (Fig. 3), a fact indicating that the plants irrigated with low B solutions suffered B deficiency. Low B treatments induced significant reductions in leaf photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E). The reductions of P_n , g_s and E in low B treatments ($\leq 0.10 \text{ mg l}^{-1}$) were 35–65, 20–45 and 15–60%, respectively, as compared with those of plants grown at 0.25 mg l^{-1} B (Fig. 3A, B and D). On the other hand, intercellular CO_2 concentration (C_i) increased almost linearly with decreasing B supply, ranging from 250 to $380 \mu\text{mol mol}^{-1}$ (Fig. 3C). In general, the leaf gas exchange features of 'Newhall' plants grafted on the two rootstocks did not differ significantly upon exposure to the

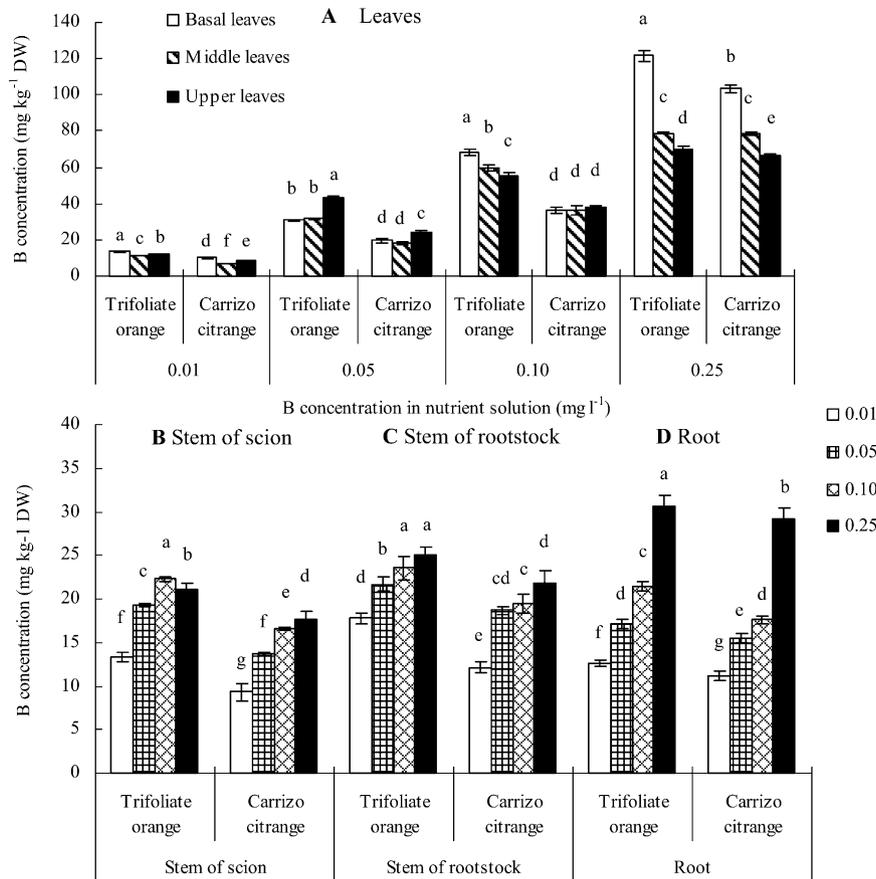


Fig. 1. Effects of B concentration (mg l^{-1}) in the nutrient solution on B concentration (mg kg^{-1} dry weight) in (A) leaves (basal, middle and upper), (B) stem of scion, (C) stem of rootstock and (D) root of 'Newhall' navel orange plants grafted on Trifoliolate orange and Carrizo citrange. Values represent the mean \pm standard errors of three replicates ($n = 3$), with three plants per each replicate. In leaves (A), bars with the same letter are not significantly different at $P < 0.05$ within each part, within the same B treatment. For each of the other plant parts (B–D), bars with the same letter are not significantly different at $P < 0.05$.

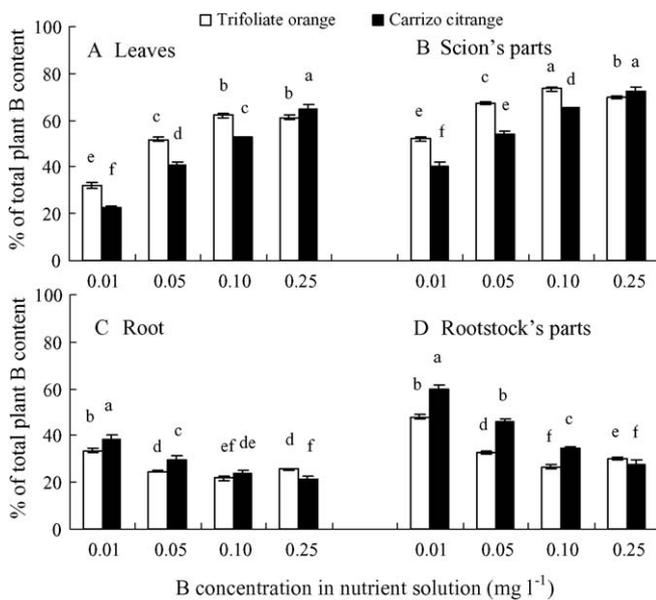


Fig. 2. Effects of B concentration (mg l^{-1}) in the nutrient solution on fractions (%) of total plant B content in (A) leaves, (B) scion's parts (leaves and stems of scion), (C) root and (D) rootstock's parts (root and stem of rootstock) of 'Newhall' navel orange plants grafted on Trifoliolate orange and Carrizo citrange. Values represent the mean \pm standard errors of three replicates ($n = 3$), with three plants per each replicate. Bars with the same letter are not significantly different at $P < 0.05$ within each calculated plant part.

same B treatment, although the average values were somewhat higher in the Carrizo citrange-grafted plants. Furthermore, it was also found that the low B-mediated reduction of P_n was associated (linear relationship) with the decreases in g_s and E, and with the increases in C_i (data not shown).

4. Discussion

4.1. Effects of low B on plant growth and leaf gas exchange parameters

In our experiment, the symptoms of water-soaked spots, wavy leaf margins and unequal leaf expansion were found in the upper-younger leaves of the plants treated with low B nutrient solutions, but no visual symptoms were detected in their mature leaves, implying that the symptoms of B deficiency took place primarily in the growing regions. This finding is in agreement with the observations of other workers (Marschner, 1995; Dell and Huang, 1997). Similar result has also been obtained in plum (*Prunus salicina*) seedlings grown in a B-free growth medium for 49 days, where although B deficiency symptoms were severe in the growing leaves, no symptom occurred in the mature leaves (Brown and Hu, 1997). The visible symptoms of B deficiency and the growth inhibition observed in our study could be explained by the predominant role of B in the formation of primary cell walls (Hu and Brown, 1994; Matoh, 1997). Indeed, B cross-links two rhamnogalacturonan II (RG-II) monomers by a borate bridge and thus provided stability to the cell wall matrix (O'Neill et al., 2004). The deficit of B reduces the rate of formation and stability of this

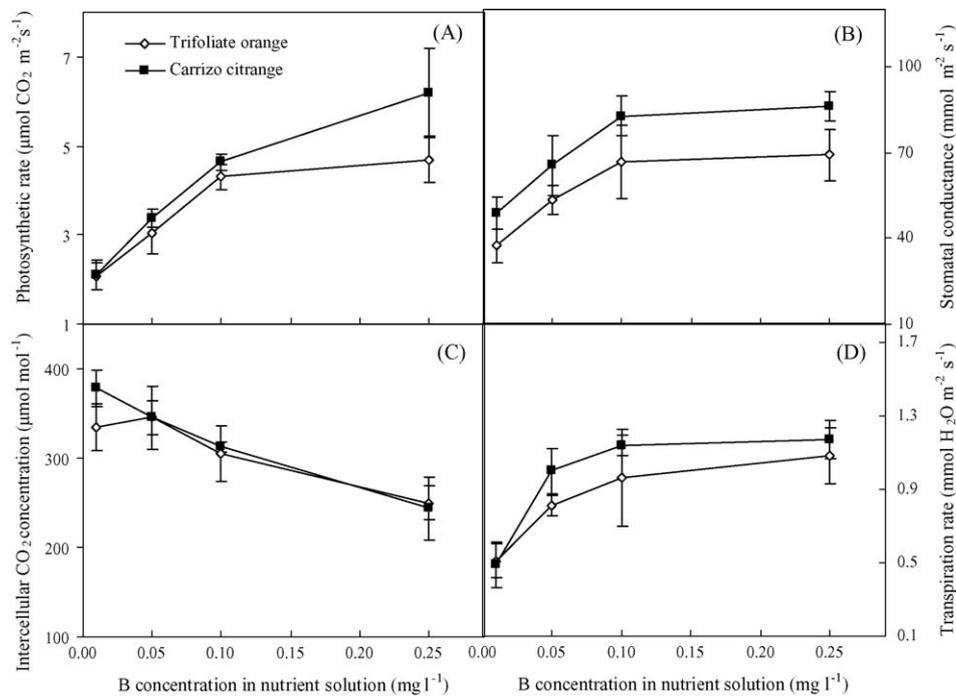


Fig. 3. Effects of B concentration (mg l^{-1}) in the nutrient solution on (A) photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (B) stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$), (C) intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$) and (D) transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of 'Newhall' navel orange plants grafted on Trifoliolate orange and Carrizo citrange. Values represent the mean \pm standard errors of nine replicates ($n = 9$).

cross-link which is essential for plant growth (O'Neill et al., 2001). Another symptom of B deficiency (corky split veins in the mature leaves), which occurred at low B availability in field research (Xiao et al., 2007), was not observed in the current study. This may be partially due to insufficient B deprivation, as the leaf symptoms can develop progressively with the severity of B deficiency (Dell and Huang, 1997). Many other factors, such as environmental stresses and soil texture, could also probably influence the appearance of the symptoms under field conditions (Shorrocks, 1997). In addition, the symptoms of corky split veins appeared in field might be resulted from the accumulation of non-structural carbohydrate in mature leaves induced by B deficiency (Zhao and Oosterhuis, 2002; Camacho-Cristóbal et al., 2004). This, however, requires further investigation.

Our data showed that the growth of scion's parts of 'Newhall' plants was inhibited by the low B treatments, irrespective of the rootstock used, which seemed to indicate that this cultivar was sensitive to low B availability (Table 1), in accordance with our previous field observation (Xiao et al., 2007). And the inhibitory effect on entire plant growth was more pronounced in the Trifoliolate orange-grafted plants under low B conditions (Table 1). However, dry weights in the rootstock's parts exhibited great differences between the two scion-rootstock combinations in response to varied B supplies. They decreased significantly in Trifoliolate orange-grafted plants with decreasing B supply in nutrient solution, but were not affected by the B treatments in Carrizo citrange-grafted plants (Table 1). These results suggest that 'Newhall' scion grafted on Carrizo citrange rootstock was more tolerant to low B availability than that grafted on Trifoliolate orange, as well as the scion's growth was mainly depended on the rootstock.

From our data, close relationships between the values of gas exchange parameters and B supply were also observed, implying that these parameters could be mediated by external B supply and in turn probably influenced the total plant growth (Table 1, Fig. 3). Furthermore, since low B treatments decreased leaf photosynthetic rate, stomatal conductance as well as chlorophyll content (data not shown), and increased intercellular CO_2

concentration of the 'Newhall' plants grafted either on Trifoliolate orange or Carrizo citrange (Fig. 3), it could be concluded that both stomatal and non-stomatal factors were implicated in the decrease of P_n under low B conditions (Sharma and Ramchandra, 1990; Yakushiji et al., 1998). Similar responses were reported in other citrus species (Papadakis et al., 2004a, b) and kiwifruit (Sotiropoulos et al., 2002) when they were grown under B toxicity. It was suggested that leaf constituents or photosynthetic enzymes that could be altered by B deficiency, may be involved in indirectly decrease of photosynthetic rate (Sharma and Ramchandra, 1990; Dell and Huang, 1997). The fact that the transpiration rate reduced by low B should partially account for the decreasing B concentrations in the leaves of the plants, since it has been widely accepted that B transport in plants is greatly influenced by transpiration rate (Raven, 1980).

4.2. Boron concentration and distribution in 'Newhall' navel orange plants

Results of B distribution in different parts of 'Newhall' plants showed that a decrease of B supply in the nutrient solution was followed by reduced B partitioning in the leaves and scion parts, increased B partitioning in the roots and rootstock's parts, irrespective of rootstock used. This finding suggest that the translocation of B from the roots to the scion tissues (stems and leaves of scion) may be restricted upon exposure to low B conditions, which is consistent with findings reported for Norway spruce (*Picea abies*) (Möttönen et al., 2001) and canola (*Brassica napus* L.) (Asad et al., 2002). This phenomenon could be ascribed partly to the impairments of vascular tissues induced by B deficiency (Dell and Huang, 1997). Such a restriction could therefore contribute to the further decreases in the leaf B concentrations observed in our study under low B treatments (Fig. 1A). Finally, under low B ($\leq 0.10 \text{ mg l}^{-1}$) Carrizo citrange-grafted plants partitioned more B to roots allowing their relatively normal growth under B deficiency conditions, a fact that may be responsible for their better performance concerning the total plant growth (Table 1).

Our results also indicate that B concentrations in the leaves of 'Newhall' plants increased as B concentrations in nutrient solution increased, as well as that leaves were the dominant sites of B accumulation (Figs. 1A and 2A). These results are in agreement with those reported for melon (*Cucumis melo* L.) (Edelstein et al., 2005), sour orange (*Citrus aurantium* L.) and Swingle citrumelo (*Citrus paradisi* Macf. × *Poncirus trifoliata* L.) plants (Papadakis et al., 2003). It was further found that variances in relation to the B concentrations among the basal, middle and upper leaves tended to decrease as the external B levels decreased (Fig. 1A), while the ratios of B concentration in upper leaves to basal leaves increased gradually (data not shown), indicating that B might be preferentially transported to upper-younger leaves. This preferential B transport to upper leaves could be responsible for the normal development of young leaves under limited B supply, which could therefore result in less decrease in dry weight of upper leaves (Table 1). It has been demonstrated that in canola (Stangoulis et al., 2001) and wheat (*Triticum aestivum* L.) (Huang et al., 2001), sufficient B transported to the growing regions of plants in order to maintain the cell wall structure and optimal plant development. In addition, B retranslocation via phloem from the mature leaves to young growing tissues (upper leaves) may be involved in allowing the relative normal growth in upper-younger leaves, since this phenomenon could occur even in plants with restricted B mobility (Brown and Shelp, 1997) such as citrus. Finally, 'Newhall' plants grafted on Carrizo citrange had lower B concentration but produced higher dry mass, compared to those grafted on Trifoliolate orange, which might be ascribed to a growth dilution effect (Jarrel and Beverly, 1981), or the other reasons that need further investigation.

5. Conclusion

Under low B conditions, a better growth performance was observed in 'Newhall' plants grafted on Carrizo citrange than in those grafted on Trifoliolate orange, suggesting that Carrizo citrange-grafted plants were more tolerant to low B compared to the plants grafted on Trifoliolate orange. Boron was preferentially translocated to the upper-younger leaves under low B conditions, contributing to normal growth of young leaves under limited B supply, which was likely due to the retranslocation of B via phloem from basal (old) leaves into young growing tissues. B distribution in different plant parts indicated that there was a restriction in translocation of B from root to scion tissues (stems and leaves of scion) under conditions of limited B availability. The changes of gas exchange parameters showed close relationships with B supply, implying that they may be mediated by external B supply and in turn they probably influenced plant growth, especially on Trifoliolate orange-grafted plants.

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References

- Asad, A., Blamey, F.P.C., Edwards, D.G., 2002. Dry matter production and boron concentration of vegetative and reproductive tissues of canola and sunflower plants grown in nutrition solution. *Plant Soil* 243, 243–252.
- Bellaloui, B., 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.
- Brown, P.H., Shelp, B.J., 1997. Boron mobility in plants. *Plant Soil* 193, 85–101.
- Brown, P.H., Hu, H., 1997. Does boron play only a structural role in the growing tissues of higher plants? *Plant Soil* 196, 211–215.
- Camacho-Cristóbal, J.J., Lunar, L., Lafont, F., Baumert, A., González-Fontes, A., 2004. Boron deficiency causes accumulation of chlorogenic acid and caffeoyl polyamine conjugates in tobacco leaves. *J. Plant Physiol.* 161, 879–881.
- Dell, B., Huang, L.B., 1997. Physiological response of plants to low boron. *Plant Soil* 193, 103–120.
- Edelstein, M., Ben-Hur, M., Cohen, R., Burger, Y., Ravina, I., 2005. Boron and salinity effects on grafted and non-grafted melon plants. *Plant Soil* 269, 273–284.
- El-motaïum, R., Hu, H., Brown, P.H., 1994. The relation tolerance of six *prunus* rootstocks to boron and salinity. *J. Am. Soc. Hort. Sci.* 119, 1169–1175.
- Forner-Giner, M.A., Alcaide, A., Primo-Millo, E., Forner, J.B., 2003. Performance of 'Navelina' orange on 14 rootstocks in Northern Valencia (Spain). *Sci. Hort.* 98, 223–232.
- Georgiou, A., Gregoriou, C., 1999. Growth, yield and fruit quality of 'Shamouti' orange on fourteen rootstocks in Cyprus. *Sci. Hort.* 80, 113–121.
- Gupta, U.C., Jame, Y.W., Campbell, C.A., Leyshon, A.J., Nicholaichuk, W., 1985. Boron toxicity and deficiency: a review. *Can. J. Soil Sci.* 65, 381–409.
- Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. In: California Agricultural Experiment Station Circular 347, The College of Agriculture, University of California, Berkeley, CA.
- Hu, H., Brown, P.H., 1994. Localization of boron in cell walls of squash and tobacco and its association with pectin. *Plant Physiol.* 105, 681–689.
- Huang, L.B., Bell, R.W., Dell, B., 2001. Boron supply into wheat (*Triticum aestivum* L. cv Wilgoyne) ears whilst still enclosed within leaf sheaths. *J. Exp. Bot.* 52, 1731–1738.
- Jarrel, W.M., Beverly, R.B., 1981. The dilution effect in plant nutrition studies. *Adv. Agron.* 34, 197–224.
- Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, New York.
- Matoh, T., 1997. Boron in plant cell walls. *Plant Soil* 193, 59–70.
- Mourão Filho, F.A.A., Espinoza-Núñez, E., Stuchi, E.S., Ortega, E.M.M., 2007. Plant growth, yield, and fruit quality of 'Fallglo' and 'Sunburst' mandarins on four rootstocks. *Sci. Hort.* 114, 45–49.
- Möttönen, M., Lehto, T., Aphalo, P.J., 2001. Growth dynamics and mycorrhizas of Norway spruce (*Picea abies*) seedlings in relation to boron supply. *Trees* 15, 319–326.
- O'Neill, Eberhard, M.A.S., Albersheim, P., Darvill, A.G., 2001. Requirement of borate cross-linking of cell wall rhamnogalacturonan II for *Arabidopsis* growth. *Science* 294, 846–849.
- O'Neill, Ishii, M.A.T., Albersheim, P., Darvill, A.G., 2004. Rhamnogalacturonan II structure and function of a borate cross-linked cell wall pectic polysaccharide. *Ann. Rev. Plant Biol.* 55, 109–139.
- Papadakis, I.E., Dimassi, K.N., Therios, I.N., 2003. Response of two citrus genotypes to six boron concentrations, concentration and distribution of nutrients, total absorption, and nutrient use efficiency. *Aust. J. Agric. Res.* 54, 571–580.
- Papadakis, I.E., Dimassi, N., Bosabalidis, A.M., Therios, I.N., Patakas, A., Giannakoula, A., 2004a. Boron toxicity in 'Clementine' mandarin plants grafted on two rootstocks. *Plant Sci.* 166, 539–547.
- Papadakis, I.E., Dimassi, N., Bosabalidis, A.M., Therios, I.N., Patakas, A., Giannakoula, A., 2004b. Effects of B excess on some physiological and anatomical parameters of 'Navelina' orange plants grafted on two rootstocks. *Environ. Exp. Bot.* 51, 247–257.
- Raven, J.A., 1980. Short- and long-distance transport of boric acid in plants. *New Phytol.* 84, 231–249.
- Sharma, P.N., Ramchandra, T., 1990. Water relations and photosynthesis in mustard plants subjected to boron deficiency. *Indian J. Plant Physiol.* 33, 150–154.
- Shorrocks, V.M., 1997. The occurrence and correction of boron deficiency. *Plant Soil* 193, 121–148.
- Smith, M.W., Shaw, R.G., Chapman, J.C., Owen-Turner, J., Lee, L.S., McRae, K.B., Jorgensen, K.R., Mungomery, W.V., 2004. Long-term performance of 'Ellendale' mandarin on seven commercial rootstocks in sub-tropical Australia. *Sci. Hort.* 102, 75–89.
- Sotiropoulos, T.E., Therios, I.N., Dimassi, K.N., Bosabalidis, A., Kofidis, G., 2002. Nutritional status, growth, CO₂ assimilation, and leaf anatomical responses in two kiwifruit species under boron toxicity. *J. Plant Nutr.* 25, 1249–1261.
- Stangoulis, J.C.R., Brown, H., Bellaloui, N., Reid, R.J., Graham, R.D., 2001. The efficiency of boron utilisation in canola. *Aust. J. Plant Physiol.* 28, 1109–1114.
- Storey, R., Walker, R.R., 1999. Citrus and salinity. *Sci. Hort.* 78, 39–81.
- Subedi, K.D., Gregory, P.J., Gooding, M.J., 1999. Boron accumulation and partitioning in wheat cultivars with contrasting tolerance to boron deficiency. *Plant Soil* 214, 141–152.
- Wojcik, P., Lewandowski, M., 2003. Effect of calcium and boron sprays on yield and quality of 'Elsanta' strawberry. *J. Plant Nutr.* 26, 671–682.
- Wojcik, P., Wojcik, M., Treder, W., 2003. Boron absorption and translocation in apple rootstocks under conditions of low medium boron. *J. Plant Nutr.* 26, 961–968.
- Wojcik, P., Cieslinski, G., Mika, A., 1999. Apple yield and fruit quality as influenced by boron applications. *J. Plant Nutr.* 22, 1365–1378.
- Xiao, J.X., Yang, X., Peng, S.A., Fang, Y.W., 2007. Seasonal Changes of mineral nutrients in fruit and leaves of 'Newhall' and 'Skagg's Bonanza' navel Oranges. *J. Plant Nutr.* 30, 671–690.
- Yakushiji, H., Morinaga, K., Nonami, H., 1998. Sugar accumulation and partitioning in Satsuma mandarin tree tissues and fruit in response to drought stress. *J. Am. Soc. Hort. Sci.* 123, 719–726.
- Zhao, D., Oosterhuis, D.M., 2002. Cotton carbon exchange, nonstructural carbohydrates, and boron distribution in tissues during development of boron deficiency. *Field Crops Res.* 78, 75–87.