

Effects of excess boron on growth, gas exchange, and boron status of four orange scion–rootstock combinations

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Abstract

Field observations indicate that boron (B)-toxicity symptoms may occur in citrus plants from inappropriate foliar spraying or overfertilizing with B especially under low-rainfall conditions, where B can accumulate to levels that become toxic to plant growth. Previous work has indicated that different rootstocks can greatly influence the scion's tolerance to B toxicity, however, little is known about the response of different citrus scion–rootstock combinations to excess-B conditions. In the present study, we investigated the effects of excess B on plant growth, gas exchange, B concentration, and distribution of four scion–rootstock combinations, Newhall and Skagg's Bonanza navel orange (*Citrus sinensis* Osb.) scions grafted on Carrizo citrange (*C. sinensis* L. Osb. × *Poncirus trifoliata* L. Raf.) and Trifoliate orange (*Poncirus trifoliata* L. Raf.) rootstocks. One-year-old plants of the four scion–rootstock combinations were grown for 183 d in sand–perlite (1 : 1, v/v) medium under greenhouse conditions. The plants were irrigated with half-strength Hoagland's nutrient solution containing two B concentrations, 0.25 (control) and 2.50 (excess B) mg L⁻¹. It was found that, apart from the combination of Newhall grafted on Carrizo citrange, the dry weights in various parts of the other three combinations were reduced by the excess-B treatment. Furthermore, the plants of Skagg's Bonanza grafted on Carrizo citrange showed the highest growth reduction amongst the four scion–rootstock combinations. In most cases, the greater reductions in dry weight were found in roots as compared to the other plant parts under excess-B conditions, indicating that roots were more sensitive to B toxicity than the other tissues. In the case of Newhall plants grafted on Carrizo citrange, the entire plant growth was increased by excess-B treatment. Boron concentrations in all plants parts increased significantly by increasing the B supply in the nutrient solution. Leaves were the dominant sites of B accumulation and showed the greatest increase in B concentration compared to the other plant parts, as B concentration in the nutrient solution increased. Our results indicate that the combination of Newhall grafted on Carrizo citrange was more tolerant to B toxicity, while the combination of Skagg's Bonanza grafted on Carrizo citrange was relatively more sensitive to B toxicity, in comparison with the other scion–rootstock combinations. However, Newhall plants contained more B in leaves and in roots than Skagg's Bonanza plants when they were both grafted on Carrizo citrange, indicating that the mechanism underlying such great differential growth responses of the two scion–rootstock combinations to B toxicity may not be associated with B exclusion from roots or reduced translocation of B to shoots. Furthermore, B distribution in different plant parts implied that the mechanism was also unlikely related to altered distributions of accumulated B in plant tissues. However, inherent ability to tolerate excessive B concentration in plant tissues may be involved in B tolerance.

Key words: boron distribution / boron toxicity / *Citrus sinensis* / gas exchange / grafting / scion–rootstock

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1 Introduction

Boron (B) toxicity is an important disorder that can limit plant growth on soils of arid and semiarid regions (Nable et al., 1997), where citrus plants are widely grown. Also, toxicity symptoms may occur in citrus plants from inappropriate foliar spraying or overfertilizing with B especially under low-rainfall conditions, since under such conditions B cannot be sufficiently leached and therefore may accumulate to levels that

become toxic to plant growth. Inappropriate application of B fertilizer occurs when navel orange trees are grown in south-east China, where B toxicity induces leaf chlorosis and senescence, decreases tree vigor as well as fruit number, size, weight, and quality, consequently resulting in a great loss of yield and commercial value (Jiang et al., 2009).

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It has been reported that growth variations in response to high concentrations of B were observed in many crop species and even among different genotypes within the same species (Ferreyra et al., 1997; Nable et al., 1997). The mechanisms underlying such variances are complicated and remain unclear. However, according to the previous studies, they are mainly associated with B exclusion from roots, reduced translocation to shoots and avoidance by means of shallow root systems. The ability to restrict B uptake into the plants can minimize the physiological impairments caused by B toxicity (Nable, 1988; Nable et al., 1997; Paull et al., 1992; Hayes and Reid, 2004). On the other hand, an inherent ability to tolerate excessive B concentration in plant tissues (Torun et al., 2006) or the differential antioxidant response that may reduce B-toxicity damage in some species (Cervilla et al., 2007) was suggested. Furthermore, many studies showed that the primary phenotypic effect of B toxicity is poorer root growth compared to that of plants grown at optimal levels of B supply (Lovatt and Bates, 1984; Nable, 1988; Reid et al., 2004; Choi et al., 2006). Moreover, the results from Choi et al. (2007) indicate this mechanism is associated with a complex control of sucrose distribution between leaf and root tips that assists in maintaining root growth under B toxicity. However, since B concentrations in roots are relatively low compared with those in leaves, even at very high levels of B supply, visible symptoms of B toxicity do not develop in roots (Nable et al., 1997). Thus, differential tissue tolerance may play an important role in plant tolerance of such nutritional disorder (Paull et al., 1988). On the other hand, it is likely that B-toxicity effects appear to be loosely correlated with accumulation of high concentrations of B in the shoots, which is a function both of the concentration of B in soil and the time of exposure (Reid, 2007). Results demonstrated for wheat suggest that symptom scoring for B tolerance may be more reliable than measuring plant B concentrations (Kalayci et al., 1998; Torun et al., 2006). Therefore, combining analysis of B status in various plant tissues with evaluation of growth responses could contribute to an understanding of the tolerance of plants subjected to B toxicity.

Whereas B deficiency can be resolved by application of B-enriched fertilizers, toxicity is a more difficult problem to manage. Previous work has indicated that the orange plants are sensitive to excess of B (Maas, 1990), and different rootstocks can greatly influence scion's tolerance to B toxicity (El-Motaium et al., 1994; Papadakis et al., 2004a, b). Therefore, screening of genotypes has identified important crop varieties that can cope with relatively high soil B concentrations (Ferreyra et al., 1997; Nable et al., 1997), which can also be used as an appropriate means for addressing potential B toxicity in navel orange production. Newhall (*Citrus sinensis* Osbeck cv. Newhall) and Skagg's Bonanza (*C. sinensis* Osbeck cv. Skagg's Bonanza) navel oranges are two important citrus cultivars that are widely grown in China, providing high-quality fresh fruits for domestic consumers. 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). On the other hand, Trifoliolate orange (*Poncirus trifoliata* L. Raf.) has been widely used as a cold-hardy and dwarfing rootstock for citrus

production in China. Moreover, little is known about the adaptability of the different citrus scion–rootstock combinations, i.e., Newhall and Skagg's Bonanza navel orange scions grafted on rootstocks of Carrizo citrange and Trifoliolate orange, to excess-B conditions.

The objectives of this work were: (1) to evaluate the effects of excess B on plant growth, gas exchange, and B concentration and distribution of Newhall and Skagg's Bonanza navel orange scions grafted on Carrizo citrange and Trifoliolate orange rootstocks, and (2) to compare the differential responses of the four scion–rootstock combinations under conditions of excess B.

2 Materials and methods

2.1 Plant materials and boron treatments

Newhall (*Citrus sinensis* Osbeck cv. Newhall) and Skagg's Bonanza (*C. sinensis* Osbeck cv. Bonanza) navel orange scions grafted on two rootstocks, either Carrizo citrange (*Citrus sinensis* L. Osb. × *Poncirus trifoliata* L. Raf.) or Trifoliolate orange (*Poncirus trifoliata* L. Raf.) were used in this experiment. These combinations are referred to as: N/CC, Newhall grafted on Carrizo citrange; N/TO, Newhall grafted on Trifoliolate orange; SB/CC, Skagg's Bonanza grafted on Carrizo citrange; SB/TO, Skagg's Bonanza grafted on Trifoliolate orange. The buds of two scion varieties did not sprout before our experiment, and the initial shoots (about 3–4 cm above the grafting unit) of the rootstock seedlings were removed. At budding time, the seedlings of both rootstocks were 1 year old and selected based on uniform stem diameter, 4–5 mm for Trifoliolate orange and 5–7 mm for Carrizo citrange, respectively. Plant materials (rootstock seedlings and buds of scions) 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.

When preparing for the experiment, the roots of the plants were severely pruned (to stimulate new root development following B treatments; Papadakis et al., 2004a, b). 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 and perlite (1 : 1, v/v). Before the transplantation, the medium had been washed with 3% hydrochloric acid (HCl) overnight and then rinsed thoroughly with deionized water, 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 set up 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 scions were about 8–10 cm in length (with seven to nine 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 two concentrations, viz. 0.25 mg B L⁻¹ (control) and 2.50 mg B L⁻¹ (to induce B toxicity; Papadakis et al., 2004a, b; Hoagland and Arnon, 1950). The plants were irrigated with the nutrient solutions every 2 d (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 drained from the bottom of the growth medium (Bellaloui and Brown, 1998). The experiment lasted for 183 d until visible typical symptoms of B toxicity appeared. During the B treatments, the plant-growth conditions were controlled as follows: 550 to 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ flux density of natural light, 33°C/25°C (day/night) of temperature, and 80% of relative humidity (measured with an Li-6400 portable measuring device, Li-COR, Lincoln, USA).

2.2 Leaf-gas-exchange measurements

At the end of the experiment, the gas-exchange parameters, i.e., the photosynthetic rate (P_n ; $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), intercellular CO₂ concentration (C_i ; $\mu\text{mol mol}^{-1}$), and transpiration rate (E ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) 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 a.m. and 12:00 noon at steady light intensity (1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) under a CO₂ concentration of (385 ± 10) $\mu\text{L L}^{-1}$ and leaf temperature between 28°C and 30°C.

2.3 Sampling, plant analyses, and boron 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 deionized water for three times. The fresh and dry weights were measured. Each dried sample was ground to fine powder and stored in an air-tight glass container for subsequent analyses.

For measurement of B concentration, 0.50 g of each sample were dry-ashed in a muffle furnace at 500°C for 6 h, followed by dissolution in 0.1 N HCl, and B was determined using ICP-AES. Boron distribution was expressed as percentage ratios (fraction, %) of B content (concentration × dry weight) in different plant parts relative to total plant B content (Subedi et al., 1999; Möttönen et al., 2001). The patterns of B distribution in leaves, roots, and stems (either of scion or rootstock) are presented to show the changes in relation to the fractions of the

total amount of B taken up by plants upon exposure to excess B.

2.4 Experimental design and statistical analyses

The experiment was set up in a completely randomized 4 × 2 factorial design with four scion–rootstock combinations (two scions of navel orange grafted on two rootstocks) and two B treatments (0.25 and 2.50 mg B L⁻¹). Three replications (three plants in each) were designed for each treatment. The experimental data were subjected to analysis of variance (ANOVA) using the SAS (SAS Institute Inc., 1996), and the differences were compared by employing the Duncan test with a significance level of $p < 0.05$.

3 Results

3.1 Symptoms of boron toxicity and plant growth

Visible symptoms of B toxicity developed when 2.50 mg B L⁻¹ (excess B) were applied to the plants of Newhall and Skagg's Bonanza navel orange scions grafted either on Carrizo citrange or on Trifoliate orange rootstocks, causing chlorosis at the tip of old leaves, which extended to the rest of the leaf blade and the middle leaves. The symptoms became more severe and apparent in Skagg's Bonanza plants grafted on Carrizo citrange at the end of the experiment.

The scions grafted on Carrizo citrange exhibited relatively higher dry weights than those grafted on Trifoliate orange, irrespectively of B treatment. Of the two scions grafted on the same rootstock, Newhall plants appeared to produce more biomass than Skagg's Bonanza (Tab. 1). Apart from the combination of Newhall grafted on Carrizo citrange, the dry weights in various parts of the other three scion–rootstock combinations were reduced by the excess-B treatment. It seemed that the reduction was more pronounced for roots. When the plants were treated with excess B, the total plant dry weights decreased by 27% in plants of Newhall grafted on Trifoliate orange, and by 31% or 27% in plants of Skagg's Bonanza when they were grafted either on Carrizo citrange or on Trifoliate orange, respectively. In the case of the combination, Newhall grafted on Carrizo citrange, dry weights in all the plant parts except in middle leaves increased when B concentration in nutrient solution was raised to 2.50 mg L⁻¹. Within this combination, the total plant dry weight increased by 10% upon exposure to excess B, compared with the 0.25 B mg L⁻¹ treatment (Tab. 1).

3.2 Boron concentration and distribution

Boron concentrations in all plant parts increased significantly as external B concentration increased from 0.25 to 2.50 mg L⁻¹, following the order: basal leaves > middle leaves > upper leaves > roots > stems of rootstock > stems of scion, for all scion–rootstock combinations. There were 4- to 10-fold, 2.5- to 3-fold, 1.5- to 2-fold, and 1.2- to 1.5-fold increases of B concentrations of leaves, roots, stems of scion as well as of rootstock stems, respectively (Fig. 1). These results suggested that leaf and root tissues were more sensitive to

Table 1: Effects of excess B (2.50 mg B L⁻¹) on dry weight (g [plant DW]⁻¹) of different plant parts of Newhall (N) and Skagg's Bonanza (SB) navel orange scions grafted on trifoliate orange (TO) and Carrizo citrange (CC) rootstocks. Values are means of three replicates ($n = 3$), with three plants per each replicate. Means within each column followed by the same letter do not significantly differ at $p < 0.05$. F values for the scion, rootstock, B treatment, and their interactions are shown.

Scion/ rootstock	B / mg L ⁻¹	Basal leaf	Middle leaf	Upper leaf	Stem of scion	Stem of rootstock	Root	Scion	Rootstock	Total plant
N/CC	0.25	4.18 b	6.98 a	9.40 a	10.8 b	7.07 b	18.0 b	31.4 a	25.1 b	56.4 b
	2.50	4.91 a	5.95 b	10.1 a	11.1 ab	9.34 a	20.6 a	32.1 a	30.0 a	62.1 a
N/TO	0.25	3.97 bc	4.63 c	6.05 c	8.43 c	3.72 d	17.4 bc	23.1 c	21.1 d	44.2 d
	2.50	2.72 d	3.88 d	5.19 d	6.54 e	2.82 ef	11.2 e	18.3 e	14.1 f	32.4 f
SB/CC	0.25	3.54 bc	5.43 b	8.67 b	11.5 a	7.13 b	15.8 c	29.2 b	23.0 c	52.1 c
	2.50	3.46 c	3.93 cd	6.15 c	8.07 c	5.50 c	8.99 f	21.6 d	14.5 ef	36.1 e
SB/TO	0.25	3.63 bc	4.20 cd	5.98 c	7.33 d	3.27 ed	13.0 d	21.1 d	16.3 e	37.4 e
	2.50	2.60 d	3.61 d	4.97 d	5.18 f	2.25 f	8.79 f	16.4 f	11.1 g	27.4 g
<i>F</i> values										
Scion		15.3**	37.6***	53.5***	91.1***	61.5***	182.4***	221***	219***	443***
Rootstock		23.7***	72.7***	319***	773***	771***	73.0***	996***	306***	1070***
B		6.10*	30.6***	29.2***	202***	4.42ns	91.1***	213***	85.4***	260***
Scion × rootstock		6.27*	16.9***	41.9***	0.06ns	20.3***	21.0***	61.8***	32.3***	86.3***
Scion × B		0.8ns	0.18ns	24.8***	64.3***	43.2***	24.3***	55.2***	45.3***	98.9***
Rootstock × B		20.1***	2.86ns	0.01ns	3.40ns	17.4***	16.3***	5.95*	25.8***	32.9***
Rootstock × scion × B		2.5ns	0.82ns	20.41***	48.7***	38.0***	56.2***	54.4***	78.6***	133***

Significance of ANOVA: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: nonsignificant

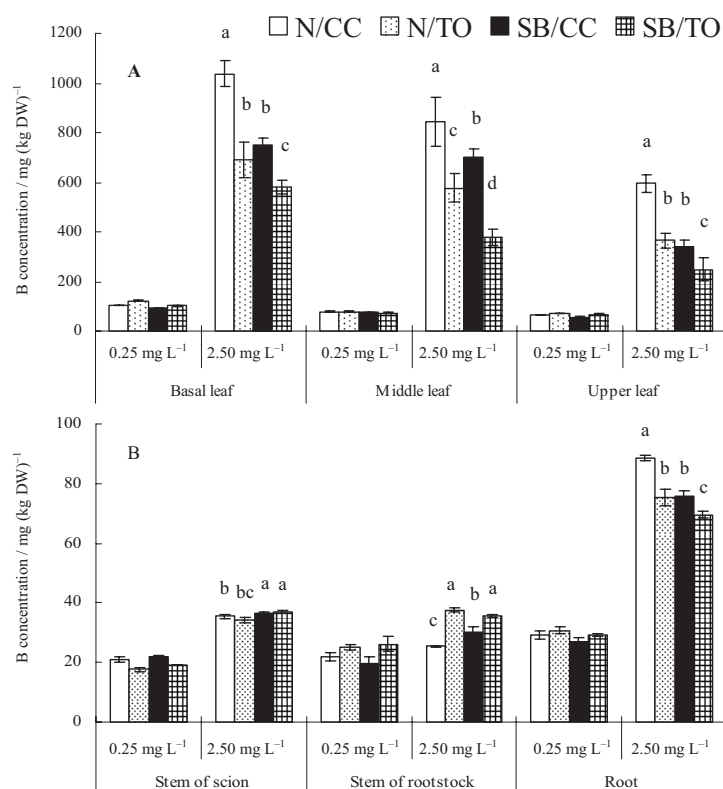


Figure 1: Effects of excess B (2.50 mg B L⁻¹) on B concentration (mg [kg dry weight]⁻¹) in (A) leaves (basal, middle, and upper) and (B) stems of scion and rootstock, and root of Newhall (N) and Skagg's Bonanza (SB) navel orange scions grafted either on Carrizo citrange (CC) or on Trifoliate orange (TO) rootstocks. Values represent the means \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 part at 2.50 mg B L⁻¹.

external high B than the other plant parts. In plants treated with 2.50 mg B L⁻¹, it was further found that leaves and roots from Newhall grafted on Carrizo citrange contained relatively more B, while those from the combination of Skagg's Bonanza grafted on Trifoliate orange contained relatively less B, as compared with the other scion–rootstock combinations.

Leaves represented the dominant sites of B accumulation, regardless of the combination and B treatment, containing from 60% to 85% with increasing external B concentration from 0.25 to 2.50 mg L⁻¹, while in the other parts the ratios decreased with the increase of external B application (Tab. 2; Fig. 2). At 2.50 mg B L⁻¹, the fractions of B distributed to leaves were more in the combinations of Carrizo citrange–grafted plants (\approx 87%) than in the combinations of Trifoliate orange–grafted plants (\approx 83%). However, the former contained less B in roots (8.0%–9.9%) than the latter (11.8%–12.3%) at 2.50 mg B L⁻¹.

3.3 Effects of excess B on leaf-gas-exchange parameters

Leaf-gas-exchange parameters were greatly influenced by B treatment (Fig. 3, Tab. 2). After treated by 2.50 mg B L⁻¹, leaf photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) decreased by about 49%–77%, 45%–63%,

and 31%–49%, respectively, as compared with those of plants grown at 0.25 mg B L⁻¹ (Fig. 3A, B, and D). These parameters were reduced to a greater extent in plants of Skagg's Bonanza grafted on Trifoliate orange. Moreover, relative higher values in P_n and g_s were recorded in leaves from the plants of Newhall grafted on Carrizo citrange, irrespective of B supply. On the other hand, intercellular CO₂ concentration (C_i) increased by about 15%–25% at 2.50 mg B L⁻¹ (Fig. 3C), which however, did not differ significantly upon exposure to the same B treatment among the four scion–rootstock combinations.

4 Discussion

In our experiment, the major effects of B toxicity on citrus plants were the decline in dry matter of various plant parts, inhibition of root growth, leaf chlorosis developing to brown spots at the tips and the edges of the leaves leading to necrosis. Symptoms were more severe in excess-B-treated Skagg's Bonanza plants grafted on Carrizo citrange (Tab. 1). The symptoms of B toxicity appeared on old (basal) leaves, at the end of the transpiration stream (Brown and Shelp, 1997), in agreement with other observations described for citrus plants when they were treated with excess B (Papadakis et al., 2004a, b). The toxicity symptoms may be caused by the metabolic disruption due to high internal concentrations of B binding to the ribose moieties of ATP, NADH, or NADPH from the metabolic pool, which inhibit

Table 2: F values and levels of significance for scion, rootstock, and B treatment and their interactions from ANOVA on B concentration and B distribution (in leaf, stem of scion, stem of rootstock as well as in root) and on gas-exchange parameters, viz. the photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO₂ concentration (C_i), and transpiration rate (E).

	B concentration				B distribution				Gas exchange			
	leaf	stem of scion	stem of rootstock	root	leaf	stem of scion	stem of rootstock	root	P_n	g_s	C_i	E
Scion	135***	39.7***	0.96ns	85.7***	2.14ns	94.2***	8.21*	7.28*	8.42**	16.6**	1.86ns	5.81*
Rootstock	196***	54.9***	139***	43.8***	12.7**	1.96ns	28.8***	42.4***	10.4**	7.28*	1.64*	7.15*
B	904***	3785***	240***	6596***	1431***	1471***	495.4***	752***	243***	353***	30.94***	207***
Scion \times rootstock	6.50*	3.89ns	2.54ns	11.2**	5.17*	28.2***	1.39ns	0.42ns	1.91ns	12.7**	1.49ns	17.8***
Scion \times B	22.7***	2.14ns	2.22ns	39.8***	0.09ns	11.4**	0.64ns	1.32ns	1.43ns	0.69ns	0.26ns	0.14ns
Rootstock \times B	49.2***	26.2***	13.0**	96.4***	8.44*	12.2**	25.4***	0.26ns	18.6***	11.0**	0.20ns	4.23*
Rootstock \times scion \times B	5.01***	0.60ns	16.8***	6.56*	9.75**	7.19*	0.06ns	11.0**	2.23ns	1.00ns	0.05ns	0.36ns

Significance of ANOVA: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: nonsignificant

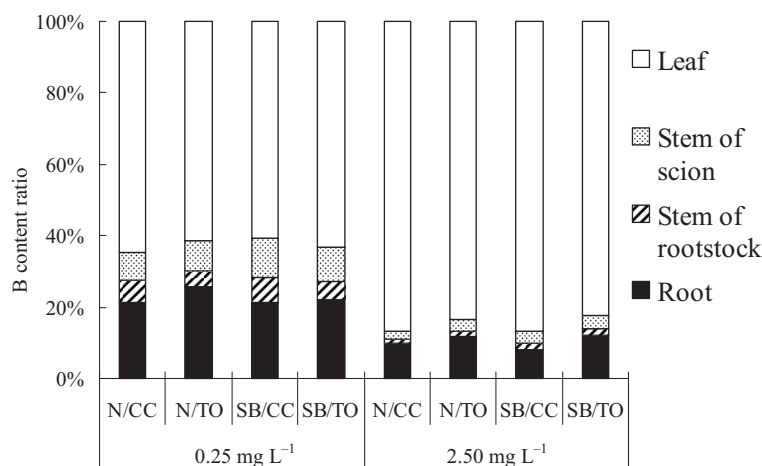


Figure 2: Effects of excess B (2.50 mg B L⁻¹) on percentage (%) of total B content partitioned to leaf, stem of scion, stem of rootstock, and root of Newhall (N) and Skagg's Bonanza (SB) navel orange scions grafted either on Carrizo citrange (CC) or on Trifoliate orange (TO) rootstocks. Values represent the mean \pm SE of three replicates ($n = 3$), with three plants per each replicate.

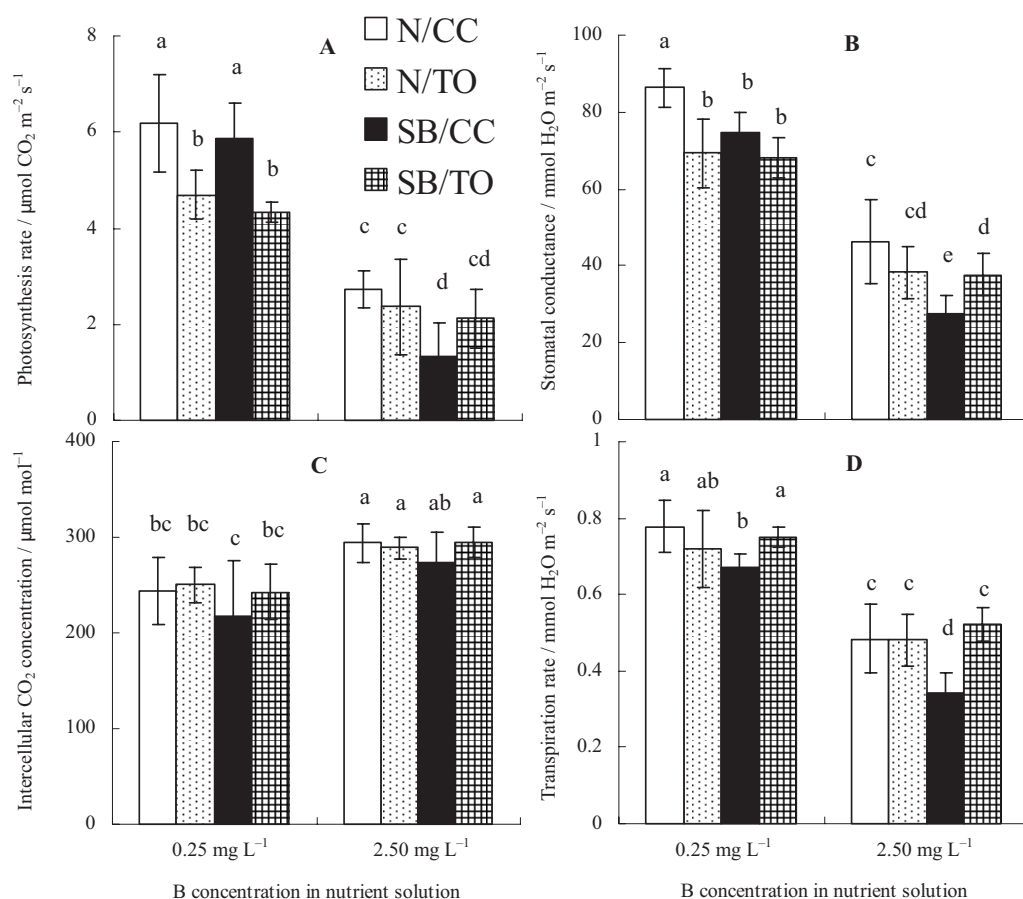


Figure 3: Effects of excess B (2.50 mg B L^{-1}) on (A) photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (B) stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), (C) intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$), (D) transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of Newhall (N) and Skagg's Bonanza (SB) navel orange scions grafted either on trifoliate orange (TO) or on Carrizo citrange (CC) rootstocks. Values represent means \pm standard errors of nine replicates ($n = 9$). Bars with the same letter are not significantly different at $p < 0.05$.

normal biosynthetic activities or energy transduction (Reid et al., 2004; Reid, 2007), consequently leading to impaired growth and development. On the other hand, excess B induces oxidative damage by lipid peroxidation and hydrogen peroxide accumulation, which may also contribute to the expression of the B-toxicity symptoms (Gunes et al., 2006; Cervilla et al., 2007).

Roots were more sensitive to external B than the other plant parts, since in most cases, the inhibitory effects of excess B on growth were more pronounced in roots (Tab. 1). The increased incidence of abnormal mitosis in root meristem, which influences cell division and ultimately decreases the rate of root elongation (Klein and Brown, 1981; Liu et al., 2000), was probably involved in decreased root growth, which would limit the ability of the plants to take up essential mineral nutrients and water, and consequently affect the growth of aboveground of the plants (Reid, 2007). Otherwise, root tips are also a site of B toxicity, and analysis of physiological and morphological changes of root tips could predict the potential occurrence of B toxicity, since the primary effect of toxic B might be on root tips when high B was applied to the rooting medium (Reid et al., 2004).

According to the growth performance of the plants after long-term exposure to excess B, only the scion-rootstock combination of Newhall grafted on Carrizo citrange exhibited better root growth, produced more new young leaves (upper leaves) and consequently had significant increases (10%) in total plant dry

weight under excess-B conditions (Tab. 1). In contrast, the other combinations showed a reduction ($\approx 30\%$) in entire plant dry weight upon exposure to excess B (Tab. 1). When Skagg's Bonanza plants were grafted on Carrizo citrange, this combination showed the greatest growth reductions in root tissues (43%) and produced less upper young leaves (Tab. 1). These results indicate that Newhall grafted on Carrizo citrange was the most tolerant combination while Skagg's Bonanza grafted on Carrizo citrange may be the most sensitive combination concerning B toxicity, amongst the four scion-rootstock combinations that were evaluated in our study.

From our data of plant dry weight and B concentration, it can be easily calculated that Carrizo citrange-grafted plants accumulated more B than Trifoliate orange-grafted plants (data not shown), implying that the roots of Carrizo citrange could take up more B than the roots of Trifoliate orange. However, at 2.50 mg B L^{-1} Newhall plants contained more B in leaves than Skagg's Bonanza plants, irrespectively of the rootstock used (Fig. 1). These results indicate that the mechanism underlying the great differential growth responses between the two scion-rootstock combinations (N/CC and SB/CC) to B toxicity cannot be explained in terms of B exclusion from roots or reduced translocation of B to shoots. However, the mechanism may be associated with the inherent ability to tolerate excessive B concentration in plant tissues. It was suggested by several studies that the internal compartmentation of B in vacuoles is a plausible explanation for B tolerance

(Torun et al., 2003, 2006; Mahalakshmi et al., 1995). Torun et al. (2006) evaluated genotypic variation in tolerance to B toxicity in 70 durum wheat (*Triticum durum*) genotypes and found that there was no relationship between shoot B concentrations and relative decreases in shoot dry weight caused by B toxicity, but the most B-sensitive genotypes generally had much lower amount of B in shoot than the genotypes showing higher tolerance to B toxicity. Similar results were also shown in bread wheat (*Triticum aestivum*; Kalayci et al., 1998) and in barley (*Hordeum vulgare* L; Mahalakshmi et al., 1995; Torun et al., 2003): The tolerant cultivars maintained high levels of B in shoots or/and roots. It was further speculated by Kaur et al. (2006a) that formation of B-polyol complexes in the vacuoles would be an effective way of storing B without damage to cellular metabolism. The B-sensitive genotype may lack this mechanism to limit toxic B concentration in the cytoplasm, with consequent decreases in tissue biomass (Kaur et al., 2006b).

Previous work has proven that different rootstocks can greatly influence scion's tolerance to B toxicity (El-Motaium et al., 1994; Papadakis et al., 2004a, b), which also can be concluded from our results that both scion and rootstock influences determine growth responses (Tabs. 1 and 2). It was further observed in our experiment that complicated scion–rootstock interactions exist in the four scion–rootstock combinations, showing different growth responses to excess-B stress (Tabs. 1 and 2). There were few reports on the combinations of scion–rootstock upon exposure to excess B in citrus plants. However, previous studies have suggested that root architecture could directly affect exploitation efficiency of nutrition and water required for plant growth (Berntson, 1994; Nielsen et al., 1994), while carbohydrate derived from the leaf photosynthesis and phytohormones synthesized in the growing shoots can affect the architecture of the root system and its function (Thaler and Pagès, 1996; Wightman and Thimann, 1980). In our experiment, excess-B treatment may have caused a significant decrease in net photosynthesis (Fig. 3), leading to negative effects on growth and the ability to supply photosynthates, which can reduce the translocation of carbohydrates to root, inhibiting growth and function of the root.

Our results show that B concentrations in the leaves of Newhall or Skagg's Bonanza plants increased as B concentrations in nutrient solution increased, and that leaves were the dominant sites of B accumulation (Figs. 1, 2), which coincided with the previous studies (Papadakis et al., 2004a, b). This could be explained by the facts that B transported through the xylem is greatly influenced by transpiration (Raven, 1980; Brown and Shelp, 1997). Moreover, effects of excess B on percentage of total B content partitioned to different plant parts showed similar changes in the distribution pattern amongst the four scion–rootstock combinations, despite the great differences in total amounts of accumulated B found among them (data not shown). This suggests that the relative B tolerance in the four scion–rootstock combinations was also unlikely to be associated with altered distributions of accumulated B in plant tissues, in accordance with the previous results in cereals (Nable et al., 1997).

Our data of gas-exchange parameters reflected close relationships with high B supply, which decreased leaf photosynthetic rate and stomatal conductance, but increased intercellular CO₂ concentration of the Newhall and Skagg's Bonanza plants grafted either on Trifoliate orange or on Carrizo citrange (Fig. 3), indicating that both stomatal and nonstomatal factors were involved in the decreases of P_n under excess-B conditions. 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. Boron toxicity can cause a decrease in chlorophyll content and structural damage of chloroplasts, which lead to a decrease in CO₂-assimilatory capacity of the mesophyll cells (Papadakis et al., 2004a, b), ultimately affecting plant growth. Furthermore, leaf-gas-exchange parameters recorded in Skagg's Bonanza plants grafted on Carrizo citrange, were greatly affected by excess-B treatments, showing the lowest values in P_n , g_s and E after exposure to excess B (Fig. 3, Tab. 2), which may be linked to the more severe negative effects of excess B on this scion–rootstock combination.

5 Conclusion

Amongst the four scion–rootstock combinations evaluated in our experiment, Skagg's Bonanza plants grafted on Carrizo citrange showed the highest growth reduction under excess B, whereas the plant growth of Newhall plants grafted on Carrizo citrange was increased by the treatment of excess B. These results indicate that the combination of Newhall grafted on Carrizo citrange was more tolerant to B toxicity, while the combination of Skagg's Bonanza grafted on Carrizo citrange was more sensitive to B toxicity. The mechanism underlying such great differential growth responses between the two scion–rootstock combinations to B toxicity was not explained in terms of B exclusion from roots or reduced translocation of B to shoots, but an inherent ability to tolerate excessive B concentration in plant tissues of Newhall navel orange.

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