

Differences in boron distribution and forms in four citrus scion–rootstock combinations with contrasting boron efficiency under boron-deficient conditions

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Abstract

Key message Boron efficiency of scion ‘Fengjie-72’ is related to its less reduced boron concentration and distribution in leaves, achieved by decreasing the ratio of available boron in roots under boron-deficient conditions.

Abstract Boron (B) deficiency is widespread in citrus orchards. Previous studies have demonstrated that the B-efficient navel orange scion ‘Fengjie-72’ (Fs) and rootstock Carrizo citrange (Cr) are more tolerant to B deficiency than the closely related B-inefficient scion ‘Newhall’ (Ns) and rootstock trifoliate orange (Tr), respectively. However, the mechanisms underlying such differences remain unclear. Here, we investigated the differences in B distribution and forms among four combinations (Fs/Cr, Fs/Tr, Ns/Cr, and Ns/Tr) under adequate (0.25 mg/L) or deficient (0.001 mg/L) B supply for 300 days in sand culture. The results showed that B concentrations in buds and leaves of Fs-grafted plants were significantly higher than the respective concentrations of Ns-grafted plants under B-deficient conditions. Moreover, B distribution of Fs-grafted plants due to B deficiency was

reduced less in leaves, but more in roots as compared to that of Ns-grafted plants. However, Ns/Cr accumulated more B in the scion stem (24 %) than the other combinations (17–19 %) when B was limited. A correlation was established between B efficiency and the ratio of B concentration in the rootstock stem or buds to the scion stem. Under B-deficient conditions, the ratio of available B (free B and semi-bound B) was significantly higher in leaves in Cr-grafted (36 %) than Tr-grafted plants (29 %), but lower in roots of Fs-grafted (22 %) than Ns-grafted plants (28 %). These results suggest that, under B-deficient conditions, differential B efficiency arises probably because Cr transports more B into scion, Fs redistributes B more efficiently within the plant, or both.

Keywords Boron deficiency · Citrus · Efficiency · Scion–rootstock combination

Introduction

Boron (B) is an essential micronutrient for higher plants (Warington 1923), and its deficiency is one of the most widespread deficiencies among all micronutrients (Loomis and Durst 1992). Boron deficiency has been reported in many agricultural crops, including citrus (Shorrocks 1997; Xiao et al. 2007; Boaretto et al. 2008). Previous studies have shown that B deficiency in citrus causes a wide range of anatomical, physiological and biological changes, including enlargement of root tips, retardation of lateral root growth, accumulation of carbohydrates and starch, and inhibition of photosynthetic capacity (Chen et al. 2012; Zhou et al. 2014).

The difference in responses to low B among genotypes within a species has long been recognized

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(Rerkasem and Jamjod 1997; Bellaloui and Brown 1998; Stangoulis et al. 2001) and is also known as the difference of B efficiency. B-efficient genotypes are those that are able to grow well in soils in which other genotypes are adversely affected by B deficiency (Graham 1984; Rerkasem and Jamjod 1997). Differential B efficiency has been reported in many crops, including citrus (Smith and Reuther 1949; Rerkasem and Jamjod 1997). In field-grown trees, Xiao et al. (2007) observed that ‘Newhall’ navel orange presented a typical symptom of B deficiency in mature leaves under low B conditions, but ‘Skagg’s Bonanza’ navel orange did not, even though both of them originated from bud mutation of ‘Washington’ navel orange. Subsequently, Sheng et al. (2009a, b) reported that genotypic variation of B efficiency within citrus existed not only in scion cultivars, but also in rootstocks. However, the mechanisms underlying those differences among different scion–rootstock combinations remain unclear.

The wide range of B efficiency among genotypes is associated with the ability to take up B from medium, to translocate B from root to shoot, and to utilize B within the plants (Bellaloui and Brown 1998; Takano et al. 2001; Marschner 2012; Pan et al. 2012). The efficiency of B uptake, transport, and utilization are closely related to B forms existing in plant tissues (Hu et al. 1996; Dannel et al. 1998; Du et al. 2002; Liu et al. 2011). Generally, B forms can be divided into two B pools: the soluble B pool (cell sap), which is a mixture of intracellular and apoplasmic fluids, and the insoluble B pool (water insoluble residue) that is mainly bound to cell wall structures (Dannel et al. 1998). Du et al. (2002) fractionated the soluble B pool into free B and semi-bound B. Recently, Liu et al. (2011) found that the citrus rootstock citrange had a much higher ratio of semi-bound B/free B than the rootstock trifoliate orange when the same scion was used. However, it is still unknown whether scions with contrasting B efficiency could affect the B forms in roots of the same rootstock, and what differences exist between the scions or the rootstocks when they influence the other grafted part.

Previous studies have shown that the navel orange scion ‘Fengjie-72’ and rootstock Carrizo citrange are more tolerant to B deficiency (efficient) than the closely related scion ‘Newhall’ and rootstock trifoliate orange (inefficient), respectively (Sheng 2009; Sheng et al. 2009b). However, little information is available on differences in B distribution and forms among these scion–rootstock combinations. Therefore, the objective of this study was to compare the differential responses of four citrus scion–rootstock combinations with contrasting B efficiency to B deficiency.

Materials and methods

Plant materials

The rootstocks and scions used here were selected based on the degree of susceptibility to low B as determined by the work of Sheng (2009) and Sheng et al. (2009b). They were as follows:

B-efficient scion ‘Fengjie-72’ navel orange [*Citrus sinensis* (L.) Osb. cv. Fengjie-72] and rootstock Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.];

B-inefficient scion ‘Newhall’ navel orange [*Citrus sinensis* (L.) Osb. cv. Newhall] and rootstock trifoliate orange [*Poncirus trifoliata* (L.) Raf.].

Four combinations were formed: Fs/Cr, ‘Fengjie-72’ scion grafted on Carrizo citrange rootstock; Fs/Tr, ‘Fengjie-72’ scion grafted on trifoliate orange rootstock; Ns/Cr, ‘Newhall’ scion grafted on Carrizo citrange rootstock; and Ns/Tr, ‘Newhall’ scion grafted on trifoliate orange rootstock.

Growth conditions and B treatments

The experiment was conducted in a greenhouse at Huazhong Agricultural University, Wuhan, China. When the scions developed three to five mature leaves, the roots of the plants were severely pruned to stimulate new root development following B treatments (Papadakis et al. 2003). All the plants were then washed with tap water to remove surface contaminants and transplanted into 10-L black plastic pots (one plant per pot) filled with B-free quartz sand and perlite (1: 1, v/v) medium. Thereafter, the plants were supplied with a modified B-free, 1/4 strength Hoagland’s No. 2 nutrient solution (Hoagland and Arnon 1950) for about 4 weeks, until three to five other leaves expanded in the scions.

B treatments commenced at the end of May 2012. The plants were irrigated with a modified Hoagland’s No. 2 nutrient solution, in which macronutrients were supplied at half strength and micronutrients, except for B, at full strength. Two B concentrations were applied, i.e., 0.25 mg/L (control) and 0.001 mg/L (to induce B deficiency). The plants were irrigated with enough solutions every 2 days, allowing some drainage from the growth medium to occur. This experiment lasted for 300 days, until the reproductive stage sensitive to B deficiency (i.e., the middle of flowering; Asad et al. 2002).

To avoid contamination with B, the medium and pots were immersed in 3 % HCl overnight and then rinsed thoroughly with deionized water (18.25 MΩ, Aquapro, USA) before transplantation. All water used for preparing

nutrient solutions and reagents for analysis was deionized water, and all chemicals were of analytical grade. To avoid salt and B accumulation, the plants were irrigated with 10 L of deionized water once a week, followed by application of 3 L nutrient solution, to ensure some excess solution leached from the pot bottom (Bellaloui and Brown 1998).

Sampling

At the end of the experiment, the scion's buds (including flower, new leaflet, and twig), leaves, and stem, and the rootstock's stem and roots were separately sampled. All the samples were initially washed with tap water and then with deionized water in 30 s. The samples were blotted dry prior to weighing (leaves and roots were subsampled), oven dried at 65 °C for 72 h, and reweighed. Each dry sample was ground to fine powder and stored in a sealed plastic bag for B analysis. The remaining fresh basal leaves and fine roots (<1 mm diameter) were used for B form analysis.

Boron analysis

For B analysis, 0.30 g of each sample was dry ashed in a muffle furnace at 500 °C for 6 h, dissolved in 15 mL HCl (1 mol/L), and then B concentration was determined by inductively coupled plasma–atomic emission spectrometry (ICP–AES, IRIS-Advan type, Thermo, USA; Zarcinas et al. 1987). Boron distribution was expressed as the percentage of B content (concentration \times dry weight) in one plant part to the total plant B content. The ratio of B concentration in different plant parts represents the ability of the grafted plant to transport B from rootstock to scion, or the ability of the scion to distribute B in various scion's parts.

Analysis of boron forms

B forms were extracted as described by Du et al. (2002) with slight modifications. A batch of 3 g of old leaves or 5 g of fine roots was cut into pieces of approximately 1 mm², put into a plastic bottle (3.0 cm inside diameter and 6.0 cm height) with 15 mL of deionized water, and shaken at 25 °C for 24 h at 100 rpm. The mixture was then filtered through a 0.15-mm mesh and centrifuged at 4 °C for 15 min at 5,000 rpm. The supernatant was collected as free B. The residue was then successively extracted with 10 mL of NaCl (1 mol/L) and 10 mL of HCl (1 mol/L) solution following the same procedure as above, and the supernatants were collected as semi-bound B and bound B, respectively.

Due to the high salt concentration in the extract of semi-bound B, ICP–AES was unable to detect its B concentration. Therefore, B concentration of the extract was determined by the colorimetric curcumin method.

Statistical analysis

Unless otherwise noted, all reported values represent means of four individual plants (replicates). Error bars indicate standard error (SE) of the means. Data were compared by one-way analysis of variance (SPSS for Windows 17.0; SPSS Inc., Chicago, IL, USA). Differences among the four scion–rootstock combinations were determined by Duncan's multiple range test ($p < 0.05$) and indicated by different letters (a, b, c, d).

Results

Dry weight

During this experiment, the dry weight in buds and roots of four combinations was not affected by B treatments (Table 1). However, as compared to Ns/Cr, the dry weight of Fs/Cr was significantly higher in rootstock stem but lower in leaves with B-adequate treatment, as well as higher in scion stem with B-deficient treatment (Table 1).

Boron concentration and distribution in various plant parts

After 300 days with B-deficient treatment, the B concentrations in buds and leaves of Fs-grafted plants were significantly higher than those of Ns-grafted plants (Fig. 1a, b). Moreover, small but significantly higher B concentration in buds was also observed in Cr-grafted plants as compared to Tr-grafted plants (Fig. 1a). The B concentration in the scion stem was significantly higher in Fs/Cr than in the other three combinations under B-deficient conditions (Fig. 1c). In addition, the B concentrations in the rootstock stem and roots of Fs/Cr were decreased relatively less than those of the other combinations when B was limited (Fig. 1d, e).

Under B-adequate conditions, B distribution in leaves of Fs-grafted plants was significantly lower than that of Ns-grafted plants, and an opposite trend was observed in roots (Fig. 2a). Under B-deficient conditions, however, no significant difference was found in leaves and roots between Fs- and Ns-grafted plants (Fig. 2b). That is, B distribution of Fs-grafted plants was reduced less in leaves, but more in roots than that of Ns-grafted plants when B was limited. Moreover, B distribution in the scion stem was

Table 1 Dry weight (DW: grams per plant) in different parts of four citrus scion–rootstock combinations under B-adequate (0.25 mg/L) and B-deficient (0.001 mg/L) conditions

Scion/ rootstock	Buds		Leaves		Scion stem		Rootstock stem		Roots	
	0.25	0.001	0.25	0.001	0.25	0.001	0.25	0.001	0.25	0.001
Ns/Tr	3.4b (0.3)	3.2b (0.4)	14.7ab (1.6)	15.2a (1.9)	15.2a (1.1)	16.5ab (1.5)	10.0ab (1.5)	10.4a (1.4)	26.4a (2.4)	29.5a (3.7)
Fs/Tr	3.7b (0.2)	3.3b (0.4)	10.3bc (1.2)	13.2a (1.3)	13.1a (1.2)	12.9b (1.2)	8.1b (1.6)	9.7a (0.7)	20.1a (1.8)	26.6a (1.8)
Ns/Cr	4.1b (0.4)	3.4b (0.9)	16.2a (3.0)	13.4a (2.6)	15.5a (0.9)	18.0a (1.2)	8.4b (0.9)	9.4a (1.4)	21.3a (2.8)	25.7a (1.8)
Fs/Cr	5.8a (0.2)	6.0a (0.4)	8.2c (0.9)	11.1a (1.4)	13.4a (0.6)	13.3b (0.6)	12.6a (0.8)	9.1a (0.3)	22.9a (1.5)	26.6a (2.4)

Values are means of four replicates with SE in parentheses. Different letters within each column indicate significant differences between combinations at $p < 0.05$

Ns ‘Newhall’ navel orange scion, Fs ‘Fengjie-72’ navel orange scion, Tr trifoliate orange rootstock, Cr Carrizo citrange rootstock

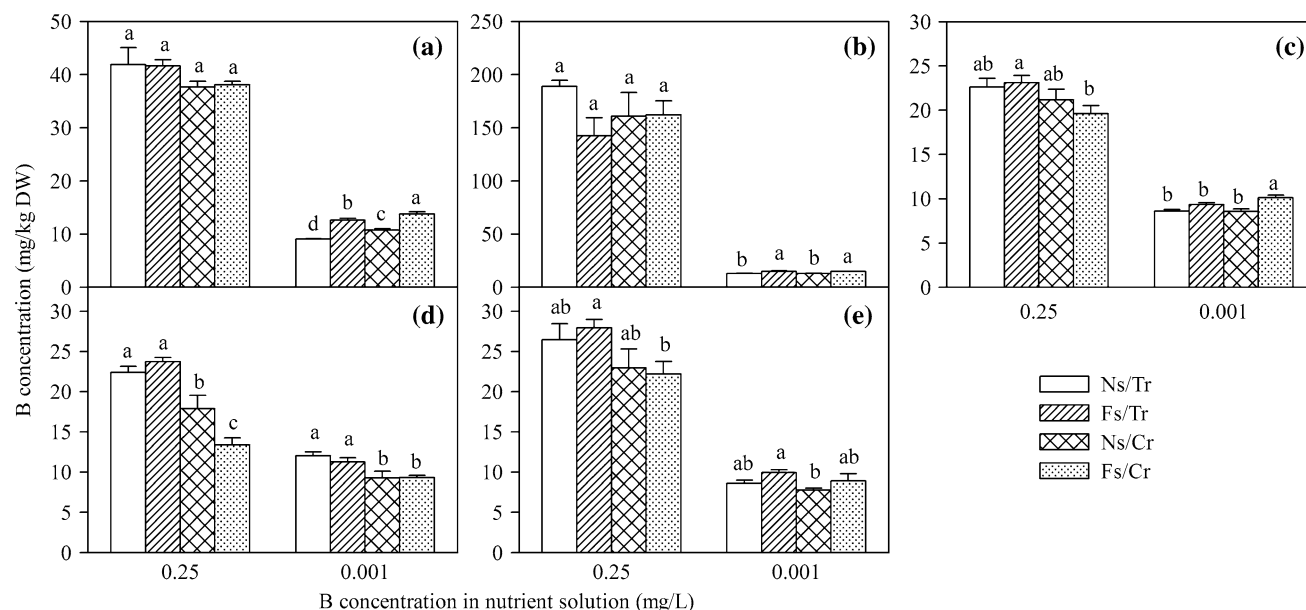


Fig. 1 Boron concentrations of **a** buds, **b** leaves, **c** stem of scion, **d** stem of rootstock, and **e** roots in four citrus scion–rootstock combinations under B-adequate (0.25 mg/L) and B-deficient (0.001 mg/L) conditions. Values are means of four replicates \pm SE.

Bars with different letters indicate significant differences between combinations for the same B level at $p < 0.05$. Ns ‘Newhall’ navel orange scion, Fs ‘Fengjie-72’ navel orange scion, Tr trifoliate orange rootstock, Cr Carrizo citrange rootstock

significantly higher in Ns/Cr (24 %) than in the other three combinations (17–19 %) under B-deficient conditions (Fig. 2b). Additionally, B distribution in the rootstock stem was higher in Ns/Tr than in Fs/Cr, with Ns/Cr and Fs/Tr being between the two (Fig. 2b).

Ratio of boron concentration between various plant parts

At adequate B supply, there was no clear relationship between B efficiency and the ratio of B concentration in different plant parts. Under B-deficient conditions, however, a correlation was established, i.e., the ratio of B concentration in the rootstock stem to the scion stem

decreased with increasing B efficiency among combinations (Table 2). Similarly, the ratio in the buds to the leaves increased as B efficiency increased. These results suggested that these two ratios might be used as selection criteria for B efficiency of different citrus scion–rootstock combinations. Moreover, Fs-grafted plants had a higher ratio of B concentration in the buds to the scion stem than Ns-grafted plants under B-deficient conditions, particularly in Tr (B-inefficient rootstock; Table 2), suggesting that this ratio could serve as an indicator for evaluating B efficiency between the citrus scions. Additionally, there were no differences in the ratios of the roots or leaves to the scion stem among combinations (data not shown).

Fig. 2 Boron distribution in different parts of four citrus scion–rootstock combinations under **a** B-adequate (0.25 mg/L) and **b** B-deficient (0.001 mg/L) conditions. Values are means of four replicates. Different letters indicate significant differences between combinations for the same plant part at $p < 0.05$. *Ns* ‘Newhall’ navel orange scion, *Fs* ‘Fengjie-72’ navel orange scion, *Tr* trifoliate orange rootstock, *Cr* Carrizo citrange rootstock

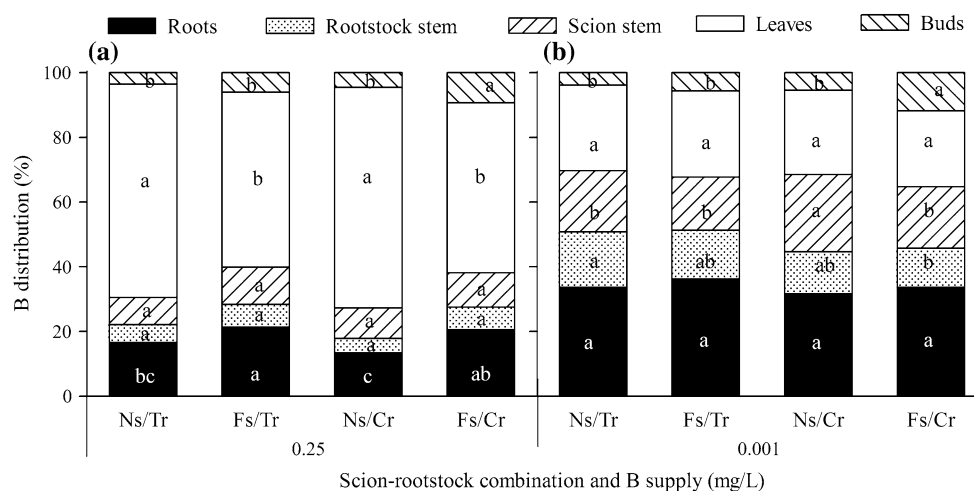


Table 2 The ratio of B concentration in various plant parts under B-adequate (0.25 mg/L) and B-deficient (0.001 mg/L) conditions

Scion/rootstock	Ratio of B concentration						B efficiency
	Rootstock stem: scion stem		Buds: leaves		Buds: scion stem		
	0.25	0.001	0.25	0.001	0.25	0.001	
Ns/Tr	0.99a	1.40a	0.22a	0.70c	1.86a	1.05c	Low
Fs/Tr	1.03a	1.20b	0.30a	0.85b	1.80a	1.35a	Medium
Ns/Cr	0.84b	1.08b	0.25a	0.83b	1.79a	1.26b	Medium
Fs/Cr	0.68c	0.93c	0.24a	0.93a	1.96a	1.36a	High

Values are means of four replicates. Different letters within each column indicate significant differences between combinations at $p < 0.05$

Low represents the grafted combination without B-efficient rootstock and scion, *medium* represents the grafted combination with either B-efficient rootstock or scion, and *high* represents the grafted combination with both B-efficient rootstock and scion

Ns ‘Newhall’ navel orange scion, *Fs* ‘Fengjie-72’ navel orange scion, *Tr* trifoliate orange rootstock, *Cr* Carrizo citrange rootstock

Concentration and ratio of boron forms in leaves and roots

The concentration of bound B in leaves was significantly lower in Fs-grafted than Ns-grafted plants with the same rootstock under B-adequate conditions (Fig. 3a). A similar pattern was also observed for free B and semi-bound B in both leaves and roots (Fig. 3a, c), though it was not statistically significant. In B-deficient treatments, however, the free B concentration in leaves of Cr-grafted plants was significantly higher than that of Tr-grafted plants, whereas the bound B concentration was remarkably higher in Fs-grafted than Ns-grafted plants (Fig. 3b). Moreover, the concentration of semi-bound B in leaves was highest in Fs/Cr, followed by Fs/Tr and Ns/Cr, and lowest in Ns/Tr (Fig. 3b). Interestingly, the concentrations in roots of free B and semi-bound B were reduced more by B-deficient treatment in Fs/Tr than in Ns/Tr, whereas those of free B and bound B were less decreased in Fs/Cr than in Ns/Cr, when compared with B-adequate treatment (Fig. 3c, d). In

addition, the concentrations of free B, semi-bound B, and bound B with B-deficient treatment were decreased by 97, 94, and 65 % in leaves and by 87, 75, and 29 % in roots, respectively, in comparison to the B-adequate treatment (Fig. 3).

The bound B ratio in leaves was significantly lower in Cr-grafted than Tr-grafted plants under B-limited conditions (Fig. 4b), although no significant difference was observed under B-adequate conditions (Fig. 4a). Furthermore, the ratio of semi-bound B in leaves was significantly higher in Fs/Cr than in the other three combinations, while that of free B was considerably higher in Ns/Cr than in the other combinations (Fig. 4b). That is, the ratio of available B (free B and semi-bound B) in leaves was higher in Cr-grafted plants (36 %) than in Tr-grafted plants (29 %) when B was limited (Fig. 4b). By contrast, the available B ratio in roots of Fs-grafted plants was significantly lower (22 %) than that of Ns-grafted plants (28 %) under B-deficient conditions, and the opposite was true for bound B ratio (Fig. 4d).

Fig. 3 Concentrations of B forms (free B, semi-bound B and bound B) in leaves **a, b** and roots **c, d** in four citrus scion–rootstock combinations under **(a, c)** B-adequate (0.25 mg/L) and **(b, d)** B-deficient (0.001 mg/L) conditions. Values are means of four replicates \pm SE. Bars with different letters indicate significant differences between combinations for the same B form at $p < 0.05$. *Ns* ‘Newhall’ navel orange scion, *Fs* ‘Fengjie-72’ navel orange scion, *Tr* trifoliate orange rootstock, *Cr* Carrizo citrange rootstock

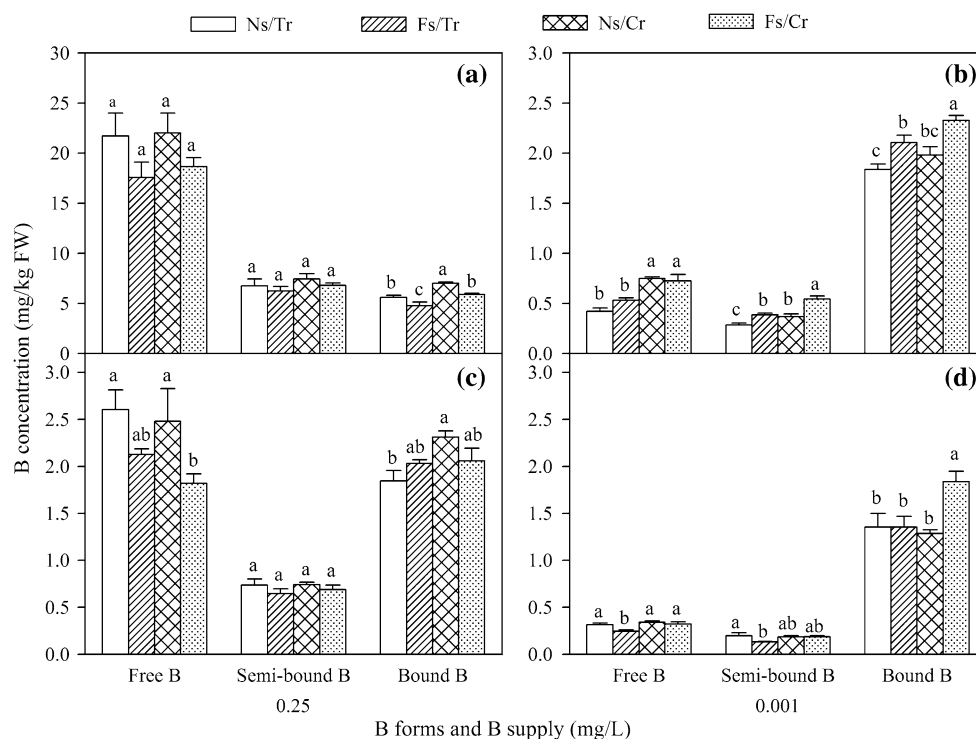
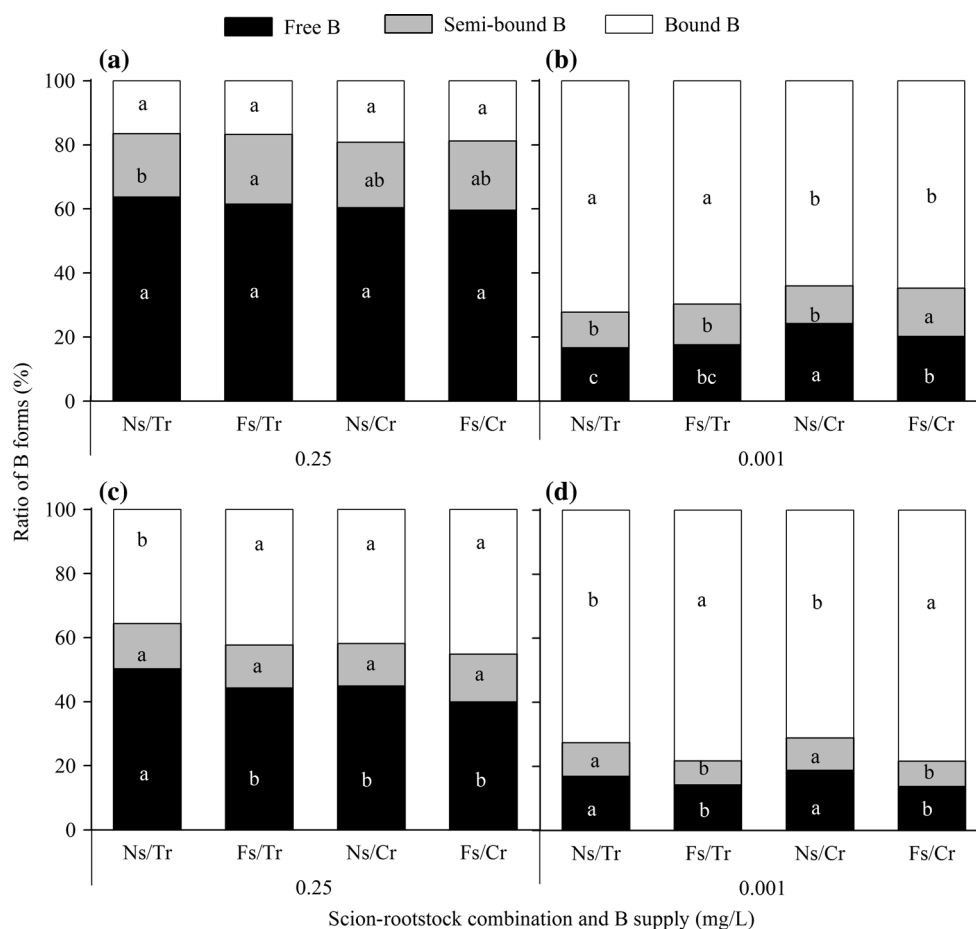


Fig. 4 Ratio of B forms in leaves **(a, b)** and roots **(c, d)** in four citrus scion–rootstock combinations under B-adequate (0.25 mg/L) and B-deficient (0.001 mg/L) conditions. Values are means of four replicates. Different letters indicate significant differences between combinations for the same B form at $p < 0.05$. *Ns* ‘Newhall’ navel orange scion, *Fs* ‘Fengjie-72’ navel orange scion, *Tr* trifoliate orange rootstock, *Cr* Carrizo citrange rootstock



Discussion

Relationship between B efficiency and B concentration and distribution

Due to the reproductive stage being more sensitive than the vegetative stage to low B supply (Asad et al. 2002), we selected the reproductive stage as the sampling period. However, no significant difference was found in the total dry weight among the four scion–rootstock combinations (calculated by Table 1), probably a consequence of the occurrence of naturally fallen leaves during the reproductive period. By contrast, the dry weight in leaves, scion stem, and rootstock stem was affected by B treatments between Fs/Cr and Ns/Cr (Table 1), indicating that Fs/Cr can distribute more dry matter into leaves from scion stem and rootstock stem than Ns/Cr under B-deficient conditions. Huang et al. (2008) demonstrated that previously acquired B in mature leaves can be retranslocated into the rapidly growing young reproductive organs under interrupted B supply. By contrast, under continuously B-deficient conditions, greater B efficiency was associated with a stronger capability for long-distance transport of B from the rooting medium into the ear of wheat via the xylem (Nachiangmai et al. 2004). In the current study, B-efficient Fs and Cr had a significantly higher B concentration in buds than B-inefficient Ns and Tr, respectively (Fig. 1a). This might be attributed to the greater amount of B distributed into the growing part (buds) that increases the opportunity for reproductive success (Nachiangmai et al. 2004). Our results also indicated that B concentration in leaves was significantly higher in Fs-grafted than Ns-grafted plants (Fig. 1b), consistent with the work of Sheng et al. (2009a). Together, these results suggest that B efficiency might be related to B concentration in buds and leaves rather than other plant organs at the reproductive stage.

Previous studies showed that within the same species B-efficient cultivars have greater ability to redistribute B than B-inefficient ones (Stangoulis et al. 2001; Liu et al. 2012). The B distribution in the scion stem of Ns/Cr was significantly higher than that of the other three combinations under B-deficient conditions (Fig. 2b). This is inconsistent with the results obtained by Sheng et al. (2009b), Liu et al. (2011), who reported that more B was distributed in the rootstock part for Ns/Cr as compared to Ns/Tr. This discrepancy can be explained by the sampling periods (i.e., vegetative stage versus reproductive stage). Due to higher B requirement for the reproductive stage (Dell and Huang 1997), the B-efficient Cr transported more B into the stem of the scion Ns. However, the B-inefficient Ns was unable to further retranslocate B efficiently into other scion parts, resulting in a higher B distribution in the

scion stem. Our results also showed that B distribution in leaves declined less in Fs-grafted than Ns-grafted plants under B-limited conditions (Fig. 2b), which coincided with our previous study (Sheng et al. 2009a). These results indicate that Fs may redistribute B more efficiently within the plant under B-deficient conditions, whereas Cr may transport more B into the scion, thereby improving B distribution to a less degree.

Indicator of B efficiency in different scion–rootstock combinations

In general, the ratio of B concentration in different parts is mainly used to evaluate the ability of B retranslocation among various species (Brown and Shelp 1997; Konsaeng et al. 2005). However, Stangoulis et al. (2001) proposed that the ratio of B concentration in younger to older leaves might be used as an indicator of B efficiency in canola. Moreover, significant differences were also observed in the B ratio of sink to source tissues between the cultivars in citrus (Boaretto et al. 2008), rutabaga (Shelp and Shattuck 1987), and broccoli (Shelp et al. 1992). In the present study, a correlation was established between B efficiency and the ratio of B concentration in different plant parts under B-deficient conditions, such as the ratio of the rootstock stem to the scion stem (Table 2). Since all commercial citrus are grafted trees, these ratios might provide an effective approach to evaluate differences in B efficiency either between scion–rootstock combinations or between the scions. The possible use of these ratios needs to be examined with a large number of scion–rootstock combinations.

Relationship between B efficiency and B forms

The free B concentration in leaves of Cr-grafted plants was significantly higher than that of Tr-grafted plants (Fig. 3b), consistent with the results of Liu et al. (2011). However, the value of free B in the present study was two to four times higher (17.6–22.0 mg/kg FW) than that obtained by Liu et al. (3.9–6.9 mg/kg FW). This could be explained by leaf age: older leaves were used in the present study (8-month-old versus 3-month-old leaves).

Using grafting experiment, Yang et al. (2013a) demonstrated that B efficiency of *Brassica napus* is controlled primarily by roots. By contrast, differences in B efficiency in the grafted citrus originated from both the rootstock and the scion. For example, the concentrations of free B and semi-bound B in roots were reduced more in Fs/Tr than in Ns/Tr under B-deficient conditions (Fig. 3d), and the opposite was true in leaves for total B concentration (Fig. 1b). These findings indicate that B-efficient Fs might facilitate B transport from B-inefficient Tr to the scion.

Similar results have been found in two tomato cultivars, Rutgers (B-efficient) and T3238 (B-inefficient; Brown and Jones 1971). However, some discrepancies existed. In tomato, no significant difference was observed in sap B concentration in roots, presumably due to the inherent differences in root traits between tomato cultivars. Because of the same rootstock used in the present study, significant difference was found in roots (Fig. 3d). Also, the concentrations of free B and bound B in roots were decreased less in Fs/Cr than in Ns/Cr under B-deficient conditions (Fig. 3d) and the same was true in leaves for total B concentration (Fig. 1b). This was attributed partly to the less B demand in Fs (Fig. 3a), and partly to the greater ability of Cr to take up B from external solution (Liu et al. 2013).

Generally, B efficiency is not only associated with the less pectin content in the cell wall (Hu et al. 1996; Yang et al. 2002; Pan et al. 2012), but also with the cell wall components (Yang et al. 2005; Liu et al. 2013). Our results indicated that bound B (B in cell wall) concentration in leaves was significantly lower in Fs-grafted than Ns-grafted plants (Fig. 3a), which can cross-link pectic polysaccharides through borate-diol bonding of two rhamnogalacturonan II molecules (Kobayashi et al. 1996; O'Neill et al. 1996). However, further work should be conducted to investigate whether differences in cell wall components exist between Fs and Ns.

The ratio of B forms can reflect the balance of subcellular compartmentation of B (Dannel et al. 2002; Du et al. 2002). Free B (B in apoplast) appears to only play a role in maintaining the concentration gradient between nutrient solution and the root symplasm through the intracellular formation of B complexes (Dannel et al. 2002). However, semi-bound B is not only responsible for synthesizing cell wall materials, but also for involving in plant metabolism (Pfeffer et al. 1999, 2001; Dannel et al. 2002; Du et al. 2002; Liu et al. 2011). In leaves, the ratio of semi-bound B was considerably higher in Fs/Cr than in the other three combinations, whereas that of free B was significantly higher in Ns/Cr than in the other combinations (Fig. 4b). These results suggest that Cr enhanced the transport of available B (free B and semi-bound B) into leaves, and that Fs employed more available B to involve in metabolism and to synthesize cell wall under B-limited conditions. The bound B ratio in roots was higher in Fs-grafted than Ns-grafted plants under B-deficient conditions (Fig. 4d). This implies that Fs may either facilitate the transport of B into the scion or maintain the integrity of cell wall in roots.

Model for responses of B forms and its insight into B-deficient diagnosis

In sunflower, the level of cell wall-bound B was less affected than that of cell sap B by short-term low B

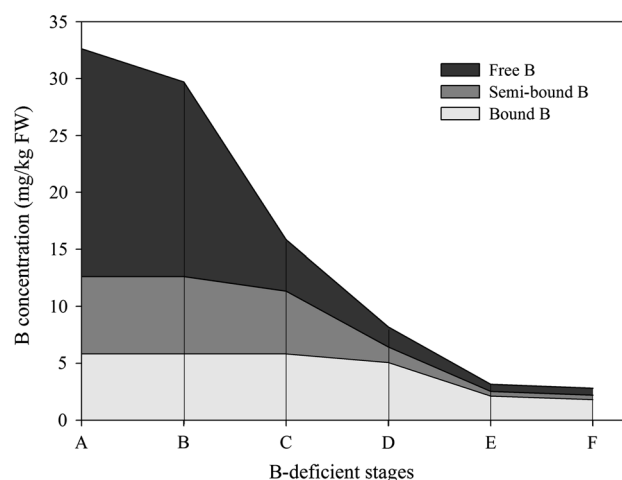


Fig. 5 Model for successive responses of B forms in citrus leaves to B deficiency: when a plant suffers from B deficiency (a), free B first slightly declines before reaching its critical level (b); then free B is sharply reduced, and semi-bound B begins to fall before reaching its critical level (c). Subsequently, semi-bound B also decreases dramatically, and bound B begins to decline before the critical level of bound B is reached (d). Finally, bound B decreases substantially, and the symptoms of B deficiency occur (e) until the plant dies (f)

treatment, irrespective of plant part and B concentration (Dannel et al. 1998; Matoh and Ochiai 2005). Similarly, after a long-term low B treatment, bound B concentrations were reduced by 65 % in leaves and 29 % in roots, respectively, being less than free B and semi-bound B (Fig. 3). Although the two species differ, these results suggest that, at least to some extent, there might be a spatial-temporal difference in the response of three B forms to low B supply. Matoh (1997) proposed that when the B supply to plant roots is high, B is initially accumulated in leaf cell walls and then may intrude into the cytoplasm, disturbing cytoplasmic metabolism, and finally result in the development of B toxicity symptoms. Here, we speculate that the relationship between B forms at a subcellular level under B-deficient conditions could be described as a continuum (Fig. 5). This model is mainly based on three aspects. First, the stability of B forms followed the order: bound B > semi-bound B > free B. Under B-deficient conditions, the most unstable free B initially declined, followed by semi-bound B, and eventually bound B decreased (Dannel et al. 2002; Matoh and Ochiai 2005; Fig. 3). Second, each B form had its own critical level (Goldbach et al. 2000; Liu et al. 2012). After suffering B deficiency, the concentration of each B form decreased sharply (provided that the decline was 70–80 % of the control) when reaching its own critical level. At other B-deficient stages, the concentration declined slightly or remained stable (provided that the decline was 5–15 % of the control). Third, the B form concentrations in leaves of B-adequate and B-deficient treatments were employed

as point A and E, respectively (Yang et al. 2013b; Fig. 3). This model presumably reflects that the sufficiency of free B contributes to the stability of semi-bound B, and thus maintains the stability of bound B (Fig. 5). It provides a possible explanation for B changes at the subcellular level.

Several researchers suggested that the soluble B pool (cell sap) might be a better indicator of the B status of plants than the total B content (Bell 1997; Goldbach et al. 2000; Dannel et al. 2002). However, soluble B can be further divided into free B and semi-bound B. Semi-bound B has a relatively stable concentration and might be involved in cytoplasmic metabolism, whereas free B shows a larger concentration fluctuation and has few direct physiological functions (Dannel et al. 2002; Match and Ochiai 2005). When semi-bound B begins to decline, that means the cytoplasmic metabolism has already been influenced by B deficiency. Therefore, semi-bound B might be a more reliable indicator of adequate B status than soluble B and a more sensitive indicator than total B.

Conclusion

Four citrus scion–rootstock combinations with contrasting B efficiency were used to investigate differential responses to B deficiency. Differences in B distribution and forms were observed between the scions or rootstocks, which resulted in differential B efficiency among scion–rootstock combinations. Our results suggested that B efficiency among combinations might be evaluated by the ratios of B concentrations in different parts under low B conditions, such as in the rootstock stem to scion stem, in buds to leaves, or in buds to scion stem. However, this requires to be examined by other citrus scion–rootstock combinations. Further investigations should be conducted to explore other B efficiency traits and help us better understand the B nutrition in grafted citrus plants.

Author contribution N. Wang performed the experiment and interpreted the results; N. Wang and Y. Liu drafted the manuscript; T. Yan and L. Fu participated in boron analysis; S. Peng and G. Zhou participated in research design; S. Peng proposed and supervised the overall project. All authors read and approved the final manuscript.

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Conflict of interest The authors declare that they have no competing interests.

References

- Asad A, Blamey F, Edwards D (2002) Dry matter production and boron concentrations of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution. *Plant Soil* 243:243–252
- Bell RW (1997) Diagnosis and prediction of boron deficiency for plant production. *Plant Soil* 193:149–168
- Bellaloui N, Brown PH (1998) Cultivar differences in boron uptake and distribution in celery (*Apium graveolens*), tomato (*Lycopersicon esculentum*) and wheat (*Triticum aestivum*). *Plant Soil* 198:153–158
- Boaretto RM, Quaggio JA, Mourão Filho FAA, Giné MF, Boaretto AE (2008) Absorption and mobility of boron in young citrus plants. *Commun Soil Sci Plan* 39:2501–2514
- Brown JC, Jones WE (1971) Differential transport of boron in tomato (*Lycopersicon esculentum* Mill.). *Physiol Plant* 25:279–282
- Brown PH, Shelp BJ (1997) Boron mobility in plants. *Plant Soil* 193:85–101
- Chen LS, Han S, Qi YP, Yang LT (2012) Boron stresses and tolerance in citrus. *Afr J Biotechnol* 11:5961–5969
- Dannel F, Pfeffer H, Römhelt V (1998) Compartmentation of boron in roots and leaves of sunflower as affected by boron supply. *J Plant Physiol* 153:615–622
- Dannel F, Pfeffer H, Römhelt V (2002) Update on boron in higher plants: uptake, primary translocation and compartmentation. *Plant Biol* 4:193–204
- Dell B, Huang L (1997) Physiological response of plants to low boron. *Plant Soil* 193:103–120
- Du CW, Wang YH, Xu FS, Yang YH, Wang HY (2002) Study on the physiological mechanism of boron utilization efficiency in rape cultivars. *J Plant Nutr* 25:231–244
- Goldbach HE, Wimmer MA, Findelee P (2000) Boron: how can the critical level be defined? *J Plant Nutr Soil Sci* 163:115–121
- Graham RD (1984) Breeding for nutritional characteristics in cereals. *Adv Plant Nutr* 1:57–102
- Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. *Calif Agric Exp Stn Circ* 347. Berkeley, CA: College of Agriculture, University of California
- Hu H, Brown PH, Labavitch JM (1996) Species variability in boron requirement is correlated with cell wall pectin. *J Exp Bot* 47:227–232
- Huang L, Bell RW, Dell B (2008) Evidence of phloem boron transport in response to interrupted boron supply in white lupin (*Lupinus albus* L. cv. Kiev Mutant) at the reproductive stage. *J Exp Bot* 59:575–583
- Kobayashi M, Matoh T, Azuma J (1996) Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. *Plant Physiol* 110:1017–1020
- Konsaeng S, Dell B, Rerkasem B (2005) A survey of woody tropical species for boron retranslocation. *Plant Prod Sci* 8:338–341
- Liu GD, Jiang CC, Wang YH (2011) Distribution of boron and its forms in young ‘Newhall’ navel orange (*Citrus sinensis* Osb.) plants grafted on two rootstocks in response to deficient and excessive boron. *Soil Sci Plant Nutr* 57:93–104
- Liu GD, Wang RD, Wu LS, Peng SA, Wang YH, Jiang CC (2012) Boron distribution and mobility in navel orange grafted on citrange and trifoliolate orange. *Plant Soil* 360:123–133
- Liu GD, Wang RD, Liu LC, Wu LS, Jiang CC (2013) Cellular boron allocation and pectin composition in two citrus rootstock seedlings differing in boron-deficiency response. *Plant Soil* 370:555–565
- Loomis WD, Durst RW (1992) Chemistry and biology of boron. *BioFactors* 3:229–239

- Marschner P (2012) Marschner's mineral nutrition of higher plants, 3rd edn. Academic press, London
- Matoh T (1997) Boron in plant cell walls. *Plant Soil* 193:59–70
- Matoh T, Ochiai K (2005) Distribution and partitioning of newly taken-up boron in sunflower. *Plant Soil* 278:351–360
- Nachiangmai D, Dell B, Bell R, Huang L, Rerkasem B (2004) Enhanced boron transport into the ear of wheat as a mechanism for boron efficiency. *Plant Soil* 264:141–147
- O'Neill MA, Warrenfeltz D, Kates K, Pellerin P, Doco T, Darvill AG, Albersheim P (1996) Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cell, forms a dimer that is covalently cross-linked by a borate ester in vitro conditions for the formation and hydrolysis of the dimer. *J Biol Chem* 271:22923–22930
- Pan Y, Wang ZH, Yang L, Wang ZF, Shi L, Naran R, Azadi P, Xu FS (2012) Differences in cell wall components and allocation of boron to cell walls confer variations in sensitivities of *Brassica napus* cultivars to boron deficiency. *Plant Soil* 354:383–394
- Papadakis I, Dimassi K, Therios I (2003) Response of two citrus genotypes to six boron concentrations: concentration and distribution of nutrients, total absorption, and nutrient use efficiency. *Anst J Plant Physiol* 54:571–580
- Pfeffer H, Dannel F, Römhelt V (1999) Isolation of soluble boron complexes and their determination together with free boric acid in higher plants. *J Plant Physiol* 154:283–288
- Pfeffer H, Dannel F, Römhelt V (2001) Boron compartmentation in roots of sunflower plants of different boron status: a study using the stable isotopes ^{10}B and ^{11}B adopting two independent approaches. *Physiol Plant* 113:346–351
- Rerkasem B, Jamjod S (1997) Genotypic variation in plant response to low boron and implications for plant breeding. *Plant Soil* 193:169–180
- Shelp BJ, Shattuck VI (1987) Boron nutrition and mobility, and its relation to the elemental composition of greenhouse grown root crops I Rutabaga. *Commun Soil Sci Plan* 18:187–201
- Shelp BJ, Penner R, Zhu Z (1992) Broccoli (*Brassica oleracea* var. *italica*) cultivar response to boron deficiency. *Can J Plant Sci* 72:883–888
- Sheng O (2009) The effects and physiological mechanisms of boron deficiency on 'Newhall' navel orange (*Citrus sinensis* Osbeck). PhD dissertation, Huazhong Agricultural University
- Sheng O, Song SW, Chen YJ, Peng SA, Deng XX (2009a) Effects of exogenous B supply on growth, B accumulation and distribution of two navel orange cultivars. *Trees* 23:59–68
- Sheng O, Song SW, Peng SA, Deng XX (2009b) 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. *Sci Hortic* 121:278–283
- Shorrocks VM (1997) The occurrence and correction of boron deficiency. *Plant Soil* 193:121–148
- Smith P, Reuther W (1949) Observations on boron deficiency in citrus. *Proc Fla State Hortic Soc* 62:21–37
- Stangoulis JCR, Brown PH, Bellaloui N, Reid RJ, Graham RD (2001) The efficiency of boron utilisation in canola. *Anst J Plant Physiol* 28:1109–1114
- Takano J, Yamagami M, Noguchi K, Hayashi H, Fujiwara T (2001) Preferential translocation of boron to young leaves in *Arabidopsis thaliana* regulated by the *BOR1* gene. *Soil Sci Plant Nutr* 47:345–357
- Warington K (1923) The effect of boric acid and borax on the broad bean and certain other plants. *Ann Bot* 37:629–672
- Xiao JX, Yan X, Peng SA, Fang YW (2007) Seasonal changes of mineral nutrients in fruit and leaves of 'Newhall' and 'Skagg's Bonanza' navel oranges. *J Plant Nutr* 30:671–690
- Yang YH, Du CW, Wu LS, Wang YH (2002) Boron distribution in the cell wall in different boron efficiency rape cultivars (*Brassica napus*). *J Plant Physiol Mol Biol* 28:339–343
- Yang YH, Yu M, Du CW, Wu LS (2005) Boron distribution in cell wall components in rape cultivars (*Brassica napus*) with different boron use efficiency. *Acta Agronomica Sinica* 31:608–611
- Yang CQ, Liu YZ, An JC, Li S, Jin LF, Zhou GF, Wei QJ, Yan HQ, Wang NN, Fu LN, Peng SA (2013a) Digital gene expression analysis of corky split vein caused by boron deficiency in 'Newhall' navel orange (*Citrus sinensis* Osbeck) for selecting differentially expressed genes related to vascular hypertrophy. *PLoS One* 8:e65737
- Yang L, Zhang Q, Dou JN, Li L, Guo LF, Shi L, Xu FS (2013b) Characteristics of root boron nutrition confer high boron efficiency in *Brassica napus* cultivars. *Plant Soil* 371:95–104
- Zarcinas B, Cartwright B, Spouncer L (1987) Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun Soil Sci Plan* 18:131–146
- Zhou GF, Peng SA, Liu YZ, Wei QJ, Han J, Islam MZ (2014) The physiological and nutritional responses of seven different citrus rootstock seedlings to boron deficiency. *Trees* 28:295–307