CrossMark

ORIGINAL ARTICLE

Boron deficiency affects root vessel anatomy and mineral nutrient allocation of *Poncirus trifoliata* (L.) Raf.

Li Mei[1] · Qiaohong Li[1] · Huan Wang[1] · Ou Sheng[2] · Shu-ang Peng[1]

Abstract Citrus plants are frequently exposed to boron (B) deficiency, which has reduced citrus production worldwide. The effects of boron deficiency on citrus rootstock mineral absorption and allocation, and cell and vessel element anatomy are very important in understanding the nutrient absorbing mechanisms and resolving the boron deficiency problems of citrus production. *Poncirus trifoliata* seedlings were grown in Hoagland's solution, which contained 0.25 μM H_3BO_3 (moderate B) and 0 μM H_3BO_3 (B deficiency). Seedling growth, root tip cell and vessel anatomy, and nutrient contents were investigated 30 days after treatment. B deficiency significantly inhibited the growth of *Poncirus trifoliata* seedlings, significantly decreased the mean lateral root length and root number, and significantly increased the root diameter and lateral root primordial (LRP) density. Ultrathin section micrographs showed a thickened root tip cell wall and more attachments on the cell wall in the boron-deficient treatment. The vessel wall of all root orders was markedly thickened and the inner vessel diameter decreased in the boron-deficient treatment. The boron and calcium contents in the leaf, stem and root were significantly decreased, and the phosphorus content decreased significantly in the leaf but not in stem or root in the boron-deficient treatment. The changes in root morphology and vessel element anatomical characteristics completely inhibited mineral nutrient absorption and transportation, resulting in a limited nutrient supply and reduced growth of *Poncirus trifoliata* in the boron-deficient treatment.

Keywords *Poncirus trifoliata* (L.) Raf. · Root morphology · Boron · Vessel

Introduction

Boron (B) is essential for the growth and development of higher plants. There are numerous reports of B deficiency affecting the productivity and quality of many agricultural crops in high precipitation areas in the past decades (Goldbach and Wimmer 2007; Han et al. 2008, 2009; Camacho-Cristóbal et al. 2008a; Ahmad et al. 2012; Bogiani et al. 2014). B deficiency causes many anatomical, physiological, and biochemical changes, such as impaired cell elongation, pollen germination and pollen tube growth. A particular role of B in cell wall formation was found to be attributed to B crosslinking with rhamnogalacturonan II molecules, and regulation of the transcript level of genes involved in cell wall synthesis and modification (Kobayashi et al. 1996; Camacho-Cristóbal et al. 2011). Although this has advanced our understanding, most of the research on physiological processes in response to B deficiency has focused on aboveground structures, and more research is required on the effect of B deficiency on root cell structure and nutrient absorption.

The roots are the main organs that penetrate the soil and are thus directly affected by B deficiency. It is, therefore,

✉ Li Mei
meili@mail.hzau.edu.cn

✉ Shu-ang Peng
179711808@qq.com; ganjuli_2002@mail.hzau.edu.cn

[1] Key Laboratory of Horticultural Plant Biology, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Ministry of Education of China, Wuhan 430070, China

[2] Institute of Fruit Tree Research, Guang Dong Academy of Agricultural Sciences, Guangzhou 510640, China

expected that the roots are the first to undergo B-deficient signals and develop corresponding symptoms (Goldbach et al. 2001). Such symptoms mainly include inhibition of root tip cell division and elongation (Dell et al. 1997), and suppression of the growth of the taproot and lateral root, which results in stubby root branching, root cracking or hollowing, or even necrosis (Martín-Rejano et al. 2011). Other researchers reported that B deficiency affects *Citrus* root morphology and physiological processes (Lu et al. 2014; Zhou et al. 2014). For example, it has been shown that B deficiency led to a decline in root and leaf nitrate contents (Camacho-Cristóbal and González-Fontes 2007) or the concentrations of other macronutrients such as magnesium, calcium, potassium or phosphate (Camacho-Cristóbal et al. 2005). However, knowledge on the changes in plant apical subcellular structure and root functions (e.g., mineral nutrient uptake) caused by the structural collapse of root cells is limited.

Boron is generally regarded as the most widespread limitation to *citrus* spp. productivity and yield stability in rain-fed production systems. Trifoliate orange (*Poncirus trifoliata*), which is the most widely used rootstock in citrus cultivation, the characteristics are of fundamental importance to soil exploration and below-ground resource acquisition, and hence are strongly related to plant adaptation to sub-optimal conditions such as B deficiency stress in citrus. The objectives of this study were (1) to determine the effects of B deficiency on root tip subcellular structure and mineral nutrient absorption, and (2) to clarify the relationships between vessel element structure and mineral nutrient absorption and allocation in response to B deficiency of trifoliate orange.

Materials and methods

Plant culture and treatments

Poncirus trifoliata seeds were surface sterilized for 20 min in 20 % sodium hypochlorite solution and then rinsed repeatedly in deionised water, placed on a porcelain tray with moistened gauze and transferred to an incubator at 30 °C. The seeds were moistened with deionised water during the entire incubation period to hasten seed germination.

Germinated seeds were transferred into plastic pots filled with vermiculite; 20 days (d) later, 200 uniform seedlings were selected, and the individual seedlings were solution-cultured in a plastic barrel covered with black plastic bags. The seedlings were fixed on a black foam board with holes and transferred to a greenhouse. The seedlings were cultured in whole Hoagland's solution for 10 d, and then the solution was replaced with 0.25 mM H_3BO_3 (control) or 0 mM H_3BO_3 (B deficiency). The

modified B-deficient full-strength nutrient solution contained 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 1 mM $NH_4H_2PO_4$, 2 mM $MgSO_4$, 9 μM $MnCl_2$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.01 μM H_2MoO_4, and 50 μM Fe-ethylenediaminetetraacetic acid or EDTA. All of the experimental studies were conducted in a greenhouse under controlled conditions of light, humidity, and temperature. The solution was ventilated for 2 h everyday and replaced once a week. The pH of all of the nutrient solutions was adjusted to 6.0 using 0.1 M KOH. The design of the experiment was completely randomized.

Plant growth parameters and mineral nutrient analysis

Six seedlings from both the control and B-deficient treatments were harvested at 0, 5, and 30 d after treatment. The seedlings were separated into roots, stems, and leaves and then weighed separately. Root morphological parameters were determined using an Epson scanner and WinRHIZO analysis system (Pro 2010a).

To determine the number of lateral root primordia (LRP), root tips without lateral roots were fixed using a formalin–acetic acid–ethanol solution (FAA) at 60 °C in 10 % HCl for 10 min, then transparented and dehydrated with a glycerol gradient series of 10, 30, 50, and 80 %. Transparent roots were placed on glass slides and photographed, and the numbers of LRP were counted using an Olympus differential interference contrast microscope.

The fresh tissues were dried in a forced oven at 70 °C for 48 h, and accurately weighed to determine the dry weight. A dried sample (0.50 g) was ashed in a muffle furnace at 500 °C for 6 h, followed by dissolution in 0.1 N HCl. B and other mineral nutrient contents of the plant samples were determined using ICP-AES (Thermo Inc, IRIS Advan, United States, Miwa et al. 2006).

Apical slice preparation and anatomical structure observation

Five days after the B-deficient treatment was imposed, small pieces of root tips (1 mm length, zone of maturation) and mature leaves were cut lengthways and initially fixed for 3 h at room temperature with 5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Ultrathin sections for electron microscopy were cut using a Reichert–Jung Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate and examined under a JEM 2000 FX II transmission electron microscope (TEM). Entire roots were observed with a Zeiss M63A stereoscope.

After washing in the buffer, some specimens were postfixed for 2 h with 2 % osmium tetroxide similarly buffered. The samples were dehydrated in an ascending ethanol

series followed by critical-point drying with carbon dioxide. Then, the dried samples were fixed on aluminum slides using double-sided adhesive tape. Finally, the samples were coated with gold and examined using a scanning electron microscope (SEM) (XL30 S-FEG, FEI).

Vessel element separation, photographs and parameter measurements

Different root orders above the root tips (zone of cell maturation) were sampled 30 days after treatment, treated with Jeffrey's Fluid (10 % chromic acid:10 % nitric acid = 1:1) at 37 °C for 72 h, then rinsed with deionized water, and stored in 70 % alcohol. Separated vessel elements were stained with safranin for 15–20 min and rinsed with deionized water, placed on microscope slides and examined under an Olympus BH-2 light microscope. A total of 100 vessel elements were randomly selected from each sample and photographed. Image-Pro Plus software was used to measure the length, diameter, wall thickness, and caliber of 100 vessel elements that were randomly selected.

Statistical analysis

The effects of B deficiency on the mineral nutrient content in different tissues were tested by factorial analysis of variance using the Systat v. 8.0 software package (SPSS Inc., Evanston, IL, USA). Significant differences between treatments were compared using the least significant difference test at the $P < 0.05$ probability level. The data are presented as mean values ± standard error (SE, $n = 6$).

Results

Plant growth and root morphology

The dry weights of the leaf, stem, and root were decreased in the B-deficient seedlings compared with the control (Table 1). The root number and total root length per plant were decreased and the lateral root diameter increased significantly compared with the control ($P < 0.05$) (Table 1; Fig. 1). The root tip swelling and growth point necrosis are shown in Fig. 1. Transparent root tissue showed that number of LRP was not affected although the lateral root number was decreased, lateral root elongation was inhibited, and the density of the LRP was increased in B-deficient seedlings.

Anatomical characteristics of root and leaf cells

SEM micrographs of the root tips showed the thickened cell wall and more attachments on the cell wall in the

Table 1 Plant dry weight (g plant⁻¹), root morphological parameters, number of lateral root primordia (LRP) (number per lateral root) and root density (No./cm) ($n = 3$, $p < 0.05$) 30 days after treatment

	Plant dry weight (g plant⁻¹)				Root morphological parameters				LRP	
	Leaf	Stem	Root	Total	Number	Length (mm)	Diameter (mm)	Total length (mm)	Number	Density
CK	0.22 ± 0.03a	0.24 ± 0.04a	0.12 ± 0.03a	0.58 ± 0.04a	116.3 ± 16.6a	24.68 ± 2.14a	2.98 ± 0.14b	3114.2 ± 582.4a	3.21 ± 0.19a	1.72 ± 0.10b
−B	0.18 ± 0.01a	0.19 ± 0.02a	0.10 ± 0.01a	0.47 ± 0.02b	93.3 ± 10.5b	11.18 ± 0.92b	3.32 ± 0.07a	1214.8 ± 164.6b	3.23 ± 0.27a	2.25 ± 0.12a

Lowercase letters indicate significant differences between the treatments ($P < 0.05$)

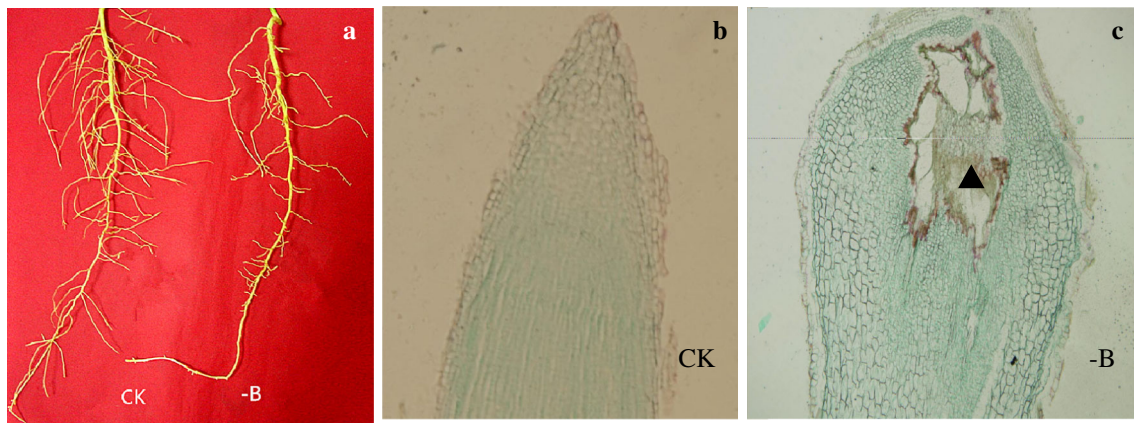CK control, −B boron deficiency; the same convention is used in the tables below

Fig. 1 Root morphology (**a**) and anatomy section (**b**, **c**) in control (*CK*) and boron deficiency (*−B*) treatments of trifoliate orange (*Poncirus trifoliata*). *CK* control, *−B* boron deficiency; the same convention is used in the figures below. *Triangle* indicates the necrosis in growth point of boron deficiency treatment

B-deficient treatment compared with the control (Fig. 2a, b). TEM micrographs of root cells showed that the cell wall thickened and separated, inside which materials accumulated in the B-deficient treatment. In addition, the cell nucleus was deformed, organelles were degraded or had disappeared, and there was a high density of electrical particles that were dispersed in the vacuole or combined with the cell wall (Fig. 2d). By contrast, the control had a thin cell wall and intact organelles that were structured and full of cytolymph (Fig. 2c). The leaf cells in the B-deficient treatment also had a thickened and separated cell wall, degraded chloroplasts, and more high-density electronic particles (Fig. 2e, f).

Root vessel element morphology and anatomical characteristics

Vessel elements that originated from the secondary xylem of the root were alternate pitted vessels with simple perforation (Fig. 3a–c). There were three different types of vessel elements: double tailed (Fig. 31, 2, 7, 8, 13, 14), single tailed (Fig. 33, 4, 9, 10, 15, 16) and those without a tail (Fig. 35, 6, 11, 12, 17, 18). In the whole root system, the proportion of double-tailed vessel elements was the largest, followed by those with a single tail and those without a tail. The proportions of vessel elements without a tail and those with a single tail increased while the proportion of those with double tails decreased from the taproot to the second-order lateral roots. Vessel length did not vary significantly between the different order roots, but the average diameter, inner diameter and vessel wall thickness decreased significantly from the taproot to the second-order lateral roots (Table 2).

The vessel length decreased in the first- and second-order roots in the B-deficient treatment. The vessel wall in all order roots thickened markedly and the inner vessel diameter of the first- and second-order roots decreased in the B-deficient treatment (Figs. 3, 4; Table 2, $P < 0.05$). TEM micrographs showed the material accumulated in the inner vessel walls, which led to the thickening of the vessel wall and a thin inner diameter in the B-deficient treatment compared with the control (Fig. 4). The proportion of double-tailed vessel elements decreased whereas those without a tail or a single tail increased in the B-deficient treatment (Table 2).

Mineral nutrient allocation

The foliar B, N, Ca, and Mg contents were significantly higher than those in the stem and root, while the Fe and Mn contents in the roots were higher than those in the stem and leaf of *Poncirus trifoliata* ($P < 0.05$). Under the condition of B deficiency, the B and Ca contents in the leaf, stem, and root were significantly decreased, while the N, Fe, and Mg contents showed no significant difference between treatments. The P content also decreased significantly in the leaf but not in the stem and root in the B-deficient treatment of *Poncirus trifoliata*. The Mg content exhibited a downward trend in all of the organs, but this was not significant in the leaf and root (Table 3, $P > 0.05$). Fe and Mn were mainly allocated to the root, while B, N, P, Ca, Mg were mainly allocated to the leaves. Under the condition of B deficiency, higher ratios of B and P were allocated to the roots (Table 3).

Discussion

B deficiency effect on root development and cell anatomy structure

The results of this research indicated that B deficiency inhibited the lateral root elongation of *Poncirus trifoliata*,

Fig. 2 Ultrathin section micrographs of root and leaf cells 5 days after treatment. Explanation of plate: **a**, **b** SEM micrographs of root cell (zone of maturation); **c**, **d** TEM micrographs of root cell (zone of maturation); **e**, **f** TEM micrographs of leaf cell (mature mesophyll cells). *CK* **a**, **c**, **e**; −B **b**, **d**, **f**. *CW* cell wall, *N* nucleus, *S* starch, *V* vacuole, *Chl* chloroplast; *triangle* indicates the isolation between cell wall; *star* indicates the accumulated materials
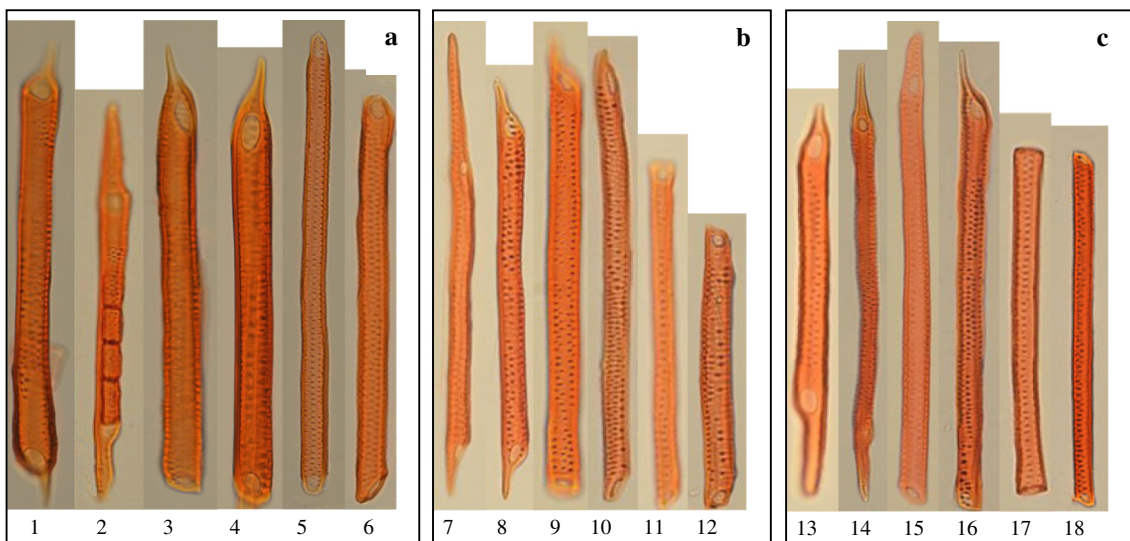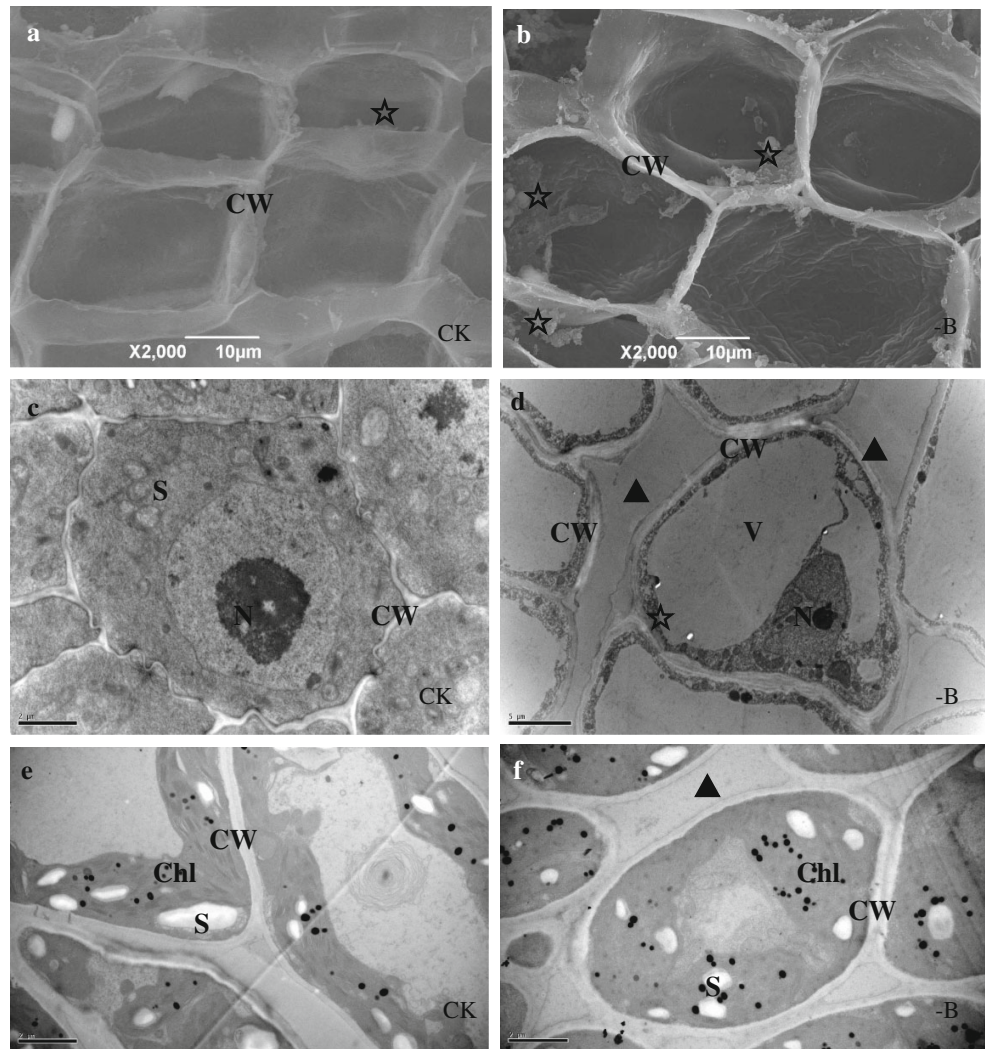

Fig. 3 Vessel elements dispersed from different order roots (zone of cell maturation) 30 days after treatment. **a** Taproot (*CK* 1, 3, 5; −B: 2, 4, 6); **b** first-order root (*CK* 7, 9, 11; −B 8, 10, 12); **c** second-order root (*CK* 13, 15, 17; −B 14, 16, 18)

Table 2 Morphological traits of the vessel elements in the control and B-deficient treatments ($n = 100$, $p < 0.05$) 30 days after treatment

	TPR (CK)	TPR (−B)	1st LR (CK)	1st LR (−B)	2nd LR (CK)	2nd LR (−B)
Vessel element length (μm)	247.99 ± 7.84ab	241.51 ± 8.47ab	274.08 ± 17.04a	228.11 ± 6.44b	273.40 ± 22.52a	242.13 ± 7.88ab
Vessel wall thickness (μm)	2.97 ± 0.09b	3.43 ± 0.11a	1.95 ± 0.06d	2.25 ± 0.08c	1.57 ± 0.05e	1.97 ± 0.05d
Inner vessel diameter (μm)	13.43 ± 0.41a	13.26 ± 0.43a	9.99 ± 0.27b	8.49 ± 0.25c	9.59 ± 0.31b	8.33 ± 0.26c
No tail (%)	4.04 ± 1.01c	7.07 ± 2.02cb	2.02 ± 2.02c	9.09 ± 1.75b	10.10 ± 2.02b	17.17 ± 2.67a
Single tail (%)	16.16 ± 1.36b	31.31 ± 5.34a	31.31 ± 4.40a	23.23 ± 2.02ab	29.29 ± 3.54a	26.26 ± 1.01ab
Double tail (%)	79.80 ± 4.40a	61.62 ± 6.14b	66.67 ± 3.50b	66.67 ± 1.75b	60.61 ± 3.03b	56.57 ± 2.67b

TPR taproot, *1st LR* first-order root, *2nd LR* second-order root
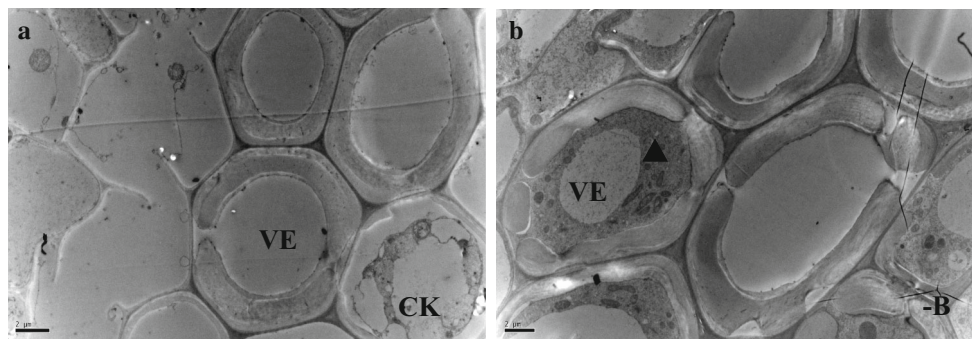
Fig. 4 TEM photographs of transaction vessel elements (VE) in root tips (zone of maturation) 5 days after treatment. **a** CK, **b** −B. *VE* vessel elements. *Triangle* indicates the accumulated materials

Table 3 Effect of B deficiency on mineral nutrient contents in different organs ($n = 3$, $p < 0.05$) 30 days after treatment

	Leaf (CK)	Leaf (−B)	Stem (CK)	Stem (−B)	Root (CK)	Root (−B)
B (μg g^{-1})	97.87 ± 6.75a	26.60 ± 6.20b	21.73 ± 0.71b	12.50 ± 0.97c	20.10 ± 0.67b	12.80 ± 0.86c
N (mg g^{-1})	2.91 ± 0.11a	2.87 ± 0.12a	1.11 ± 0.09c	1.33 ± 0.08c	1.78 ± 0.09b	1.82 ± 0.04b
P (mg g^{-1})	3.25 ± 0.10a	2.42 ± 0.31b	1.93 ± 0.26b	2.09 ± 0.15b	3.10 ± 0.36a	3.26 ± 0.10a
Ca (mg g^{-1})	12.51 ± 0.33a	10.31 ± 1.09b	7.16 ± 0.25c	5.29 ± 0.18d	5.26 ± 0.12d	4.07 ± 0.02e
Mg (mg g^{-1})	2.29 ± 0.16a	2.11 ± 0.20ab	1.78 ± 0.20b	1.34 ± 0.04c	1.13 ± 0.05c	1.01 ± 0.04c
Fe (μg g^{-1})	175.76 ± 4.31b	206.89 ± 17.66b	47.23 ± 6.01c	47.69 ± 0.90c	1218.80 ± 245.34a	1395.91 ± 359.57a
Mn (μg g^{-1})	65.44 ± 8.50b	62.47 ± 7.35b	28.59 ± 3.95c	22.47 ± 0.49c	265.19 ± 59.13a	231.18 ± 35.36a

Lowercase letters indicate significant differences between organs and treatments ($P < 0.05$)

which is consistent with the research results on other citrus root stocks (Mei et al. 2011; Zhou et al. 2014). In addition, our results showed that the root tips swelled and coarsened, and the number of LRP did not decrease in the B-deficient treatment (Table 1). The research results of Martín-Rejano et al. (2011) also indicate that a low B supply caused an inhibition of primary root (PR) growth without altering the number of lateral roots (LRs). These results showed that the effect of B deficiency on root elongation was greater than that on the number of lateral roots.

It is well known that B is a necessary material for the construction of the cell wall, which combined with pectic polysaccharides, contributes to the structure and function of the cell wall (Liu et al. 2013). B deficiency symptoms in the leaves and root tip cells include nuclear pycnosis, the disappearance of organelles, and pectin in the areas where the cell wall collapses (Fig. 2b, d, f). It is well known that considerable B is needed to construct the cell wall for the rapid cell division of the growing root tip. If B is not supplied promptly, the root tip cells will be severely limited by B, resulting in a reduction in the formation of pectic polysaccharide compounds at the growing point, which in turn affects cell wall construction (Läuchli 2002). Consequently, necrotic areas first

appear at the growing point. It has recently been shown that B deficiency causes a rapid decrease in the expression of several AGP genes, which have been suggested to be putative B-binding structures in *Arabidopsis* roots (Camacho-Cristóbal et al. 2008b, 2011). Therefore, it is important to point out that B might exert its action in membranes not only by stabilizing membrane molecules (Bolãnos et al. 2004), but also by regulating the expression of genes involved in membrane structure and function.

B deficiency effect on root vessel element structure

Vessel elements are the main type of cells involved in the transport of water and nutrients, and the structure of the vessel directly affects the efficiency of water and nutrient transport. Our results showed that the vessel cell wall had sediment, and the cell wall thickness was greater and the inner vessel diameter was smaller in the B-deficient treatment than in the control. These structural changes will reduce the functioning of the vessels in transporting nutrients and water. Covalently cross-linked pectin networks cannot form without B; therefore, the cell wall structure and function will be affected, such as cell wall thickening. Research conducted by Ishii et al. (2001) indicated that considerable callose and carbohydrate accumulation occurred in the sieve tubes and vessels, and the sieve pores were blocked under B deficiency. The accumulation of substances or an embolism in the vessel elements will hinder the upward transport of mineral nutrients, i.e., mineral nutrients absorbed by roots cannot be transported to the aboveground plant parts even if the culture medium is nutrient rich.

A single perforation in the pitted vessel of the *Poncirus trifoliata* root is a type of evolved trait. The pits of the vessel elements are the main channel for the horizontal transport of water and mineral nutrients, and the perforation plates on the side wall are also involved in the horizontal transport of water and mineral nutrients (Muhammad and Sattle 1982). Vessel elements without a tail or those with a single tail are characteristics of the advanced evolutionary process of the vessel element system (Bailey 1944), which contribute to the water- and mineral nutrient-conducting ability of vessel elements. In this study, the proportion of double-tailed vessel elements was the largest. The longer and greater the diameter of the vessel elements is, the higher the conducting efficiency (Frost 1930). Under B-deficient conditions, the length and diameter of the vessel elements were reduced in the taproot and second-order lateral root, which reduced water and mineral nutrient absorption and transport, limiting the nutrient supply and reducing plant growth.

B deficiency effect on mineral nutrient absorption and allocation

The B content initially decreased significantly ($P < 0.05$) in the leaves (10 d), and then decreased significantly in the stems (20 d) and roots (30 d) after *Poncirus trifoliata* was subjected to B-deficient conditions (data not shown). Though there is increasing evidence to support B retranslocation in several plant species (Brown and Shelp 1997; Lehto et al. 2000; Hajiboland et al. 2013), the fact that the B content decreased in the leaves initially showed that B retranslocation to the aboveground part was still restricted to a certain degree when B was limiting.

Mutual promotion or antagonism in plant nutrient absorption will affect the absorption of other mineral nutrients. Research has shown that B deficiency inhibited the absorption of phosphorus and hindered the transfer of phosphorus in the plant, and conversely, phosphorus deficiency inhibited the absorption of B (Kaya et al. 2009). The current study offers further evidence that phosphorus uptake was significantly inhibited in the B-deficient treatment. Calcium is a main chemical component of the cell wall, and more than 90 % of B in plants is present in the cell wall. There are reports that indicate that Ca and B promote each other (Ramon et al. 2000), whereas others believe there is mutual antagonism between them. Several Ca^{2+} channel/transporter genes were upregulated in response to short-term B deficiency in *Arabidopsis* roots (Quiles-Pando et al. 2013).

B as well as other mineral nutrients, such as Ca and P, was decreased in the aboveground components in the B-deficient treatment. In addition, B deficiency decreases the photosynthetic rate (Xiao 2001; Pandey and Archana 2013). Stomata appeared closed, collapsed, and sunken underneath the epidermis of soybean (Will et al. 2011) and stomatal conductivity decreased; therefore, the transpiration rate is suppressed in B-deficient plants. The reason for the mineral nutrient content decrease in the aboveground components is, therefore, not only related to the decline in the absorption and transportation functions of the root system, but may also be associated with a slower transpiration stream.

Author contribution statement   Li Mei carried out most of the experiments and drafted the manuscript. Shu-ang Peng and Ou Sheng participated in the design of the study. Li Mei, Qiaohong Li and Huan Wang performed parts of the experiment. All authors have read and approved the final manuscript.

References

Ahmad W, Zia MH, Malhi SS, Niaz A, Saifullah (2012) Boron deficiency in soils and crops: a review. In: Aakash Goyal (ed) Crop plant. InTech, pp 77–114

Bailey IW (1944) The development of vessels in angiosperms and its significance in morphological research. Am J Bot 31(7):421–428

Bogiani JC, Sampaio TF, Abreu-Junior CH, Rosolem CA (2014) Boron uptake and translocation in some cotton cultivars. Plant Soil 375:241–253

Bolãnos L, Lukaszewski K, Bonilla I, Blevins D (2004) Why boron? Plant Physiol Biochem 42:907–912

Brown PH, Shelp BJ (1997) Boron mobility in plants. Plant Soil 193:85–101

Camacho-Cristóbal JJ, González-Fontes A (2007) Boron deficiency decreases plasmalemma H+-ATPase expression and nitrate uptake, and promotes ammonium assimilation into asparagine in tobacco roots. Planta 226:443–451

Camacho-Cristóbal JJ, Maldonado JM, González-Fontes A (2005) Boron deficiency increases putrescine levels in tobacco plants. J Plant Physiol 162:921–928

Camacho-Cristóbal JJ, Rexach J, González-Fontes A (2008a) Boron in plants: deficiency and toxicity. J Integr Plant Biol 5(10):1247–1255

Camacho-Cristóbal JJ, Herrera-Rodríguez MB, Beato VM, Rexach J, Navarro-Gochicoa MT, Maldonado JM, González-Fontes A (2008b) The expression of several cell wall-related genes in Arabidopsis roots is down-regulated under boron deficiency. Environ Exp Bot 63:351–358

Camacho-Cristóbal JJ, Rexach JM, Herrera-Rodríguez B, Navarro-Gochicoa MT, González-Fontes A (2011) Boron deficiency and transcript level changes. Plant Sci 181:85–89

Dell B, Brown PH, Bell RW (1997) Boron in soils and plants: reviews. Kluwer, Dordrecht

Frost FH (1930) Origin of vessels. Bot Gaz 89:67–94

Goldbach HE, Wimmer M (2007) Boron in plants and animals: is there a role beyond cell-wall structure? J Plant Nutr Soil Sci 170:39–48

Goldbach HE, Yu Q, Wingender R, Schulz M, Wimmer M, Findeklee P, Baluška F (2001) Rapid response reactions of roots to boron deprivation. J Plant Nutr Soil Sci 164:173–181

Hajiboland R, Bahrami-Rad S, Bastani S, Tolra R, Poschenrieder C (2013) Boron re-translocation in tea (Camellia sinensis (L.) O. Kuntze) plants. Acta Physiol Plant 35:2373–2381

Han S, Chen LS, Jiang HX, Smith BR, Yang LT, Xie CY (2008) Boron deficiency decreases growth and photosynthesis, and increases starch and hexoses in leaves of citrus seedlings. J Plant Physiol 165:1331–1341

Han S, Tang N, Jiang HX, Yang LT, Li Y, Chen LS (2009) CO_2 assimilation, photosystem II photochemistry, carbohydrate metabolism and antioxidant system of citrus leaves in response to boron stress. Plant Sci 176:143–153

Ishii T, Matsunaga T, Hayashi N (2001) Formation of rhamnogalacturonan II-borate dimer in pectin determines cell wall thickness of pumpkin tissue. Plant Physiol 126:1698–1705

Kaya C, Tuna AL, Dikilitas M, Ashraf M (2009) Supplementary phosphorus can alleviate boron toxicity in tomato. Sci Hortic 121:284–288

Kobayashi M, Matoh T, Azuma J (1996) Two chains of rhamnogalacturonan II is cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol 110:1017–1020

Läuchli A (2002) Functions of boron in higher plants: recent advances and open questions. Plant Biol 4:190–192

Lehto T, Kallio E, Aphalo PJ (2000) Boron mobility in two coniferous species. Ann Bot 86(3):547–550

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

Lu YB, Yang LT, Li Y, Xu J, Liao TT, Chen YB, Chen LS (2014) Effects of boron deficiency on major metabolites, key enzymes and gas exchange in leaves and roots of Citrus sinensis seedlings. Tree Physiol 34:608–618

Martín-Rejano EM, Camacho-Cristóbal JJ, Herrera-Rodríguez MB, Rexach J, Navarro-Gochicoa MT, González-Fontes A (2011) Auxin and ethylene are involved in the responses of root system architecture to low boron supply in Arabidopsis seedlings. Physiol Plant 142:170–178

Mei L, Sheng O, Peng S, Zhou G, Wei Q (2011) Growth, root morphology and boron uptake by citrus rootstock seedlings differing in boron-deficiency responses. Sci Hortic 129:426–432

Miwa K, Takano T, Fujiware T (2006) Improvement of seed yields under boron-limiting conditions through overexpression of BOR1, a boron transporter for xylem loading, in Arabidopsis thaliana. Plant J 46:1084–1091

Muhammad AF, Sattle R (1982) Vessel structure of Gnetum and the origin of angiosperms. Am J Bot 69:1004–1021

Pandey N, Archana (2013) Antioxidant responses and water status in Brassica seedlings subjected to boron stress. Acta Physio Plant 35(3):697–706

Quiles-Pando C, Rexach J, Navarro-Gochicoa MT, Camacho-Cristóbal JJ, Herrera-Rodríguez MB, González-Fontes A (2013) Boron deficiency increases the levels of cytosolic Ca^{2+} and expression of Ca^{2+}-related genes in Arabidopsis thaliana roots. Plant Physiol Bioch 65:55–60

Ramon OC, Elvira E, Jose SM (2000) Boron and calcium distribution in nitrogen-fixing pea plants. Plant Sci 151:163–170

Will S, Eichert T, Fernández V, Möhring J, Müller T, Römheld V (2011) Absorption and mobility of foliar-applied boron in soybean as affected by plant boron status and application as a polyol complex. Plant Soil 344:283–293

Xiao Y (2001) Effects of boron on shoot development. Dissertation, University of Sheffield, UK

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