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Full Length Article

Boron and Calcium Homeostasis Affects Trifoliate Rootstock (*Poncirus trifoliata*) Root Configuration and Nutrient Utilization

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Abstract

One of the most important factors for plant growth is to balance the essential nutrients of a plant. The plant growth, root characteristics and element content of rootstock at different levels of boron (B) and calcium (Ca) were studied in hydroponics to evaluate the interactions between B and Ca in trifoliate rootstock [*Poncirus trifoliata* (L.) Raf.] through systematic analysis. The results showed that the treatments +B-Ca (10 μM B and 0 m*M* Ca), -B+Ca (0 μM B with 1.23 m*M* Ca), -B-Ca (0 μM B and 0 m*M* Ca) severely inhibited the root length, total root surface area (SA) and the proportion of the middle root (mid-root) of the trifoliate rootstock as compared to +B+Ca (10 μM B with 1.23 m*M* Ca as CK). The B deficient treatment considerably improved the absorption and utilization of Ca by rootstock. Compared to the control, the biomass of rootstock and B accumulation in -B+Ca or +B-Ca treatments were noticeably decreased. The Fourier-transform infrared analysis (FTIR) analysis demonstrated that Ca promoted B transport to the shoot by the form of B-polyols compounds. It was also observed from the principal component analysis (PCA) that the proportion of the mid-root in the root system might be the main factor for the absorption of the two essential nutrients. In summary, B and Ca absorption exhibited a negative correlation in trifoliate roots. © 2019 Friends Science Publishers

Keywords: Boron; Calcium; FTIR; Nutrient utilization; Root configuration; Trifoliate rootstock

Introduction

The necessity of boron (B) for plant growth was reported in 1923 (Warington, 1923). Subsequent studies confirmed that B is involved in many biochemical and physiological processes of plants. One of the most convincing functions of B is to maintain the stability of cell wall through borate cross-linking of apiosyl residues of the pectin polysaccharide rhamnogalacturonan II (RG-II) (O'Neill *et al*., 2004). Moreover, B takes part in nitrogen metabolism (Gonzálezfontes and Camacho-Cristobal, 2007), root development (Martín-Rejano *et al*., 2011; Iqbal *et al*., 2017) and even in gene expression (Beato *et al*., 2008, 2010). The interaction between B and other elements showed that B addition improved potassium (K) uptake in rice shoot (*Oryza sativa* L.) (Kumar *et al*., 1981; Rehman *et al*., 2018), wheat (Yadav and Manchanda, 1979) and lentils (*Lens culinaris* L.) (Singh and Singh, 1983). Trees sprayed with 200 mg/L manganese (Mn), zinc (Zn) and B were the most effective treatment for the enhancement of foliar B levels (Chatzistathis *et al*., 2017). B deficiency is not conducive to the utilization of K in cotton (Wu *et al*., 2016) and B foliar application increased Ca content in *Vitis vinifera* (Güneş *et al*., 2015). In addition, a reduction in leaf Mn concentration was found by B application in the study of Aref (2014). The recent research revealed that the phosphorus fertilization of soil increases B deficiency in soils (Muhlbachova *et al*., 2017).

B and Ca exhibit similar functions in the cell wall through developing stable structure and signalling across the cell membrane (Gonzálezfontes and Camacho-Cristobal, 2007). Tanaka (1967) observed that the increasing Ca application in acidic soil reduces B uptake and accumulation in plant tissues, this effect can be corrected by applying B fertilizer (Braekke, 1983). Ca^{2+} also plays a hormone-like role in regulating the absorption and the movement of nutrients (Siddiqui *et al*., 2013). B deficiency symptoms like early infection or nodule organogenesis could be partially alleviated by the supply of Ca^{2+} (Redondo-Nieto *et al*., 2012). Interestingly, B deficiency can increase the cytoplasmic Ca^{2+} and the expression of Ca^{2+} related genes (Redondo-Nieto *et al*., 2012; Quilespando *et al*., 2013).

The roots of higher plants are sensitive to B deficiency and roots are the main part of plants to absorb water and nutrients through rhizosphere (Liu *et al*., 2012; Canadell and Zedler, 1995). China has the largest citrus cultivation area in the world and covers 22 provinces and municipalities (Rizza *et al*., 2007). *Poncirus trifoliata* is the main rootstock of citrus in China. However, citrus producing areas are generally distributed in acidic soils of Southern China with

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low content of available B and Ca (Ren *et al*., 2009; Zhao *et al*., 2014). Therefore, understanding the growth law of plants under different B and Ca conditions is of great significance for promoting rootstock growth and producing high quality citrus. The purpose of this study was to evaluate the interaction between B and Ca on the growth parameters and nutrient utilization and configuration of roots in trifoliate rootstock.

Materials and Methods

Plant Material and Experimental Treatment

The experiment was carried out in a greenhouse under natural conditions at Huazhong Agricultural University, Wuhan. The uniform sized citrus (*P. trifoliata*) seedlings with 10–12 leaves and 5–6 cm root length were selected and grown in the hydroponic condition. Prior to transplanting to nutrient solution, all plants were washed with distilled water to remove surface contaminants, then subsequently soaking in tap water for 2 d. The seedlings were transplanted to plastic black pots (one plant per pot) in a 3-L of nutrient solution. The pots were immersed in 1 *M* HCl and thoroughly washed with distilled water before transiting seedlings. The modified culture solution from Hoagland and Arnon (1950) was used in the following concentrations: 2 m*M* KNO_3, 0.5 m*M* $MgSO_4$, 0.14 m*M* Na_2HPO_4, 0.32 m*M* NaH_2PO_4, 4.45 μM $MnCl_2$, 0.8 μM $ZnSO_4$, 0.16 μM $CuSO_4$, 0.18 μM Na_2MoO_4 and 28.7 μM Fe-EDTA. This experiment was comprised of four treatments: +B+Ca (CK), +B-Ca, -B+Ca and -B-Ca and each treatment was replicated four times. B and Ca were applied in the form of H_3BO_3 (10 μmol·L^{-1}) and $Ca(NO_3)_2$ (1.23 mmol·L^{-1}), respectively. In +B-Ca treatment, the NO_3^- was supplied in the form of $NaNO_3$ (2.46 m*M*). The pH of the nutrient solution was maintained at 6.0 ± 0.2 with NaOH or H_2SO_4. The nutrient solution was applied to the seedlings in a 1/4 strength for 5 days without B, then 1/2 of ionic strength for next 5 days, and finally full-strength (Fernando *et al*., 2011). The nutrient solution was renewed once a week. Purified water was obtained by a system consisting of three units (active charcoal, ion exchanger and reverse osmosis), with electric conductivity lower than 0.06 μS cm^{-1} (B concentration < 0.5 μM). The solution was aerated for 10 min every 4 h interval. Analytical-grade reagents were used to prepare nutrient solutions.

Plant Sampling and Determination of B and Ca Contents

After 50 days of growing in the nutrient solution, the plants were harvested and all samples were washed repeatedly with ultrapure water and green-killing was carried out at 105°C for 30 min. Each plant was divided into three parts: root, stem and leaves, and subsequently dried at 70°C to a constant weight. The plant samples were grounded to fine powered and dry ashed at 500°C for 4 h in a muffle furnace. Ca content was determined by atomic absorption spectrophotometry (Yeung *et al*., 2010) and B concentration by curcumin colourimetric method (Dible *et al*., 1954). The root related parameters, B and Ca ratios in root and shoot were calculated by following equations:

$$R/S = \text{root DW (g)}/\text{shoot DW (g)}$$

Where, R/S: Root-Shoot ratio

$$B\ (Ca)\ A\ (\mu g) = B\ (Ca)\ (\mu g.g^{-1}) \times DW\ (g)$$

Where B(Ca)A: B (Ca) accumulation

$$B\ (Ca)\ UE\ (g.\ mg^{-1}) = \text{whole plant DW (g)}/\text{total B(Ca)A (mg)} \times 100$$

Where B (Ca) UE: the utilization efficiency of B (Ca)

$$PRTE = \text{root parameter}/TRE$$

Where, PRTE: The proportion of root parameters in total diameter within each diameter range: TRE: total root parameter within each diameter range.

Measurement of Root Morphology and Growth Parameters

The roots were scanned by Epson Perfection V700 root analyzer (WinRHIZO root analysis system) to measure the root growth parameters. The measured parameters were included total root length, root surface area (SA), root volume, root diameter, apical number and lateral root number. Plant height was measured with measuring ruler (index value 1 mm).

FTIR Analysis of Roots

The roots were subjected to FTIR analysis according to Liu *et al*. (2014).

Statistical Analysis

FTIR spectrum of different samples was normalized and baseline was corrected by Omnic 8.0. The data were processed by Microsoft Excel 2010 and Origin 8.6 (Origin Lab Corporation, USA), PCA analysis and one-way ANOVA ($P < 0.05$) were carried out by Duncan's multiple range test on SPSS software (IBM Corporation, USA).

Results

Effect on Plant Height and Leaves Number

The results showed that there were significant differences in leaves number and plant height under different treatments of B and Ca. The +B+Ca and -B+Ca treatments exhibited substantial differences in number of leaves, while +B-Ca and -B-Ca were without discrepancy. It showed that -B+Ca or +B-Ca treatments inhibited the plant growth in terms of number of leaves (Fig. 1A). The plant height was basically followed the same trend as number of leaves (Fig. 1B),

Table 1: Effect of different treatments on the accumulation of dry matter of trifoliate rootstock

Treatments	Root DW (g·plant^{-1})	Stem DW (g·plant^{-1})	Leaf DW (g·plant^{-1})	Shoot DW (g·plant^{-1})	Total plant DW (g·plant^{-1})	R/S
+B+Ca	0.12 ± 0.01 a	0.16 ± 0.02 a	0.16 ± 0.02 a	0.32 ± 0.03 a	0.45 ± 0.04 a	0.38 ± 0.02 b
+B-Ca	0.09 ± 0.03 b	0.12 ± 0.02 a	0.09 ± 0.02 b	0.21 ± 0.04 b	0.30 ± 0.07 b	0.40 ± 0.07 ab
-B+Ca	0.12 ± 0.01 a	0.14 ± 0.01 a	0.11 ± 0.02 b	0.25 ± 0.03 b	0.37 ± 0.04 b	0.49 ± 0.03 a
-B-Ca	0.06 ± 0.01 b	0.12 ± 0.03 a	0.09 ± 0.01 b	0.22 ± 0.04 b	0.28 ± 0.05 c	0.29 ± 0.06 c

DW: dry weight, R/S: root/shoot ratio. +B+Ca (10 μM B with 1.23 m*M* Ca as control), +B-Ca (10 μM B and 0 m*M* Ca), -B+Ca (0 μM B with 1.23 m*M* Ca), -B-Ca (0 μM B and 0 m*M* Ca). Values followed by the same letters within the same column indicate non-significant, results are expressed as mean ± SE ($P < 0.05$, Duncan's multiple range test)

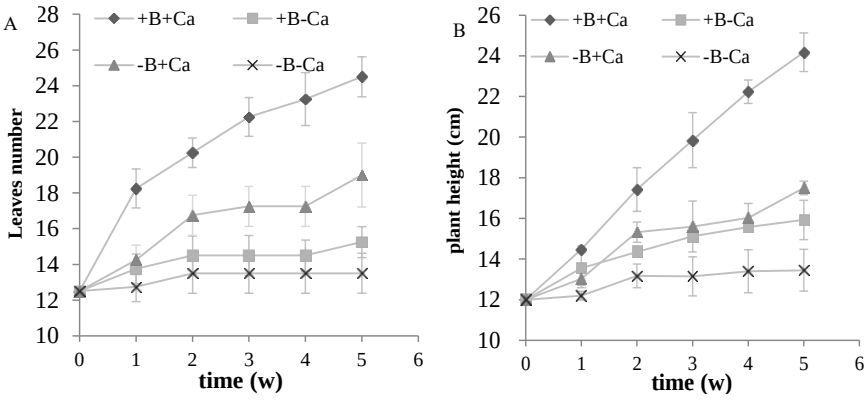

Fig. 1: Changes of leaves number and plant height in different treatment. **A**: leaves number, **B**: plant height. +B+Ca (10 μM B with 1.23 m*M* Ca as control), +B-Ca (10 μM B and 0 m*M* Ca), -B+Ca (0 μM B with 1.23 m*M* Ca), -B-Ca (0 μM B and 0 m*M* Ca). Values are means of four replicates ± SE ($P < 0.05$, Duncan's multiple range test)

indicating that B and Ca play a sensitive role in promoting the growth of plants, and Ca application can reduce the adverse effects caused by B deficiency.

Effect on the Accumulation of Dry Matter

The dry weight (DW) of each part varied in response to nutrition deficiency. The control treatment exhibited the highest root (0.12 g), stem (0.16 g), leaves (0.16 g), shoot (0.32 g) and total plant (0.45 g) DW at the end of this experiment (Table 1). Furthermore, other treatments of +B-Ca, -B+Ca and -B-Ca experienced a severe DW reduction. Root DW of +B-Ca and -B-Ca treatments decreased by 25 and 50% respectively compared with +B+Ca, but there was no significant change in -B+Ca treatment. Moreover, the +B-Ca, -B+Ca and -B-Ca decreased DW of shoot by 34.4, 21.9 and 31.3%, respectively in contrast to +B+Ca treatment. The order of the R/S was -B+Ca > +B-Ca> +B+Ca > -B-Ca, indicating that -B-Ca treatment was the most detrimental to the growth of trifoliate roots to +B+Ca.

Effect on the Root Morphological Characteristics

Some root growth parameters were found to be severely affected under the treatment of +B-Ca, -B+Ca and -B-Ca. The total root length and root SA of +B+Ca was the highest among all treatments (Table 2) while the root length of +B-Ca, -B+Ca and -B-Ca treatments decreased by 46.5, 52.4 and 67.6% in comparison to +B+Ca. In summary, the results showed that deficiencies of B and Ca can lead to a decline in the viability of the root.

Effect on Root Growth of Different Diameter Range

The diameter of roots and root systems was divided into three groups for further investigation: coarse root (> 1.2 mm), mid-root (0.4 ~ 1.2 mm) and fine root (0 ~ 0.4 mm). The root length, root SA and root volume of fine roots treated with +B-Ca, -B+Ca and -B-Ca were considerably decreased as compared with the control treatment. Interestingly, there were slight differences among those treatments. The root length of +B-Ca, -B+Ca and -B-Ca decreased by 66.4, 66.8 and 68.3%, whereas the root SA reduced by 76.4, 76.4 and 79.4% respectively, similarly, the fine root volume of +B-Ca, -B+Ca and -B-Ca declined by 80% when compared with +B+Ca. The changing trend of the mid-root was the same as mentioned above parameters. However, it was noted that the index of coarse root was significantly increased under -B-Ca, -B+Ca and -B-Ca treatments (Table 3).

The results of PRTE showed that, there was no difference in root length proportions (fine, mid-root) among all treatments (Table 4). The fine root SA ratio of +B-Ca, -B+Ca and -B-Ca was considerably lower than +B+Ca. However, the ratio of length, SA and the volume of coarse root were prominently greater than those of +B+Ca.

Table 2: Effect of different B and Ca treatments on the root morphological characteristics of trifoliate rootstock

Treatments	Total length (cm)	Total SA (cm^2)	Average diameter (mm)	Total volume (cm^3)	Apical number ($N \cdot plant^{-1}$)	Lateral root number ($N \cdot plant^{-1}$)
+B+Ca	243.82 ± 10.65 a	42.18 ± 2.02 a	0.55 ± 0.03 c	0.58 ± 0.05 a	589.00 ± 84.16 b	333.25 ± 53.38 a
+B-Ca	130.45 ± 3.12 b	31.81 ± 1.61 b	0.78 ± 0.02 a	0.62 ± 0.05 a	579.00 ± 56.33 b	374.00 ± 15.18 a
-B+Ca	116.09 ± 16.78 b	27.12 ± 2.54 c	0.75 ± 0.06 ab	0.51 ± 0.04 a	764.67 ± 78.73 a	314.75 ± 50.93 a
-B-Ca	78.92 ± 14.59 c	16.17 ± 2.45 d	0.66 ± 0.05 b	0.27 ± 0.05 b	568.67 ± 71.72 b	201.00 ± 48.60 b

SA: surface area. +B+Ca (10 μM B with 1.23 m*M* Ca as control), +B-Ca (10 μM B and 0 m*M* Ca), -B+Ca (0 μM B with 1.23 m*M* Ca), -B-Ca (0 μM B and 0 m*M* Ca). Values followed by the same letters within the same column indicate non-significant, results are expressed as mean ± SE ($P < 0.05$, Duncan's multiple range test)

Table 3: Effect of different B and Ca treatments on root system parameters in the different diameter range of trifoliate rootstock

Treatments	Root length (cm)			Root SA (cm^2)			Root volume (cm^3)		
	$0 < L \leq 0.4$	$0.4 < L \leq 1.2$	$L > 1.2$	$0 < SA \leq 0.4$	$0.4 < SA \leq 1.2$	$SA > 1.2$	$0 < V \leq 0.4$	$0.4 < V \leq 1.2$	$V > 1.2$
+B+Ca	91.06 a	160.33 a	6.37 c	6.74 a	28.18 a	3.08 b	0.05 a	0.42 a	0.15 b
+B-Ca	30.61 b	74.73 b	17.85 a	1.59 b	15.06 b	10.65 a	0.01 b	0.26 b	0.52 a
-B+Ca	30.19 b	74.36 b	12.17 b	1.59 b	15.75 b	7.03 a	0.01 b	0.29 b	0.37 a
-B-Ca	28.89 b	42.67 b	9.26 c	1.39 b	9.28 c	3.99 b	0.01 b	0.17 c	0.18 b

L: length, SA: surface area, V: volume. +B+Ca (10 μM B with 1.23 m*M* Ca as control), +B-Ca (10 μM B and 0 m*M* Ca), -B+Ca (0 μM B with 1.23 m*M* Ca), -B-Ca (0 μM B and 0 m*M* Ca). Values followed by the same letters within the same column indicate non-significant, results are expressed as mean ± SE ($P < 0.05$, Duncan' s multiple range test)

Table 4: Effect of different B and Ca treatments on the ratio of root system parameters in different diameters of trifoliate rootstock

Treatments	Root length ratio			Root SA ratio			Root volume ratio		
	$0 < L \leq 0.4$	$0.4 < L \leq 1.2$	$L > 1.2$	$0 < SA \leq 0.4$	$0.4 < SA \leq 1.2$	$SA > 1.2$	$0 < V \leq 0.4$	$0.4 < V \leq 1.2$	$V > 1.2$
+B+Ca	0.35 a	0.62 a	0.02 b	0.18 a	0.74 a	0.08 b	0.09 a	0.68 a	0.24 b
+B-Ca	0.25 a	0.61 a	0.14 a	0.06 b	0.55 a	0.39 a	0.01 b	0.32 b	0.66 a
-B+Ca	0.26 a	0.64 a	0.10 a	0.07 b	0.65 a	0.29 a	0.01 b	0.43 b	0.55 a
-B-Ca	0.36 a	0.53 a	0.11 a	0.10 ab	0.63 a	0.27 a	0.02 b	0.48 b	0.50 a

L: length, SA: surface area, V: volume. +B+Ca (10 μM B with 1.23 m*M* Ca as control), +B-Ca (10 μM B and 0 m*M* Ca), -B+Ca (0 μM B with 1.23 m*M* Ca), -B-Ca (0 μM B and 0 m*M* Ca). Values followed by the same letters within the same column indicate non-significant, results are expressed as mean ± SE ($P < 0.05$, Duncan's multiple range test)

Table 5: Differences of CaUE and BUE in different treatments

Treatments	CaA ($\mu g \cdot plant^{-1}$)	BA ($\mu g \cdot plant^{-1}$)	CaUE ($g \cdot mg^{-1}$)	BUE ($g \cdot mg^{-1}$)	Ca/B
+B+Ca	6,538.11 b	17.12 a	0.07 b	24.43 d	381.93
+B-Ca	3,871.66 c	8.94 b	0.08 a	33.67 c	433.07
-B+Ca	7,633.52 a	5.20 c	0.06 c	71.47 a	1467.98
-B-Ca	3,765.28 d	6.14 bc	0.08 a	46.00 b	613.23

CaA: Ca accumulation, BA: B accumulation, CaUE: Ca utilization efficiency, BUE: utilization efficiency, Ca/B: Calcium-Boron ratio. +B+Ca (10 μM B with 1.23 m*M* Ca as control), +B-Ca (10 μM B and 0 m*M* Ca), -B+Ca (0 μM B with 1.23 m*M* Ca), -B-Ca (0 μM B and 0 m*M* Ca). Values followed by the same letters within the same column indicate non-significant, results are expressed as mean ± SE ($P< 0.05$, Duncan's multiple range test)

Root Morphological Parameters through PCA

Radar chart was used to describe the PCA of the relative values of 16 traits, such as total root length, total root SA, total root volume, etc. (Fig. 2). The cumulative contribution rate of the first three principal components exhibited a higher explanatory rate for the total variation of the data set, accounting for 89.23%. In PC1, the larger eigenvectors were associated with the mid-root SA, root volume, total root SA and mid-root length and scored 50.45%, and collectively referred to as the mid-root evaluation factor. The evaluation index of the PC2 corresponding to the larger eigenvectors were linked to coarse root SA, volume and length, named as coarse root evaluation factor. The eigenvectors associated with PC3 included the fine root volume and fine root SA, called as fine root evaluation factor. Thus, the total SA of plant root is an important index to reflect the nutrient absorption of roots, especially the index of mid-root indicated the greatest influence on the absorption of nutrients, followed by coarse roots and fine roots.

Effects on Plant B and Ca Content

It was observed that -B+Ca inhibited the B accumulation (BA) in the plant parts, similarly, +B-Ca treatment exhibited reduced Ca accumulation (CaA) in comparison to +B+Ca. However, -B+Ca treatment exhibited higher CaA as compared to the +B–Ca treatment (Table 5) and +B-Ca treatment favored the BA in the plant parts as compared to the –B treatment (Fig. 3A and B) indicating that the -B+Ca treatment exhibited the sufficient amount of Ca translocation in the roots, and the BA in the shoot was slightly increased with +B-Ca treatment. Under -B-Ca

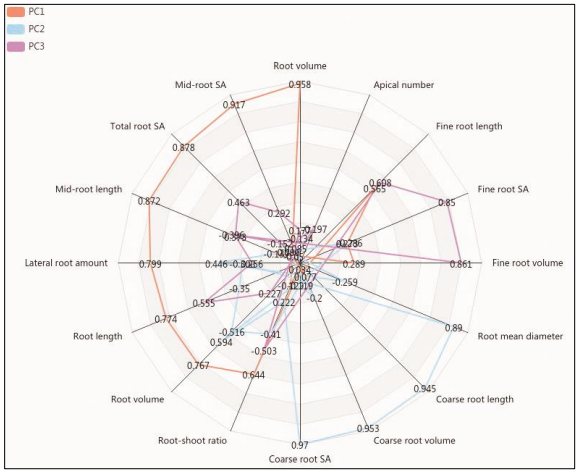

Fig. 2: Principal component load matrix showed by radar chart of root morphologic response factor. Note: PC1, PC2 and PC3 represent the first principal component, the second principal component and the third principal component, respectively. We represent the evaluation factor of this principal component with the line of the outer layer of the same color: correlation value (C-value > 0.8)

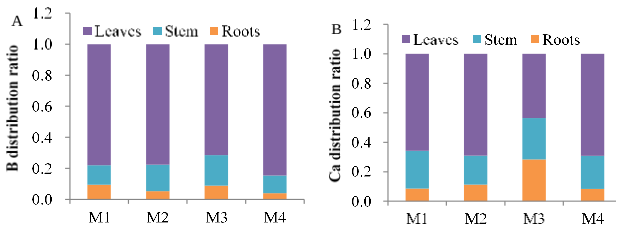

Fig. 3: Difference of absorption and distribution of Ca and B in different treatments. Note: The meaning of each picture is B distribution ratio (A), Ca distribution ratio (B) among different treatments. Columns with different lower case letters (a, b, c, d) are significantly different, values are means of four replicates ± SE ($P < 0.05$, Duncan's multiple range test)

treatment, B and Ca were almost accumulated in the shoots indicating that the -B+Ca or +B-Ca treatments might transfer B and Ca to the shoots. The effect was even more pronounced in the -B-Ca treatment.

Differences of CaUE and BUE in Different Treatments

Nutrient accumulation and use efficiency were depicted in Table 5. The CaA and BA in different treatments showed significant differences. The results showed that +B-Ca and -B-Ca treatments increased the utilization of Ca, while the CaUE was reduced under –B+Ca treatment.

There was a significant difference among the four treatments. The highest BUE was observed in the -B+Ca treatment among all treatments. The BUE of +B-Ca, -B+Ca and -B-Ca was higher than that of the +B+Ca treatment (1.4, 2.9 and 1.9 times, respectively), indicating a significant increase of BUE under +B-Ca, -B+Ca and -B-Ca conditions.

FTIR Analysis of Roots under B and Ca Treatments

Although the IR approximately exhibited same wavelengths, there were obvious differences in the number of characteristic peaks and the relative absorbance values in some regions (Fig. 4A; 4000 ~ 3500 cm^{-1}, 1800 ~ 1000 cm^{-1}). The vibration of the free hydroxyl (-OH) in the range of 4000 ~ 3500 cm^{-1} was mainly expressed in some carbohydrates. There was no enormous difference between the relative absorbance of 3500 and 1800 cm^{-1}, so the wavelength of 1800~1000 cm^{-1} was selected for detailed analysis (Fig. 4B).

The characteristic peaks near 1697, 1683 and 1670 cm^{-1}, indicate the content of secondary metabolites like fatty acids, saponins, flavonoids and protein structure. A change in the composition of these metabolites was observed in the –B+Ca treatment. The absorption peak of -B+Ca was found to be located near 1543, 1523 and 1384 cm^{-1} and exhibited higher intensities of relative absorption than that of +B-Ca, while the -B-Ca intensity was the lowest, indicating that Ca could not completely reverse the loss of the total protein, lipid and cellulose caused by -B+Ca treatment. Compared with the absorption intensity near 1340 and 1319 cm^{-1}, it was observed that the absence of both elements decreased the synthesis of nucleic acids and other related substances. The analysis of the spectra near 1155 cm^{-1}, 1078 cm^{-1} and 1035 cm^{-1} showed that the absorption intensity of -B-Ca treatment was greater than that of -B+Ca, indicating that Ca reduced the carbohydrate content under -B+Ca.

Discussion

The previous studies indicated that nutrients uptake ability of fine and mid-root is stronger than coarse root, and the thickness of roots can reflect the condition of nutrient absorption (Sullivan *et al*., 2000). B and Ca are essential nutrients for plant growth and development. Both nutrients deficiencies can affect the physiological functions: inhibit normal growth, development and dry matter accumulation. Previously, it has been reported that B deficiency inhibited the cell division and extension of the root apex (Dell and Huang, 1997) and suppressed the cotton root elongation and biomass accumulation (Zhu *et al*., 2001). Moreover, B deficiency remarkably decreased the growth of citrus roots to +B+Ca, the root length, root SA and root number (Mei *et al*., 2011). The same results were also observed in *Arabidopsis thaliana* (Cramer and Jones, 1996) and tomato under Ca deficiency (Dong *et al*., 2003). The ratio of Ca/B has been used to assess the B deficiency status of tomato (Tanaka, 1967), radish (Tariq and Mott, 2007) and other crops, and the normal Ca/B ratio exists between 300 ~ 500 in plants. The larger the ratio, the more serious the B deficiency will be. This can be seen from the experimental data, the evaluation of this method is also applicable to trifoliate roots to +B+Ca seedlings. The results of present

Table 6: Characteristic peaks of infrared spectra

Wave number (cm^{-1})	Vibrations	Compound source
3500 ~ 4000	–OH and N–H stretching vibration	carbohydrates
1683 ~ 1735	C=O stretching vibration	fatty acid , saponins ,flavonoids
1647~1680	amide I (C=O telescopic vibration in –CO N H–)	reflects the total protein conformation
near 1543	amide II (N–H deformation and C–N stretching vibrations)	reflects the total protein content
1380 ~ 1420	C–H deformation vibration	lipids and cellulose
1200 ~ 1340	amide III (N–H deformation and C–N stretching vibrations)	nucleic acid
1000 ~ 1200	C–O and C–C stretching vibrations	carbohydrates (alcohols, ether groups, ester groups, or phenols)

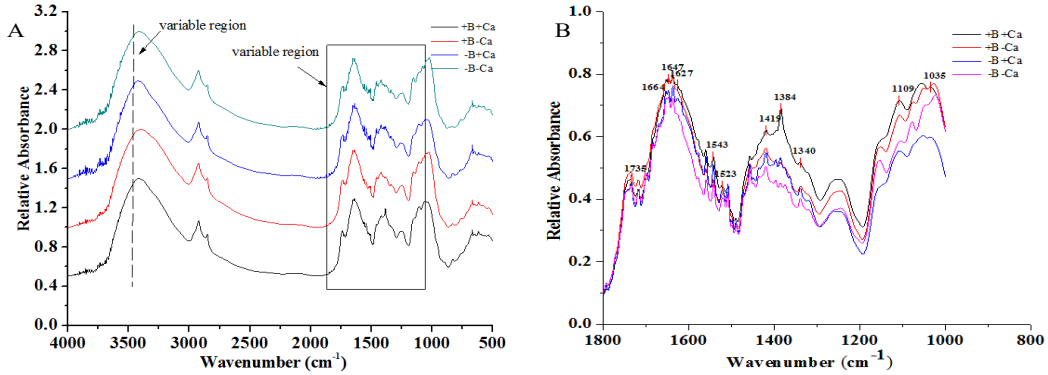

Fig. 4: FTIR analysis of root system of different treatments. A: Absorption FTIR spectra in the 4000–400 cm^{-1} region in the root of trifoliate seedlings with different B treatments, B: Detailed analysis were carried out in the 1800–1000 cm^{-1} region. +B+Ca (10 μM B with 1.23 m*M* Ca as control), +B-Ca (10 μM B and 0 m*M* Ca), -B+Ca (0 μM B with 1.23 m*M* Ca), -B-Ca (0 μM B and 0 m*M* Ca)

study showed that -B+Ca and +B-Ca treatments alone or in combination severely hampered the plant growth, dry matter accumulation, root length and root SA, whereas B deficiency led to the increase of Ca/B ratio and average root diameter. These results are in agreement with the findings of Mei *et al*. (2011). Our study results showed that the root length, root volume, root SA and proportion were remarkably inhibited, which might have accelerated the senescence of roots under both nutrient deficiencies. Factor analysis results indicated that the mid-root length might play an important role in the absorption of nutrient elements in trifoliate rootstock to +B+Ca.

Nutrition use efficiency was defined as the dry matter produced per unit nutrient accumulation in plants and the BUE and CaUE can be defined likewise (Rengel and Damon, 2008). B deficiency can increase the absorption of Ca (Koshiba *et al*., 2010) in tobacco and Ca supply leads to the decrease of B absorption (Gupta and Macleod, 1981). The study results showed that Ca and B were transferred to shoot from the root, which improved the BUE and CaUE and also proved that -B+Ca can increase the absorption of Ca in trifoliate rootstock to +B+Ca. Therefore, in B deficient soils, Ca fertilizer application should be reduced.

FTIR (simple and high sensitive technology) not only identifies functional groups but also deduces the chemical composition on the basis of the difference of infrared spectrum. Each chemical bond has its own characteristic absorption peak in a range of 4000 ~ 400 cm^{-1} (Table 6) (Yang and Yen, 2002; Barth, 2007; Kasprzyk *et al*., 2017) within the infrared spectrum (IR). B deficiency can reduce the pectin content of cell wall and the proportion of RG-II, which also decreases cell wall stability and increases the content of cellulose and hemicellulose (Ishii and Hayashi, 2001; Liu *et al*., 2014). Similarly, Ca deficiency also weakens the stablility of cell wall and membrane (Liu *et al*., 2008). This study showed that intense vibration located near 1384 cm^{-1} was attributed to C–H stretching vibration and originates from lipid and cellulose, indicating Ca supply can increase cell wall materials like cellulose and semi-cellulose to relieve cell injury caused by B deprivation. Spectra from B-deficient root showed the weaken peak value of C–O, C–C and amide III, indicating the reduction of carbohydrate and nucleic acid in root system due to those matters transported to the aboveground parts of the plant, the addition of Ca promoted this effect. It was suggested that the presence of Ca promoted B transport in the form of B-polyols compounds (Brown and Hu, 1994; Brown and Hening, 1996) to the shoots.

Conclusion

The present study results showed that -B+Ca and +B-Ca treatments resulted in the increase of coarse root length while decreased the root SA and root volume. The factor analysis results indicated that mid-root might be the main root type of trifoliate rootstock to +B+Ca for B and Ca absorption. Moreover, -B+Ca and +B-Ca treatments severely hindered the normal growth of trifoliate roots to

+B+Ca and DW accumulation of plants, improving the absorption of Ca and B simultaneously and the transported to the shoots, eventually causing a great change of metabolism. Thus, the presence of Ca may alleviate some of the root damage caused by B deficiency. However, the interaction mechanism between Ca and B is needed to be further studied.

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