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Short communication

Improving the boron uptake of boron-deficient navel orange plants under low boron conditions by inarching boron-efficient rootstock

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ABSTRACT

Inarching is often used to correct nutrient deficiency, but the characteristic of boron (B) uptake in inarched citrus remains unknown. One-year-old 'Newhall' navel orange plants on trifoliate orange were inarched with Carrizo citrange seedlings. After a 15-month B deficiency, the plants were resupplied with low B for 35 days to investigate the B uptake and the contribution of inarching rootstock to B absorption in different parts by using ^{10}B labeling and split-root methods. When resupplying low B to B-deficient plants, the B concentration and proportion of newly absorbed B in new leaves, new twigs, and old leaves were higher in inarched than non-inarched plants. By contrast, the B concentration in original rootstock roots of inarched plants was lower than that of non-inarched plants. Regardless of B treatment, the fresh weight in original rootstock roots was significantly lower in inarched than non-inarched plants. Interestingly, the enriched ^{10}B abundance was detected in the original rootstock from split-root treatments in which only the inarching rootstock was supplied with labeled ^{10}B, implying that newly acquired B can be retranslocated from scion to rootstock. The newly-absorbed-B contribution of inarching rootstock was higher for the scion but lower for both rootstocks under low B conditions when compared with B-adequate conditions. These results suggest that, under low B conditions, inarching B-efficient Carrizo citrange onto B-deficient navel orange improves the plant's B uptake, increases the B concentration in young scion parts, and thus enhances the tolerance of the whole plant to B deficiency.

1. Introduction

Boron (B) is an essential micronutrient for vascular plants (Warington, 1923), and its deficiency is worldwide in many agricultural crops, including *Citrus* species (Shorrocks, 1997; Xiao et al., 2007; Boaretto et al., 2008). Boron deficiency in citrus may inhibit root growth, depress the photosynthetic capacity and disturb the metabolism in leaves, and thus result in substantial loss of fruit yield and quality (Chen et al., 2012).

Boron fertilization is used extensively to correct B deficiency in citrus production (Jiang et al., 2009; Boaretto et al., 2011). However, B application to B-deficient citrus plants not only raises cultivation cost, but also easily causes B toxicity because the range of B concentration between deficiency and toxicity is narrow in citrus (Gupta et al., 1985; Zhuang et al., 1991; Nable et al., 1997; Ling et al., 2010). Another more environment-friendly and sustainable approach is to screen the B-efficient rootstock genotypes, which are able to grow well in soils with low B availability where most cultivars are adversely affected (Rerkasem and Jamjod, 1997; Mei et al., 2011; Zhou et al., 2014). Trifoliate orange [TO; *Poncirus trifoliata* (L.) Raf.] and Carrizo citrange [CC; *Citrus sinensis* (L.) Osb. × *P. trifoliata* (L.) Raf.] are two important genotypes of citrus rootstocks that are used widely throughout the world (Forner-Giner et al., 2003; Cantuarias-Aviles et al., 2010; Hussain et al., 2013). Our previous studies suggested that CC tends to be tolerant to B deficiency (B-efficient), while TO is sensitive to B deficiency (B-inefficient) in both seedlings and grafted plants (Sheng et al., 2009; Mei et al., 2011). However, B deficiency mainly occurs in mature fruit-bearing citrus trees under field conditions (Xiao et al., 2007), which makes the replacement of B-inefficient rootstock with a B-efficient cultivar difficult because the operation is time-consuming and costly.

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Fortunately, the inarching technique can be used to change the original rootstock to a tolerant cultivar immediately. Inarching used here refers to a grafting method of planting young seedling(s) beside a grafted tree and then approach-grafting them onto the trunk of the scion (Burns et al., 1964; Bové and Ayres, 2007). In practice, inarching with resistant rootstocks is frequently used to improve the tolerance of grafted plants to a variety of abiotic and biotic stresses (Li et al., 1990; Nakajima et al., 1992; Román et al., 2004). Undoubtedly, plants with two root systems of different genotypes should possess a more complicated procedure of nutrient absorption in comparison with plants with a single root. However, little is known about the B uptake of inarched citrus plants and the contribution of inarching rootstock to B absorption.

Therefore, the aims of this study were to investigate the B uptake of 'Newhall' navel orange on TO inarched with a CC seedling through a ^{10}B labeling experiment, and to assess the contribution of inarching rootstock to B absorption using a split-root system in which only the inarching root was supplied with ^{10}B under low or adequate B conditions.

2. Materials and methods

2.1. Preparation of plant materials

One-year-old B-inefficient trifoliate orange [TO; *P. trifoliata* (L.) Raf.] seedlings were budded with 'Newhall' navel orange [*C. sinensis* (L.) Osb. cv. Newhall] in early October 2009. One year later, fifteen of the twenty-four grafted plants were further inarched with B-efficient Carrizo citrange [CC; *C. sinensis* (L.) Osb. × *P. trifoliata* (L.) Raf.] seedlings at Huazhong Agricultural University, Wuhan, China. A budded plant was shaved flat at 2 cm above the graft union, down to the cambium, and a similarly shaved seedling with 6–8 mm stem diameter was approach-grafted to this area. The grafted stems were wrapped together by using transparent-plastic film. One half-year later, the seedling shoots were cut at 1 cm above the inarched position. Successful inarching was characterized by the stem diameter below the union being appreciably larger than the diameter above (Burns et al., 1964). The inarched and non-inarched plants were then transplanted into 10-L black plastic pots (one plant per pot) filled with B-free quartz sand and perlite (1:1, v/v) medium in a greenhouse. The plants were fertilized using a modified Hoagland's No. 2 nutrient solution (Hoagland and Arnon, 1950): 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-EDTA. The plants were irrigated with nutrient solution every two days. To avoid salt and B accumulation in the growth medium, 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 drained out of the pot bottom (Wang et al., 2014).

2.2. Boron treatments

After late January 2012, half the non-inarched and inarched plants were irrigated with nutrient solution containing 0.25 mg/L B (served as B-adequate), and the other received 0.001 mg/L B (B-deficient) in a sand culture. All the treatments replicated three times, with one plant for each non-inarched replication and two plants for each inarched replication. Before the treatments, the medium was flushed with deionized water three times to eliminate the accumulated B in the pot; three non-inarched and inarched plants were harvested separately for the measurement of fresh weight and B concentration as basic samples. Fifteen months after B treatments, the fresh weight of each plant and the B concentration in leaves were measured again.

2.3. The ^{10}B labeling and split-root experiment

There were six treatments as follows: (1) non-inarched with adequate B (i.e., the B-adequate plants were still supplied with 0.25 mg/L B); (2) inarched with adequate B; (3) split-root and inarched with adequate B; (4) non-inarched with low B (i.e., the B-deficient plants were resupplied with 0.01 mg/L B to mimic the low B status under field conditions); (5) inarched with low B; and (6) split-root and inarched with low B (Fig. 1). All the roots were supplied with labeled $H_3{}^{10}BO_3$ (99% atom ^{10}B, Aldrich, USA), except for the original roots in treatments (3) and (6) as H_3BO_3. Each treatment had three replicates.

The solution culture was initiated on May 26, 2013 and performed for 35 days. All the sand-grown plants from B treatments were soaked in tap water for 2 days, and washed with deionized water to remove surface contaminants. The shoots of the plants were then pruned uniformly down to twenty to twenty-five remaining leaves to maintain a similar photosynthetic capacity. Six of the twelve inarched plants were split-root by manually separating the inarching root from the original root. Thereafter, all the six non-inarched plants and half the twelve inarched plants were transferred into 40-L black plastic boxes (60 cm × 30 cm × 30 cm) and the other half of inarched plants (split-root) were transferred into 10-L black plastic boxes (20 cm × 20 cm × 30 cm), containing 1/2 strength nutrient solution, and supplied with the same natural abundance of B concentration as the sand culture for 14 days. Before the ^{10}B treatments, the containers were washed with deionized water three times. To minimize environmental differences, two trees with one non-inarched plant and one inarched plant were placed in one 40-L box. Half the inarched trees were split-root, with the original root kept in one 10-L box and the inarching root in the other 10-L box (Fig. 1). The edges of these two boxes were removed to make them close enough for split-root cultivation. The boxes were fully covered with two pieces of black foam plate, and the plants were properly anchored with overhead wires against the lodging. The nutrient solution was continuously aerated and renewed every 7 days. The pH of nutrient solution was adjusted to 5.8–6.2 with 1 mol/L NaOH or H_2SO_4.

The proportion of ^{10}B derived from nutrient solution (%Bdfs) was calculated by the following equation (Liu et al., 2012):

$$\%\text{Bdfs} = \left[\frac{\left(\%^{10}\text{B in sample} - \%^{10}\text{B in background}\right)}{\left(\%^{10}\text{B}_{\text{H3BO3}} - \%^{10}\text{B in background}\right)}\right] \times 100$$

where the $\%^{10}B$ in sample is the ^{10}B proportion in different plant parts, the $\%^{10}B_{H3BO3}$ is 99, and the $\%^{10}B$ in background is 19.66 ± 0.025 based on the leaf samples prior to the experiment ($n = 6$).

The ^{10}B accumulation in plant parts (Bdfs) was calculated as follows:

$$\text{Bdfs} = \frac{\%\text{Bdfs}}{100} \times \text{total B in sample}$$

In addition, the contribution of inarching roots to B uptake was the proportion of the ^{10}B abundance in split-root inarched plants to that in non-split-root inarched plants for the same part.

2.4. Sampling

At harvest, the plants were separated into new leaves and twigs (with emergence after the beginning of the experiment), old leaves and twigs (with emergence before the experiment), scion stem, rootstock stem, and roots. For inarched plants, the roots and rootstock stem of the original rootstock and inarching rootstock were separately sampled (Fig. 1). All the samples were initially washed with tap water and then with deionized water in 30 s. They were