Effects of boron nutrition on arsenic uptake and efflux by rice seedlings

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ABSTRACT: The impacts of boron (B) through root application and foliar spray on arsenic (As) uptake and efflux by/in rice seedlings (*Oryza sativa* L.) were investigated in three hydroponic experiments. Addition of B in culture medium didn't alter concentrations of arsenite [As(III)], arsenate [As(V)] and total As in rice seedlings under either As(III) or As(V) exposure. Foliar B supply decreased root As concentrations by 20.9% under As(V) treatment and by 12.6% under As(III) treatment, respectively, yet didn't significantly decrease shoot As concentrations. Concentrations of As(V) were positively related to B concentration in rice root under As(V) treatment following foliar B supply ($P < 0.05$). Rice seedlings extruded 105.2% more As after As(III)-pretreatment than after As(V)-pretreatment. Foliar B supply increased the amount of As excreted by As(III)-pretreated rice root by 14.0%–16.9% ($P > 0.05$) and made no effect on As efflux for As(V)-pretreatment seedlings. These results indicate that root application of B at four times the concentration of As can hardly decrease As accumulation by rice, whereas foliar B supply is conducive to a decline in As acquisition by rice root. It is likely that B channel is at least not the main pathway for As(III) entering into rice root, and As(V) distribution mechanism in rice plant may share with B.

1 INTRODUCTION

Boron (B) mainly occurs in soils or organisms as $B(OH)_3$ which is an uncharged molecule structurally similar to $As(OH)_3$. Many studies have shown that NIP channel proteins such as OsNIP3;1, AtNIP5;1, AtNIP6;1 which facilitate As uptake or transport are also $B(OH)_3$ transporters essential for B uptake by rice or thale cress roots (Hanaoka *et al.*, 2014; Miwa and Fujiwara, 2010). PIP channel proteins (OsPIP2;4 and OsPIP2;7) from rice mediated not only B but also As transport (Kumar *et al.*, 2014). Furthermore, the expression of these aquaporins can be regulated by B level in the environment (Kumar *et al.*, 2014). The tissue specificity of OsNIP3;2 expression is similar to that of B transporter OsNIP3;1 (Chen *et al.*, 2017). It remains unknown whether As uptake and efflux are influenced by B addition.

2 METHODS/EXPERIMENTAL

2.1 Effect of culture solution B-level on As uptake

After seedlings of rice were pre-cultured in a full strength nutrient solution for one week, 6 treatments including three B levels (0, 10 and $40 \mu\text{mol L}^{-1}$) with $10 \mu\text{mol L}^{-1}$ As(III) or As(V) in solution were imposed. Plants were harvested after As exposure for 2 d. Shoot and root were quickly separated, washed with deionized water, blotted dry and ground in a

mortar and pestle with liquid N for further measurements after the fresh weight was recorded.

2.2 Effect of foliar B supply on As uptake

Rice seedlings subjected to preculture in a nutrient solution without B for one week were transferred to nutrient solutions containing $10 \mu\text{mol L}^{-1}$ As(III) or As(V) whilst no B addition. Boron was applied through foliage including foliar sprays of deionized water (control), and 9, 36 mmol L^{-1} H_3BO_3 solution. Foliar sprays (3 mL per pot) were applied once a day. Plants were harvested as described above after As exposure and foliar B supply for one week. Specially, shoot was rinsed with deionized water containing Triton X-100 three times first to remove B adhering on the surface of leaves.

2.3 Effect of foliar B supply on As uptake

Rice seedlings were pre-cultured in a nutrient solution without B for one week, and $10 \mu\text{mol L}^{-1}$ As(III) or As(V) was added into culture solution afterwards. After As exposure for one week, plant roots were rinsed in an ice-cold desorption solution, and then soaked in the same ice-cold desorption solution for 10 min to remove apoplastic As. After roots were washed with deionized water, plants were transferred to 50 mL lightproof centrifuge tubes containing 40 mL nutrient solution without As and B, and foliar B supply

Figure 1. Effects of B level of culture solution on As(III) and As(V) concentrations in rice shoot.

Table 1. Total As and B concentrations in shoot and root of rice seedlings grown in culture solutions with As (III) or As(V) under foliar sprays of different B levels.

As species	Foliar B supply	Shoot As (µg g ⁻¹)	Root As (µg g ⁻¹)	Shoot B (µg g ⁻¹)	Root B (µg g ⁻¹)
As(V)	B0	7.4	27.7	4.2	2.5
	B9	7.0	23.1	21.7	2.1
	B36	6.5	21.9	70.1	1.7
As(III)	B0	19.2	57.2	5.8	1.1
	B9	19.6	60.0	24.9	1.2
	B36	18.2	50.0	70.4	1.1
Significance of					
As species		***	***	ns	***
Foliar B supply		ns	*	***	*
As × B		ns	ns	ns	*

commenced as described above once plants were transferred. One week later, plants were harvested.

3 RESULTS AND DISCUSSION

3.1 Effect of B level in culture solution on As uptake

With increasing B concentration in culture solution, up to four times that of As, concentrations of both As(III) and As(V) in shoots and roots of rice under either As(III) or As(V) exposure were not changed statistically (Fig. 1). It may be attributed to the highly strict control of the permeability to B(OH)₃ by ar/R selectivity filter in comparison to As(III).

3.2 Effects of foliar B supply on As uptake

Root As concentrations declined significantly by 20.9% under As(V) treatment and by 12.6% under As(III) treatment, respectively ($P < 0.05$), with increasing foliar B level supply (Table 1). However, foliar B supply didn't decrease shoot As concentrations ($P < 0.05$) under either As(V) or As(III) exposure. Root B concentrations decreased by up to 47.1% ($P < 0.05$) with increasing foliar B levels in As(V) treatment but not in As(III) treatment. Root As(V) concentration decreased by up to 29.8% following foliar

Figure 2. Relationship between root B concentration vs root As(V) concentration of rice seedlings under foliar sprays of different B levels.

Figure 3. Everyday amount of As excreted into culture solutions by rice seedlings under foliar sprays of B.

B supply in As(V) treatment ($P < 0.05$). Further analysis showed significant positive relationships between root B concentration versus root As(V) concentration (Fig. 2) under As(V) exposure ($P < 0.05$).

3.3 Effects of foliar B supply on As efflux

Rice seedlings extruded 105.2% more As after As(III)-pretreatment than after As(V)-pretreatment. Foliar B supply increased the amount of As excreted by As(III)-pretreated rice root by 14.0%–16.9% ($P > 0.05$) and made no effect on As efflux for As(V)-pretreatment seedlings (Fig. 3).

4 CONCLUSIONS

Boron level in culture solution made no effect on the uptake of As(III) and As(V) by rice roots. Foliar B supply decreased concentrations of total As and As(V) in root significantly but didn't markedly decrease shoot As concentrations. There was a significant relationship between B and As(V) concentrations in rice root under As(V) exposure following foliar B supply. Foliar B supply didn't increase As efflux under As(III)- or As(V)-pretreatment. It was likely that B and As(V) share identical regulatory mechanism inside

rice plant, and B channels contributed little to As entering into rice roots.

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