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Brassinosteroids and iron plaque affect arsenic and cadmium uptake by rice seedlings grown in hydroponic solution

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Abstract

Brassinosteroids (Brs) have drawn a wide attention due to their protective role against toxicity of heavy metals in plants. To better understand the role of Br in arsenic (As) and cadmium (Cd) uptake by rice plants, a hydroponic experiment was conducted to investigate the combined effect of 24-epibrassinolide (Br24) or 28-homobrassinolide (Br28) and iron plaque (IP) on As and Cd uptake and accumulation in rice seedlings. Six-week-old seedlings were sprayed with 0.2 or 0.02 μM Br24 or Br28 and grown in nutrient solution for 3 d, and then 20 or 60 mg Fe^{2+} dm^{-3} was used to induce root IP formation for 3 d. These seedlings with or without Br and with or without IP were exposed to solution containing 0.5 mg dm^{-3} As^{III} or Cd for 9 d. The results showed that rice growth decreased when Br24 were applied, but it increased when a combination of Br24 and IP was applied. Fe concentrations in dithionite-citrate-bicarbonate (DCB) extracts were increased after 0.2 or 0.02 μM Br24 application in the absence of IP, but decreased by Br24 in the presence of IP. In the absence of IP, As and Cd content in leaves was significantly reduced by 0.02 μM Br24 and 0.2 μM Br28, respectively. The As content in leaves was also reduced by the combination of 0.02 and 0.2 μM Br28 and IP, and the Cd content in leaves was reduced by the combined effect of 0.2 μM Br24 and IP. These results indicate that Br24 and Br28 could impede As and Cd accumulation, and the interactions between Br and IP may have a potential in restricting the transport of As and Cd into rice shoots.

Additional key words: 24-epibrassinolide, 28-homobrassinolide, heavy metals.

Introduction

Arsenic is a toxic element classified as a carcinogen to humans (Smith *et al*. 2002). Cadmium is one of the most toxic elements in the environment. Most bioavailable As or Cd is derived from anthropogenic sources, namely fertilizer application and waste (Clemens and Ma 2016).

Rice is one of the most widespread food crops. Rice can accumulate higher concentrations of As compared to wheat, maize, or barley owing to the increased bioavailable As in flooded soil (Meharg *et al*. 2009, 2013, Li *et al*. 2011). However, Cd exhibits higher bio-availability in sufficiently aerated soils because of the deposition of CdS under anaerobic conditions (Fan *et al*. 2010). Therefore, controlling aerobic and anaerobic environments by regulating flooding conditions cannot efficiently block As or Cd uptake by rice plants when they simultaneously occur.

Iron plaque (IP), widely distributed on root surfaces of wetland plants, is generally precipitated *via* oxidation of ferrous to ferric ions by the release of oxygen or oxidants from roots (Armstrong 1964). IP is a strategy for wetland plants to survive under flooding or anaerobic conditions (Khan *et al*. 2016). As other wetland plants, IP can be formed on the root surfaces of rice plants grown in wet soil or in hydroponic culture (Liu *et al*. 2008, Luo *et al*. 2015, Xu *et al*. 2015). IP can immobilize heavy metals (As, Al, Cd, and Zn), retard their uptake by plants,

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Abbreviations: Br - brassinosteroid; Br24 - 24-epibrassinolide; Br28 - 28-homobrassinolide; DCB - dithionite-citrate-bicarbonate; IP - iron plaque.
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and so ameliorate their toxicity (Liu *et al*. 2004a, Chen *et al*. 2006, Xu and Yu 2013). Previous studies mostly focus on single element As or Cd; little information has been obtained on the effect of IP on As and Cd uptake by rice plants when they coexist. Anaerobic conditions result in As mobilization and higher As bioavailability to rice in paddy soil but at the same time, these conditions cause low Cd solubility due to Cd reaction with S forming CdS. However, the increased oxygen concentration in drained soil can convert CdS to $CdSO_4$ which is more bio-available (Fan *et al*. 2010). Therefore, translocation of As and Cd into rice plants when they simultaneously occur cannot be efficiently controlled *via* field water management. Hence, exploring more effective approaches to reducing their uptake and accumulation in rice are particularly important in soils contaminated by both elements.

Brassinosteroids (Brs) play critical roles in plant growth and development (Jin *et al*. 2015) and their analogues can be artificially synthesized, *e.g.,* 24-epibrassinolide (Br24) or 28-homobrassinolide (Br28). At present many BRs are often applied to protect plants against heavy metal stress (Yusuf *et al*. 2012, Ramakrishna and Rao 2013a,b, Fariduddin *et al*. 2014). It was reported that Br28 could regulate ion uptake, reduce heavy metals accumulation and alleviate their toxicity to plants (Sharma *et al*. 2011, Yusuf *et al*. 2011, Fariduddin *et al*. 2014). However, the effect of Brs on As and Cd uptake by rice plants is still not clear. Limited information is available on effects of Br on IP formation and the interactions of Br and IP on uptake of As and Cd in rice plants. Therefore, the aim of the present paper was to determine the contribution of IP to blocking heavy metal uptake by plants and the pivotal role of Brs in protecting plants against heavy metals. We hypothesized that Br24 or Br28 application may help root IP impede As and Cd uptake and accumulation in rice seedlings.

Materials and methods

Seeds of rice (*Oryza sativa* L.) cv. Yiyou 673 (local cultivar in Fujian Province, China) were disinfected in 30 % (v/v) H_2O_2 for 15 min and washed thoroughly with double distilled water. The seeds were geminated in moistened quartz for 20 d. Uniform seedlings were selected and grown in pots with 1/3-strength nutrient solution (pH 5.5). The composition of full-strength nutrient solution was as follows [mM]: NH_4NO_3, 5; K_2SO_4, 2; $CaCl_2$, 4; $MgSO_4 \cdot 7\,H_2O$, 1.5; KH_2PO_4, 1.3; H_3BO_4, 0.01; $ZnSO_4 \cdot 7\,H_2O$, 10^{-3}; $CuSO_4 \cdot 5\,H_2O$, 10^{-3}; $MnSO_4 \cdot H_2O$, 5×10^{-3}; $Na_2MoO_4 \cdot 2\,H_2O$, 5×10^{-4}; $CoSO_4 \cdot 7\,H_2O$, 2×10^{-4}; Fe(II)-ethylenediaminetetra-acetic acid (EDTA), 0.05. The nutrient solution was changed every 3 d and the experiment was carried out in a naturally lit greenhouse with a photoperiod about 12 h and a temperature between 25 and 35 °C.

After 3 weeks, 48 seedlings sprayed with Br24 (0.2 or 0.02 μM) or Br28 (0.2 or 0.02 μM) and 12 seedlings without Br were grown in nutrient solution for 3 d to test the effect of Br on IP formation or combined effect of Br and IP on As and Cd uptake. Then, $FeSO_4 \cdot 7\,H_2O$ (20 or 60 mg dm^{-3}) was added to the seedlings to induce IP formation during next 3 d. Afterwards, seedlings were grown in normal 1/3-strength nutrient solution for 3 d and then subjected to a mixture of 0.5 mg dm^{-3} As ($NaAsO_2$) and 0.5 mg dm^{-3} Cd [$Cd(NO_3)_2 \cdot 4\,H_2O$] for 9 d. On the third day of As and Cd treatments, another 24 seedlings without IP were sprayed with Br24 or Br28 in both concentrations and continuously grown in nutrient solution containing 0.5 mg dm^{-3} As and 0.5 mg dm^{-3} Cd for another 6 d for testing the effect of Br on As and Cd uptake. In addition, there were 3 seedlings without Br, IP and As and Cd, and 3 seedlings with only As and Cd treatments. There were 30 different combinations of treatments, each replicated three times to give a total of 90 pots (Table 1 Suppl.).

At harvest, all seedlings were divided into two parts: leaves and roots. Iron plaque on roots was characterized by the dithionite-citrate-bicarbonate (DCB) method (Taylor and Crowder 1983). The whole fresh root was incubated in 50 cm^3 of a solution containing 0.03 M sodium citrate ($Na_3C_6H_5O_7 \cdot 2\,H_2O$), 0.125 M sodium bicarbonate ($NaHCO_3$), and 1 g of sodium dithionite ($Na_2S_2O_4$) at 25 °C for 60 min. Roots were washed 3 times using deionized water, which was added to a DCB extracts, and the volume of solution was brought to 100 cm^3. The solutions were then filtered using 0.45-μm membrane filters and stored at 4 °C for analyses of Fe, As, and Cd. Then, leaves and roots were oven-dried at 70 °C for 72 h after which dry masses were recorded. After that, these leaf and root samples were ground to a fine powder and digested to determine the content of Fe, As, and Cd according to Williams *et al*. (2007). The content of Fe, As, and Cd in plant samples and DCB extracts were measured by Induced Couple Plasma-Mass Spectrometer (ICP-MS, *NexION 300X*, Perkin Elmer, Norwalk, USA).

Analysis of variance (*ANOVA*) and independent *t*-tests were carried out using *SPSS* software (*v.19.0*, Chicago, IL, USA). Presented data are means ± SDs (n = 3), and they were also analyzed using least significant difference (LSD) at the 5 % level.

Results

In the present study, IP, Br, As, and Cd treatments had significant effects on leaf and root biomass of rice seedlings (Table 2 Suppl.). Compared to the control seedlings, Br24 in concentration of 0.02 μM ($Br24_L$) and

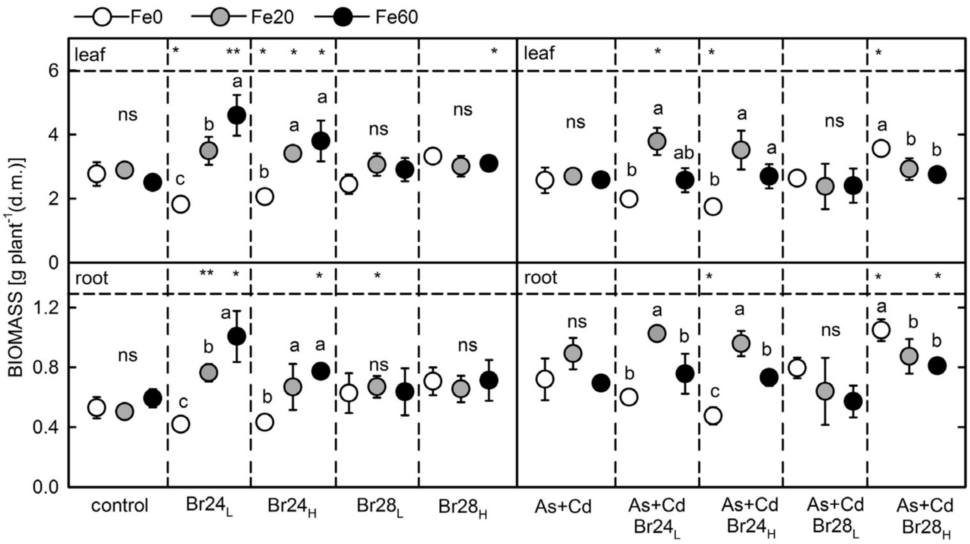

Fig. 1. Leaf and root biomass of rice seedlings exposed to 0 or 0.5 mg dm^{-3} As and Cd for 9 d as affected by 0.2 and 0.02 μM 24-epibrassinosteroid ($Br24_L$ and $Br24_H$) or 28-homobrassinosteroid ($Br28_L$ and $Br28_H$), as well as iron plaque (IP; 0, 20, and 60 mg dm^{-3} Fe^{2+}). Data are means ± SDs, $n = 3$. Different letters indicate significant differences at $P < 0.05$ among IP levels and * between control and Br treatments.

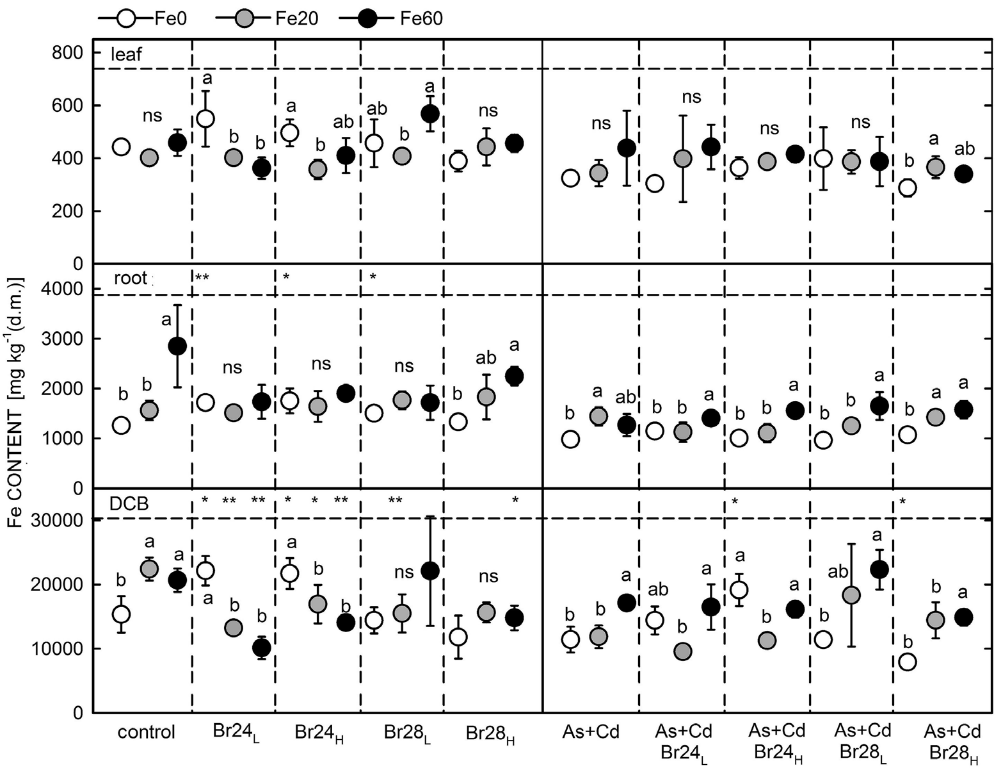

Fig. 2. Iron content in leaf, root and dithionite-citrate-bicarbonate (DCB) extract of rice seedlings exposed to 0 or 0.5 mg dm^{-3} As and Cd for 9 d as affected by 0.2 and 0.02 μM 24-epibrassinosteroid ($Br24_L$ and $Br24_H$) or 28-homobrassinosteroid ($Br28_L$ and $Br28_H$), as well as iron plaque (IP; 0, 20, and 60 mg dm^{-3} Fe^{2+}). Data are means ± SDs, $n = 3$. Different letters indicate significant differences at $P < 0.05$ among IP levels and * between control and Br treatments.

0.2 µM ($Br24_H$) significantly reduced leaf biomass in the absence of IP, but $Br24_L$, in the presence of iron plaque, significantly increased leaf biomass of rice seedlings exposed to As and Cd treatments.

Root biomass was significantly increased by Br24 ($Br24_L$ and $Br24_H$) in the presence of IP in comparison with control group (Fig. 1). Under As and Cd treatments, root biomass was significantly decreased and increased by $Br24_H$ and $Br28_H$ in the absence of IP, respectively, and only $Br28_H$ with Fe60 significantly increased root biomass.

The Fe content in leaves, roots, and DCB extracts was significantly affected by As and Cd treatments, IP, and Br respectively (Table 2 Suppl.). Compared to the control, Fe content in roots and DCB extracts significantly was increased by Br24 ($Br24_L$ and $Br24_H$) without IP, but DCB-Fe concentrations were decreased by Br24 with IP (Fig. 2). Under As and Cd treatments, $Br24_H$ increased and $Br28_H$ decreased Fe concentrations in DCB extracts in the absence of IP. In addition, compared to the control group, As and Cd treatments generally reduced Fe content in leaf, root and DCB extract regardless of IP (Fig. 3).

The content of As and Cd in roots was affected by IP (Table 2 Suppl.). As content in roots was increased by IP at $Br24_L$ or $Br28_H$ (Fig. 3). The leaf As content varied widely at different Br supply. IP increased As content in leaves at $Br24_L$, but decreased at $Br28_L$ and $Br28_H$ (Fig. 3). In the absence of IP, compared with control, As content in leaves was significantly reduced by $Br24_L$, but was increased at $Br28_L$ and $Br28_H$ (Fig. 3).

At $Br24_H$ group, Cd content in roots and leaves was decreased by IP (Fig. 4). But IP (Fe20) increased content in roots of $Br28_L$ and $Br28_H$ groups (Fig. 4). Cd content in roots and leaves was decreased by IP at $Br24_H$. However without IP, compared with control, leaf and root Cd content was significantly increased at $Br24_H$; and leaf Cd content was decreased at $Br28_H$ (Fig. 4).

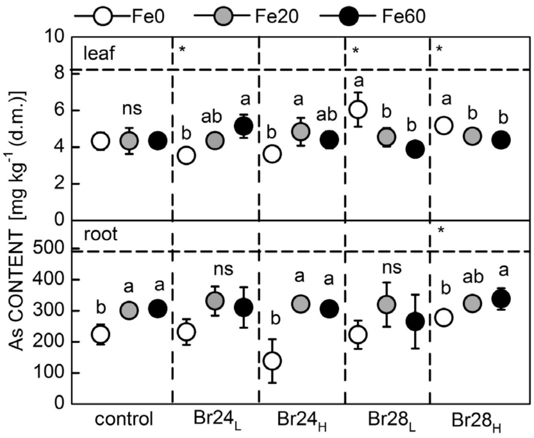

Fig. 3. Arsenic content in leaf, root, and dithionite-citrate-bicarbonate (DCB) extract of rice seedlings as affected by 0.2 and 0.02 µM 24-epibrassinosteroid ($Br24_L$ and $Br24_H$) or 28-homobrassinosteroid ($Br28_L$ and $Br28_H$), as well as iron plaque (IP; 0, 20, and 60 mg dm^{-3} Fe^{2+}). Data are means ± SDs, n = 3. Different letters indicate significant differences at $P < 0.05$ among IP levels and * between control and Br treatments.

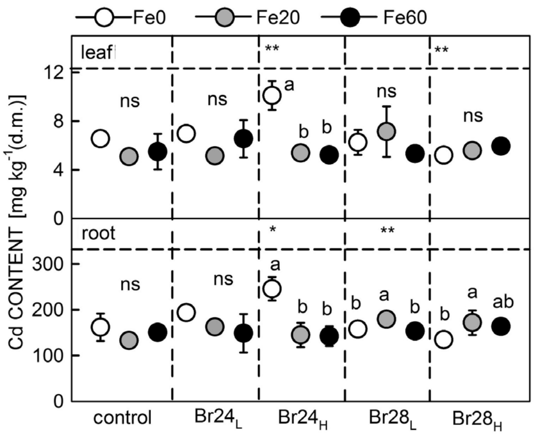

Fig. 4. Cadmium content in leaf, root, and dithionite-citrate-bicarbonate (DCB) extract of rice seedlings as affected by 0.2 and 0.02 µM 24-epibrassinosteroid ($Br24_L$ and $Br24_H$) or 28-homobrassinosteroid ($Br28_L$ and $Br28_H$), as well as iron plaque (IP; 0, 20, and 60 mg dm^{-3} Fe^{2+}). Data are means ± SDs, n = 3. Different letters indicate significant differences at $P < 0.05$ among IP levels and * between control and Br treatments.

Discussion

In this study, the results showed that Fe content in roots and DCB extracts was significantly increased by supplied Fe (Fe20 and Fe60), which is consistent with reports in the literature (Liu *et al*. 2004b, 2007a); however, the opposite results were found in DCB extracts in the presence of $Br24_L$ and $Br24_H$ (Fig. 2). The significant increase in root biomass could explain this (Fig. 1). Ye *et al.* (1998) showed that more Fe was sequestered on root surface in plants with lower root biomass. As and Cd treatments consistently reduced Fe content in leaf, root, and DCB extracts, which agrees with the conclusions of Liu *et al*. (2010), who found that Cd in soil (10 mg kg^{-1}) can significantly reduce Fe content in rice plants and Liu *et al*. (2004b) found that Fe content in shoots and roots of rice seedlings with 0.5 mg dm^{-3} As was lower than without As. However, Liu *et al*. (2007b) reported no significant difference in Fe content in rice plants with or without supplied Cd. Different genotypes may be the main factor affecting Fe uptake and transport in rice plants (Liu *et al*. 2010).

Our study showed that IP increased As content in rice leaves at $Br24_L$ and $Br24_H$, but decreased it at $Br28_L$ and $Br28_H$ (Fig. 3). Compared to the control, in the absence of IP, $Br24_L$ significantly reduced As content in rice leaves, but $Br28_L$ and $Br28_H$ increased it (Fig. 4). Our results showed that $Br28_L$ and $Br28_H$ increased As content in rice leaves, but the interactions of IP (Fe20 and Fe60) and Br28 decreased it. Kaim *et al*. (2016) showed that As^V accumulation was markedly lowered by Br24 in *Cicer arietinum*, which partially explained the decreasing As in rice leaf in our present study. At present, no research has been reported on the effect of Br28 on As uptake and translocation in plants, and whether or not Br28 can be used to reduce As uptake by plants or to promote the plant. Our findings are consistent with those showing that the content of Cu and Ni in *Brassica juncea* (Kanwar *et al*. 2013, Sharma and Bhardwaj 2007), B in *Arabidopsis thaliana* (Surgun *et al*. 2016), and Cr in tobacco (Bukhari *et al*. 2016) are significantly decreased by proper concentrations of Br24. Bukhari *et al*. (2016) found that Br24 significantly reduced Cr content in leaves of tomato genotypes Meiyu2-1 and NC107, but increased it in leaves of genotype 2010-38. These results imply that Br24 can decrease uptake of heavy metals by plants and ameliorate their toxicity.

Our results showed that in the absence of IP, Cd content in leaves was increased by $Br24_H$, but decreased by $Br28_H$. Different metal elements, Br concentrations, plant species, and genotypes may have led to the observed differences. Ramakrishna and Rao (2013b) and Sharma *et al*. (2008) reported that Br28 significantly reduced content of Zn in radish seedlings and Ni in *Brassica juncea*. They pointed out that Br28 can alleviate Zn and Ni stresses by increasing the activities and metabolism of glutathione and other antioxidative enzymes (Ramakrishna and Rao 2013a). However, Sharma *et al*. (2011) found that Br28 can promote Cr uptake by *Raphanus sativus* and increased activities of antioxidant enzymes (Sharma *et al*. 2011). Different metal elements, Br concentrations, plant species, and genotypes may have led to the observed differences, and further research is needed to elucidate the molecular mechanisms of Br in mediating plant responses to heavy metals.

Ekinci *et al*. (2012) showed Br24 (1, 2, and 3 μM) could decrease Cd content in leaves of lettuce under 50 mM NaCl, but increases it under 0 or 100 mM NaCl. Our results showed that $Br24_H$ increased Cd content in leaves without IP. But the interactions of IP and $Br24_H$ reduced Cd content in leaves in comparison with single action of $Br24_H$. This could be due to the increasing Fe content, which could decrease Cd accumulation in rice shoot (Liu *et al*. 2008, 2010). He *et al*. (2017) also showed that Fe supply inhibits Cd uptake by *Arabidopsis thaliana*. Br24 decreases Fe content in shoots of rice, cucumber, and lettuce (Ekinci *et al*. 2012, Wang *et al*. 2012, 2015), but increases Fe content under salt stress or Fe deficiency (Ekinci *et al*. 2012, Song *et al*. 2016), which is consistent with our results. Actually, Cd exposure can induce Fe deficiency in plants (Astolfi *et al*. 2014). In this respect, Br24 probably alleviated Fe loss under salt or heavy metals and prevented Cd uptake by plants.

The present study suggests that $Br24_L$ can decrease As content, and $Br28_H$ can reduce Cd content in leaves of rice plants without IP. The combined effects of Br28 and IP decreased As content in rice leaves, and the combined effects of $Br24_H$ and IP decreased leaf Cd content, indicating that the interactions between Br and IP could play pivotal roles in reducing As and Cd uptake by rice plants. Further studies are still required to detect the effects of three-way interactions between Br24, Br28, and IP on As and Cd uptake and accumulation in rice root, leaf, and grain tissue, and elucidate the molecular mechanisms of Br in mediating plant responses to heavy metals.

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