Pol. J. Environ. Stud. Vol. 28, No. 5 (2019), 3951-3958
DOI: *10.15244/pjoes/95036* ONLINE PUBLICATION DATE: 2019-05-03

Original Research

Influence of Br24 and Gr24 on the Accumulation and Uptake of Cd and As by Rice Seedlings Grown in Nutrient Solution

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Received: 13 July 2018
Accepted: 10 September 2018

Abstract

Plant hormones are trace organic molecules that play important roles in plant growth, development and stress resistance. 24-epibrassinolide (Br24) and strigolactones (Gr24) were reported to alleviate the toxicity of heavy metals and restrict their translocation into plants. In this study, the effects of Br24 and Gr24 with or without iron plaque (IP) induction on the uptake and accumulation of cadmium (Cd) and arsenic (As) were examined in rice seedlings exposed to 6-day treatments of 2 mg L^{-1} Cd or 5 mg L^{-1} As in nutrient solution. The results showed that the growth of rice seedlings was decreased by Cd or As treatment, but Br24 or Gr24 with or without IP alleviated this adverse effect. Br24 or Gr24 can improve root length, average diameter, surface area, and volume, and tip the number of roots of rice plants with or without IP. Cadmium contents in rice shoots were significantly reduced by Br24 with IP or Gr24, but Cd accumulation and translocation from root to shoot were not decreased. The interaction of Br24 or Gr24 and IP dramatically increased As contents in rice root, and reduced As translocation from root to shoot. Br24 or Gr24 with IP induction significantly increased Fe content, but decreased the contents of mineral elements (Mn, Cu and Zn) in rice plants. Our results suggested that the exogenous application of Br24 or Gr24 could be advantageous against the toxicity of Cd or As, and a potential way to effectively inhibit Cd or As uptake by rice plants.

Keywords: 24-epibrassinolide, strigolactones, iron plaque, heavy metals, rice seedlings

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Introduction

Heavy metal pollution in the environment has been widely concerning [1, 2]. The pollution of Cd and As has attracted much attention due to its widespread nature and harmfulness [3-6]. Cd and As are not essential elements for plant growth and will have negative effects on the growth and yield of crops when they exceed a certain concentration in the environment, which poses a potential threat to human health through the transmission and accumulation of the food chain [7-10]. Reports have shown that the contents of Cd and As in rice on the market exceeded the required standards, resulting in their accumulation in the human body [11, 12]. Long-term consumption of rice with high levels of Cd or As may induce various diseases like Itai-itai disease, bladder cancer, lung cancer, skin cancer, etc. [13, 14].

Rice is one of the most widely grown crops in the world and a staple for about 3 billion people [15]. Over the past several decades, a large amount of arable land has been polluted by heavy metals from frequent industrial activities [16, 17]. Recent studies have shown that plant hormones can affect the uptake and accumulation of heavy metals in plants [18]. Brassinolide (Br) as a plant hormone was discovered in the 1970s and plays an important role in the regulation of plant growth and development [19, 20]. In addition, several studies have found that Br can decrease the translocation of heavy metals (Cu, Cr, Zn, Pb, and Cd) into plants and alleviate their toxicity [18, 21-24]. Nevertheless, limited information can be obtained on whether Br can decrease the uptake of heavy metals by rice plants. Strigolactone as a plant hormone found in the 1970s can stimulate the germination of parasitic plant seeds, promote mycelial growth of arbuscular mycorrhizal fungi, directly or indirectly inhibit germination of plant lateral bud and coexist with host plants, etc. [25, 26]. Recent studies have uncovered the fact that synthetic strigolactones (Gr24) can effectively reduce the translocation of Cd to switchgrass [27]. However, it is not clear whether Gr24 will also play a positive regulatory role in response to heavy metal stress in rice plants.

At present, there have been many reports on the effect of iron plaque (IP) on retarding accumulation and uptake of heavy metals by wetland plants. The IP is an iron oxide formed by the oxidation of Fe^{2+} around the rhizosphere of wetland plants by the oxygen or oxidizing substances secreted from the roots [28]. Iron plaque can adsorb Cd and As on root surface of rice plant and decrease their uptake by rice plants to some extent [29, 30]. But whether the interaction between plant hormones and IP can decrease the uptake and accumulation of Cd or As in rice plants still needs further verification. Therefore, the main aims of this study were to explore two questions on plant hormones application and IP induction to investigate the distribution of Cd or As in rice seedlings: (1) Could exogenous Br24 or Gr24 application effectively reduce Cd or As uptake by rice plants with or without IP? (2) What are the possible mechanisms for the observed effects? To explain these questions, a hydroponic experiment was designed to test the uptake and translocation of Cd or As by rice seedlings by soaking seeds in Br24 solution or adding Gr24 into nutrient solution.

Materials and Methods

Rice Growth and Experiment Treatment

Rice seeds (Yongyou1540 purchased in Shufang Town, Nanping City, Fujian Province) were soaked in 30% H_2O_2 for 15 minutes and rinsed thoroughly with deionized water. Half of the seeds were soaked in deionized water and the other half in a 0.1 nM Br24 solution. After 24 h, the rice seeds were evenly spread in the quartz sand for germination. After 16 days, the rice seedlings were transferred to a 20 × 200 mm glass tube wrapped in aluminum foil containing 1/4-strength Hoagland solution, which was changed every 3 days and the pH was adjusted to 5.5. After 10 days, the rice seedlings were transferred to deionized water for 12 hours, and then IP was induced: (1) rice seedlings without IP induction: grown in 1/4-strength Hoagland solution (-P) for 3 days to avoid precipitation of P and Fe; (2) rice seedlings with IP induction: grown in 1/4-strength Hoagland solution including 30 or 70 mg L^{-1} Fe^{2+} (-P, -Fe(II)-EDTA) for 3 days. Iron plaque induced by 30 or 70 mg L^{-1} Fe^{2+} were called IP30 and IP70, respectively. After that, these rice seedlings were grown in 1/4-strength Hoagland solution for 5 days for recovery growth. Then, rice seedlings were grown in 1/4-strength Hoagland solution, including 5 mg L^{-1} As ($Na_3AsO_4·12H_2O$) or 2 mg L^{-1} Cd ($Cd(NO_3)_2·4H_2O$) with or without 1μM Gr24 addition for 6 days. The total combinations of Br24 or Gr24 with or without IP gave 15 treatments, each in triplicate (Table 1). The experiment was carried

Table 1. Experiment treatment.

No Br24 or Gr24 treatment	Br24 treatment		Gr24 treatment	
Control	Br24+Cd	Br24+As	Gr24+Cd	Gr24+As
Cd	Br24+IP30+Cd	Br24+IP30+As	Gr24+IP30+Cd	Gr24+IP30+As
As	Br24+IP70+Cd	Br24+IP70+As	Gr24+IP70+Cd	Gr24+IP70+As

out in a greenhouse at 25-35ºC with light exposure of 12-14 h d^{-1}.

Tissue Elements Analysis

At harvest, the rice seedlings were divided into two parts of root and shoot, and washed with deionized water. The fresh roots were scanned in a root scanner, and then placed in a 70ºC oven with shoots for 72 h. Afterward, oven-dried root and shoot samples were ground and soaked in a microwave digestion tube with 8 mL HNO_3 overnight. Then 1 mL H_2O_2 (30%) was added into a microwave digestion tube and the digestion process was carried out for 4 hours at 95ºC. The digestion solution was transferred to a volumetric flask and filtered using a 0.45 μm filter. Reagent blank and standard reference material (bush twigs and leaves, GBW07603, Chinese National Certified Reference Material) are used for quality control and analysis of the digestion procedure. Concentrations of Cd, As, Fe, Mn, Cu and Zn in roots and shoots were determined using induced couple plasma mass spectrometry (ICP-MS, NexION 300X; Perkin Elmer, NY).

Statistical Analysis

One-way ANOVA (analysis of variance) was carried out using SPSS software (19.0, SPSS, Inc., Chicago, IL, USA). The effects of Br24 and Gr24 with or without IP were tested on rice seedling biomass, root parameters and the concentrations of Cd, As, Fe, Mn, Cu and Zn in rice plants. Data presented are means ± SE (n = 3), and were analyzed using least significant difference (LSD) at the 5% level.

Results and Discussion

To examine whether Cd or As affected the growth of rice seedlings under Br24 or Gr24 with or without IP, we analyzed plant biomass and root parameters, including length, surface area, volume, diameter and tips of root (Figs 1 and 2). Biomass of rice shoot was decreased by Cd, but increased by Br24 or Gr24 with or without IP (Fig. 1). With respect to the control, Br24 or Gr24 also significantly increased root biomass of rice seedlings exposed to Cd stress (Fig. 1). Arsenic exposure did not

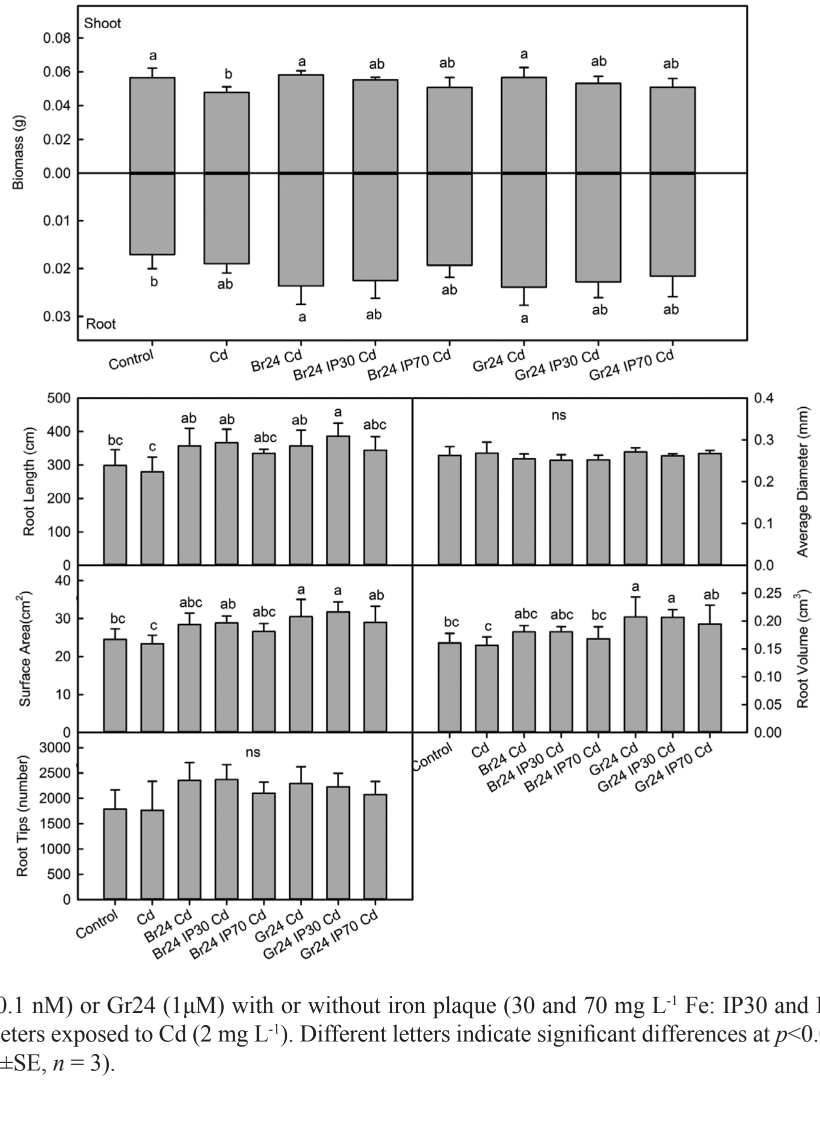

Fig. 1. Effects of Br24 (0.1 nM) or Gr24 (1μM) with or without iron plaque (30 and 70 mg L^{-1} Fe: IP30 and IP70) on biomass of rice seedlings and root parameters exposed to Cd (2 mg L^{-1}). Different letters indicate significant differences at $p<0.05$ using least significant difference (LSD) (means±SE, n = 3).

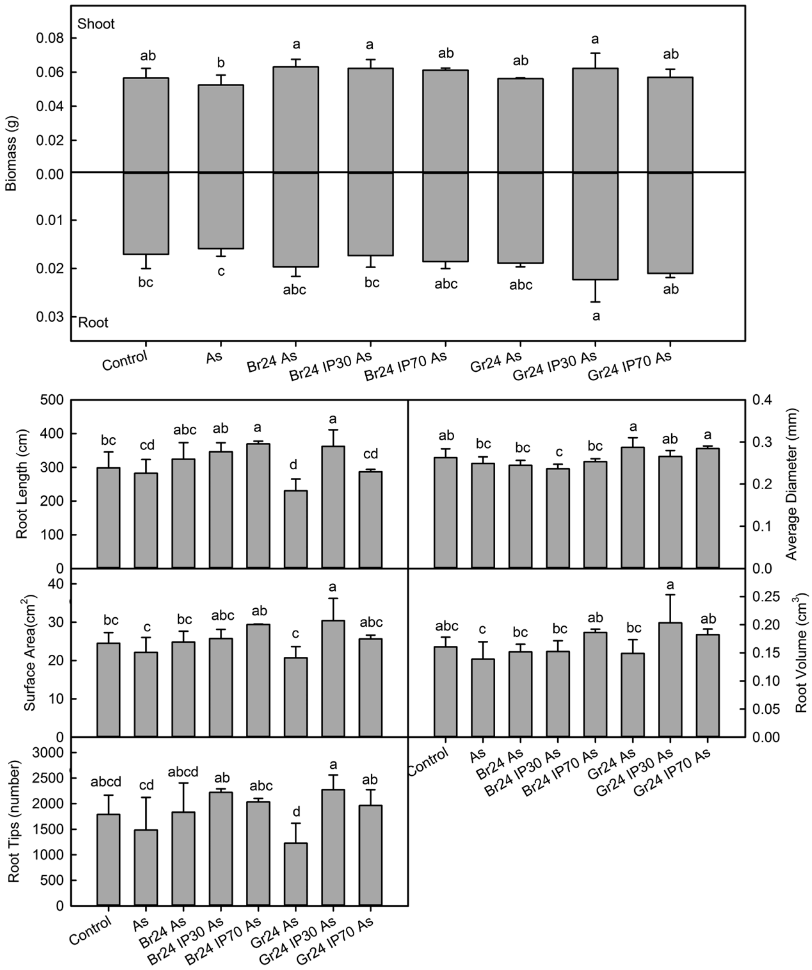

Fig. 2. Effects of Br24 (0.1 nM) or Gr24 (1μM) with or without iron plaque (30 and 70 mg L^{-1} Fe: IP30 and IP70) on biomass of rice seedlings and root parameters exposed to As (5 mg L^{-1}). Different letters indicate significant differences at $p<0.05$ using least significant difference (LSD) (means±SE, $n = 3$).

affect biomass of rice seedlings (Fig. 2). In comparison with As treatment, Gr24 with IP30 promoted biomass of shoot and root, and Br24 with or without IP30 increased shoot biomass (Fig. 2). Cadmium and As are not essential elements for higher plant growth, and excessive Cd or As exposure has been reported to cause adverse effects on growth and yield of rice plant [31, 32]. Our previous work indicated that 0.02 μM Br24 alleviated Cd and As negative effects on biomass of rice with IP20 [33]. A recent study showed that Gr24 can alleviate Cd toxicity to switchgrass seedlings [27]. These results were in line with the present study, revealing that 0.1 μM Br24 or 1 μM Gr24 promoted rice growth under As or Cd treatment (Figs 1 and 2).

Compared with the control group, Cd treatment did not affect the length, surface area, diameter, volume and tip numbers of rice roots (Fig. 1). In comparison with Cd treatment, Br24 or Gr24 markedly increased the root length with or without IP30 (Fig. 1). Gr24 with or without IP30 or IP70 significantly increased root surface area and volume (Fig. 1). In comparison with the control group, the root parameters were also not affected by As treatment (Fig. 2). Br24 with IP30 or IP70 and Gr24 with IP30 significantly stimulated root length in comparison with As treatment (Fig. 2). Root diameter, surface area, volume and tips were increased by Gr24 with or without IP30 or IP70, Br24 with IP70 and Gr24 with IP30, Br24 with IP70 and Gr24 with IP30 or IP70, and Br24 with IP30 and Gr24 with IP30 or IP70, respectively (Fig. 2). These results indicated that Br24 or Gr24 with or without IP neutralized the decrease in root parameters of rice seedlings under Cd or As stress, which was supported by Kapoor et al. [34] showing that the decline in root length of *Raphanus sativus* seedling under Cd stress was alleviated by Br24 (0.1, 0.01 and 0.001 μM), and Tai et al. [27] reporting that 1 μM Gr24 increased root length, surface area, volume and tips of switchgrass seedlings under Cd stress. Br24 also can relieve the toxic effect of Ni, Hg and Cr on the growth of *Raphanus sativus* L., chickpea seedlings and *Oryza sativa* L. by protecting photosynthesis and improving antioxidant enzyme activity [18, 35, 36].

Cadmium content in shoots was significantly decreased by Br24 with IP30 and Gr24 (Fig. 3). Root Cd content was obviously decreased by Gr24 with or without IP30 or IP70. However, Br24 or Gr24 did not decrease Cd translocation to rice shoot (Fig. 3). The total amount of Cd ranged from 1.63-1.90 μg in shoots and from 10.24-13.40 μg in roots (Fig. 3). Root Cd content was increased by Br24, and decreased by Gr24 with IP70. Br24 or Gr24 with or without IP did not markedly affect the amount of Cd in shoots. The highest proportion (85%-88%) of Cd was concentrated in rice roots (Fig. 3).

Our results were partly consistent with those reported by RADY et al. [23] and Tai et al. [27], who observed Br24 and Gr24 significantly decreased Cd contents in *Phaseolus vulgaris* L. and switchgrass seedlings. Iron plaque can inhibit Cd uptake by rice seedlings under low-dose Cd exposure (0.1 mg L^{-1}), but not under 1.0 mg L^{-1} [37]. However, in the present study the results showed that IP30 obviously decreased Cd uptake by rice seedlings exposed to 5 mg L^{-1} Cd in the presence of Br24 or Gr24. This indicates that a positive role of Br24 with IP occurred in decreasing Cd uptake by rice plants. Our previous study also demonstrated that Cd in rice plants was markedly decreased by the interaction of Br24 and IP [33]. It was speculated that Br24 and Fe could play a certain defensive role in coping with Cd stress. Br24 can decrease shoot Fe content and increase root Fe content of rice seedlings with Fe-sufficient or Fe-deficient medium [38]. Our previous study also showed that Br24 increased Fe content in rice roots, but not in shoots without Cd stress, and did not affect Fe content in rice plants with Cd treatment [33]. This contrary result can be attributed to different rice varieties. In this study, our results showed no significant effect of Br24 on Fe content in rice plants in the presence of Cd or As treatment. The different application method of Br24 may be one of the reasons for these dissimilar results. Soaking seeds or foliar spraying or foliar spraying at different plant growth stages all determined the Br's effects on Cd uptake by plants [22, 39]. In addition, nitric oxide (NO) was reported to be involved in the reduction of Cd in plants [40], but did not increase Fe content in plants. It can be seen that reducing Cd uptake by plants is not only due to the increasing Fe supply, but a very complicated process, although Liu et al. [29] reported that increasing Fe supply decreased Cd content in rice plants. This was in line with our results showing that Gr24 decreased Cd uptake by rice plants in the absence of IP (Fig. 3). At present, there are few reports on the effect of Gr24 on the translocation and uptake of heavy metals in plants. Only Tai et al. [27] showed that

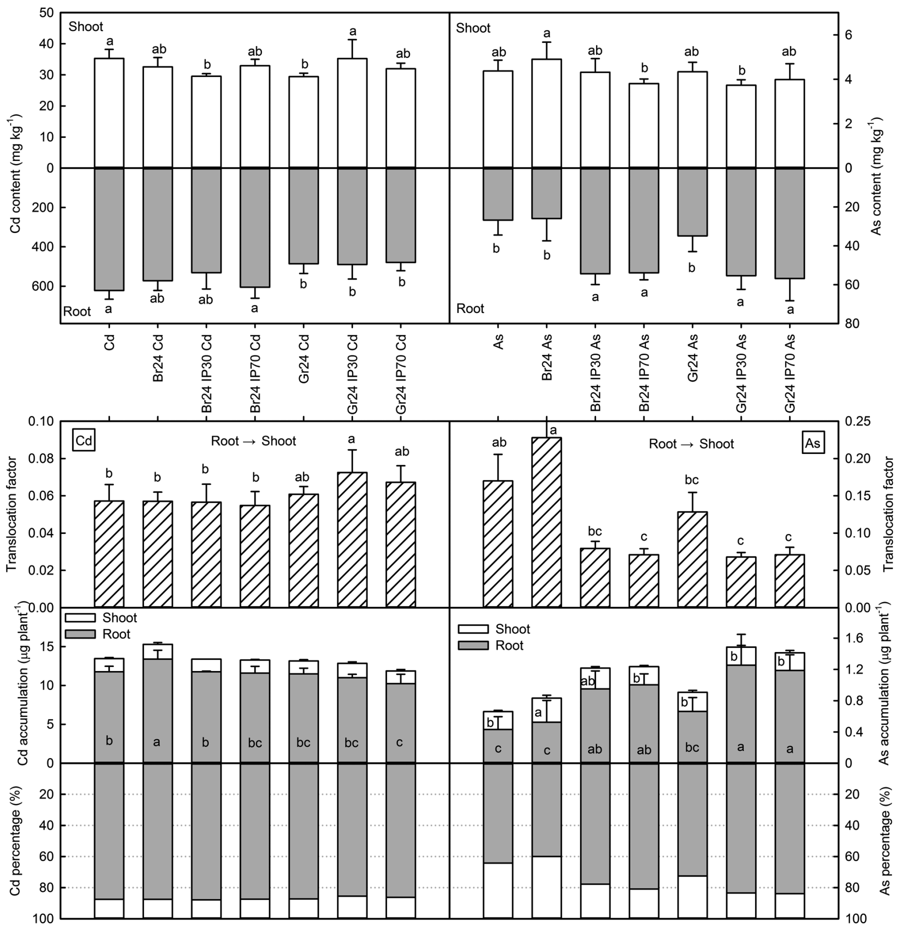

Fig. 3. Effects of Br24 (0.1 nM) or Gr24 (1μM) with or without iron plaque (30 and 70 mg L^{-1} Fe: IP30 and IP70) on contents, translocation factor and accumulation of Cd or As in shoots and roots of rice seedlings exposed to Cd (2 mg L^{-1}) or As (5 mg L^{-1}) stress. Different letters indicate significant differences at $p<0.05$ using least significant difference (LSD) (means±SE, n = 3).

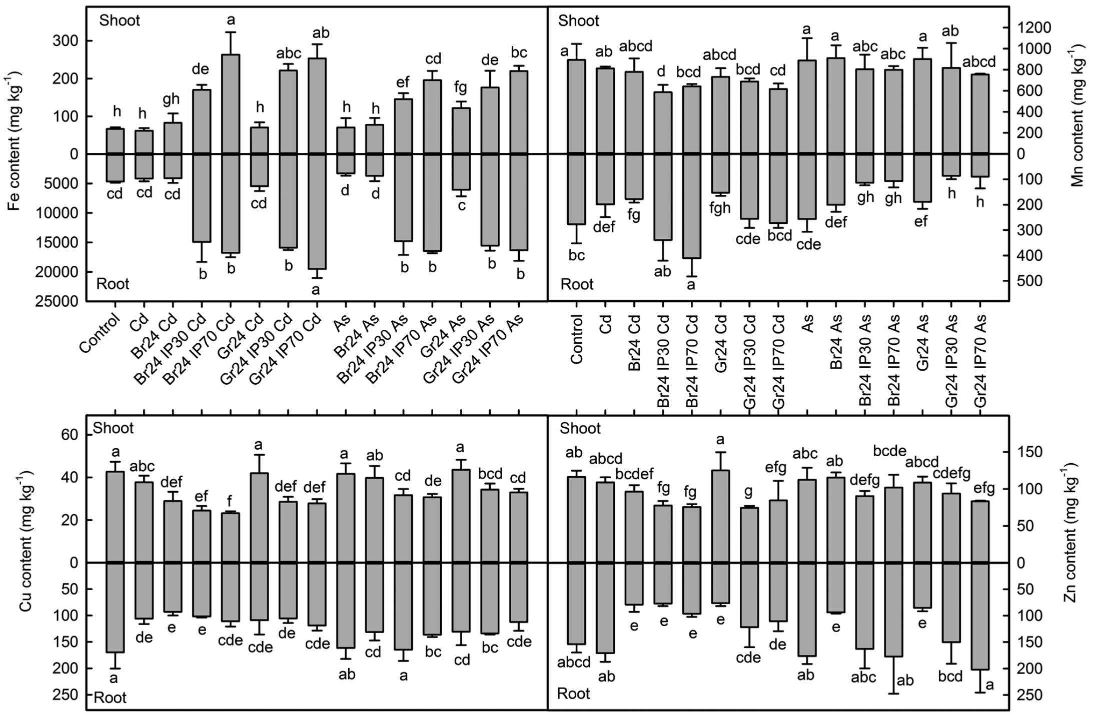

Fig. 4. Effects of Br24 (0.1 nM) or Gr24 (1μM) with or without iron plaque (30 and 70 mg L^{-1} Fe: IP30 and IP70) on contents of Fe, Mn, Cu and Zn in shoots and roots of rice seedlings exposed to Cd (2 mg L^{-1}) or As (5 mg L^{-1}) stress. Different letters indicate significant differences at $p<0.05$ using least significant difference (LSD) (means±SE, $n = 3$).

the reason for Gr24 reducing Cd uptake by switchgrass seedling might be attributed to the improvement of some essential elements (Fe, Mn, Cu and Zn). However, no significant increase in these elements in rice plants was found in our results (Fig. 4). It is noticed that the contents of Mn, Cu and Zn in shoots and roots of rice plant were reduced by IP induction (Fig. 4). Recent studies reported that NO and Gr24 interacted in plants inducing stomatal closure [41] and root elongation [42], and Gr24 can stimulate a significant increase in NO contents. Therefore, it can be inferred that Gr24 possibly regulated the response of plants to Cd stress by stimulating NO-enhancing antioxidant enzyme activity.

Br24 or Gr24 did not affect As content in shoots of rice seedlings with or without IP, but markedly increased As content of rice roots with IP30 or IP70 (Fig. 3). Br24 or Gr24 significantly decreased As translocation to rice shoots with IP30 or IP70 (Fig. 3). The total amount of As in shoots of rice ranged from 0.23-0.31 μg and from 0.43-1.25 μg in roots (Fig. 3). Arsenic content in rice shoots was increased by Br24, and root As content was enhanced by Br24 or Gr24 with IP. The proportion of As was 64% in roots and 36% in shoots, respectively. Br24 or Gr24 with IP decreased the proportion (16-22%) of As in rice shoots. Our previous study showed that As content in rice shoots was decreased by 0.02 μM Br24, but not by 0.2 μM Br24; and the interaction of IP induction and 0.02 or 0.2 μM Br24 can increase shoot As content [33]. This indicated that As uptake by rice plants was determined by different Br24 concentrations. Our results showed that IP induction also did not affect As uptake by rice shoots, but increased As uptake and accumulation in rice roots (Fig. 3), which was supported by Liu et al. [43, 44], who found a significant positive relationship between Fe and As concentrations in shoots or plaque. Singh et al. [45] reported that NO can decrease As content in rice plants, probably by down-regulating the expression of *OsLsi1* and *OsLsi2* and enhancing the Fe accumulation. In this study, although Fe content was increased by Gr24 under As stress, As content in rice plants was not affected. The reason for this dissimilar result may be due to the different NO supply. Farnese et al. [46] found that 0.1 mg L^{-1} NO did not affect As content in *Pistia stratiotes.* In addition, As concentrations in environmental media also affected NO's effect on As contents in plants. For example, Namdjoyan et al. [47] showed that NO can significantly decrease As content in shoots of watercress plants under 50, 75 and 100 μM As treatments, but not under 25 μM As. However, the reasons why Gr24 or Br24 with or without IP can affect or reduce As or Cd translocation and uptake in rice plants still needs to be studied and discussed in the future.

Conclusions

In this study, plant hormone-Br24 or Gr24 with or without IP induction was applied to a rice-nutrient solution system to investigate its potential effect on Cd or As uptake by rice seedlings. The results showed that Br24 or Gr24 can improve the growth of rice seedlings with or without IP. Br24 with IP or Gr24 significantly decreased Cd content in rice shoots, but Cd accumulation and translocation from root to shoot were not reduced. Although arsenic content in rice plants was not decreased, As translocation from root to shoot was markedly reduced by the interaction of Br24 or Gr24 and IP. Although we have discussed the uptake and accumulation of Cd and As in rice seedlings and explained the reasons for that, many of them cannot be confirmed due to the lack of research in this field at present, especially for Gr24's effect. Subsequent studies are still needed to further verify the effect of Br24 or Gr24 on the accumulation of Cd or As in rice grains and explore the possible molecular mechanism.

Acknowledgements

This study was financed by the National Science Foundation of China (31500319).

Conflict of Interest

The authors declare no conflict of interest.

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