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Full Length Article

Effect of Brassinosteroid and Selenium on Uptake and Accumulation of Chromium in Yellow Flag (*Iris pseudacorus*)

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Abstract

A hydroponic experiment was conducted to investigate the effect of brassinosteroids (Br) and selenium (Se) on iron plaque (IP) formation and chromium (Cr) accumulation and uptake by *Iris pseudacorus* L. (yellow flag). Yellow flag seedlings (4 week old seedlings) were grown under 0.1 mg L^{-1} Br (24-epibrassinolide (Br24) or 28-homobrassinolide (Br28)) or 0.5 mg L^{-1} Se (Se(IV) or Se(VI)) for 2 weeks. Then seedlings with or without IP induction (60 mg Fe^{2+} L^{-1} for 3 days) were exposed to 5 mg L^{-1} hexavalent chromium [Cr(VI)] for 1 week. The results showed that Cr reduced Fe accumulation and uptake by yellow flag with or without IP; however, this effect was significantly neutralized by Br, but not by Se. IP significantly enhanced Cr accumulation in DCB extract and the whole yellow flag seedling, and increased Cr content by 36%. Br24 and Br28 significantly enhanced Cr accumulation and uptake by yellow flag and increased Cr enrichment by 36 and 51% in the absence of IP, and 7 and 21% in the presence of IP, respectively. Se(IV) markedly improved Cr accumulation in the root, but Cr accumulation and uptake in the shoot and DCB extract were not affected by Se(IV) and Se(VI). Our study suggests that Br24 and Br28 can alleviate Fe loss caused by Cr stress and increase Cr accumulation and uptake by yellow flag seedlings.

Keywords: Chromium; Phytoremediation; Brassinosteroid; Iron plaque; Selenium

Introduction

Soil and water contamination by chromium (Cr) received widespread attention due to its abundance and toxicity (Gao and Xia, 2011; Mashkoor *et al*., 2016; Ballesteros *et al*., 2017). Cr mainly consists of two valence states: Cr(III) and Cr(VI) in the environment (Rai *et al*., 1989). It has been classified as a Group I carcinogen, and Cr(VI) shows much more soluble and toxic to plants and human in comparison with the relatively immobile Cr(III) (Richard and Bourg, 1991; Smith *et al*., 1997). Cr contamination often occurs via anthropogenic pathways, including electroplating, leather, mining, manufacturing of pigments, dyes, textiles, etc (Gao and Xia, 2011). Consequently Cr could enter the human food chain by translocation, accumulation and uptake in soil, water, plants and animals, and pose potential health threat to humans (Xia *et al*., 2016). In this regard, it is extremely urgent to solve this environment problem. Recently, phytoremediation has gradually become a promising, economic and environment-friendly remediation technology to remove heavy metals in the environment (Singh *et al*., 2003; Hashim *et al*., 2011). *Iris pseudacorus* L. (yellow flag) as one of ornamental hydrophyte possesses characteristics of macrophytes with high efficiency to accumulate heavy metals (Mang *et al*., 2007; Xu *et al*., 2015). In addition, yellow flag, like most wetland plants, can form iron plaque (IP) on root surfaces under saturated or anaerobic condition.

Ferrous ion is oxidized by oxygen or antioxidant secreted from roots and is precipitated on root surfaces of wetland plants (Armstrong, 1964; Taylor *et al*., 1984). The Fe precipitation is called IP and is composed mainly of amorphous Fe oxyhydroxides and small amounts of crystalline Fe oxides (Hansel *et al*., 2001; Weiss *et al*., 2004; Xu and Yu, 2013). Several studies have reported that IP could adsorb metal(loid)s and decide their fate by blocking or improving their translocation into plants (Ye *et al*., 1998; Batty *et al*., 2000; Ye *et al*., 2003; Liu *et al*., 2004; Liu *et al*., 2007; Liu *et al*., 2008b; Xu and Yu, 2013; Xu *et al*., 2015). Various factors (such as amount of IP, metal(loid) type and concentration, pH, etc.) could affect metal(loid) translocation and accumulation in plants. The effects of IP on Cr translocation and uptake in plants showed that IP could immobilize Cr on root surface of rice and yellow flag and significantly increased Cr uptake by yellow flag (Hu *et al*., 2014a; Xu *et al*., 2015).

Brassinosteroids (Br) as vital regulators in plant development and growth can alleviate heavy metal toxicity through increasing antioxidant enzyme activity (Clouse and Sasse, 1998; Fariduddin *et al*., 2014). It was reported that Br could increase photosynthesis (Yu *et al*., 2004; Ahammed *et al*., 2013), which enhanced radial oxygen loss contributing

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to IP formation (Lai *et al*., 2012; Wu *et al*., 2012). Therefore, we hypothesized that Br might promote IP formation; and the combined effects of Br and IP could enhance Cr accumulation and uptake by yellow flag.

Selenium (Se), a nonessential element for plant growth, can also alleviate heavy metal (Cd, Pb, Hg) toxicity to plants possibly through forming Se-metal complexes (Sasakura and T. Suzuki, 1998; Belzile *et al*., 2006; Fargasova *et al*., 2006; Hu *et al*., 2014b). Most studies focus on the role of Se in decreasing translocation and uptake of heavy metals in plants, but little information has been obtained on Se effect on phytoremediation in heavy metals in macrophytes. Qing *et al*. (2015) found that Se(IV) could protect plant from Cr stress through increasing antioxidants activity but did not affect Cr translocation in cabbage leaves. Besides, Se supply promoted As and Al uptake by *Pteris vittata* L. (Srivastava *et al*., 2009) and ryegrass (Cartes *et al*., 2010). Taking together the above information, therefore, our objectives in the present study were (1) to test the influence of Br (24-epibrassinolide (Br24) and 28-homobrassinolide (Br28)) or Se (Se(IV) and Se(VI)) on IP formation and (2) to investigate the effect of Br or Se on Cr translocation and uptake by yellow flag with or without IP.

Materials and Methods

Plant Culture and Experiment Treatment

Seeds of yellow flag, obtained from Guanyin Lake, Fujian agriculture and forestry university, Fujian Province, China, were soaked in 30% v/v H_2O_2 for 60 min and then were rinsed thoroughly using deionized water. These seeds were placed on moist quartz sand for 30 days for germination in greenhouse with temperature from 25–35°C. Uniform seedlings were transferred to 1/3 strength Hoagland nutrient solution (pH 5.5) for 30 days. Then these seedlings were grown in nutrient solution with Br24 (0.1 mg L^{-1}), Br28 (0.1 mg L^{-1}), Se(IV) (0.5 mg L^{-1}) and Se(VI) (0.5 mg L^{-1}), respectively. After two weeks, all seedlings were transferred in deionized water for 12 h and induced to form IP by adding 60 mg Fe^{2+} L^{-1} ($FeSO_4 \cdot 7H_2O$) into nutrient solution without P for 3 days. Afterwards these seedlings were grown in normal 1/3 strength Hoagland nutrient solution for 3 days and then exposed to 5 mg L^{-1} Cr ($K_2Cr_2O_7$) for one week. A total of 16 treatments were as follows: control, IP, Cr, Br24, Br28, Se(IV), Se(VI), IP + Br24, IP + Br28, IP + Se(IV), IP + Se(VI), IP + Cr, IP + Br24 + Cr, IP + Br28 + Cr, IP + Se(IV) + Cr, IP + Se(VI) + Cr. The nutrient solution was changed twice a week and the experiment was carried out in a greenhouse at 15–25°C with natural day/night sunlight. The full-strength Hoagland solution was composed of (mmol L^{-1}): KH_2PO_4 (1.0), KNO_3 (5.0), $Ca(NO_3)_2 \cdot 4H_2O$ (5.0), $MgSO_4 \cdot 7H_2O$ (2.0); $CuSO_4 \cdot 5H_2O$ (3.2×10^{-4}), $ZnSO_4 \cdot 7H_2O$ (7.7×10^{-4}), $MnCl_2 \cdot 4H_2O$ (9.2×10^{-3}), H_3BO_3 (4.6×10^{-2}), $H_2MoO_4 \cdot 4H_2O$ (3.85×10^{-4}), Fe(II)-ethylenediamine tetraaceticacid (EDTA) (2.0×10^{-2}).

Chemical Analysis of Plant Samples

At harvest, the whole plant of yellow flag was divided into shoot and root. The root was washed thoroughly using deionized water and was incubated in 50 mL dithionite-citrate-bicarbonate (DCB) solution containing 0.03 M sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 0.125 M sodium bicarbonate ($NaHCO_3$), and 1 g sodium dithionite ($Na_2S_2O_4$) for 60 min at 25°C (Taylor and Crowder, 1983). Then washed the roots three times using deionized water and transferred these solution into 100 mL volumetric flask. DCB extracts solution was filtered using 0.22 μm membrane filters and stored in 4°C for analysis. The shoots and roots were oven-dried at 65°C for 72 h and then were weighed. The dry plant samples were ground and transferred into digestion tubes for digestion according to Xu *et al*. (2015). Transferred digestion solution to a 100 mL volumetric flask and filtered the solution through 0.22 μm membrane filters. A reagent blank and a standard referencematerial (bush twigs and leaves, GBW07603, Chinese National Certified Reference Material) were included as a quality control for digestion procedure and subsequent analysis.

The concentrations of Fe, Cr, Cu, Zn, Mn and B in plant samples were measured by Induced Couple Plasma-Mass Spectrometer (ICP-MS, NexION 300X; Perkin Elmer, NY), and the concentrations of Fe and Cr in DCB extracts were measured using a flame atomic absorption spectrophotometer (FAAS Solaar M6, Thermo Electron Corp., Waltham, MA, USA).

Statistical Analysis

Independent-sample T tests and ANOVA were conducted to test the effects of Br or Se with or without IP on biomass, distribution of Fe, Cr, Cu, Mn, Zn and B in leaves, roots, DCB extracts and the whole yellow flag seedlings exposed to Cr stress. Data presented are means ± SD (n = 3) and analyzed using least significant difference (LSD) at the 5% level. The statistical analyses were carried out using SPSS software (19.0, SPSS, Inc., Chicago, IL, USA).

Results

Plant Growth

The shoot biomass of yellow flag was significantly increased by the combined effects of Br24 and IP, but not by other treatments. The root biomass was reduced by the combined effects of Cr, IP and Br24 or Se (Se(IV) or Se(VI)) (Fig. 1). Compared to Cr treatment, Br24 and Br28 markedly increased shoot growth of yellow flag exposed to Cr stress in the absence of IP, but not in the presence of IP.

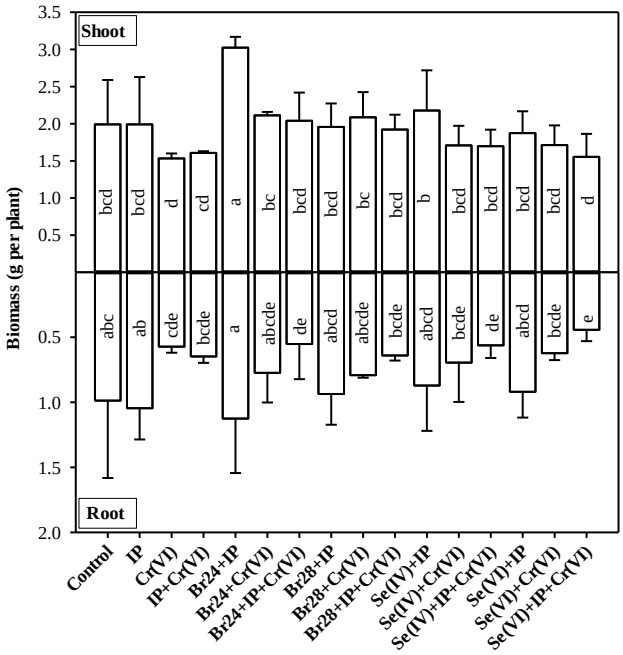

Fig. 1: Biomass of shoot and root of yellow flag (*Iris pseudacorus* L.) seedlings with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP) (Fe^{2+}: 0 or 60 mg L^{-1}) under 0 or 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) stress. Different letters indicate significant differences at $p < 0.05$ in biomass of shoot and root of yellow flag seedlings. Data are means ± SD, $n = 3$

Analysis of Fe

In the control group, 60 mg Fe^{2+} L^{-1} addition did not significantly affect Fe concentrations in DCB extracts; however, Fe addition increased DCB-Fe concentrations under Cr stress with or without Br (Br24 and Br28) or Se (Se(IV) and Se(VI)) (Fig. 2). The Fe concentrations in root tissues were significantly increased by IP and the combined effects of IP and Br (Br24 and Br28) with or without Cr, but not by Se(IV) and Se(VI) (Fig. 2). However, shoot Fe concentrations were not affected by IP and other treatments except at Br28. In the absence of IP, the combined treatments of Se(IV) and Cr decreased Fe concentrations in DCB extracts (Fig. 2). In the presence of IP, however, DCB-Fe concentrations were significantly increased by the combined effects of Br (Br24 and Br28) and Cr. Under Cr-free treatment, Br did not affect Fe concentrations in shoots, roots and DCB extracts, but root Fe concentrations were significantly reduced by Se(IV) and Se(VI); and Se(VI) also decreased DCB-Fe concentrations (Fig. 2). Under Cr-free condition, Se(IV) supply markedly reduced Fe accumulation in DCB extract, root and whole yellow flag plant in the presence of IP (Fig. 3). Under Cr treatment, Fe accumulation in DCB extracts and whole plant was significantly enhanced by Br24 and Br28 regardless of formation or not of IP (Fig. 3). In comparison with the control seedlings, Fe accumulation in DCB extracts and whole plants was dramatically increased by IP or the combination of IP and Br24 under Cr-free treatment (Fig. 3). Similarly, when compared to the control, Br (Br24 and Br28) with IP enhanced Fe accumulation in DCB extracts under Cr treatment; and Br28 with IP promoted Fe uptake by whole plant (Fig. 3). Under Cr treatment, additionally, IP formation dramatically increased Fe accumulation in roots and DCB extracts, but not in shoots (Table 1). However, compared to Cr-free treatment, Cr stress significantly reduced Fe accumulation in roots and DCB extracts in the presence of IP; and Br (Br24 and Br28) and Se (Se(IV) and Se(VI)) neutralized this effect (Table 1). The percentage of Fe in DCB extracts, roots and shoots was 5–19%, 10–27% and 54–83%, respectively (Fig. 4).

Table 1: Distribution of iron (Fe) in shoot, root, dithionite-citrate-bicarbonate (DCB) extract and the whole plant of *I. pseudacorus* (yellow flag) seedlings with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP) (Fe^{2+}: 0 or 60 mg L^{-1}) under 0 or 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) stress

Treatment				Fe content (mg)				
				—	Br24	Br28	Se (IV)	Se (VI)
DCB-Fe	A	+IP	+Cr	3.78	6.27	7.22	4.77	4.94
	B	-IP	+Cr	1.58 **	2.41 *	2.78 **	1.20 **	1.96 *
	C	+IP	-Cr	6.88 **	6.32	5.76	4.39	6.26
Root-Fe	A	+IP	+Cr	1.11	1.04	1.15	0.75	0.60
	B	-IP	+Cr	0.43 **	0.64	0.65 *	0.59	0.49
	C	+IP	-Cr	1.85 *	1.37	1.22	0.90	1.39 *
Shoot-Fe	A	+IP	+Cr	0.42	0.69	0.60	0.47	0.42
	B	-IP	+Cr	0.37	0.37	0.40*	0.43	0.44
	C	+IP	-Cr	0.51	0.89	0.67	0.52	0.50
Total-Fe	A	+IP	+Cr	5.31	8.01	8.96	6.00	5.96
	B	-IP	+Cr	2.38 **	3.43 *	3.83 **	2.22 **	2.89
	C	+IP	-Cr	9.24 **	8.57	7.64	5.81	8.15

The "*" or "**" labeled bars mean significant differences between A and B group or A and C group at $p < 0.05$ or $p < 0.01$ in shoot, root, DCB extract and the whole plant of yellow flag seedlings. Data are means ± SD, $n = 3$

Analysis of Cr

The Cr concentrations in yellow flag were not affected by IP with or without Br or Se except Cr concentrations in DCB extracts under Se(VI) supply (Fig. 5). The Cr concentrations in shoots and roots were significantly increased by Br24 and Se(IV), respectively (Fig. 5). The results of enrichment content of Cr in yellow flag showed that compared to the seedlings without IP, Cr accumulation in DCB extract and whole plant was markedly increased by IP, but not by the combination of IP and Br (Br24 and Br28) or Se (Se(IV) and Se(VI)) (Fig. 6). In comparison with the control seedlings, Br28 and Br24 increased Cr accumulation in

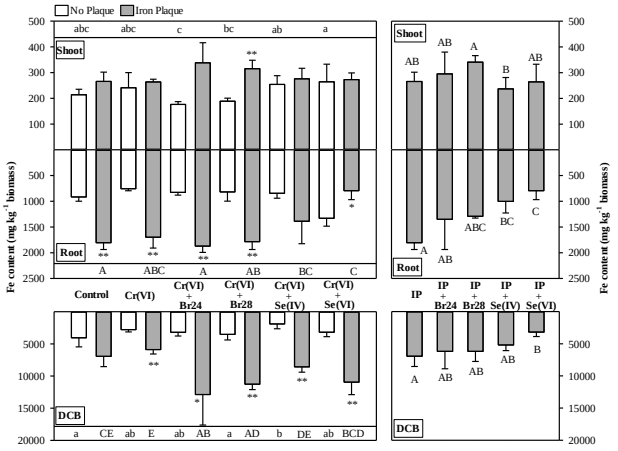

Fig. 2: Distribution of iron (Fe) in shoot, root and dithionite-citrate-bicarbonate (DCB) extract of *I. pseudacorus* (yellow flag) seedlings with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP) (Fe^{2+}: 0 or 60 mg L^{-1}) under 0 or 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) stress. Contents of Fe in shoot, root and DCB extract were presented as mg kg^{-1} biomass weight. Different letters indicate significant differences at $p < 0.05$ in shoot, root and DCB extract of yellow flag seedlings (capital letters for no plaque group and lowercase letters for IP group). The "*" or "**" labeled bars mean significant differences between no plaque group and IP group at $p < 0.05$ or $p < 0.01$. Data are means ± SD, $n = 3$

DCB extract and root tissue, respectively; and Br (Br24 and Br28) enhanced Cr accumulation by whole plants (Fig. 6). In the absence of IP, compared to the control plants, Br24, Br28, Se(IV) and Se(VI) increased enrichment content of Cr by 36%, 51%, 19% and 27%, respectively; and they increased that by 7%, 21%, 8% and -6% in the presence of IP (Fig. 6). IP formation increased Cr enrichment content by 36% compared to the control seedlings (Fig. 6). The percentage of Cr in shoots, roots and DCB extracts was 2–7%, 13–23% and 73–84%, respectively (Fig. 7).

Analysis of Cu, Zn, Mn and B

Compared to the control seedlings, the concentrations of Zn, Mn and B in root tissues were markedly reduced by Cr; and Mn and B concentrations in shoots were significantly decreased by IP (Fig. 8). Mn loss in root of yellow flag exposed to Cr stress was significantly neutralized by IP or the combined effects of IP and Se(IV); and Zn loss was neutralized by the combined effects of IP and Br28; and B loss was counteracted by the combined effects of IP and Br (Br24 and Br28) (Fig. 8).

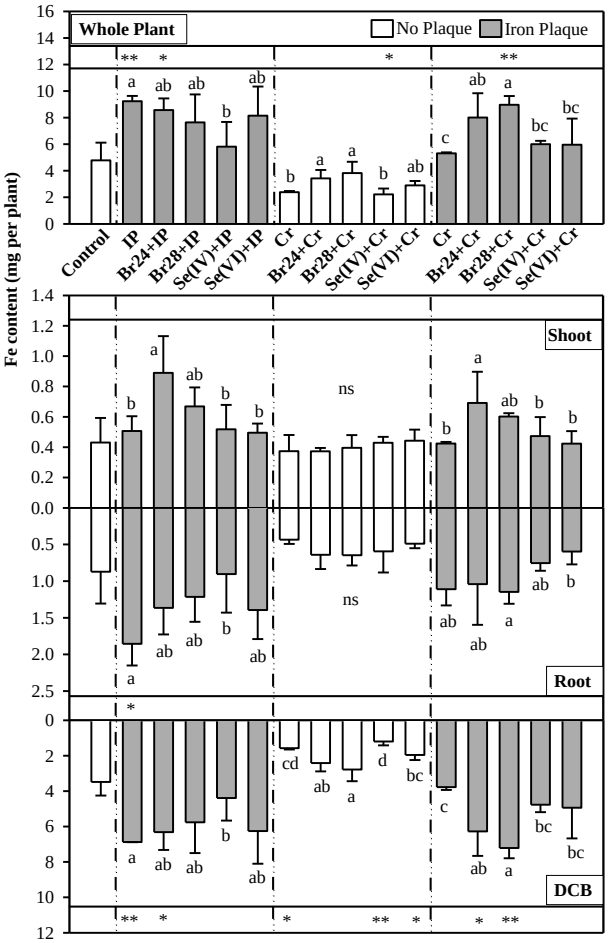

Fig. 3: Distribution of iron (Fe) in shoot, root, dithionite-citrate-bicarbonate (DCB) extract and the whole plant of *I. pseudacorus* (yellow flag) seedlings with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP) (Fe^{2+}: 0 or 60 mg L^{-1}) under 0 or 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) stress. Contents of Fe in shoot, root and DCB extract were presented as mg each plant. Different letters indicate significant differences at $p < 0.05$ at IP, no plaque or IP + Cr group in shoot, root, DCB extract and the whole plant of yellow flag seedlings. The "*" or "**" labeled bars mean significant differences between control and other treatment at $p < 0.05$ or $p < 0.01$. Data are means ± SD, $n = 3$.

Discussion

Cr(VI) stress could cause Fe deficiency in plants through competing electron carriers of Fe and Cu to inhibit electron transport (Dixit *et al*., 2002). Gopal *et al*. (2009) reported that Fe concentrations in spinach were significantly reduced by Cr(VI), which led to a decrease in chlorophyll and heme biosynthesis. Our results showed that Cr(VI) stress

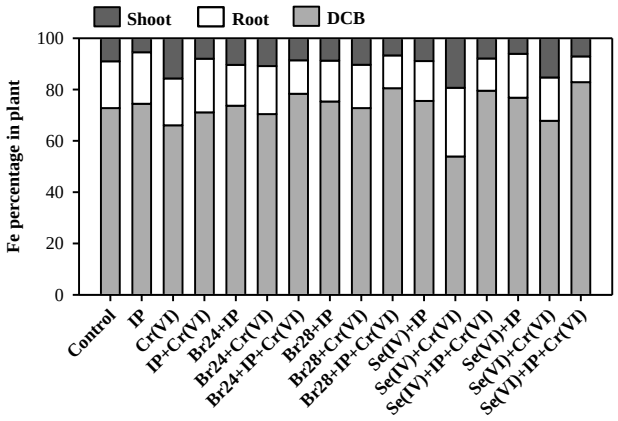

Fig. 4: Percentages of iron (Fe) in shoot, root and dithionite-citrate-bicarbonate (DCB) extract *I. pseudacorus* (yellow flag) seedlings with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP) (Fe^{2+}: 0 or 60 mg L^{-1}) under 0 or 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) stress. Data are means ± SD, $n = 3$

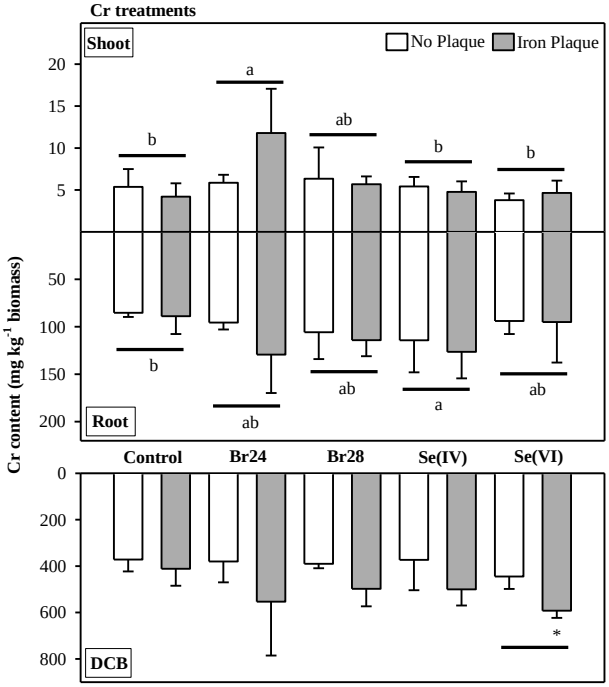

Fig. 5: Distribution of chromium (Cr) in shoot, root and dithionite-citrate-bicarbonate (DCB) extract of *I. pseudacorus* (yellow flag) seedlings exposed to 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP) (Fe^{2+}: 0 or 60 mg L^{-1}). Contents of Cr in shoot, root and DCB extract were presented as mg kg^{-1} biomass weight. Different letters indicate significant differences at $p < 0.05$ among different treatments in shoot, root and DCB extract of yellow flag seedlings. The "*" labeled bars mean significant differences between no plaque and IP group at $p < 0.05$. Data are means ± SD, $n = 3$

markedly reduced Fe content in yellow flag in the presence of IP (Table 1). We also found that Br24 and Br28 counteracted the loss of Fe in DCB extracts and the whole plants exposed to Cr(VI) with or without IP (Fig. 3). Song *et al*. (2016) showed that Br24 could enhance Fe content and positively regulate peanut growth under Fe deficiency. In addition, our results revealed that Cr(VI) stress reduced the concentrations of nutrient elements (Zn, Mn and B) in yellow flag (Fig. 8). These finding were in line with the reports of previous study showing that Cr(VI) resulted in reduced Cu and Zn in *A. viridis*, rice and barley (Liu *et al*., 2008a; Sundaramoorthy *et al*., 2010; Ali *et al*., 2011), and B in *Spartina argentinensis* (Redondo-Gomez *et al*., 2011). However, in the present study, Br (Br24 and Br28) ameliorated Zn and B loss in root of yellow flag with IP (Fig. 8). Talaat and Abdallah (2010) reported that Br24 and Br28 could enhance the concentrations of Fe and other nutrient elements in faba bean. The reason for neutralization in loss of Fe and other microelements may be attributed to the role of Br in regulating or keeping ion homeostasis in pants with or without heavy metal stress. For example, Br24 could keep cell normal metabolism, restore ATP content and maintain ion balance (K, P, Na, Mg, Cl, Fe and Mn) in cucumber plants (Yuan *et al*., 2015). Br significantly enhanced content of chlorophyll, protein and monosaccharides and number of cells of *Chlorella vulgaris* under Cd, Pb and Cu stress (Bajguz, 2011).

In the present study, IP enhanced Cr accumulation and uptake in DCB extracts and whole plants (Fig. 6). Our results agreed with the findings of Hu *et al*. (2014a) who found that IP could immobilize Cr but did not affect its uptake and translocation in rice plants, and Xu *et al*. (2015) who reported that IP improved Cr adsorption in DCB extracts and increased Cr uptake by yellow flag. Our results showed that Br24 and Br28 could increase Cr uptake by yellow flag (Fig. 6). Many previous studies reported that Br24 and Br28 could reduce the concentrations of metal(loid)s (Ni, Zn, Cu and B) and alleviate their toxicity in plants (Sharma and Bhardwaj, 2007; Sharma *et al*., 2008; Kanwar *et al*., 2013; Ramakrishna and Rao, 2013; Surgun *et al*., 2016). Their results suggested that Br (Br24 and Br28) could decrease metal (loid)s uptake by plants via enhancing the activities of glutathione metabolism and biosynthesis and antioxidant enzymes. However, Bukhari *et al*. (2016) found that Cr concentrations in the leaves of the Meiyu2-1 and NC107 genotype tomato plants were significantly reduced by Br24, but were increased in 2010-38 genotype.

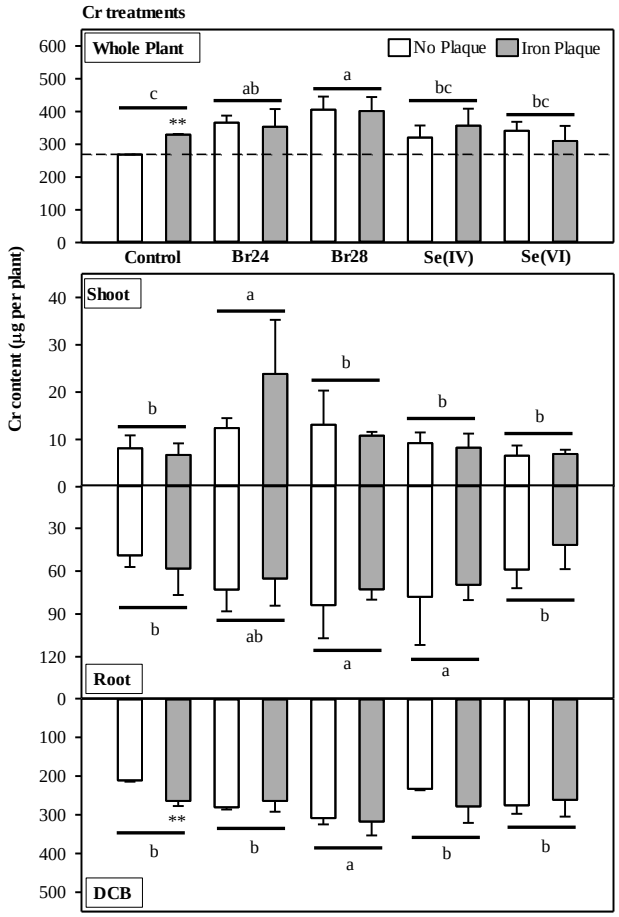

Fig. 6: Distribution of chromium (Cr) in shoot, root, dithionite-citrate-bicarbonate (DCB) extract and the whole plant of *I. pseudacorus* (yellow flag) seedlings exposed to 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP) (Fe^{2+}: 0 or 60 mg L^{-1}). Contents of Cr in shoot, root, DCB extract and the whole plant were presented as μg each plant. Different letters indicate significant differences at $p < 0.05$ among different treatments in shoot, root, DCB extract and the whole plant of yellow flag seedlings. The "**" labeled bars mean significant differences between no plaque and IP group at $p < 0.01$. Data are means ± SD, $n = 3$.

Sharma *et al*. (2011) also reported that Br28 markedly increased Cr uptake by *Raphanus sativus* L. Thus it can be seen that different element type and plant genotype may result in the observed difference.

Meanwhile, we found that Se(IV) and Se(VI) could improve Cr accumulation and uptake by yellow flag (Fig. 5 and 6). Our results were in line with the findings of Qing *et al*. (2015) who reported that Se(IV) did not affect Cr translocation and uptake by cabbage leaves, but increased Cr

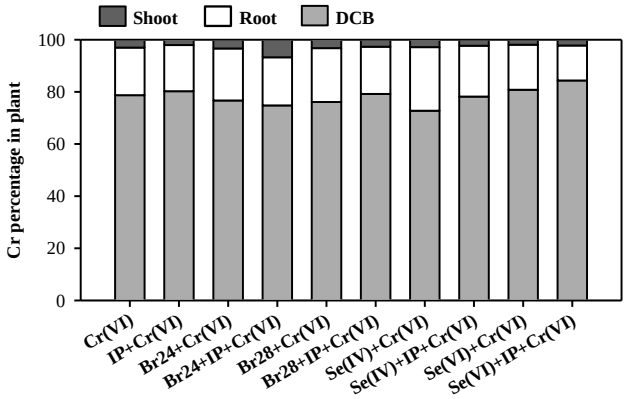

Fig. 7: Percentages of chromium (Cr) in shoot, root and dithionite-citrate-bicarbonate (DCB) extract *I. pseudacorus* (yellow flag) seedlings exposed to 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP). Data are means ± SD, $n = 3$.

content by 20% at least. Se possibly combined with Cd, Hg and Ag and formed Se-metal complexes, which alleviated their toxicity to plants (Shanker *et al*., 1996; Sasakura and T. Suzuki, 1998; Belzile *et al*., 2006; Fargasova *et al*., 2006; Feng *et al*., 2013). However, Bluemlein *et al*. (2009) showed that no complexes or peptides of Se(IV) and As(V) were found, although Se significantly enhanced As uptake by *Thunbergia alata*. The difference may occur partly as a result of poor sulfur assimilation. The transport of Se(VI) into plant cells could be mediated by sulfate carriers (Maruyama-Nakashita *et al*., 2007; Schiavon *et al*., 2007). Se can compete with sulfur for the binding site of sulfate transporters and repress its transport and uptake by plants (Ellis and Salt, 2003; Schiavon *et al*., 2007). In the present study, yellow flag was grown in nutrient solution with 0.5 mg L^{-1} Se for 1 week before Cr treatment. Hence, Se supply possibly substituted sulfur for proteins to some extent, resulting in the reduced synthesis of important amino acids such as cysteine, methionine, and glutathione (Michela and Mario, 2008), which could significantly decrease Cr uptake by plants (Lay and Levina, 1996; Yu *et al*., 2007; Zeng *et al*., 2012). Consequently, more Cr could be translocated into plants due to the reduced synthesis of these amino acids, which could explain the increased Cr accumulation by yellow flag with Se supply in our study.

Conclusion

Cr stress decreased Fe accumulation and uptake by yellow flag, but Br significantly neutralized this effect regardless of formation or not of IP. Cr exposure reduced the concentrations of microelements (Zn, Mn and B) in yellow flag; and IP, Br, Se and the combined effects of IP

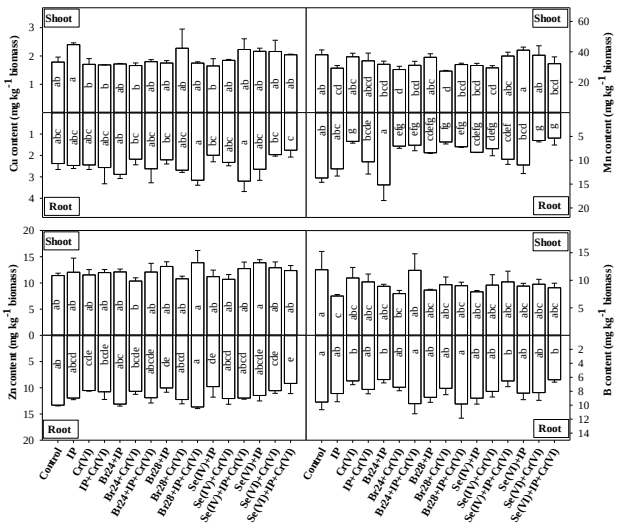

Fig. 8: Distribution of copper (Cu), manganese (Mn), zinc (Zn) and boron (B) in shoot and root tissue of *I. pseudacorus* (yellow flag) seedlings with brassinosteroid (24-epibrassinolide (Br24): 0.1 mg L^{-1} and 28-homobrassinolide (Br28): 0.1 mg L^{-1}) and selenium (Se(IV): 0.5 mg L^{-1} and Se(VI): 0.5 mg L^{-1})) with or without iron plaque (IP) (Fe^{2+}: 0 or 60 mg L^{-1}) under 0 or 0.5 mg L^{-1} hexavalent chromium (Cr(VI)) stress. Contents of Cu, Mn, Zn and B in shoot, root and DCB extract were presented as mg kg^{-1} biomass weight. Different letters indicate significant differences at $p < 0.05$ in shoot and root tissue of yellow flag seedlings. Data are means ± SD, $n = 3$

and Br or Se counteracted their loss to some extent. IP markedly increased Cr enrichment by 36%; Br24 and Br28 significantly enhanced Cr accumulation and uptake and increased Cr enrichment by 36% and 51% in the absence of IP, and 7% and 21% in the presence of IP. Se also improved Cr accumulation by yellow flag although the difference was not statistically significant. To what extent the application of exogenous Br or Se can more effectively increase Cr accumulation in yellow flag requires further studies.

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