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The Role of Iron Plaque in Transport and Distribution of Chromium by Rice Seedlings

X.-Z. Yu*, M.-R. Lu and X.-H. Zhang

The Guangxi Key Laboratory of Theory & Technology for Environmental Pollution Control,
College of Environmental Science & Engineering, Guilin University of Technology,
Guilin 541004, People's Republic of China

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Hydroponic studies were conducted to investigate the role of iron plaque on transport and distribution of chromium (Cr) by rice seedlings. Microscopical observations indicate that iron plaque developed quickly at the root surface of rice seedlings, but the distribution of iron plaque was more intense near root base and less towards root tip. Results showed that rice seedlings exposed to Cr(III) depicted significantly higher capacity for Cr accumulation in plant tissues than Cr(VI) in the presence of iron plaque. However, transport of Cr within plant cells was more evident in Cr(VI) treatment with iron plaque than Cr(III) treatment. Results also showed that there are significant impact on transport of K, Mn and Zn in rice seedlings treated with Cr(VI) in the presence of iron plaque, while significant effect on transport of Mn and Zn were observed in Cr(III)-treated rice seedlings. Results from detached root test provide additional evidence to confirm the presence of iron plaque, that had different impact on Cr uptake when Cr(VI) or Cr(III) was supplied.

Keywords: chromium, translocation, iron plaque, rice, DCB extract

Introduction

In order to avoid anoxia, development of iron plaque on the root surface has been considered as a survival mechanism for plants living in anoxic and flooded environments (Chabbi 1999; Xu and Yu 2013), in which oxygen supply to the roots is able to function through a well-equipped internal aeration system in the reduced environment (Gibberd et al. 2001; Povidisa et al. 2009). Indeed, iron plaque has been observed in various species of plants, including freshwater, wetland and salt marsh plants, as well as mangrove seedlings (Povidisa et al. 2009). Iron plaque is a layer of crystalline or amorphous iron or manganese (hydr)oxides (Jiang et al. 2009; Liu et al. 2016), that the oxygen has been released to form a protective oxic zone around the rhizosphere to prevent the entrance of any toxic substances into the plants (Povidisa et al. 2009; Mei et al. 2012). It was evident from previous reports that there are some sorptive functional groups of iron oxides/hydroxides on iron plaque (Liu et al. 2016), that may adsorb and/or precipitate metal ions,

*Corresponding author; E-mail: yuxiaozhang@hotmail.com; Phone: (+86) 773-5897016;
Fax: (+86) 773-5896285

which may affect their phyto-availability (Otte et al. 1989; Liu et al. 2016). Plenty of evidence shows that the formation of iron plaque at root surface may serve as a barrier against heavy metals, which are entering to the root cells (Batty et al. 2002). The development and extent of iron coatings at the root surface was driven by various abiotic and biotic parameters (Xu and Yu 2013). Certainly, the oxidizing capacity of plant roots and the availability of Fe^{2+} are the most important biotic and abiotic elements, respectively (Xu and Yu 2013). Additionally, species morphological (e.g., root length) and anatomical (e.g., root porosity, structure of root epidermis) features are able to affect and control oxygen supply and oxygen release to the root system (Sand-Jensen et al. 2005; Povidisa et al. 2009).

The formation of iron plaque on root surfaces was able to decrease Cu, Ni, Zn and Al toxicities to rice (Greipsson 1994, 1995; Chen et al. 2006) and to alter transport and translocation of Cd, Se and As (Liu et al. 2004, 2007; Zhou et al. 2007). It may also act as storage compartment for Sb(V) and Sb(III) at the rice rhizosphere (Huang et al. 2011; Okkenhaug et al. 2012). This study was conducted to clarify the role of iron plaque on Cr uptake and accumulation by rice seedlings. Despite the fact that the presence of iron plaque on roots is common and most likely could be beneficial for rice, little information is available on the effect of iron plaque on Cr uptake by rice seedlings.

Materials and Methods

Preparation of rice seedlings

Preparation of rice seedlings was identical to our previous investigation (Yue et al. 2015). Seeds of rice (*Oryza sativa* L. cv. XZX 45) was planted in sand at 25 °C and irrigated with a modified ISO 8692 nutrient solution (Yue et al. 2015). After 10–18 d of pre-growth, depending on specific tests, young and equal size seedlings were collected in a buffer solution containing 1 mM $CaCl_2$ + 2 mM MES-Tris buffer (pH 6.0) for 4 h to clean up extra ions from the cell wall (Ebbs et al. 2008). Then, pre-treated seedlings were used for the subsequent tests.

Experiment 1: Induction of iron plaque formation on root surface

After 10, 12, 14, 16 or 18 d of growth, pre-treated seedlings were collected and rinsed with deionized water. The excised roots were precisely weighted (1.0 g fresh weight) and placed in 50 mL glass container. Iron plaque was extracted from fresh root surface using cold dithionite-citrate-bicarbonate (DCB) solution (Otte et al. 1989) with a slight modification. Excised roots were incubated in 30 mL DCB solution at room temperature (22–25 °C) for 30 min. After that the extracts were transferred into 50 mL glass flasks. Roots were rinsed with deionized water twice that was amended with DCB extract. Finally, the solution was made up to 50 mL with deionized water. The concentration of Fe in DCB extraction was analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Experiment 2: Determination of extraction time of iron plaque from roots

In order to clarify the effects of extraction time on extraction efficiency of iron plaque from root surface by DCB solution, excised roots of 16 d old rice seedlings were precisely weighted (1.0 g fresh weight) and kept in 50 mL glass container. After that iron plaque was extracted using DCB solution in intervals of 10 min over 60 min. The remaining procedure was identical to Experiment 1.

Experiment 3: Cr uptake and distribution in rice seedlings

After 16 d of growth, ten pre-treated seedlings with similar height and weight were exposed to 50 mL Cr-spiked solution containing 1.70, 6.98 and 13.66 mg Cr/L Cr(VI) or 3.31, 13.32 and 31.89 mg Cr/L Cr(III). Potassium chromate (K_2CrO_4) and chromium nitrate ($Cr(NO_3)_3 \cdot 9H_2O$) of analytical grade with ≥ 95% purity was used. Each Cr treatment was conducted in 4 replicates. After 24 h of exposure, seedlings were collected and divided into roots and shoots. Iron plaque was extracted using cold DCB solution after 30 min. The resulting DCB extraction solution was made up to 50 mL with deionized water. Additionally, roots after DCB extraction, and also shoots were oven dried at 90 °C for 2 d. Dried materials were digested with 4:1 HNO_3-$HClO_4$ solution. The content of total Cr and other nutrient elements (K, Ca, Mg, Cu, Fe, Mn and Zn) in plant materials and in DCB extracts was analyzed by ICP-AES.

Experiment 4: Cr uptake by detached rice roots

Exposure regime of Cr uptake by detached roots was identical to our previous work (Yu et al. 2007). Excised roots (1.0 g fresh weight) were placed in 50 ml solution containing 1.72, 7.11, and 14.39 mg Cr/L Cr(VI) or 3.41, 13.87, and 34.57 mg Cr/L Cr(III). Each Cr treatment was conducted in 4 replicates. After 24 h of incubation, excised roots were collected and carefully rinsed with deionized water. Iron plaque at the root surfaces was extracted using cold DCB solution for 30 min. The resulting DCB extraction solution was made up to 50 mL. Detached roots after DCB extraction were oven dried. The remaining procedure was identical to Experiment 3. The concentration of total Cr in roots and in DCB extraction was measured.

In order to clarify the effect of iron plaque on Cr uptake by detached roots, iron plaque was preliminarily removed from roots using DCB solution. After DCB extraction, roots were rinsed with deionized water and blotted with tissue paper. Excised roots (1.0 g fresh weight) without iron plaque were placed in 50 mL solution containing Cr(VI) or Cr(III) with similar doses to the tests (Experiment 4) with iron plaque, respectively. After 24 h of exposure, excised roots were rinsed with deionized water and oven dried. Total Cr in detached roots was also determined.

Statistical analyses

All data obtained were subjected to analysis of Tukey's multiple range test. The statistical significance was performed at 0.05 levels.

Results

Induction of iron plaque formation in roots

Reddish iron plaques were observed at the surface of rice roots (Fig. 1), indicating that the roots were quickly coated with iron plaque. Compared with the seedlings without iron plaque, higher amounts of Fe in the plaque were accumulated by rice seedlings after analysis of Fe in DCB extracts. Figures 2 gives the changes of Fe concentrations in DCB extracts over growth time, in which a linear increase ($R^2 = 0.98$) in Fe concentration was observed with increasing growth duration of rice seedlings.

Determination of extraction time of iron plaque from roots

DCB solution was used to extract iron plaque. After DCB treatment, rice roots lost their red-colour and became white (Fig. 1). Figures 3 depicts the changes of Fe concentration in DCB extracts versus extraction time. It can be observed that the curve showed a monotonic change in Fe concentrations, in which de-sorption of Fe by DCB solution from the

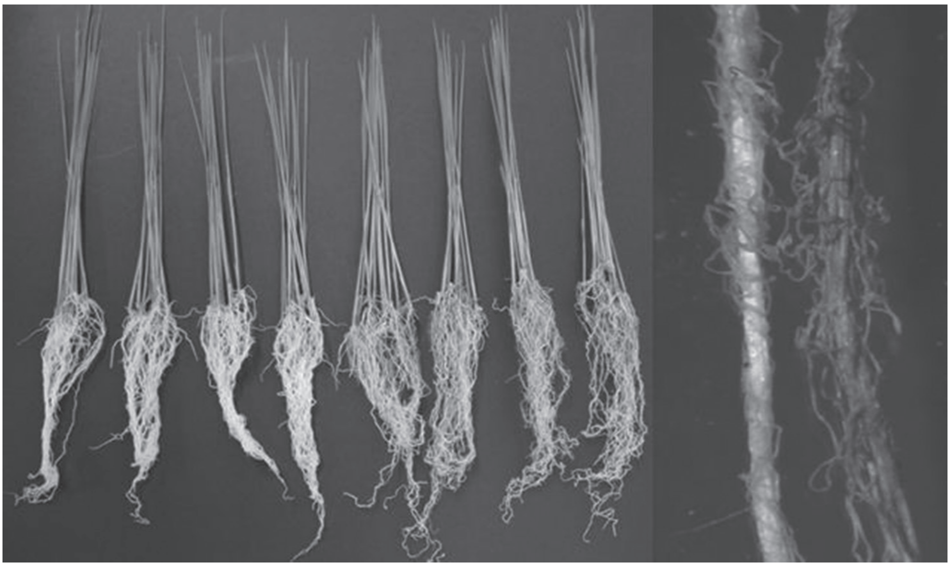


Figure 1. Rice roots with or without iron plaque. White roots were without iron plaque after DCB extraction and reddish roots were with iron plaque. The right part of figure was microscopic view (magnification W10X/23) for roots before and after DCB extraction

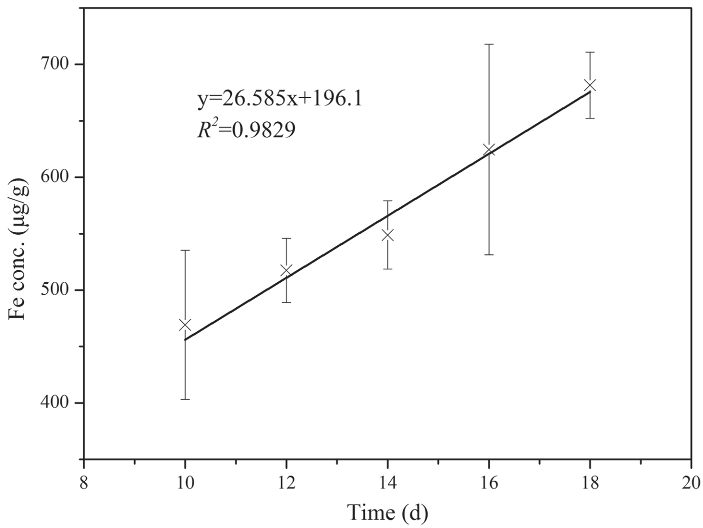


Figure 2. Effect of growth time on formation of Fe plaque on root surfaces of rice seedlings (the values are the mean of four replicates; vertical lines represent standard deviation)

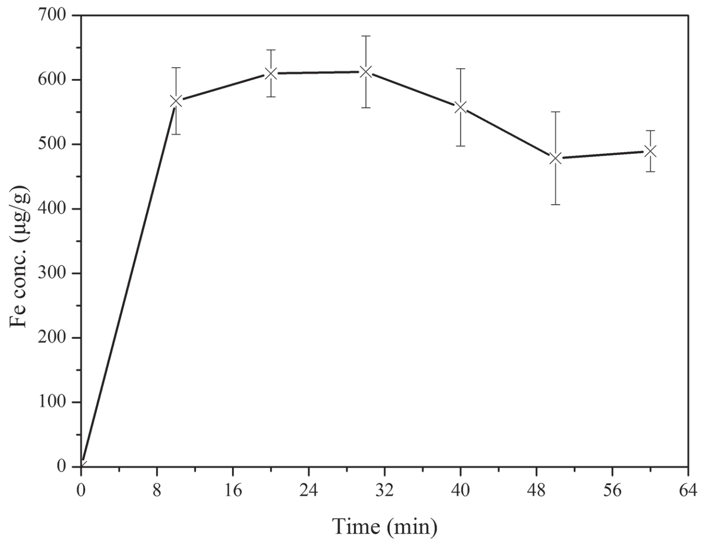
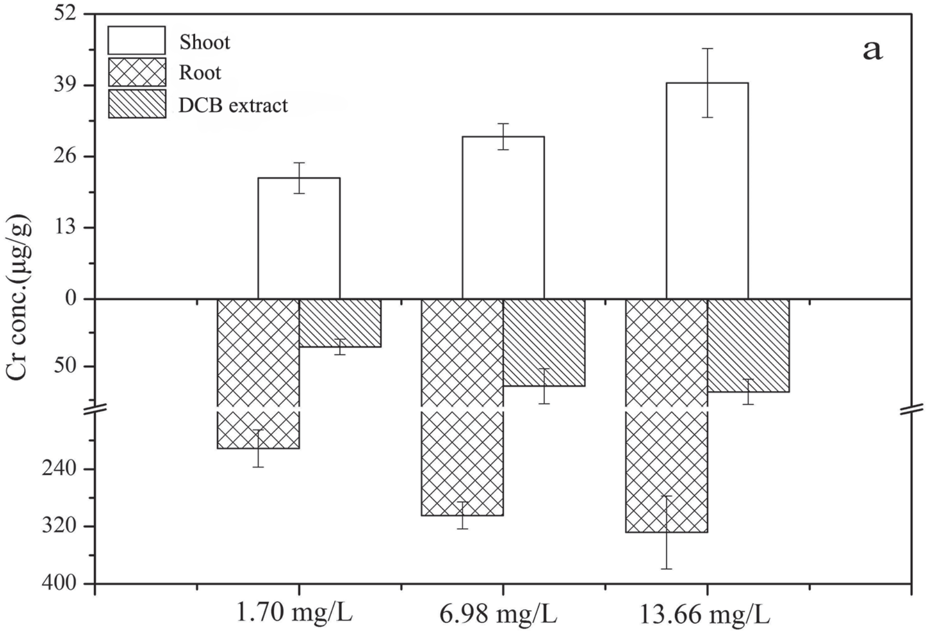


Figure 3. Effect of DCB extraction time on de-sorption of Fe from the plaque (the values are the mean of four replicates; vertical lines represent standard deviation)



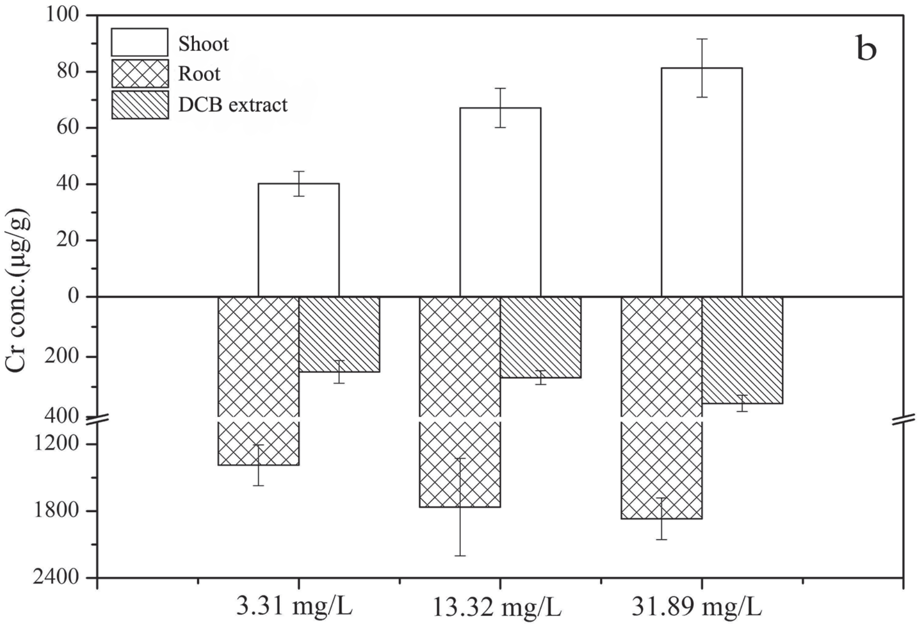


Figure 4. Measured Cr concentrations in DCB extracts, roots and shoots of rice seedlings exposed to Cr(VI) (a) or Cr(III) (b) (the values are the mean of four replicates; vertical lines represent standard deviation)

root surface was very quick at the first 10 min, after that more gradual until equilibrium was obtained at 20–30 min.

Uptake of Cr by rice seedlings

The concentrations of Cr in DCB extracts, roots and shoots of rice seedlings exposed to Cr(VI) or Cr(III) are shown in Fig. 4. Cr concentrations were measured below detection limit in roots and shoots of rice seedlings without Cr supply (data not shown). Concentrations of Cr in roots of rice seedlings exposed to Cr(VI) were significantly higher than those in DCB extracts ($p < 0.05$) (Fig. 4a). However, approximately 75.1% (SD 1.74, $n = 4$) of the total Cr accumulation was detected in roots of rice seedlings exposed to Cr(VI), whereas 14.8% was found in DCB extracts. It was interesting to note that the concentration of Cr in shoots was significantly lower than those in DCB extracts and roots ($p < 0.05$) (Fig. 4a), which accounted for 10.1% of the total amount recovered from plant materials. For the Cr(VI)-treatment, concentration of Cr followed the trend Root-Cr > DCB extract-Cr > Shoot-Cr.

At the Cr(III)-treatment, concentration of Cr in roots detected was almost 5.8-fold higher than those in DCB extracts ($p < 0.05$) (Fig. 4b). The Cr concentration in shoots was determined to be 40.14–81.28 μg/g, which accounted for 3.8% of the Cr accumulation in rice seedlings exposed to Cr(III). Concentration of Cr followed the same trend as the Cr(VI) treatment.

Uptake of other nutrient elements by rice seedlings

The concentration of K, Mn in roots (Fig. S1a*) and shoots (Fig. S1b) decreased significantly in Cr(VI)-treatment ($p < 0.05$) compared with control, more or less effect was observed in Fe, Ca, Mg and Cu concentrations in both roots and shoots ($p > 0.05$). Exposure to Cr(VI) had positive influence on Zn concentration in roots ($p < 0.05$), but significant decrease was observed in shoots ($p < 0.05$).

Mn and Zn in roots was (Fig. S2a) decreased significantly in rice seedlings exposed to Cr(III) ($p < 0.05$) compared with control, while remarkable decrease was only observed in case of Zn concentration in shoots ($p < 0.05$) (Fig. S2b). Exposure to Cr(III) had more or less effect on other nutrient elements in both roots and shoots ($p > 0.05$).

The two Cr ions had different effects on distribution of nutrient elements in DCB extracts (Fig. S3a and b). The concentrations of Ca, Cu and Zn in DCB extracts decreased significantly in Cr(VI)-treatment (Fig. S3a) ($p < 0.05$), but noticed positive effect on Fe concentrations ($p < 0.05$) (Fig. S3a). It was interesting to note that Ca and Mn in DCB extracts decreased significantly in rice seedlings exposed to Cr(III) (Fig. S3b) ($p < 0.05$). Both Cr species had more or less effect on other nutrient elements, but the trend was slightly different ($p > 0.05$).

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

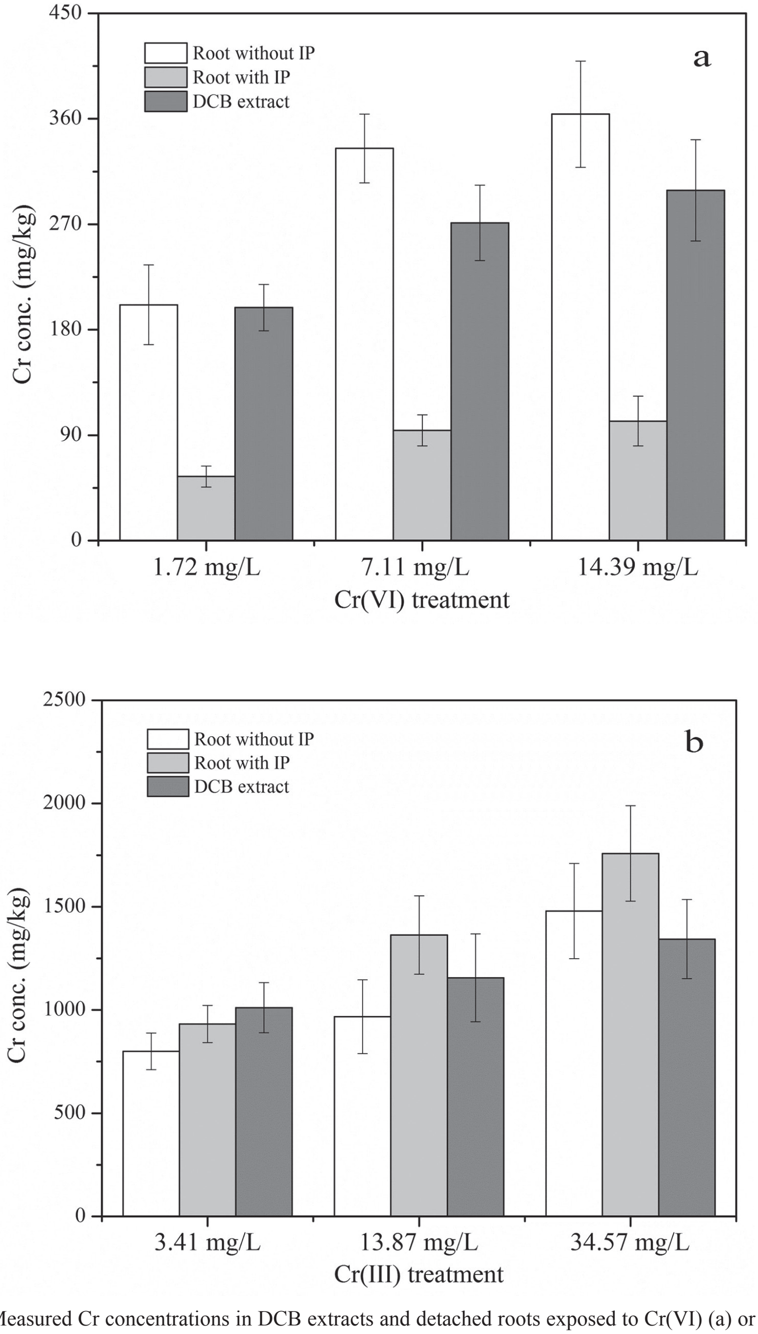


Figure 5. Measured Cr concentrations in DCB extracts and detached roots exposed to Cr(VI) (a) or Cr(III) (b) (the values are the mean of four replicates; vertical lines represent standard deviation)

Cr uptake by detached rice roots

The concentrations of Cr in DCB extracts and detached roots exposed to Cr(VI) or Cr(III) are shown in Fig. 5a and b. Concentrations of Cr in –IP roots exposed to Cr(VI) were significantly higher than those in +IP roots ($p < 0.05$) (Fig. 5a). It was interesting to note that concentration of Cr in DCB extracts in +IP roots exposed to Cr(VI) were significantly higher than those in roots (Fig. 5a). However, approximately 24.3% (SD 2.34, $n = 4$) of the total Cr accumulation was detected in roots in +IP roots exposed to Cr(VI), where 75.7% was found in DCB extracts in +IP roots.

At the Cr(III)-treatment, concentrations of Cr in –IP roots were slightly lower than those in +IP roots ($p > 0.05$) (Fig. 5b). A negligible difference existed in Cr concentrations between DCB extracts and roots in +IP roots exposed to Cr(III), in which approximately 47.1% (SD 4.49, $n = 4$) of the total Cr accumulation was detected in DCB extracts in +IP roots.

Discussion

It has been reported that plants are easily able to uptake both Cr(VI) and Cr(III) species. In this investigation, more Cr accumulation was detected in rice seedlings exposed to Cr(III) than those in Cr(VI) treatment ($p < 0.05$). Since different doses of Cr were used, here it is interesting to compare the concentration sensitivity of total Cr accumulation rate between the two Cr treatments using linear fit curves. Based on our experimental data, the linear plots between the total Cr accumulation rate and initial Cr concentration were all significant (figure not shown, $R^2 > 0.87$). The difference between the two variants was 1.49, indicating that Cr(III) was more bioavailable to rice seedlings than Cr(VI). A same conclusion was also made by Shahandeh and Hossner (2001). Although more Cr(III) was removed from the hydroponic solution by rice seedlings than Cr(VI). Cr(VI) is more mobile in plants, judged by the translocation efficiency (TE), which is defined as the ratio of metal accumulation in upper parts to metal accumulation in total plant materials. Our results showed that TE values between the two Cr treatments were quite different ($p < 0.05$). However, the mean TE value was 10.07% (± 0.96) for rice seedlings exposed to Cr(VI), whereas significantly lower value, i.e. 3.77% (± 0.72) was observed in case of Cr(III) treatment.

Development of iron plaque started on root surface was typically in between 12–72 h in numerous wetland plants (Xu and Yu 2013). In this study, we also found that iron plaque formed quickly on the root surface of rice seedlings grown in sand. Fe measurement from Fe extracts, together with microscopical observation, provided convincing evidence to prove the formation of iron plaque at the root surface. The photo of rice roots (Fig. 1) before DCB extraction depicted a reddish layer of Fe deposits, whereas after DCB extraction, the roots lost their red-colour and the epidermal cells were visible without coatings. We also noted that the distribution of iron plaque on the root surface was not homogeneously. A similar observation was also reported previously (Povidisa et al. 2009), in which the iron plaque was located more at the root base and less towards the root tip. However, a completely different conclusion was obtained by Taylor et al. (1984), where the iron plaque on the roots of macrophytes was more evident at the root tips. Additionally, Chabbi (2003) found that iron plaque mainly accumulated in rhizodermis, exodermis and endodermis of *Juncus bulbosus* L., and less detected in cortex and parenchyma regions. This information collected suggests that the distribution pattern of iron plaque on the root surface were variable with species of plants (Liu et al. 2016). In our current experiment, the same dose of Fe in the form of Fe-EDTA was used in plant growth nutrient solution for both Cr-treated rice seedlings. However, the difference in Fe concentrations in DCB extracts, roots and shoots between the two Cr treatments was remarkable ($p < 0.05$). However, it is important to compare the distribution of Fe and Cr in DCB extracts, roots and shoots using the ratio of Fe to Cr. Thus, the calculated Fe/Cr ratio was determined to be 1.54 (± 0.60), 68.87 (± 11.6) and 10.04 (± 2.66) for DCB extracts, roots and shoots of rice seedlings exposed to Cr(VI), while Fe/Cr ratio from Cr(III) treatment was estimated to be 0.25 (± 0.03), 10.29 (± 0.90) and 4.40 (± 1.30), respectively.

Several studies have been reported that the presence of iron plaque inhibited uptake of heavy metals by plants. Ye et al. (1998) revealed that seedlings of *Typha latifolia* with iron plaque could reduce Cd accumulation at the root surface significantly, compared with the plant seedlings without iron plaque. However, a different result was obtained by Liu et al. (2007), where iron plaque had little impact on uptake and accumulation of Cd by rice. Ye et al. (1997) also observed that Cu uptake by *Typha latifolia* was independent on the presence of iron plaque, but the plaque had positive effect on Ni uptake. Less accumulation of Cr was observed in *Lobelia dortmanna* and *Phragmites australis* in the presence of iron plaque (Christensen and Jensen 1998; Batty et al. 2000). In our present experiment with detached roots, roots without iron plaque showed significant higher concentrations of Cr than roots with iron plaque in Cr(VI) treatment ($p < 0.05$), indicating that the inhibition of Cr(VI) uptake in presence of iron plaque. However, a complete different result was obtained in case of Cr(III) treatment, in which roots with iron plaque had slightly higher concentrations of Cr than roots without iron plaque ($p > 0.05$). We also noticed that the presence of iron plaque also significantly affect the distribution of Cr in root and in iron plaque. In case of Cr(VI) treatment, more Cr was detected in iron plaque rather than root ($p < 0.05$). However, the distribution of Cr(III) in roots and iron plaque was slightly different ($p > 0.05$).

In our present study, we observed that the presence of iron plaque played a barrier role in uptake of both Cr species by rice seedlings. Subsequently, it may affect transport of Cr in plant materials. We also noticed that the TE values for Cr(III) treatment were determined by 3.77% ± 0.72 and 4.39% ± 0.84 for rice seedlings with and without iron plaque, respectively. The difference was marginal ($p > 0.05$). A similar result was also obtained in case of Cr(VI) treatment, in which the TE values was calculated by 10.07% ± 0.96 and 11.82% ± 1.13 ($p < 0.05$). This result was similar to other previous works. Less Cu and Ni was also found in the leaves of rice plants with ion plaque than in the plants without plaque (Greipsson 1995). Ma et al. (2013) found iron plaque was the barrier to Pb translocation from rice roots to shoots. However, different conclusions were also reached by other studies, in which root tissue rather than iron plaque was the barrier for Pb and Cd transport in *Typha latifolia* L. (Ye et al. 1998) and Cd transport in rice (Liu et al. 2007).

In the present study, we also noticed that the presence of iron plaque significantly affect K, Mn and Zn translocation in rice seedlings exposed to Cr(VI), while significant impact on TE values for Mn and Zn was observed in Cr(III)-treated rice seedlings. Our result suggests that the effect of iron plaque on heavy metals uptake and transport by plants was significantly related to different plant species.

In conclusion, our results indicate that the rice seedlings had capacities to form iron plaque on the root surface. The distribution of iron plaque was not uniform and plaque deposits were mainly present at the root base and less at the root tip. More Cr(III) was eliminated from the nutrient solution by rice seedlings than Cr(VI). However, the effect of iron plaque on the transport of Cr was more evident in the case of Cr(VI) than Cr(III) treatment. The presence of iron plaque also affected transport of other nutrient element within plant materials of rice seedlings exposed to both Cr(III) and Cr(VI).

Acknowledgements

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Figure S1*. Hierarchical cluster analysis of the distribution of nutrient elements in rice seedlings exposed to Cr(VI) (a: roots; b: shoots). Cluster analysis (CA) was conducted using uncentered correlation as similarity metric, based on the normalized data. The linkage distance is determined as average linkage. CA was performed to elucidate similarities in measured nutrient elements from DCB extracts, roots and shoots of Cr-exposed rice seedlings. Hierarchical CA was conducted by the program MeV (MultiExperiment Viewer) v.4.9.0. The cluster colour bar was displayed as fold change relative to control

Electronic Supplementary *Figure S2*. Hierarchical cluster analysis of the distribution of nutrient elements in rice seedlings exposed to Cr(III) (a: roots; b: shoots). CA calculations are described at Fig. S1

Electronic Supplementary *Figure S3*. Hierarchical cluster analysis of the distribution of nutrient elements in DCB extracts from Cr(VI) (a) and Cr(III) treatment (b). CA calculations are described at Fig. S1