Combined effects of chromium and arsenic on rice seedlings (*Oryza sativa* L.) growth in a solution culture supplied with or without P fertilizer

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The growths of two rice genotypes (Jin23A and CDR22) under the coexistence of As and Cr in solution culture with and without P were investigated. The result showed that rice shoot dry weight decreased due to the complex contamination of As and Cr, however, the influences on plant height, root length and root dry weight were insignificant.

Complex contamination of As and Cr, P, Fe, rice

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Arsenic (As) is an element which naturally occurs in the Earth's crust, and it exists in an organic or inorganic form. Its abundance in soils is 5–10 mg kg⁻¹ [1,2]. Arsenic pollution occurs in areas with the release of As into soil and/or water due to arsenic-bearing mineral weathering. Arsenic pollution may also result from such human activities as mining and smelting, the use of As-containing wastewater, and the application of arsenic-bearing fertilizer and pesticides [3].

Arsenic-bearing mines are widely distributed in the provinces of middle-south and southwest China, e.g., Hunan, Yunnan, Guangdong and Guangxi Zhuang Autonomous Region. The soils and water systems near such mines may be polluted by the activities of mining and smelting. For example, according to a report by Song *et al.* [4], the Jindong village area of the Diaojiang basin in Guangxi is experiencing severe As pollution: the As concentrations in topsoils (0–15 cm) and subsoils (15–30 cm) are over 200 and 500 times the As concentrations according to Level III of Chinese Soil Environmental Quality Standard. A large expanse of rice fields are polluted by As in Changning county of Hunan

Province, and the As concentrations in soils are between 92 and 840 mg kg⁻¹. The soil As concentration in more than 8000 hectares of rice fields in Hubei Province is much higher than the background value in the area [5]. Ure and Berrow found that the long term use of arsenic-bearing pesticides is associated with As accumulation in soils, and the concentration may reach 366–732 mg kg⁻¹ [2].

Rice is one of the staple food crops in China and other Asian countries. About 90% of international rice production is grown in Asia. In the primary Asian rice producing nations, e.g., Bangladesh, China and Thailand, the farmland and groundwater have been polluted by different levels of As. Meharg and Rahman [6] were the first researchers who investigated the effect of As on rice growth in Bangladesh. It was found that As concentrations in rice grains increased with the use of arsenic-bearing groundwater. Similar results were obtained in Bangladesh, and west and middle India [7,8] by other researchers. Abedin found that long-term irrigation of rice plants with arsenic-bearing wastewater not only caused rice field pollution but also led to as high as 200 mg kg⁻¹ of As in rice straw, which may be used as cattle food [9].

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With the wide application of chromium (Cr) in industries and due to the absence of proper treatment of Cr-bearing wastewater before flowing into agricultural fields, the soil Cr concentration in certain areas has exceeded the regulated standard. The harmfulness of chromium to the soil-plant system has been identified by scientists and aroused public attention. Zayed *et al.* [10] investigated the uptake and accumulation of Cr^{3+} and CrO_4^{2-} by 11 vegetable species. They found that the above-ground parts and the under-ground parts of the plants had different Cr accumulation behavior, and different species behaved differently. Compared to the control treatment, the Cr uptake by chickpeas inoculated with nodule bacteria respectively decreased by 14%, 34% and 29% in the roots, shoots and grains [11]. Gardea-Torredey *et al.* [12] also investigated the uptake of Cr^{3+} and Cr^{6+} by tumbleweeds.

However, in practice, soil pollution often arises from multiple pollutants rather than from any one individual pollutant. It has been found that soils around mines and smelting works generally have complex contamination by several heavy metals, e.g., As, Cr, Cd, Cu and Zn [13]. The investigation of a single pollutant inadequately reveals the true mechanism of the transference, the accumulation and the biological toxicity of pollutants under coexistence. In this study, we focused on the combination of As^{3+} and Cr^{3+} to investigate their effect on the growth of two rice genotypes (JinA23 and CDR22) and on the plant uptake and accumulation of Fe, P, As and Cr in a solution culture with and without phosphorous (P) addition.

1 Materials and methods

1.1 Plant culture

Two genotypes of rice (*Oryza sativa* L.), Jin23A and CDR22, were used in this study. Seeds were surface sterilized with 10% H_2O_2 for 10 min and washed with deionized water three times. Germinated seeds were transferred into perlite and allowed to grow for 2–3 weeks until the 3–4 leaf stage. The seedling growth environment was controlled as follows: temperature $28^\circ\text{C}/20^\circ\text{C}$ (d/n), photoperiod 14 h, light intensity $260\text{--}350\text{ mmol m}^{-2}\text{ s}^{-1}$, relative humidity 60%–70%.

The seedlings were transplanted into polyvinyl chloride (PVC) containers (7 cm high and 15 cm in diameter) with 500 mL of nutrient solution (constituents: KNO_3 2.0, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 2.0, $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ 0.8, KH_2PO_4 0.7, NaCl 0.05 (in mmol L^{-1}), and Fe-EDTA 50.0, H_3BO_3 10.0, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 5.0, ZnCl_2 1.0, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ 1.0, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.5 and $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 (in $\mu\text{mol L}^{-1}$) for 15 d (one seedling per PVC container), and were divided into two groups: one group continued to grow in the cultural solution with phosphorous (+P) ($0.7\text{ mmol L}^{-1}\text{ KH}_2\text{PO}_4$) and the other group was grown in solution without phosphorous

(–P). Two weeks later, Cr^{3+} ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$) and As^{3+} (NaAsO_2) were added. Three combinations of Cr^{3+} and As^{3+} were used: $0\text{ mg L}^{-1}\text{ As}^{3+} + 1.0\text{ mg L}^{-1}\text{ Cr}^{3+}$; $0.5\text{ mg L}^{-1}\text{ As}^{3+} + 1.0\text{ mg L}^{-1}\text{ Cr}^{3+}$; $1.0\text{ mg L}^{-1}\text{ As}^{3+} + 1.0\text{ mg L}^{-1}\text{ Cr}^{3+}$. Each treatment was conducted in quadruplicate. All cultures were carried out in a greenhouse and the condition was the same as in the seedling culture. 10 days after the addition of As and/or Cr, the plants were harvested.

1.2 Plant analysis

When the plants were harvested, the plant height and the root length were measured. The iron plaque on the root was extracted by dithionite-citrate-bicarbonate (DCB) [15]. Before extraction, the roots were washed with deionized water, and the roots were immediately put into containers with 40 mL solution of 0.03 mol L^{-1} sodium citrate ($\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) and 0.125 mol L^{-1} sodium bicarbonate (NaHCO_3) and kept for 10 min. 1 g of $\text{Na}_2\text{S}_2\text{O}_4$ was added and the extraction continued for one hour. After that, the roots were removed, washed with deionized water, made up to 100 mL and filtered for analysis. The roots and shoots were dried in an oven at 70°C for 72 h, after which their weights were measured. The amount of iron plaque was expressed as the iron content extracted per unit root dry weight.

The shoots and roots were separately ground, before 0.25 g of each ground sample was placed into a digestion can and 5 mL of nitrate was added. Digestion was allowed in a microwave digestion oven (Mars 5, CEM Corporation, USA) at 180°C for 10 min. During the course, standard reference material GBW07605 was applied for the quality control of the entire digestion and analysis process. After that, the digested sample was made up to 50 mL with deionized water and filtered. The concentrations of Fe, P and Cr in DCB extraction solution and in the digestion solution were analyzed by ICP-MS, and the arsenic was analyzed by AFS-2202E atomic fluorescence. The recovery rate was about 95%.

1.3 Data analysis

Iron plaque-to-shoot transference factors (TF_{shoot}) and iron plaque-to-root transference factors (TF_{root}) were calculated based on the following equations:

$$\text{TF}_{\text{shoot}} = [\text{C}_{\text{x-shoot}}]_{\text{dry}} / [\text{C}_{\text{x-iron-plaque}}], \quad (1)$$

$$\text{TF}_{\text{root}} = [\text{C}_{\text{x-root}}]_{\text{dry}} / [\text{C}_{\text{x-iron-plaque}}], \quad (2)$$

where $[\text{C}_{\text{x-shoot}}]_{\text{dry}}$ and $[\text{C}_{\text{x-root}}]_{\text{dry}}$ respectively represent the concentration of element X (Fe, P, As and Cr) in shoots and roots, and $\text{C}_{\text{x-iron-plaque}}$ represents the concentration of element X in the iron plaque.

Distribution coefficients (DC) of Fe, P, As and Cr are defined as the ratio of the total content of the corresponding element in shoots to that in roots.

Two-way analysis of variance (ANOVA) was conducted using microsoft windows-based genstat package (6th edition, NAG Ltd., England).

2 Results

2.1 Shoot height, root length and biomass

Table 1 shows the height, root length and dry weight of the shoots and roots of the two rice genotypes grown in solutions with 1.0 mg L⁻¹ Cr and different concentrations of As. The table shows that the plant height, root length and the weight of the shoots and roots largely differed between genotypes ($P<0.001$) and Jin23A grew better than CDR22. The addition of As to the cultural solution had considerable influence on the rice shoot biomass, while the impacts on the rice root biomass, plant height and root length were insignificant. In the absence of P, the shoot weights of both rice genotypes (Jin23A and CDR22) under treatment of 1.0 mg L⁻¹ As³⁺+1.0 mg L⁻¹ Cr³⁺ were respectively 11.2% and 13.1% lower than that under 0.5 mg L⁻¹ As³⁺+1.0 mg L⁻¹ Cr³⁺, and with P addition, the weights were respectively 13.8% and 6.0% lower. The addition of P significantly stimulated rice growth so that the weight of shoots and roots increased ($P<0.001$), but root length exhibited no significant change.

2.2 Fe, P, As and Cr accumulation in plant tissues

The concentrations of Fe in the shoots and roots of both rice genotypes significantly decreased with the increase of As

concentration in the nutrient solution ($P<0.001$). Adding P or not brought about a significantly different Fe concentration in plant tissues (Tables 2 and 3). Compared to the control treatment, the treatment of 1.0 mg L⁻¹ As³⁺+1.0 mg L⁻¹ Cr³⁺ resulted in a respective decrease of the Fe concentration in Jin23A shoots and roots of 9.4% and 27.1% in the absence of P, and in the presence of P, the respective decrease was 25.7% and 32.1%. The Fe concentration decreases in CDR22 shoots and roots were respectively 29.8% and 35.0% in the absence of P, and respectively 28.5% and 27.5% in the presence of P. The concentration of P in plant tissues was significantly correlated with P addition ($P<0.001$). Compared to the treatment without P, 0.7 mmol L⁻¹ P in solution led to an increase of P concentration in Jin23A shoots and roots by 6.1–7.4 and 3.7–5.1 times, and the increases in CDR22 shoots and roots were 6.4–8.8 and 3.6–7.7 times. The addition of As and Cr to the solution did not significantly influence the concentration of P in rice shoots and roots.

With the increase of As in the cultural solution, the root As concentration in both rice genotypes Jin23A and CDR22 increased, while the shoot As concentration did not increase, or slightly decreased. When P was applied, the root As concentration was significantly affected ($P<0.001$), but the shoot As concentration showed no significant difference. Under the treatment of 0.5 mg L⁻¹ As³⁺+1.0 mg L⁻¹ Cr³⁺ and 1.0 mg L⁻¹ As³⁺+1.0 mg L⁻¹ Cr³⁺, the As accumulation in roots of Jin23A was respectively 7.7% and 11.2% higher in the presence of P than in the absence of P, and for roots of CDR22, the accumulation was respectively 35.5% and 31.1% higher.

Table 1 Height, root length, and dry weight (DW) of shoots and roots of two rice genotypes grown in solutions with Cr (1.0 mg/L) and different concentrations of As^{a)}

As (mg/L)	Genotypes	Height (cm)		Root length (cm)		DW of shoot (g)		DW of root (g)	
		−P	+P	−P	+P	−P	+P	−P	+P
0	Jin23A	52.38	62.53	23.38	23.13	2.13	4.06	0.69	1.13
		(0.55)	(1.81)	(1.39)	(1.03)	(0.13)	(0.17)	(0.05)	(0.14)
	CDR22	38.38	41.38	21.10	20.88	1.09	1.50	0.46	0.46
		(0.95)	(1.89)	(0.86)	(0.63)	(0.02)	(0.21)	(0.01)	(0.04)
0.5	Jin23A	52.90	62.00	23.38	23.13	2.41	3.76	0.84	1.04
		(0.67)	(0.98)	(0.38)	(0.97)	(0.08)	(0.10)	(0.05)	(0.07)
	CDR22	37.50	43.50	20.75	20.38	0.99	1.67	0.47	0.51
		(0.61)	(0.54)	(0.32)	(0.43)	(0.10)	(0.16)	(0.04)	(0.05)
1.0	Jin23A	52.68	58.80	20.20	23.20	2.14	3.24	0.81	0.92
		(0.32)	(0.39)	(0.81)	(0.56)	(0.06)	(0.20)	(0.08)	(0.06)
	CDR22	35.45	42.93	20.38	19.88	0.86	1.57	0.48	0.72
		(1.05)	(0.87)	(0.94)	(0.69)	(0.07)	(0.15)	(0.03)	(0.09)
Analysis of variance									
As		NS		NS		<i>P</i> <0.05		NS	
Genotypes (G)		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.001	
P		<i>P</i> <0.001		NS		<i>P</i> <0.001		<i>P</i> <0.001	
As×G		NS		NS		NS		NS	
As×P		NS		NS		NS		NS	
G×P		<i>P</i> <0.05		NS		<i>P</i> <0.001		NS	
As×G×P		<i>P</i> <0.05		NS		<i>P</i> <0.05		<i>P</i> <0.05	

a) Data in brackets represent the standard error.

Table 2 Concentrations of Fe, P, As and Cr in shoots of two rice genotypes grown in solutions with Cr (1.0 mg L⁻¹) and different concentrations of As^{a)}

As (mg L ⁻¹)	Genotypes	Fe (mg kg ⁻¹)		P (g kg ⁻¹)		As (mg kg ⁻¹)		Cr (mg kg ⁻¹)	
		-P	+P	-P	+P	-P	+P	-P	+P
0	Jin23A	290.10 (19.45)	209.27 (16.50)	1.05 (0.02)	7.44 (0.28)	0.00 (0.00)	0.00 (0.00)	4.34 (0.31)	5.71 (0.25)
	CDR22	365.94 (10.69)	326.46 (10.41)	1.10 (0.03)	10.70 (0.32)	0.00 (0.00)	0.00 (0.00)	7.03 (0.43)	5.77 (0.63)
0.5	Jin23A	257.13 (17.02)	181.68 (7.19)	0.97 (0.02)	8.15 (0.11)	8.82 (1.37)	12.00 (1.96)	3.96 (0.11)	3.96 (0.32)
	CDR22	287.88 (2.53)	253.36 (13.78)	1.02 (0.03)	9.02 (0.37)	16.19 (0.64)	21.34 (2.54)	7.24 (0.70)	4.63 (0.59)
1.0	Jin23A	262.74 (20.33)	155.41 (4.37)	1.01 (0.01)	8.16 (0.33)	9.90 (0.91)	12.35 (2.50)	5.09 (0.28)	4.49 (0.10)
	CDR22	336.97 (9.80)	233.51 (3.20)	1.19 (0.02)	8.73 (0.55)	18.75 (1.17)	15.63 (2.09)	8.09 (0.79)	6.20 (0.25)
Analysis of variance									
As		<i>P</i> <0.001		NS		<i>P</i> <0.001		<i>P</i> <0.01	
Genotypes (G)		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.001	
P		<i>P</i> <0.001		<i>P</i> <0.001		NS		<i>P</i> <0.01	
As×G		NS		<i>P</i> =0.001		<i>P</i> =0.001		NS	
As×P		<i>P</i> <0.05		NS		NS		NS	
G×P		NS		<i>P</i> <0.001		NS		<i>P</i> <0.001	
As×G×P		NS		<i>P</i> =0.001		NS		NS	

a) Data in brackets represent the standard error.

Table 3 Concentrations of Fe, P, As and Cr in roots of two rice genotypes grown in solutions with Cr (1.0 mg/L) and different concentrations of As^{a)}

As (mg kg ⁻¹)	Genotypes	Fe (mg kg ⁻¹)		P (g kg ⁻¹)		As (mg kg ⁻¹)		Cr (mg kg ⁻¹)	
		-P	+P	-P	+P	-P	+P	-P	+P
0	Jin23A	306.90 (14.93)	233.53 (34.63)	0.41 (0.01)	2.23 (0.11)	0.00 (0.00)	0.00 (0.00)	36.76 (1.97)	58.35 (9.09)
	CDR22	421.46 (26.52)	324.54 (7.12)	0.43 (0.00)	3.77 (0.38)	0.00 (0.00)	0.00 (0.00)	54.16 (1.06)	58.03 (7.73)
0.5	Jin23A	207.85 (3.84)	217.05 (12.01)	0.39 (0.02)	2.38 (0.05)	161.98 (9.13)	174.41 (14.32)	46.63 (2.52)	48.62 (5.23)
	CDR22	329.76 (25.20)	286.75 (22.16)	0.43 (0.03)	3.01 (0.15)	141.84 (4.81)	192.18 (11.89)	72.11 (1.58)	38.99 (4.91)
1.0	Jin23A	223.58 (2.39)	158.56 (5.63)	0.48 (0.01)	2.24 (0.10)	351.25 (25.48)	390.58 (10.82)	87.82 (8.01)	41.81 (3.43)
	CDR22	273.77 (5.79)	235.34 (21.37)	0.60 (0.02)	2.75 (0.18)	253.42 (15.23)	332.34 (16.93)	207.77 (10.02)	147.45 (33.56)
Analysis of variance									
As		<i>P</i> <0.001		NS		<i>P</i> <0.001		<i>P</i> <0.001	
Genotypes (G)		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> =0.001		<i>P</i> <0.001	
P		<i>P</i> <0.05		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.01	
As×G		NS		<i>P</i> <0.05		<i>P</i> <0.001		<i>P</i> <0.001	
As×P		NS		<i>P</i> =0.01		<i>P</i> <0.01		<i>P</i> =0.001	
G×P		NS		<i>P</i> <0.001		NS		NS	
As×G×P		NS		<i>P</i> <0.05		NS		NS	

a) Data in brackets represent the standard error.

The concentrations of Cr in rice shoots and roots were significantly influenced by P and As addition (*P*<0.01, Tables 2 and 3). Generally, rice Cr concentration decreased with P addition while it increased with the increase of As in a cultural solution, especially in roots. Compared to the control treatment, 1.0 mg L⁻¹ As³⁺+1.0 mg L⁻¹ Cr³⁺ in the solution respectively resulted in an increase of Cr concentration in Jin23A roots by 40.2% (with P) and 138.9% (without P), and an increase of Cr in CDR22 roots by 154.1% (with P) and 283.6% (without P).

2.3 Distribution and transference of Fe, P, As and Cr in rice plants

The transference coefficients of Fe from the iron plaque to shoots (TF_{shoot}) and roots (TF_{root}) were respectively 0.09–0.42 and 0.08–0.46 for Jin23A, and 0.08–0.19 and 0.08–0.23 for CDR22 in different treatments. As and P addition to nutrient solution reduced TF_{shoot} and TF_{root} of Fe, and the impact was significant for both genotypes of rice (*P*<0.001,

Tables 4 and 5). The TF_{shoot} and TF_{root} of P were respectively 4.96–7.01 and 1.36–3.17 for Jin23A, and 2.71–4.31 and 1.29–1.55 for CDR22 in different treatments. The effect of As and Cr on TF_{shoot} of P was significant but not on TF_{root} of P.

There was a large difference between the transference of As from the iron plaque to the roots and shoots: the trans-

ference coefficients were respectively 6.10–10.80 and 1.73–12.38 in roots of Jin23A and CDR22, and respectively 0.17–0.76 and 0.13–1.47 in shoots. Compared to the transference coefficients of Fe, P and As, the TF_{shoot} of Cr was the smallest, less than 0.07 in all treatments, and TF_{root} of Cr was in the range of 0.22–0.78. Additionally, the addition of As and P significantly influenced the TF_{shoot} of Cr.

Table 4 Iron plaques-to-shoot transference factors (TF_{shoot}) for Fe, P, As and Cr in two rice genotypes grown in solutions with Cr (1.0 mg/L) and different concentrations of As ^{a)}

As (mg L ⁻¹)	Genotypes	Fe		P		As		Cr	
		-P	+P	-P	+P	-P	+P	-P	+P
0	Jin23A	0.43 (0.06)	0.13 (0.00)	5.34 (0.22)	6.03 (0.38)	—	—	0.05 (0.01)	0.07 (0.01)
	CDR22	0.19 (0.02)	0.09 (0.01)	3.87 (0.37)	4.28 (0.25)	—	—	0.03 (0.00)	0.03 (0.01)
0.5	Jin23A	0.35 (0.01)	0.11 (0.01)	7.01 (0.13)	6.48 (0.29)	0.35 (0.05)	0.76 (0.17)	0.04 (0.00)	0.04 (0.00)
	CDR22	0.12 (0.03)	0.08 (0.01)	3.11 (0.48)	4.21 (0.29)	0.36 (0.09)	1.47 (0.48)	0.02 (0.01)	0.04 (0.00)
1.0	Jin23A	0.24 (0.06)	0.07 (0.00)	6.69 (0.14)	4.96 (0.07)	0.17 (0.02)	0.29 (0.07)	0.02 (0.00)	0.04 (0.00)
	CDR22	0.11 (0.01)	0.09 (0.02)	2.71 (0.20)	4.31 (0.36)	0.13 (0.01)	0.39 (0.03)	0.01 (0.00)	0.02 (0.00)
Analysis of variance									
As		<i>P</i> <0.001		<i>P</i> <0.05		<i>P</i> =0.001		<i>P</i> <0.001	
Genotypes (G)		<i>P</i> <0.001		<i>P</i> <0.001		NS		<i>P</i> <0.001	
P		<i>P</i> <0.001		NS		<i>P</i> =0.001		<i>P</i> <0.001	
As×G		NS		<i>P</i> <0.01		NS		<i>P</i> <0.05	
As×P		<i>P</i> <0.05		NS		<i>P</i> <0.05		NS	
G×P		<i>P</i> <0.001		<i>P</i> <0.001		NS		NS	
As×G×P		NS		<i>P</i> <0.001		NS		NS	

a) Data in brackets represent the standard error.

Table 5 Iron plaques-to-root transference factors (TF_{root}) for Fe, P, As and Cr in two rice genotypes grown in solutions with Cr (1.0 mg L⁻¹) and different concentrations of As ^{a)}

As (mg L ⁻¹)	Genotypes	Fe		P		As		Cr	
		-P	+P	-P	+P	-P	+P	-P	+P
0	Jin23A	0.46 (0.07)	0.20 (0.07)	2.09 (0.11)	1.82 (0.15)	—	—	0.46 (0.06)	0.78 (0.20)
	CDR22	0.23 (0.03)	0.09 (0.01)	1.54 (0.18)	1.55 (0.28)	—	—	0.26 (0.02)	0.32 (0.04)
0.5	Jin23A	0.29 (0.02)	0.13 (0.02)	2.81 (0.08)	1.90 (0.11)	6.57 (0.36)	10.80 (0.63)	0.42 (0.02)	0.55 (0.05)
	CDR22	0.13 (0.02)	0.09 (0.01)	1.29 (0.14)	1.41 (0.14)	3.11 (0.74)	12.38 (2.19)	0.22 (0.05)	0.29 (0.03)
1.0	Jin23A	0.20 (0.03)	0.07 (0.00)	3.17 (0.12)	1.36 (0.07)	6.10 (0.42)	9.19 (1.05)	0.35 (0.02)	0.36 (0.02)
	CDR22	0.09 (0.01)	0.08 (0.01)	1.38 (0.13)	1.42 (0.27)	1.73 (0.28)	8.62 (0.87)	0.28 (0.01)	0.50 (0.03)
Analysis of variance									
As		<i>P</i> <0.001		NS		<i>P</i> <0.05		NS	
Genotypes (G)		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.05		<i>P</i> <0.001	
P		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> =0.001	
As×G		<i>P</i> <0.05		<i>P</i> <0.05		NS		<i>P</i> =0.001	
As×P		<i>P</i> <0.05		<i>P</i> <0.01		NS		NS	
G×P		<i>P</i> <0.01		<i>P</i> <0.001		<i>P</i> <0.01		NS	
As×G×P		NS		<i>P</i> <0.01		NS		NS	

a) Data in brackets represent the standard error.

Table 6 Distribution coefficients (DCs) of Fe, P, As and Cr between shoots and roots of two rice genotypes grown in solutions with Cr (1.0 mg L⁻¹) and different concentrations of As^{a)}

As (mg L ⁻¹)	Genotypes	Fe		P		As		Cr	
		−P	+P	−P	+P	−P	+P	−P	+P
0	Jin23A	0.94	0.84	2.56	3.34	−	−	0.12	0.10
		(0.03)	(0.23)	(0.06)	(0.09)			(0.01)	(0.01)
	CDR22	0.88	1.01	2.54	2.95	−	−	0.13	0.11
		(0.05)	(0.04)	(0.08)	(0.38)			(0.01)	(0.02)
0.5	Jin23A	1.23	0.84	2.51	3.42	0.05	0.07	0.09	0.08
		(0.06)	(0.04)	(0.11)	(0.07)	(0.01)	(0.01)	(0.00)	(0.01)
	CDR22	0.89	0.89	2.38	3.00	0.11	0.11	0.10	0.09
		(0.08)	(0.04)	(0.14)	(0.09)	(0.01)	(0.02)	(0.01)	(0.00)
1.0	Jin23A	1.18	0.98	2.12	3.66	0.03	0.03	0.06	0.05
		(0.10)	(0.01)	(0.08)	(0.20)	(0.00)	(0.01)	(0.01)	(0.01)
	CDR22	0.94	1.02	1.99	3.24	0.08	0.05	0.04	0.05
		(0.03)	(0.10)	(0.10)	(0.36)	(0.01)	(0.01)	(0.00)	(0.01)
Analysis of variance									
As		<i>P</i> <0.05		NS		<i>P</i> <0.001		<i>P</i> <0.001	
Genotypes (G)		NS		<i>P</i> <0.05		<i>P</i> <0.001		NS	
P		<i>P</i> <0.05		<i>P</i> <0.001		NS		NS	
As×G		NS		NS		<i>P</i> <0.001		<i>P</i> <0.001	
As×P		NS		<i>P</i> <0.01		NS		<i>P</i> <0.01	
G×P		NS		NS		NS		NS	
As×G×P		NS		NS		NS		NS	

a) Data in brackets represent the standard error.

Both shoots and roots accumulated a large amount of Fe, while P mainly accumulated in the shoots. Without the addition of P, the distribution coefficients (DCs) of P in Jin23A and CDR22 were respectively 2.12–2.56 and 1.99–2.54, and with P addition, the DCs of P were respectively 3.34–3.66 and 2.95–3.24 (Table 6). The DC of Fe in shoots and roots was significantly affected by the addition of As and P to the solution (*P*<0.05), but the DC of P suffered little impact.

Arsenic mainly accumulated in rice roots. The DC of As was 0.03–0.07 for Jin23A and 0.05–0.11 for CDR22 under different As concentrations in the solution (Table 6). The DC of As in shoots and roots of both rice genotypes significantly decreased with the increase of As in the nutrient solution (*P*<0.001), while P did not influence the DC of As. Similarly as with As, Cr also primarily accumulated in rice roots. The DC of Cr was 0.05–0.12 for Jin23A and 0.04–0.13 for CDR22 (Table 6). The DC of Cr in shoots and roots of both rice genotypes decreased with the increase of As concentration in nutrient solution (*P*<0.001), but showed little response to P.

3 Discussion

As and Cr are not necessary elements for plant growth, and excess exposure to them might poison plants. According to our experiments, the impact of complex contamination of As and Cr on plant height, root length and root biomass did not seem high, but shoot biomass vigorously responded (Table 1). The biomass of the plant parts under and above ground both significantly reduced due to As poisoning [17].

The rice biomass reduction caused by Cr poisoning was also reported by scientists [18]. However, some studies showed that the addition of a low concentration of As and Cr stimulated the growth of crops and thus increased crop yield [19,20].

There is a complicated interaction between As and P in plants. As and P have a similar atomic structure. When As combines with 3-P-glyceraldehydes, ATP production is inhibited and hence the processes of phosphorylation and oxidative phosphorylation of cells are influenced. Some reports indicated that As poisoning may close the channel of plants for P uptake so that plant P uptake is inhibited [21,22]. However we found that As did not influence the concentration of P in rice roots and shoots in our experiments. The reason might lie in the more complicated mechanism of complex contamination than with a single contaminant. P might influence As uptake and accumulation in plants. According to Geng *et al.* [23], the application of enough P counteracted the poisoning effect of As and thus eliminated the symptoms of As poisoning. In our experiments, the plant height and dry weight of rice roots and shoots were significantly higher under the treatments with P addition than without P, indicating that P relieves the poisoning effect of As and Cr. For treatment of 0.5 mg L⁻¹ As³⁺+1.0 mg L⁻¹ Cr³⁺ and 1.0 mg L⁻¹ As³⁺+1.0 mg L⁻¹ Cr³⁺, the As concentrations in rice roots were 7.7%–35.5% and 11.2%–31.1% higher in the presence of P than without P addition. Similarly, Geng *et al.* found that As concentrations in the shoots and roots of the two rice genotypes 94D-54 and 94D-64 largely increased with the increase of P in the solution [23].

The mechanism of plant Cr uptake is more complicated,

and other elements influence Cr uptake and accumulation. Plants uptake both Cr(III) and Cr(VI) [10,24], however the uptake of Cr(III) is by a passive process, while Cr(VI) uptake proceeds through a sulfate carrier [25]. In our experiments, the addition of Cr(III) in solution resulted in a high Cr uptake and accumulation in rice, and the shoot Cr concentrations under the cultures with P addition were lower than without P. The application of As facilitates the uptake and accumulation of Cr in shoots and roots. Shewry and Peterson reported that plant Cr uptake is inhibited by hexavalent ions, such as SO_4^{2-} , but Ca^{2+} promotes plant Cr uptake [26]. Similarly, chromium contamination also influences the uptake of other elements. Mora *et al.* found that Cr(III) significantly reduced the Fe and Cu uptake and accumulation by tomatoes [27]. The effects of Cr(III) on the uptake of major elements (P, Ca, K and Mg) and minor elements (Fe, Cu and Zn) in tumbleweed were reported by Gardea-Torrestedey *et al.* [12]. Similarly, the effects of Cr(III) on the uptake and accumulation of Fe, P, As and Cr in rice were investigated in this study, but the mechanism of As (III) and Cr(III) complex contamination might be more complicated than that of single Cr(III) contamination.

Phosphorous application in a cultural solution significantly increases the concentrations of Fe and P in the iron plaque, and reduce As and Cr concentrations. The concentrations of As and Cr in iron plaque of rice increased with the increase of As concentration in solution, but the concentrations of Fe and P in the iron plaque were not significantly influenced, indicating that the impact of the iron plaque on plant element uptake mechanism may be very complicated. Through X-ray fluorescence microscopy, Batty *et al.* found that the iron plaque mainly consists of Fe and Mn oxides and their hydroxides [28]. Iron plaque has a special electrochemical characteristic so that it's an amphoteric colloid. It changes the existing form of anions and cations of heavy metal around plant roots through actions of adsorption and desorption, oxidation and reduction, and organic or inorganic complexation, thus it influences the biological availability of the ions [29,30]. Rice iron plaque strongly induces the formation of arsenate, which forms a buffer zone in the root microenvironment to inhibit the accumulation of toxic chemicals in rice tissues [31]. Therefore, investigation of the uptake and accumulation of As and Cr by rice in the presence of a root surface iron plaque provides a scientific basis for phytoremediation by heavy metals.

4 Conclusion

This study indicated that the effect of the complex contamination of As and Cr on rice shoot dry weight is significant, although not so on plant height, root length and root dry weight. The concentration of P (or As) in rice shoots is significantly increased with P (or As) application. Application of P and As in a cultural solution significantly reduces

the concentrations of Fe in the shoots and roots of the two rice genotypes. The root As concentration is significantly affected by the addition of P in the solution, while P concentrations in roots and shoots are not significantly influenced by As addition. Application of P in the solution results in a decrease of rice shoot Cr concentration, but the application of As leads to an increase of shoot Cr concentration.

Concentrations of As and Cr in the iron plaque of the two rice genotypes are significantly affected by the addition of As in the solution. Phosphorous addition in the solution largely increases the concentrations of Fe and P while significantly decreasing the concentrations of As and Cr in the iron plaque. Through the comparison of elemental transference factors (TFs), it is evident that phosphorous transfers from the iron plaque to rice roots and shoots, but the transferences of Cr and Fe are lower, and As is also lower. The transferences of As and Cr from the iron plaque to rice roots and shoots are influenced by P management. The influence of P addition on As and Cr in rice grains was not found in this study, because rice was harvested before experiencing an entire growing season. If P management reduces the accumulation of As and Cr in rice grains, it would provide a fundamental guiding role for As and Cr contamination prevention in rice fields.

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