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Using iron fertilizer to control Cd accumulation in rice plants: A new promising technology

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Effects of two kinds of iron fertilizer, $FeSO_4$ and EDTA·Na_2Fe were studied on cadmium accumulation in rice plants with two rice genotypes, Zhongzao 22 and Zhongjiazao 02, with soil culture systems. The results showed that application of iron fertilizers could hardly make adverse effects on plant growth and rice grain yield. Soil application of EDTA·Na_2Fe significantly reduced the Cd accumulation in rice roots, shoots and rice grain. Cd concentration in white rice of both rice genotypes in the treatment of soil application of EDTA·Na_2Fe was much lower than 0.2 mg/kg, the maximal Cd permission concentration in cereal crop foods in State standard. However, soil application of $FeSO_4$ or foliar application of $FeSO_4$ or EDTA·Na_2Fe resulted in the significant increase of Cd accumulation in rice plants including rice grain compared with the control. The results also showed iron fertilizers increased the concentration of iron, copper and manganese element in rice grain and also affected zinc concentration in plants. It may be a new promising way to regulate Cd accumulation in rice grain in rice production through soil application of EDTA·Na_2Fe fertilizers to maintain higher content of available iron and ferrous iron in soils.

rice (*Oryza sativa* L.), cadmium, iron fertilizer, EDTA·Na_2Fe

Cadmium, the main heavy metal pollutant, brings a grave threat to human health due to its high mobility in soil-plant systems and its world-wide pollution in food production systems. Cd uptake and accumulation in crop plants are determined by various factors including soil pH, and soil phytoavalable Cd content. Soil phytoavailable Cd content is the essential content of these environmental factors. Therefore, lots of agricultural or chemical technologies focusing on reducing soil phytoavailable Cd concentration have been developed to reduce crop Cd accumulation, which includes enhanced soil pH and reduced soil redox potential. Those factors reducing soil phytoavailable Cd concentration to control Cd accumulation in crop plants were the so-called “extrinsic factors ”, which could affect not only Cd status in soils, but also other mineral metal element status and might cause adverse effects on crop growth[1−3]. For example, enhanced soil pH may lead to significantly decreased phytoavailable iron, manganese, copper and zinc content essential to crop, and ultimately is adverse to crop growth and crop production[4]. Therefore, it may be reasonable to control Cd accumulation through those “intrinsic factors” that inhibit the processes of Cd uptake into root systems and transport to harvestable parts through regulation of plant metabolic pathways involved in Cd uptake or transportation. There is a good example that spraying a given amount of ABA on crop plants could markedly reduce Cd accumulation in shoots through induction of leaf stomatal closure and alleviation of transpiration process coupled with Cd transport[5−7]. Recently, it was well documented at mo-

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lecular levels that Cd could be taken up into cell via iron metabolic systems in plant roots depending on iron status in soils[8−19]. These interesting findings imply that Cd accumulation in crops might be controlled through regulation of plant iron metabolic systems by alteration of soil iron status.

Iron is an essential element for plant growth. Although abundant in most soils, Fe predominates as insoluble Fe^{3+} precipitates largely unavailable to plants, especially at neutral or alkaline pH. Plants have developed multi-mechanisms involved in iron uptake into plant roots from soils relying on soiliron status during the evolutionary process[8,9]. Under iron-sufficient conditions, all plants reduce Fe^{3+}-chelates and transport the resulting Fe^{2+} through the plasma membrane via low-affinity iron transport systems, still uncharacterized at the molecular level[8,9]. Under iron-deficient conditions, plants use two distinct strategies to assimilate Fe from the environment. The graminaceous monotyledonous plants release low-molecular-weight, high-affinity $Fe(^{3+})$-chelating compounds called phytosider-ophores (PS), which solubilize ferric Fe in the rhizosphere and are recognized for uptake by specific membrane receptors and ultimately reduced to Fe^{2+} in the cytoplasm. This mechanism, a kind of high-affinity iron transport system, was called strategy II. While, the dicotyledonous and non-graminaceous monotyledonous plant species acidify the rhizosphere to enhance the iron availability, reduce Fe^{3+} to Fe^{2+} by plasma membrane-bound ferric reductase at the cell membrane and transport ferrous ions (Fe^{2+}) into cytoplasm via membrane-bound iron transporter, which is defined as strategy I, another kind of high-affinity iron transport system[8,9]. Naimatullah et al. (2002), Ishimaru et al. (2006) and Nakanishi et al.(2006) isolated and characterized a third iron-deficiency-induced high-affinity iron transport system in rice plants which was different from strategy II, but similar to strategy I[11−13], named "similar strategy Ⅰ" in this paper. According to the above studies, rice plants at least possess three types of iron-transport systems, ie. low affinity iron transport system and two high-affinity iron transport systems. Previous studies also showed there was a dynamic balance between low-affinity iron transport system and high-affinity iron transport systems relying on soil iron status[8−19]. The high-affinity iron transport systems were strongly induced by iron deficiency and inhibited by the high available iron content in soils. It was also suggested that the mechanisms involved in root assimilation of iron from soil could be regulated by shoot iron nutritional status[14,15].

It was reported that Cd uptake of higher plants was closely related to the inductive mechanisms involved in iron transport systems depending on soil iron status. Iron deficiency in environment could strongly induce Cd uptake by roots and transport to shoot in dicotyledonous and non-graminaceous monotyledonous plant species[13,16−19]. The membrane iron transporter, IRT1, in strategy I strongly induced by iron deficiency has been characterized as the main factor at the molecular level for high accumulation of Cd in dicotyledonous plants because IRT1 could not only transport Fe^{2+} across cell membrane into cell, but also Cd^{2+} and some other bivalent metal elements such as zinc effectively[13,16−19]. Non-graminaceous monotyledonous plants were also found to accumulate much more Cd in plants under iron deficiency in environment, and it was hypothesized that Cd accumulation in grass plants might involve the iron transport system in roots[20,21]. Ishimaru et al. (2006) found that membrane iron transporters, OsIRT1 and OsIRT2, in similar strategy I in rice plants could directly transport Cd at the molecular level, besides Fe^{2+}[12,13], which strongly expressed and played a critical role in Cd accumulation in rice plants under iron-deficient conditions. According to the expression of these two membrane transporters among the plant parts, it was speculated that OsIRT1and OsIRT2 might play the main roles in roots and OsIRT2 also in shoots and grains[11−13].

With comprehensive consideration of the relations between iron and Cd uptake mechanisms in rice plant at the molecular levels, it was hypothesized that it might be effective to control Cd accumulation in crop plants, especially in harvestable parts through enhanced available soil iron content or improved iron nutritional status in crop shoots[21]. The object of the present study was to confirm this hypothesis at the agronomical level and establish a new agronomical technology to control Cd accumulation in rice plants.

1 Matierials and methods

1.1 Materials preparation

1.1.1 Cd-contaminated soil preparation. Cd-contaminated soil was incubated with purple paddy soil by addition of $CdCl_2$ at the fuyang base of China National

Rice Research Institute, Hangzhou, China in 2004. The soil has been used for rice cultivation for two years and was homogeneous. The total soil Cd content was 5.70 mg/kg and DTPA-extractable Cd content 1.76 mg/kg. Cd-contaminated soil was dried in the shade and ground homogeneously and prepared.

1.1.2 Rice seedling preparation. Two early-*indica* rice genotypes, viz., Zhongjiazao 02 and Zhongzao 22 were used as materials. The seeds were soaked in de-ionized water containing 0.5 μg ml^{-1} SCN-(CH$_2$)$_2$-SCN for 2 d and germinated at 30℃－35℃ for 1 d, and then seeded on sandy bed which was previously washed with dilute sulfuric acid.

1.2 Treatment

This study was carried out with soil cultivation system with porcelain cups containing 11 kg dry soil per porcelain cup. There were 5 iron fertilizer treatments as below: (1) CK; (2) soil application of $FeSO_4$: 0.55 g Fe was added to soil per porcelain cup and homogenized with $FeSO_4$ as Fe source before rice transplant. 0.2 g Fe was added to soil per porcelain cup with $FeSO_4$ solution as Fe source at the booting stage and the initial grain-filling stage, respectively; (3) soil application of EDTA·Na$_2$Fe: 0.55 g Fe was added to soil per porcelain cup, and homogenized with EDTA·Na$_2$Fe as Fe source before rice transplant. 0.2 g Fe was added to soil per porcelain cup with EDTA·Na$_2$Fe solution as Fe source at the booting stage and the initial grain-filling stage, respectively; (4) foliar application of $FeSO_4$: $FeSO_4$ solution containing 0.5 g/L Fe was sprayed on rice shoot every 10 d after initiation of the booting stage, 5 times in total; (5) foliar application of EDTA·Na$_2$Fe: EDTA·Na$_2$Fe solution containing 0.5 g/L Fe was sprayed on rice shoots every 10 d after initiation of the booting stage, 5 times in total. The seedlings with 5 leaves were selected with uniformity and transplanted with two seedlings per porcelain cup. The experiment was designed as random block with 5 replications and there were 50 porcelain cups in total.

1.3 Sampling and measurements

SPAD value was measured on the flag leaf using a chlorophyll meter (Minolata SPAD-502, Japan) with 10 replications at the grain-filling stage. Rice plants and soils were sampled at the mature stage and rice growth-related parameters and yield-related parameters were recorded. Rice roots were immerged in a solution containing 1.0 mmol/L EDTA for 2 h, and then rinsed with de-ionized water twice. The plants were separated into grains which further milled into brown rice and white rice, leaves, shoots and roots, and dried up to a constant weight at 105 ℃ for 1 h and then at 60℃ for 48 h in an oven, weighed and then ground. Soil samples were dried in the shade. 0.500 g soil was mixed with 25 ml de-ionized water and agitated for 1 h, the supernatant to be measured for soil pH. 1.000 g soil was mixed with 50 ml solution containing 1.0 mmol/L DTPA and agitated for 4 h. The supernatant after filtration was measured for DTPA-extractable element content. After microwave digestion, Cd in brown and white rice was measured using GFAAS (Solaar M6，Thermo Elemental，America) and other elements in brown and white rice and all elements in rice leaves, shoots, roots and soils were measured using a simultaneous inductively coupled argon-plasma emission spectrometre (ICAP 61E trace analyzer, Themo-Jarrell Ashe, Franklin, MA).

1.4 Data analysis

All data presented are the mean values. Statistical analysis was carried out by two-way ANOVA and Duncan's multiple-range test (SSR) to test significance of the difference between means with post hoc least significant difference test (LSD) at $P\leqslant0.05$.

2 Results

2.1 Effects of iron application on DTPA-extractable Fe and Cd concentration and in soil

Soil pH and soil DTPA-extractable Cd concentration are two important environmental factors affecting Cd uptake by plant roots, and soil DTPA-extractable Fe concentration plays a critical role on which mechanisms are principally used by plants to assimilate iron from soils. The results showed that soil pH and soil phytoavailable Cd concentration did not change when iron fertilizer was applied compared with the control irrespectively of iron forms or application methods (Table 1). However, Soil application of EDTA·Na$_2$Fe significantly increased DTPA-extractable iron concentration by 7.96%, and the other treatments did not alter DTPA-extractable iron content compared with CK.

2.2 Effects of iron application on Cd and Fe concentration in rice plants

2.2.1 Cd and Fe concentration in rice grain. Cd accumulation in rice grain was affected by iron fertilizers dependent on iron species and application methods (Table 2). Cd accumulations in both brown rice and white rice were much stimulated by soil application of $FeSO_4$ and foliar application of $FeSO_4$ or DTA.Na_2Fe by at least more than 35% compared with CK, whereas significantly inhibited by soil application of EDTA·Na_2Fe by more than 66.7% to 75%, and Cd concentration in white rice of both rice genotypes was much lower than 0.2 mg/kg, the maximal permission concentration in cereal crop foods in China. In addition, there was hardly difference in Cd accumulation in brown rice between the two genotypes at the CK treatment, but Zhongjiazao 02 had much higher Cd accumulation in both brown rice and white rice than Zhongzao 22 after iron fertilizer application.

Iron fertilizer application affecting iron concentration in rice grains was dependent on iron species and its application methods (Table 2). Application of $FeSO_4$ did not affect iron concentration in brown rice of both genotypes, but stimulated iron concentration in white rice of both genotypes on the whole compared with CK, while EDTA·Na_2Fe could significantly stimulate iron accumulation in brown rice and white rice of both rice genotypes.

2.2.2 Cd and Fe accumulation in rice shoots and roots.

Iron fertilizers could affect Cd concentration in rice plants dependent on iron species and application methods (Table 3). Cd concentration in shoots and roots of both rice genotypes was markedly enhanced by soil application of $FeSO_4$ or foliar application of $FeSO_4$ or EDTA·Na_2Fe. However, soil application of EDTA·Na_2Fe significantly reduced Cd concentration in shoots and roots of both rice genotypes by 75.78% to 80.52% with both the genotypes representing the similar trend. There was no difference in Cd concentration in plants between the two genotypes.

Iron concentration in rice plants was also affected by iron fertilizers. All iron fertilizer treatments significantly elevated iron concentration in shoots of Zhongjiazao 02, but for Zhongzao22, shoot iron concentration increased markedly by soil application of $FeSO_4$ or EDTA·Na_2Fe and decreased by foliar application of $FeSO_4$ or EDTA·Na_2Fe. In addition, there was a significant difference in shoot iron concentration between the two application methods with higher iron concentration in both rice shoots under soil application than under foliar application. Soil application of $FeSO_4$ resulted in enhanced iron concentration in both rice roots, and foliar applica-

Table 1 Effects of iron treatment on DTPA-extractable iron and cadmium content and pH value in soil

Treatment		pH	DTPA-extractable element concentration	
			Cd concentration (mg/kg)	Fe concentration (mg/kg)
CK		7.38a	1.761a	180.8b
Soil application	$FeSO_4$	7.61a	1.753a	184.4b
	EDTA·Na_2Fe	7.36a	1.762a	195.2a
Foliar application	$FeSO_4$	7.51a	1.775a	182.2b
	EDTA·Na_2Fe	7.39a	1.790a	183.8b

All data presented are the mean values of 10 data consisting of two five- replications for two rice genotypes respectively due to no difference between the means of the two five- replications. Within each column, the values followed by the same letter have no difference at the 5% level.

Table 2 Effects of iron application on iron and cadmium concentration in brown rice and white rice

Treatment		Cd (mg/kg)		Fe (mg/kg)	
		Zhongjiazao 02	Zhongzao 22	Zhongjiazao 02	Zhongzao 22
Brown rice					
CK		1.072c	1.045c	32.3b	37.7bc
Soil application	$FeSO_4$	1.471ab	1.330ab	33.6b	37.5c
	EDTA·Na_2Fe	0.241d	0.193d	54.8a	40.3a
Foliar application	$FeSO_4$	1.583a	1.473a	35.8b	38.8b
	EDTA·Na_2Fe	1.392b	1.242b	56.3a	39.5ab
White rice					
CK		0.383c	0.370c	11.2b	13.3b
Soil application	$FeSO_4$	0.526ab	0.457b	11.9b	13.7ab
	EDTA·Na_2Fe	0.084d	0.067d	19.5a	14.8a
Foliar application	$FeSO_4$	0.539a	0.523a	12.6b	14.6a
	EDTA·Na_2Fe	0.489b	0.437b	19.1a	14.4a

All data presented are the mean values of 5 replications. Within each column，the values followed by the same letter have no difference at the 5% level.

tion of $FeSO_4$ presented hardly effects on iron concentration in the roots of Zhongzao 22 but increased significantly iron concentration in the roots of Zhongjiazao 02. Both soil application and foliar application of $EDTA\cdot Na_2Fe$ reduced significantly iron concentration in the roots of Zhongjiazao 02, but elevated slightly iron concentration in the roots of Zhongzao 22.

2.3 Effects of iron application on Cu, Mn and Zn accumulation in rice plants

2.3.1 Cu, Mn and Zn concentration in brown rice and white rice. Cu, Mn and Zn are three important essential micro-elements for animal and plants. Iron application had significant effects on Cu, Mn and Zn accumulation in rice grains (Table 4). Both the two application methods with iron fertilizers could significantly stimulate Cu, Mn accumulation in brown rice and white rice of both rice genotypes. Considering the iron species, application of $EDTA\cdot Na_2Fe$ had more obvious effects than application of $FeSO_4$ on Cu accumulation in rice grains, but the case was opposite for Mn accumulation. Soil application of $FeSO_4$ or foliar application of $FeSO_4$ or $EDTA\cdot Na_2Fe$ showed no significant effects on Zn content in both brown rice and white rice, whereas soil application of $EDTA\cdot Na_2Fe$ inhibited Zn accumulation in rice grains with the same case between the two genotypes.

2.3.2 Cu, Mn and Zn concentration in rice shoots and roots. Iron fertilizers played important roles in Cu, Mn and Zn accumulation in rice shoots and roots depending on irons species and application methods (Table 5). Compared with CK, all the four iron treatments significantly elevated Cu concentration in rice shoots and roots, especially in the roots with more than one time higher than CK. Application of $FeSO_4$ stimulated Mn accumulation in rice shoots and roots irrespectively of application methods, while application of $EDTA\cdot Na_2Fe$ showed different effects on Mn accumulation in rice plants relying on application methods. Soil application of EDTA·

Table 3 Effects of iron application on iron and cadmium accumulation in rice plants

Treatment		Cd (mg/kg)		Fe (mg/kg)	
		Zhongjiazao 02	Zhongzao 22	Zhongjiazao 02	Zhongzao 22
Shoot					
CK		69.00c	79.50c	331.3c	440.7b
Soil application	$FeSO_4$	130.00a	121.17a	754.3a	514.3a
	$EDTA\cdot Na_2Fe$	25.33d	23.83d	727.3a	558.7a
Spray application	$FeSO_4$	92.17b	109.67b	471.7b	417.0b
	$EDTA\cdot Na_2Fe$	128.33a	129.50a	411.7b	380.3bc
Root					
CK		1725.00c	1745.67c	19233.3b	8083.3c
Soil application	$FeSO_4$	3250.00a	2272.17b	27766.7a	21833.3a
	$EDTA\cdot Na_2Fe$	633.25d	550.33d	16700.0c	9676.7c
Foliar application	$FeSO_4$	2304.25b	2585.50b	19100.0b	15600.0b
	$EDTA\cdot Na_2Fe$	3208.25a	3880.17a	13033.3d	8120.0bc

All data presented are the mean values of 5 replications. Within each column, the values followed by the same letter have no difference at the 5% level.

Table 4 Copper, manganese and zinc concentration (mg/kg) in brown rice and white rice

Treatment		Zhongjiazao 02			Zhongzao 22		
		Cu	Mn	Zn	Cu	Mn	Zn
Brown rice							
CK		3.668c	26.653c	22.273c	3.677c	22.208d	23.897a
Soil application	$FeSO_4$	3.997b	33.703a	24.690a	3.847bc	28.953a	23.657a
	$EDTA\cdot Na_2Fe$	5.122a	33.593a	21.747c	4.418a	26.725b	21.830b
Foliar application	$FeSO_4$	4.368b	27.720b	23.248b	3.920b	22.377d	23.972a
	$EDTA\cdot Na_2Fe$	4.938a	27.872b	23.050b	4.607a	24.668c	22.810ab
White rice							
CK		2.348c	9.209b	19.592b	2.294c	8.503d	18.728a
Soil application	$FeSO_4$	2.718b	12.876a	21.344a	2.552bc	10.834a	18.760a
	$EDTA\cdot Na_2Fe$	3.386a	12.738a	17.222c	2.932a	10.278a	17.282b
Foliar application	$FeSO_4$	2.834b	9.882b	18.374bc	2.584b	8.962c	18.880a
	$EDTA\cdot Na_2Fe$	3.356a	9.975b	18.136c	2.904a	9.874b	17.768b

All data presented are the mean values of 5 replications. Within each column, the values followed by the same letter have no difference at the 5% level.

Table 5 Copper, manganese and zinc concentration (mg/kg) in rice plants

Treatment		Zhongjiazao 02			Zhongzao 22		
		Cu	Mn	Zn	Cu	Mn	Zn
Shoot							
CK		2.73c	472.63b	47.55bc	2.65c	453.83b	46.85b
Soil application	$FeSO_4$	4.68b	1145.33a	74.02a	3.08b	980.50a	76.45a
	EDTA·Na_2Fe	4.48b	489.00b	41.87c	3.18b	461.67b	39.04c
Foliar application	$FeSO_4$	3.13c	529.23b	48.98b	2.93bc	476.97b	50.46b
	EDTA·Na_2Fe	5.68a	408.33c	51.19b	4.00a	295.17c	39.50c
Root							
CK		13.60e	352.93c	62.76d	15.30c	169.70d	53.70d
Soil application	$FeSO_4$	36.60c	1002.27a	97.29b	27.53b	516.63a	85.28b
	EDTA·Na_2Fe	58.48a	441.73b	139.17a	43.40a	214.07c	68.01c
Foliar application	$FeSO_4$	27.50d	382.30c	83.24c	26.08b	369.77b	99.68a
	EDTA·Na_2Fe	40.45b	253.97d	69.44d	45.93a	170.80d	69.09c

All data presented are the mean values of 5 replications. Within each column, the values followed by the same letter have no difference at the 5% level.

Na_2Fe could elevate Mn concentration, and these stimulative effects were much lower than that caused by application of $FeSO_4$. On the whole, foliar application of EDTA·Na_2Fe showed significant negative effects on Mn accumulation in both rice shoots and roots. While, application of iron fertilizers had more complicated effects on Zn accumulation in rice plants. Soil application of EDTA·Na_2Fe decreased Zn accumulation in rice shoots, but increased Zn accumulation in rice roots of both rice genotypes. Foliar application of EDTA·Na_2Fe decreased Zn accumulation in shoots of Zhongzao 22 and stimulated Zn accumulation in roots of Zhongzao 22 and in the shoots and roots of Zhongjiazao 02. Soil application or foliar application of $FeSO_4$ stimulated significantly Zn accumulation in shoots and roots of both rice genotypes.

4 Discussion

Cadmium is one of the most toxic heavy metals for animal and plant, which possesses high capability to move among the rice production systems. Previous studies showed that Cd uptake into plant roots and its transport to shoots was subject to the iron metabolic pathways in plants which had been elucidated at the molecular levels[8–19]. The aim of the present study was to develop an agronomical technology to reduce Cd accumulation in rice plants, especially in rice grain based on the great achievements on the relations between Cd and Fe metabolic pathways and its regulative mechanisms at the molecular level. Cd uptake and transport in rice plants could be achieved through iron metabolic pathway which was regulated by soil iron status and shoot iron status. We considered the following three aspects important to control Cd accumulation in rice plants: (1) to increase soil phytoavailable iron concentration to inhibit the expression of similar strategy Ⅰ in rice roots and reduce Cd uptake via similar strategy Ⅰ; (2) to elevate ferrous ions (Fe^{2+}) in soils to not only increase soil phytoavailable iron concentration, but also compete Cd^{2+} for ferrous ion membrane transporter such as OsIRT1and OsIRT2, and ultimately reduce Cd uptake into rice roots; (3) to improve iron nutrition status in rice shoots to inhibit the expression of high-affinity iron transport systems (similar strategy I mainly) in roots. Based on the above three aspects, we carried out the present study using $FeSO_4$ and EDTA·Na_2Fe as iron fertilizers to control Cd accumulation in rice plants due to EDTA·Na_2Fe's high stability in soils.

The present study was conducted in 2006 and 2007 for two years using the same rice genotypes. This paper presents the results from the study in 2007 due to no difference in the results between the two years. The results showed that iron fertilizer could significantly affect Cd concentration in rice plants. Soil application of EDTA·Na_2Fe reduced significantly Cd concentration in rice plants including rice grain, shoots and roots compared with CK. Cd concentrations in the white rice of Zhongjiazao 02 and Zhongzao 22 were 0.084 mg/kg and 0.067 mg/kg respectively which were much lower than 0.2 mg/kg, the maximal permission of Cd concentration in cereal crop foods in China and 0.1 mg/kg, the recommended maximal permission of Cd concentration by the international food standards organization, Codex Alimentarius Commission[22]. However, Cd concentration in rice plants including rice grains was heavily intensified by soil application of $FeSO_4$, foliar application of $FeSO_4$ or EDTA·Na_2Fe. Comparison of Cd concentration among plant tissues showed that rice roots had higher Cd concentration than shoots and shoots higher than rice grain. The same trend was observed in both rice genotypes in all iron treatments. But Cd concentration in plant parts was much lower in the treatment of soil application of EDTA·Na_2Fe than that of the corresponding plant parts in other treatments. Based on Cd concentration of rice roots and rice shoots, it might be hypothesized that the decrease of Cd concentration of brown rice and white rice in soil application of EDTA·Na_2Fe was mainly caused by lower Cd uptake capability of rice roots, and the increase of Cd concentrations in other iron treatments was mainly caused by increased Cd uptake capability of rice roots. As described previously, rice had two types of iron-deficiency-induced high-affinity iron transport systems, i.e. strategy II and similar strategy I[11−13]. The cell membrane iron transporters of similar strategy I, OsIRT1 and OsIRT2 which are expressed mainly in rice roots and OsIRT2 which might also be expressed in rice shoots and grain, could not only transport directly Fe^{2+} across cell membrane, but also Cd^{2+} effectively[12,13]. As we know, EDTA·Na_2Fe was an effective iron fertilizer which could persistently provide enough ferrous ion in soils. Meanwhile, soil application of EDTA·Na_2Fe could elevate the phytoavailable iron content due to its high stability in soils as showed in Table 1. Therefore, soil application of EDTA·Na_2Fe could significantly reduce the Cd uptake by rice roots and subsequent transportation to shoots and grain at least in part because of increased phytoavailable iron content as ferrous forms which could inhibit the expression of the high-affinity iron transport systems including similar strategy I. On the other hand, Fe^{2+} could compete with Cd^{2+} for the membrane iron transporter, OsIRT1and OsIRT2, in rice roots which lead further to inhibition of Cd uptake into roots. Whereas, $FeSO_4$ was easy to be oxygenated into ferric forms and immobilized in soils, therefore, soil application with $FeSO_4$ did not obviously change the soil DTPA-extractable iron content compared and could not provide effective iron nutrition for rice growth. Meanwhile, much ferromanganese oxide would deposit at the rhizosphere or surface of rice roots due to the characteristic of oxygen secretion by rice roots and intermittent irrigation, which could absorb high content of Cd, Mn and Cu and cause increased Cd uptake into rice roots. Soil application of $FeSO_4$ might further accelerate the formation of ferromanganese oxide at the rhizosphere or surface of rice roots, and ultimately stimulate Cd, Mn and Cu accumulation. Unexpectedly, foliar application of iron nutrition significantly enhanced the Cd concentration in rice plants compared with the control. It was well documented and practiced by agricultural production that foliar application of iron nutrition could availably improve the iron nutrition states in crop shoots[25,26], and shoot-to-root signal of iron nutrition state played a critical role in iron utilization in roots[14,15]. Sufficient iron nutrition in shoots would inhibit the expression of high-affinity iron transport systems and attenuate the iron uptake into rice roots[14,15]. However, in our studies, foliar application of nutrition did improve the nutrition state in rice plants (Tables 2 and 3), but Cd accumulation was also significantly enhanced in all rice plant tissues and the increased Cd accumulation was apparently caused by the enhanced Cd uptake capability of rice roots. It implied that rice plants utilizing iron from soils might be involved in much more complicated signal regulation processes needed for further research. The above results showed that soil application of ferrous chelate might be a new promising technology to control Cd accumulation in rice plants through reining the diversity of iron uptake mechanisms. A small quantity of iron fertilizers might enter soils during the process of foliar spraying or rainfall in the foliar application treatments. Iron concentration in solution used for foliar spray was only 0.5 g/L and the dosage of iron solution was very small (an advantage of fertilizer for foliar application), and therefore, it could be thought that foliar application would make no significant effects on iron species and iron valence state in soils.

Of course, there were many environmental factors affecting Cd uptake by rice roots such as soil pH value, soil phytoavailable Cd content. Low soil pH value would dissociate the immobile Cd from soil particles into mobile forms and increased soil phytoavailable Cd content (DTPA-extractable Cd content) and consequentially strengthened the Cd accumulation in plants[27]. Soil phytoavailable Cd content was the essential content of most environmental factors affecting Cd uptake from soils by plant roots[4,19]. In our study, application of iron fertilizer, irrespectively of iron forms or application methods, did not pose obvious impact on soil pH and DTPA-extractable Cd content. The results implied that application iron fertilizers could significantly affect Cd uptake and accumulation in rice plants through the alteration of plant iron metabolism processes.

Summarily, soil application of $\mathrm{EDTA{\cdot}Na_2Fe}$ could significantly reduce Cd uptake and accumulation in rice plants through the alteration of plant iron metabolism processes by enhanced phytoavailable iron content and increased ferrous content in soils. Therefore, from this point of view, it should be effective to control Cd accumulation in rice plants as long as high phytoavailable iron content and high ferrous ions were maintained in field soils. It was suggested that under submerged conditions, the low soil redox potential (Eh value) could deoxidize Fe^{3+} to Fe^{2+} with increased iron content as ferrous forms[] and most dissociative Cd was transformed as cadmium sulfide (CdS), a slightly soluble form[13]. These two kinds of factors could strongly reduce Cd uptake and accumulation in rice plants. However, it would consume a large quantity of irrigational water to maintain submerged paddyfields, and which was not feasible because of world-wide water deficiency at present day and which was also adverse to rice growth. Soil application of $FeSO_4$ and foliar iron nutrition would not be suitable for control Cd accumulation in rice plants.

In the present study, we also characterized the chlorophyll content of the flag leaf at the grain-filling stage, plant height and shoot dry weight at the harvest stage to estimate the effects of iron fertilizer application on rice growth. The results showed the three growth-related parameters did not represent significant difference among all iron treatments, which indicated that application of iron fertilizer would not be adverse to rice growth. It was important for rice production to achieve high grain yields. Therefore, we measured four yield-related parameters, i.e. panicle number per pot, total grains per panicle, total filled-grains per and kilo-grain weight in all iron treatments and the results also showed application of iron fertilizer would not obviously influence the rice yield compared with the control. Furthermore, application of iron fertilizer could strongly improve rice nutrition quality with enhanced iron, copper and manganese content in white rice. In fact, it was widely used in crop production to apply iron fertilizer to rectify iron deficiency in crop plant, and which also confirmed indirectly the case that application of iron fertilizer was not adverse to rice growth and rice production[25,26]. The present study shows that it might be a new promising technology to control Cd accumulation in rice plants using iron fertilizer with appropriate iron forms and application methods.

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