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Influence and interaction of iron and cadmium on photosynthesis and antioxidative enzymes in two rice cultivars

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HIGHLIGHTS

- Cd has negative influence on the photosynthesis and the formation of biomass in rice.
- Fe can alleviate the Cd-induced changes at early and later growth stages of two rice varieties.
- Fe is greatly beneficial for the rice development under Cd-polluted environment.

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ABSTRACT

In this study, a soil pot experiment was conducted to investigate the changes in photosynthesis and antioxidative enzymes in two rice varieties (Shendao 6 and Shennong 265) supplied with iron (Fe), cadmium (Cd), and Fe and Cd together. The concentrations of Fe and Cd in the soil were 0, 1.0 g Fe$\cdot$kg^{-1} and 0, 2.0 mg Cd$\cdot$kg^{-1}, respectively. Photosynthetic indices and antioxidative enzyme activities were recorded at different rice growth stages. At the early stage, Cd showed a transient stimulatory effect on the photosynthetic rate of Shennong 265. For Shendao 6, however, Cd showed a transient stimulatory effect on photosynthetic rate, intercellular CO_2 concentration, stomatal conductance and transpiration efficiency. In addition, the results show that Cd can also enhance the superoxide dismutase (SOD) and peroxidase (POD) activities, but reduce the malondialdehyde (MDA) and soluble protein contents in the two rice cultivars. Subsequently, Cd starts to inhibit photosynthesis and SOD activity until the ripening stage, causing the lowest photosynthetic rate and SOD activity at this stage. In contrast, Fe alleviates the Cd-induced changes at earlier or later growth stage. Notably at the later growth stage, the results show that the interaction between Fe and Cd increases the SOD and catalase (CAT) activities, while decreasing the lipid peroxidation and promoting photosynthesis. As a result, it ultimately increases the biomass. The results from this study suggest that Fe (as Fe fertilizer) is a promising alternative for agricultural use to enhance the plant development and, simultaneously, to reduce Cd toxicity in extensively polluted soils.

1. Introduction

Iron (Fe) is an important and essential micronutrient for plant growth, acting as co-factors for a wide range of plant reactions (Cairo et al., 2002; Hänsch and Mendel, 2009; Balk and Pilon, 2011). Fe actively participates in vital metabolic processes such as photosynthetic and respiratory electron transport chains in chloroplasts and mitochondria. Therefore, Fe imbalance is known to be dangerous to photosynthesis and respiration (Busi et al., 2006; Vigani et al., 2013), and also can induce oxidative stress in cells by enhancing the generation and accumulation of reactive oxygen species (ROS) (Karlheinz et al., 1995; Busi et al., 2006; Bashir et al., 2010).

Cadmium (Cd) is one of the most toxic elements to all living organisms, even at low concentrations. In plants, Cd can severely influence several physiological and biochemical processes such as

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water uptake, nutrient assimilation, photosynthesis, and respiration (Singh and Tewari, 2003; Daud et al., 2015). It has been found that Cd toxicity can interfere with electron transport chains or block antioxidant enzyme structures in chloroplast and mitochondria (Sheoran et al., 1990; Heyno et al., 2008), which result in accumulation of ROS and lipid peroxidation (Chaoui et al., 1997; Srivastava et al., 2014).

Both Fe and Cd are closely associated together in plants (Rizwan et al., 2016) because they have similar chemical properties and share the same transporters (Ogo et al., 2014). It was reported that Cd could be absorbed and transported by the transporters which belong to essential metals. These transporters show broad substrate specificity towards divalent metals including Fe^{2+}, Zn^{2+}, Mn^{2+}, and Cd^{2+} (Eide et al., 1996; Cohen et al., 1998; Vert et al., 2002). The existence of Cd in plants induces an Fe deficiency symptom where Cd inhibits not only Fe absorption (Sharma et al., 2004), but also Fe transportation from underground parts to aboveground parts (Yoshihara et al., 2006; Solti et al., 2011; Xu et al., 2015). When Fe is deficient, plants can absorb more Cd through the Fe uptake systems which are activated due to Fe deficiency (Cohen et al., 1998; Thomine et al., 2000; Lombi et al., 2002). It was observed that the overexpression of Fe transporter (NtPIC1) could increase the Fe concentration in the shoot and, as a result, reduce Cd uptake by tobacco (Gong et al., 2015). An exogenous Fe supplement can decrease Cd concentration in rice and, hence, alleviate Cd toxicity. Therefore, this is greatly favorable for rice growth and yield (Liu et al., 2007, 2008; Shao et al., 2007; Sebastian and Prasad, 2015a).

Many researchers have reported the interaction between Fe and Cd in rice, especially in photosynthesis and in the antioxidative system (Shao et al., 2007; Ogo et al., 2014; Khavari-Nejad et al., 2014; Sebastian and Prasad, 2015a, 2015b). These physiological processes are the major target sites for both Fe and Cd to play roles in plants. Despite the progress in understanding Fe and Cd homeostasis, little insight has been gained concerning the effects of Fe nutritional status on Cd metabolism in rice. The present study is designed to explore photosynthesis and antioxidative enzyme changes in the leaves of two rice cultivars treated with different amount of Fe and Cd. Results are expected to provide theoretical support for the use of Fe fertilizer in agricultural land to control Cd accumulation in rice grains.

2. Materials and methods

2.1. Soil culture and experiment setup

Soil used in this experiment was collected from a rice field in Liaoning Province, Northeast China, and is classified as a Meadow soil. Root debris and stones were removed and the soil was air-dried and sieved (3 mm). The soil has the following properties: pH (10:1 distilled water:soil) 6.7, organic matter ($K_2Cr_2O_7$–H_2SO_4) 20.8 g kg^{-1}, total N (semi-quantitative titration) 1.98 g kg^{-1}, Olsen-P (0.5 M $NaHCO_3$) 19.9 mg kg^{-1}, available K (1.0 M NH_4OAc) 46 mg kg^{-1}, DTPA-Fe (2:1 extract solution:soil) 72.3 mg kg^{-1}, and DTPA-Cd 0.135 mg kg^{-1}. The soil was supplied with 0.2 g N kg^{-1} as $(NH_4)_2SO_4$, 0.15 g $P_2O_5 \cdot kg^{-1}$ as KH_2PO_4 and 0.20 g $K_2O \cdot kg^{-1}$ as K_2SO_4 as basal fertilizers. Subsequently, the soil was amended with Fe and Cd. Fe was supplied at a rate of 0 and 1.0 g kg^{-1} (as $FeSO_4 \cdot 7H_2O$) and Cd at a rate of 0 and 2.0 mg kg^{-1} (as $3CdSO_4 \cdot 8H_2O$). All chemical reagents were dissolved in deionized water and then mixed thoroughly with the soil. Fe and Cd were then added to the soil to form a total of four treatments, which are Fe0Cd0, Fe1Cd0, Fe0Cd2, and Fe1Cd2, respectively. The round plastic pots with a mean diameter of 10 cm and a height of 20 cm were used in the experiment. Each pot was filled with 600 g soil. The soil was then moistened with deionized water and left to reach equilibrium for two weeks. There were three replicates for each treatment.

2.2. Plant materials and growth conditions

Two varieties of rice, Shendao 6 and Shennong 265, were used in the experiment that was conducted in a greenhouse at College of Land and Environment, Shenyang Agricultural University, Shenyang. The average day/night regime was 15/9 h with a temperature regime of 30/15 °C. Two pre-cultivated three-leaf rice seedlings were transplanted in each pot that was then flooded with deionized water. The water level was maintained at about 1–2 cm above the soil surface during the entire plant growth period between 26 May and 28 September 2013.

2.3. Determination of the photosynthetic parameters

During the plant growth period in 2013, SPAD (Soil and Plant Analyzer Development) values of the newly mature leaves were determined on June 22 (tillering stage), July 20 (jointing stage), August 9 (blooming stage), and September 3 (ripening stage) using a chlorophyll meter (SPAD 502Plus, Konica Minolta, Inc.). Then, the net photosynthetic rate, intercellular CO_2 concentration, transpiration rate, and stomatal conductance of the same leaves were determined using a portable photosynthesis system (Li-6400XT Open 6.1; Li-Cor Biosciences, Lincoln, NE, USA). Lastly, the leaves, except for those at tillering stage were cut off and sealed in plastic ice-filled bags and were brought back to the lab and kept in −80 °C refrigerator.

2.4. Estimation of MDA, soluble protein, and antioxidant enzymes

The leaf samples were taken out from the −80 °C refrigerator. An amount of 0.9 g of leaf samples was homogenized in 3.0 ml of 0.05 mmol L^{-1} phosphate buffer (pH 7.8) under chilled conditions. The crude extracts were centrifuged at 12,000 g for 15 min at 4 °C. The supernatants were used to determine the MDA, soluble protein, and various antioxidative enzymes.

MDA was determined as a thiobarbituric acid reactive substance according to the method in Dhindsa et al. (1981). The soluble protein was determined using the bovine serum as a standard following the method described in Bradford (1976). SOD (EC 1.15.1.1) activity was assayed by inhibition of photochemical reduction due to nitro blue tetrazolium (NTB) (McCord and Fridovich, 1969). POD (EC 1.11.1.7) activity was determined by the decrease absorbance at 470 nm because of the formation of guaiacol polymerization (Beffa et al., 1990). CAT (EC 1.11.1.6) activity was measured using the decrease absorbance at 240 nm based on the disappearance of H_2O_2 in the reaction mixture (Aebi, 1984). All these measurements were repeated twice for every leaf sample, and the average value was used for analysis.

2.5. Plant harvest

At harvest, the rice plants were removed from each plastic pot and separated into root, shoot, and grain. The fresh samples were rinsed with deionized water three times and the moisture on the samples was gently removed with blotting paper. Then, these samples were heated at 105 °C for 20 min and dried at 70 °C for three days. When the samples reached a constant weight, they were removed and weighed for their dry weights.

2.6. Statistical analyses

Two-way analysis of variance (ANOVA) was performed on the data using SAS for Windows (version 8.2, SAS Institute Inc., Cary, NC, USA). Data were presented as means ± SD (n = 3), and the means were compared using least significant difference (LSD) at the 5% level.

3. Results

3.1. Dry weights of root, shoot and grain

Generally, the dry weights of shoot, root and grain of Shendao 6 were decreased slightly when Cd was added to the soil (Fe0Cd2) and were increased slightly when Fe was added (Fe1Cd0). When both Fe and Cd were added, the values of biomass were between the previous two treatments and closed to the Fe0Cd0 treatment (Table 1). For the Shennong 265, the dry weights of shoot, root and grain were increased with addition of either Cd or Fe to the soil, most notably when Fe was added. It was found that the shoot and grain dry weights of Shennong 265 after Fe1Cd0 treatment were the highest, while the root dry weights after Fe1Cd1 treatment were the highest. These results suggest that Cd potentially inhibits the growth of Shengdao 6, while Fe alleviates the adverse effects induced by Cd in this rice variety. Fe and the interaction of Fe and Cd were determined to be beneficial for Shennong 265 growth.

3.2. The SPAD values of leaves

The SPAD values of the leaves from the two rice varieties (Shendao 6 and Shennong 265) gradually decreased from the tillering stage to the ripening stage. It was observed that the SPAD values of Shennong 265 were always higher than that of Shendao 6 during the whole growth period (Fig. 1). For the Shendao 6, when Fe was added to the soil, the SPAD values were higher than that without Fe addition. For Shennong 265, the SPAD values were higher when either Fe was added or no Fe and Cd were added to the soil. Cd reduced the SPAD values of the two rice varieties from the jointing stage (Shendao 6) or the blooming stage (Shengnong 265) until the ripening stage. The trend due to the effect of Fe and Cd on SPAD values was more apparent at the later stages of the two rice varieties. These results indicate that Fe plays an important role in increasing the content of chlorophyll because Fe participates in the biological synthesis of chlorophyll in terms of Fe-containing proteins located in plastid subcompartments (Briat et al., 2007). However, Cd inhibits the chlorophyll formation by replacing and interfering with the functions of Fe, Mg and Zn (Muneer et al., 2014).

3.3. Photosynthetic rate, intercellular CO_2 concentration, stomatal conductance, and transpiration efficiency

The photosynthetic rate, intercellular CO_2 concentration, stomatal conductance, and transpiration efficiency in the leaves of Shendao 6 and Shengnong 265 all increased from the tillering stage to the blooming stage and then decreased until the ripening stage. At the tillering stage, the four indices of Shendao 6 and the photosynthetic rate of Shennong 265 were significantly increased by Cd supplement, but were decreased by adding Cd and Fe together (Fig. 2). This result indicates that Cd stimulates the photosynthesis, but Fe weakens the influence induced by Cd at the early growth stage of the rice. From the jointing stage, the photosynthetic rate of Shendao 6 became the lowest when Cd was added to the soil, whereas, the photosynthetic rate of Shennong 265 was the highest when both Fe and Cd were added to the soil. Until the ripening stage, the photosynthetic rate of Shendao 6 was significantly decreased by the Cd supplement and slightly increased by the Fe supplement. The photosynthetic rate of Shennong 265, however, increased with the addition of Cd and Fe simultaneously, but decreased when Cd was solely supplied. These results suggest that long-term Cd stress can destroy the normal photosynthesis process in rice, but Fe is helpful in restoring the physiological metabolism.

The intercellular CO_2 concentration in both rice varieties presented a reverse trend when compared to the photosynthetic rate at the ripening stage (Fig. 2). The stomatal conductance and transpiration efficiency at this stage showed no obvious difference between the treatments. Generally, the metabolism reactions in plants slow down at the later growth period. Therefore, water and gas exchanges become decreased. Due to this occurrence, the stomatal conductance and transpiration efficiency keep a similar level between the treatments while the intercellular CO_2 concentration decreases due to higher CO_2 consumption at the treatments with a higher photosynthetic rate.

3.4. Contents of MDA and soluble protein in leaves

At the jointing stage, the MDA content in the leaves of Shendao 6 and Shennong 265 increased with the addition of Fe to the soil, but declined with the addition of Cd to the soil. When both Fe and Cd were added to the soil together, the MDA content had a median value as shown in Fig. 3. At the ripening stage, the MDA content in Shendao 6 was highest when no Fe and Cd were added to the soil, but was lowest when Fe was added to the soil. For Shennong 265, the MDA content showed highest when Fe was added to the soil, and was lowest when both Fe and Cd were added to the soil. Between the joining and the ripening stages, the MDA content of the two varieties had no significant difference except that the MDA content by Fe1Cd2 treatment was higher than that by Fe0Cd0 treatment. From these results, we can conclude that the formation of MDA can be inhibited by Cd, but stimulated by Fe when rice suffers from a short time exposure of Cd and Fe. After a long time exposure, MDA in Shendao 6 can have a reverse effect by Fe treatment, while MDA in Shennong 265 will remain unchanged.

At the jointing stage, the soluble protein content in the leaves of Shendao 6 and Shennong 265 decreased significantly with the addition of Cd to the soil, followed by the addition of Fe and Cd

Table 1
Mean dry weights of shoot, root, and grain of two rice varieties.

Treatments		Shendao 6			Shengnong 265		
		Dry weights of root	Dry weights of shoot	Dry weights of grain	Dry weights of root (g)	Dry weights of shoot	Dry weights of grain
Cd0	Fe0	7.12 ± 0.25a	35.8 ± 3.35a	17.7 ± 3.96a	5.59 ± 1.06b	18.9 ± 2.86c	9.87 ± 2.01b
	Fe1	7.78 ± 0.88a	36.1 ± 6.66a	17.2 ± 4.67a	6.81 ± 0.33ab	36.8 ± 1.95a	18.6 ± 2.25a
Cd2	Fe0	6.04 ± 0.36a	26.5 ± 2.95a	10.9 ± 2.12a	5.95 ± 0.41b	28.6 ± 1.52b	15.4 ± 1.39a
	Fe1	6.65 ± 1.31a	34.8 ± 1.71a	15.0 ± 2.20a	7.37 ± 0.49a	33.0 ± 1.40a	16.4 ± 0.31a

Notes: The plants of the two rice varieties (Shendao 6 and Shennong 265) were exposed to 0 or 2 mg kg^{-1} Cd (Cd0 and Cd2) in the presence (1 g kg^{-1} Fe1) or absence (0 g kg^{-1} Fe0) of Fe in soil. The mean dry weights were gained at harvest. The data were subjected to two-way analysis of variance. Data presented are means ± SD (n = 3), different lower case letters represent significantly different by least significant difference at the 5% level.

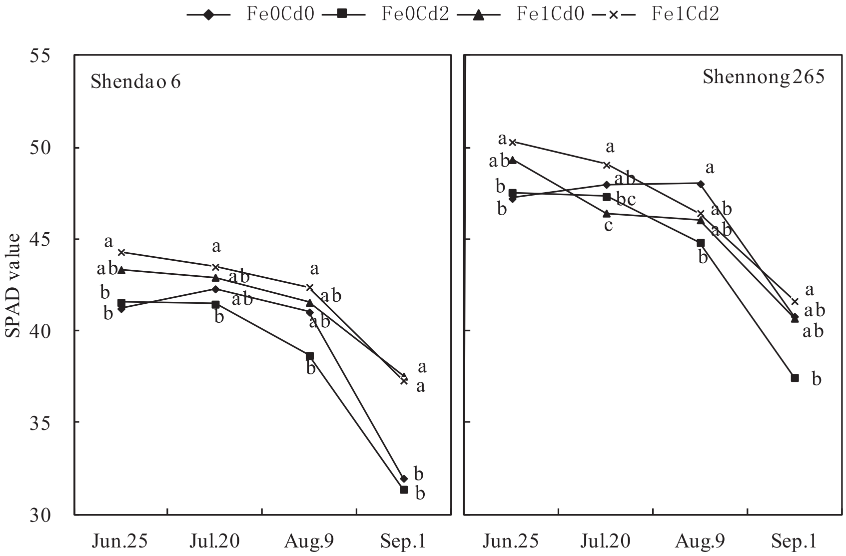

Fig. 1. SPAD values in the leaves of two rice varieties grown in soil amended with different levels of Fe (0, 1 g kg^{-1}) and Cd (0, 2 mg kg^{-1}). The treatments were Fe0Cd0, Fe0Cd2, Fe1Cd0, and Fe1Cd2. The SPAD values were measured on June 25, July 20, August 9, and September 1, 2013, respectively. Different lowercase letters denote significant differences between treatments on the same measurement date at the 5% level.

together, and then by the addition of Fe. The Fe0Cd0 treatment had the highest protein content. At the ripening stage, the soluble protein content was the highest when both Fe and Cd were added to the soil (Fig. 3). This indicates that Fe and/or Cd can decrease the soluble protein in rice at the beginning of the supplement, but Fe and Cd can interact to increase the protein content after a long time period of addition to the soil.

3.5. *Activities of SOD, POD and CAT in leaves*

At the jointing stage, the SOD activity in the leaves of Shendao 6 and Shennong 265 were significantly increased when Fe and Cd were added separately or in combination to the soil. At the ripening stage, the SOD activities of two varieties were the highest when Fe and Cd were added to the soil (Fig. 4). This indicates that Fe and/or Cd can increase the SOD activity in rice shortly with supplement. However, after a long time period of addition, it was only seen that the SOD activity was increased when Fe and Cd acted jointly together.

At the jointing stage, the POD activity in the leaves of Shendao 6 and Shennong 265 was significantly higher when Cd was added to the soil. However, at the ripening stage the POD activities of the two varieties were lowest when Fe and Cd were added to the soil (Fig. 4). It is concluded that the short-term Cd pollution can increase the POD activity, while Fe and Cd together can decrease the POD activity for a long time after addition.

In most cases, the CAT activity in the two rice varieties did not show significant differences between the treatments. It is notable that the CAT activity by the Fe1Cd1 treatment was highest for Shendao 6 during the whole growing period and for Shennong 265 at the ripening stage (Fig. 4). This pattern suggests that the interaction between Fe and Cd tends to increase CAT activity.

4. Discussions

One of the significant negative impacts caused by Cd toxicity is the retardation of plant development and eventually the decrease of biomass (Guo et al., 2009; Sebastian and Prasad, 2015a; Daud et al., 2015). In this study, the dry weights of root, shoot and grain of Shendao 6 were found decrease with the addition of Cd to the soil, but slightly increase with the addition of Fe. However, for Shennong 265, the dry weights of root, shoot and grain increased with the addition of Cd, Fe and Cd + Fe to the soil. The changing pattern of biomass for the two rice varieties is most likely attributed to the photosynthetic difference in their leaves. The photosynthetic rate of Shendao 6 treated with Cd was very slow from the jointing stage to the ripening stage even though it showed a fast rate for a short time period at the beginning when Cd was added to the soil. However, the photosynthetic rate of Shennong 265 fed with Cd, Fe and Cd + Fe were all higher than that without the addition of Cd and Fe. Moreover, this pattern was observed for a relatively long time period from nearly the tillering stage to the blooming stage. The present results support the statement that photosynthesis accounts for biomass productivity in plants (Beadle and Long, 1985). This is because the biomass production depends on photosynthetic assimilating accumulation, which provides the carbon skeleton for plant growth (Imsande and Touraine, 1994).

It is seen that the photosynthesis was significantly influenced by Cd and Fe at different growth stages of the two rice varieties. At the earlier stage, Cd significantly stimulated photosynthesis by increasing the photosynthetic rate, intercellular CO_2 concentration, stomatal conductance as well as transpiration efficiency. Subsequently, Cd reversely inhibited the photosynthesis. This stimulatory effect is in agreement with the results from other studies (Singh and Tewari, 2003; Hassan et al., 2005). For example, Hassan et al. (2005) reported that Cd slightly increased rice height at the tillering stage when Cd was fed to rice at a low concentration in solution (0.1 mol L^{-1}). At the booting stage, plant height was reduced by the same Cd treatment. In addition, they also found that the rice height was notably inhibited when 1.0 or 5.0 mol L^{-1} Cd was present in the solution, irrespective of growth stages (Hassan et al., 2005). Therefore, it was found that extended and intensive Cd pollution was very harmful to plant photosynthesis and biomass formation (Sheoran et al., 1990; Siedlecka and Krupa, 1996a, 1996b; Sandalio et al., 2001). One of the crucial reasons for negative effect of Cd is that it can inhibit chlorophyll production as shown in Fig. 1. This could also be explained as Cd distorting the chloroplasts, reducing the size and number of grana stacks (Hakmaoui et al., 2007; Qadir et al., 2004) or inhibiting the activities of PSI and PSII (Faller et al., 2005; Timperio et al., 2007). Differently from Cd, Fe

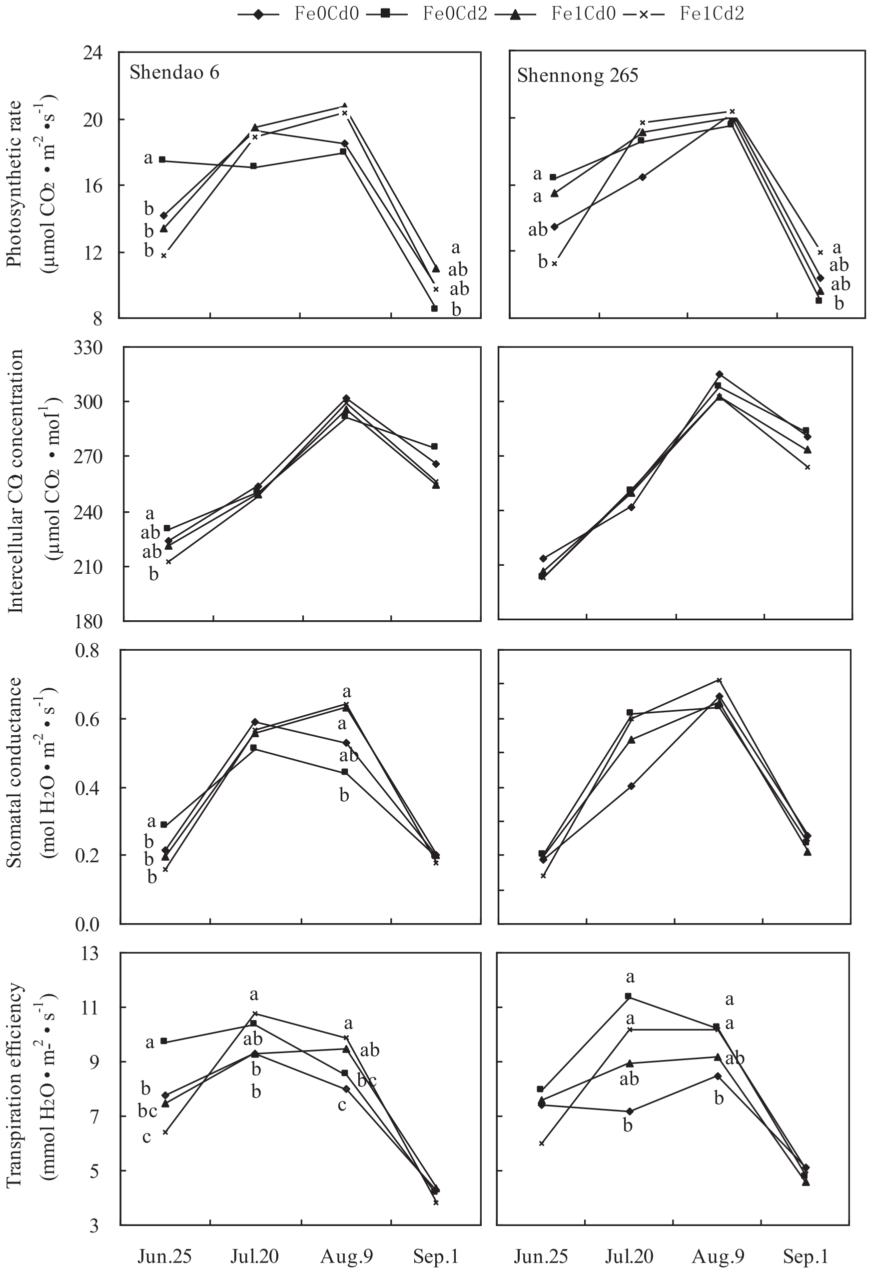

Fig. 2. Photosynthetic rate, intercellular CO_2 concentration, stomatal conductance, and transpiration efficiency in the leaves of two rice varieties grown in soil amended with different levels of Fe (0, 1 g kg^{-1}) and Cd (0, 2 mg kg^{-1}). The treatments were Fe0Cd0, Fe0Cd2, Fe1Cd0, and Fe1Cd2. The SPAD values were measured on June 25, July 20, August 9, and September 1, 2013, respectively. Different lowercase letters denote significant differences between treatments on same measurement date at the 5% level.

slightly promoted photosynthesis of Shendao 6 and Shennong 265 after the jointing stage and before the blooming stage, respectively. This is because Fe is necessary for the synthesis of pigments, chloroplast structure, photosynthetic enzyme activity, and photosynthetic electron transport chains (Vigani et al., 2013).

The influence of Cd and Fe on MDA, soluble protein, and antioxidative enzymes in leaves also depended on both cultivars and growth stages. Cd decreased the MDA and soluble protein contents and increased the SOD and POD activities at the earlier stage. This indicates low ROS productivity and lipid peroxidation with transient Cd exposure. This result is consistent with the Cd-induced improvement of photosynthesis at beginning of Cd pollution as shown above. However, after a long-term presence in the soil, Cd did not decrease the MDA and soluble protein content anymore and slightly decreased the SOD activity. Generally, Cd toxicity is proved to induce the formation of various ROS, increase the MDA content as a result of lipid peroxidation (Chaoui et al., 1997; Hassan et al., 2005; Srivastava et al., 2014), decrease the soluble protein content (Chaoui et al., 1997; Singh and Tewari, 2003; Daud et al., 2015), and increase (Singh and Tewari, 2003; Srivastava et al., 2014) or decrease (Hassan et al., 2005; Uraguchi et al., 2006) the SOD, POD, or CAT activities depending on stress intensity and duration, plant species and ages (Piquery et al., 2000). However, in this study, although Cd inhibited the photosynthesis after a long-term existence in the soil, Cd did not significantly change the MDA and soluble protein contents, and POD and CAT activities. Unlike Cd, Fe increased the MDA content and SOD activity at the earlier stages of the two rice varieties. At the later stage, the same effect on Shendao

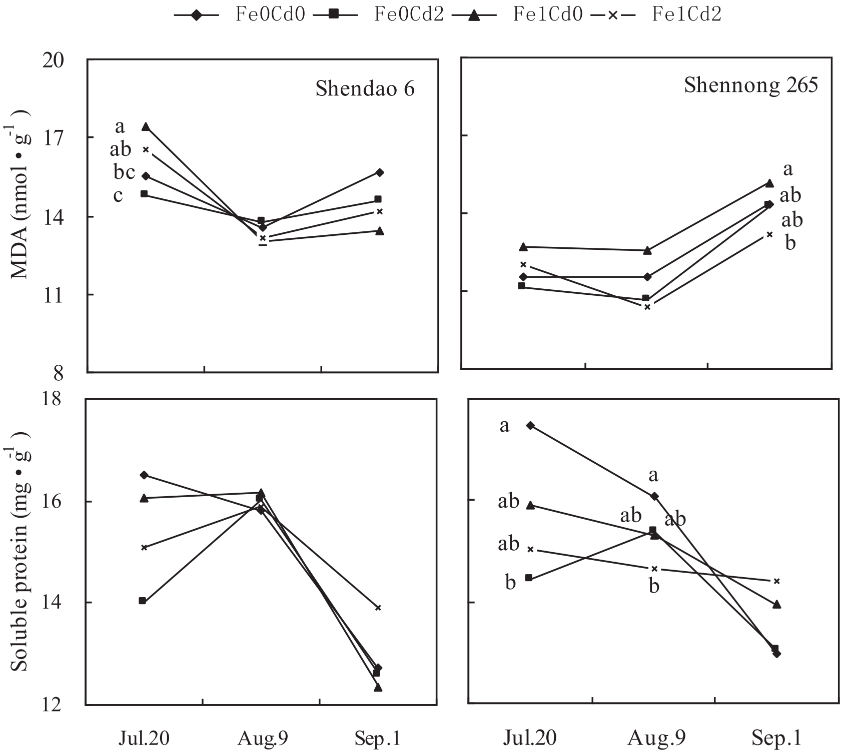

Fig. 3. Contents of MDA and soluble protein in the leaves of two rice varieties grown in soil amended with different levels of Fe (0, 1 g kg^{-1}) and Cd (0, 2 mg kg^{-1}). The treatments were Fe0Cd0, Fe0Cd2, Fe1Cd0, and Fe1Cd2. The contents of MDA and soluble protein were measured on July 20, August 9, and September 1, 2013, respectively. Different lowercase letters denote significant differences between treatments on same measurement date at the 5% level.

6 did not show obviously. However, the effect on MDA is seen in Shennong 265. The data presented here indicates that Fe can motivate the lipid peroxidation in rice in a short or long time period, even though it can promote the photosynthesis and increase the biomass.

There are obviously different responses to the Fe and(or) Cd treatments between the two rice varieties. The growth of Shennong 265 was promoted by Fe and was not inhibited by Cd, but the growth of Shendao 6 got the inhibition from Cd and slightly promotion from Fe. The positive effect of Fe on the photosynthetic rate in Shennong 265 remained continuous during the whole growth period. Furthermore, Fe can alleviate the Cd-induced adverse influence on the photosynthetic rate at the later stages. However, for Shendao6, the stimulating effect of Fe on the photosynthetic rate began later than for Shennong 265. Although Fe can alleviate the Cd toxicity in Shendao 6 similar to that in Shennong 265, the extent is less. These results show that Shennong 265 is more tolerant to Cd than Shendao 6, which may be because Shennong 265 has more active vitality as illustrated by the higher SPAD values and the lower MDA contents in its leaves. In order to keep the active vitality, Shennong 265 needs more amount of Fe to uptake. Thus, the promoting effect of Fe is more prominent in Shennong 265. Differently from Shennong 265, Shendao 6 is more vulnerable to Cd. Therefore, it has relatively low SPAD value, photosynthetic rate and SOD activity when Cd was individually added to the soil.

Iron has a profound influence on alleviating the Cd-induced changes in rice. As observed in this study, Fe prominently alleviated the Cd-induced decrease of biomass and SPAD in Shendao 6. Fe also weakened the Cd-induced enhancement of photosynthesis and decline of MDA and soluble protein contents at the earlier stage, as well as restored the Cd-induced decline of photosynthesis and SOD activity at later stages of the two rice varieties. These results are in agreement with that from other researchers (Siedlecka and Krupa, 1996b; Sharma et al., 2004; Sárvári et al., 2011; Khavari-Nejad et al., 2014; Sebastian and Prasad, 2015a). For example, Sebastian and Prasad (2015a) reported that the photosynthetic electron transport chain was severely affected by Cd, which resulted in reduced rice production under Cd stress. However, exogenous Fe restored the photosynthetic electron transport. In addition, ascorbic acid (AsA), reduced glutathione (GSH), SOD, CAT, and POD also decreased with Cd treatment. A supplement of Fe prevented the loss of activities of these reduction–oxidation reaction regulators and enzymes. The antagonism of Fe to Cd could be partly attributed to the reduction of Cd intake and availability by Fe competition for membrane transporters and Fe–S clusters in plants (Bashir et al., 2015). More notably, Fe interacted with Cd to upregulate the SOD and CAT activities, increase soluble protein content, and decrease the MAD content, thus promoting photosynthesis especially at the later growth stage and eventually increasing the biomass of the two varieties. SOD and CAT were emphasized as important enzymes to scavenging ROS. O_2^- is converted by SOD to hydrogen peroxide which can then be removed by CAT (Dhindsa et al., 1981). Both SOD and CAT worked together to decrease the ROS in rice in the experiment, which provides a highly favorable condition for the photosynthesis process. In the process, Fe plays an extremely important role. On the one hand, Fe is indispensable for the formation of some antioxidative enzymes such as Fe-SOD (Hänsch and Mendel, 2009). On the other hand, Fe is necessary to keep these enzymes active. Therefore, Fe can protect rice from heavily oxidative stress induced by Cd in a relatively long growth period, which further enables higher photosynthesis and finally greater biomass. Based on the results, the use of Fe fertilizer in agriculture is suggested as a promising way to improve rice growth and raise the yield in case of inevitable and extensive Cd pollution in paddy fields.

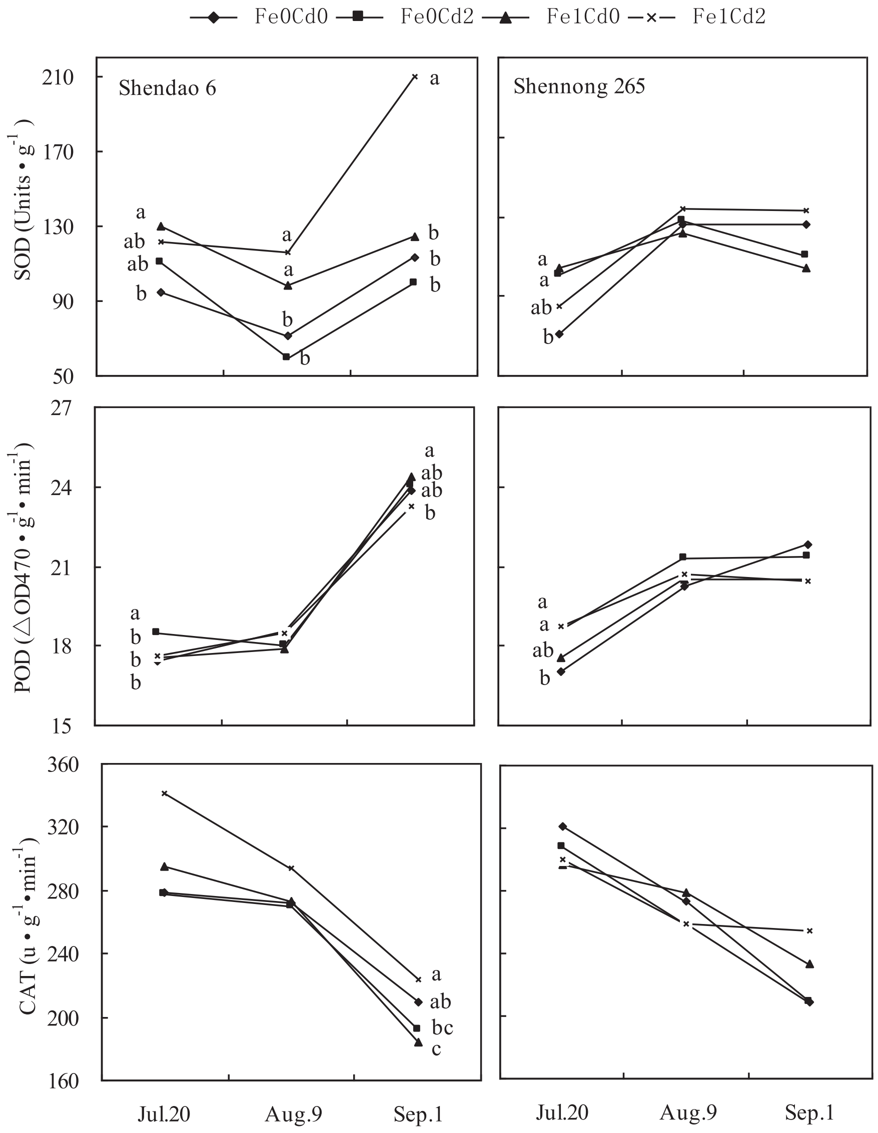

Fig. 4. Activities of SOD, POD, and CAT in the leaves of two rice varieties grown in soil amended with different levels of Fe (0, 1 g kg^{-1}) and Cd (0, 2 mg kg^{-1}). The treatments were Fe0Cd0, Fe0Cd2, Fe1Cd0, and Fe1Cd2. The activities of SOD, POD, and CAT were measured on July 20, August 9, and September 1, 2013, respectively. Different lowercase letters denote significant differences between treatments on same measurement date at the 5% level.

5. Conclusions

This study shows that Cd has a crucial negative influence on rice development and biomass production by inhibiting the chlorophyll synthesis and photosynthetic metabolism even though Cd presents a transient stimulating effect. On the contrary, Fe alleviates the Cd-induced changes in rice. Fe and Cd interact to up-regulate the SOD and CAT activities and increase soluble protein content, thus promoting photosynthesis and eventually raising the biomass of both rice varieties. The results suggest that Fe fertilizer can be used in agriculture to resist Cd toxicity in plants.

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