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Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd stress

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ABSTRACT

Cadmium contamination in soil has become a serious issue in sustainable agriculture production and food safety. A pot experiment was conducted to study the influence of four N fertilizer forms on grain yield, Cd concentration in plant tissues and oxidative stress under two Cd levels (0 and 100 mg Cd kg^{-1} soil). The results showed that both N form and Cd stress affected grain yield, with urea-N and NH_4^+-N treatments having significantly higher grain yields, and Cd addition reducing yield. NO_3^--N and NH_4^+-N treated plants had the highest and lowest Cd concentration in plant tissues, respectively. Urea-N and NH_4^+-N treatments had significantly higher N accumulation in plant tissues than other two N treatments. Cd addition caused a significant increase in leaf superoxide dismutase (SOD) and peroxidase (POD) activities for all N treatments, except for NO_3^--N treatment, with urea-N and NH_4^+-N treated plants having more increase than organic-N treated ones. The results indicated that growth inhibition, yield reduction and Cd uptake of rice plants in response to Cd addition varied with the N fertilizer form.

1. Introduction

Rice is a staple food crop in the world, second only to wheat in the planting area [1]. In China rice is the crop with the largest planting area. Currently, in many regions, paddy fields have been to different extent contaminated by cadmium (Cd), causing a health hazard to people. Clear evidence has linked human renal tubular dysfunction with soil Cd contamination in rice farm families in Asia [2]. In Japan, rice is a leading source of Cd burden for human [3]. Cd toxicity may cause essential nutrient deficiency and changes in the concentration of basic nutrients such as N and P in plant tissues [4], therefore better understanding of the mechanism of heavy metal toxicity in terms of nutrient supply is needed. It has been revealed that Cd is strongly phytotoxic, and causes growth inhibition and even plant death due to its interaction with photosynthesis, respiration and nitrogen assimilation in plants [5].

Cd influences N metabolism in the plant directly or indirectly [6]. It has been argued that proper N application may alleviate toxic effect of Cd in real soil condition, by increasing the amounts of stromal proteins, photosynthetic capacity of leaves and the plant growth [7]. Nitrate (NO_3^-) and ammonium (NH_4^+) ions are the two major forms of nitrogen taken up by the plants, while nitrate taken up from the medium should be reduced to ammonium before its assimilation into the organic nitrogen compounds. It has long been observed that ammonium and nitrate differ in their effects on the growth and chemical composition of plants [8–10]. Moreover, NO_3^- and NH_4^+ induce a net release of OH^- and H^+ ions, respectively [11,12]. Hence they will change the rhizosphere pH in different way and pose the distinct influence on Cd availability in soil.

Meanwhile, Cd toxicity also causes oxidative stress, changing the activities of various antioxidant enzymes [5,13]. It is found that Cd toxicity enhances lipid peroxidation in plant cells, reflected by increased melondialdehyde (MDA) content [14]. One of the protective mechanisms is the enzymatic antioxidant system, which involves the sequential and simultaneous action of a number of enzymes including superoxide dismutase (SOD), peroxidase (POD). In fact, activities of antioxidant enzymes are inducible by oxidative stress due to exposure to abiotic or biotic stresses [15,16], and therefore, represent a general plant response to adverse conditions. However, the direction and size of the response varies with plant species and tissues analyzed, and the kind and intensity of stress treatment [17]. It could be hypothesized that the difference in stress tolerance among plant species and genotypes within a species is intrinsically associated with the development of the enzymatic antioxidant system and the type of N nutrition supplied under the stress conditions.

There is little information about the response of growth and Cd uptake in rice plants to Cd toxicity under different N sources. To our knowledge, this experiment is the first of its kind studies to be conducted under soil condition and to be done until the plants fully mature to produce grains. Here we report the influence of

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different forms of N fertilizer on antioxidant enzyme activity and Cd concentration in the Cd-stressed rice plants.

2. Materials and methods

2.1. Experimental design

The experiment was conducted in the experimental farm of Huajiachi Campus, Zhejiang University (Hangzhou, China; 31°16′N, 120°12′E) during the late rice-growing season (June–October) in 2006. The soil was sandy loam with pH 6.8. The results of soil fertility analysis before sowing were as follows: organic matter content, 26.2 g kg^{-1}; available N, P and K contents, 152.1, 36.4 and 46.5 mg kg^{-1}, respectively. The soil used for the experiment was dried under natural condition and grinded and then divided into two parts. Into one of them, Cd was added in the form of $CdCl_2$ at a rate of 100 mg kg^{-1}, and thoroughly mixed. The other part of the soil had no Cd addition, used as the control. Before sowing, the seeds were surface sterilized with 0.1% H_2O_2 for 20 min, rinsed thoroughly with deionized water, and soaked overnight in sterile water at room temperature, and then germinated in sterilized moist quartz sand in a greenhouse. After 9 days when the seedlings were at 2-leaves age, the plants were selected uniformly and transplanted onto plastic pots containing 10 kg of soil and in each pot, four seedlings were planted. The soil was soaked for 1 week prior to rice seedling transplanting. Diethylene triamine penta-acetic acid (DTPA, 0.005 M)-extractable Cd content in the soils of the control and Cd treatment was 0.12 and 0.86 mg kg^{-1}, respectively. A Cd-sensitive rice cultivar Xiushui 63, identified in a previous experiment [18] was used. There were four N fertilizer forms at a rate of 180 kg ha^{-1} N, i.e. $CO\ (NH_2)_2$ (urea), $Ca\ (NO_3)_2$ (calcium nitrate), $(NH_4)_2SO_4$ (ammonium sulphate) and organic fertilizer, which is organic liquid fish concentrate containing N of 2.2–2.4% (w/v), and P of 7.3 g L^{-1}. Fertilizers were applied with four splits, 40, 20, 20, and 20% at before transplanting, tillering, stem elongation, and booting stages, respectively. The experiment was arranged with completely randomized block design with six replications. Each block (replication) consisted of eight treatments (combination of 2 Cd levels and 4 N forms), and each treatment had three pots. Thus totally the experiment contained 144 pots.

2.2. Sampling and measurements

The second fully expanded leaves of the plants were sampled for enzymatic analysis at heading and milking stages. Samples (leaves) were homogenized in 0.05 M phosphate buffer (pH 7.8) by grinding with a mortar and pestle under chilled condition with liquid nitrogen. The homogenate was filtered through four layers of muslin cloth and centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatants were used for enzyme assays. SOD, POD activities and MDA concentration were determined according to Zhang [19].

At maturity, the plants were separated into leaf, stem and panicle and then dried at 80 °C for 24 h and weighted. The separated plant tissues were ground into powder, digested with HNO_3 and Cd concentration was measured by atomic absorption spectroscopy (Shimadzu, AA 6300, Japan).

2.3. Statistical analysis

Data are the average of at least three independent replicates. ANOVA was conducted for all data and LSD used for testing the difference between Cd levels or N forms by using the statistical package SAS 8.0 for Windows.

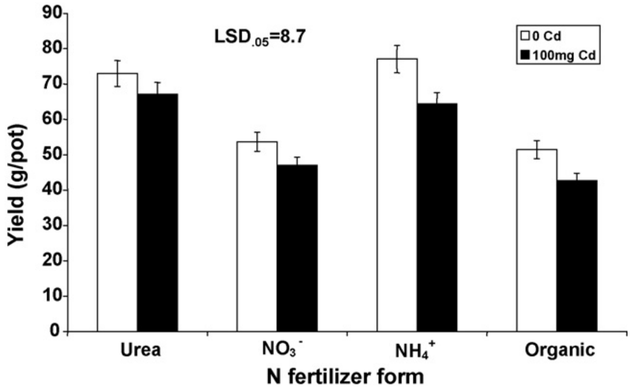

Fig. 1. Effects of different N forms on rice yield under two Cd levels. Vertical bars represents standard errors ($n = 3$).

3. Results

3.1. Grain yield

There was a significant difference in grain yield among the four N forms (Fig. 1). Without Cd addition (control), urea- and NH_4^+-N treatments had significantly higher grain yields than the other two treatments. Cd addition caused a significant reduction of grain yield relative to the control. Moreover, the reduced extent varied with N form. Thus, there was no significant difference for urea and NO_3^- treatments between the two Cd levels, while the difference was significant for the other two N forms.

3.2. Cadmium concentration and nitrogen accumulation

When the plants grew in the soil without Cd addition, Cd was not detected in the NH_4^+- and organic-N treated plants, while urea-treated plants had the highest Cd concentration (Table 1). Cd addition into the soil significantly increased Cd concentrations in plant tissues. However, the extent of the increase varied with N form. NO_3^--N treated plants had significantly higher Cd concentrations in all plant tissues than those treated with other N forms. In addition, the difference in Cd concentration among N forms was tissue-dependent. For leaf, no significant difference was found among N treatments. While for stem, the difference was significant. Moreover, a significant interaction between Cd and N form could be found for leaf and panicle Cd concentrations, but not for stem Cd concentration.

There was a significant difference between the two Cd treatments in leaf and panicle N accumulation, but not in stem N accumulation. In general, Cd addition reduced tissue N accumulation. There was a significant difference in tissue N accumulation among the four N forms, with urea- and NH_4^+-N treated plants having more N accumulation.

3.3. Activity of superoxide dismutase

At heading stage, there was no significant difference among four N fertilizers in leaf SOD activity for the plants grew under normal condition (Fig. 2). While for the plants exposed to Cd stress, the significant difference among four N fertilizers in leaf SOD activity could be found, with NH_4^+-N and NO_3^--N treated plants having the highest and lowest SOD activity, respectively. In addition, except for NO_3^--N treatment, other three N treatments showed the higher SOD activity in Cd-stressed plants relative to the control. At milking stage, SOD activity in each treatment was obviously lower than that at heading stage. There was a significant

Table 1
The effect of N forms on Cd concentration and N accumulation

Cd levels (mg/kg)	N form	Cd concentration ($\mu g\,g^{-1}$)			N accumulation (mg/plant)		
		Leaf	Stem	Panicle	Leaf	Stem	Panicle
100	Urea	0.56 b[a]	4.61 c	1.98 b	18.5 ab	32.8 ab	74.6 b
	NO_3^-	1.65 a	7.64 a	5.19 a	7.0 c	18.9 e	40.9 d
	NH_4^+	0.26 b	2.56 d	0.62 d	17.3 ab	36.2 ab	74.2 b
	Organic	0.44 b	6.26 b	1.68 bc	6.7 c	17.5 e	42.5 d
0	Urea	0.15 b	0.09 e	0.35 de	20.0 a	29.4 c	80.8 ab
	NO_3^-	0.09 b	0.06 e	0.11 ef	10.5 bc	22.7 d	55.8 bc
	NH_4^+	0.00 b	0.00 e	0.00 f	21.5 a	43.3 a	86.6 a
	Organic	0.00 b	0.07 e	0.00 f	8.5 c	17.2 e	54.7 bc
Difference between Cd levels		**[b]	**	**	*	NS	*
Interaction		**	NS	**	NS	NS	NS

[a] The same letter after data within a column represents no significant difference at 95% probability.

[b] NS, * and ** represent no significant, significant at 95% probability and highly significant at 99% probability.

difference among the four N fertilizers in SOD activity for both control and Cd-stressed plants. However, for the control, urea- and NO_3^--N treatments had higher values than the other two treatments. While for the Cd-stressed plants, urea-treated plants had the highest SOD activity.

3.4. Peroxidase activity

At heading stage, there was a significant difference among four N forms in POD activity for the control plants, with NH_4^+- and organic-N treatments having the highest and lowest activities, respectively (Fig. 3). Cd addition significantly increased POD activity for all N treatments, except for NO_3^--N treatment. At milking stage, a significant difference could be found among the four N forms in POD activity for both the control and Cd-stressed plants. For the control, urea- and organic-N treatments had the highest and lowest activities, respectively. However, for the Cd-stressed plants, NH_4^+-N treatment had the highest value, although organic-N treatment remained the lowest. In addition, Cd addition significantly increased POD activity in comparison with the control.

3.5. MDA content

MDA content may reflect the extent of oxidative stress. At heading stage, there was a significant difference among the four N forms in leaf MDA content, irrespectively of Cd treatment. For the control, NH_4^+-N treatment had the highest activity, being significantly higher than the other three N treatments (Fig. 4). Cd addition increased MDA content of all N treatment, except for NO_3^--N treatment. At milking stage, no significant difference was found in leaf MDA content among the four N treatments for the control plants. However, for the Cd-stressed plants, there was a significant difference in leaf MDA content between urea- and NH_4^+-N treatments, although there was no significant difference between NO_3^- and organic-N treatments. In addition, Cd stress caused a significant

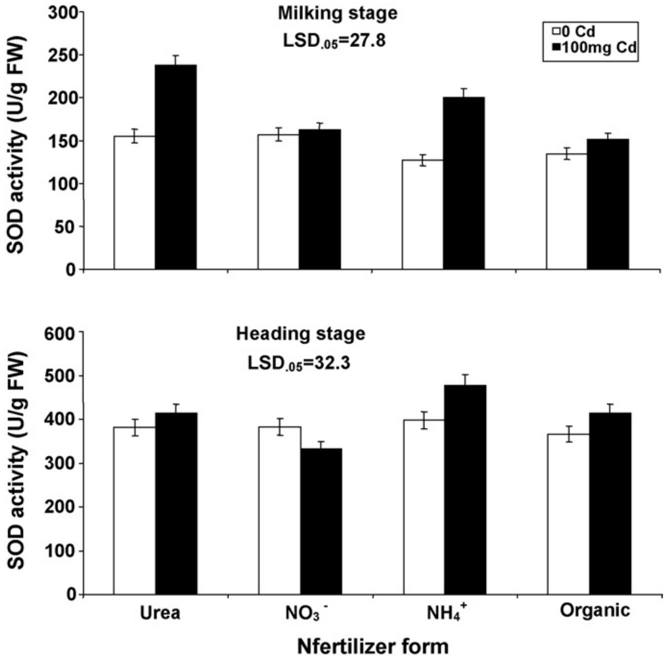

Fig. 2. Effects of different N forms on SOD activity in rice leaves at heading and milking stages. Vertical bars represents standard errors ($n = 3$).

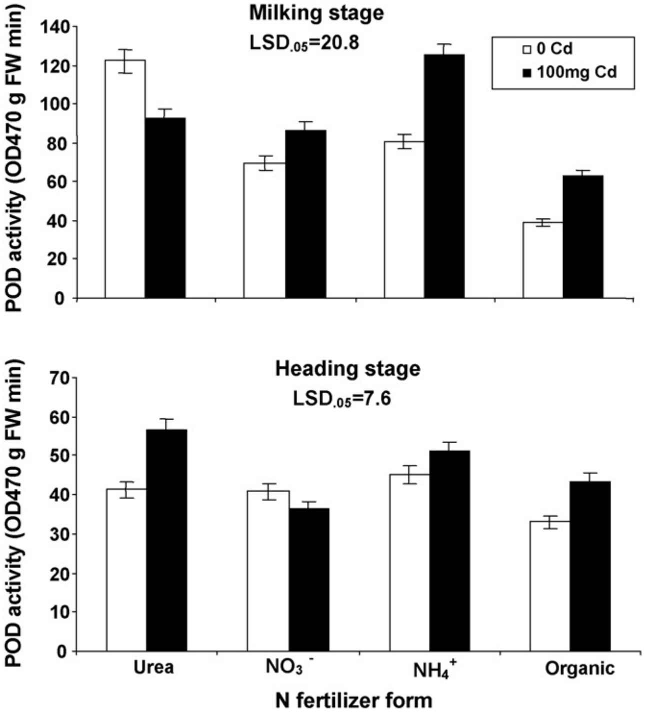

Fig. 3. Effects of different N forms on POD activity in rice leaves at heading and milking stages. Vertical bars represents standard errors ($n = 3$).

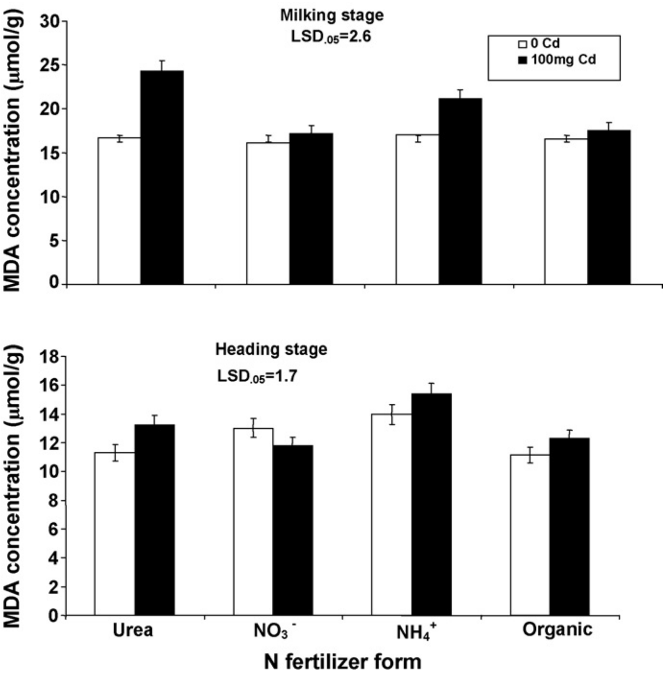

Fig. 4. Effects of different N forms on MDA content in rice leaves at heading and milking stages. Vertical bars represents standard errors (*n* = 3).

increase in leaf MDA content for the urea- and NH_4^+-N treated plants relative to the control.

4. Discussion

Nitrogen source (N form) had a significant effect on Cd and N concentration and accumulation in both roots and shoots. In the previous reports, the relationship between Cd and N accumulation in the plants was contradictory. According to Mitchell et al. [20], N and Cd accumulation was positively associated, while Bhandal and Kuar [21] found the opposite results. In the current study, it can be observed that the NO_3^--N treated plants had higher Cd concentration and less N accumulation than the NH_4^+-N treated ones, suggesting an antagonistic effect between NH_4^+-N and Cd, and a synergistic effect between NO_3^--N and Cd. This result confirmed the findings of Hassan et al. [22]. In addition, grain yield differed among the four N fertilizers, with NH_4^+-N fed plants producing the highest yield, followed by urea-N fed plants. Under Cd stressed condition, however, NH_4^+-N fed rice was mostly affected, with a 10% decrease in yield, followed by organic fertilizer (9.4% reduction). The result was inconsistent with previous findings [23–27].

Antioxidant enzymes and certain metabolites play an important role in adaptation and ultimate survival of plants under stress conditions. In fact, activities of anti-oxidative enzymes are inducible by oxidative stress [15,16], which reflects a general strategy required to overcome stress. One of the protective mechanisms is the enzymatic antioxidant system, which involves the sequential and simultaneous action of a number of enzymes. Superoxide dismutase and peroxidase are most commonly used to demonstrate the status of antioxidant capacity [28]. The results of this study indicate that SOD and POD activities significantly increased when plants were exposed to Cd stress and the increase was more pronounced in urea and NH_4^+-N treated plants.

Dixit et al. [29] reported that the increase in level of lipid peroxidation may be a consequence of generation of ROS, and as a result, SOD activity showed a marked increase in leaves of the Cd-treated plant. The response of SOD activity to Cd or other stresses is reported to be dependent on stress intensity, species and age of the plants exposed to stress [30]. The current results showed that anti-oxidative level caused by Cd addition was higher in urea and NH_4^+-N treated plants than those treated with the other two N forms, thus posing the plants to less damage by oxidative stress. As reported by Hegedus et al. [31], it is possible that the enhanced POD activity could be resulted from either ionic microenvironment or tissue specific gene expression in leaves. Moreover, POD participating in lignin biosynthesis could build up a physical barrier against toxic heavy metals [32]. It should be mentioned that POD activity is also used as a potential biomarker for metal toxicity in examined plant species [33]. These results further suggests that due to elevated activity of SOD and POD, mechanisms of anti-oxidative defense were active and that these parameters can serve as a better intrinsic defense tool to resist Cd-induced oxidative damage in rice plants.

The results of the current research showed that there was a significant increase in MDA content in urea- and NH_4^+-N treated plants when subjected to Cd stress at milking stage. It indicated that Cd induced the increasing oxidative damage and that these two N forms indirectly leads to production of superoxide radicals, resulting in increased lipid peroxidation and oxidative stress in the rice plants. However, the effects of Cd on MDA content under different N forms were dependent on the growth stage. As Cd is not an oxide-reducing metal leading to oxidative damage directly (such as iron), the oxidative stress induced by Cd in the present study could be an indirect effect of Cd interacting with the different N forms, leading to increased production of O_2^- and lipid peroxidation.

It may be concluded from this study that the oxidative damage and yield reduction of rice plants exposed to Cd toxicity are dependent on N form, and in general, the plants supplied with NH_4^+-N have less oxidative damage and yield reduction caused by Cd stress in comparison with the plants supplied with NO_3-N. The results indicated the possibility of alleviating Cd stress through improving fertilization.

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