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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.

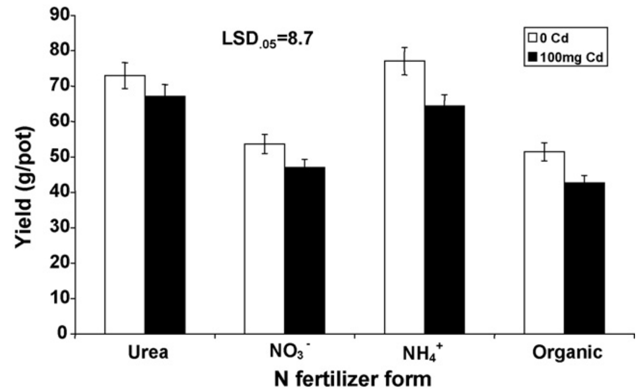

Fig. 1. Effects of different N forms on rice yield under two Cd levels. Vertical bars represents standard errors ($n = 3$).

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