# Biochemical and physiological responses of rice cadmium

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**Abstract:** Cadmium (Cd) is a non-essential heavy metal that is recognized as a major environmental pollutant. Cd enters the ecosystem chiefly as the result of human activities. Present investigation was conducted to assess the biochemical and physiological responses of rice to different concentrations of Cd. Seeds of rice were sterilized and were allowed to germinate under various Cd concentrations. Cadmium caused a gradual decrease in vigor index, root length, shoot height and amylase activity. Germination indexes of treated seeds were decreased. Root length decreased under all treatments. Cadmium uptake by seedlings increased with increasing Cd concentration and followed Michaelis-Menten kinetics. The average total amylolytic activities of rice seeds did not have a significant change in less than 4mg Cd l-1 medium compared to the control, but activity was progressively depressed with increasing concentration of Cd (6–10 mg l-1). Antioxidative responses of rice seedlings were also explored. Antioxidant enzyme activity in rice leaves generally showed no significant changes at low levels of Cd exposure. But high levels of Cd stimulated enzyme activity. These results suggested that prompt antioxidative responses might be necessary for the reduction of Cd-induced oxidative stress in roots but not in leaves.

**Keywords:** cadmium, rice, *Oryza sativa*, antioxidative response

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## Introduction

Environmental pollution caused by the release of a wide range of compounds as a consequence of industrialization has now assumed serious proportions (Jain et al., 2005). Industrial effluents are responsible for serious water (Otokunefor and Obiukwu, 2005) and soil pollution (Konwar and Jha, 2010), which is considered as one of the major factors responsible for low productivity of crops. A considerable number of reports are available on the effect of different industrial effluents on different crops (Cabral et al., 2010; Naaz and Pandey, 2010).

Cadmium (Cd) is one of the most serious pollutants, and massive amounts are released into the environment by human activities (Nriagu and Pacyna, 1988) whereas In areas little affected by human activities, Cd is only released during the weathering of rocks. Cd is a heavy metal that enters the environment mainly from industrial processes and phosphate fertilizers and is transferred to animals and humans through the food chain. In the natural environment, the cadmium content in igneous rocks is generally low and has no clear relationship with the concentration of other chemical elements other than Zn. Cd does not have any known biological functions and is highly toxic, for humans, animals, and plants, and is one of the widespread pollutants with a long biological half-life (Chirila et al., 2009). Cadmium has recently been shown to be an endocrine-disrupting chemical with estrogenic properties and a potential prostate carcinogen (Benbrahim-Tallaa et al., 2007). Cd toxicity effects in plants include inhibition of physiological processes such as respiration, photosynthesis, interference with electron-transport chains, plant–water relationship, nitrogen and mineral nutrition. Thus Cd toxicity leading to reduced plant growth, even to death (Pál et al., 2006). Cd is a nonredox metal unable to participate in Fenton and/or Haber–Weiss reactions, but it causes oxidative stress by generating reactive oxygen species (ROS) .

Rice (*Oryza sativa* L.) is an important crop worldwide and is the staple food in the diet of more than one third of the world's population (Jung et al., 2005; Konwar and Jha, 2010). Rice is also considered to be a model plant among monocots for biological research because of its small genome size (Goff et al., 2002; Yu et al., 2002). The growth and yield of rice depends upon various factors, one of which is the soil quality. Soil pollution is a major cause of change in the quality of the soil.

The increased level of ROS induced by Cd is connected with its toxicity, and Cd-related phenomena also include effects on nucleic acids, proteins and gene expression (Deckert, 2005). Cd has a high affinity for protein–thiol groups, thereby inhibiting some essential enzymes (Baudouin-Cornu and Labarre, 2006).. Recently, several literatures focused on specific organs such as barley vacuoles (Schneider et al., 2009), Indian mustard roots (Alvarez et al., 2009) to describe the Cd effects in stressed plants. Detailed proteome analyses of rice have also been done for deeper insights into complex cellular processes (Agrawal et al., 2009; Ahsan et al., 2009; Aina et al., 2007).

Cd causes to inhibition of photosynthesis, such as biosynthesis of chlorophyll and functioning of photochemical reactions . Photosystem II (PSII) is extremely sensitive to Cd and its function was inhibited to a much greater extent than that of Photosystem I (PSI). Recent studies have also indicated that Cd exerts multiple effects on both donor and acceptor sites of PSII. On the donor site, the presence of Cd inhibits the oxygen evolving cycle and, consequently, oxygen evolution; on the acceptor site, it inhibits electron transfer from $Q_A^-$ to $Q_B^-$ (Sigfridsson et al., 2004).

The aim of the present study was to investigate the effect of Cd stress on germination and seedling growth of rice. The germination parameters, Cd concentration, and activities of total amylase, α-amylase, and β-amylase in seeds were determined.

## Methods

### Plant material, growth conditions and growth and germination assay

Rice (*Oryza sativa* L. cv. Tarom Atri) seeds were surface-sterilized and soaked in distilled waterfor 24 h and then sited on filter papers placed in Petri dishes moistened with distilled water containing various Cd concentrations (0, 2, 4, 6, 8, and 10 mM), supplied as $CdCl_2$. After germination seedlings were grown on pots filled with vermiculite saturated with Hoagland nutrient solution (Hoagland and Arnon, 1950) supplemented with 0, 2, 4, 6, 8 and 10 mM $CdCl_2$. root and shoot lengths of 12-d-old seedlings were measured. Germination percentage, germination index, and vigor index were calculated according to (Liu et al., 2005; Mhatre and Chaphekar, 1982). Seedling biomass was also determined.

## Cd determination

The washed roots were immersed in 20 mM EDTA solution for 10 min, re-washed and dried in an oven. The samples were ground, weighed, and digested with a mixture of $HNO_3$–$HClO_4$ (He et al., 2006). The Cd concentration in the digestion solution was analyzed by atomic absorption spectroscopy (Hitachi Z- 81001).

## Determination of amylase activity

Total amylolytic activity, α-amylase activity and β-amylase activity of 12 d germinated seeds were measured according to the method of Swain and Dekker described (Swain and Dekker, 1966) .

## Determination of antioxidative enzymes

Protein extracts of plant material prepared by a method slightly modified from the one described by Böddi et al. (1996) . The rice leaves were ground at 95°C in an extraction buffer of 10% (v/v) glycerol, 4% (w/v) sodium dodecyl sulphate (SDS), 0.3 M dithiothreitol, 0.001% bromophenol blue and 250 mM Tris–HCl, pH 6.8. The proteins were quantified by a colorimetric assay for protein determination using the Bio-Rad DC Protein Assay kit based on the well-documented Lowry assay (Bio-Rad, Richmond, CA)..

SOD was assayed on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), according to the methods of Beauchamp and Fridovich (1971)  and Beyer and Fridovich (1987) . Peroxidase (POD, (EC 1.11.1.7) activity was measured on the basis of determination of guaiacol oxidation at 470 nm (Bergmeyer, 1974; Lagrimini, 1991).

The activity of CAT was measured by the method of Aebi (1984)  and was determined by monitoring the disappearance of $H_2O_2$ at 240 nm.

## Chlorophyll a fluorescence measurements

Chlorophyll a fluorescence measurements were performed with a chlorophyll fluorometer (PAM-2000; Heinz-Walz, Effeltrich, Germany) according to the manufacturer's instructions.

## Results

### Effect of Cd stress on rice germination

The response of rice germination to Cd is summarized in Figure 1. The germination percentage was decreased significantly only by the greatest Cd concentration tested (10 mM) and it was not significantly inhibited by lower amount of Cd. Under 2 mM Cd, germination indexes of treated seeds were not inhibited significantly when compared with the control, while it was decreased significantly at 4, 6, 8 and 10 mM Cd. The vigor index was significantly affected by different Cd concentrations (Figure 1). Decrease of germination and vigor index indicates that the quantity of germination was affected (Figure 1).

### Effect of Cd stress on the root and shoot length and biomass

The length of root and shoot as well as the biomass were measured. No significant reduction of shoot length was recorded with the increase of Cd concentrations of 2 and 4 mM Cd. Higher concentrations of Cd, however had significant effect on length of shoots. Root length decreased significantly under all treatments (Figure 2). The extent of biomass also decreases significantly under Cd stress (Figure 2).
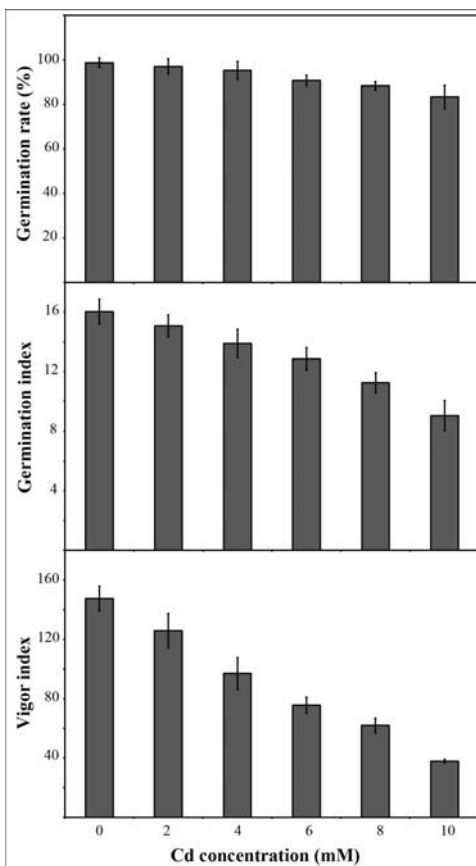




**Figure 2.** The length of root and shoot and dry weight biomass of rice seedlings growing under various Cd concentrations. Data are shown as mean ± SE of three replicates.



**Figure 1.** Germination percentage, germination index, and vigor index of rice seeds at various Cd concentrations. Data are shown as mean ± SE of three replicates.
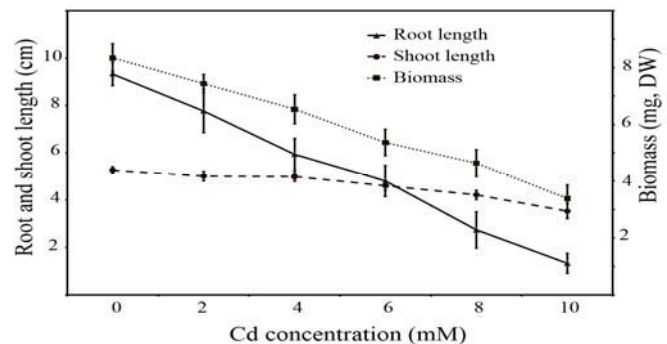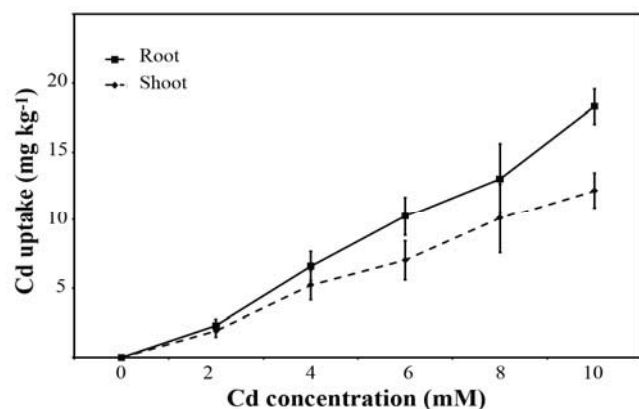
**Figure 3.** Cadmium accumulation in different parts of rice seedlings grown under various Cd concentrations. Data are shown as mean ± SE of three replicates.

### Heavy metal concentrations in plants

Cd concentrations in different parts of rice plants were significantly higher for the plants grown at the solution supplemented by $CdCl_2$ compared to those grown in the untreated solution (Figure 3). The highest concentration of Cd recorded at the plant grown in the solution supplemented with 10 mM Cd. As Figure 3 showes Cd concentration were higher in the roots than the shoots.

### Effect of Cd on amylases

Average total amylolytic activity and α-amylase and β-amylase activities in germinated seeds with different concentrations of treatment are presented in Figure 4. The average total amylolytic activities of rice seeds did not have a significant change in less than 4mg Cd $L^{-1}$ medium

compared to the control, but activity was progressively depressed with increasing concentration of Cd (6–10 mg L$^{-1}$). Similar patterns of response were observed for α-amylase and β-amylase activities. Increasing the Cd concentration resulted in decline of the total amylolytic activity, α-amylase activity and β-amylase activity of germinated seeds up to 26.8%, up to 34.7%, up to 24.7% respectively (Figure 4).

### Effects of Cd on antioxidant enzymes

Antioxidant enzyme activity in rice leaves generally showed no significant changes at low levels of Cd exposure. But high levels of Cd stimulated enzyme activity. Significant changes in SOD activity were observed in rice seedlings with the increase in level of Cd treatment when compared with control (Table 1). With the increase in level of Cd treatment, a gradual increase in SOD activity was observed. The activity of POD increased gradually with increasing Cd concentration up to the highly toxic Cd level (Table 1). Increasing Cd concentration also resulted in a gradual increasing of the activity of CAT (Table 1).

### Effect of Cd on fluorescence parameters

Exposing rice seedlings to different levels of Cd resulted in changes of the chlorophyll fluorescence parameters, $F_0$ and $F_M$ (Table 2). Increasing Cd concentrations resulted in increase of $F_0$ values. In contrast, $F_M$ values decreased significantly for the whole range of Cd concentrations. The $F_V/F_M$ values of the controls were about 0.84 (Table 2). $F_V/F_M$ ratios decreased by increasing Cd level. Examination of $F'_V/F'_M$ and $\Phi$PSII values clearly showed that these parameters had a high correlation with $F_V/F_M$ and had similar responses (Table 2). $\Phi$PSII and $F'_V/F'_M$ diminished at all Cd concentrations. A significant decrease for $q_P$ was observed at all Cd levels. On the other hand, variation of $q_{NP}$, reflecting the nonradioactive energy dissipation was non-significant for all Cd concentrations (Table. 2).

Increasing Cd level to 4 mM had no significant effect on ETR. The 6 mM concentration of Cd, however decreased significantly the ETR (Figure 5).

**Table 1:** Effects of Cd treatments on the activities of the leaf antioxidant enzymes, CAT (mU mg$^{-1}$ protein), POD ($\Delta OD_{470}$ min$^{-1}$ g$^{-1}$ FW) and SOD (U mg$^{-1}$ protein). The results are presented as means ± *SD*.

| Characteristics | Cd concentration (mM) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Catalase | 56.6 ± 8.4 | 54.5 ± 8.1 | 50.8 ± 7.2 | 52.0 ± 4.7 | 53.1 ± 6.2 | 55.6 ± 5.9 |
| Peroxidase | 19.3 ± 3.4 | 54.9 ± 5.7 | 80.3 ± 4.1 | 108.9 ± 8.2 | 120.8 ± 10.4 | 131.9 ± 7.5 |
| Superoxide dismutase | 50.9 ± 7.2 | 102.8 ± 10.9 | 153.9 ± 10.6 | 285.7 ± 13.5 | 318.3 ± 14.1 | 359.2 ± 14.3 |

**Table 2:** Effects of Cd treatments on photochemical parameters of rice seedlings. The results are presented as means ± *SD*.

| Characteristics | Cd concentration (mM) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| $F_0$ | 175.55 ± 15.32 | 188.47 ± 19.49 | 194.79 ± 19.28 | 196.70 ± 21.72 | 233.58 ± 26.21 | 256.38 ± 27.9 |
| $F_M$ | 1050.37 ± 92.46 | 998.74 ± 94.72 | 997.28 ± 104.13 | 854.16 ± 131.24 | 819.06 ± 121.96 | 446.19 ± 116.93 |
| $F_V/F_M$ | 0.84 ± 0.02 | 0.76 ± 0.02 | 0.71 ± 0.01 | 0.64 ± 0.04 | 0.63 ± 0.06 | 0.54 ± 0.09 |
| $Fv'/Fm'$ | 0.75 ± 0.01 | 0.72 ± 0.01 | 0.70 ± 0.04 | 0.68 ± 0.02 | 0.64 ± 0.08 | 0.61 ± 0.03 |
| $q_P$ | 0.98 ± 0.01 | 0.97 ± 0.01 | 0.96 ± 0.01 | 0.95 ± 0.02 | 0.94 ± 0.02 | 0.93 ± 0.01 |
| $q_{NP}$ | 0.19 ± 0.01 | 0.18 ± 0.01 | 0.18 ± 0.01 | 0.18 ± 0.01 | 0.20 ± 0.02 | 0.22 ± 0.01 |
| $\Phi$PSII | 0.78 ± 0.04 | 0.73 ± 0.04 | 0.64 ± 0.03 | 0.63 ± 0.05 | 0.60 ± 0.07 | 0.58 ± 0.05 |
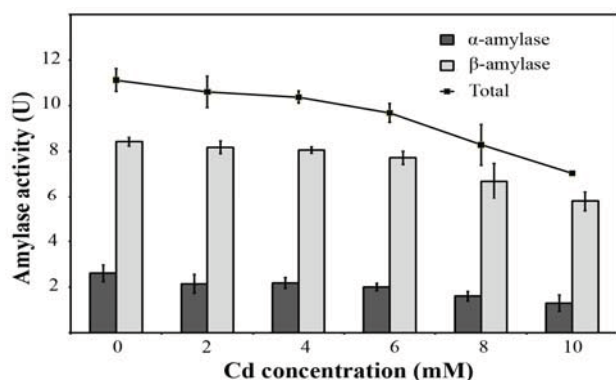


**Figure 4.** Amylolytic activity in rice grown in solution with different concentrations of Cd. Data are shown as mean ± SE of three replicates.
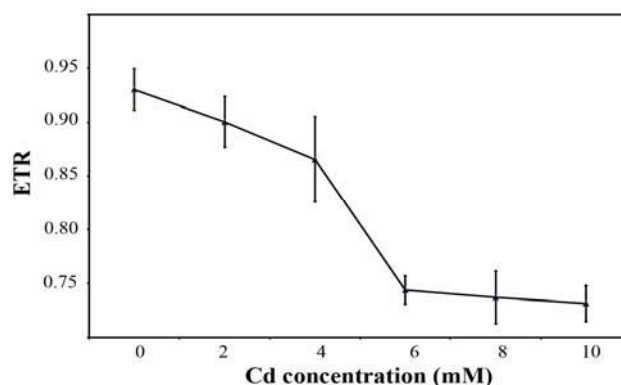
**Figure 5.** Differential ETR values detected in 7-d-old leaves of rice after treated with different concentrations of Cd. Data are shown as mean ± SE of three replicates.

### Discussion

In this study, Cd was applied at external concentrations of 2–10 mM. The results show that the germination of rice seeds was inhibited considerably with increasing Cd concentrations. Therefore, under field conditions, soil Cd pollution may have adverse effects on seed germination. In higher plants, Cd toxicity generally inhibits the growth and reduce the biomass production. In the present study germination did not decrease in response to Cd exposure (Figure 1). Cd stress caused a significant inhibition of root elongation and seedling biomass. Less toxicity has been seen in shoot (Figures 1, 2). These results are in agreement with the studies accomplished oncucumber and wheat (Munzuroglu and Geckil, 2002). Blum (1997) also found root length to be the most sensitive parameter to Cd treatment. Inhibition of root elongation is considered to be the first evident effect of Cd toxicity in plants due to the fact that plant roots are the first point of contact with the toxic Cd in the growth medium (Liu et al., 2003; Munzuroglu and Geckil, 2002). Cell division at the root tip and cell elongation in the extension zone were affected by the presence of heavy metals. Drazic et al. reported that Cd-induced inhibition of root growth was associated with a decrease of K, Mg, Ca, and Fe concentrations in roots (Drazic et al., 2006).

Cd was found accumulated in the root and leaf. The accumulation of Cd in the root and leaf depends on binding to the extracellular matrix and on the transport efficiency (Marchiol et al., 1996). Results of this research showed that the accumulation of Cd in roots was higher than in leaves (Figure 3). Most of the Cd that entered the plant system accumulated in the roots. A first barrier against Cd stress, operating mainly at the

root level, can be immobilization of Cd by means of the cell wall and extracellular carbohydrates . These results indicated that rice has a great ability to accumulate Cd, primarily in roots, and to prevent the transfer of excess Cd to the leaves. Similar results were reported by Wang et al. (2007). The varying levels of increments in heavy metal also recorded concentrations have been reported in wheat and corn grains (El-Naim et al., 2004). The variations in heavy metal concentrations in various parts of plants have been ascribed to compartmentalization and translocation through the vascular system (Kim et al., 2003).

Minimum concentrations of heavy metals were found in stem as it is a transporter organ (Korboulewsky et al., 2002). Plants may accumulate and store metals in root and stem in non-toxic forms. Binding of toxic metals at cell walls of roots and leaves away from sensitive sites within the cell or storing them in a vacuolar compartment are known avoidance mechanisms of heavy metal tolerance in plants (Memon et al., 2001).

It is well known that mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrates essential to growth of the root and plumule (Pritchard et al., 2002). Starch is quantitatively the most abundant storage material in seeds and available evidence indicates that in germinating seed starch is degraded predominantly via the amylolytic pathway (Juliano and Varner, 1969). $\alpha$-amylase is the major enzyme involved in the initial degradation of starch into more soluble forms while phosphorylase and $\beta$-amylase assist in the further conversion to free sugars which affords the nutrition of seed germination (Juliano and Varner, 1969).In the present study the average total, $\alpha$- and $\beta$-amylase activities were significantly depressed by higher Cd concentrations (Figure 4), which is consistent with the effects of high concentrations of $Cu^{2+}$, and $Hg^{2+}$ on rice (He et al., 2010), and arsenate and arsenite for wheat germination (Liu et al., 2005). Thus, the inhibition of root growth by Cd is likely to be attributable in part to a lack of products of endosperm mobilization.

The presence of toxic metals in the cell leads to the formation of AOS, which cause further severe oxidative damage to different cell organelles and biomolecules . To scavenge ROS, plants possess a well-organized antioxidative defense system comprising enzymatic and nonenzymatic antioxidants. The cooperative function of these antioxidants plays an important role in scavenging ROS and maintaining the physiological redox status of organisms (Cho and Seo, 2005). In this study, a significant increase of SOD activity in leaves was observed with increasing Cd concentration. This may be attributed to the increased production of superoxide, resulting in the activation of existing enzyme pools or increased expression of genes encoding SOD (Mishra et al., 2006). Increased SOD activity caused by heavy metals has been previously observed in several plant species, and is routinely considered to be an adjustment response to stress (Verma and Dubey, 2003). A slight drop or no change in SOD activity under low levels of Cd stress indicates no excess accumulation of superoxide anion in seedlings, since SOD activity is mediated by superoxide level. Wu et al. (2003) also found a slight decrease in SOD activity accompanied by a reduction in products of lipid peroxidation in barleys with a low-level Cd treatment.

POD activity reflects the modified mechanical properties of the cell wall and cell membrane integrity of plant leaves under stress conditions. The activity of POD increased with increasing Cd concentration. The increase in SODand POD activities indicated that seedlings had the capacity to adapt to moderate Cd concentrations by developing an antioxidative defense system. A similar result was obtained by Wu et al. (2003) who observed the increase in SOD, POD and CAT activities for barley under Cd stress.

Physiological processes such as photosynthesis are very sensitive to heavy metals in higher plants (Tanyolac et al., 2007). This research has shown that the photo activation of PSII is inhibited by Cd . Increasing Cd concentration resulted in slightly increase of $F_0$ but progressively decrease of $F_M$ (Table 2). The increase in $F_0$ with Cd treatment can be attributed to an impact on the PSII reaction centre, or a reduction in the energy transfer from the antennae to the reaction center (Ralph and Burchett, 1998). It is generally accepted that the $F_M$ intensity expresses the state of PSII when all QA molecules are in the reduced stage (Mallick and Mohn, 2003). The decline in the $F_M$ suggests a change in the ultrastructure of thylakoid membrane, affecting the ETR. Both $F_0$ and $F_M$ caused a decrease in $F_V/F_M$ when exposed to increasing Cd concentrations. The ratio of $F_V/F_M$ is often used as a stress indicator and describes the potential yield of the photochemical reaction (Mallick and Mohn, 2003). It is reported that $F_V/F_M$ may decrease if reoxidation of QA was limited by the decrease or partial block of electron transport from PSII to PSI Mallick and Mohn (2003). In parallel, photochemical parameters showed that Cd treatment likely caused more oxidative damage and did not indicate cessation of PSII activity, even though plant death was not observed at the highly toxic Cd level. Also, decreased $F_V/F_M$ resulted in a decrease in the quantum efficiency of excitation energy trapping of the open PSII reaction center ($F'_V/F'_M$) and in the quantum efficiency of PSII open centers in a light-saturated state, $\Phi$PSII. However, the 10mM Cd concentration caused the maximum decrease in $F_V/F_M$, $F'_V/F'_M$ and $\Phi$PSII (Table 2). Increasing of $q_{NP}$ with increase of Cd concentration, indicating a rise of the thermal dissipation in PSII antennae, often results in a decrease in $F'_V/F'_M$ (Lu and Zhang, 2000; Lu et al., 2000). Despite decrease of $F'_V/F'_M$ in the presence of Cd, $q_{NP}$ did not change considerably with increasing Cd concentration (Table 2). It was suggested that the Cd-induced decrease in $F'_V/F'_M$ was probably not involved in the process of $q_{NP}$. On the other hand, $q_P$ decreased with increasing Cd concentration. The decreased qP suggested a down regulation of the open PSII reaction centers (Genty et al., 1989).

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