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Full Length Research Paper

Effects of Cadmium on Wheat Growth and Some Physiological Factors

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Abstract: The aim of this study is to examine the effects of Cd on the growth, oxidative stress, and antioxidative enzymes as well as on photosynthetic performance of early wheat seedlings. The possible mechanisms of wheat seedlings, response to Cd stress involving free radical metabolism and antioxidant changes are also discussed. Effects of cadmium (Cd) on seed germination, seedling biomass, root length and shoot height, Cd uptake, amylase activity and the activities of antioxidative enzymes as well as photosynthetic activities, soluble protein and malondialdehyde (MDA) concentrations in wheat seedlings were investigated. Seedlings were exposed to different CdCl₂ concentrations for 7 days. Seed germination and seedling biomass and root and shoot elongation decreased with increasing of Cd. Root was the most sensitive parameter. Seedlings took more Cd up with increasing Cd in the test solutions. Fluorescence measurements showed that F_0 was slightly increased but F_M was decreased progressively with increasing Cd. Total amylolytic, α -amylase and β -amylase activities were depressed by the higher Cd concentrations. A significant increase of MDA contents and peroxidase POD and superoxide dismutase SOD activity in Seedlings were observed with increasing concentration of Cd. The MDA content was linearly and positively correlated with seed germination frequency, biomass increment, root length and shoot height elongation, suggesting that MDA may be useful as a biological indicator of Cd toxicity in wheat.

Key words: cadmium, stress, wheat, *Triticum aestivum*

Abbreviation: Cd-cadmium; MDA- malondialdehyde; POD- superoxide dismutase; SOD- superoxide dismutase; PSII-Photosystem II; ROS-reactive oxygen species

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INTRODUCTION

Nowadays, developing has become synonymous with deforestation and progress has become synonymous with pollution. Because of the increased mining and industrial activities in the late 19th and early 20th the pollution due to heavy metals has increased considerably world wide. Plants do not have the ability to sense physical properties of metals, e.g. specific weight. The term "heavy metal" was defined mainly by the specific weight of metals. The definition was often connected with the expectation that the substance should be toxic. This definition is not acceptable and also inconsistent in use as already stressed in literature. However, in Plant Sciences, the term is so widely used that it is hardly possible to eliminate it (Appenroth, 2010). Heavy metal contamination is a serious environmental problem that limits plant productivity and threatens human health. Cadmium (Cd) is one of the most highly toxic environmental pollutants in the atmosphere, soil and water. This is a growing concern since Cd in soil can be transferred to plants, resulting in phytotoxicity and threats to animal and human health through the food chain. Cd enters the environment mainly through industrial processes, irrigation with wastewater and application of metal-containing pesticides municipal based composts and phosphatic fertilizers and atmospheric deposition (Ranieri, et al., 2005).

Although Cd is generally considered to be a highly toxic element and has negative effects on plant development and growth (Maksymiec, Wojcik, & Krupa, 2007), a positive effect of low concentration of Cd on plant growth has also been reported in plants such as rice, soybean and barley in hydroponic experiments. Regardless of different concentration ranges, the phenomenon that a low level of Cd stimulates plant growth seems repeatable. Cd concentration of uncontaminated soils is usually below 0.5 mg kg⁻¹, but can reach up to 3 mg kg⁻¹

depending on the soil material. At low concentrations Cd is not toxic to plants, but it is toxic at higher concentrations and characteristically inhibits root growth and cell division. Cd is not an essential nutrient for plants but it is easily taken up by roots and translocated to different plant parts. The toxic symptoms of metals in plants can be recognized by changes in biochemical and physiological processes or by organ and intact plant responses such as growth inhibition, reduction in yield, chlorosis, alterations of anatomical, morphological and several metabolic activities in different cell compartments, especially chloroplasts (Maksymiec, et al., 2007).

These damaging effects include 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, Bernat, Mamedov, & Styring, 2004).

Evidences indicate that plants respond to heavy metal toxicity in three different molecular mechanisms due to chemical and physical properties of metals: (i) production of reactive oxygen species (ROS) by autoxidation, (ii) blocking of essential functional groups in biomolecules, and (iii) displacement of essential metal ions from biomolecules (Schützendübel & Polle, 2002). The toxicity of Cd which is a non-redox metal may be associated with oxidative damage caused by ROS such as superoxide radicals ($O_2^{\cdot-}$), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$). Cd, Depending on its concentration, can either inhibit or stimulate the activity of several antioxidative enzymes before any visible symptoms of toxicity appear (Correa, et al., 2006).

These ROS are highly reactive and damage membrane lipids, proteins, pigments and nucleic acids, resulting in dramatic reductions of growth and productivity, and eventually causing the death of plants. These species are inevitable by-products of all aerobic lives under normal physiological conditions due to leakage of the electron transport chain and subsequent cascades of reaction (Verma & Dubey, 2003) but they generally stay at an acceptable level and do not cause oxidative damage. Stress environments such as drought, heat, and heavy metal exposure may increase the generation of ROS in plants, and oxidative stress would arise if the balance between ROS generation and removal was broken (Cho & Seo, 2005). Cd stress might cause oxidative damage such as lipid peroxidation and induce alterations of the antioxidant system in various plants. ROS production induced by Cd is usually deduced from changes in the antioxidant system (Ranieri, et al., 2005). The response of antioxidant enzymes to Cd, and in general to metals, can vary among species and among different tissues (Tiryakioglu, Eker, Ozkutlu, Husted, & Cakmak, 2006).

Plants adopt different strategies to reduce Cd-induced oxidative damage. Increasing the activities of antioxidative enzymes increases tolerance of plants to stress. The antioxidative enzyme system constitutes superoxide dismutase (SOD; EC 1.12.1.1) as the primary step of cellular defence. It dismutates $O_2^{\cdot-}$ to H_2O_2 and O_2 . Further, the accumulation of H_2O_2 is restricted by the action of the ascorbate–glutathione cycle, where ascorbate peroxidase (APX; EC 1.11.1.11) reduces it or by the action of catalase (CAT; EC 1.11.1.6) or glutathione peroxidase (GPX; EC 1.11.1.9) in peroxisomes.

The purpose of this study is to examine the effects of Cd on the growth, oxidative stress, and antioxidative enzymes as well as on photosynthetic performance of early wheat (*Triticum aestivum* L.) seedlings. The possible mechanisms of wheat seedlings, response to Cd stress involving free radical metabolism and antioxidant changes are also discussed.

Similar seeds were placed in dishes and incubated at 23 ± 2 °C. After germination seedlings were grown in the pots filled with vermiculite saturated with Hoagland nutrient solution supplemented with 0, 2, 4, 6, 8 and 10 mM $CdCl_2$. The seedlings were grown in a growth chamber under 16/8 h day/night light period and at 23 ± 2 °C day/night temperature and under $400 \mu mol m^{-2} s^{-1}$ light. The Cd solutions were prepared according to Liu et al. (Xiaoli Liu, Zhang, Shan, & Christie, 2007).

Chlorophyll a fluorescence measurements were performed according to experimental protocol of Genty (Genty, Briantais, & Baker, 1989). The photochemical quenching calculated as done by Schreiber (Schreiber, Bilger, & Neubauer, 1994).

The amounts of Chl were calculated according to Brouers and Michel-Wolwertz (1983). The Cd concentration in the digestion solution was analyzed by atomic absorption spectroscopy (Hitachi Z- 81001). SOD and POD were assayed on the basis of ability of SOD to inhibit the photochemical reduction of NBT and determination of guaiacol oxidation at 470nm. The activity of CAT determined by monitoring the disappearance of H_2O_2 at 240 nm. The level of lipid peroxidation was measured by estimation a decomposition product of peroxidized polyunsaturated fatty acid component of membrane lipid, Malondialdehyde.

RESULTS

Effect of Cd on Seed Germination and Growth Parameters

Effects of different concentrations of Cd (0, 2, 4, 6, 8, and 10 mgL⁻¹) on seed germination frequency, seedling biomass and root and shoot elongation were evaluated. As figure 1 shows, seed germination, seedling biomass and root and shoot elongation decreased with increasing concentrations of Cd. A positive relationship (P<0.01) could be found between the repression of seed germination(%), seedling biomass, root and shoot elongation and the concentrations of Cd in the growth medium. The slope coefficient of the equations for germination inhibition rates and Cd concentrations was 3.41. Seedling biomass also decreased significantly (P<0.05) with increasing concentration of Cd (Fig. 1). The slope coefficient was 3.38.

Effects of Cd on root and shoot elongation are also presented in Fig. 1. Increasing Cd concentrations resulted in significant reductions in root length and shoot height. The equation slope coefficients were 7.15 and 4.45 for root length, and shoot height respectively.

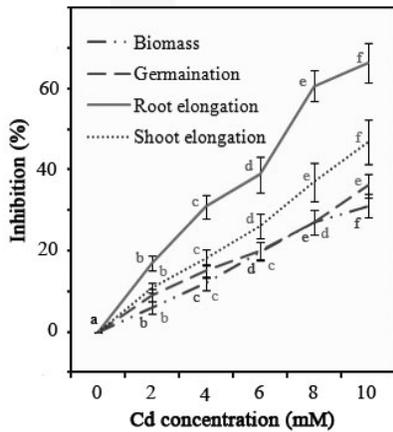


Figure 1. Inhibitory effect (%) of cadmium concentrations on seed germination, seedling biomass and root and shoot elongation. The results are presented as means ± SD.

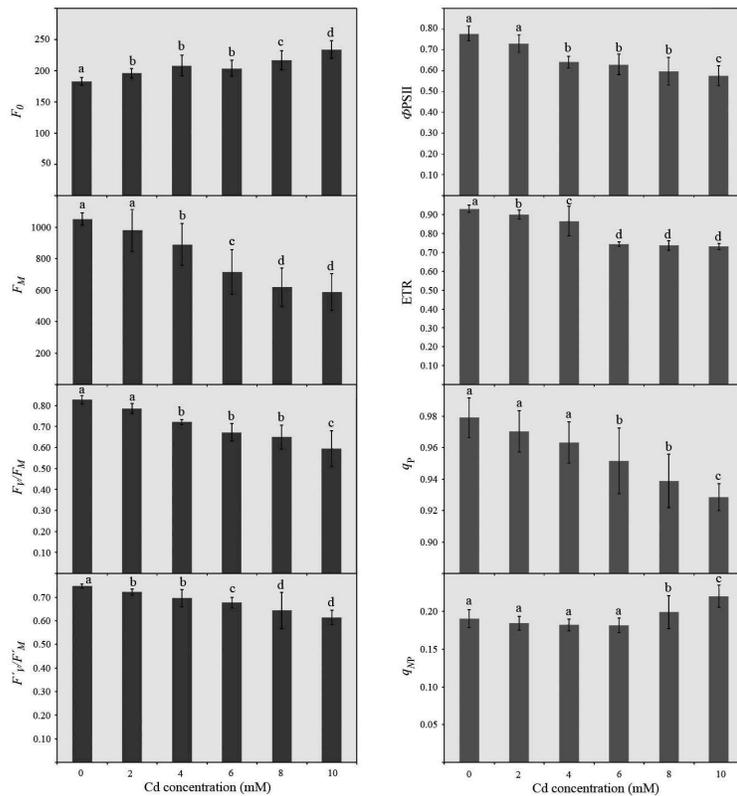


Figure 2. Effect of Cd on photochemical parameters of wheat seedlings. (A) Minimum fluorescence, (B) maximum fluorescence, (C) maximum quantum efficiency of PSII, (D) quantum efficiency of excitation energy trapping of PSII, (E) quantum efficiency of PSII, (F) electron transport rate, (G) photochemical quenching and (H) non-photochemical quenching. The results are presented as means ± SD.

Effect of Cd on Fluorescence Parameters

Exposing wheat seedling to different levels of Cd resulted in changes of the chlorophyll fluorescence parameters, F_0 and F_M , (Fig. 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. In control leaves, F_v/F_M values were approximately 0.83 (Fig. 2). F_v/F_M ratios decreased by 28% at 10 mg L⁻¹ Cd level. Examination of F_v'/F_M' and Φ PSII values clearly showed that these parameters had a high correlation with F_v/F_M and had similar responses (Fig. 2). Φ PSII and F_v'/F_M' diminished at all Cd concentrations (Fig. 2). The Cd concentration of 10 mM caused the maximum decrease in these fluorescence parameters. Cd induced qP determined by the redox state of Q_A , the primary electron acceptor of PSII. A significant decrease for qP was observed at all Cd levels (Fig. 2). On the other

hand, variation of q_{NP} , reflecting the nonradioactive energy dissipation was non-significant for all Cd concentrations (Fig. 2).

Effect of Cd on Pigment Contents

Cd treatment affected the chlorophyll amount of leaves (Fig. 3). The reduction of chlorophyll *a* and *b* contents of leaves was detected with enhanced Cd accumulation in leaves. Light-induced chlorophyll accumulation was inhibited by increasing Cd concentration in the nutrient solution (Fig. 3). Under Cd stress, the chlorophyll *b* content of leaves was more affected than the chlorophyll *a* content (Fig. 3). Total chlorophyll content of the leaves decreased significantly with increasing Cd concentration. Total chlorophyll content decreased approximately by 44% at the lowest Cd concentration (0.2 mM). The Cd concentration of 10 mM caused the maximum (84%) decrease of Chl amounts. The highest carotenoid content was measured in control plants and it decreased with increasing Cd concentration. Carotenoid content was decreased by 67% with high amount of Cd (Fig. 3).

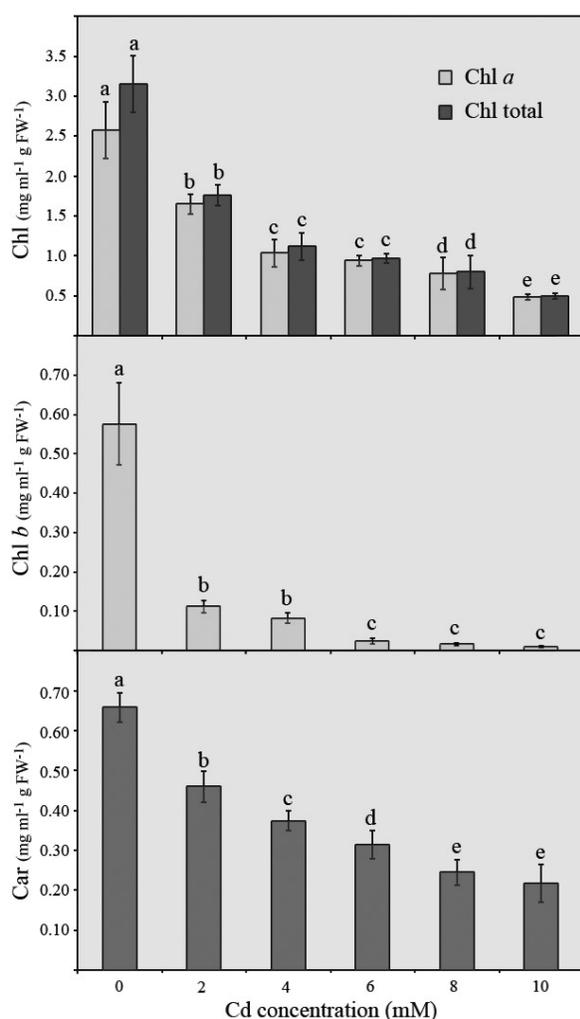


Figure 3. Effects of Cd on chlorophyll *a* and total chlorophyll (A), chlorophyll *b* (B) and carotenoid contents (C) of wheat seedlings.

The results are presented as means \pm SD.

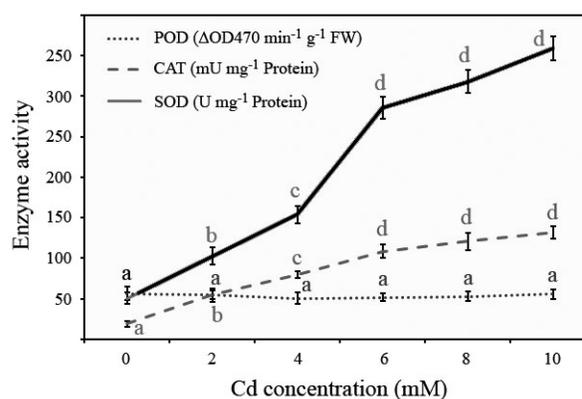


Figure 4. Effects of Cd treatments on the activities of SOD, CAT and POD in the leaves of wheat seedlings. . The results are presented as means \pm SD.

Effect of Cd Concentration on Cd Accumulation

Results indicated an increase in Cd accumulation in leaves and roots with increasing Cd concentration in the growth medium. As figure 2 shows Cd concentrations in wheat roots were about 10 times greater than those in leaves. At the highest level of Cd treatment, Cd reached 5.8 and 0.3 mg kg⁻¹ FW in roots and leaves, respectively (Table 1).

Table 1. Cadmium accumulation and amylolytic activity in wheat grown in solution with different concentrations of Cd

Cd in solution mM	Cd accumulation (mg kg ⁻¹ FW)		Amylase activity	
	Root	Leaf	α -amylase	β -amylase
0	0 ^a	0 ^a	2.27 ± 0.13 ^a	8.60 ± 0.30 ^a
2	0.7 ± 0.05 ^b	0.07 ± 0.01 ^b	2.23 ± 0.26 ^a	8.41 ± 0.16 ^a
4	1.1 ± 0.09 ^c	0.08 ± 0.01 ^c	2.19 ± 0.25 ^a	8.37 ± 0.10 ^a
6	1.7 ± 0.32 ^d	0.10 ± 0.01 ^d	1.88 ± 0.17 ^b	7.36 ± 0.43 ^b
8	3.4 ± 0.22 ^e	0.15 ± 0.03 ^c	1.49 ± 0.21 ^c	6.68 ± 0.40 ^c
10	5.8 ± 0.40 ^f	0.30 ± 0.08 ^f	1.11 ± 0.09 ^d	5.97 ± 0.40 ^d

Letters a–e indicate significant differences ($P < 0.05$) within each column using a least significant difference (LSD) test.

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 wheat seeds in ≤ 4 mg Cd L⁻¹ medium were comparable 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 the total amylolytic activity of germinated seeds declined up to 26.8%, α -amylase activity up to 34.7%, and β -amylase activity up to 24.7% (Table 1).

Effects of Cd on Antioxidant Enzymes

Antioxidant enzyme activity in leaves generally showed no significant difference compared to the control at low levels of Cd exposure. But high levels of Cd stimulated enzyme activity. Significant changes in SOD activity were observed in wheat with the increase in level of Cd treatment when compared with control (Fig 4). 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 (Fig 4).

Increasing Cd concentration also resulted in a gradual increasing of the activity of CAT (Fig 4).

Effects of Cd on Lipid Peroxidation

The levels of lipid peroxidation in wheat leaves were measured in terms of MDA content (Fig. 5). Cd exposure resulted in an accumulation of lipid peroxidation products in leaves, but the accumulation was only significant at the 10 mg l⁻¹ Cd treatment level, where it was 63% more than that of the control.

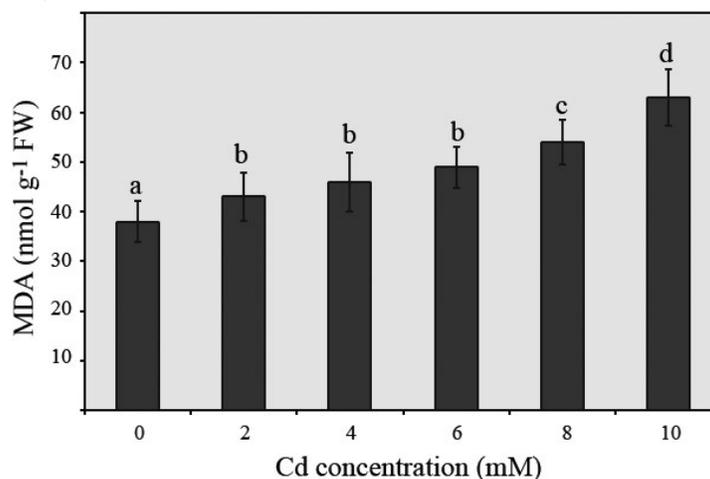


Figure 5. MDA contents in the leaves of wheat seedlings under Cd exposure. The results are presented as means ± SD.

DISCUSSION

Seed Germination and Growth Parameters

Inhibitory effects of Cd on seed germination frequency showed significant linear relationships ($P < 0.01$). Seedling biomass, root and shoot elongation and concentrations of Cd in the seedlings had also linear relationships. Root length had more reduction in response to increases in the concentrations of Cd in the growth medium than the other parameters (Fig 1). The degree of inhibition followed the order: root length > shoot height > biomass, germination frequency. It seems that root length to be the most sensitive indicator among the growth parameters. The study accomplished by Liu et al (2005), however, showed that root length and shoot height had similar sensitivities heavy metal. Cadmium damage occurs in the roots first, because Cd can readily penetrate the root cortex.

Seed germination relies almost solely on seed reserves for the supply of metabolites for respiration as well as other anabolic reactions. Quantitatively starch is the most abundant storage material in seeds. Evidences indicate that seed starch is degraded predominantly via the amylolytic pathway in germinating (Juliano & Varner, 1969). In the present study the average total amylolytic, α - and β -amylase activities were significantly depressed by the higher concentrations of Cd. α -amylase is the major enzyme involved in the initial degradation of starch into more soluble forms while phosphorylase and β -amylase assist in the further conversion to free sugars which affords the nutrition of seed germination. Reduction of amylase activity may therefore be the major factor involved in the depression of seed germination.

Fluorescence Parameters

Physiological processes such as photosynthesis are very sensitive to heavy metals in higher plants. This research has shown that the photoactivation of PSII is inhibited by Cd. Increasing Cd concentration resulted in slightly increase of F_0 but progressively decrease of F_M (Fig. 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. It is generally accepted that the F_M intensity expresses the state of PSII when all QA molecules are in the reduced stage. 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. 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 (Mallick & 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, Φ_{PSII} . However, the 10mM Cd concentration caused the maximum decrease in F_v/F_M , F'_v/F'_M and Φ_{PSII} (Fig. 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 . Despite decrease of F'_v/F'_M in the presence of Cd, q_{NP} did not change considerably with increasing Cd concentration (Fig. 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 q_P suggested a down regulation of the open PSII reaction centers.

Pigment Contents

Leaf chlorosis is one of the most commonly observed consequences of Cd toxicity. The leaves were significantly affected by Cd treatment. Chlorophyll contents (a , b and $a+b$) abruptly declined with increasing Cd concentrations (Fig 3). Similar results have been reported previously (Wu, Zhang, & Dominy, 2003). Light-induced chlorophyll accumulation was inhibited by increasing Cd concentration. Under Cd stress, the chlorophyll b content of cultivars was more affected than the chlorophyll a content (Fig. 3). Total chlorophyll content decreased approximately by 44% at the lowest Cd concentration (2 mM). A decrease in the photosynthetic activity may be partly due to the decreased chlorophyll content. Additionally, the decrease in photosynthetic activity significantly reduced the biomass of seedlings grown at different Cd concentrations

Carotenoids act as light-harvesting pigments as well, and can protect chlorophyll and membrane destruction by quenching triplet chlorophyll and removing oxygen from the excited chlorophyll– oxygen complex. In this work, carotenoid content of cultivars decreased with increasing Cd concentrations (Fig. 3). As a result, the lowest carotenoid content could not facilitate detoxification of toxic oxidation radicals formed in response to Cd treatments.

Cd Accumulation

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. Results of this research showed that the accumulation of Cd in roots was higher than in leaves (Table 1). 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 wheat 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).

Amylolytic Activity

Seed germination relies almost exclusively on seed reserves for the supply of metabolites for respiration as well as other anabolic reactions. 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 & Varner, 1969). In the present study the average total amylolytic, α -amylase and β -amylase activities were significantly depressed by the higher concentrations of Cd. α -amylase activity was the most sensitive parameter (Table 1). α -amylase is the major enzyme involved in the initial degradation of starch into more soluble forms while phosphorylase and β -amylase assist in the further conversion to free sugars which affords the nutrition of seed germination (Juliano & Varner, 1969). Reduction of amylase activity may therefore be the major factor involved in the depression of seed germination.

Antioxidant Enzymes

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 (Radotic, Ducic, & Mutavdzic, 2000). 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 & 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 & 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 (Somasekaraiah, Padmaja, & Prasad, 1992). 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 SOD and POD activities indicated that seedlings had the capacity to adapt to moderate Cd concentrations by developing an antioxidative defence 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.

The results showed that the activity of CAT decrease in seedlings under Cd stress. This is in agreement with the findings of Shah *et al.* (2001) and Sandalio *et al.* (2001) who studied rice and pea plants. CAT is present in peroxisomes and mitochondria but not in chloroplasts (Mishra, et al., 2006). CAT is sensitive to O_2^- radicals and thus its increasing content under Cd stress may result in inactivation of the enzyme (Cakmak, 2000). The decrease may also be associated with degradation caused by induced peroxisomal proteases or may be due to photoinactivation of enzyme (Sandalio, et al., 2001).

Lipid Peroxidation

Lipid peroxidation is the consequence of free radical mediated reactions and is a good indicator of prevalence of oxidative. Increasing the Cd resulted in increase in the MDA level.

Increased production of toxic oxygen free radicals was generated by Cd. The unsaturated fatty acid components of membrane lipids are highly susceptible to these free radical attack and are peroxidized in its presence of which MDA is an indicator (Kappus, 1985). In the present study MDA increased with increasing Cd concentrations, indicating that the degree of damage to membrane lipids depended on the Cd concentrations in the culture medium.

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