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Original Research Paper

Cadmium Chloride Induced Biochemical Changes in *Triticum aestivum* (L.) Var. MP LOK 1

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ABSTRACT

The seeds of *Triticum aestivum* var. MP LOK1 were sown in earthen pots and cadmium chloride treatment was given. The plants were treated with increasing concentrations of cadmium chloride i.e., 25, 50 and 100 ppm. The aim of this work was to determine the effects of cadmium chloride on the photosynthetic pigments, polyphenol, proline and antioxidative response in *Triticum aestivum*. It is evident from the results that chlorophyll content of the wheat was not much affected by the lower levels of cadmium chloride but significantly affected at 100 ppm level. It was slightly increased only at 25 ppm by 5.10%. The polyphenol content of the leaves was increased at 25 and 50 ppm of the cadmium chloride but decreased by 16.54% at 100 ppm of the metal salt. Maximum increase in proline content was found as 27.36% at 100 ppm of cadmium chloride. The increased levels of polyphenols at elevated levels of cadmium chloride might be to tolerate higher levels of cadmium stress. The elevated activities of catalase and peroxidase under metal salt probably came for maintenance of cellular membranes while decrease at elevated levels of cadmium chloride might be due to the toxic nature of cadmium chloride. The details of the parameters studied are discussed in the present paper.

INTRODUCTION

Environmental pollution is one of the most significant problems that the world faces today. Mining and smelting of metalliferous ores, the use of pesticides, fertilizers, increased liquid and solid waste disposals, landfill leachates, fossil fuel burning, paints, batteries, industrial wastes and land application of industrial or domestic sludge can result in heavy metal contamination of urban and agricultural soils (Blaylock & Huang 2000). The threat that heavy metals pose to human and environmental health is aggravated by their long-term persistence in the environment (Ensley 2000). Additional sources of heavy metals that can cause harm to plants are surface runoff, traffic density, use of oil or fossil fuels for heating, atmospheric dusts, plant protection agents and fertilizers, which could be absorbed through the leaf blades (Atrouse et al. 2004). It is well established fact that abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide. Although many metals are essential for cells, all of them are toxic at higher concentrations (Marschner 1995). One reason, metals may become toxic is that they may cause oxidative stress, especially redox active transition metals, which can take up or give off an electron (e.g., Fe^{++} or Fe^{+++}, Cu^{+} or Cu^{++}) can give rise to free radicals that cause damage (Jones et al. 1991). Another reason why metals may be toxic is that they can replace other essential metals in pigments or enzymes, disrupting the function of these molecules (Rivetta et al. 1997).

Cadmium is a relatively rare element (0.2 mg/kg in the earth crust) and is not found in the pure state in the nature. It occurs mainly in association with the sulphide ores of zinc, lead and copper. It presents a serious hazard to public health and is a threat to most life forms (Breakman et al. 1997). Metal trace elements, including Cd, cause severe damage at each level in living organisms, from populations and communities to cell elements (Schutzendubel et al. 2001). It has anthropogenic effects in activities such as the non-ferrous metal industry, mining, the production, use, and disposal of batteries, and disposal of metal-contaminated waste and sludge. Application of pesticides and phosphate fertilizers leads to dispersion of Cd (Alloway 1995). Nowadays, cadmium-nickel battery manufacture consumes 55% of the cadmium output and it is expected that this application will expand with the increasing use of rechargeable batteries and their potential use for electric vehicles. In many respects cadmium has become a vital component of modern technology, with countless applications in the electronics, communications, power generation and aerospace industries (Yates 1992).

The aim of this work was to determine the effect of the cadmium chloride on the photosynthetic pigments, polyphenol, proline and antioxidative enzymes in *Triticum aestivum.* It is evident from the results that chlorophyll content of the wheat was not much affected by the lower levels of cadmium chloride but significantly affected at 100 ppm level while carotenoid content of the leaves of the wheat was slightly increased only at 25 ppm by 5.10%. The elevated activities of catalase and peroxidase under metal salt probably came from an increased capacity for oxygen radical scavenging and maintenance of cellular membranes while decrease at elevated levels of cadmium chloride might be due to the toxic nature of cadmium chloride. The details of the results obtained on biochemical parameters of the wheat are discussed in the present paper.

MATERIALS AND METHODS

The seeds of the *Triticum aestivum* var. MP LOK1 were obtained from the market of Pune. The seeds were sown in the earthen pots and allowed to grow naturally for a week and then the cadmium chloride treatment was commenced. The plants were treated with increasing concentrations of cadmium chloride i.e., 25, 50 and 100 ppm. Every alternate day, they were watered with a double amount of water to maintain the uniform salt concentration in the pots and to cope up with the loss of water by evaporation from the soil surface and by transpiration from the plant surface.

1. Photosynthetic pigments: (a) Chlorophylls: The chlorophylls of the mature leaves were estimated following the method of Arnon (1949). **(b) Carotenoids:** The carotenoids were obtained by using the method suggested by Kirk & Allen (1965).

2. Polyphenols: The polyphenols content of the leaves was estimated by the method of Folin & Denis (1915).

3. Proline: Proline was estimated from the oven dried leaves by the method described by Bates et al. (1973).

4. Enzymes: (a) Catalase (E.C. 1.11.1.6): The activity of Catalase in the fresh mature leaves was determined by the method described by Sadasivam & Manickam (2008). **(b) Peroxidase (E.C. 1.11.1.7):** Peroxidase from fresh mature leaves was determined the method described by Maehly (1954).

Statistical analysis of the data was carried out by using GraphPad software. Mean, standard deviation and percent variation were calculated. 'One Way Analysis of Variance' (ANOVA) was tested in order to see the statistical difference among the means. Tukey-Kramer multiple comparison test of significance was carried out which suggested the variation among the column means is significant or not at different levels of significance. The data were analysed for three different levels of significance based on the 'p' values as 1) * significant ($p < 0.01$ to 0.05), 2) ** very significant ($p < 0.001$ to 0.01) and 3) *** extremely significant ($p < 0.001$).

RESULTS AND DISCUSSION

Chlorophylls: The effect of cadmium chloride on chlorophyll content of the mature leaves of *Triticum aestivum* is shown in Table 1. It is evident from the results that chlorophyll content of the wheat is not much affected by the lower levels of cadmium chloride but significantly affected at 100 ppm level. It is observed that the total chlorophyll content of the mature leaves was slightly increased due to increasing concentrations of $CdCl_2$ at 50 ppm. It is also evident that chl. *a*/chl. *b* ratio was decreased considerably in the leaves of *Triticum* and showed a perfect negative correlation with the increasing level of the cadmium chloride to the pot soil culture. The chl. *b* content of the leaves was observed to be increased significantly at all treatments of cadmium chloride with highest increase by 37.57% at 25 ppm, while chl. *a* appears to be more sensitive to cadmium chloride than chl. *b*.

Chlorophyll content in plants correlates directly to the healthiness of plant (Zhang et al. 2005). Exposure to cadmium significantly decreases the chlorophyll content (Azevedo et al. 2005). Cadmium chloride decreases chlorophylls and chl. *a*:*b* ratio (Jana & Choudhury 1982). Kupper et al. (1996) were of the opinion that cadmium has an ability to replace magnesium, a central atom of chlorophyll molecule. Oancea (2006) observed the significant decrease in concentration of chlorophylls and carotenoids under the influence of cadmium.

From the present investigation, it is clear that the total chlorophyll content in the leaves of wheat was increased at lower levels of cadmium chloride. Such an increase in the chlorophyll content might be due to the osmotic adjustment mechanism developed by the plants while a decrease at 100 ppm level might be associated with the disruption in cellular functions, membrane deterioration, and damage to photosynthetic electron transport chain due to accumulated ions. The drastic reduction in chl. *a*:*b* ratio at higher levels of metal salt indicates that chl. *a* might have been replaced by chl. *b* which is in accordance with the results obtained by Sandalio et al. (2001).

Carotenoids: The effect of cadmium chloride on carotenoid content of mature leaves of the *Triticum aestivum* is shown in Table 2. It is evident from the results that carotenoid content of the wheat is affected by the cadmium chloride. It was slightly increased only at 25 ppm by 5.10 %.

According to Armstrong (1996) carotenoids have two major functions in photosynthesis. They protect chloroplast from photo-oxidative damage and also act as accessory light harvesting pigments. Oancea (2006) observed that cadmium produces a significant decrease in concentration of carotenoids.

It appears that the total carotenoid content in the leaves of *Triticum* was reduced due to higher level of cadmium chloride, which might seem to be toxic to this accessory pigment while the slight increase at lower level of cadmium chloride might be due to the protective nature of the carotenoids.

Polyphenols: The influence of cadmium chloride on polyphenol content of the leaves of *Triticum aestivum* is shown in Table 2. It is evident that polyphenol content of the leaves was increased at 25 and 50 ppm of the cadmium chloride but decreased at by 16.54% at 100 ppm of the metal salt.

Very little attention has been paid towards the influence of metal salts on the polyphenol metabolism in plants. Treatment of the barley plants with Cd (1.10-6 mol/L) in a nutrition solution caused the increase in the total polyphenols in all parts of the plant (Dudjak et al. 2004). Stress caused by Cd affects the level of polyphenol compounds as protective secondary metabolite compounds in barley

(Wu et al. 2003). The increase of total polyphenols content as a response to Cd stress was also observed by Baisakhi et al. (2003) in the tolerant clone of *Chloris barbata* Sw.

From the present investigation, it is clear that the increased levels of polyphenols at elevated levels of cadmium chloride induce accumulation of secondary metabolites in the experimental species in order to tolerate higher levels of cadmium chloride stress and adverse conditions aroused.

Proline: The effect of cadmium chloride on proline content of the leaves of *Triticum aestivum* is given in Table 2. It is clear from the results that the level of proline in the leaves was considerably increased and showed a perfect positive correlation with the increasing levels of cadmium chloride to the pot soil culture. Maximum percent increase in proline content was found to be 27.36% at 100 ppm of cadmium chloride.

Proline accumulation in response to environmental stresses has been considered by number of authors as an adaptive trait concerned with stress tolerance and it is generally assumed that proline is acting as a compatible solute in osmotic adjustment (Larher et al. 1993). Proline production decreases the osmotic potential of cells, which can increase the uptake of water (Hare et al. 1998). Additionally, increased level of free proline is an indicator of protein degradation (Yoshiba et al. 1997). A large body of data for proline suggests significant beneficial functions under metal stress and, in general, the molecule has three major functions namely metal binding, antioxidant defence and signalling (Sharma & Dietz 2006).

The increased levels of proline in the leaves of wheat might be to maintain osmoregulation, stabilization of proteins and conservation of energy for a post-stress period.

Antioxidants: In plants, the links between reactive oxygen species (ROS) production and photosynthetic metabolism are particularly important (Rossel et al. 2002). A high concentration of ROS, such as superoxide radical (O_2^-), singlet oxygen ($1/2O_2$), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) at cellular level causes oxidative stress to biomolecules such as lipids and proteins (Vajpayee et al. 2002). High concentrations of heavy metals elicit oxidative stress in plant cells (Rengasamy & Doran 2002). Greater accumulation of Cd, Ni, and Pb results in greater catalase activity (Fayigaa et al. 2004). Cadmium has no effect on superoxide dismutase activity but increases ascorbate peroxidase, syringaldazine peroxidase and guaiacol peroxidase and the activities are observed to be always higher in roots than in leaves (Milone et al. 2003).

The activities of enzymes involved in detoxification of hydrogen peroxide in chloroplasts, were markedly enhanced in barley cultivar Hamidiye by increasing Cd supply (Tiryakioglu 2002). Wu et al. (2003) reported that Cd-stress induced a concentration and gene type dependent oxidative stress response in barley leaf blades, characterised by an accumulation of malondialdehyde and the alteration pattern of antioxidative enzymes.

Catalase (E.C. 1.11.1.6): The activity of this enzyme was observed to be stimulated by cadmium chloride and was highest by 208.0% at 25 ppm level (Table 3).

Catalase is a common enzyme found in nearly all living organisms, where it functions to catalyse the decomposition of hydrogen peroxide to water and oxygen. The reaction of catalase in the decomposition of hydrogen peroxide is as follows (Scandalios 1994).

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Hydrogen peroxide is a harmful by-product of many normal metabolic processes and to prevent damage, it must be quickly converted into other less toxic substances. To end this, catalase is

Table 1: Effect of cadmium chloride on chlorophyll content of the leaves of *Triticum aestivum.*

Sr. No.	Chlorophyll	Cadmium chloride (ppm)			
		Control	25	50	100
1.	Chl. *a*	115.28 0.94	102.79*** (±3.82)-10.83	86.00*** (3.69)-25.40	58.05** (3.05)-49.65
2.	Chl. *b*	118.47 (3.30)	162.98*** (3.68)+37.57	156.72*** (1.84)+32.28	136.79*** (5.04)+15.46
3.	Total chl.	233.68 (2.76)	259.23*** (7.29)+10.93	242.64*** (5.47)+3.83	194.77*** (6.24)-16.65
4.	Chl. *a* : Chl. *b* ratio	0.97 (0.03)	0.63*** (0.01)-35.24	0.55 (0.02)-43.65	0.42 (0.03)-56.38

Table 2: Effect of cadmium chloride on polyphenol, carotenoid and proline content of the leaves of *Triticum aestivum.*

Sr. No.	Name of the Component	Cadmium chloride (ppm)			
		Control	25	50	100
1.	Polyphenol	493.78 20.48	602.65*** (14.68)+22.05	670.69*** (15.43)+35.83	412.13*** (23.57)-16.54
2.	Carotenoid	154.67 2.01	162.56*** (1.86)+5.10	107.73*** (2.44)-30.34	93.71*** (1.09)-39.41
3.	Proline	16.21 (0.39)	17.87 (0.92)+10.23	18.63* (0.78)+14.91	20.65*** (0.97)+27.36

Table 3: Effect of cadmium chloride on catalase and peroxidase activity of the leaves of *Triticum aestivum.*

Sr. No.	Name of the component	Cadmium chloride (ppm)			
		Control	25	50	100
1.	Catalase	0.467 (0.013)	1.438*** (0.061)+208.0	1.065** (0.037)+128.14	0.822** (0.036)+76.03
2.	Peroxidase	0.772 (0.036)	2.078*** (0.054)+169.07	1.227*** (0.053)+58.94	1.146*** (0.033)+48.38

Each value is expressed as mg/100 g fresh tissue
Values in parenthesis indicate standard deviation
Each value is mean of three determinations

*Significant (p = 0.01 to 0.05)
**Very Significant (p = 0.001 to 0.01)
***Extremely Significant (p < 0.001)

frequently used by cells to rapidly catalyse the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani et al. 1996).

Tiryakioglu (2002) observed that compared to SOD, GR and AP, activity of catalase was less affected by the Cd treatments in two barley cultivars. However, the difference in catalase activity between both cultivars became minimal with increasing Cd supply. Generally, increasing Cd supply tended to reduce and enhance activity in Tokak and Hamidiye, respectively.

The elevated activities of catalase under metal salt probably came from an increased capacity for oxygen radical scavenging and maintenance of cellular membranes, which indicates the relationship between metal tolerance antioxidant defence system, while decrease at elevated levels of cadmium chloride might be due to the toxic nature of cadmium chloride.

Peroxidase (E.C. 1.11.1.7): The influence of cadmium chloride on the activity of peroxidase (POD) in the leaves of ***Triticum aestivum*** is given in Table 3. It is clear from the results that the activity of this enzyme was considerably increased by 169.07% over the control especially at 25 ppm cadmium chloride in the leaves of the wheat.

POD is widely distributed in higher plants where it is involved in various processes, including lignification, auxin metabolism, salt tolerance and heavy metal tolerance (Passardi et al. 2005). Peroxidase typically catalyses a reaction of the form:

$$ROOR' + \text{electron donor } (2\, e^-) + 2H^+ \rightarrow ROH + R'OH$$

The increase in the activity of peroxidase under salinity stress may be regarded as an inhibition of stimulated secondary metabolism. It may also be involved in scavenging the reactive oxygen species, particularly in plants grown under stress condition.

CONCLUSION

In the present study sensitivity of wheat to Cd toxicity and its effect on photosynthetic pigments, polyphenols, proline and for the levels of antioxidant enzymes was studied. From the present investigation, it is clear that the total chlorophyll content in the leaves of wheat was increased at lower levels of cadmium chloride and such an increase in the chlorophyll content might be due to the osmotic adjustment mechanism developed by the plant. However, the drastic reduction in chl. ***a***:***b*** ratio at higher levels of metal salt indicates that chl. ***a*** might have been replaced by chl. ***b***. The total carotenoid content in the leaves of ***Triticum*** was reduced due to higher level of cadmium chloride which might seem to be toxic to this accessory pigment while the slight increase at lower level of cadmium chloride might be due to the protective nature of the carotenoids.

The increased levels of polyphenols at elevated levels of cadmium chloride might be to tolerate higher levels of cadmium stress and to minimise the adverse conditions aroused, while the increased levels of proline in the leaves of wheat might be to maintain osmoregulation and stabilization of proteins. The elevated activities of catalase and peroxidase under metal salt probably came from an increased capacity for oxygen radical scavenging and maintenance of cellular membranes while decrease at elevated levels of cadmium chloride might be due to the toxic nature of cadmium chloride.

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