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Cadmium Induced Changes in Pigment Content, Ion Uptake, Proline Content and Phosphoenolpyruvate Carboxylase Activity in *Triticum Aestivum* Seedlings

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Abstract: Heavy metals are important environmental pollutants and their toxicity is a proplem increasing for ecological, evolutionary, and environmental reasons. In Wheat seedlings (*Triticum aestivum*), uptake of ions, chlorophyll content, shoot and root growth were inhibited by adding 0.05mM cadmium to the nutrient medium. Our results also depicted an inverse relationship between biomass (dry mass data) and proline accumulation in the tested plant under stressed conditions. Cd stress also enhanced the activity of PEP carboxylase (E.C, 4.1.1.31) in leaves while it was slightly inhibited in root tissue.

Keywords: Wheat, pigment content, ion uptake, proline, cadmium, Phosphoenolpyruvate carboxylase activity.

INTRODUCTION

Pollution of the environment by heavy metals underpins much interest in their action on plants. Consequently, there is considerable published information about the action of Cd^{2+} on plant growth and on physiological and biochemical processes. Cadmium is readily taken up and accumulated in trace quantities, but is not essential for plant growth (Sanita and Gabbrielli, 1999). Accumulation of Cd^{2+} has been reported for plants grown in hydroponics, pot culture and under field conditions (Hernandez *et al.,* 1996 and 2004 and Benavides *et al.,* 2005). Cadmium negatively affects plant growth and development; it is recognized as an extremely significant pollutant due to its high toxicity and large solubility (Hernandez *et al.,* 1996; Pinto *et al.,* 2004). The mechanisms of Cd^{2+} toxicity are not completely understood yet. Harmful effects produced by Cd^{2+} might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins (Gouia *et al.,* 2000 and 2004). Cadmium can alter the uptake of nutrients by plants through its effects on the availability of minerals from the soil (Ramon *et al.*, 2003). Several studies have suggested that an oxidative stress could be involved in Cd^{2+} toxicity , by either oxygen free radical production, or by decreasing enzymatic and non-enzymatic antioxidants (Sandalio *et al.,* 2001; Fornazier *et al.,* 2002; Cho and Seo, 2004; Kuo and Kao, 2004; Cho and Seo, 2004; Surjenru *et al.,* 2007). The metal is taken up into the plants more readily from solutions than from soil (Sanita and Gabbrielli, 1999), chlorosis, leaf rolls and stunting are easily visible symptoms of cadmium toxicity in plants.

In this work, the effect of imposing cadmium (0.05mM) in the nutrient medium on growth, ion uptake, chlorophyll and proline contents as well as on the activity of PEP carboxylase has been investigated in order to assure a readjustment of the co-ordination between N and C metabolism via the modulation of PEP carboxylase.

MATERIALS AND METHODS

Wheat grains were sterilized with 2.5% sodium hypochlorite for 15 min. and washed extensively with distilled water. They were then germinated in Petri dishes with wetted filter papers at 35°C in the dark. After 48 hr. incubation, uniformly germinated seeds were selected and cultivated in a 250 ml beakers containing complete Hoagland solution. The hydroponically cultivated seedlings were grown-on under illumination of $90 w/m^2$ using fluorescent lamps, with a 14-hr. photoperiod and temperature of 25°C. Ten-day-old seedlings were used to study the effect of Cd^{2+}. Cadmium acetate was added to the nutrient medium to make a concentration of 0.05mM. In preliminary experiments this concentration was shown to provide obvious retardation of shoot growth. Physiological measurements were done 6, 24 and 48 hours after addition of Cd^{2+} to the nutrient medium.

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Determination of Pigments, Proline and Soluble Protein Contents:

Chlorophyll Determination:

About 1.0g of fresh leaf tissue was ground using 10 ml of 80 % acetone. The mixture was then centrifuged for 15 min. at 3000rpm. The supernatant was used to determine chl. A, chl. b and carotenoids by reading the absorbance at 663, 646 and 470 nm against acetone.

Proline determination: Proline was measured according to the method described by Bates (1973). Protein content was determined by the method of Bradford (1976) with BSA as the slandered protein.

Element Analysis:

Ten mg of dry matter (of shoots or roots) were digested in 1 ml of 60 % ultra pure nitric acid at 300°C for 6 hours (Netondo *et al.,* 2004). Elements were then quantified using atomic absorption spectrophotometer (Model AA-6800, Shimadzu) Nitrate content was measured according to the method described by Cataldo *et al.* (1975).

Extraction of PEP Carboxylase:

Approximately 1.0g of leaf tissue was ground in a prefilled mortar with purified sand and 10 ml of ice cold homogenization buffer, which has the following composition: 0.1M Tris.Hcl (pH 7.8), 0.5mM EDTA, 1mM MgSO4 and 1mM DTE (freshly prepared). The extract was centrifuged in a Beckman-centrifuge (model 2-21) for 25 min. at 16.000 rpm. Approximately 0.3 g of solid PVP was added to 3ml of the supernatant and mixed vigorously with a stirrer for 3 sec., then the mixture was centrifuged at 4°C for 10 min. at 8000 rpm. The clear supernatant was used as the source of the enzyme.

Assay of PEP Case:

Activity of PEPCase was determined spectrophotometrically as described by Blanke *et al*. (1986) at 340nm by coupling the reaction to the oxidation of NADH in the presence of malate dehydrogenase (MDH). The standard assay medium contained the enzyme extract, 10 units of MDH, 0.1mM NADH, 2.5mM MgSO4 and 5mM $NaHCO_3$ in a total volume of 2.95ml 50mM Tricine buffer (pH 8.8). The reaction was started by the addition of 50µl of PEP at 2.2 mM final concentration. The rate of oxidation of NADH was measured 15 sec. after the addition of PEP over 3 min. The reaction was observed using the visual display of the spectrophotometer (Cecil CE 7200 split-beam spectrophotometer) to confirm the adequate mixing of the cuvette contents and that NADH oxidation caused by the reaction was linear. Assays were done in duplicate.

RESULTS AND DISCUSSIONS

Results:

Wheat seedlings grown in the presence of 0.05mM Cd^{2+} in the nutrient medium showed significant growth reduction within the time of exposure in both shoots and roots (Table 1).

Table 1: Effect of Cadmium in the nutrient medium (0.0 and 0.05mM) on both shoot and root fresh and dry weights of *Triticum aestivum* after 6, 24 and 48 hours of exposure.

	Shoots (mg)				Roots (mg)			
	0.0 Cd		0.05mM Cd		0.0 Cd		0.05mM Cd	
Hours after exposure	F.wt.	D.wt.	Fwt	D.wt.	F.wt.	D.wt.	Fwt	D.wt.
6	187±12	18.2±0.2	181±11	17.3±0.1	111±10	10.8±0.2	119±11	11.3±0.2
24	217±22	20.2±0.3	199±12	18.7±0.2	126±9.0	11.6±0.2	122±13	10.8±0.1
48	247±21	23.4±0.2	208±21	20.2±0.3	140±9.4	12.8±0.1	128±9.9	10.2±0.1

Values represented means ±SE, n = 4

In the controls fresh weight of both roots and shoots increased linearly over the 48 hr. experimental period, the growth rates were 0.15 and 0.30g fresh weight d^{-1} for roots and shoots respectively. Cadmium did not affect the growth of either roots or shoots over the first 6 hours .Thereafter, the fresh weights increased linearly with time, though with lower rates than in the controls: 0.06 and 0.09 g fresh weight d^{-1} for roots and shoots respectively. At the end of the experimental period, the leaves of Cd^{2+}-treated plants showed symptoms of chlorosis and necrotic areas began to be evident at their tips. Roots did not show any apparent damage. Fig. (1) shows the time course of Cd^{2+} accumulation over a 48-hr. period. Cadmium was linearly accumulated in roots up to 48 hr. In shoots, Cd^{2+} concentration increased greatly in the first 6 hours, although to a lesser

extent than in roots, and then remained constant till 48 hr. The concentration of Cd^{2+} in roots was about 2-fold higher than in shoots during the time of exposure. During the first 6 h after adding cadmium to the nutrient medium, a clear inhibition of ion uptake was observed (Fig. 2). It accounted for 53%, 38%, 71% and 37% for K^+, Mg^{2+}, NO_3^- and Ca^{2+} respectively.

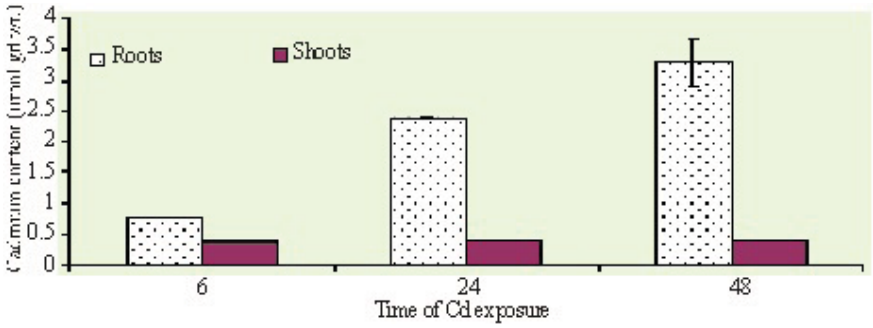

Fig. 1: Time course of Cd^{2+} accumulation in roots and shoots of wheat seedlings grown in a complete nutrient solution supplemented with (or without) 0.05mM cadmium acetate.

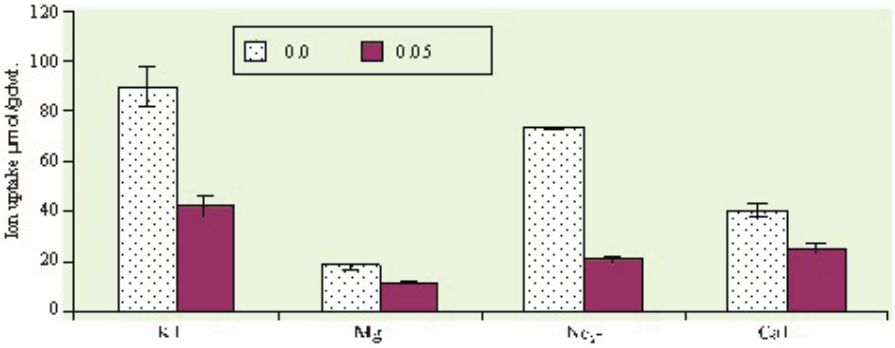

Fig. 2: Ion uptake by control and Cd-treated wheat seedlings 6 hours after exposing the plants to 0.05mM Cd in the nutrient medium.

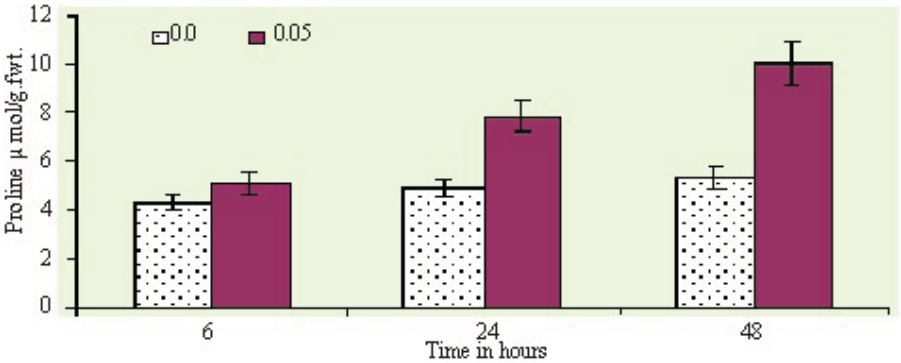

Fig. 3: The effect of cadmium on proline accumulation in wheat plants.

The treatment resulted in a significant increase in proline content on the account of biomass (dry mass data). Proline accumulated in Cd^{2+} treated plants and reached 50% increase at the end of experiment (Fig.3). A decrease of total chlorophyll content by 14% and 25% as compared to the control was recorded after 24 hr. and 48hr. of exposure respectively (Table 2).

Cadmium stress enhanced the activity of phosphoenolpyruvate carboxylase (PEPC) in leaves while it was slightly retarded in roots (Fig. 4). Cd^{2+} caused a decrease in root PEPC activation state after 24 hr. of exposure (16.5 µmol NADH/gfwt./hr.) whereas in shoots about 73% increase in enzyme activity was recorded in the treated plants (Fig. 5).

Table 2: Effect of 0.05mM Cd^{2+} in the nutrient medium on pigments content of wheat.

Concentration of Cd (mM) in the nutrient medium	Pigment contents (mg /g F.wt.)								
	Time after exposure to Cd								
	6 h			24 h			48 h		
	Chl.a	Chl.b	Cart.	Chl.a	Chl.b	Cart.	Chl.a	Chl.b	Cart.
0.0	13.80	3.80	1.60	13.50	3.40	1.70	13.60	3.60	1.70
0.05	13.00	3.20	1.20	11.50	2.88	1.00	10.60	2.22	0.90

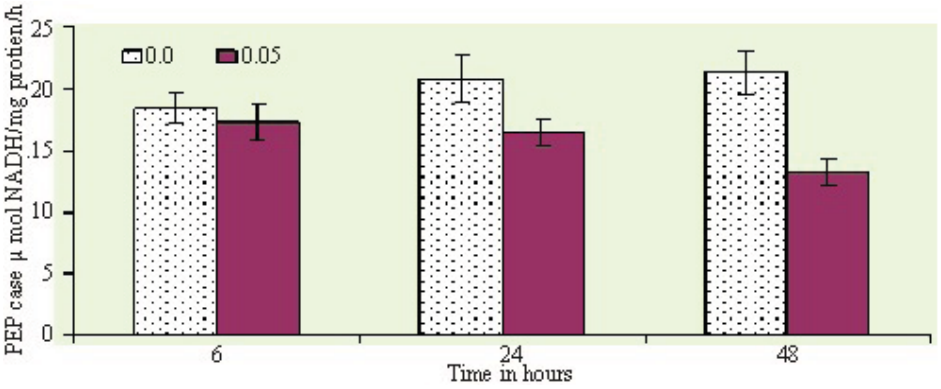

Fig. 4: Activity of PEPCase in roots of wheat seedlings subjected to 0.0 and 0.05mM Cd in the nutrient.

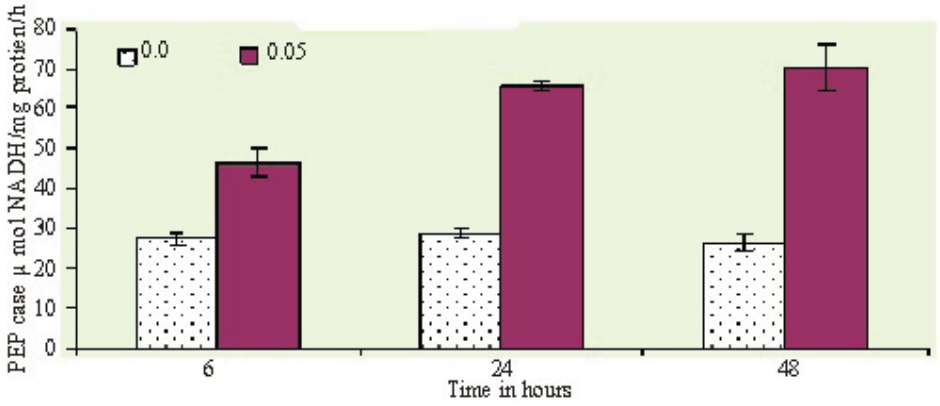

Fig. 5: Activity of PEPCase in shoots of wheat seedlings subjected to 0.0 and 0.05mM Cd in the nutrient.

Discussion:

Cadmium treatment led to an inhibition of growth rate and ion uptake in wheat seedlings. Inhibition of root function was evident in terms of reduction of ion uptake. This may not be surprising since roots are the first to come in contact with the injurious cadmium. Chlorophyll and carotenoids are the central part of the energy manifestation of every green plant system and therefore, any significant alteration in their levels is likely to cause a marked effect on the entire metabolism of the plant.

In the present study, Cd^{2+}exposure significantly decreased the total chlorophyll content of the tested plant; a slight decrease in carotenoids was also detected. .Reduction of chlorophyll content by excess Cd^{2+} has been reported in *Riccia* sp. by Prasad *et al.* (2004) who concluded that high Cd^{2+} inhibits the formation of chlorophyll by interfering with protochlorophyllide production. Carotenoides protect chlorophyll from photooxidative destruction and therefore, a reduction in carotenoid could have a serious consequence on chlorophyll pigments. Cd^{2+} also reduced the absorption of nitrate by about 70%, this agrees with the data obtained by Hernandez *et al.* (1996) who recorded a reduction of nitrate uptake which was due to inhibition of nitrate reductase activity in the roots of pea seedlings. Exposing plants to heavy metals give raise to a series of reactions which generate numerous free radicals which may be reflected by altered levels of major anion and accumulation of proline.

In this experiment, proline, an osmoprotectant, accumulated upon exposing wheat seedlings to Cd^{2+}. Proline is supposed to participate in the reconstruction of chlorophyll, activates the Krebs cycle and constitutes an energy source (Ramon *et al.,* 2003). It is also an important part of structural proteins and enzymes and participates in repair processes. Proline accumulation under heavy metal stress has also been reported earlier in some higher plants (Parada, 1991; Alia *et al.,* 1991 and 1993). In wheat leaves, PEP carboxylase fulfils a variety of physiological roles. In the anaplerotic pathway, the enzyme contributes to the replenishment of Krebs cycle intermediates when organic acids are directed towards other metabolic pathways such as amino acid and protein synthesis (Melzer and OLeary, 1987; Latzko and Kelly, 1983; Jeanneau *et al.,* 2002; Netondo *et al.,* 2004 and Sebei *et al.,* 2006).

In this investigation, Cd^{2+} induces a 4-fold increase in shoot PEPCase activity after 24 hr. of exposure. Our data agree with those obtained by Gouia *et al*. (2004) who studied the effect of Cd^{2+} on the activities of root and leaf enzymes involved in carbon and nitrogen metabolism in bean seedlings. They concluded that the increase in PEPCase activity by Cd^{2+} was due to *de novo* synthesis of the enzyme polypeptide and also modification of the phosphorelation state of the enzyme. Cadmium may have modified, via a modulation of PEPCase activity, the C flow towards the amino acid biosynthesis. In leaves of *Triticum aestivum*, Cd^{2+} markedly modified specific amino acid content. Proline significantly accumulated compared to those of the control plants.

The present study suggests that Cd^{2+} stress is a part of the syndrome of metal toxicity, we also conclude that I- although *Triticum aestivum* shows slight reduction in growth, it is quite good in resisting the stress caused by cadmium. II- increasing the activity of PEPCase could be due to the readjustment of the co-ordination between N and C metabolism via modulation of the enzyme PEPCase that could therefore avoid the accumulation of toxic levels of ammonium (confirming the results of Astolfi and Passera, 2004 on Maize seedlings). III- Proline, a universal protectant of various stresses, may be used for bioremediation. More knowledge of cadmium uptake and/or partitioning within the plant is of immediate use in reducing Cd^{2+} ingestion by humans. In the longer term, knowledge of the physiological basis of cadmium uptake and partitioning may lead to the development of varieties that contain less Cd^{2+} in the edible portion. This would allow cultivation to continue in moderately polluted soils.

REFERENCES

Alia, P., 1991. Proline accumulation under heavy metal stress. J. Plant Physiol., 138: 554-558.

Alia, P. and P. Mohanty, 1993. Proline in relation to free radical production in seedlings of *Brassica juncea* raised under sodium chloride stress. Plant and Soil, 155/156: 497-500.

Astolfi, S., S. Zuchi and C. Passera, 2004. Role of sulpher availability on cadmium-induced changes of nitrogen and sulpher metabolism in Maize leaves. Plant Physiol., 161: 795-802.

Bates, L., R. Waldrem and I. Teare, 1973. Rapid determination of free praline for water stress studies. Plant and Soil, 39: 205-207.

Benavides, M., S. Gallego and M. Tomaro, 2005. Cadmium toxicity in plants. Brazillian Journal of Plant Physiology, 17: 677-697.

Blanke, M., B. Notton and D. Hucklesby, 1986. Physical and kinetic properties of photosynthetic PEP carboxylase in developing apple fruit. Phytochem., 25: 601-606.

Bradford, M., 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem., 72: 248-254.

Cataldo, D., M. Haroon, L. Shrader and V. Youngs, 1975. Rapid calorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commune Soil Sci. and Plant Anal., 6: 71-80.

Cho, U. and N. Seo, 2004. Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation. Plant Sci., 168: 113-120.

Fornazier, R., R. Ferreira, A. Vitoria, S. Molina, P. Lea and R. Azevedo, 2002. Effects of cadmium on enzyme activities in sugar cane. Biol. Plant., 45: 91-97.

Gouia, H., M. Ghorbal and C. Meyer, 2000. Effect of cadmium on activity of nitrate reductase and other enzymes of the nitrate assimilation pathway in bean.Plant Physiol. and Biochem., 38: 629-638.

Gouia, H., A Suzuki, J Brulfert and H. Ghorbal, 2004. Effect of cadmium on the co – ordination of nitrogen and carbon metabolism in bean seedlings. Plant Physiol., 160: 367-376.

Hernandez, L., A. Garate and R. Carpena, 2004. Effect of cadmium on the uptake, distribution and assimilation of nitrate in *Pisum sativum*. Plant and Soil, 189: 97-106.

Hernandez, L., R. Carpena and A. Garate, 1996. Alterations in the mineral nutrition of pea seedlings subjected to cadmium. J. Plant Nutr., 19: 1581-1598.

Jeanneau, M., J. Vidal, G. Dupont, B. Lebouteiller, M. Hodges, D. Gerentes and P. Perez, 2002. Manipulating PEPC levels in plants. Journal of experimental botany, 53: 1837-1845.

Kuo, C. and H. Kao, 2004. Antioxidant enzyme activities are up regulated in response to cadmium in sensitive, but not in tolerant, rice (*Oryza sativa*) seedlings. Bot. Bull. Acad. Sin., 45: 291-299.

Latzko, E. and G. Kelly, 1983. The many – faced function of PEPCase in C_3 plants .Plant physiol., 21: 805-813.

Melzer, E. and M. O Leary, 1987. Anaplurotic CO2 fixation by PEPCase in C_3 plants. Plant physiol., 84: 58-60.

Netondo, G. J. Onyango and E. Beck, 2004. Sorghum and salinity. Crop Sci., 44: 797-805.

Prasad, S., R. Dwivedi, M. Zeeshan and R. Singh, 2004. UV-B and cadmium induced changes in pigments, photosynthetic electron transport activity, antioxidant levels and antioxidative enzyme activities of *Riccia* sp. Acta Physiol. Plant., 26: 423-430.

Ramon, O., E. Vazquez, M. Fernandez, M. Felipe and P. Zornoza, 2003. Cadmium –stress in white lupine: effects on nodule structure and functioning. Plant Physiol., 161: 911-919.

Sandalio, L., H. Dalurzo, M. Gomez, P. Romerio and D. Riola, 2001. Cadmium- induced changes in growth and oxidative metabolism of pea plants. J. Exp. Bot., 52: 2115-2126.

Sanita, D. and R. Gabbrielli, 1999. Response to cadmium in higher plants. Env. Exp. Bot. 41: 105-130.

Sebei, K., Z. Ouerghi, H. Kallel and S. Boukhchina, 2006. Evolution of Phosphoenolpyruvate carboxylase activity and lipid content during seed maturation of *Brassica napus* L. Plant Biology and Pathology, 329: 719-725.

Surjendu, K., D. Jayashree, P. Sanjukta and P. Debasmite, 2007. Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. Braz. J. Plant Physiol., 19: 219-229.