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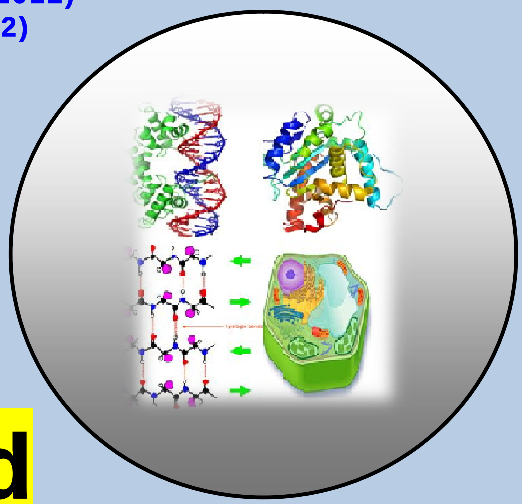

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RESEARCH PAPER

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Cadmium Induced Toxic Effects on Growth and Metabolism of Sugarbeet (*Beta vulgaris* L.) Plants

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ABSTRACT

Stimulation at lower concentration and suppression at higher concentration of cadmium chloride was observed in sugarbeet plants. Concentrations of both chlorophyll and total sugar were also found to be decreased at higher doses of cadmium. However, an enhancement in protein concentration and elevation in the activity of enzyme catalase was observed at 0.25 and 1.0 mM doses of $CdCl_2$ and that of peroxidase activity at increasing doses of cadmium.
Keywords: Cadmium, Toxic effect, Beta vulgaris L., Growth and Metabolism.

INTRODUCTION

Metals can be divided into essential and non-essential types. Essential metal deficiency or excess beyond certain thrash hold concentration give rise to differential effects. However, some non-essential heavy metals are beneficial when they are supplied to plants in low amounts. Animals and plants possess a resistance power against adverse conditions and thus they have a capacity to prevent excessive accumulation of potentially toxic metal species but beyond a certain limit, they lose this resistance power and metal species get accumulated in their bodies causing deleterious effects.

Cadmium (Cd) comes under the category of toxic non-essential heavy metal. Cd exposure to both humans and animals can cause adverse health effects leading to itai-itai disease. Cd accumulates essentially in kidney, liver and gill and cause hypertension, liver damage and is carcinogenic. Godbold and Hutterman et al. 1985, Breckle 1991, Nies 1999, reported that solubility and mobility of metals is affected by absorption, desorption and complexation processes which are dependent on the soil type.

Heavy metals can be transported, eroded or fixed within the soil. The ecological hazards related to the heavy metals are not dependent on their total content in soil but rather on their form of bonding and therefore their bioavailability. The plant's heavy metal absorption is dependent on plant and genus related factors as well as leaching and the concentration of heavy metals in the solution. Thus these toxic metals may enter the human body through plants by becoming a part of food chain. Above facts in view, the present investigation was carried out to study the phenomenon of uptake and translocation of heavy metal and its toxic effects on growth and metabolism of sugar beet plants.

MATERIAL AND METHODS

Experiment was carried out in sand culture conditions. Plants of sugar beet were grown in controlled glass house condition in pots having purified sand in replicates. Controlled plants were provided only basal nutrient solution whereas test plants were treated with different doses of cadmium chloride along with basal nutrient solution. Plants were observed daily for some abnormal changes in the form of chlorosis, necrosis, browning of leaf tissue and other typical toxic symptoms resulted due to cadmium chloride exposure.

The composition of basal nutrient solution was the same as given by Hewitt, 1966. Macro and micronutrient solutions were prepared by using A.R. (Analytical Reagents) grade chemicals. Graded levels of cadmium chloride were super imposed on basal nutrient solution and were supplied in the doses of 0.25, 0.5, 1.0 and 2.0 mM. The basal nutrient solutions (control) along with respective treatment of cadmium chloride were supplied regularly for proper growth of plants. As far as morphological parameters were concerned, numbers of plant branches were counted in each pot and their mean values were taken. Plants were than harvested for taking fresh and dry matter yield. For evaluating the fresh weight of plants, they were taken out from plastic containers, washed with running water followed by distilled water. After taking fresh weight of plants, they were cut into pieces and kept in oven at 70 °C for 3 days after which the dry weight of each plant was measured with the help of electronic balance. For metabolic parameters, fresh leaf was ground with sand in ice chilled pestle and mortar kept in ice bath. 1 g of leaf tissue was extracted in 10 ml of glass distilled water. The homogenate was filtered through two folds of muslin cloth with the help of Buchner funnel and stored at freezing temperature in refrigerator. Leaf extract was used for estimation of various metabolic parameters. Chlorophyll concentration was measured by the method of Ptering, 1940, Protein and total sugars were estimated by the methods of Lowry et al. 1950 and Dubias et al. 1956 respectively.

The activities of antioxidative enzymes were measured in the fresh leaf extracts viz., Catalase was assayed by the method of Bisht, 1972, a modified method of Euler and Josephson 1927, while that of peroxidase was measured by the modified method of Luck (1963).

RESULTS AND DISCUSSION

Growth parameters and visible symptoms

Toxic effects on plant growth were observed at higher doses of $CdCl_2$ treatment (Table 1), although lower concentration, i.e. 0.25 mM of $CdCl_2$ showed stimulatory effects in branching of sugar beet plants. Thickness of stem and chlorosis of leaf and growth along with inter and intra venial necrotic patches were observed at excess amount of cadmium.

Rate of basal leaf shedding was found to be faster at higher doses. Fresh and dry weights of plants were also found to be decreased. Overall growth of plants was found to be adversely affected by higher doses of $CdCl_2$.

Reduced growth of plant at higher doses of cadmium was already reported by number of workers (Dixit et al., 2001, Liao et al., 2003 and Singh et al., 2006). Factors responsible for reduced growth of plants might be associated with abnormal transport of some essential nutrients such as Zn, K and Fe. Deficiency of Zn caused by excess amount of Cd might be a reason of reduced growth as Zn deficiency is reported to be involved in the reduction of auxin through its involvement in the synthesis of tryptophan, a precursor of auxin (Tsui, 1948). In some cases, displacement of chemically related metal ion with heavy metals might be a responsible factor for suppressed growth of plants.

Metabolic parameters

Concentrations of chlorophyll and total sugar were found to be significantly reduced at higher doses of cadmium (Table 2) as compared to controlled treatment. Inhibition in the biosynthesis of chlorophyll in leaves under Cd^{+2} stresses as also found in this study was already reported by Stobart et al. 1985 and Shukla et al. 2003. They reported Cd^{+2} interference with the formation of chlorophyll. Keshan and Mukherjee, 1992 also reported inhibition of chlorophyll biosynthesis due to cadmium. Singh et al. (2005; 2006) have already reported reduced sugar concentration at excess supply of Cd and Cr. This is in conformity with the findings of this study.

Heavy metals such as Cd are responsible for developing water stress conditions to plants and as a result of relative water content (RWC) of plants gets decreased which negatively affect the process of photosynthesis causing reduced sugar content in plants. Lang et al. 1995 reported reduced CO_2 assimilation due to Cd stress condition which is responsible for decreased sugar content in leaves. However, protein concentration was found to be increased at increasing doses of $CdCl_2$. The reason attributed to enhanced protein content in the leaf tissue is probably the synthesis of phytochelatins in response to heavy metal stress. The nature has provided a tendency in plants to protect themselves against heavy metal stress conditions by generating a resistance protein called proline.

Protein content was also found to be increased at increasing doses of both cobalt and lead in gram seeds (Tandon and Gupta, 2002). Kaneta et al. 1983, suggested that increase in protein content might be due to the synthesis of metal binding protein by Cd stress. The results of this study are in agreement with the earlier studies reported above. The activities of two iron enzymes viz. catalase and peroxidase were differentially affected by different doses of cadmium. 0.25 and 1.0 mM doses of $CdCl_2$ caused enhancement in catalase activity of sugar beet plants while highest dose of 2.0 mM $CdCl_2$ caused significant reduction in the activity of other antioxidative enzyme. Peroxidase was found to be stimulated at given doses of cadmium chloride.

The activity of both antioxidative enzymes viz., POD and CAT showed variable trends during the present investigation. In some cases, the activity was found to be enhanced while in other cases it was found to be suppressed. Heavy metal pollution induces plant to produce more peroxidase and enhances POD activity.

Higher POD activity reflects more serious damages happened on plant organs. Liao et al. (2003) observed increased POD activity in *Vicia faba* plant in heavy metal stress condition. Enhanced activity of these antioxidative enzymes might be due to the generation of metal ion induced H_2O_2 and ROS (Reactive oxygen species). An elevated activity of antioxidative enzymes indicates the excessive heavy metal stress conditions in plants and the changes in the activity of enzymes can be correlated with the plant species and heavy metal type.
Catalase activity might be suppressed due to the reduced supply of iron for the synthesis of Catalase enzyme. This might be due to the fact that during heavy metal stress conditions, the transport of essential elements gets blocked which might have resulted into reduced catalase activity. A decrease in catalase activity was also reported by Somashekaraiah et al. 1992.

Table 1. Effect of different doses of cadmium on the growth and biomass of sugarbeet (*Beta vulgaris* L.) plants.

Treatments	Number of shoot branches	Fresh weight (gms)	Dry weight (gms)
Control	14 ±1.00	185 ±16.50	33.5 ±3.70
0.25 mM $CdCl_2$	16 ±1.00	245 ±6.10	50.5 ±2.80
1.0 mM $CdCl_2$	11 ±1.00	140 ±10.60	22.5 ±3.10
2.0 mM $CdCl_2$	8 ±1.00	95 ±5.50	13 ±1.20
CD at 5% P	06.15	31.29	06.56

Table 2. Effect of different treatments of cadmium on the metabolism of sugarbeet (*Beta vulgaris*.L.) plants.

Treatments	Chlorophyll mg/g fresh weight	Protein µg/ g fresh weight	Sugar mg/g fresh weight	Catalase µmoles H_2O_2 split $100mg^{-1}$ fresh weight	Peroxidase $\Delta OD/100mg^{-1}$ fresh weight
Control	2.66 ±0.03	233.20 ±35.48	1.77 ±0.10	152.5 ±2.50	3.37 ±0.17
0.25mM $CdCl_2$	2.79 ±0.02	501.27 ±18.37	1.38 ±0.06	227.5 ±2.50	4.55 ±0.00
1.0 mM $CdCl_2$	2.40 ±0.01	417 ±42.45	0.82 ±0.05	202.5 ±7.50	4.67 ±0.00
2.0 mM $CdCl_2$	2.17 ±0.01	397.33 ±50.06	0.72 ±0.02	112.5 ±2.50	5.03 ±0.10
CD at 5% P	000.15	237.85	00.19	29.45	0.69

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