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Impact of chromium on crop plants with remedial approach

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Abstract: Plant growth, chlorophyll and sugar content of Urad plant were reduces with increasing chromium toxicity in plants. At lower doses of chromium plant showed a significant stimulation in protein content and inhibition at 2.0mM of chromium. While 2.0mM chromium combine with Zn and Fe was able to compensate the ill effects of chromium on the protein synthesis of plants. A significant enhancement was observed on the activity of enzyme catalase at lower dose of chromium except at 2.0mM dose of this metal. However, peroxidase activity also showed stimulatory effect with chromium activity.

Keywords: Urad (*Vigna mungo L.*), Chromium, Chlorophyll, Sugar content and Activity of enzymes

Introduction

Chromium is the 7^{th} most abundant element on the earth and 21^{st} in the crustal rock (Mc. Grath and Smith., 1990). Its abundance in earth crust ranges from100 to 300 micro gram / gram. Soil may contain between 5 to 3000 micro gram / gram of chromium per gram (Shewry and Peterson, 1979). Chromium has manr oxidation state among them chromium VI is most abundant form followed by Cr III species. Chromium VI is considered to be most toxic among different valences, which is usually associated with oxygen as chromate (CrO_4) or dichromate (Cr_2O_7) ions. While the Cr III in the form of oxide, hydroxide or sulphate is less mobile, exist usually bound to organic matter in soil & aquatic animals (Mc Grath & Smith, 1990). Chromium III is sparingly soluble & less toxic while Cr VI being more soluble in H_2O and is highly toxic to living system (Nieboer and Richardson, 1980). Several metabolic processes also interrupted by Cr in such cases it exhibit retarded growth, biomass & photosynthesis and shows the symptoms of chlorosis and necrosis followed by death. (Mc Grath, 1982; Satyakala and Jamil, 1992; Sinha *et al.*, 1993 Gaur *et al.*, 1994 ; Sharma *et al.,* 1995). Different level of Cr accumulate in plant differentially in which maximum accumulation takes place in roots followed by leaves and rhizome of Nymphea alba L. (Vajpayee *et al.*, 2000). He further observed induced Cr toxicity at 1micro m resulting in the buildup of aminolaevulinic acid (ALD) and reduced activity of alpha aminolaevulinic acid dehydratase (ALAD), nitrate reductase (NR), total chlorophyll & protein content. Both chlorophyll 'a' & 'b' decreased at 1micro m concentration of Cr but chlorophyll 'a' is more sensitive to chlorophyll 'b' at lower concentration of Cr (1.25 mg/L).

Germination % of seed derived from mine waste dumps and control site (uncontaminated) were 96.4% and 78.45% respectively. With doubling of Cr concentration 1.25 to 2.5 mg/L. The percent of seed germination decrease with 13% (Gyana et al., 2000).When the plants of cauliflower were exposed to heavy dose of Cr, Co and Cu in refined sand, the plant produce characteristic phyto-toxicity symptoms. Excess supply of each heavy metal reduces biomass in cauliflower. These metal also decline Fe chlorophyll 'a' and 'b' , protein content and activity of catalase in the leaves. Transport of Cr in aerial part of the plant was also reduced & every heavy metal caused reduction in micro and macro nutrient content in different part of the plant. Transfer of nutrient was found to be least effective by Cr as compared to Co and Cu (Chatterji and Chatterji, 2000). An appropriate amount of Cr was seen in Date palm leaves, which was consumed by live stock and finally enters into the food chain. Excess accumulation of Cr in leaves through soil cause chlorosis. Fruit of Date palm was devoid of Cr and can be consumed by animals (Pillary *et al.*, 2003). harma *et al.*, (2003) conducted an experiment in maize plant to show the visible symptoms and some metabolic parameters in plants. Visible toxicity symptoms include inter-veinal chlorosis, vein clearing in young leaves, marginal leaf curling with yellowish nacrotic patches. The concentration of chlorophyll 'a' and 'b' were decreased on exposure to Cr, while the enzymatic activity like phenyl-phosphate ribo-nuclease increase & other iron enzyme catalase decreases. The activity of amylase was reduced on exposure of Cr. Reduction in grain production and protein content was observed in Cr treated plants at 0.05,0.1,0.25 and 1.0mM dose. Inter-veinal chlorosis and curling of leaves was also reported by Bonet *et al.,* (1991). Cr VI decrease activity of ribo-nuclease & acid phosphate in germinating pea seeds grown in media containing Potassium dichromate (Dua and Sawhney, 1991).

Material and Mathods

Short-term experiments were carried out by using petridishes culture. Such experiments were performed initially by washing these petridishes with some common detergent (Vim). Tap water washing was followed by Hcl washing and thus making these glass petridishes free from contaminations. Petridishes were finally washed with de ionised and glass distilled water. Experimental seeds were soaked in controlled nutrient solution and solution of different treatment of heavy metal (s). High quality of whatman filter paper were placed in petridishes then soaked with controlled solution and solution of various treatment in replicates in the temperature range of 20 to 30^{0}C. Thirty five seeds were germinated in each petridishes and after 12 hours of soaking nutrient solution were changed leaving enough nutrient solution to cover a height of about 2mm. The subsequent were made at intervals of 24 hours. Solution of respective heavy metal was superimposed on the basal solution.

Graded level of Cr supply to urad and use petridishes culture technique. The nutrient solution had the following composition as- Ca (No_3) - 8 Meq/L, KNo_3 - 4 Meq/L, $MgSo_4$ - 4Meq/L, NaH_2Po_4 - 4, Fe - 5.6 ppm, Mn - 0.55 ppm, Cu - 0.046 ppm, Zn - 0.065 ppm, B - 0.37 ppm, Mo - 0.05 ppm, Cr - 0.006 ppm (Hewitt, 1966 and Jacobson, 1941). Catalase activity was measured by Bisht (1972) and a modified method of Euler and Josephson (1927). Peroxidase activity measured by Luck (1963). Chlorophyll content was measured by Petering (1940). Total sugar content was estimated by Dubais *et al.* (1953) and protein content was estimated by Lowery *et al.* (1951).

Result and Discussion

Plant growth and visible symptoms: Increasing doses of Cr caused reduction in the root and shoot length of urad plants. However a combined dose of 2.00mM Cr with 56ppm iron caused slight recovery in shoot and root length of urad seedling. However when the same dose of Cr was combined with 13ppm of Zn it gave a significant enhancement in the root lengths of the plants (Table 1). Thickness of the stem of urad plants was adversely affected with increasing level of Cr in plants. Damage of root tip was the common symptom of Cr toxicity in plants. Damage of root tip was also observed at highest dose of Cr.

Chlorophyll and sugar content: Chlorophyll and sugar concentration was found to be reduced at increasing doses of Cr and also even at combined doses of Cr with Fe and Zn (Table 1).

Protein content: In protein concentration a significant stimulation was observed at 0.50 and1.00 mM of Cr. However, a dose of 2.00mM Cr alone was proved to be quite toxic for this parameter. Encouraging results were obtained when this toxic dose of Cr (2.00mM) was combined either with 56ppm Fe or 13ppm Zn. Thus Fe and Zn in when combined with 2.00mM Cr was able to be negate the ill effect of excess Cr, rather these essential elements (Fe and Zn) caused stimulation in the synthesis of protein in urad plants (Table 1).

Anti-oxidative Enzymes: The activity of other iron *viz.* catalase was also found to be significantly enhanced at different doses of this heavy metal. Fe and Zn was able to stimulate the enzyme activity when combined with other wise irresponsive dose of 2.00mM Cr. (Table 1). Other anti-oxidative enzyme peroxidase also showed stimulatory effect with Cr toxicity. Maximum stimulation in the activity of this enzyme was observed at lowest dose of Cr. Combination of highest dose of Cr with Fe and Zn was also found to be helpful in increasing the activity of this enzyme, as compared to a dose of only 2.00mM Cr in Urad plant.

Typical phytotoxic symptoms of heavy metals (Cd,Co,Cr) include chlorosis first in younger and than in older leaves and also reduced plant growth. The chlorotic reponce of plants has been attributed to interference in iron metabolism. Heavy metals such as Mn, Cu, Zn, Co and Ni used to produced visible symptoms in plants. Some of these symptoms resembled with the symptoms of iron deficiency, while others are somewhat specific to a particular heavy metal (Agarwala *et al.*, 1977).Reduction in plant growth was more vigorous at higher doses (2 and 4 mM) of cobalt, cadmium and chromium. Reduced growth of plants might be due to abnormal transport of essential nutrients including zinc. Asheavy metal interfere in iron metabolism and reduce the transport of essential nutrients like K, Fe to meristmatic (Foliar and bud) S regions of plants. Absence of some essential nutrients in the meristmatic regions of plants may also caused reduced plant growth. Lack of growth might be due to deficiency of Zn, which help in the synthesis of auxin. Some essential nutrients are also known to be constituents of cytoplasm and enzymes. Stunted growth of plant due to excess amount of Cd was reported by Larbi *et al.*, (2002) and due to Co and Cr was reported by different workers (Bisht, 1972; Vazquez *et al*., 1987; Bisht and mehrotra,1989; Tripathi and Tripathi,1999; Gopal *et al*.,2003). Severe chlorosis red brown coloration, necrosis and retarded growth of appendages were observed during the experimental period. Chlorosis is also a symptom of Fe deficiency and abnormality in the synthesis of chloroplastic pigments in plants Chlorosis may also be due to some enzymes which do not work properly in absence of mineral elements such as K, Ca, Mg, Mn and Zn. Heavy metal may disrupt membrane of the root tips and other cells that affect absorption and transport of some essential nutrients such as Fe, Mg etc. (Bisht, 1972; Yang *et al*., 2001; Zornoza *et al*., 2002; Tandon

Table-1: Effect of different treatments of chromium on physical and biochemical parameters in urad (*Vigna mungo* L.) plants

Treatments	Shoot length (cm)	Root length (cm)	Chlorophyll (mg/g fresh weight)	Sugar content (mg/g fresh weight)	Protein content (mg/g fresh weight)	Catalase activity (micro mole H_2O_2 split /100mg fresh weight)	Peroxidase activity (OD/100mg fresh weight)
0.50mM Cr	18.400+0.424	6.350+0.070	1.580+0.014	0.725+0.106	28.750+0.339	40.000 +7.071	1.450+0.070
0.50mM Cr	13.450+0.070	6.115+0.120	1.450+0.000	0.500+0.000	35.880+0.566	280.000+7.071	2.175+0.035
1.00mM Cr	12.315+0.403	5.250+0.070	1.235+0.007	0.400+0.000	31.360+0.000	190.000+0.000	1.825+0.035
2.00mM Cr	9.695+0.049	4.600+0.141	0.035+0.007	0.150+0.070	15.365+0.672	147.500+3.536	1.725+0.035
2.00mM + Fe	12.910+0.014	5.465+0.021	1.155+0.021	0.360+0.014	31.390+0.014	190.000+0.000	1.910+0.014
2.00mM + Zn	13.005+0.007	6.700+0.028	1.205+0.007	0.380+0.000	31.825+0.016	207.500+3.536	1.940+0.000
C.D. at 5%	1.05	0.379	0.050	0.227	1.684	19.854	0.167

* Values represented the mean + SD of three replicates

and Gupta, 2002; Larbi *et al*., 2002). It appears that all the heavy metals viz. Co, Cd and Cr taken in this study cause reduction in iron concentration in the leaves. It appears that heavy metal in excess amounts are responsible for causing adverse effect on iron metabolism in plants. This might have resulted due to reduced transport of iron from roots to shoots Iron is known to be involved in the biosynthesis of chlorophyll and carotenoids (Pascal *et al*., 1995). Alteration in sugar concentration also took place in heavy metal treated plant. In the present study sugar content showed negative trends at excess doses of Cd, Co and Cr in different plants. Heavy metal such as Cr reduces leaf area and number of leaves in tomato and brinjal (Purhit, 2003; Tripathi and Tripathi, 1999).Heavy metal also cause water stress like condition (Pandey and Sharma, 2002) and decrease relative water content (Bhattacharya and Chaudhuri, 1995). It is well known that water has great importance in the process of photosynthesis, thus it reduced water content may also be potent reason of sugar synthesis in plants. Gopal *et al.,* (2003) reported decreased sugar and starch with the increase of Co due to the disturbance in the activity of enzyme either causing synthesis of carbohydrate and degradation of higher molecular carbohydrate essential for incorporation in bio-molecule. Reduced sugar synthesis in plants by Cr might be due to lower synthesis or diversion of metabolite to other synthesis processes. Finding of this study is in agreement with some earlier reports (Tripathi and Tripathi, 1999; Hemalatha, 1999; Bazzaz *et al*., 1975; Prince *et al*., 2002).

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