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EFFECTS OF HEXAVALENT CHROMIUM (VI) ON ROOT GROWTH AND CELL DIVISION IN ROOT TIP CELLS OF *AMARANTHUS VIRIDIS* L.

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Abstract

The effects of different concentrations of Cr(VI) (10^{-6} M to 10^{-3} M) on root growth and cell division in root tips of *Amaranthus viridis* L., were studied. Chromium (VI) had toxic effects on the root tip cells during mitosis, such as colchicine mitoses, anaphase bridges, chromosome stickiness. Chromosome stickiness implied the high toxicity of Cr(VI). The mitotic index decreased with increased concentration of Cr(VI), duration of treatment time and the ratio of anomalous dividing cells reversed. The results also indicated that the root growth was completely stopped by 10^{-3} M Cr(VI) after 24 h treatment time, and slightly inhibited by 10^{-4} M Cr(VI) during the whole experiment. Chromium (VI) had a stimulatory effect on the root growth of *A. viridis* L. exposed to 10^{-5} M Cr(VI) during the entire experiment. The mitotic index in the present study can be correlated with rate of root growth, suggesting that the inhibition of root growth resulted from inhibition of cell division.

Introduction

Chromium (Cr) occurs in nature in bound forms that constitute 0.1-0.3 mg/kg of the Earth's crust and has several oxidation states from Cr(-II) to Cr(+VI) (Zayed & Terry, 2003). It is unique among the heavy metals because of its existence in two environmentally important oxidation states: trivalent (Cr III) and hexavalent (Cr VI) (Srivastava *et al.*, 1999). Chromium is recognized as an essential element for humans and animals (Mertz, 1967), but not for plants (Huffman & Allaway, 1973; Liu *et al.*, 1992), although some investigations report that it is beneficial to plant growth (Zheng *et al.*, 1987). Emphasis has become more prevalent towards the problems of Cr pollution with the development of modern industry. Chromium has been used on a large scale in many different industries, including metallurgical, electroplating, production of paints and pigments, tanning, wood preservation, Cr chemicals production, and pulp and paper production (Zayed & Terry, 2003). These industrial processes discharge large quantities of Cr compounds in liquid, solid and gaseous wastes into the environment and can ultimately have significant adverse biological and ecological effects. Chromium can be toxic to plants in its common oxidation states, Cr(III) and Cr(VI) (Mortvelt & Giordano, 1975; Bartlett & James, 1979). Cr(VI) is considered as the most toxic form of Cr, up to 10-100 times more toxic than Cr (III) compounds (Katz & Salem, 1994, Kotaś & Stasicka, 2000). There are several reports on the toxic effects of Cr on plants (Liu *et al.*, 1992; Kleiman & Cogliatti 1998; Zayed & Terry, 2003). Visible symptoms of Cr toxicity have been described mainly in plants growing in Cr-containing nutrient solution or potted soil amended with tannery sludge (Pratt, 1966; Foroughi *et al.*, 1976; Joshi *et al.*, 1999).

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Seedling roots in contact with Cr(VI) collapse and seem unable to absorb water (Mukherji & Roy, 1977; Bishnoi *et al.*, 1993; Corradi *et al.*, 1993). Excess supply of Cr(III) inhibits the uptake of Fe, induces the Fe deficiency type response and changes in plant water relations, resulting in decrease in physiological availability of water (Pandey & Sharma, 2003).

Tianjin is a big industrial city in P.R. China with many discarded chemical plants there. The waste Cr(VI) residue heap within one chemical plant contains 1941.82 mg/kg Cr, 417.69 mg/kg Mn, 19254.79 mg/kg Fe, 34.00 mg/kg Cu and 819.50 mg/kg Zn (Data has not been published). The age of the waste heap is estimated about 40 years. It is covered by specific vegetation with abundant occurrence of *Plantago asiatica* L., *Phragmites australis* (Cav.) Trin., *Kochio scoparia* (L.) Schrad., *Scirpus planiculmis* F. Schmidt, *Amaranthus viridis* L., and others (Wang *et al*., 2002).

The objective of this investigation was to understand the cytological effects of hexavalent Cr(VI) on root growth and cell division in *Amaranthus viridis* L.

Materials and Methods

The seeds of *Amaranthus viridis* L., used in the present investigation were collected from plants growing in the soil nearby the waste Cr(VI) residue heap within a discarded chemical plant which produced potassium dichromate. The soil is about 15 meters from the heap.

Seeds were soaked in tap water for 6 h before starting the experiments, and were allowed to germinate in Petri dishes in the dark at a constant temperature of 25 °C for 18 h, then they were treated with different concentrations of Cr(VI) solutions at 25°C for 24, 48 and 72 h. Cr(VI) was provided as potassium dichromate ($K_2Cr_2O_7$). The Cr solutions were prepared in deionized water. Different concentrations of Cr(VI) ranging from 10^{-3} M to 10^{-6} M were added to the 1/2 strength Hoagland nutrient solution (Stephan & Prochazka, 1989). The Hoagland solution comprised of 5 mM $Ca(NO_3)$, 5 mM KNO_3, 1 mM KH_2PO_4, 50µM H_3BO_3, 1 mM $MgSO_4$, 4.5µM $MnCl_2$, 3.8µM $ZnSO_4$, 0.3µM $CuSO_4$, 0.1µM $(NH_4)_6Mo_7O_{24}$ and 10µM FeEDTA at pH 5.5. The 1/2 strength Hoagland solution was used for the control experiment. The test liquids were changed regularly every 12 h.

Macroscopic observations were made at the end of each time interval. In each treatment, 20 treated roots were examined and the length of roots was measured. For the cytological studies, 20 roots in each treatment group were cut and fixed in 95% ethanol: acetic acid 3:2 for 4 to 5 h and hydrolyzed in 1 M hydrochloric acid: 95% ethanol: glacial acetic acid 5:3:2 for 6-6.5 min at 60°C. For the observation of chromosomal morphology, 20 root tips were squashed in Carbol Fuchsin solution (Li, 1982). Data for measuring root length were analyzed by standard statistical software (Sigmaplot 8.0).

Results

Effects of Cr(VI) on root growth: The effects of Potassium dichromate on root growth of *Amaranthus viridis* L., varied with the different concentrations used and duration of treatment. (Fig.1). The root growth was completely stopped by 10^{-3} M Cr(VI) since 24 h, and slightly inhibited in the 10^{-4} M Cr(VI) during the whole experiment. However, 10^{-5} M Cr (VI) showed stimulatory effect on the root growth of *A. viridis* L., during the entire experiment.

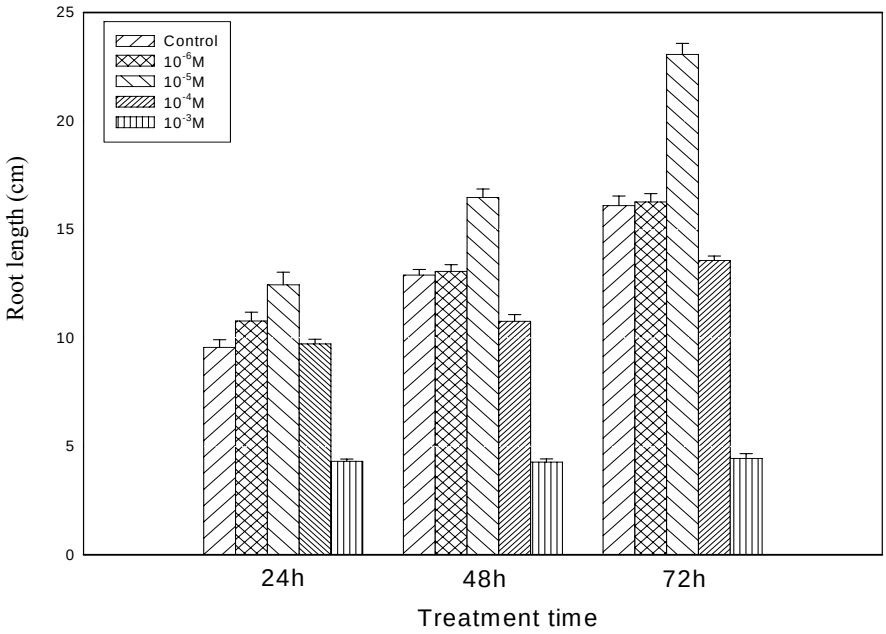

Fig. 1. Effects of different concentrations of Cr(VI) on root length of *A. viridas* L., vertical bars denote SE (n=20).

In comparison to control plants, the roots exposed to 10^{-4} M ~ 10^{-6} M Cr (VI) proved more or less normal during the whole treatment. Roots treated with 10^{-3} M Cr(VI) showed slightly twisted appearance after 24 h treatment, were partly yellow in root-hair zone after 48 h and became more abnormally stubby and stiff with duration of treatment.

Effects on cell division and chromosome morphology

Mitotic index: The mitotic index reflects the frequency of cell division and is regarded as an important parameter when determining the rate of root growth. The mitotic index decreased progressively with increased Cr(VI) concentration, except for the seedlings exposed to 10^{-5} M Cr(VI) (Table 1). In 10^{-6} M Cr(VI), the mitotic index was slightly higher than control 24 h after treatment, but lower than control with increasing the duration of time. In 10^{-5} M Cr(VI), the mitotic index was higher than the control during the entire experiment. At 10^{-3} M Cr(VI), the mitotic index was extremely low, because there were no dividing cells after treating for 72 h.

Effects on chromosome morphology: C-mitoses, chromosome bridges and chromosome stickiness were observed in the root tip cells of all treated groups after treatment with Cr(VI)(Table 1).The frequency of c-mitoses increased with increasing concentration of Cr(VI) and treatment time. Highly condensed chromosomes were randomly scattered in the cell (Fig. 2a). Chromosome bridges involving one or more chromosomes (Fig. 2b-c) were found after Cr(VI) treatment, even some chromosome bridges developed to chromosome stickiness. The frequency of cells with anaphase bridges increased as

Table 1. Effects of potassium dichromate ($K_2Cr_2O_7$) on cell division in the root tip of *A. viridis* L.

Time (h)	CrVI (M)	Mitotic Index	Number of cells	Normal dividing cells in %		Anomalous dividing cells in %			Anomalous Mitoses in %
				Metaphases	Anaphases	C-mitosis	Chromosome bridges	Chromosome stickiness	
24	CK	19.4	500	50.4	39.2	0.2	0.2	10.0	10.4
	10^{-6}	20.2	500	51.8	34.6	0.4	0.8	12.2	13.4
	10^{-5}	21.3	500	48.6	39.4	0.6	0.4	11.0	12.0
	10^{-4}	19.0	500	43.8	32.4	0.8	1.6	21.4	23.8
	10^{-3}	4.2	117	23.1	15.4	5.1	0.9	55.6	61.6
48	CK	22.2	500	49.8	38.4	0.2	0.4	11.2	11.8
	10^{-6}	21.4	450	47.1	36.7	0.4	0.7	15.1	16.2
	10^{-5}	24.1	500	46.4	39.3	0.5	0.5	13.3	14.3
	10^{-4}	18.1	500	40.8	30.8	2.6	1.8	24.0	28.4
	10^{-3}	3.2	97	16.5	12.4	2.1	0	69.0	71.1
72	CK	22.6	366	48.6	36.1	0.8	0.5	13.9	15.2
	10^{-6}	21.6	257	45.1	33.5	1.9	1.2	18.3	21.4
	10^{-5}	23.0	500	45.2	39	1.0	1.2	13.6	15.8
	10^{-4}	16.5	500	36.0	33	3.2	2.0	25.8	31.0
	10^{-3}	NDC							

NDC=no dividing cells

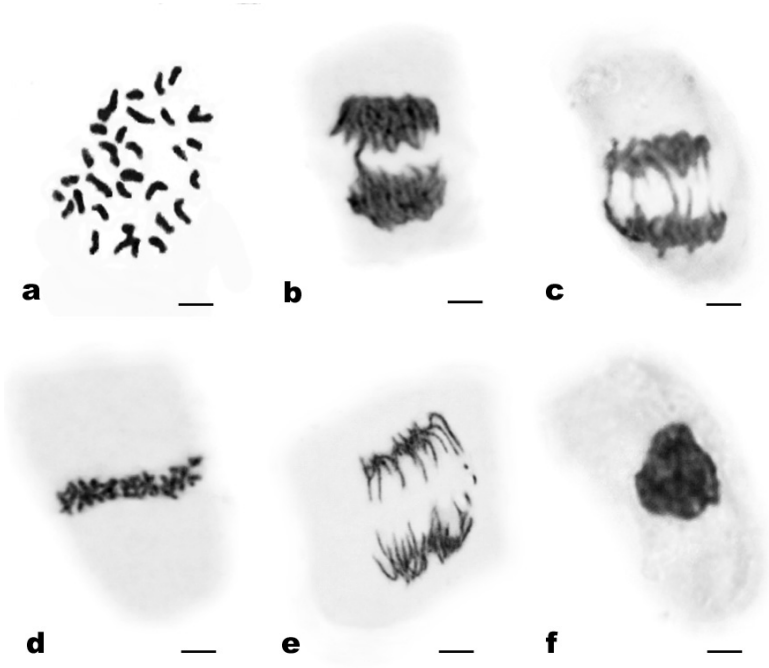

Fig. 2. The effects of Cr(VI) on root tip cell division of *Amaranthus viridis* L.
a c-metaphase. **b-c** Chromosome bridges**.** **d** Chromosome stickiness. **e** Chromosome fragment. **f** nucleolar swelling. Bars = 2.5 µm.

Cr(VI)) concentration and duration of treatment increased (Table 1). Chromosome stickiness, usually being irreversible, reflects highly toxic effects and probably leads to cell death (Fig. 2d). In the three types of anomalous mitoses, chromosome stickiness dominated, especially in the treatments of 10^{-3} M Cr(VI). In contrast to the control, the frequency of chromosome stickiness in 10^{-6} M Cr(VI) and 10^{-5} M Cr(VI) was slightly higher than that in control, but at the concentration of 10^{-5} M Cr(VI) the frequency was lower than at 10^{-6} M Cr(VI). In addition to the aberrations mentioned above, chromosome fragments (Fig. 2e) in anaphase cells and nucleolar swelling (Fig. 2f) at interphase were observed.

Discussion

The results in the present investigation indicated that Cr inhibited the root growth of *Amaranthus viridis* L., at higher concentrations of Cr (VI) (10^{-3} – 10^{-4} M). The low concentration (10^{-5} M Cr(VI)) had a stimulatory effect on root growth of *A. viridis* L., during the entire experiment. The mitotic index in the present study can be correlated with rate of root growth, suggesting that the inhibition of root growth resulted from inhibition of the cell division. Cr toxicity on plants depends on experimental conditions, plant species and Cr species. Barley seeds germinated and grew well at Cr(VI) levels up to 100 mg/kg in soil but were always later in development due to Cr inhibition, which is responsible for mobilizing the reserve starch necessary for initial growth (Zayed &

Terry, 2003). In our earlier study on effects of trivalent and hexavalent chromium on root growth and cell division of *Allium cepa*, both trivalent and hexavalent chromium inhibited the root growth at the given concentrations (Liu *et al.*, 1992). Report by Parr (1982) showed that at high levels of Cr(VI) in soil (500 mg/kg Cr) germination and growth of bush bean were substantially affected. In the present investigation, *Amaranthus viridis* grew well in the soil at high concentration of Cr (1454 mg/kg Cr) (Figs. 3-4).

Many studies concerning chromium interference with uptake of Ca, K, Mg, Pb, B and Cu in soybeans (Turner & Rust, 1971), in sugar beet (Terry, 1981) and in barley seedlings (Shewry & Peterson, 1974; Skeffington *et al*., 1976) have been reported. The results observed by Barceló *et al*., (1985), who studied the effect of chromium (VI) on mineral element composition of bush beans, revealed that P, K, Zn, Cu and Fe translocation in bean plants exposed to Cr(VI) could be inhibited. Also, the report by Terry (1981) indicated that at toxic concentrations of Cr(VI) (>2 mg/kg Cr), sugar beet plants absorbed very little Ca and were Ca-deficient. The interference of mineral nutrients especially Ca results in the imbalance of Ca, disturbs the activity of CaM in cells, then affects movement of chromosomes in cell mitosis. Cr(VI)-induced alteration of nuclear structure and inhibition of cell division in plant roots has been observed by several authors (Levan, 1945; Corradi *et al.*, 1991; Villalobos-Pietrini *et al.*, 1993). In the present investigation, Cr(VI) induced toxic effects on chromosomes during cell division such as c-mitosis, anaphase bridges and chromosome stickiness, are in agreement with the findings of Levan (1945) and Liu *et al*., (1992). The inhibitory effects of Cr on root growth and its toxic effects on cell division may result from the changed transport of Ca^{2+} across the plasma membrane into cytoplasm, and lead to imbalance of Ca^{2+} in the cells, which, in turn, disturbs and destroys the physiological activity and regulation of calmodulin (CaM). The importance of CaM lies in its ability to activate a number of key enzymes such as phospholipase (Leshem *et al.*, 1984) and nicotinamide adenine dinucleotide kinase. Means & Dedman (1980) found that calmodulin (CaM) was specially located in the mitotic spindle, implying its involvement in the process of chromosome movement through regulation and control of depolymerization and polymerization of the microtubules (Li & Sun, 1991).

The mechanisms of Cr uptake and translocation in plants differ with the lapse of time that Barceló & Poschenrieder (1997) divided into three stages: Early investigations with wheat revealed that only Cr (VI) is taken up by plants (Bourque *et al.*, 1967). Further studies using rice suggested that Cr (VI), before penetrating plant roots, is reduced to Cr (III) (Myttenaere & Mousny, 1974). Nowadays, both forms, Cr (VI) and Cr (III), are thought to be taken up by plants. However, the two ions do not share a common uptake mechanism (Zayed & Terry, 2003). Uptake of Cr (III) seems to be passive, while that of Cr (VI) is considered to be active (Barceló & Poschenrieder, 1997). The uptake of Cr(VI) is mediated by the sulfate carrier but with lower affinity (Skeffington *et al.*, 1976) Cr (III) tightly binds to carboxyl groups of amino acid in proteins forming binuclear complexes (Schlösser, 1991). It was reported that, following uptake, Cr(VI) is immediately reduced in cells to Cr (III). Once inside the cell, Cr (III)is located in the cytosol (Sayato *et al.*, 1980; Yanmamoto *et al*., 1981).

Amaranthus viridis showed high tolerance to Cr(VI) in the present investigation. There is higher toxicity of Cr in solution culture because the Cr supply is soluble and is thus available for plant uptake, whereas in soil major portions of Cr become unavailable due to adsorption, reduction, and precipitation processes (Zayed & Terry 2003).

Fig. 3. *Amaranthus viridis* L., grown in soil at high concentration of Cr (1454 mg/kg Cr), in Tianjin, China.

Fig. 4. *Amaranthus viridis* L., grown in soil at high concentration of Cr (1454 mg/kg Cr), in Tianjin, China.

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