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In-situ monitoring of chromium cytotoxicity in sugarcane

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Abstract

The potential of *in-situ* monitoring of cytotoxic effects of chromium through root-tip assay was studied in a sugarcane cultivar CoLk 8102 (*Saccharum* spp. hybrid). Sugarcane setts supplied with graded concentrations of chromium (VI), exhibited a reduction of 85.92 and 95.10 % in mean root length at 40 and 80 ppm Cr dosages along with 61.25 and 82.50 % reduction in mean root number/node respectively. Mitotic index of root tip cells of treated setts declined and the frequency of aberrant mitotic phases increased *pari passu* to the increasing chromium concentration. To compare and quantify the effect of graded chromium dosages on frequency of chromosome aberrations *vis-à-vis* inhibition of mitotic activity, a 'Decretion factor' (D.F.) has been used for the first time. The value of DF increased with the increase in the chromium dosages. The increase in chromosome aberration frequency was low at low chromium dosages (1 or 2 ppm), but the high Cr dosages (40 and 80 ppm), induced sharp reduction in mitotic efficiency of root system along with anomalies in the process of cell division and induced chromosome aberrations in sugarcane root meristem, which in turn affected the over all plant growth.

Key words

Chromosome aberrations, Decretion factor, Heavy metals, Mitotic index, Root-tip assay, *Saccharum species*

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Introduction

Indiscriminate use of fertilizers and pesticide, and unsystematic disposal of industrial effluents enhance the possibility of heavy metal toxicity in environment and biological system (Jain *et al*., 2004). In addition, industrial wastes containing heavy metals affect genetic systems by producing various types of chromosomal abnormalities. Heavy-metal pollutants have a high bioaccumulation rate and they are slowly released in an organism, causing a number of damages. In line with other heavy metals like As, Cu, Cd, Co, Ni, Sn and Zn, Chromium is a broadline heavy metal, and is phytotoxic either at all concentrations or above certain threshold levels (Nieboer and Richardson, 1980). It can affect growth, water balance, pigment content, and initiate lipid peroxidation causing oxidative damage to plants (Panda and Choudhury, 2005). This metal entered into agro-ecosystem through the use of municipal waste-based composts and through irrigation with sewage water from chrome plating and steel industries (Nriagu, 1988). Soil and aquatic ecosystems in the vicinity of Cr-releasing sources are adversely affected by it, making arable land unproductive and unfertile. Chromium is considered strongly toxic because chromium compounds in the soil are more or less insoluble as the metal ions are tightly bound to humus and clay particles (Jamal *et al*., 2006). The presence of chromium is significant in the tannery effluents, which farmers often use to fulfill their irrigational requirements (Sharma and Mehrotra, 1993). Use of tannery and industrial effluents, municipal waste-based composts and biosolids application on sugarcane fields and production of sugarcane on metal polluted fills might create the problem of heavy metal toxicity for sugarcane cultivation (Barry *et al*., 1998; Liu *et al*., 1994).

Quick and precise methods for detection and evaluation of toxic effects of environmental pollutants are seriously warranted. Due to the highly conserved nature of the genetic material, a broad variety of genera are used for such tests. The most widespread organisms used include bacterial indicator species, yeasts, fungi, insects, mammalian cells or laboratory rodents and plants (Pavlica *et al*., 1992). Response of plants to mutagenic treatment can be evaluated at different levels of organization: from DNA, chromosome and genome, to the whole organism. Chromosome aberration and micronuclei tests have been conducted with plant species such as

Vicia faba (Cavusoglu *et al*., 2010), *Crepis capillaris* (Grant and Owens, 1998), *Hordeum vulgare* (Gecheff, 1996) etc.; however, the literature on this aspect with reference to sugarcane is scanty. Since the potential of *in-situ* monitoring of heavy metal toxicity especially of chromium has long been recognized in other crop plants, the present investigation was aimed to study the cytotoxic effects of graded concentrations of chromium (VI) on a sugarcane cultivar CoLk 8102 (*Saccharum* species hybrid) through root-tip assay at an early stage of growth .

Materials and Methods

Sugarcane (*Saccharum* spp. hybrid cultivar CoLk 8102) plants were raised from single bud setts (pieces of cane stem having one bud each, used for vegetative propagation) in soil with pH of about 6-7 at ambient temperature under tray culture conditions. Potassium dichromate was mixed to the soil for differential dosages of Cr (1, 2, 20, 40 and 80 mg Cr kg^{-1} soil or ppm Cr) supply before planting along with the control, which did not receive any treatment. Each treatment contained two replicates. Root tip assay was done to find out the effect of chromium dosages on mitotic efficiency and chromosomal aberrations. For cytological analysis, 1-1.5 cm long root tips from germinating setts were fixed directly in carnoy's 6:3:1 fluid (6 alcohol: 3 chloroform: 1 glacial acetic acid) as well as after pretreatment with 1:1 v/V aqueous solution of saturated para dichlorobenzene and 0.002 M 8 hydroxyquinoline for 3-3$^1/_2$ hr (Srivastava, 1995). Root tip assay, mitotic index (MI) analysis and frequency determination of chromosomal aberrations in root-tip cells due to *in-situ* application of different dosages of chromium was done as described by Srivastava and Jain (2010). Changes in the MI due to different Cr treatments were expressed as a Factor (F) representing the factor of the mean MI from Cr treated setts over the mean MI from control and calculated as mean MI% of treated group divided by mean MI% of control. Root growth parameters with reference to root length and number of roots/node in settlings (plants regenerated from buds of setts) were recorded at 20 days after planting (DAP). A 'Decretion factor' (DF) has been coined as a new term in the present study as an absolute value to compare and quantify the effect of treatment on chromosome aberration frequency *vis-à-vis* inhibition of mitotic activity. The DF has been calculated as a proportional measure of % aberrant cells to the total mitotic index and converted into percentage.

Results and Discussion

It was observed that *in situ* application of chromium inhibited the process of growth in terms of retarded root development in treated setts. Such effect was more pronounced at 40 and 80 ppm levels of Cr as compared to the lower levels. There was a reduction of 85.92 and 95.10% in mean root length at 40 and 80 ppm Cr dosages along with a reduction of 61.25 and 82.50% in mean root number/node, respectively. General response of decreased root growth due to Cr toxicity could be due to inhibition of root cell division/ root elongation or to the extension of cell cycle in the roots (Shanker *et al*., 2005). Inhibition of root growth may also be due to the reason that Cr (VI) functions as a hill reagent and can inhibit electron transport both in the photosynthetic and mitochondrial apparatus, thus accounting for reduced NADPH pool (Shanker, 2008), which may also result in apoptosis or cell death. Visible symptoms of excess Cr dosages were observed after 25 DAP as growth depression and leaf chlorosis at 40 and 80 ppm. Chromium was found toxic affecting the growth of root, shoot and seedling length in two varieties of wheat *viz*. Anmol and Kiran by Jamal *et al*., 2006. Chlorosis in the upper plant parts has been considered primarily a manifestation of toxic effects of chromium in roots and stem (Sharma *et al*., 1995). Interestingly, the symptoms were more prominent at higher chromium dosages.

A decrease in mitotic index was observed at all concentrations of chromium (Table 1), indicating thereby a mitodepressive effect of chromium treatment on cell division activities in root-tip cells. The mitotic index decreased with increasing concentrations of chromium. The reduction in mitotic index % was largely dose dependent since it showed a steady decline with increasing chromium dose from 16.45% in control (no added chromium) to 1.34% at 80 ppm Cr dose. A decreased cell division rate and deviations from the normal mitosis along with serious anomalies in the process of cell division and induced chromosome aberrations were noticed in the *Allium cepa* root meristem as an effect of the heavy-metal and cyanide contaminated waters (Staykova *et al*., 2005). The increasing concentrations of the chromium induced a marked reduction in the mitotic activity of root meristems of *Vicia faba* (Chidambaram *et al.,* 2009). The decrease in mitotic index indicates the loss of dividing cells, which may be attributed to chromium interference in the normal sequences of mitosis leading to disturbance of spindle function. A factor 'F' governing the changes in Mitotic Index (MI) % was calculated (Table 1) as the ratio of mean MI% of treated group *vs*. mean MI % of control to help ascertain the extent of reduction in mitotic efficacy as a result of increase in chromium dosages. This factor showed more than ten times reduction in MI% at 80 ppm Cr dose in comparison to the 1 ppm Cr dose. This clearly indicates that at 80 ppm Cr dose, the mitotic index drastically fell to as low as 1.34% and the value of the factor 'F' was reduced to 0.082 which was a reduction of more than10 times to that of 1 ppm Cr dose (13.6% and 0.826, respectively). In other words, the lower the value of factor 'F' is, the higher would be the effect of mitodepressive activity of Cr application.

The effects of different dosages of chromium upsetting the mitotic efficiency of root tip cells of sugarcane *cv*. CoLk 8102 in terms of frequency of normal and aberrant cell division phases are given in Table 1. The chromium treatment affected the spindle function and decreased metaphase and anaphase stages. Analysis of these two stages of cell division revealed a reduction of more than 90% in metaphase and anaphase stages at 80 ppm Cr dose and of nearly 80% at 40 ppm dose as compared to the control. In addition, the percentages of abnormal mitotic phases increased with increasing concentration of chromium (Table 1). A 'Decretion factor' (DF) has been coined as a new term in the present study as an absolute value to compare and quantify the effect of graded chromium

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Table - 1: Influence of different dosages of chromium (Cr) application *in situ* on mitotic cell division phases of root tip cells of sugarcane cultivar CoLk 8102

Treatment (ppm)	Total cells studied	Total dividing cells	% cells exhibiting change				Numerical decrease over control (%)		Mitotic index (MI) %	Changes in MI% factor (F) *	** D.F. for mitotic efficiency
			Metaphase		Anaphase						
			Normal	Aberrant	Normal	Aberrant	Metaphase	Anaphase			
0.0	1197	197	8.43	0.08	7.85	0.08	-	-	16.45	-	0.98
1.0	2257	307	5.72	0.53	6.65	0.71	26.56	13.61	13.60	0.826	9.12
10.0	2498	298	4.36	1.28	5.08	1.20	28.84	26.71	11.93	0.725	20.78
20.00	2740	200	2.19	1.46	2.77	0.88	54.03	57.16	7.29	0.443	32.10
40.00	2877	87	0.73	0.59	1.04	0.66	83.38	80.05	3.02	0.184	41.39
80.00	2534	34	0.28	0.32	0.36	0.39	92.57	91.19	1.34	0.082	52.98

* Factor (F) calculated as mean MI % of treated group divided by mean MI % of control, ** D.F. = Decretion factor calculated as proportional measure of % aberrant cells to the total mitotic index and converted into percentage

Table - 2: Frequency of chromosomal aberrations in root-tip cells of sugarcane cultivar CoLk 8102 following *in-situ* application of different dosages of chromium (Cr)

Treatment (ppm)	Frequency of various somatic aberration											
	At metaphase						At anaphase					
	Total dividing cells	Number of aberrant cells	% cells showing aberration				Total dividing cells	Number of aberrant cells	% cells showing aberrations			
			Spindle inhibition	Stickiness	Laggards fragments	C-mitosis			Delayed anaphase	Multipolar anaphase	Micronuclei	Bi/Multinucleate cell
0.00	102	1	-	-	0.98	-	95	1	1.05	-	-	-
1.00	141	12	2.84	2.13	2.83	0.71	166	16	3.61	1.81	3.01	1.21
2.00	141	32	5.67	7.09	7.09	2.84	157	30	3.82	6.37	6.37	2.55
20.00	100	40	8.00	12.00	12.00	8.00	100	24	6.00	6.00	8.00	4.00
40.00	38	17	7.89	13.16	15.78	7.89	49	19	16.33	4.08	14.29	4.08
80.00	15	8	13.33	26.67	6.66	6.67	19	10	10.52	10.53	15.79	15.79

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dosages on frequency of chromosome aberrations *vis-à-vis* inhibition of mitotic activity (Table 1). The DF has been calculated as a proportional measure of % aberrant cells to the total mitotic index and converted into percentage. The value of DF increases with the increase in the chromium dosages. The correlation coefficient between the chromium application dosages (ppm) and the DF are highly significant (r= 0.93). This shows that the term DF may be used to compare and quantify the cytotoxic effects of any chemical or pollutant in plants.

The wide spectrum of cytotoxic effects induced by different dosages of chromium included spindle inhibition, chromosome stickiness, laggards, C-mitosis, delayed anaphases, multipolarity and bi/multi nucleate cells, chromosome fragmentation and micronuclei formation etc. (Table 2). Chidambaram *et al.* (2009) also observed increased number of chromosomal aberrations like stickiness, laggards, chromosome bridges, C-metaphase, fragmentation and binucleate cells with the increasing concentrations of chromium. It may be due to the effect of hexavalent chromium on spindle apparatus. The formations of chromosomal stickiness could also be observed at high frequency owing to the disturbance in nucleic acid metabolism of the cell. The high micronuclei frequency observed in treated samples could be induced either by the lagging of whole chromosomes or the immobility of large acentric fragments. The formation of acentric fragments might have resulted from different chromosome aberrations, and the lagging of chromosomes was caused by disturbances in the mitotic spindle/ centromere or the failure of a chromosome to get attached to spindle fibre. Spindle inhibition can be explained by disturbance in the synthesis of proteins and nucleic acids resulting in a change of nucleoprotein configuration of chromosomes. C- mitosis has also been considered as reliable indices of spindle inhibition. Partial C- mitosis may lead to abnormal anaphases resulting in the formation of many poles (multipolar anaphases). Multipolarity, binucleate cells and delayed anaphases were the most common aberrations present at anaphase in the present study. Such chromosomal irregulations can affect the vigour, fertility, yield or competitive ability of the exposed plants (Kara *et al*., 1994).

At present, plant bioassays are well-established systems and are used for screening and monitoring environmental chemicals with mutagenic and carcinogenic potential (Ma, 1999). Cytogenetic tests analyze the frequency and type of chromosome aberrations in mitotic cells and the frequency of micronuclei in interphase cells. Using plant bioassays for testing and monitoring environmental chemicals or pollutions has many advantages. They are easy to handle, inexpensive and in many cases more sensitive than other available systems (Constantin and Owens, 1982). Mostly higher plant bioassays are based on the detection of chromosomal aberrations, micronuclei test, sister chromatid exchanges, comet assay and recently, on the analysis of DNA strand breaks using Fluorescent *in situ* hybrydization (FISH). Work done in other crops reflects the toxic effects of chromium on plant growth. In seedlings of blackgram, a gradual decrease of germination percentage, root length, shoot length, fresh weight, dry weight, total chromosome length, absolute chromosome length and average chromosome length was observed with the increase of chromium concentrations (Chidambaram *et al.,* 2009). Sen *et al*. (1994) showed that chromium hampered uptake of other metals in *Salvinia natans* L. Reduction in uptake of manganese, zinc and copper in response to chromium application at 0.05, 0.1, 0.25, 0.5 and 1.0 mM in maize (*Zea mays* L. CV. Ganga 5) was observed after 56 days of growth (Sharma and Pant, 1994). In a pot culture experiment by Singh (2001), chromium (VI) applied at 30 mg kg^{-1} reduced the spinach yield to a greater extent than Cr (II). The results from the present experiment showed that although low chromium dosages (1 or 2 ppm) may not be cytotoxic, they encourage a certain increase in aberration frequency. The excess Cr supply (especially 40 and 80 ppm Cr) induces sharp reduction in mitotic efficiency of root system in sugarcane and provoked serious anomalies in the process of cell division leading to chromosome aberrations in sugarcane root meristem, which in turn would affect the over all plant growth. Since sugarcane is a highly complex polyploid hybrid with inherent chromosome mosaicism and endoreduplication cycles, the multiplication of chromosomal divisional errors during cell division may further enhance the problem. These findings suggests a caution while growing sugarcane on soils with high heavy metal contamination especially chromium. Moreover, sugarcane root-tip assay proved successful for monitoring the toxic effects of chromium.

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