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Genotoxic Effects of Chromium (Cr) in *Eleusine coracana* - Mitotic analysis

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Abstract

The purpose of the current work was to assess the genotoxic effect of Chromium (Cr) in one of the most economically important crop plant of Garhwal, *Eleusine coracana*. Two accessions of *E. coracana* were subjected to detailed root mitotic studies. The effect of Cr on root growth varied with different concentrations used. No roots were available in 10^{-1} M and 10^{-2} M during the whole experiment. At 10^{-3}M, the Mitotic Index (MI) was extremely low (0.017 in E1 and 0.008 in E2). In the course of mitosis various anomalies viz. fragmentation, clumping, stickiness of chromosomes, random grouping at anaphase, chromosome bridges at anaphase, and c-metaphase were observed mostly. Most of the anomalies were observed at 10^{-3} M Cr concentrationinboth the accessions of *E. coracana*.

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Introduction

Contamination of soil in cultivated fields by industrial effluents loaded with toxic heavy metals has emerged as a new threat to agriculture. Most of the effluents and wastes contain heavy metals in an amount sufficient enough to cause toxicity to crop plants (Hutchinson and Whiteby, 1974, Temple and Bisessar, 1981; Khan *et al*., 2006). Excessive accumulation of heavy metals like nickel, copper, cobalt, etc. in soil originating from metal mining and processing and other technological human activities have been reported on a number of occasions (Foy *et al.,*1978; Maliszewaska *et al.,* 1958).

Von Rosen (1954) grouped Cr into very strong active heavy metal category. The chemical symbol of Chromium is Cr. The atomic number is 24. The relative atomic mass is 51.996g. Its density is 7.19g/cm^3 at 20°C. The boiling point and melting point of Cr is 2672°C and 1857°C respectively. The vapor pressure is 10^{-6}Pa at 844°C and it is solublein diluted hydrochloric and sulphuric acid. Cr is present everywhere and can be found in three forms: - metal ore, trivalent chromium (Cr III)and hexavalent chromium (Cr VI). Chromium is used tomake steel and other alloys, for chrome plating, and as an additive to limit corrosion. Its compounds are colored hence given the name chromium. Hexavalent chromium occurs naturally, notably in water-saturated conditions and it is an indicator of human pollution. This form is relatively soluble and can move more readily from soil to groundwater.

Materials and Method

The *Eleusine coracana* (Finger millet) is commonly known as Mandwa or Kodu in Garhwal, belongs to the family Poaceae. *Eleusine coracana,* a species native to tropical East Africa is a short stemmed, dry land adapted, millet with excellent storage characteristics and an outstanding mineral content (Gaur, 1999).

For the present study, the seeds were procured from two local sources as follows:

Accessions	Place
E1	Chaubattakhal, 1800m asl
E2	Uttarkashi, 1200 m asl

Table 1. Different accessions of *E. coracana.*

asl = above sea level

The seeds of each accession of *E. coracana* (E1, E2) were first surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 3-4 minutes and washed thoroughly and then with distilled water. The seeds were placed on double layered filter papers (3 mm, Whattman filter papers) with cotton pads 'sandwiched' between them equidistantly in various treatments in petri dishes and incubated under white light at 22°C. Ten molar solutions of $K_2Cr_2O_7$ ranging between 10^{-10}M to 10^{-1}M were prepared. The control sets were raised in distilled water and treated sets were raised in various molar concentrations of $K_2Cr_2O_7$. Only a fixed amount (about 50 ml) of distilled water or Cr solutions was added in petri dishes. The various molar concentrations of Cr were prepared in Hoagland's solution

For mitotic studies, 20 root tips of control and treated seedlings of *E. coracana* were fixed in 3:1 absolute ethanol and glacial acetic acid having a pinch of ferric chloride. The fixed root tip samples were stored in 70% ethanol in refrigerator. After 24 hours of fixation, the root tips were boiled in 3% aceto-carmine and left for overnight. After that these were smeared and squashed in 1.5% aceto-carmine. The following parameters were used for the analyses:

a) Number of non-dividing cells,
b) Number of dividing cells,
c) Number of actively dividing cells, and
d) Percent frequency of mitotic anomalies.

Mitotic index was also calculated by using following formula:

Number of cells in mitosis (actively dividing cells)

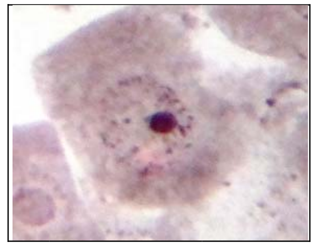
$$\text{Mitotic index} = \frac{\text{Number of cells in mitosis (actively dividing cells)}}{\text{Total number of cells (non-dividing cells + dividing cells + actively dividing cells)}}$$

Results and Discussion

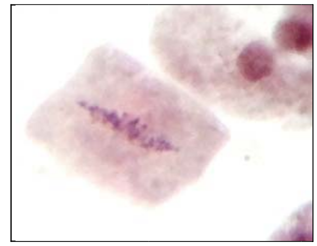
Both the accessions of *E. coracana* were subjected to detailed root mitotic studies in control and treated plants. The effect of Cr on root growth varied with different concentrations used. No roots were available in 10^{-1} M and 10^{-2} M during the whole experiment. The mean data related to non-dividing cells (NDC), dividing cells (DC) and actively dividing cells (ADC) is given in table 2.

Mitotic Index (MI) was also calculated by formula given in materials and methods. The mitotic index (MI) reflects the frequency of cell division and is regarded as important parameter for determining the rate of root growth. At 10^{-3}M, the MI was extremely low (0.017 in E1 and 0.008 in E2). The graphical representation of mitotic index in different concentrations in both the accessions of *E. coracana* is shown in figure 1. In the course of mitosis, various anomalies were observed. The frequency distribution of different anomalies in different concentrations of Cr in both the accessions of *E. coracana* is shown in table 3. Following mitotic anomalies were observed in treated plants:

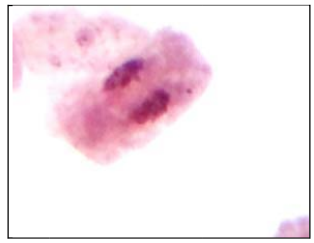
a) Fragmentation (plate 1 g),
b) Stickiness of chromosomes (plate 1c,d,e),
c) Random grouping at anaphase (plate 1 h),
d) Chromosome bridges at anaphase (plate 1 f), and
e) C-metaphase (plate 1 n, i).



(a) Prophase

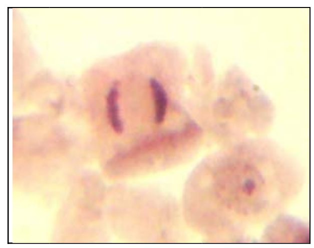


(b) Metaphase

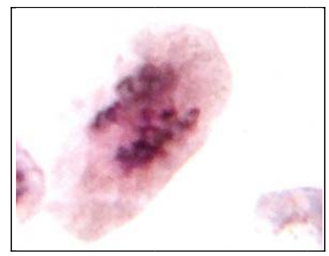


(c) Stickiness at anaphase

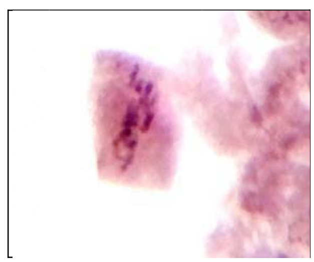
(d) Stickiness at anaphase & metaphase



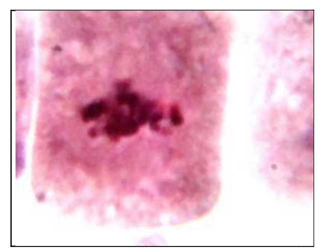
(e) Stickiness at anaphase



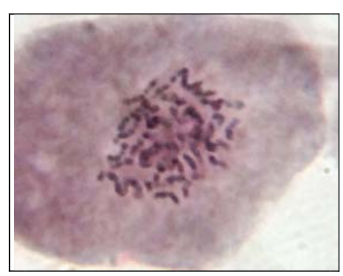
(f) Bridge at anaphase



(g) Fragmentation



(h) Random grouping at metaphase



(i) C-metaphase

Plate 1 (a-i): Microphotographs showing different mitotic anomalies in *Eleusine coracana*

Cr Concentration	Parameter	E1						E2					
		Mean	**±**	**SE**	**Range**			**Mean**	**±**	**SE**	**Range**		
Control	**NDC**	44.10	±	1.19	32.00	-	61.00	44.43	±	1.13	32.00	-	58.00
	DC	1.43	±	0.10	1.00	-	3.00	1.47	±	0.10	1.00	-	3.00
	ADC	1.59	±	0.15	1.00	-	4.00	1.87	±	0.16	1.00	-	4.00
10^{-10}M	**NDC**	40.25	±	1.82	18.00	-	65.00	45.38	±	1.17	35.00	-	60.00
	DC	1.92	±	0.17	1.00	-	4.00	1.50	±	0.10	1.00	-	3.00
	ADC	1.56	±	0.12	1.00	-	3.00	2.00	±	0.17	1.00	-	4.00
10^{-9}M	**NDC**	36.93	±	1.95	15.00	-	62.00	47.10	±	1.04	35.00	-	60.00
	DC	1.81	±	0.17	1.00	-	4.00	1.06	±	0.09	0.00	-	2.00
	ADC	1.57	±	0.12	1.00	-	3.00	1.65	±	0.19	0.00	-	4.00
10^{-8}M	**NDC**	40.68	±	1.85	22.00	-	64.00	48.30	±	0.86	37.00	-	60.00
	DC	2.00	±	0.16	1.00	-	4.00	0.88	±	0.09	0.00	-	2.00
	ADC	1.52	±	0.09	1.00	-	3.00	1.31	±	0.19	0.00	-	4.00
10^{-7}M	**NDC**	36.43	±	1.33	19.00	-	56.00	49.95	±	0.80	37.00	-	60.00
	DC	1.73	±	0.15	1.00	-	4.00	0.71	±	0.09	0.00	-	2.00
	ADC	1.50	±	0.11	1.00	-	3.00	1.00	±	0.15	0.00	-	3.00
10^{-6}M	**NDC**	46.60	±	1.26	35.00	-	66.00	50.65	±	0.90	37.00	-	65.00
	DC	2.19	±	0.17	1.00	-	4.00	0.76	±	0.11	0.00	-	2.00
	ADC	1.39	±	0.08	1.00	-	2.00	1.04	±	0.14	0.00	-	3.00
10^{-5}M	**NDC**	48.08	±	1.43	32.00	-	66.00	51.10	±	0.90	37.00	-	65.00
	DC	1.95	±	0.17	1.00	-	4.00	0.76	±	0.11	0.00	-	2.00
	ADC	1.75	±	0.14	1.00	-	4.00	0.77	±	0.13	0.00	-	3.00
10^{-4}M	**NDC**	52.35	±	1.32	40.00	-	70.00	51.60	±	0.88	40.00	-	65.00
	DC	1.60	±	0.19	0.00	-	4.00	0.73	±	0.11	0.00	-	2.00
	ADC	1.58	±	0.13	1.00	-	3.00	0.55	±	0.11	0.00	-	2.00
10^{-3}M	**NDC**	56.30	±	1.19	41.00	-	70.00	52.70	±	0.74	45.00	-	65.00
	DC	1.16	±	0.19	0.00	-	4.00	0.82	±	0.12	0.00	-	2.00
	ADC	1.00	±	0.14	0.00	-	3.00	0.42	±	0.10	0.00	-	2.00

Table 2: Mean data related to non-dividing cells (NDC), dividing cells (DC) and actively dividing cells (ADC) in different Accessions of *Eleusine coracana.*

Accession	Cr Concentration	a	b	c	d	e	f	g	h	i	j
E1	Control	8.33	4.17	4.17	0.00	8.33	0.00	0.00	0.00	29.17	0.00
	10^{-10}M	14.29	0.00	0.00	0.00	8.33	0.00	0.00	0.00	16.67	0.00
	10^{-9}M	0.00	4.17	0.00	0.00	8.33	0.00	0.00	0.00	41.67	0.00
	10^{-8}M	18.75	4.17	12.50	0.00	8.33	0.00	8.33	0.00	20.83	0.00
	10^{-7}M	36.36	4.17	0.00	0.00	12.50	0.00	0.00	0.00	12.50	0.00
	10^{-6}M	28.57	0.00	12.50	0.00	8.33	0.00	8.33	0.00	12.50	0.00
	10^{-5}M	31.25	4.17	12.50	0.00	8.33	0.00	8.33	0.00	12.50	0.00
	10^{-4}M	21.74	8.33	20.83	0.00	4.17	0.00	12.50	0.00	29.17	0.00
	10^{-3}M	16.13	29.17	29.17	0.00	0.00	0.00	20.83	0.00	29.17	0.00
E2	Control	0.00	4.17	0.00	0.00	8.33	0.00	0.00	0.00	29.17	0.00
	10^{-10}M	0.00	0.00	0.00	0.00	8.33	0.00	0.00	0.00	33.33	0.00
	10^{-9}M	0.00	0.00	0.00	0.00	4.17	0.00	0.00	0.00	29.17	0.00
	10^{-8}M	0.00	4.17	0.00	0.00	8.33	0.00	0.00	0.00	29.17	0.00
	10^{-7}M	0.00	8.33	20.83	0.00	8.33	0.00	0.00	0.00	25.00	0.00
	10^{-6}M	11.76	8.33	20.83	0.00	4.17	0.00	0.00	0.00	29.17	0.00
	10^{-5}M	16.67	4.17	20.83	0.00	8.33	0.00	0.00	0.00	20.83	8.33
	10^{-4}M	13.04	12.50	20.83	0.00	0.00	12.50	0.00	8.33	20.83	8.33
	10^{-3}M	11.11	12.50	33.33	4.17	0.00	12.50	0.00	8.33	20.83	8.33

Table 3: Mean data related to percent frequency of different mitotic abnormalities in different accessions of *Eleusine coracana*.

a. Clumping
b. Restitution Nucleus
c. C- Metaphase
d. Random Grouping of Chromosomes
e. Late movements of chromosomes to metaphase plate
f. Chromatin erosion
g. Chromatin fragmentation
h. Laggards
i. Binucleate condition
j. Trinucleate condition

Cr Concentration	E1	E2
Control	0.034	0.039
10^{-10}M	0.036	0.041
10^{-9}M	0.039	0.033
10^{-8}M	0.034	0.026
10^{-7}M	0.038	0.019
10^{-6}M	0.028	0.020
10^{-5}M	0.034	0.015
10^{-4}M	0.028	0.010
10^{-3}M	0.017	0.008
10^{-2}M	0.000	0.000

Table 4: Mitotic index (MI) in various concentrations in different accessions of *Eleusine coracana*

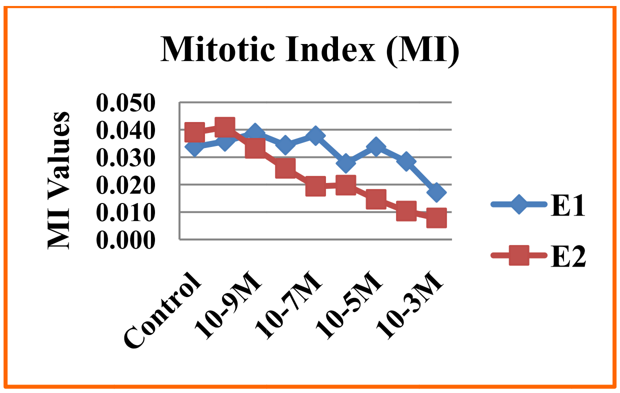


Fig. 1 Graphical representation of mitotic index (MI) in various concentrations in different accessions of *Eleusine coracana*

At 10^{-3} M Cr concentration, most of the anomalies like chromosome stickiness, clumping, chromosome bridges and c-metaphase were observed in both the accessions of *Eleusine*.

Cr induced alteration of nuclear structure and inhibition of cell division in roots has been observed by many authors (Levan, 1945; Corradi *et al*., 1991; Villalobos-Pietrini *et al*., 1993). The most appropriate reason for the increase in abnormalities may be due to malfunction and inactivation of number of key enzyme for ion uptake and spindle control.

The formation of bridges could be attributed to chromosome stickiness and to chromosome breakage and reunion. The induction of lagging could be attributed to the failure of the normal organization and function of the spindle apparatus. Such type of abnormalities is due to the loss of microtubule of spindle fibers. This was supported by previous reports of several authors (Patil and Bhat, 1992; Salam *et al*., 1993; Chidambaram *et al*., 2009).

The mitotic index (MI) reflects the frequency of cell division and is regarded as important parameter for determining the rate of root growth. Heavy metal accumulation in soil and its importance on the morphological, biochemical and cytological aspects of plants have received more attention in recent times by many workers (Abbasi *et al*., 1992; Premkumar *et al*., 2001& 2014; Prakash *et al*., 2004).

In *E.coracana,* the mitotic index decreased progressively with the increase in Cr concentrations in both the accessions. In treated roots the mitotic index was found to be lower than the control. At 10^{-3}M, the mitotic index was extremely low (0.017 in E1 and 0.008 in E2).

In the present studies, 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 precocious movement of the chromosome might have been caused by the early terminalization, stickiness of chromosome or because of the movement of the chromosome ahead of the rest during anaphase (Permjit and Grover, 1985; Chidambaram *et al*., 2009).

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