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Analysis of Nickel Induced Genotoxicity in Root Meristem of *Allium cepa*

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Abstract

Nickel induced genotoxic effect on *Allium cepa* root meristem was evaluated after treating onion root tips with various concentrations of $NiCl_2$ in different time intervals. Mitotic index was higher (9.87 ± 1.96) at initial concentration of 10µg/l in three time intervals (1h,3h and 5h) tested and decreased progressively when $NiCl_2$ concentration and exposure time was increased. Chromosomal gap was found to be very less (1.00) in 30µ/l concentration in all time intervals and found to be increased slightly in higher concentrations. Chromosomal break was found to be highest (36.00) in 100µg/l in all time intervals. Total aberration was found to be increased significantly as concentration increased.

Keywords: genotoxic; *Allium cepa;* root meristem; Nickel

Introduction

Heavy metals constitute one of the major groups of genotoxic environmental pollutants posing serious threat to human as well as environmental well-being (Panda and Panda, 2002). The elevated levels of heavy metals in plants may suppress the metabolism and translocation of reserve materials to the growing regions and their subsequent utilization. Heavy metals like arsenic, chromium, cadmium, nickel, iron and lead are carcinogenic in human and epidemiological studies on genotoxic effects of these heavy metals have been reported in various biological systems (Bemba Meka *et al.,* 2005; Sik *et al*., 2009; Samuel *et al.,* 2010; Olorunfemi *et al.,* 2011; Sharma and Vig, 2012). Toxicity through heavy metals including arsenic, cadmium, lead, mercury, chromium, nickel, manganese and iron, is a widespread problem in India and other developing countries (Khurshid and Zaheeruddin, 2000). Excess heavy metal stress causes oxidative damage, but some reactive oxygen species (ROS) can participate in signal transduction pathways. Nickel in the form of several alloys and compounds has been in wide spread commercial use over 100 years. Several million workers worldwide are exposed to airborne fumes, dust and mists containing nickel and its compounds. Nickel has been attributed to its deleterious effect on photosynthesis, respiration and mineral nutrition (Parida *et al.,* 2003). Carcinogenic effects of nickel in different levels were reported in experimental rats (Wealkes *et al.,* 1987). Occupational exposure to nickel is reported to elevate sister chromatid exchanges (Peltonen, 1979) and chromosomal aberration (Nishimura and Umeda, 1979).

Ni is classified as an essential trace element (Brown *et al.,*1987) and although it is found everywhere in the environment, it usually occurs only in trace amounts. Vascular plants have been found to be highly effective for recognizing and predicting metal stress in the environment (growth, inhibition, reduction of biomass production, water absorption and translocation).

Hyper-accumulation of toxic heavy metal ions by plants is thought to be dependent on three physiological mechanisms: high rates of uptake from soil; efficient translocation from the roots to the shoots; and safe deposition of heavy metals in appropriate comportments of the shoot (Lasat *et al.,* 2000). The elevated levels of heavy metals in plants may suppress the metabolism and translocation of reserve materials to the growing regions and their subsequent utilization thus heavy metals at supra. Optimal concentrate affect the agronomic traits of plants (Sinha and Gupta, 2005) nickel is an essential element for plants and in small quantities, has been reported to improve crop yield and quality (Brown *et al.,* 1990; Atta-aly,1999) phytotoxicity of nickel has been attributed to its deleterious effect on photosynthesis, respiration mineral nutrition, plant water status and transport of assimilates (Parida *et al.,* 2003; Liamas *et al*., 2008) metal contamination reflects natural as well as anthropogenic sources in which many metals

occur in association with various metals including arsenic, cadmium. lead, mercury chromium nickel manganese and iron, is a widespread problem in India metal like arsenic, chromium, cadmium, nickel, iron and lead are carcinogenic in human and epidemiological studies on the genotoxic effects of nickel compounds have been published (Bemba Meka *et al.*,2005) Nickel is a well known carcinogen carcinogenicity of nickel was reported in rats occupational exposure to nickel is reported to elevate sister chromatic exchanges (Peltonen, 1979) and chromosomal aberration (Nishimura and Umeda, 1979). Though several reports suggest deleterious effect of heavy metals, simple assays and specific mechanism to ascertain the level of genotoxicity of these metals in occupationally exposed population are inadequate. Therefore, in the present study an assessment was made to compute the level of genotoxicity in the root meristem of *Allium cepa* L. exposed with nickel chloride through chromosomal aberration assays.

Materials and Methods

Healthy and equal size small onions (*Allium cepa L.*) were placed for germination in wet sand after removing dry scales and roots. After 36 hours, onion bulbs with freshly formed roots were removed from the sand, washed and placed on the crown top of containers (small bottle) filled with aqueous $NiCl_2$ solution in different concentrations (10,20,30,40,50,100µg/ml). Basal portion of onion bulb was placed on mouth of the bottle close to the level of $NiCl_2$ solution. Three sets were kept for each concentration and incubated in different time interval in complete darkness at room temperature. A bottle with distilled water was used as control. After the incubation period, onion bulbs were removed from $NiCl_2$ solution, washed thoroughly with distilled water. Root tips were removed carefully and used as the source of meristem. Root tips in each set of treatment were collected separately and treated with0.03% aqueous colchicine solution for 3 hours. After this treatment, root tips were fixed in ethanol acetic acid solution (3:2), kept for 4 to 5 hours and hydrolyzed in 1N HCl for 5 minutes. Hydrolyzed root tips were stained with safranin solution for a few minutes, squashed in a clean microscopic slide overlaid with a cover slip and observed under compound bright filed microscope. About 400 meristematic cells were scored and mitotic index was calculated as the number of dividing cells per total cells scored in the microscopic field. Cytological defects such as chromosomal break, chromosomal gap, chromosomal fragments, abnormal metaphase and multiple break were scored for each treatment sets, mean values were calculated (Table-1).

Table-1: Chromosomal aberration in *Allium cepa* root tip treated with $NiCl_2$ in different concentration and duration (400 cells scored)

1hour

Conc. µ/ml	Mitotic index (mean±SD)	Abnormal metaphase (mean±SD)	Chromosomal break (mean±SD)	Chromosomal fragments (mean±SD)	Chromosomal gab (mean±SD)	Exchange (mean±SD)	Multiple break
control	11.24±1.73	2.95	1.00	0.50	1.5	0.50	0
10	9.87±1.96*	7.25	4.50	1.75	1.50	0.75	0
20	7.95±1.78*	9.15	6.75	2.00	1.50	0.75	0
30	6.35±0.87*	9.75	5.50	2.75	1.00	2.75	.50
40	5.88±2.71*	16.75	19.50	3.50	2.00	1.75	1.75
50	4.98±1.98*	26.50	23.25	3.75	7.25	2.25	3.75
100	5.12±2.78*	34.50	29.25	5.75	5.75	4.75	5.75

3 hour

Conc. µ/ml	Mitotic index (mean±SD)	Abnormal metaphase (mean±SD)	Chromosomal break (mean)	Chromosomal fragments (mean±SD)	Chromosomal gab(mean±SD)	Exchange (mean±SD)	Multiple break
control	10.76±0.16	4.75	1.25	1.75	1.00	0.50	0
10	10.43±2.25 *	5.50	1.50	2.25	2.75	0.50	0
20	9.75±1.38*	6.25	2.50	2.75	2.75	0.75	0
30	8.34±2.56*	12.00	7.25	2.00	3.75	2.75	1.75
40	7.34±2.45*	19.75	18.75	2.75	2.75	3.00	4.50
50	6.25±2.98*	47.50	31.25	4.25	3.50	9.50	8.75
100	5.23±1.75*	45.50	29.25	9.25	5.75	8.75	9.50

5 hour

Conc. µ/ml	Mitotic index (mean±SD)	Abnormal metaphase (mean±SD)	Chromosomal break (mean±SD)	Chromosomal fragments (mean±SD)	Chromosomal gab(mean±SD)	Exchange (mean±SD)	Multiple break
control	11.95±1.00	4.50	1.75	1.50	0.50	0,25	0
10	7.46±2.08*	9.50	4.75	1.50	0.50	3.50	0
20	6.26±2,43*	10.00	4.25	1.50	2.50	3.75	0
30	7.15±2.88*	11.50	5.25	3.75	2.00	2.50	0
40	6,95±2.16*	18.50	10.75	3.75	5.00	4.75	2.50
50	5.36±3.76*	48.25	30.50	9.75	4.75	5.25	11.00
100	4.98±2,16*	56.75	36.00	12.75	7.00	7.75	12.25

*Statistically significant when compared to untreated control
Statistically significant at P<value, a=0.005 when compared with previous concentration

Results and Discussion

Nickel is one among the micronutrients for the growth and development of plants and in small quantities, it has been reported to improve crop yield and quality. However, similar to other micro elements, at excess concentration this metal becomes toxic for most of the plant species. Phytotoxicity of nickel has been attributed to its deleterious effects on photosynthesis, respiration and mineral nutrition (Parida *et al.*,2003; Llamas *et al.*, 2008; Llamas and Sanz, 2008) as well as its ability to induce oxidative stress. Nickel toxicity and the following detoxification in plants is still explored less compared to other heavy metals such as aluminum and cadmium (Prasad, 2004). Hence, a clear insight over nickel toxicity on plant as well as human genome is needed. In the present study, $NiCl_2$ was exposed to onion root meristem in different concentrations and time interval to evaluate nickel induced genotoxicity. Mitotic index was higher (9.87 ± 1.96) at initial concentration of 10µg/l in all time intervals (1,3,5 hours) tested and decreased progressively when $NiCl_2$ concentration (20, 30,40,50,100µg/l) and exposure time was increased (Table. 1). Heavy metal induced genotoxic effects on plants and other biological systems depend on the oxidation state of metal, its concentration and duration of its exposure. In general, effects are more pronounced at higher concentrations and at longer duration of exposures. Plant species could respond differently to exposure to the same metal depending on the number of diploid chromosomes, total length of the diploid complement and the number of metacentric chromosomes (Ma *et al.*, 1995).

Epidemiological studies have clearly implicated Ni(II) compounds as human carcinogens on the basis of an increased mortality from respiratory tract malignancies in refinery workers chronically exposed to nickel containing dust and fumes. Other health effects of inhalation exposure to soluble and insoluble nickel compounds are also reported (IARC, 1990). Hence, the specific level for exposure with this nickel containing dust and other products need to be evaluated by means of a suitable assay. The result of this present study is in par with this report (Olorunfemi and Ehwre, 2010; Sharma and Vig, 2012). Among various concentrations and exposure levels tested, chromosomal gap was found to be very less (1.00) in 30µ/l concentration in all time intervals (1,3,5 hours) and found it was increasing in higher concentrations of 20, 30,40,50 and 100µg/l (Table- 1). Bhowmik (2000) was reported such a genotoxicity with different biological materials when treated other heavy metals in different concentrations (Bhowmik, 2000).

Nickel compounds have been shown to produce single-strand breaks in cellular DNA, as well as chromosomal aberration and DNA-protein cross links (Patierno and Costa, 1985). It has been suggested that nickel was not directly involved in the DNA-protein cross links but was catalyzing it through indirect mechanisms, which may include the formation of oxygen radicals (Kasprzak.,1991). To evaluate the level of such DNA break and damage over the cross links, assessment on chromosomal breakage is being a preliminary assay. In the present study, chromosomal break was assessed in all sets of samples (root meristem) treated with different concentrations (10,20,30,40,50,100µg/l) of NiCl2 in different time intervals (1h,3h,5h). Highest chromosomal break (36.00) was observed in 100µg/l in all time intervals. This observation is substantiating the previous reports (Sik *et al.*, 2009; Olorunfemi *et al.*, 201l; Sharma and Vig, 2012). Further, the total aberration increased significantly as concentration increased.

Conclusion

Nickel is a useful heavy metal, used extensively in electroplating, manufacture of steel, alloys, batteries and electronic devices. Nickel containing compounds are released into the

atmosphere during mining, smelting, and refining operations. Nickel and its compounds have been reported to be potent carcinogens and toxic agents in human and experimental animals. However, the precise molecular mechanism of nickel carcinogenesis is undefined. In this context, chromosome based bioassays on nickel induced genotoxicity will be useful to establish a specific molecular strategy for further understanding of nickel toxicity on genomes. Mitotic index, chromosomal break, chromosomal gap, multiple break assays in this present study will be used for the development of such molecular strategies for the assessment of genetic damage by heavy metals like nickel.

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