International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 6(2): 197-201 June 2013 .

Biotechnology

Effect of Sr^{2+} on Mitotic Activity and Chromosomal Behavior in Root Meristem of *Allium cepa* L.

Desha Meena[1*], Sarvesh K Singh[2] and S.K.Chaudari[3]

[1]Forest Genetics and Tree Breeding Division, Arid Forest Research Institute, Jodhpur, Rajasthan, India
[2]Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India
[3]Defence Research Laboratory, Defence Research and Development Organization, Jodhpur, Rajasthan, India

[*]**Email:** desha@icfre.org

Paper No. 103 Received: December 13, 2012 Accepted: March 13, 2013 Published: June 1 ,2013

Abstract

The effect of Strontium chloride ranging from 5, 10, 20, 40 and 80 ppm on the mitotic activity and chromosomal behavior in root meristem of *Allium cepa* for 24h, 48h and 72h was studied. Accumulation of Sr^{2+} in the root meristem reduced the root growth of *Allium cepa* which was determined using Atomic absorption spectrophotometer (Perkin Elmer) using strontium lamp-68 at 460 nm. Concentrations higher than 20 ppm of Sr^{2+} applied for 24h were toxic for *Allium cepa*. The non-lethal concentrations of Sr^{2+} showed an inhibitory effect on cell division in root tips of *Allium cepa* and caused a decrease in their mitotic index values. All treatments changed the frequency of mitotic phases as compared with the control values. Sr^{2+} treatments produced a number of mitotic abnormalities in dividing cells in root tips of plants resulting from its action on the spindle apparatus such as C-metaphases, lagging chromosomes and multipolar anaphases and Binucleate. The induction of chromosomal stickiness and chromosomal aberrations such as bridges and breaks indicates its action on the chromosome. These abnormalities (chromosome breaks and chromosomal bridges at ana–telophases) indicate true clastogenic potential of the ions tested.

Highlights

- *Allium cepa* cells treated with Sr^{+2} ion ranging from 5, 10, 20, 40 and 80 ppm for 24h, 48h and 72h showed reduced growth, mitotic abnormalities due to the accumulation of the ion in the root meristem which indicates its clastogenic potential.

Keywords: atomic absorption analysis, *Allium cepa* L., chromosomal abnormalities, mitotic index, strontium analysis.

A rapid industrialization and its use in agriculture had led to regional and global redistribution of metals with consequent environmental pollution (Gupta *et al.,* 2009). Estimation of the migration ability of any pollutant in the natural environment is considered to be a necessary stage for predicting the ecological situation (Jayakumar and Jaleel 2009). The metal trace elements pollution is a big problem for the large parts of the world. They usually occur as a result of natural and anthropogenic activities such as urbanization, nuclear explosion, military exercise, atomic blast (Choudhary *et al*., 2006). Heavy metals are the most hazardous non-degradable pollutants, get accumulated and become toxic both to plants and animals (Sharma *et al.*, 2005). In trace amount, they are essential for various metabolic processes in living organisms, and create physiological stress leading to generation of free radicals when in high concentration (Meena *et al*., 2011). The occurrence and behavior of strontium in the biosphere have acquired increasing interest since the advent of its long-lived radioactive isotopes which are products of nuclear

fission. Small quantities of native strontium are widely distributed in soils, plants and animals, where its abundance is usually compared to that of the homologous element calcium. The radiation hazard from food contaminated with radioactive fall-out is the internal radiation from fission products entering the bodies of animals and humans. The principal source of internal radiation immediately following a nuclear explosion is the external contamination of edible plants resulting from fresh fission products being deposited on agricultural areas. As time passes and the initially contaminated food has been discarded, the principal sources of internal radiation become indirect. From the contaminated soils the radioactive isotopes are absorbed through plant roots into food- and feed crops. Radioactive strontium has been shown to be accumulated in above-ground parts of the plant, to a greater extent than any other of the long-lived fission products. The radioactive strontium can lead to various bone disorders and diseases, including bone cancer (Reginster *et al.,* 2005). Therefore the knowledge on plant uptake of radio strontium will be important for devising effective strategies and developing techniques, such as agriculture counter measures and phyto-remediation to minimize the transfer of radio strontium from soil to humans (Brummer *et al*., 1986).

Disturbances in growth and morphogenesis are one of the visible symptoms of stressor action. Most compounds penetrate into the plant through root system; therefore root is the place of primary responses to their action. The root is characterized by a very high growth rate and this determines its very high sensitivity to various factors. Variation in the root growth is easy to measure, therefore cytological assay become very convenient method for investigation of toxicity of various chemical compounds (Kozhevnikova *et al.,* 2008).

The first study on mitosis was carried out by Levan (1938) by using colchicin on *Allium cepa* root meristem cells. Allium test is the most common test in order to determine the toxicity in the labs because of the storage and easy growing pecularities of Allium (Rank *et al*., 2002; Yüzbaþýoðlu *et al*., 2003). Therefore the present study was designed to examine the effect of strontium chloride on cell divisions in the root meristems of *Allium cepa* to reveal the cytotoxic effect and chromosomal abnormalities under the laboratory conditions.

Materials and methods

Study area: A laboratory experimental test was performed at Defence Research Laboratory, Defence Research and Development Organization Jodhpur.

Plant material, growth conditions and metal treatments: Healthy and equal-sized bulbs of the common onion *Allium cepa* L. (2n = 16) were used for the experiment. The experimental set up was similar to that of Fiskesjö (1988). 100 onions bulbs with the dry scales removed were used in each series were washed and directly placed in a container with strontium solution ranging from 5, 10, 20, 40 and 80 ppm prepared in distill water (pH = 6.5) for 24h, 48h and 72h**,** respectively. Control roots were maintained in distill water (pH = 6.5). Onion bulbs were allowed to germinate and produce roots in beakers at room temperature (20°C) and were protected from direct sunlight. The length of the roots for each series of concentrations was measured respectively for 24h, 48h and 72 h. The 9 best onion bulbs within each set of treatments for 24h, 48h and 72h respectively were selected for strontium analysis as well as mitotic activity.

Strontium Analysis

One ml of sulphuric acid and 15 ml of double distilled water were added to a Kjeldahl flask containing 1 g of dried and powdered root samples of treated onion bulbs and incubated at 80°C for overnight. After that 5 ml of acid mixture (nitric acid, 3: perchloric acid, 1) was added and digested until the nitric acid and perchloric acid were driven off. The digest was cooled, diluted, filtered through Whatman No.42 filter paper and made up each to 50 ml. The digested samples were then analyzed for accumulation of strontium content after 2^{nd} and 5^{th} day of treatment (mg g^{-1} DW) using Atomic absorption spectrophotometer (Perkin Elmer) of strontium lamp -68 at 460 nm.

Mitotic Activity and Chromosomal behavior

27 root tips in each treatment group were washed with tap water and distilled water, and excised at 24h, 48h and 72 h, respectively and fixed in 1:3 acidic alcohols at 10.30 a.m. and preserved in acetyl alcohol. Root tips squashes were made using 2% Acetocarmine stain. Different phases of mitosis were counted and chromosomal abnormalities were observed to calculate mitotic index and total abnormality percentage at different phases.

MITOTIC INDEX = Number of actively dividing cells / Total number of cells *100

Results and discussion

Phytotoxic effects of strontium on root growth of *Allium cepa*

Exposure to 5, 10, 20, 40 and 80 ppm strontium chloride solution caused drastic changes and significantly reduced the growth of the root of *Allium cepa*. The effect of the strontium on the growth of the root is shown in figure 1.

The growth of *Allium cepa* was adversely affected when the plants were exposed to different concentrations of strontium at 24h, 48h and 72 h, respectively. The root length of the *Allium cepa* was decreased on enhancing the strontium concentration and maximum was recorded with treatment of 5ppm for 24h. Strontium stresses cause multiple direct and indirect effects on plant growth and also alter some physiological processes. Plant height decreased due to decrease in mitotic frequency and accumulation of strontium in cell wall components (Tomar *et al*., 2000). As a result, phytotoxicity of strontium on growth parameters has been drastically reduced. The mechanisms behind this hyper accumulation and detoxification include chelation to organic acids or proteins (Oven *et al*., 2002) or it may be due to its larger biomass apart from the stronger metal uptake ability.

Strontium Analysis

The Atomic absorption spectrophotometer (AAS) studies revealed the accumulation of strontium primarily in the roots

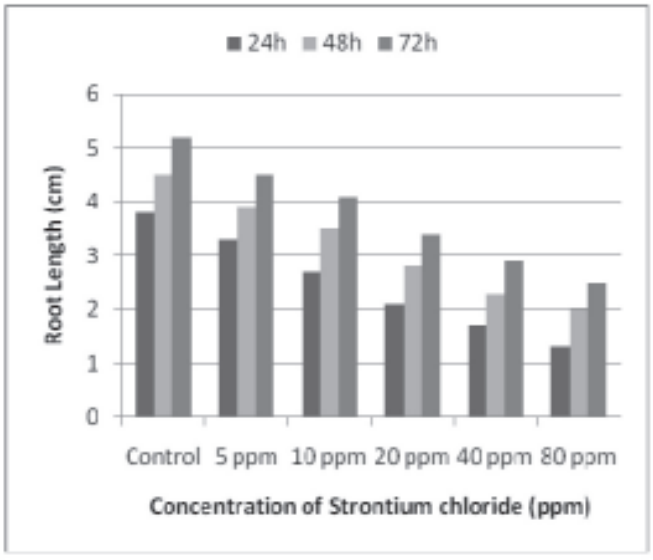

Figure 1: Phytotoxic effects of strontium on root length (cm) of *Allium cepa*

because of the absorption of nutrition from the treated nutrient sources. As the duration of the treatment level was increased the accumulation of the strontium in the root

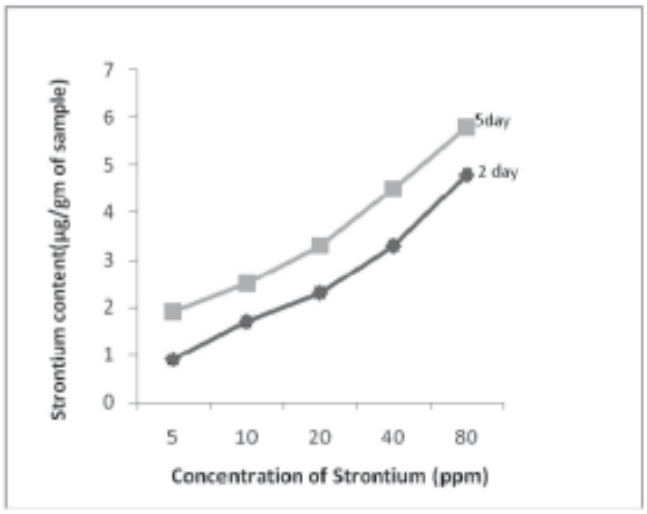

Figure 2. Accumulation of strontium in root tissue of *Allium cepa*

tissues also got enhanced resulting in the strontium content high in 5th day root tissue compared to 2nd day. (Figure 2)

Effects of strontium on mitotic index of *Allium cepa* root meristem

The mitotic index reflects the frequency of cell division and it is regarded as an important parameter. The mitotic index decreased progressively as a function of increased strontium concentration and exposure time (Table 1**)** thus can be concluded that higher concentration and longer duration of treatment is toxic to cells.

There have been a number of differences in mitotic index in all concentrations and all times. The highest values in mitotic difference were obtained in 72h application of 80ppm concentration and the lowest in 24h examination of 80 ppm strontium. Decrease of mitotic index level shows mitodepressive effect of the experimental material resulting in the inhibition of cells access to mitosis. It shows that *Allium cepa* disturbs the normal cell cycle process by preventing the biosynthesis of DNA and/or microtubule formation (Sadia and Vahidy, 1994).

As the concentration of Strontium was increased the abnormal dividing cells were observed. Clumping of chromosomes was most frequently encountered. C-metaphases, lagging chromosomes, chromosomal bridges

Table 1: Mitotic Effects of strontium chloride in root tips of *Allium cepa* root meristems

Concentrationof Strontium chloride(ppm)	Number of cells	Mitotic Index(%)	Normal Dividing cells	Anamolous dividing cells			
				C-metaphases	Lagging chromosomes	Binucleate	Chromosomal breaks
Control- 24hr	150	75.3	113	-	-	-	-
5	150	70.6	106	-	-	-	-
10	150	61.3	92	-	-	-	-
20	150	56.6	85	-	-	-	-
40	150	42	63	-	1	-	1
80	150	48	72	1	2	2	2
Control-48hr	150	62.6	94	1	-	2	-
5	150	56	80	1	1	1	1
10	150	53.3	72	1	2	2	3
20	150	46.6	61	1	1	3	4
40	150	42.6	48	4	3	3	5
80	150	44.6	53	3	2	4	5
Control-72hr	150	47.3	71	1	1	-	2
5	150	43.3	58	1	2	2	2
10	150	38.6	46	2	3	3	4
20	150	35.3	39	2	3	4	5
40	150	33.3	33	3	4	4	6
80	150	34.6	28	4	3	5	5

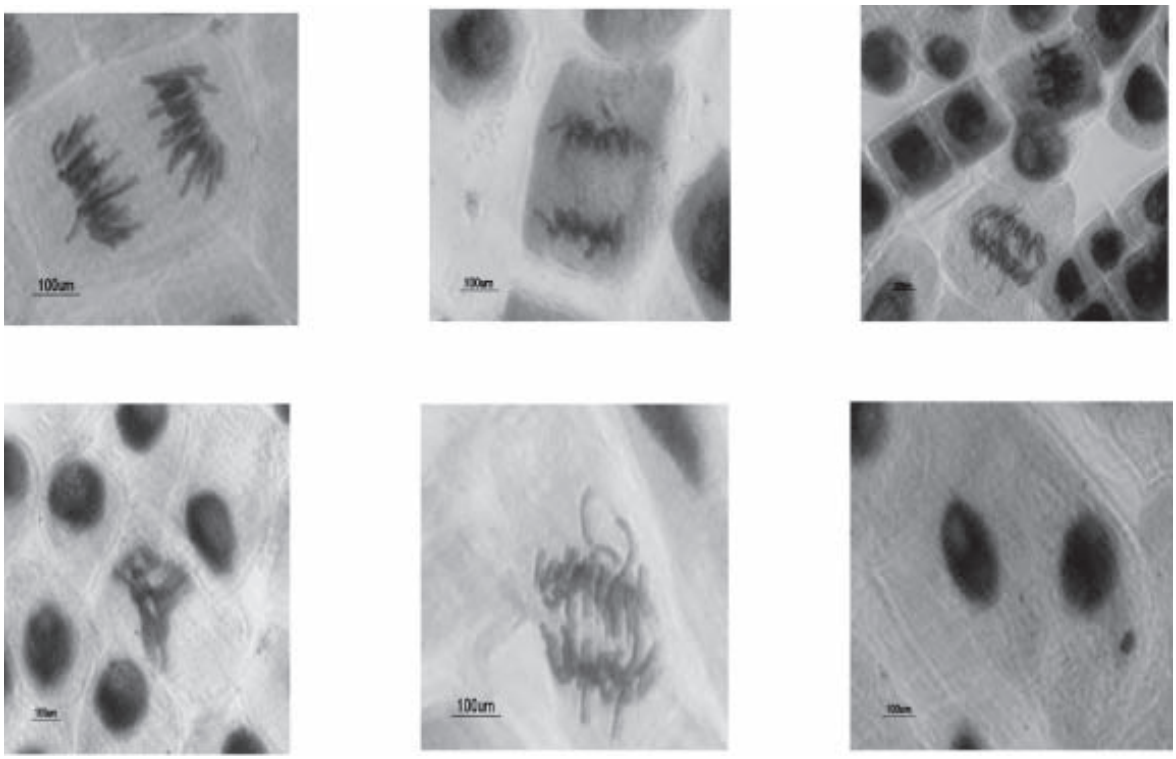

Figure 3: Chromosomal abnormalities observed in *Allium cepa L*. under different treatment of Strontium chloride (A) Lagging Chromosome (B) Chromosomal Breaks (C) Clumped chromosomes in Metaphase and Chromosome Bridge (D) Chromosome stickiness (E) C-Metaphase (F) Binucleate

and breaks, Multipolar anaphases, Binucleate and Chromosomal stickiness were also observed during the divisions. When the growth inhibition on root was more than 45%, it indicated the presence of a substance which was either sub lethal or toxic to the examined organism.

The other anomalies such as disturbed anaphase-telophase and stickiness were also observed by the effect of strontium on microtubule formations. Stickiness occurs because of sub-chromatid linkage between chromosomes or chromosomes lose their movement abilities, due to which they get stuck in anywhere, and cannot go to final destination. Stickiness is accepted as an indicator of toxicity which results in cell death (El-Ghamery *et al*., 2000) where as Binuclear cells are accepted as the inhibition of cytokinesis in any control points of the cellular cycle (Ateeq *et al*., 2002). Very few numbers of Anaphase bridges were also observed. This could happen during the translocation of the unequal chromatid exchange or due to dicentric chromosome presence and bridges caused structural chromosome mutations. (El-Ghamery, El-Nahas and Mansour, 2000).

Conclusion

Hence, it is concluded that higher concentration and longer duration of treatment is toxic to cells. As the concentration of strontium chloride is increased the growth of the roots of *Allium cepa* reduced gradually from 2nd to 5th day and revealed the accumulation of strontium in the root cells and observed increase in strontium content from 2nd to 5th day. Different cytological changes produced by the strontium chloride were investigated in root meristem of *Allium cepa* and showed mitodepressive effect in lower concentration of strontium. Therefore, the present study revealed the cytotoxic and clastogenic properties of strontium chloride.

References

Ateeq, B., M. Abul Ferah, Niamat, M. Ali, and M. Ahmad. 2002. Clastogenicity of pentachlorophenol, 2, 4-D and butachlor evaluated by *Allium* root tip test. *Mutation Research* **514**: 105-113.

Brummer, G., J. Gerth, and U. Herms. 1986. Heavy metal species, mobility and availability in soils. Z. P flanzenernaehr Bodenkd **149**:382-398.

Choudhary, M., U. Kumar, Jetley, M. A. Khan, S. Zutshi, and T. Fatma. 2006. Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the Cyanobacterium *Spirulina platensis*-S5. *Ecotoxic Environment Safety* **66**:204-209.

El-Ghamery, A. A., A. I. El-Nahas, and M. M. Mansour. 2000. The action of atrazine herbicide as an indicator of cell division on chromosomes and nucleic acid content in root meristems of *Allium cepa* and *Vicia faba*. *Cytologia* **65**: 277-287.

Fiskesjo, G. 1988. The Allium test - An alternative in environmental studies: the relative toxicity of metal ions. *Mutation Research* **197**: 243-260.

Gupta, D.K., A. Srivastava, and V.P. Singh. 2008. Phytoremediation of lead induced toxicity in *Vigna mungo*(L) Hepper by Vetiver Grass. In Proceedings of The Vetiver System for Environmental Protection and Natural Disaster management. Pp 126-135. Cochin, India.

Jayakumar, K., and C. A. Jaleel. 2009. Uptake and Accumulation of Cobalt in Plants: A Study Based on Exogenous Cobalt in Soybean. *Botany Research International* **2** : 310-314.

Kozhevnikova, A. D., I. V. Seregin, E. I. Bystrova, A. I. Baelyeva, M. N. Kataeva, and Ivanov,V.B. 2009. The effect of Lead, Nickel and Strontium Nitrates on Cell Division and Elongation of Maize Roots. *Russian Journal of Plasma Physics* **56** :242-250.

Levan, A. 1938. The effect of colchicine on root mitosis in Allium. *Hereditas* **24**: 471-486.

Meena, D., H. Singh, and Chaudhari,S.K. 2011. Elucidating Strontium Response on Growth Dynamics and Biochemical Change in *Phaseolus mungo* L. *International Journal of Agriculture, Environement and Biotechnology* **4** :107-113.

Oven, M, E. Grill, A. Golan-Goldhirsh, T. M. Kutchan, and Meinhart,H.Z. 2002. Increase of free cysteine and citric acid in plant cells exposed to cobalt ions. *Phytochemistry* **60**:467-474.

Reginster, J.Y., E. Seeman, M.C. DeVernejoul, S. Adami, J. Compston, C. Phenekos, J. P. Devogelaer, Diaz , M. Curiel. A. Sawicki, S. Goemaere, O.H. Sorensen, D. Felsenberg, and P. J. Meunier. 2005. Strontium ranelate reduces the risk of non vertebral fractures in postmenopausal women with osteoporosis: treatment of peripheral osteoporosis (TROPOS) study. *J Cl Endocrinology and Metabolism* **90**: 2816-2822.

Rank, J., Lopez,L.C., Nielsen,M.H. and Moretton,J 2002. Genotoxicity of maleic hydrazide, acridine and DEHP in *Allium cepa* root cells performed by two laboratories. *Hereditas* **136:**13-18.

Sadia, K. B. and Vahidy, A.A. 1994. Cytotoxic effect of herbicide ronstar on meristamic cells of *Allium cepa*, L. *Pakistan J ournal of Botany* **26**: 69-74.

Sharma, R.K.,Agrawal, M. and Marshall,F. 2005. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicology and Environment Safety* **66**: 258-266.

Tomar, M., I. Kaur, Neelu, and Bhatnagar,A.K. 2000. Effect of enhanced lead in soil on growth and development of *Vigna radiate* (L.) Wilezek. *Indian Journal of Plasma Physics* **5**(1):13-18.

Yuzbasýoglu, D., F. Unal, C. Sancak, and Kasab, R. 2003. Cytological effects of herbicide race flurochloridone on *Allium cepa*. *Caryologia* **56**: 97-105.

