Allium Cepa Root Chromosomal Aberration Assay: A Review

Namita Khanna*and Sonia Sharma

Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, India.

**Department of Physiology, Guru Gobind Singh Medical College, Baba Farid University of Health Sciences, Faridkot, Punjab, India.*

Received 05-08-2013; Revised 14-08-2013; Accepted 20-08-2013

Abstract

Higher plants, an important material for genetic tests to monitor various pollutant present in the environment. Among the plant species, *Alium cepa* has been used to evaluate chromosome aberrations and disturbances in the mitotic cycle. Now days, it has been used to assess a great number of genotoxic/antigenotoxic agents, which contributes to its increasing application in environmental monitoring. The *A. cepa* is commonly used as a test organism because it is cheap, easily available and handled and has advantages over other short-term tests. Among the endpoints of *A. cepa* root chromosomal aberrations, detection of chromosomal aberration have been the most used one to detect genotoxicity/ antigenotoxicity along the years. The mitotic index and chromosomal abnormalities are used to evaluate genotoxicity and micronucleus analysis used to verify mutagenicity of different chemicals. The *Allium cepa* root chromosomal aberration assay is widely used to determine genotoxic and antigenotoxic effects of different plant extracts.

Keywords: *Allium cepa*, genotoxicity, clastogenic, mitotic index.

1.Introduction

There are number of toxic chemicals in the environment, they are mostly discharged by industries into water, air and soil. The continuous use of chemicals, led the world to establish various chemicals industries. The chemicals enter in our environment through both natural and anthropogenic ways. Once they enter in our biological process, it's really difficult to eliminate them from the environment and disturb various biochemical processes, leading to fatal results. Numerous potentially mutagenic chemicals have been studied because they can cause mutagenic, damaging and inheritable changes in the genetic material. Many thousands of toxic chemicals including pharmaceuticals products, domestic and industrial wastes, pesticides and petroleum products are present in the environment and new chemicals are being introduced every year. No doubt, rapid progress of chemical industry has provided economic and social benefits but at the same time it has accentuated the environmental and social problems. Environmental biologists are presently concerned to safeguard the human beings from exposure to chemicals.

Genotoxicity is to determine the magnitude of genetic risk to man by an environmental agents/ chemicals under a specified level of exposure. Unfortunately, the direct assessment in human is not feasible because of ethnic, logistic and practical considerations.

***Corresponding Author: Dr. Namita Khanna,** Department of Physiology, Guru Gobind Singh Medical College, BFUHS, Faridkot, Punjab, India. **E-Mail Id:** dr.namitakhanna@yahoo.com, soniasharma.bot@gmail.com **Mobile No.** +91-9417392924

Even the epidemiological approaches used to detect genotoxic and carcinogenic chemicals have limitationsbecause detection is possible systems. There are many employing wide variety of organisms ranging from viruses, bacteria, plants and insects to human cell cultures and intact mammals to evaluate the mutagenicity of environmental chemicals. In order to identify the harmful effects of substances in different concentrations and time of exposure, a variety of tests have been employed, such as cytogenetic tests. These tests are commonly used for biomonitoring the extent of pollution and to evaluate the effects of toxic and mutagenic substances in the natural environment [1,2]

Higher plants constitute an important material for genetic tests to monitor environmental pollutants. However this feature is due to the possibility of assessing several genetic endpoints range from point mutation to chromosomal aberrations in cells (Table 1).

Among the higher plants species, the most frequent ones used to evaluate environmental contamination are *Allium cepa*, *Vicia faba*, *Zea mays*, *Tradescantia*, *Nicotiana tabacum*, *Crepis capillaris* and *Hordeum vulgare*. But, still among these species, *Allium cepa* (Onion) has been considered an efficient test organism to indicate the presence of mutagnic chemicals [3,4] due to its kinetic characteristic of proliferation and chromosome suitable for this type of study [1,2]. *A. cepa* root chromosomal aberration assay was described as an efficient test system routinely used to evaluate the genotoxic potential of chemicals in the environment, due to its sensitivity and good correlation with mammalian test systems [5,6]. Thus *A. cepa* is an efficient test organism for environmental monitoring, especially in contaminated aquatic environments [7-9].

2.Modification of *Allium cepa* root chromosomal aberration assay

Higher plants, an important material for genetic tests to monitor various pollutant present in the environment. The *A. cepa* test was first introduced by Levan [3] to examine the effect of colchicines on mitotic spindles and has been in frequent usage since then. The procedure of the original test implied germinating onion bulbs in distilled water at room temperature after removing dry scales of bulbs. When the roots tips grown out to a length of 1-2 cm in water, and thereafter exposed to specific treatments followed by macroscopic and microscopic observations after a certain time period. However, weak contaminations in naturally occurring water, as in water from rivers or other supplies of water for human use, often gave very little effects in the original form of the test [10]. Since then, technical modifications in the *A. cepa* test have been designed in order to enable a more comprehensive assessment of weak and unknown contaminations, as the complex mixtures, which comprehend most of the environmental and the pure samples [4-5,11].

The first adaptations of the *A. cepa* test were made by Fiskesjo [4] by designing it a test organism for environmental monitoring. For this purpose, he proposed modifications that allowed both the evaluation of soluble and insoluble compounds in water and the assessment of the effects of complex mixtures. Series

of onions bulbs were being allowed to directly germinate in the chemical to be tested and the final observations being made within few days. Since no initial treatment with pure water was included, so this method of "treatment" is more similar to conditions in nature. Even small amounts of toxic contaminations by chemicals produced effects on the differences in root length among the different experimental series of bulbs. More severe toxic effects of chemicals influence the shape and color of the root tips also. For further extending the significance of the results, microscopic analysis can be performed. This new modification of the *Allium cepa* root chromosomal aberration assay has also been convenient for studying the action of different concentrations of known toxic chemicals.

Later, Rank and Nielsen [12] proposed new modifications to the *Allium* test, making it even more efficient to analyze various known complex mixtures. However, all the modifications proposed by the authors were related to the evaluation of chromosomal aberrations (CA), which detects various genotoxic agents. The test was modified to assess the mutagenic effects by observing the micronuclei (MN) induction in the roots cells of *A. cepa* exposed to different environmental pollutants. It is known that CA, such as chromosomal breaks, fragments and chromosome losses, result in the formation of micronucleated cells, since both fragments and entire chromosomes cannot be incorporated into the main nucleus during the cell cycle [13]. Nevertheless, Rank [11] presented a different opinion from the above authors, because according to them, the CA analysis, besides estimating the genotoxic effects of tested agents, also enables the evaluation of their clastogenic and aneugenic actions. Since several authors have demonstrated the efficiency of the analysis of CA in *A. cepa as* to be more advantageous to investigate the action mechanisms of tested agents on DNA, which enables a better understanding of the effects promoted by such agents [2, 14]. It may be an advantage to use the modified *Allium* test as it needs lower concentrations to give specific response as compared to older methods, which means that under certain conditions it is more sensitive than the original test. The modified test is also especially well suited for the photographic display of the macroscopic and microscopic responses.

3.Materials and methods

Test organism

Healthy and equal sized bulbs of common onion (*Allium cepa* L.:2n=16), are chosen and series of bulbs are grown in test chemicals. For experiments, dried and diseased bulbs should not be used.

Test procedure

The loose outer scales of bulbs and old roots were removed with the help of sharp and pointed forceps so as to expose the root primodia. A series of bulbs were then placed on coupling jars containing test liquid at a temperature of 25±1°C. The experiment should be performed at relatively constant temperature and protected against direct sunlight. Test chemical should be stored in refrigerator (Figure 1)

Cytological investigations.

Fixation

After treatment, the bulbs were washed thoroughly under running tap water. The root tips from each bulb were plucked and fixed in Farmer's fluid (glacial acetic acid: ethanol:: 1:3) for 24 hours.

Squash preparation

For chromosomal analysis, the root tips were hydrolyzed in 1N HCl at 60^0C for 1

minute and transferred to a watch glass containing aceto-orcien and 1N HCl (9:1). They were then heated intermittently for 5-10 minutes, covered and kept aside for 20-30 minutes. The tip of the root was then cut with sharp blade and placed on a glass slide in a drop of 45% glacial acetic acid and covered with coverslip. The root tip was squashed by tapping with matchstick and sealed with DPX. The cells were scored under the microscope for different types of chromosomal aberrations.

3.1 Advantages of *Allium cepa* root chromosomal aberration assay

The main conclusion of all the investigations made by many authors was that plant assays are efficient and reliable test systems for the rapid screening of chemicals for mutagenicity and clastogenicity. Among these assays *Allium cepa* L. chromosomal aberration assay have been proven to be effective, sensitive, less costly and used for testing the potential mutagens in both mitotic and meiotic cells [15-16]. The *Allium/Vicia* root chromosomal aberration assay has also been adopted by the International Program on Plant Biossays (IPPB) for the evaluation of the environmental pollutants [17]. This assay has also been used to monitor the antigenotoxic nature of various plants and plant products.

Different parameters of *Allium cepa* such as root shape, growth, mitotic index and chromosomal aberrations can be used to estimate the cytotoxicity, genotoxicity and mutagenicity of environmental pollutant [18]. The *Allium* test has many advantages as genotoxicity screening assay, one being that root cells of *Allium cepa* posses the mixed function oxidase system which is capable of activating promutagens or genotoxic chemicals. In the *Allium* test, inhibition of root growth and the appearance of stunted roots indicate cytotoxicty, while wilting of root explains toxicity [5]. Nevertheless both these observations are due to the suppression of mitotic activity.

Figure 1: Schematic representation of *Allium cepa* root chromosomal aberration assay.

Table 1. Summary on use of *Allium cepa* root chromosomal aberration assay for environmental monitoring.

S. No	Agent/s studied	Nature	Type of aberrations	Reference
1.	Hospital effluents	Chemica l mixture of pollutants	chromosomal disruptions, anaphasic bridge/s and micronuclei	[19]
2.	Coal fly ash	Mixture of chemicals	root growth and mitotic indices inhibition; binucleated cells formation.	[20]
3.	Industrial wastewater	Wastewater	mitotic division reduction; mitotic anomalies	[21]
4.	Lead	Heavy metal	decrease root growth and mitotic index; Induce chromosome bridge/s, laggard chromosome/s and micronuclei.	[22]
5.	Nano-silver	Anti-bacterial	mitotic index decrease, c-metaphase, stickiness, bridge/s, laggard/s and micronuclei	[23]
6.	Magnesium sulphate	Fertilizers	cytostatic and clastogenic properties	[24]
7.	Industrial effluents contaminated with azo dyes	Mutagenic chemicals	mitotic index reduce; bridge/s, laggard/s, c-metaphase, binucleated cells; loss of chromosomes	[25]
8.	Lead	Heavy metal	root growth and mitotic index reduced; chromosome bridge/s, laggard chromosome/s and micronuclei	[22]
9.	Maleic hydrazide	Herbicide	chromosomal aberrations like bridge/s, laggard/s etc.	[26]
10.	Petroleum hydrocarbon	Complex chemical mixture	nuclear bud, micronuclei, mini cells, polynucleated cells, chromosomal bridge/s, c-metaphase and break/s	[14]
11.	Extracts of *Psychotria* (*P. myriantha* and *P. leiocarpa*)	Herbal medicine	chromosomal aberrations, inhibition of cell division was more in *P. leiocarpa* than *P. myriantha*	[27]
12.	Quizalofop-P-ethyl	Herbicide	stickiness, bridge/s, vagrant/s, c-anaphase, multipolarity, micronuclei	[28]
13.	Cadmium	Metal	inhibition of mitotic index; CA, MA	[29]

			and micronucleus	
14.	Maleic hydrazide	Metal	mutagenic events reduce and induce translocation of chromosomes	[30]
15.	Atrezine	Herbicide	inhibit mitotic index; micronucleus, chromosomes and mitotic aberrations	[31]
16.	Aluminium	Metal	oxidative stress, damage DNA and cell death	[32]
17.	Aqueous extracts of *Azadirachta indica, Morinda lucida, Cymbopogon citratus, Mangifera indica* and *Carica papaya*	Medicinal plants	mitotic spindle disturbance, inhibitory, mitodepressive, turbagenic and inhibition of root growth	[33]
18.	Curcumin	Antimutagen	chromosome break/s, gap/s and fragment/s	[34]
19.	Potassium metabisulp-hite	Food preservative	mitotic index reduce; break/s, gap/s	[35]
20.	Sodium benzoate, boric acid, citric acid, potassium citrate and sodium citrate	Food preservative	mitotic division reduce, anaphase bridge/s, c-mitosis, micronuclei, break/s, lagging, stickiness, and unequal distribution	[36]
21.	*Plantago lanceolata*	Medicinal plant	decrease mitotic index; induce breaks, bridges, stickiness	[37]
22.	Vanadium	Metal	chromosomal aberrations	[38].
23.	Avenoxan	Herbicide	abnormal cell increased, stickiness, bridge/s, laggard/s	[39]
24.	Acetaminophen	Analgesic	roots did not grow at high concentration, mitotic index declined	[40]
25.	Fumonisins	Toxic	genetic damage occurs, chromosomal aberrations, sister chromatid exchanged	[41]
26.	Lechates from solid waste	Heavy metal contamination	mitotic index inhibition, chromosomal aberrations and	[42]

			micronuclei	
27.	Heavy metal contaminated river water	Heavy metal	decreased cell reproduction; bridge/s, fragment/s, laggard/s, c-mitosis, micronuclei	[43]
28.	Dinocap	Fungicides	stickiness, c-mitosis, laggard/s, multipolarity, micronuclei, polyploidy fragment/s	[44]
29.	Air pollution	Cytotoxic substance	mitotic cell division decreased, genotoxic substance found	[45]
30.	Diuron contaminated soil	Urea herbicide	break/s, micronucleated and binucleated cells; mitotic index declined	[46]
31.	Atrazine	Pesticide	break/s	[47]
32.	BDE-99	Flame retardant	chromosomal aberrations	[48]
33.	Industrial wastewater from Shawa, Meet EI, Akrad, Telbana, Belgay	Industrial wastewater	mitotic division inhibition, chromosome ring/s, fragment/s, bridge/s, disturbed metaphase	[49]
34.	Sewage water	Toxic metals	growth inhibition occur, wilting appears on root tip/s, abnormal dividing cell increased	[27]
35.	Aqueous extract of *Aristolochia triangularis, Cayaponia bonariensis, Solanum granulsolepros-um*,	Antihypertensive agents	micronuclei, asynchronic divisions	[49]
36.	Sodium metabisulfite	Food preservatives	mitotic index decreased, c- mitosis, stickiness	[50]
37.	*Azadirachta indica*	Insecticide	micronucleus, multinucleated cells, bridge/s, stickiness, laggard/s	[51]
38.	Lead	Metal	mitotic activity inhibition, level of DNA synthesis declined, c-mitosis	[52]
39.	Sewage and industrial effluents from the Amritsar	Domestic and industrial wastewater	high number of micronuclei and anaphase aberrations	[53]

40.	Cypermeth-rin and fenvalerate	Insecticides	mitotic index inhibition; chromosomal and mitotic aberrations	[6]
41.	Cs and Sr	Radioisotopes	germination rate of onions decrease; aberrations like stickiness, vagrant	[54]
42.	Waste, surface and ground water	Toxic substances	root growth inhibition; metaphase and anaphase aberrations	[55]
43.	Polluted water sample	Industrial and municipal wastewater, water from treatment plant	fragment/s, c-mitosis, stickiness	[15]
44.	Alkyl benzene, sulphonate and citowett	Surfactants	root length declined; mitotic index decreased; chromosomal aberrations	[56]
45.	Wastewater samples	Mixture of toxic substances	inhibition of mitotic activity, chromosomal and genomic aberrations	[57]
46.	Phosphine gas	Fumigative agent	root length and viability of seeds reduced, frequency of aberrated cells increase	[58]
47.	Carbetamide	Pesticide	c-mitosis, break/s and bridge/s	[59]
48.	Chlorophenoxy acids	Herbicide	c-tumors, stickiness, vagrant/s, fragment/s; mitotic index decreased	[60]
49.	Carboxin, Oxycarboxin	Pesticide	micronuclei	[61]
50.	2, 4, 5-T	Herbicides	cell enlargement and chromosome aberrations; duration of mitotic cycle increased	[62]

3.2 Different endpoints analysed by the *Allium cepa* root chromosomal aberration assay

The *Allium* test has been used for monitoring the genotoxic, cytotoxic and mutagenic nature of different test chemicals. Following are the genetic categories of different parameters analyzed by this test system.

I. **Root form**: The roots exhibited highest sensibility, with significant effects even at the lower concentration of test chemical. This parameter is observable after 3-5 days of treatment that show swelling, bending and discoloration of the root tips or roots.

II. **Root length and EC_{50} determination**: Root growth decrease over 45% indicates the presence of toxic

nature of substances [4] having sublethal effects on plants [52]. For the determination of EC_{50} a series of bulbs were grown on coupling jars containing distilled water at a temperature of 25±1˚C. After 24 hours, the bulbs with uniform root growth were selected and placed on coupling jars filled with different concentrations of both test chemical and distilled water (negative control). This set of onion bulbs was termed as day one. On the fourth day, root lengths were measured for each group (control as well as treatment group) and mean values were calculated. Taking mean root length of control as 100%, lengths of different treatment groups were plotted against test concentrations and the point on the graph which showed 50% growth was designated as EC_{50} concentration.

III. Mitotic index (MI): The cytotoxic level of a test chemical/compound can be determined based on the increase or decrease in the mitotic index (MI), which can be used as a parameter of cytotoxicity in studies of environmental biomonitoring [15]. Significant reduction in MI, noted in the present study may be due to the inhibition of DNA synthesis or the blocking in the G_2 phase of the cell cycle [63]. Several other chemicals have been reported to inhibit mitosis [36]. Inhibition of mitotic activities is used for tracing cytotoxic substances. The cytotoxic level can be determined by the decreased rate of mitotic index. A mitotic index decrease below 22% of negative control causes lethal effects on test organism while a decrease below 50% has sublethal effects [64] and is called cytotoxic limit value. Several investigators have used MI as an endpoint for the evaluation of genotoxicity or antigenotoxicity of different chemical treatments [65,66].

IV. Chromosomal aberrations (CAs): CAs are characterized by change in either total number of chromosomes or in chromosomal structure which occur as a result of the exposure of chemical treatment. To evaluate the different chromosomal abnormalities, several types of CAs are considered in different stages of cell cycle (Prophase, metaphase, anaphase and telophase). CAs were grouped into 2 types, clastogenic and physiological aberrations. Clastogenic aberrations include chromatin bridge/s, chromosomal break/s and ring chromosome/s where as physiological aberrations include c-mitosis, vagrant/s, stickiness, delayed anaphase and laggard/s.

The term c-mitosis was coined by Levan [3] and described that colchicines prevents the assembly of the spindle fibres and results in scattering of the chromosomes over the cells. There are number of pesticides which are c- mitotic agents like mercury, carbamates, dieldrin etc. the propham, chlorpropham, carbaryl, benomyl etc. are extremely active c- mitotic chemicals. In physiological aberrations, frequency of cells with c- mitosis was found to be maximum then other aberrations. Several investigators were able to induce C-mitosis in plant cells using different types of food additives [36, 50].

In delayed anaphase, the two anaphasic chromosomal groups lie close to each other near the equatorial plate. The frequency of aberrated cells with delayed chromosomes was very high and increased with increasing concentration of test chemicals.

Lagging chromosomes resulted due to failure of the chromosomes to get attached to the spindle fibre and to move

to either of the two poles. Turkoglu [36] also reported the induction of lagging chromosomal aberration also called laggard/s following treatment with food additives.

Stickiness of chromosomes has resulted from increased chromosomal contraction and condensation or might from the depolymerization of DNA and partial dissolution of nucleoproteins. Chromosome stickiness reflects toxic effects, usually of an irreversible type and probably leading to cell death. Same results are in line with the results of many research groups that examined the effects of different chemicals on different materials [36, 50]. In vagrant chromosome/s, a chromosome moves ahead of from its chromosomal group toward poles and leads to the unequal separation of number of chromosomes in the daughter cells. Vagrant chromosomes have been observed by many workers in different studies [30].

The clastogenic effects were noticed in the form of chromatin bridge/s, chromatin break/s and ring chromosomes. Ring chromosomes are the result of loss of chromosomes from the telomeric side. Chromatin bridges could happen during the translocation of the unequal chromatid exchange and cause structural chromosome mutation. This type of anomaly was also observed in the mitosis of *Vicia faba* and *Allium cepa* after treatments with food additives [67, 36].

V. **Micronuclei (MN)**: MN can be spontaneously originated due to the development of the isolated chromosome that results from an unequal distribution of genetic material. However, their induction is commonly used to detect genetic damages derived from exposure to mutagenic chemicals. According to some authors, MN can be a formed as a result of acentric fragments or entire chromosomes not incorporated to the main nucleus during the cell cycle. Therefore, any substance that is able to promote micronuclei formation is said to be clastogenic or aneugenic [68]. MN test is considered to be one of the most promising tests for the evaluation of environmental mutagenicity/ genotoxicity, since it is efficient, simple and fast assay Cells bearing micronuclei were observed at different stages of cell cycle, although most of them involved in interphase and prophase stages. Most often, the MN observed was synchronic to the division of main nuclei. However, in some cases such synchrony was not present. Based on analyses of human lymphocytes, some authors have suggested that the exceeding DNA of a cell may originate a bud and which gives rise to a micronucleus and it is subsequently expulsed as a mini cell. Mini cells constitute small cytoplasmatic portions bearing a small nuclear content. The formation of micronuclei (MN) in root tip cells has been widely studied in the evaluation of various chemical agents [36].

VI. Other abnormalities

Ghost cells were observed by the Celik and Aslanturk [16] while evaluating the cytotoxicity and genotoxicity of leaf extract of *Inula viscosa* with *Allium cepa* test. Ghost cell is a dead cell in which the outline is visible but nucleus and cytoplasmic structure is not stainable. Cell death or apoptosis is a biological process of living organisms. The cell death was induced by high concentrations

of toxic chemicals and other. Univalent chromosomes may result from low chiasma frequency or by the presence of asynaptic or desynaptic genes in prophase 1 stage of cell cycle. The presence of binucleated cells was reported by several investigators in several genera following chemical treatments [67]. The occurrence of binucleated cells was the result of inhibition of cytokinesis process of cell division.

4. Conclusion

From the information provided in the review, it is concluded that among different plant assays, the *A. cepa* test is an easy, fast and very sensitive assay to detect environmental genotoxicity/ antigenotoxicity of chemicals or natural plant products. This assay is related to the study of effect of chemicals at the genetic level which includes both microscopic and macroscopic parameters.Thus, this test provides an important method for the screening of environmental toxicity caused by toxicants.

Conflict of interest statement: We declare that we have no conflict of interest.

Acknowledgments
Authors are thankful to Guru Nanak Dev University, Amritsar, India for providing the necessary laboratory facilities for the work. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Matsumoto ST, Study on the influence of potentially genotoxic tannery effluents on the contamination of water resources in the region of Franca-SP, Ph.D. Thesis. State University of Sao Paulo/ Sao Jose do Rio Preto- SP. 216, 2004.
2. Matsumoto ST., Mantovani MS., Malagutti MI., Dias AL., Fonseca I.C., Marin-Morales MA., Assessment of the genotoxic and mutagenic effects of chromium residues present in tannery effluents using the micronucleus and comet assay in *Oreochromis niloticus* and chromosome aberrations in of *Allium cepa. Genetics and Molecular Biology*, 2006; 29: 148-158.
3. Levan A. The effect of colchicine on root mitosis in *Allium. Hereditas* 1938; 24: 471-486.
4. Fiskesjo G. The *Allium* test as a standard in environment monitoring. *Hereditas*, 1985; 102: 99-112.
5. Grant WF., Chromosome aberration assays in *Allium*. A report of U.S. Environmental Protection Agency Gene- Tox Program. *Mutation Research*, 1982; 99: 273-91.
6. Chauhan LKS., Saxena PN., Gupta SK.Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of *Allium cepa. Environmental and Experimental Botany*, 1999; 42: 181-189.
7. Parry JM., Tweats DJ., Al-Massaur, Monitoring the marine environment for mutagens. *Nature*, 1976; 264: 538-540.
8. Rank J., Nielson M.H. Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutation Research*, 1994;312; 17-24.

9. Jiang W.S., Liu D.H. Effects of Pb^{2+} on root growth, cell divisions and nucleolus of *Brassica juncea* L. Israel. *Journal* of *Plant Science,* 1999; 47: 153-156.
10. Fiskesjo G. Mercury and selenium in a modified *Allium* test. *Hereditas*, 1979; 91: 169-178.
11. Rank J. The method of *Allium* anaphase-telophase chromosome aberration assay. *Ekologija*, 2003; 1: 38-42.
12. Rank J., Nielson MH. Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutation Research,* 1994; 312: 17-24.
13. Fenench M. The *in vitro* micronucleus technique. *Mutation Research,* 2002; 455: 81-95.
14. Leme DM., Angelis D.F., Marin-Morales M.A., Action mechanisms of petroleum hydrocarbons present in waters impacted by an oil spill on the genetic material of *Allium cepa* root cells. *Aquatic Toxicology*, 2008; 88: 214-219.
15. Smaka-Kincl V., Stegner P., Lovka M., Toman, M.J. The evaluation of waste, surface and ground water quality using the *Allium* test procedure. *Mutation Research*, 1996; 368: 171-179.
16. Celik T.A., Aslanturk, O.S., Anti-mitotic and anti-genotoxic effects of *Plantago lanceolata* aqueous extract on *Allium cepa* root tip meristem cells. *Cellular and Molecular Biology,* 2006; 61: 693-697.
17. Ma T.H. The international program on plant bioassays and the report of the follow-up study after the hands-on workshop in China. *Mutation Research*, 1999; 426: 103-106.
18. Amin A.W., Cytotoxicity testing of sewage water treatment using *Allium cepa* chromosome aberration assay. *Pakistan Journal of Biological Sciences*, 2002; 5: 184-188.
19. Bagatin M.D., Vasconcelos T.G., Laughinghouse IV H.D., Martins A.F., Tedesco S.B. Biomonitoring hospitals effluents by the *Allium cepa* L. test. *Bulletin of Environmental Contamination and Toxicology,* 2009; 82: 590-592.
20. Chakraborty R., Mukherjee A.K., Mukherjee A. Evaluation of genotoxicity of coal fly ash in *Allium cepa* root cells by combining comet assay with the *Allium* test. Environmental *Monitoring and* Assessment, 2009; 153: 351-357.
21. Sik L., Acar O., Aki, C. Genotoxic effects of industrial wastewater on *Allium cepa* L. *African Journal of Biotechnology,* 2009; 8: 1919-1923.
22. Carruyo I., Fernandez Y., Marcano L., Montiel X., Torrealba, Z. Correlation of toxicity with lead content in root tip cells (*Allium cepa* L.). *Biological Trace Element Research*, 2008; 125: 276-285.
23. Babu K., Deepa M.A., Shankar S.G., Rai, S. Effect of nano-silver on cell division and mitotic chromosomes: a prefatory siren. *The Internet Journal of Nanotechnology*, 2008; 2: 2.
24. Bhatta P., Sakya S.R. Study of mitotic activity and chromosomal behaviour in root meristem of *Allium cepa* L. treated with magnesium sulphate. *Ecoprint*, 2008; 15: 83-88.
25. Carita R., Marin-Morale M.A. Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. *Chemosphere*, 2008; 72: 722-725.
26. Jabee F., Ansari M.Y.K., Shahab, D. Studies on the effect of maleic

hydrazide on root tip cells and pollen fertility in *Trigonella foenum-graecum* L. *Turkish Journal of Botany*, 2008; 32: 337-344.
27. Lubini G., Fachinetto JM., Laughinghouse IV HD., Paranhos JT., Silva ACF., Tedesco SB. Extracts affecting mitotic division in root-tip meristematic cells. *Biologia,* 2008; 63: 647-651.
28. Mustafa Y., Arikan ES., Genotoxicity testing of quizalofop-P-ethyl herbicide using the *Allium cepa* anaphase-telophase chromosome aberration assay. *Caryologia*, 2008; 61: 45-52.
29. Seth C.S., Misra V., Chauhan L.K.S., Singh, R.R., Genotoxicity of cadmium on root meristem cells of *Allium cepa*: cytogenetic and comet assay approach. *Ecotoxicology and Environmental Safety*, 2008; 71: 711-716.
30. Sondhi N., Bhardwaj R., Kaur S., Kumar N., Singh, B. Isolation of 24-epibrassinolide from leaves of *Aegle marmelos* and evaluation of its antigenotoxicity employing *Allium cepa* chromosomal aberration assay. *Plant Growth Regulation*, 2008; 54: 217-224.
31. Srivastava K., Mishra, KK. Cytogenetic effects of commercially formulated atrazine on the somatic cells of *Allium cepa* and *Vicia faba*. *Pesticide Biochemistry and Physiology*, 2009; 93: 8-12.
32. Achary VMM., Jena S., Panda KK., Panda BB. Aluminium induced oxidative stress and DNA damage in root cells of *Allium cepa* L. *Ecotoxicology and Environmental Safety*, 2007; 70: 300-310.
33. Akinboro A., Bakare AA. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *Journal of Ethnopharmacology*, 2007; 112: 470-475.
34. Irulappan R., Natarajan P. Antimutagenic potential of curcumin on chromosomal aberrations in *Allium cepa. Journal of Zhejiang University Science B,* 2007; 8: 470-475.
35. Kumar LP., Pannverselvan, N. Cytogenetic studies of food preservative in *Allium cepa* root meristematic cells. *Facta Universitatis*, 2007; 14: 60-63.
36. Turkoglu S., Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutation Research/ Genetic Toxicology and Environmental Mutagenesis*, 2007; 626: 4-14.
37. Celik T.A., Aslanturk O.S. Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. *Biologia,* 2007; 62: 292-296.
38. Marcano L., Carruyo I., Fernandez Y., Montiel X., Torrealba Z. Determination of vanadium accumulation in onion root cells (*Allium cepa* L.) and its correlation with toxicity. *Biocell,* 2006; 30: 259-267.
39. Kaymark F., Muranli FDG. The cytogenetic effects of avenoxan on *Allium cepa* and its relation with pollen sterility. *Acta Biologica Hungarica,* 2005; 56: 3-4.
40. Rathore H.S., Chowbey P. Prevention of acetaminophen-induced mitodepression with myrobalan in *Allium cepa* model. *Iranian Journal of Pharmacology and Therapeutics*, 2005; 4: 100-104.
41. Lerda D., Bistoni M.B., Peralta N., Ychari S., Vazquez M., Bosio G. Fumonisins in foods from Cordoba (Argentina), presence and

genotoxicity. *Food and Chemical Toxicology,* 2005; 43: 691-698.

42. Chandra S., Chauhan L.K.S., Murthy R.C., Saxena P.N., Pande P.N., Gupta S.K. Comparative biomonitoring of leachates from hazardous solid waste of two industries using *Allium* test. *Science of the Total Environment,* 2005; 347: 46-52.
43. Ivanova E., Staikova T.A., Velcheva I. Cytogenetic testing of heavy metal and cyanide contaminated river water in a mining region of southwest Bulgaria. *Journal of Cell and Molecular Biology*, 2005; 4: 99-106.
44. Celik M., Yuzbasioglu D., Unal F., Arslan O., Kasap R. Effects of dinocap on the mitosis of *Allium cepa* L. *Cytologia,* 2005; 70: 13-22.
45. Glasencnik E., Ribaric-Lasnik C., Savinek K., Zalubersek M., Mueller M., Batic F., Impact of air pollution on genetic material of shallot (*Allium cepa* L. var. *ascalonicum*) exposed at differently polluted sites in Slovenia. *Journal of Atmospheric Chemistry*, 2004; 49: 363-376.
46. Saxena P.N., Chauhan L.K.S., Chandra S., Gupta S.K. Genotoxic effects of Diuron contaminated soil on the root meristem cells of *Allium sativum*: A possible mechanism of chromosome damage. *Toxicology Mechanisms and Methods*, 2004; 14: 281-286.
47. Bolle P., Mastrangelo S., Tucci P., Evandri M.G., Clastogenicity of atrazine assessed with the *Allium cepa* test. *Environmental and Molecular Mutagenesis,* 2004; 43: 137-141.
48. Evandri M.G., Mastrangelo S., Costa L.G., Bolle P., *In vitro* assessment of mutagenicity and clastogenicity of BDE-99, a pentabrominated diphenyl ether flame retardant. *Environmental and Molecular Mutagenesis,* 2003; 42: 85-90.
49. EI-Shahaby O.A., Migid H.M.A., Soliman M.I., Mashaly I.A., Genotoxicity screening of industrial wastewater using the *Allium cepa* chromosome aberration assay. *Pakistan Journal of Biological Sciences,* 2003; 6: 23-28.
50. Rencuzogullari E., Kayraldiz A., ILA H.B., Cakmak T., Topaktas, M. The cytogenetic effects of sodium metabisulfite, a food preservative in root tip cells of *Allium cepa* L. *Turkish Journal of Biology*, 2001; 25: 361-370.
51. Soliman M.I. Genotoxicity testing of neem plant (*Azadirachta indica* A. Juss.) using the *Allium cepa* chromosome aberrations assay. *Journal of Biological Sciences,* 2001; 1021-1027.
52. Wierzbicka M. The effect of lead on the cell cycle in the root meristem of *Allium cepa L. Protoplasma,* 1999; 207: 186-194.
53. Grover I.S., Kaur S. Genotoxicity of wastewater samples from sewage and industrial effluent detected by the *Allium* root anaphase aberration and micronucleus assays. *Mutation Research*, 1999; 426: 183-188.
54. Kovalchuk O., Kovalchuk I., Arkhipov A., Teljuk P., Hotin B., Kovalchuk L. The *Allium cepa* chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in the Ukraine contaminated by the Chernobyl accident. *Mutation Research*, 1998; 415: 47-57.
55. Kincl V.S., Stegnar P., Lovka M., Toman M.J. The evaluation of waste, surface and ground water quality

using the *Allium* test procedure. *Mutation Research,* 1996; 368: 171-179.

56. Bellani L.M., Rinallo C., Bennici, A. Cyto-morphological alternations in *Allium* roots induced by surfactants. *Environmental and Experimental Botany*, 1991; 31: 179-185.
57. Vidakovic Z., Papes D., Toxicity of waste drilling fluids in modified *Allium* test. *Water, Air and Soil Pollution,* 1993; 69: 413-423.
58. Younis S.A., Al-Hakkar Z.S., Al-Rawi F.I., Hagop E.G. Physiological and cytogenetic effects of phosphine gas in *Allium cepa*. (L.). *Journal of Stored Products Research*, 1989; 25: 25-30.
59. Badr A. Mitodepressive and chromotoxic activities of 2 herbicides in *Allium cepa*. *Cytologia,* 1983; 48: 451-458.
60. Fiskesjo G., Lassen C., Renberg L. Chlorinated phenoxy acetic acids and chlorophenols in the modified *Allium* test. *Chemico -biological* Interactions, 1981; 34: 333-344.
61. Sakamoto H.T.E., Takahashi C.S., Action of benlate fungicide on Tradescantia stamen hairs and *Allium cepa* root tip cells. *Revista Brasileira de Genetica*, 1981; 4. 367-382.
62. Grant W.F., The genotoxic effects of 2,4,5-T. *Mutation Research*, 1979; 65: 83-119.
63. Sudhakar R., Gowda N., Venu G., Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa. Cytologia,* 2001; 66: 235-239.
64. Sharma S., Vig, A.P., Antigenotoxic effects of Indian mustard (*Brassica juncea* (L.) Czern.) aqueous seeds extract against mercury (Hg) induced genotoxicity. *Scientific Research and Essay*, 2012; 7: 1385-1392.
65. Sharma S., Nagpal A., Vig A.P. Genoprotective potential of *Brassica juncea* L. Czern. against mercury induced genotoxicity in *Allium cepa*. *Turkish Journal of Biology,* 2012; Doi: 10.3906/biy-1110-18.
66. Panda B.B., Sahu U.K. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphorus insecticide fensulfothion. *Cytobios,* 1985; 42: 147-155.
67. Gomurgen A.N. Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. *Cytologia,* 2005; 70: 119-128.
68. Meng Z., Zhang L. Cytogenetic damage induced by sodium bisulfite. *Mutation Research*, 1992; 298: 63-69.

Cite this article as: Namita Khanna and Sonia Sharma. *Allium Cepa* Root Chromosomal Aberration Assay: A Review. **Indian J. Pharm. Biol. Res. 2013; 1(3):105-119.**

All © 2013 are reserved by Indian Journal of Pharmaceutical and Biological Research.