World Applied Sciences Journal 22 (3): 342-344, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.22.03.2977

Genetic Alterations Revealed in *Allium cepa*-Test System under the Action of Some Xenobiotics

[1,2]Maxim V. Trushin, [3]Andrey Yu. Ratushnyak,
[1]Irina A. Arkharova and [3]Anna A. Ratushnyak

[1]Kazan Federal University, Kremlyovskaya 18, 420008 Kazan, Russia
[2]Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, 420111 Kazan, Russia
[3]State Budgetary Establishment Research Institute for Problems of Ecology and Mineral Wealth Use, Tatarstan Academy of Sciences, Daurskaya 28, 420089 Kazan, Russia

Abstract: Since the beginning of the industrialization, heavy metal pollution of soil and aquatic ecosystems has been significantly increased. The aim of the present study was to investigate genetic effects of lead and salicylic action in *Allium cepa*-test system. Onion bulbus were obtained commercially and were placed in small jars with the tested solutions of lead, salicylic acid and their combination. For the negative control it was used distilled water. When roots reached lengths of 1 cm, they was cut of and fixed with solution containing ethanol and acetic acid and then fixed roots were placed into staining solution and visualized. In this work it was presented that lead and salicylic acid have the opposite action toward a mitotic index in cells on onion roots. Salicylic action and lead discretely have a weak mutagenic potential that is less evident in the case of combined action of these xenobiotics.

Key words: Genetic alterations % Chromosomal aberrations % Micronucleus % Lead

INTRODUCTION

Heavy metals are metals with specific gravity greater than 5 g/cm^3 [1]. Since the beginning of the industrialization, heavy metal pollution of soil and aquatic ecosystems has been significantly increased [2]. The primary reason for actuality of this problem is the human health risk resulting from intake of contaminants through drinking water or through the food chain. Heavy metals were reported to be able to provoke many diseases in humans [3-5]. Heavy metals may cause acute toxic effects and cancer in mammals, which are due to DNA damage [6]. Some bioassays can effectively detect the genotoxic effects of heavy metals and many studies have shown that plant tests are highly sensitive [7-9]. *Allium cepa* roots are regarded as one of the fisrt organisms for use in cytogenetic studies to evaluate toxicity and genotoxicity of xenobiotics [10, 11]. *Allium cepa*-test system has been commonly used for environmental monitoring as well as for the toxicity assessment of heavy metals, pesticides and industrial wastewaters [12-15]. This method is cost-effective, easy to perform and sensitive enough to respond to low concentrations of toxicants [16, 17]. Therefore, we used this test in our work to test genetic effects of some xenobiotics.

The aim of the present study was to investigate genetic effects of lead and salicylic acid action in *Allium cepa*-test system.

MATERIALS AND METHODS

Onion bulbus were obtained commercially and were placed in small jars with the tested solutions of lead (in form of lead acetate, 2.5 mg/L), salicylic acid (10^{-4} mol/L) and their combination. For the negative control it was used distilled water. When roots reached lengths of 1 cm, they was cut of and fixed with solution containing 96% ethanol and acetic acid (in proportion 3:1). 2 h later, fixed roots were placed into staining solution (2% acetocarmine). Later, root tips were squashed in slides and visualized with AxioLab A1 (Carl Zeiss, Germany).

Corresponding Author: Trushin, Depertment of Genetics, Kazan Federal University, Kremlyovskaya 18, 420008 Kazan, Russia.

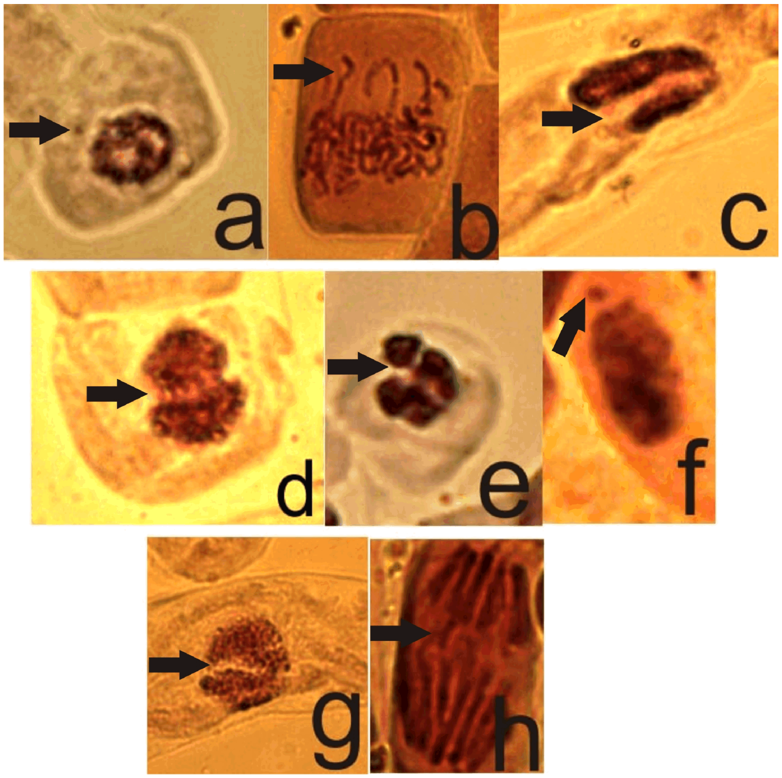

Fig. 1: Examples of genetic alterations in different experimental variants: Pb alone - a and b - micronucleus and metaphase with chromosome loss, respectively; salicylic acid (SA) alone - c and e - lobulated nuclei, d - nucleus with nuclear constriction, f - micronucleus; Pb + SA - g and h - lobulated nucleus and anaphase with chromosome bridge, respectively.

RESULTS AND DISCUSSION

In this work we analyzed 3531 cells from roots of *Allium cepa* plants. We investigated the effect of lead, salicylic acid and their combination on the appearance of chromosomal aberrations and nuclear alterations as well as changes in mitotic index. We detected that lead and salicylic acid influenced mitotic index (Table 1).

As a result of analysis, it was found that salicylic acid alone inhibited mitotic index. In combination with lead we detected that mitotic index was higher than in control variants. Lead alone also stimulated cell division: mitotic index was increased. Considering the inhibiting action of salicylic acid (variant 2, Table 1) and stimulating effect of lead, we can conclude that the observed stimulation of cell division (increased mitotic index) in variant 3 was connected namely with lead action. Our previous work with another plant, *Pisum sativum* L. also indicated that lead may promote growth-enhancing effect [18].

Examples of genetic alterations in different experimental variants are presented at Figure 1.

Table 1: Values for mitotic index in cells of Allium cepa roots exposed to lead and salicylic action

Experimental variants	Mitotic index
I-Control	8.626
II-SA alone	5.66
III-Pb+SA	10.61
IV-Pb alone	11.79

Concerning chromosomal aberrations and nuclear alterations, it should be noted that salicylic acid have a weak genotoxic effect that is in agreement with previously published data [19]. Lead in comparison with other variants had a highest genotoxicity resulting in formation of micronuclei at different stages of cell cycle (interphase and metaphase) as well as to loss of chromosomes during formation of metaphase platelet (Figure 1 a,b). Reduction of genotoxic potential of lead at combination with salicylic acid (variant 3) might be mediated with formation in root cells of onion plants compounds preventing the hazardous action of heavy metal toward karyotype and cell nucleus. The detected phenomenon is in agreement with previously reported data on the ability of salicylic acid, a signal phytohormone, to reduce the toxic effect of various genotropic toxicants [20].

Thus, in this work it was presented that lead and salicylic acid have the opposite action toward a mitotic index in cells on onion roots. Salicylic action and lead discretely have a weak mutagenic potential that is less evident in the case of combined action of these xenobiotics.

REFERENCES

1. Nies, D.H., 1999. Microbial heavy-metal resistance. Appl. Microbiol. Biotechnol., 51: 730-750.
2. Nriagu, J.O., 1996. A history of global metal pollution. Science, 272: 273-274.
3. Brown, M.J. and S. Margolis, 2012. Lead in drinking water and human blood lead levels in the United States. MMWR Surveill Summ., 61: Suppl 1-9.
4. Foster, M. and S. Samman, 2012. Zinc and regulation of inflammatory cytokines: implications for cardiometabolic disease. Nutrients, 4(7): 676-694.
5. Brewer, G.J., 2012. Copper toxicity in Alzheimer's disease: cognitive loss from ingestion of inorganic copper. J. Trace Elem. Med. Biol., 26(2-3): 89-92.
6. Hughes, K., M.E. Meek, R. Newhook and P.K.L. Chan, 1995. Speciation in health risk assessments of metals: Evaluation of effects associated with forms present in the environment. Reg Toxicol Pharmacol., 22: 213-220.
7. Sandhu, S.S., T.H. Ma, Y. Peng and X. Zhou, 1989. Clastogenicity evaluation nite: A possible mechanism of its comutagenesis. Mutat Res., 224: 437-445.
8. Ma, T.H., M.M. Harris, V.A. Anderson, I. Ahmed, K. Mohammad and J.L. Bare, 1979. Tradescantia-micronucleus (Trad-MCN) tests on 140 health related agents. Mutat Res., 138: 157-167.
9. Grant, W.F., 1994. The present status of higher plant bioassays for detection of environmental mutagens. Mutat Res., 310: 175-185.
10. Fiskesjo, G., 1993. The Allium test-a potential standard for the assessment of environmental toxicity. In: Gorsuch JW, ed., *ASTM.*
11. Levan, A., 1938. The effect of colchicine on root mitoses of Allium. Hereditas, 24: 471-486.
12. Chauhan, L.K.S., P.N. Saxena and S.K. Gupta, 1999. Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of *Allium cepa*. Environ Exper Bot., 42(3): 181-189.
13. Fiskesjo, G., 1988. The Allium test-an alternative in environmental studies-the relative toxicity of metal-ions. Mutat Res., 197: 243-260.
14. Kovalchuk, O., I. Kovalchuk, I. Arkhipov, P. Telyuk, B. Hohn and L. Kovalchuk, 1998. The *Allium cepa* chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in the Ukraine contaminated by the Chernobyl accident. Mutat Res., 415: 47-57.
15. Grover, I.S. and S. Kaur, 1999. Genotoxicity of wastewater samples from sewage and industrial effluent detected by the Allium root anaphase aberration and micronucleus assays. Mutat Res., 426: 183-188.
16. Yildiz, M. and E.S. Arikan, 2008. Genotoxicity testing of quizalofop-P-ethyl herbicide using the *Allium cepa* anaphase-telophase chromosome aberration assay. Caryologia, 61(1): 45-52.
17. Yildiz, M., I.H. Cierci, M. Konuk, A. Fatih Fidan and H. Terzi, 2009. Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. Chemosphere, 75(7): 934-938.
18. Ratushnyak, A. Yu., A.A. Ratushnyak, M.G. Andreeva, A.R. Kayumov, M.I. Bogachev and M.V. Trushin, 2012. Effect of Lead and Salicylic Acid on Some Plant Growth Parameters in Pisum sativum L. World Applied Sciences Journal, 19(8): 1157-1159.
19. Kuboyama, N. and A. Fujii, 1992. Mutagenicity of analgesics, their derivatives and anti-inflammatory drugs with S-9 mix of several animal species. J Nihon Univ Sch Dent., 34(3): 183-195.
20. Patra, J., M.K. Sahoo and B.B. Panda, 2005. Salicylic acid triggers genotoxic adaptation to methyl mercuric chloride and ethyl methane sulfonate, but not to maleic hydrazide in root meristem cells of *Allium cepa* L. Mutat Res., 581(1-2): 173-180.