Studii şi Cercetări	Martie 2019	Biologie	28/1	90-93	Universitatea"Vasile Alecsandri" din Bacău

CYTOGENETIC RESPONSE OF EXOGENOUS ADITTION OF SILVER NITRATE IN CULTURE MEDIA DESIGNED FOR MASS PROPAGATION OF *ALLIUM CEPA* PLANTS

Tina Oana Cristea, Maria Calin, Silvia Ambarus, Creola Brezeanu, Petre Marian Brezeanu, Gabriel-Alin Iosob, Nelly Fânaru, Ana Maria Nechita, Maria Prisecaru

Key words*: organogenesis, embryogenesis, regeneration, plants*

INTRODUCTION

Allium cepa L. is a monocot plant belonging to the family Liliaceae having 2n=16 long chromosomes.

Ag toxicity and tolerance in plants have been investigated by Delhaize and Ryan (1995) mainly on modulation of plant responses, but the effect of low doses of Ag has been rather limited on antioxidative enzyme response and oxidative damage on chromosome structure.

Various types of abnormalities were reported by the treatment of heavy metals at the cell and tissue levels, affecting apical elongation zones of the root apex. In particular, Ag toxicity primarily targets the cell wall, cytoskeleton, nuclear materials and plasma membrane. The strong binding affinity of Ag with oxygen donor ligands affects retardation, cell division and growth (Mossor-Pietraszewska 2001).

On the other hand, the beneficial influence that AgNO3 exerts on the regeneration processes in the tissues grown in vitro has determined the intensification of the research regarding its inclusion in the culture substrate. In the literature, silver nitrate is used to improve somatic embryogenesis in numerous plant species: Coffea sp. (Fuentes et al. 2000; Giridhar et al. 2004), Carrot (Nissen, 1994), White spruce (Kong and Yeung, 1994), Triticum durum (Fernandez et al. 1999), and Zea mays (Vain Hortand Flament, 1989) ; Vain Hort et al. 1989; Songstad et al. 1991).

Also, numerous studies have shown the beneficial influence of silver nitrate on: axillary sprouting with multiple shoots formation, somatic organogenesis, root formation, delayed tissue senescence, inhibition of calus formation and promotion of sprouting in the tissues grown on culture media supplemented with different concentration of silver nitrate.

Ever since the discovery of chromosomes, the elaboration of methods and techniques for researching the material substrate of heredity has become a major concern for cytologists and, subsequently, for geneticists. Continuous refinement of these techniques and methods now allows for a wide range of investigations on chromosomes during mitotic and meiotic (reductive) division, including: Since the discovery of chromosomes, the development of methods and techniques for researching the material substrate of plant heredity has become a concern. of great interest to cytologists and subsequently to geneticists. Continuous refinement of these techniques and methods now allows for a wide range of investigations on chromosomes during mitotic and meiotic (reductive) division, including: determining the number, shape and size of chromosomes in mitosis, and the karyotype of the respective species; the study of intra- and interspecific transfer of chromosomes or chromosome segments (genes respectively); determining the degree of ploidy of intraspecific, interspecific, intergeneric hybrids, or of plants subjected to the action of polyploidizing substances; the study of the numerical or structural chromosomal changes induced by the action of chemical or physical mutagenic agents; study of aneuploid organisms and how genes are placed on chromosomes; determining the degree of homology of the chromosomes by studying the metaphase I of the meiosis and their disjunction in the other phases of the division cycle, at interspecific or intergeneric hybrids, etc.

Thus, the present study aims to perform a screening on the main cytogenetic aspects of Allium cepa L. seedlings, obtained on in vitro cultivation variants that have been included in the AgNO3 composition at different concentrations.

MATERIAL AND METHODS

The experiments were accomplished at Vegetable Research and Development Station Bacau, in The Laboratory of Tissue Culture. The biological material is represented by seeds belonging to Orizont genotype, which were aseptically germinated on tissue culture media *in vitro.*

The seeds were surface sterilised by immersion in mercuric chloride solution ($HgCl_2$) 0.1% for 10 minutes, followed by repeated washing with sterile distilled water. The sterile seeds were cultivated on a basic medium Murashige Skoog, 1962. On this medium the seeds sprout rapidly and the germination capacity surpasses 95 %.

One-week old seedlings were used as source of explants: tips of the small plants in cotyledonary stage, and hypocotyls.

The excised explants were cultivated on MS medium solidified with 8.0 g/l of agar, having sucrose (25 g/l) as carbon source, zeatine 16,8 µM as PGR; the medium was supplemented with different concentration of $AgNO_3$, (5 µM, 10 µM and 15 µM). The pH was adjusted to 5.8 prior to the addition of the agar and autoclaved at 121°C (1.06 kg/cm^2) for 25 min.

Thus, the tested variants were: V0 - control without silver nitrate, V1 - 5 µM, V2 - 10 µM, V3 - 15 µM. Cultures were then incubated at 26±1°C, a 16-h photoperiod, and 5000 lx light intensity.

After the regeneration of shoots, the newly formed plantlets were used for the accomplishment of cytogenetic studies.

Cytogenetic studies

The roots were excised and fixed in three parts of absolute alcohol and one part of glacial acetic during 24 hours in room temperature, then preserved in 70% alcohol and kept in refrigerator for future cytogenetic studies.

The preparation of biological material for cytogenetic studies was performed according to the data presented by the specialized literature, namely the staining method with Carr Reagent.

After hydrolysis, the HC1 is removed from the vials and 2-3 ml of Schiff or Carr reagent are added. Chromosome examination and photographing were performed under the Hund microscope, at a magnitude of 1000x.

Statistical analysis

The data were analyzed by ANOVA (analysis of variance). The means were compared using the Duncan multiple comparison test at $P < 0.05$.

RESULTS AND DISCUSSIONS

Mitotic index is a best biomonitor to assess the effects of various chemicals on cell division (Abdel, 2013). The mitotic index was observed for more than 1000 cells. The silver nitrate determined a wide range of mitotic disturbances in the Allium root tips from plants regenerated in vitro when compared to control fig.1. Still, on variant with a low concentration of silver nitrate, variant V1, the mitotic index was found to be higher than all variants, including control.

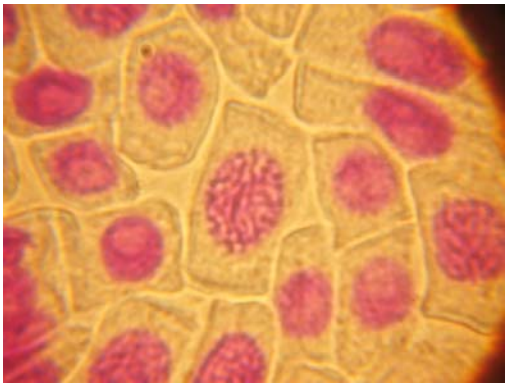

Fig. 1. *Allium* cells in active mitotic division

This finding is also reflected in the results obtained in the study regarding the morphogenetic reaction of Allium on media supplemented with silver nitrate, in which the number of shoots obtained were significatively higher. It was found that the mitotic index decreased had a significantdecrease in mitotic index in high doses, 10 and 15 µM (Table 1, fig.2).

Table 1. Effect of different concentration of $AgNO_3$ on mitotic index at Allium plants regenerated in vitro

Variant	Total no. of cells	No. of dividing cells	Mitotic Index
Control	1021	108	10.57±0.31
V1	1153	134	11.62±0.36
V2	1078	111	10.29±0.16
V3	1145	104	9.08±09

Mean ± SE

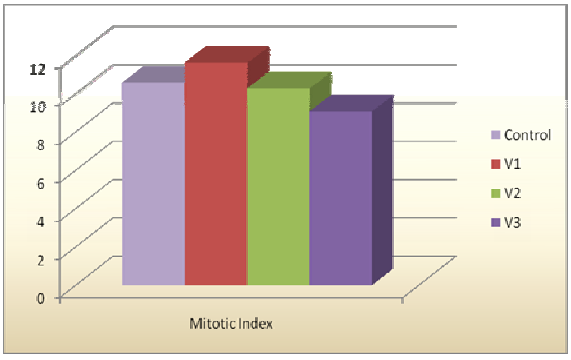

Fig. 2. Variation of mitotic index dpending on the concentration of silver nitrate in culture media

The highest proportion was recorded by the prophasic index, whose value was between 38.9 and 42.06, significantly higher than the other indexes - fig. 3 (metaphasic, anaphasic and telophasic index). The lowest value was observed at the telophase index which had values between 4.11 and 6.02.

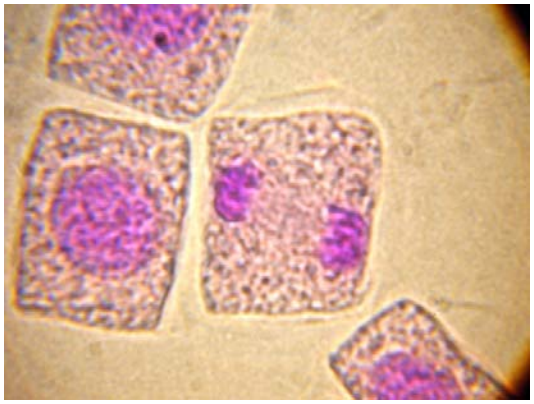

Fig. 3. Late anaphase in *Allium* cells

The present study showed that the chromosomal aberrations were observed in all the concentrations with values that exceed the control. Chromosomal aberration showed a maximum at 15 μM $AgNO_3$ –variant V3 when compared to the others and the lowest value was recorded in control variant (Table.2).

Table 2**.** Effect of different concentration of silver nitrate on chromosomal anomalies of *A. cepa*

Variant	Abnormality	Metaphase	Anaphase
Vo	04.67±1.22	02.11±3.24	02.45±4.13
V1	04.24±1.70	01.36±1.88	01.39±1.34
V2	06.62±2.97	02.60±3.07	04.29±2.95
V3	09.80±1.47	06.63±3.30	03.56±3.44

Among the most frequent abnormalities identified are: disturbed metaphase chromosomes, single and multiple bridge formation with fragmentation, anaphase chromosome protruded out, multipolar anaphase and sticky anaphase.

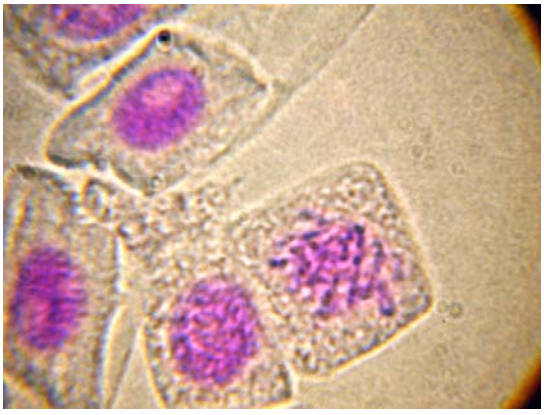

Fig. 4. Disturbed metaphase with clumping chromosomes

According to some researchers, various chromosomal abnormalities in metaphase and anaphase are due to the shifting of poles by depolymerizationof spindle fibers.

Chromosome bridges is either due to chromosome stickiness producing abnormal anaphase separation or may be attribute to unequal translocation or inversion of chromosome segments and also due to breakage and fusion of chromosome or chromatids (Patlolla et al, 2012).

CONCLUSIONS

The current study showed that silver nitrate induced cytotoxic and genotoxic effects on Allium cepa root tip cells depending on its concentration in culture media. Still, on variant with a low concentration of silver nitrate, variant V1, the mitotic index was found to be higher than all variants, including control. This finding is also reflected in the results obtained in the study regarding the morphogenetic reaction of Allium on media supplemented with silver nitrate, in which the number of shoots obtained were significatively higher.

The results obtained demonstrated that AgNO3 determined in onion plant grown on media suplimented with silver nitrate the production of secondary metabolits that damaged the somatic chromosomes, leading to formation of spindle abnormalities (multipolarity, sticky bridge in anaphase, multiple bridges in ana-telophase, early separation, clumping of chromosomes, late separation, laggard chromosome).

ABSTRACT

Silver nitrate is a powerful tool for any tissue culture protocol as it is an inhibitor of ethylene action which negatively affects callus growth, shoot regeneration and somatic embryogenesis *in vitro*. Various types of abnormalities were reported by the treatment of heavy metals at the cell and tissue levels, affecting apical elongation zones of the root apex. In particular, Ag toxicity primarily targets the cell wall, cytoskeleton, nuclear materials and plasma membrane. The strong binding affinity of Ag with oxygen donor ligands affects retardation, cell division and growth (Sudhakar, 2001).

The results of our study demonstrated that the addition of silver nitrate in culture medium influences both the morphogenetic reaction of explants and the cytogenetic structure of regenerated plants. The observations showed agradual decrease in mitotic index, with increasing concentration of the silver nitrate. This antimitotic effect may be due to the arrest of cell division due to changes at in the chromosomal morphology or spindle orientation. A thorough screening of the chromosomalabnormalities showed that the total number of abnormal cells increased with increasing concentrations of silver nitrate in a dose dependent manner.

ACKNOWLEDGMENT

This work was cofinanced from the European Social Fund through the project: ADER 25.2.2: „Cercetare cu privire la proiectarea unui echipament inteligent horticol de analiză, predic□ie □i ac□iune biodinamică", grant of the Romanian Ministery of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III, The BRESOV project GA no 7742 44, European Union's HORIZON 2020 research and innovation programm and presented in frame of FARMRES Conference.

REFERENCES

1. ABDEL-AZEEM EA and A ELSAYED BA., 2013 - Phytotoxicity of silver nanoparticles on Vicia faba seedlings. New York Sciences Journal, 6(2):148-156;
2. ARYA, S. K. and MOKHERJEE, A., 2014 - Sensitivity of *Allium cepa* and *Vicia faba* towards cadmium toxicity. J. Soil Sci. Plant Nutr. 14:447–458;
3. KUMARI M, MUKHERJEE A, CHANDRASEKARAN N., 2009 - Genotoxicity of silver nanoparticles in Allium cepa, Sci. Total Environ. 407, 5243–5246;
4. KUMARI, M.; KHAN, S.S.; PAKRASHI, S.; MUKHERJEE, A.; CHANDRASEKARAN, N., 2011 - Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of Allium cepa. J. Hazard Mater. 90, 613-621;
5. LIU, D. H., JIANG, W. S., WANG, W. AND ZHAI, L. 1995 - Evalution of metal ion toxicity on root tip cells by the *Allium* test. Isr. J. PlantSci. 43: 125–133;
6. MURASHIGE, T. & SKOOG, F., 1962 - A revised medium for rapid growth andbioassays with tobacco tissue cultures. *Physiologia Plantarum,* 15:473-497;
7. PATLOLLA AK, BERRY A, MAY L and TCHOUNWOU PB., 2012 - Genotoxicity of silver nanoparticles in Vicia faba:A pilot study on the environmental monitoring of nanoparticles. Int. J. Environ. Res. Public Health, 9, 1649-1662; doi:10.3390/ijerph9051649;
8. SUDHAKAR R, GOWDA N, VENU G., 2001 - Mitotic abnormalities induced by silk dyeing industry effluents in the cells of Allium cepa. Cytologia.; 66; 235–239.

AUTHORS` ADDRESS

CRISTEA TINA OANA, CALIN MARIA, AMBARUS SILVIA, BREZEANU CREOLA, BREZEANU PETRE MARIAN, FÂNARU NELLY, NECHITA ANA MARIA - Vegetable Research and Development Station Bacau, Calea Barladului, No. 220, Bacau, code: 600388, e-mail: tinaoana@yahoo.com;

IOSOB GABRIEL-ALIN - Vegetable Research and Development Station Bacau, Calea Barladului, No. 220, Bacau, code: 600388 and „Vasile Alecsandri", University of Bacau, Faculty of Science, Department of Biology, Marasesti Street, no. 157, Bacau, Romania, e-mail: iosob.gabriel@gmail.com;

PRISECARU MARIA - „Vasile Alecsandri", University of Bacau, Faculty of Science, Department of Biology, Marasesti Street, no. 157, Bacau, Romania, e-mail: prisecaru_maria@yahoo.com.