

ASPECT OF CYTOGENETIC ANALYSIS OF POTATOES MITOSIS UNDER COLCHICINE INFLUENCE

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Abstract

This experiment was performed in order to study the changes in cell division, as a result of colchicine influence on potatoes root meristems. Same concentration (0.5%) in four times of exposure (0.5h; 1.0h; 1.5h; 2.0h) along a control (distilled water) for each variant were used with the aim to identify the types of modifications in cells division and chromosomes behavior. Mitotic abnormalities were identified both in the cells from the control material and to those subjected to colchicine influence for shorter and longer time. Percent of normal mitosis decreased once with the increase in time exposure and as mitotic abnormalities were identified chromosomal fragments, chromosomal bridges, micronuclei and C mitosis.

Key words: root meristems, chromosome abnormalities, *Solanum tuberosum* L., colchicine, mitosis, cell division

INTRODUCTION

The *Solanaceae* family comprises the largest group of angiosperms in the world. Within this family, the most economically important species is the cultivated potato, *Solanum tuberosum*, which is autotetraploid ($2n=4x=48$) and the diploids account for 70% of the species. Hawkes (1990) recognized 232 species divided into 21 series. The tetraploid species cover a wide range of distribution, from the southern part of the U. S. to the most southern region of Chile. However, the diploid level has greater genetic diversity associated with resistance to various biotic and/or abiotic factors and consequently, it is of great use in the genetic breeding of the cultivated species and has a greater concentration of diversity in the Andes (Ordoñez, Benny et al., 2017).

The tetraploid *Solanum tuberosum* has four homologues, which include 12 unique chromosomes each, thus showing tetrasomic inheritance (Bradshaw, J.E., 2007).

Common potatoes are the fourth most consumed foods in the world and second for the quantity of proteins/hectare. Romania occupy third place between EU states as cultivated surface and seventh from the point of view of yield (<http://agrotrends.ro>). It is a crop which has become increasingly important for food security due to its nutritional value and high production capacity (Soare Rodica et al., 2020).

The development of the bioeconomy on regional/national levels is heavily reliant on the establishment of precise and efficient governance frameworks (Fieroiu Daniela et al., 2023).

Solanum tuberosum belongs to *Solanaceae* family which include 90 genera and 2800 species. *S. tuberosum* has two subspecies: *andigena* (diploid) adapted to short day conditions and *tuberosum* (tetraploid) adapted to longer day length.

Variation in chromosome features is believed to have accompanied evolutionary

divergence in plant species. Potatoes presents high cariological variability. It has diploid species ($2n=24$), triploids ($2n=36$), tetraploids ($2n=48$), pentaploids ($2n=60$) and hexaploids ($2n=72$) (Baciu Anca et al., 2009). Autopolyploids are polyploids arising within or between populations of single species, while allopolyploids contain two or more distinct genomes and can arise via hybridization of two different species concomitant with genome doubling (Iancu Paula, 2018). Polyploids are organisms whose genomes consist of more than two complete sets of chromosomes. Polyploidy represented initially, an unusually tangible genetic feature that could be directly observed and manipulated not only from plant breeders, but also from a broad spectrum of empirical and theoretical geneticists (Ramsey, J. and Ramsey S. Tara, 2014). Also, polyploidy is associated with rapid and extensive restructuring of the genome, including profound changes in chromosome number and structure (translocations, deletions) (Gaeta, R.T. and Pires, J.C., 2010).

Cytogenetic analysis has been widely used in genetic studies. Chromosome identification was a challenge in many plant species with small chromosomes such as potatoes (Chen, Q., and Li, H.Y., 2005), but obtaining the chromosomes from root tip cells became a common procedure so that these were subjected to the influence of different reagents and the analysis of mitotic division indicated many effects.

Colchicine is a natural alkaloid with an antimitotic activity, obtained from *Colchicum autumnale* L. plant. In higher concentrations is a powerful poison, but in smaller concentrations (0.01-0.1%) can modify cells division (Voica, N., et al., 1984). There are a lot of publications which

dealed with the influence of phytohormones on root initiation. Colchicine as a non-hormonal factor or microtubule polymerization inhibitor is able to stop/inhibit/alter cell division and it has been used in a variety of methodologies involving the treatment of seeds or apical meristems of germinated seedlings. A large bibliography concludes that chromosomal number in plant cells are frequently doubled after treatment with colchicine, so polyploidy is a consequence of contact with this substance. Almost universally, living cells respond to colchicine after one basic pattern and new tests extend knowledge into other areas of science. The colchicine mitosis (C-mitosis) is built upon the principle of metaphase arrest. This implies control over cells division. Mitotic blocking, induction of micronuclei and chromosome doubling after potatoes treatments with colchicine was reported by Ramulu, K.S. et al., 1991. Potatoes crop is very popular and attacked by a series of diseases and insects, so that is often sprayed with chemical substances, both in vegetation and after so, genotoxic substance research is of great importance for the protection of the environment because it enables an insight into the influence of genotoxic substances on organisms (Peter Firbas and Tomaz Amon, 2014).

The aim of the present study was to evaluate the effect of the colchicine on potatoes roots.

MATERIALS AND METHODS

Mitosis is the cell division that underlies the growth of living organisms. Root meristems were used to highlight mitotic chromosomes. Thus, potato bulbs from the Tâmpa variety (obtained from Heidrun x Colina) were put to sprout in Petri dishes with water. When the roots were 0.8-1 cm

long, they were harvested and kept in colchicine for different times (0.5h; 1.0h, 1.5h; 2.0h). Later, they were processed according to the Feulgen-Rossenback method. The microscopic slides were realized in accordance with squash method. Their study was made using CELESTRON microscope at 40x. After the analyzes performed on the cells, the percentage of normal mitoses and the types of aberrations that appeared as a result of the influence of colchicine were determined.

RESULTS AND DISCUSSIONS

Normally, during mitosis, the formation of the two daughter cells is expected and these are identical to mother cell. Also, it is expected that all phases of the cycle occur perfectly, especially DNA replication, orientation of the chromosomes on the metaphase and segregation of chromatids. Any change can affect the duration of this process and the formation of the daughter cells (Lima, D.C. et al., 2015).

The chromosomes were small and difficult to investigate because there are very similar.

Colchicine applied in small doses (0,5%) to plants produces a retarding effect on mitosis in the cells of either growing vegetative or germinal tissue.

In the cells of Tampa cultivar, the highest frequency of abnormalities observed in the cell cycle occurred mostly during prophase. In the later stages of the cell cycle, there was a reduction of this amount and that could be caused by some of the non-oriented chromosomes observed during this phase. The same effect was seen in the late anaphase chromosomes. This observation reflects part of the cell cycle

control system, which ensures the stability and regularity of cell division. So, mitosis was mostly normal in control materials raised in distilled water, with few exceptions, possible as influence of the substances sprayed in vegetation period or during winter keeping.

Mitotic abnormalities were identified even in the cells from control material and those subjected to colchicine influence for shorter and longer time. So, micronuclei, fragments, single, double and multiple bridges were observed in the root meristems held in colchicine for longer time. Increase in the cells with deformed chromosome was thought to relate directly to the exposure time. Although few vagrant/sticky chromosomes were identified.

A constant decrease in normal mitosis percentage is observed once with the increasing time of exposure, as well as chromosomal aberrations in metaphase, anaphase and telophase (Fig. 1) (Table 1). It is thought that colchicine determines the over-contraction of the chromosomes and that why fragments appears (table 2).

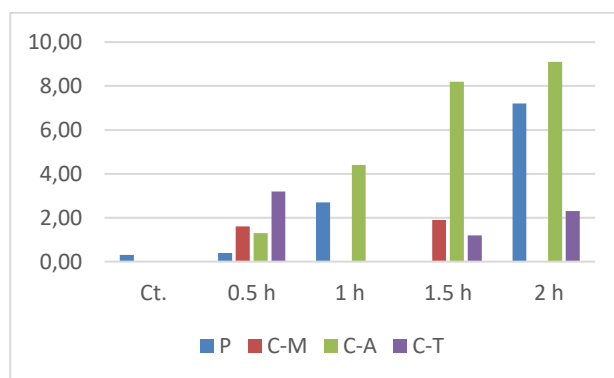


Fig. 1. Percent of each phases of mitosis

Table 1. Values of normal mitosis and chromosome aberrations after treatment of the root meristems of potatoes with colchicine

Treatment time (h)	Normal mitosis (%)	Prophase (%)	Metaphase (%)		Anaphase (%)		Telophase (%)	
			CA	C-M	CA	C-A	CA	C-T
Ct.	97.3	0.3	-	-	-	-	2.2	-
0.5	90.6	0.4	-	1.6	1.3	-	2.9	3.2
1,0	91.4	2.7	0.5	-	4.4	-	1.0	-
1.5	80.8	5.0	0.7	1.9	8.2	1.8	0.4	1.2
2.0	74.9	7.2	0.8	-	9.1	2.3	2.4	3.3

Legend: CA - chromosomal aberrations; C-M - C-metaphase; C-A - C-anaphase; C-T - C-telophase

Examined cells – 200 for each slide

Table 2. Percent of the modifications types appeared as a result of colchicine influence

Treatment time (h)	Micronuclei (%)	Chromosomal fragments (%)	Chromosomal bridges (%)	Vagrant/sticky chromosomes (%)
Ct.	-	1.13	-	-
0.5	0.2	4.50	2.64	-
1,0	0.3	7.27	6.86	-
1.5	0.4	12.33	11.54	0.02
2.0	1.4	16.31	11.60	0.04

Examined cells – 200 for each slide

Increase time of exposure lead to increases the percentage of metaphase with un-oriented chromosomes. Generally, in this study, cells behave as real polyploid cells showing both normal mitotic cell division and few types of aberrations. Also, sodium chloride has negative effects on rye seeds on concentrations of 0.5, 1, 1.5 and 2 g/l (Pascaru Adina et al., 2014). Lead affects the germination of coriander seeds and cytological study indicated affectations on mitotic activity and nucleoli increase (Petrescu Irina et al., 2015). The observations made by Sărac, I. et al., 2015, in order to test the effect of cadmium (Cd) on root and foliar system growth of *Allium sativum* L. (garlic) showed that this element as heavy metal has significant mutagenic activity.

In potatoes, other authors, using colchicine, reported increases in the

number of leaves or in plant height depending on genotype (Mahbube, M.A et. al., 2011).

Interphases with micronuclei, metaphases with retarded chromosomes, anatelophases with chromosomal bridges, retarded chromosomes and chromosomal fragments, with percentage low in all tested genotypes of potatoes were reported by Ciobanu Iustina Brândușa et al., 2012.

Mitosis is generally, the process whereby genetic material, previously duplicated, is distributed giving rise to two genetically identical cells. The number of chromosomes remains constant through successive cell divisions, with an exact chromosomal distribution in the newly formed cells, maintaining the original ploidy.

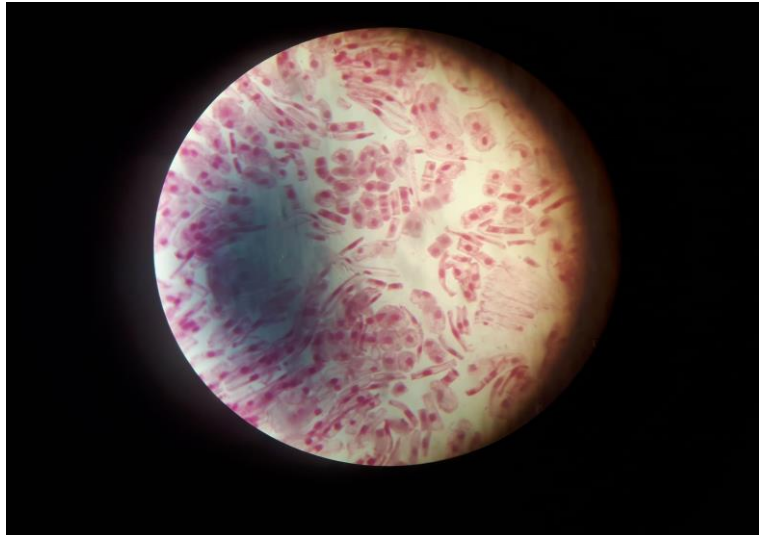


Foto. 1. Diverse phases of normal cells mitosis (Ct.)

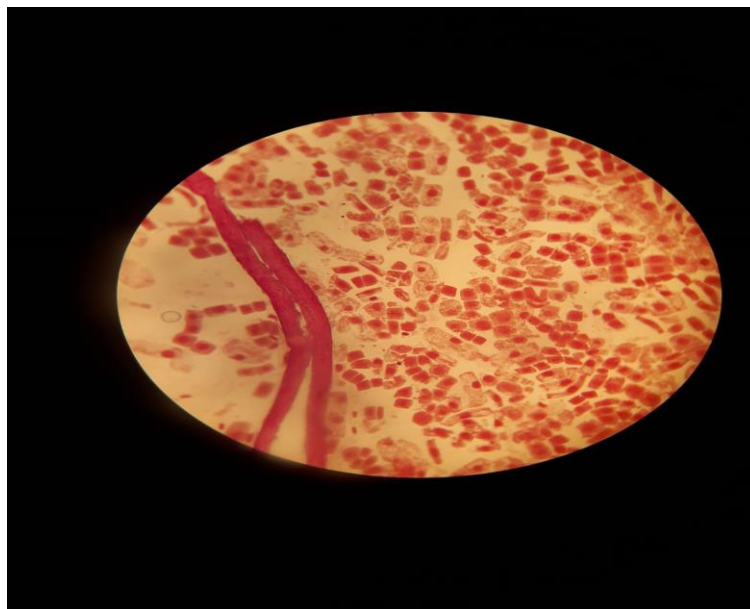


Foto. 2. C-telophases (1.5 h)

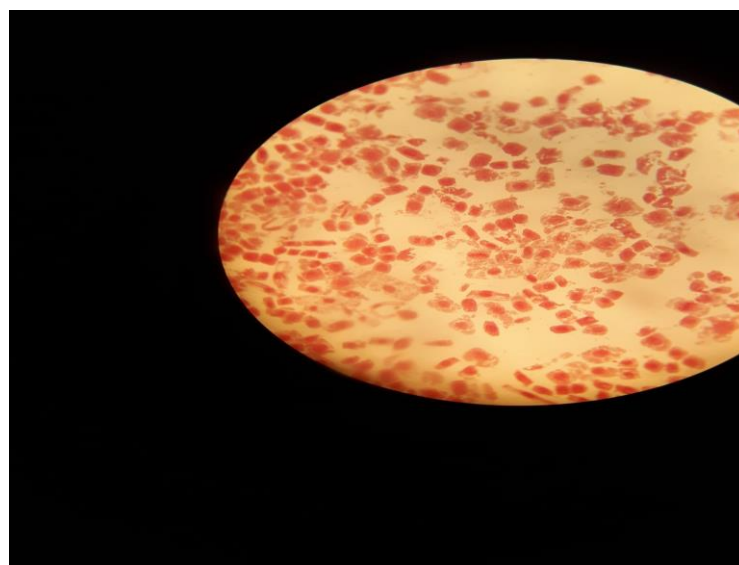


Foto. 3. Chromosomes fragmentation (2.0 h)

CONCLUSIONS

In the present investigation, the conclusion is that Tâmpa variety is stable and colchicine effects are reduced upon the percent and types of modifications and also chromosome aberrations appearance.

Longer time of exposure lead to increases the percentage of metaphase with un-oriented chromosomes.

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