STUDIES REGARDING THE CYTOGENETIC CHARACTERIZATION OF TWO PERSPECTIVE LINES OF *ALLIUM URSINUM*

STUDII PRIVIND CARACTERIZAREA CITOGENETICĂ A DOUĂ LINII DE PERSPECTIVĂ DE *ALLIUM URSINUM*

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Abstract. *The size of chromosomes and their morphology are important indicators used to identify the evolutionary relationships of different species of plants. The scope of genetic studies is based on very broad areas of fundamental studies (taxonomy), reaching up to fields applied (eg plant breeding). Genetic characterization is important to identify the species or hybrid population analysis. Counting chromosomes and genetic determinations include determining karyotype, mitotic index, determination and analysis of mitotic abnormalities. According with the literature, chromosome aberrations have been used as a measure of reproductive success in plants for many years and have been correlated with morphological changes, fertility-sterility relationships, mutations etc. The study focused toward the cytogenetic characterisation of two perspective lines of Allium ursinum from Vegetable Research and Development Station Bacau field collection. The investigations were focused toward the determination of the main cellular indexes (mitotic index, prophase index, metaphase index, telophase index and anaphase index) as indicators of growth and development processes speed. Another objective of the present paper is screening of some aspects regarding the type and frequency of chromosomes aberrations that appeared at Allium ursinum plants. The main types of aberrations identified at these plants are: contraction, stickiness, fragmentation, inter-chromatin bridges, ring chromosomes, C-mitosis.*

Key words: chromosomes, mitotic, index, genetic, aberrations.

Rezumat. *Mărimea cromosomilor şi morfologia lor sunt indicatori importanţi utilizaţi în identificarea relaţiilor evoluţionare ale diferitelor specii de plante. Aria de aplicabilitate a studiilor de genetică este extrem de amplă plecând de la domenii de studii fundamentale (taxonomie), ajungând până la domenii aplicative (de exemplu ameliorarea plantelor). Caracterizarea genetică este importantă pentru identificarea speciei sau pentru analiza populaţiilor hibride. Determinările genetice includ numărarea cromosomilor şi stabilirea cariotipului, determinarea indexului mitotic şi analiza anomaliilor mitotice. Conform literaturii de specialitate, aberaţiile cromozomiale au fost utilizate ca o măsură a succesului de multiplicare a plantelor de mulţi ani şi aceste aberaţii fiind corelate cu schimbări în morfologia plantelor, în relaţia fertilitate-sterilitate, mutaţii etc. Prezentul studiu s-a concentrat pe caracterizarea citogenetică a două linii de perspectivă de Allium ursinum aflate în colecţia în câmp de la Staţiunea de Cercetare Dezvoltare pentru Legumicultură Bacău. Investigaţiile au vizat determinarea principalilor indici celulari (indicele mitotic, indicele profazic, metafazic, anafazic şi telofazic), ca indicatori ai vitezei proceselor de creştere şi dezvoltare. Un alt obiectiv subsidiar a fost realizarea unui screening privind*

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prezența şi tipul aberațiilor cromosomiale din ana-telofaza mitozelor plantelor de Allium ursinum.

Cuvinte cheie: cromozomi, mitotic, index, genetic, aberații

INTRODUCTION

Cell cycle is a unidirectional process in which cells passes through different phases, without omission or returning to a previous one (Harbage, 2011). Once inside the cell cycle, the cell must undergo division. Proportionality of cell division in different genotypes intensity is kept constant during the different phases of plant development, so determining the mitotic activity in roots of 1-2 cm (2-3 days after germination), could help to reveal the peculiarities of cell division genotypes studied.

Genetic characterization is important to identify the species or to analyse a hybrid population. The genetic determinations include counting chromosomes, karyotype determination, mitotic index and analysis of mitotic abnormalities (Li, 1991).

With these studies it is possible to determine the following parameters (Hassell, 2004): determine the number, shape and size of chromosomes in mitosis and karyotype species composition; study the influence of physical and chemical mutagens on the karyotype; determining the ploidy of the plant; determining the degree of homology of the chromosomes in metaphase 1 of meiosis, as well as chromosomes disjunction in other phases at plants with different degrees of ploidy and interspecific hybrids; study of aneuploid organisms and the placement of genes on the chromosome; the intra and interspecific transfer of genes and chromosomes or chromosomal segments etc..

Cytogenetic studies in *Allium ursinum* species focused on the following specific objectives:

- establishing the mitotic index (represented by the percentage of mitotic cells (M) over the total number of cells, expressed as a percentage basis.

- in order to establish the main cell indices for each of these plants the percentage of cells in various stages of division: prophase, metaphase, anaphase and telophase were calculated.

- observations about the presence and type of chromosomal aberrations present in the cell, knowing that these aberrations in various stages of division are an indicator of the stability of studied genotypes.

MATERIAL AND METHOD

The biological material is represented by root tips from germinated seeds of two perspective lines of *Allium ursinum,* originated from the field collection from Vegetable Research and Development Station Bacau, Romania.

The cytogenetic studies were accomplished in meristematic root cells, stained in Carnoy fixing solution for 24 hours at 4^0C then hydrolyzed with HCl for 7 minutes and colored with the basic coloring solution Carr. The root meristems were displayed using squash technique and for each genotype 4000 cells were counted.

Chromosome slides were then observed microscopically. Numbers of dividing cells at different levels of mitosis were recorded. Mitotic data were subjected to statistical analysis by calculating the mitotic index (% cells in division per total number of examined cells), prophasic index (% cells in prophases per total number of examined cells), metaphasic index (% cells in metaphases per total number of examined cells), anaphasic index (% cells in anaphase per total number of examined cells) and telophasic index (% cells in telophase per total number of examined cells). In the same time we monitored the incidence of abnormalities in ana-telophasic stage.

RESULTS AND DISCUSSIONS

After the seeds germinated, the root tips of about 1-1.5 mm were used for chromosome studies. After excision, the tips were placed in Carnoy stain for at least 24 h, in refrigerator. After fixation, the roots were repeatedly washed with sterile distilled water, hydrolyzed in HCl and stained with Carnoy solution.

One of the objectives of the present study was the establishment of the main division indexes (mitotic index, prophasic index, metaphasic index, anaphasic index, telophasic index) The results, calculated for each variant (variant 1 – plants from perspective breeding line I and variant 2 – plant from breeding line II) are shown in table 1, 2.

Table 1

The number of cells identified in different phases of mitotic cycle at *Allium ursinum* plants

Variant	Total no of cells analyzed	Interphase	No. of cells in active division	Repartition of cells in different division phases			
				P*	M*	A*	T*
Variant 1	4226	3602	624	239	163	114	108
Variant 2	4564	3813	751	259	204	180	108
Media	*4395*	*3707*	*687*	*249*	*183*	*147*	*108*

***P – prophase, M – metaphase, A – anaphase, T - telophase**

Table 2

The values of the main indexes registered in the meristematic cells of *Allium ursinum* plants

Variant	IM	Repartition of cells percentage/phases of division			
		% P	% M	% A	% T
Variant 1	14,76	38,30	26,12	18,26	17,30
Variant 2	16,45	34,48	27,16	23,96	14,38
Media	***15,6***	***36,39***	***26,64***	***21,11***	***15,84***

As it is illustrated in the previous tables, the values obtained are similar, which denotes the fact the values of media calculated for each type of index are the correct one that represents the characteristic of the repartition of cell phases.

Regarding the repartition of cells per each phases of division the results obtained showed that most of the cells are in prophase (36,39%), followed by metaphase (26,64%), anaphase (21,11%) and telophase (15,84%) (fig. 1-5).

The value of the mitotic index was 15,6, which denotes that the plant was in a phase of active growth.

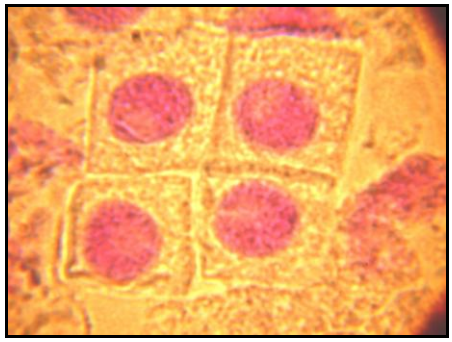

Fig. 1 – Cells in interphase

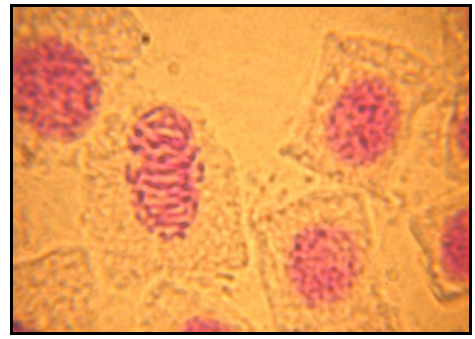

Fig. 2 – Cells in prophase

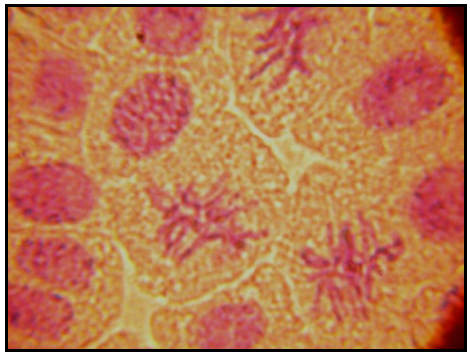

Fig. 3 – Cells in methaphase

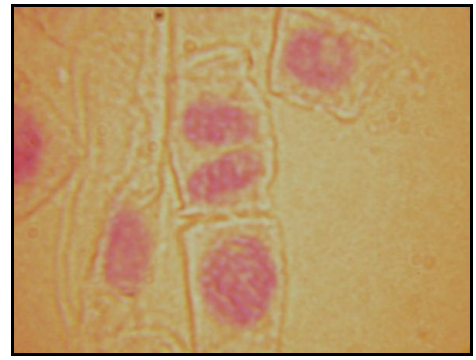

Fig. 4 – Cells in anaphase

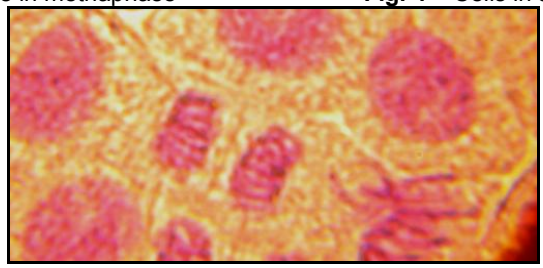

Fig. 5 – Cells in late telophase

A subsidiary objective of our study was the determination of the main types of abnormalities observed in the root cells of *Allium ursinum*. The results are presented in table 3 and graphically represented in figure 6.

Table 3

The frequency of cells with chromosomal aberrations and their spectrum identified in the ana-telophase of *Allium ursinum* plants

Variant	Total A-T studied	A-T aberrance %	$\bar{x} \pm s_{\bar{x}}$ %	from which	
				A-T with bridges%	A-T with fragments%
Variant 1	287	24	8,36	58,7	40,2
Variant 2	460	42	9,13	60,2	39,8

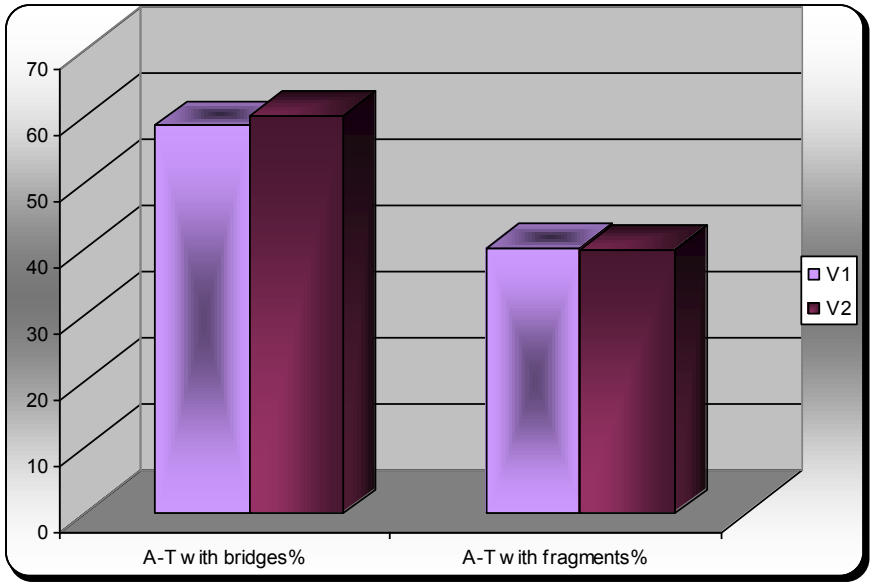

Fig. 6 – Graphical representation of frequency of cells with chromosomal aberrations at *Allium ursinum* lines

The chromosomal aberrations observed can be classified as: aberrations that affect the chromosome, aberrations that affect the chromatid, mixed or others. Thus, among chromatid-type aberrations we underline the following ones: single fragments, two or more fragments of unequal size; single bridges, two or more bridges with or without fragments of unequal size. Chromosome-type aberrations comprised of one or more double fragments; one or more double bridges with or without double fragments. Where both chromatid and chromosome-type aberrations were observed within a cell, the aberration was classified as mixed.

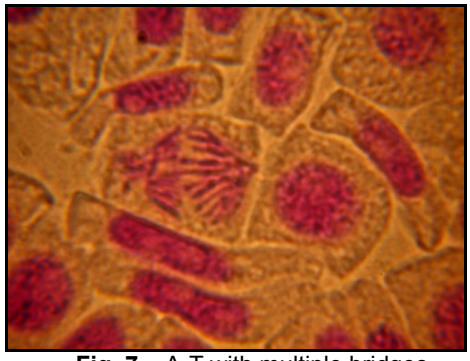

Fig. 7 – A-T with multiple bridges

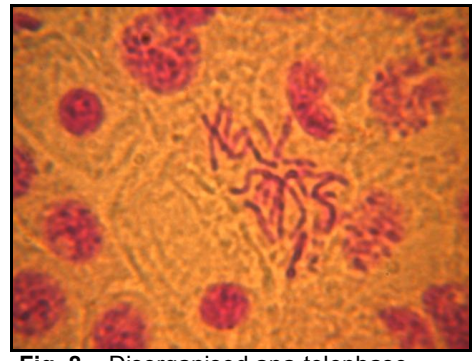

Fig. 8 – Disorganised ana-telophase

A small category, other damage including lagging chromosomes with or without any of the other groups was also observed.

CONCLUSIONS

1. The value of the mitotic index was 15,6 which denotes that the plant was in a phase of active growth.

2. The results related with the repartition of cells per each phases of division showed that most of the cells are in prophase (36,39%), followed by metaphase (26,64%), anaphase (21,11%) and telophase (15,84%).

3. The chromosomal aberration observed in the ana-telophases of cells was mainly ana-telophases with simple or multiple bridges and ana-telophases with fragments, but also expelled or late chromosomes and multipolar ana-telophases. All the aberattion observed could be classified as: aberrations that affect the chromosome, aberrations that affect the chromatid, mixed or others. But we also observed metaphases with lagging chromosomes, expelled chromosomes or ring chromosomes, multipolar ana-telophases, as well as binucleate cells and interphases with micro-nucleuses.

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