

Comparative Study on the Genetics Importance of Onion (*Allium cepa*) Cell Division under Salt Stress

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ABSTRACT

Genetic material is the most important component in the structure of a cell as it contains genetic information which all along the cell division must be correctly distributed and propagated in the next generations, this process being faultless. Thus, genetic material is compressed and stocked with the help of some histone and non-histone proteins, forming the chromosomes. The species of the *Allium* genus have got a variety of cytogenetic characteristics, genomes of different sizes, karyotypes with a different number of chromosomes and polyploidies. Within this type, there are significant genome variations due to the activity of DNA sequences which can change their place in the genome. The main factors influencing these factors are the transposable elements, these ones playing an important role in genome evolution due to the link between their high number and the chromosome size wherein they move to, with the help of which they form control and elimination mechanisms.

Keywords: cell division, genetic material, genome, cytogenetic characteristics, salt environment

INTRODUCTION

The *Allium* genus includes a multitude of species among which the following: onion (*Allium cepa*, L), garlic (*Allium sativum*, L), leek (*Allium porrum*, L) and other similar species with bulbs or underground stem (Frunză & Bârsan, 2021).

The most popular plants of this genus are onion and garlic, plants grown by people, noticeable due to their strong, a little pungent aroma as well as to their medicinal benefits. Their cultivation begins in the Middle and Far East, being spread in time all over the world. Over time

it has been discovered that all these species are beneficial to human health, being intensively used in medicine (Fenwik *et al.*, 1985).

The *Allium* genus includes biennial species which grow as bulbs and are generally used in gastronomy and medicine, being resistant plants, easy to adapt to soil and climate conditions. They contain nutritious substances such as vitamins C and B6, and together with antioxidants, provide the human body with a multitude of benefits (Vânătoru *et al.* 2019). The main nutritional compounds are sodium, zinc, copper, manganese, selenium, followed by lipids and acids (Avantor *et al.* 2019).

The most used organ in food of these species is their metamorphosed underground stem, known as well under the name of bulb, playing a role in storing the reserve substances but also of the first vegetable structures. Another role of the bulbs is that of adaptation and survival in severe winter conditions. They proliferate by chive or direct seeding (Vânătoru *et al.* 2019).

In these species, the radicular meristems can be easily obtained to see the stages of mitotic division. The genotoxicity test of mitotic division of species of *Allium* genus, named also genotoxicity test for *Allium cepa* is widely used to determine the influence of different substances on the development degree of a species or to discover their mutagenic potential. (Bonciu *et al.* 2018).

Also, the test performed on *Allium cepa* is an in vivo experimental model which is used to determine the genotoxicity of a certain substance (Banti & Hadjikakou, 2019).

This test is used as well because it has a high correlation with the testing systems on mammals (Sainis *et al.* 2016; Chrysouli *et al.* 2018a and 2018b; Milionis *et al.* 2018; Stathopoulou *et al.* 2018).

After the treatment with different substances, the influencing or mutagenic degree is quantified by calculating the mitotic index and by observing some possible DNA lesions, such as chromosomal aberrations, nuclear anomalies or the occurrence of micronuclei (Milionis *et al.* 2018; Stathopoulou *et al.* 2018).

Salt stress is one of the main factors which influence plants' growth and productivity, leading to significant changes at cell and molecule levels. Cell division is an essential process for plants' growth and development, and salt stress can influence this process by different mechanisms: by perturbing the cell stem or by deforming the chromosomes and cytoskeleton.

A study published in 2023 shows that salt stress is one of the most common obstacles influencing most crops and production at world level. Cultivated onion is sensitive to salt stress, the latter one affecting both its growth and development as well as the productivity at world level. (Regessa,*et al.* 2018).

One of the main objectives of genetic improvement research is the development of resistant genotypes to stress environment. This plant is vulnerable even from the first stage of life and up to maturity to salt stress. (Alam,*et al.* 2023).

To study the salt stress effects on a species, this one can be introduced in the cultivation of tissues by the addition of some salts in the plant's growing environment. Based on their physico-chemical properties, stress indicators are classified into categories of osmotic and non-osmotic penetration, ionic and non-ionic penetration and cell penetration respectively.

Chromosomes are packages of DNA and proteins which include and compress the genetic material to store high quantities of genetic information in a limited space as the nucleus is. Each species has got a specific number of chromosomes. For example, in onion (*Allium cepa* L.), ($2n=16$) there are 16 chromosomes distributed in eight pairs, and which are essential for the plant's growth and development (Varuzhanyan & Chan, 2020).

Peruzzi *et al* (2017), made a study on the chromosomal evolution and diversity of *Allium* genus (*Allioideae: Amaryllidaceae*) in which the number of somatic chromosomes, basic chromosomes, total length of haploids were included, and three distinct measurements which describe the structure of a karyotype thus determining its asymmetry. The main analyses regarding the evolution of karyotypes were made by the aid of phylogeny.

Due to asymmetry, differences and similarities between karyotypes were discovered, one of the factors determining these discoveries is the difference between karyotypes' lengths, whereas the similarity between the karyotypes of the *Allium* genus is provided by the conclusions of a primitive character, explained by the DNA introduction according to the length of chromosomes' arms (Peruzzi *et al.* 2017).

Polyploidy is the process through which organisms get supplementary sets of chromosomes. This helps in the development of the genome, together with the natural selection influencing genome growth by getting it adapted to abiotic stresses such as drought and salinity (Ricroch *et al.* 2005).

MATERIALS AND METHODS

The research topic approaches the comparative study of cell division in three onion cultivars which brings into discussion the identification of mitotic index of these ones, under the influence of salt stress. By means of comparative analysis, information on these plants' capacity for adapting to unfavorable conditions of environment, will be provided.

The present paper aims to evaluate the influence of different degrees of salinity on the intensity of cell division in radicular meristems, derived from three onion cultivars, which were grown in three different salinity conditions.

Study Objectives

- To establish the number of chromosomes specific to all the three cultivars of onion analyzed.
- To calculate the mitotic index noticed for each cultivar of onion analyzed under distinct salinity conditions.
- To present potential numerical or structural changes of chromosomes in the onion cultivars.

Cultivars of Onion Studied:

Onion is a quite resistant plant, due to its roots that can reach a length of 6-8 cm; it is available in most cultivating areas, but preponderantly in the temperate one. Seeds germination can last between 4-5 days, and the optimum temperature is 3-4°C, but the seeds are able to resist to temperatures up to – 8°C.

To reach maturity, the temperature must range between 25 – 30 °C, its growth being extremely fast as its leaves grow in 8 – 12 hours per day, and the bulbs between 16 – 18 hours respectively. There fore, it requires permanent light. Another important factor is humidity because it requires higher humidity to germinate (Vînătoru *et al.* 2019).

The cultivar *Golden of Buzau* is one of the most well-known cultivars in our country, reaching even the American territory. This plant has a bulb under oblong or oblate shape, with a weight between 120–300 grams, height of 6-8 centimeters and diameter 5-7 centimeters.

The bulb is goldish yellow and is covered by leaves when the harvesting is done too early, and when the harvesting is done too late the number of leaves decreases significantly (fig.1). The plant can reach a height of almost 50-60 centimeters at maturity and can have between 6-8 dark green leaves. The period of vegetation can last between 150-160 days.



Fig. 1: Golden Onion of Buzau

The cultivar *Red of Fagaras* is one of the most cultivated cultivars in our country, being from the Fagaras homeland, often appreciated for its intense taste and resistance to the environmental conditions. This cultivar has got a purple-colored bulb, with a diameter varying between 7-8 centimeters and a weight between 150-160 grams. The bulb has a spherical-oblate shape, and its vegetation can last up to 130 days (fig. 2). It is a more fastidious cultivar which prefers the soils of optimum humidity content, rich in organic matter, easily permeable and aerated. It is cultivated especially in rivers' meadows. The harvesting of this cultivar is done when its leaves are dried.



Fig. 2: Red Onion of Fagaras

The *Gladstone* cultivar is a lasting onion with a vegetative productivity of 125 days. The bulbs are big, round and uniform which display a thin layer tunica of pearly white (fig. 3).

It has high resistance to diseases, and it is significantly affected by the environmental conditions. The plant has dark green leaves.



Fig. 3: White Onion Gladstone
(<https://www.bing.com/>)

Cell Division and Cytogenetic Characteristics of Allium Genus:

Cell division is the process by which a cell divides itself into two or more daughter cells. Two predominant categories of this process are: mitosis and meiosis. Mitosis is the division by which cells replicate identically, thus contributing to the tissue growth, replacement and repair; the second type of division is meiosis which happens in the reproductive cells and generates sexed cells called gametes (Isacescu, 2019).

Cell cycle consists of two main stages: interphase (stage in which the cell occupies its role in the organism and in which metabolic processes take place) and cell division (stage in which the cell divides, and which can be of two types: mitotic and meiotic).

The Interphase is the stage in which the cell grows and replicates, defining the role it plays in the organism. During this stage both anabolic and catabolic processes take place in the cell membrane, but also the processes necessary through which the cell gets ready for division, by growing and replicating the genetic material (Gavrilă, 2003).

The interphase consists of three main subphases:

Phase G1 – is the phase in which the cell grows and fulfills normal functions. Also, G1 plays an important role in cell cycle as in the end there comes the checking point when the cell must have met some conditions to get into phase S. If not, the cell enters a repose state called G 0.

Phase S or Synthesis – is the stage in which DNA replicates, doubling its quantity, following the semi-conservative model, and chromosomes are copied so as each daughter cell receives a complete copy of the genetic information.

Phase G2- the cell continues to grow and to get ready for division, the DNA is being checked to see if it was correctly replicated under favorable conditions. In this stage proteins are preponderantly synthesized which further on help form new daughter cells.

The interphase is one of the most important stages of the process through which the cell gets ready for mitosis or meiosis by the help of which accurate checks are being made (control

points, the transition between phase G1 and S, as well as the transition between G2 and division) to avoid the occurrence of possible errors that might affect the correct transmission process of genetic information to daughter cells (Gould & King, 1984).

Mitotic Division:

It is the most often encountered cell division and can be seen in somatic cells. Mitosis has the role of ensuring growth, replacement of worn out and damaged cells and regeneration of tissues in multicell organisms and consists of four main phases:

In mitosis prophase the chromosomes become visible and get thickened, the nucleole disappears, centrioles are carried towards the opposite poles of the cell, generating division spindle fibers between them (fig.4). At the beginning of this stage the nucleus grows up, while the chromosomes coil up and shorten, thus the two chromatids are formed (Gavrilă, 2003).

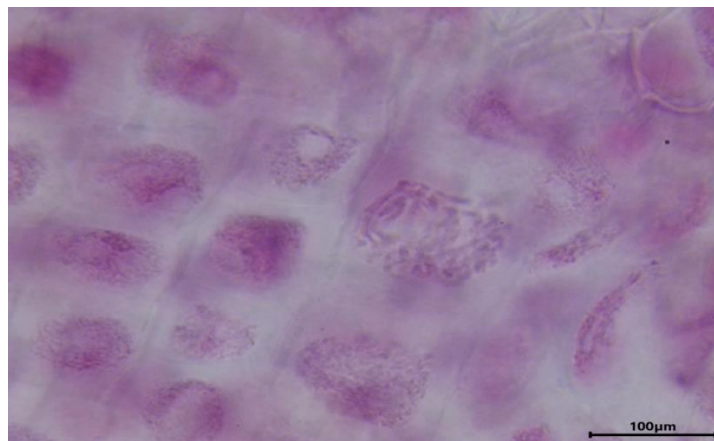


Fig. 4: Prophase *Allium cepa* (original photography)

Metaphase - the chromosomes are put into the cell center and get attached to the spindle fibers with the aid of centromere (fig. 5). Thus, a correct repartition of the genetic material in the daughters' cells is provided (Gavrilă, 2003).

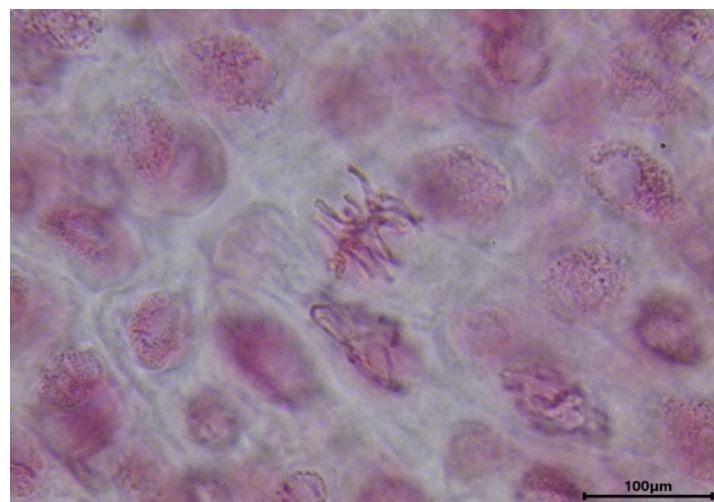


Fig. 5: Metaphase *Allium cepa*, (original photography)

Anaphase - the chromosomes divide themselves inside the centromere into two equal shares, called chromatids that migrate towards the cell's opposite poles (fig. 6).



Fig. 6: Anaphase *Allium cepa*, (original photography)

Telophase - the chromosomes reach the cells opposite poles (fig. 7), there begins the formation of nuclear membranes around the chromosomes, generating the two son nuclei.

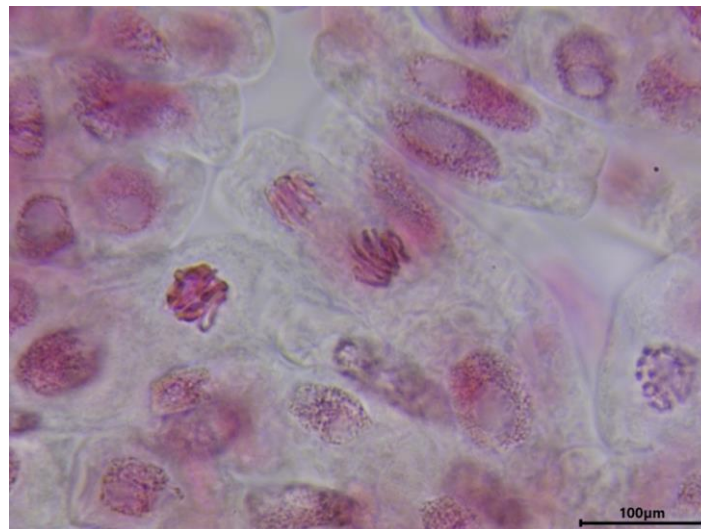


Fig. 7: Telophase *Allium cepa*, (original photography)

Cytokinesis is the last stage of cell cycle in which cytoplasm division takes place, when two distinct daughter cells are generated. During mitosis, the moment when the chromosomes separate and reach the cell's opposite poles in anaphase, the division process of cytoplasm starts (fig. 8), and during meiosis this one takes place after the two successive divisions, leading thus to the formation of the four daughter cells.

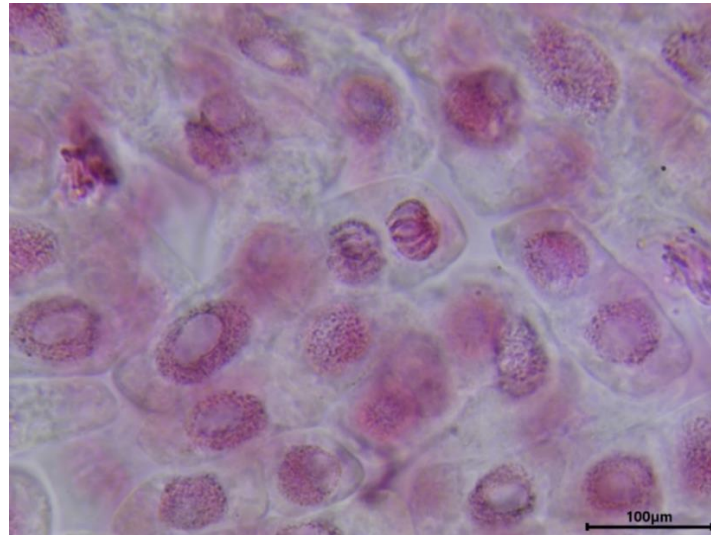


Fig. 8: Cytokinesis *Allium cepa*, (original photography)

This process involves the building of a structure called „cleavage furrow” in the animal cell or the forming of a cellulose plate in plant cells and divides the cell into two ones, being sure that each cell benefits from a correct share of cytoplasm and cell organs (Gavrila, 2003).

The mitosis role is to keep the number of chromosomes in daughter cells constant, thus providing genetic correctness and uniformity in the transmission of genetic information from one generation to another (Hațieganu & Pascale, 2015).

Cytogenetics Test at *Allium cepa* L., Uses:

The cytogenetics test at *Allium cepa* has numerous applications. It analyzes the discrepancies and concordances in terms of cell cycle, of cell division and the toxicity aspects of different substances and the way in which they influence cell division (Isacescu, 2019).

The cytogenetics test at *Allium cepa* can be used for the following applications:

- Expression of the genes involved in the adjustment of cell cycle
- Influence of chemical substances or environmental factors on cell division
- Variability and frequency of cell division in plant tissues and organs
- Description and comparison of cultivars by emphasizing morphological and genetical characteristics and how they influence the division processes.
- Use of molecular biology to show as accurately as possible cell division (Isacescu, 2019).

The biological studies on many cultivars of the species *Allium cepa* L., bring to the fore chromosomal anomalies caused by more factors, among which residual water is one of the most important ones. Toxins are the enemies that affect both the human body and the plants' health and crops. They have a major negative influence on soils as well by destroying mineral salts which are the plants' feed. In this research the species *Allium cepa* stands out by its chromosomal characteristics as being one of the best indicators of the damage caused at DNA level and the anomalies resulted from. The most exposed cells of this species are the root tips,

the latter ones getting into contact with the toxins in soil(Wijeyaratne &Wickramasinghe, 2020).

Wijeyaratne &Wickramasinghe (2020) studied the influence of three different affluents, with different pollution degrees on root meristems in onion. After each batch was watered by three different types of water, the rootlets were harvested to see the influence exerted by toxins on cell division in the root meristems examined.

At the same time, this study shows that the mitotic index and phase index of meristem cells of *Allium cepa* roots can be affected by used water, leading to chromosomal modifications. This stands for the fact that in the used water of the experiment there were also mutagenic substances (Wijeyaratne &Wickramasinghe, 2020).

Stages of Sample Preparation Process

1. Between 5 – 15 rootlets from each onion bulb were taken and placed in 1.5 ml centrifuge tubeswith acetic acid: ethanol solution3:1 for 24h.
2. They underwent through 70% alcohol and kept in the refrigerator until the preparation cytogenetic samples.
3. The process of hydrolysis withHCl0,1N solution was carried on. The solution rootlets were placed on
4. In-solution rootlets were placed on plates and put to boil at 60°C for 1 minute. Then, the rootlets were washed with distilled water to remove HCl solution.
5. Rootlets coloration. The samples were put to boil with acetic carmine for30 secondsat a temperature varying between 83-100°C to get an in-depth coloration of the material.
6. After waiting some time, the material was rinsed with distilled water to remove the coloring agent.
7. A drop of 45% acetic acid was added to the samples, and if there were noticed some traces of coloring agent, the medium was rinsed again by distilled water and a new drop of solution was added.
8. The cytogenetic preparation was made by the “Squash” method. A portion of rootlet was placed on a glass plate, then it was pressed uniformly and strongly with the aid of a clean napkin to define the cell display in a single layer at the optical microscope (fig. 9).
9. The samples were analyzed by an optical microscope, and the photos of cytogenetic preparations were taken by using **Optika Vision Prosoft**, 20x, 40x and100x lens.



Fig. 9: The Materials used at cytogenetic preparations: (original photography)

RESULTS AND DISCUSSION

Calculating the Mitotic Index of Three Cultivars of Allium Genus Under Salt Stress

The division stages were observed, and the occurrence of chromosomal anomalies of number and structure was studied. Cell divisions were counted, and the mitotic index was calculated according to the formula:

$$I = \frac{\text{number of cells in division}}{\text{total number of cells}} * 100$$

I = mitotic index

At the end, the number of cells in different stages of division was noted, then the mitotic index was calculated for at least five rootlets harvested from each bulb of each batch. For each rootlet more than 300 cells were counted.

The statistical parameters and graphs were made with the aid of statistical functions implemented in Microsoft Office Excel (version 2021) and in Post v. 4.17 statistical program (Hammer et al. 2001). The arithmetic averages and standard deviation were calculated. To compare the mitotic index of the three onion cultivars and the salinity degree, the following tests were used: Kruskal-Wallis non-parametric test, post-hoc Dunn test and then, a linear regression was used to check if there is a correlation between the two parameters, also in Post v 4.17 program.

All the mitotic division phases were observed. A total of 24.179 cells studied, out of which 1.425 were in division. On average, for the Gladstone cultivar without water addition, 1.000 cells, out of which 61 were in prophase, 10 in metaphase, 4 in anaphase, and 4 in telophase (figures 14,15,16, 17). In the Gladstone batch watered with 100um slat solution, an average of 43 cells in prophase and 16 cells in metaphase, 4 cells in anaphase and 38 cells in telophase were registered (tab.1). For the Golden of Buzau cultivar without salt addition the following values were registered: 52 divisions, in 1.880 of cells counted, most cells being in prophase (24) (tab.1).

For the Red of Fagaras cultivar, the highest values of divisions were registered also in the batch without salt addition, whereas for the other two batches, 100 mM and 200 mM salinity Red of Fagaras, respectively, no rootlets were found.

Table 1: Number of cells in the division phases of the 9 batches

Parametric	The cultivar used	No. of rootlets	Total No. of cells observed	No. of cells in division	Prophase	Metaphase	Ana-phase	Telophase
N	Gladstone without salt addition	5.00	3700.00	274.00	208.00	38.00	14.00	14.00
Average			1000.00	80.00	61.60	10.40	4.00	4.00
DS			308.22	30.63	24.90	3.26	1.41	1.41
N	Gladstone 100mM	5.00	2950.00	342.00	219.00	83.00	21.00	19.00

Average			590.00	68.40	43.80	16.60	4.20	3.80
DS			231.43	27.09	15.28	10.59	1.47	1.47
N	Gladstone200mM	5.00	3075.00	231.00	215.00	12.00	4.00	0.00
Average			615.00	46.20	43.00	2.40	0.80	0.00
DS			212.65	27.23	26.58	1.02	0.40	0.00
N	Golden of Buzau without salt addition	4.00	1880.00	52.00	24.00	6.00	14.00	8.00
Average			470.00	13.00	6.00	1.50	3.50	2.00
DS			120.00	6.00	6.00	0.50	0.50	0.00
N	Goldenof Buzau 100 mM	7.00	4620.00	150.00	124.00	8.00	8.00	10.00
Average			660.00	21.43	17.71	1.14	1.14	1.43
DS			340.00	4.03	5.36	0.99	0.64	1.68
N	Goldenof Buzau 200 mM	3.00	1800.00	114.00	114.00	0.00	0.00	0.00
Average			600.00	38.00	38.00	0.00	0.00	0.00
DS			0.00	0.00	0.00	0.00	0.00	0.00
N	Red of Fagaraswithout salt addition	4.00	1700.00	177.00	126.00	36.00	12.00	3.00
Average			425.00	44.25	31.50	9.00	3.00	0.75
DS			108.97	13.55	10.40	3.08	0.00	0.43
	Red of Fagaras 100 mM	5.00	1500.00	0.00	0.00	0.00	0.00	0.00
	Red of Fagaras 200 mM	5.00	1654.00	0.00	0.00	0.00	0.00	0.00

No chromosomal anomalies were noticed in any of the preparations. The number of chromosomes in the three cultivars is the same – standard $2n=16$ (figures 10, 11, 12).

The MI was calculated in all the 9 onion batches (table 1). The values of the mitotic index in the batches where rootlets were generated varied between MI=14.25 in the Red of Fagaras batch, without salt addition, and MI=2.00, in the Golden of Buzau batch (tab.2).



Fig.10: Microscopic field, 20x lens of cells in division for the Gladstone cultivar without salt addition. (the arrows were used to highlight the cells in division)

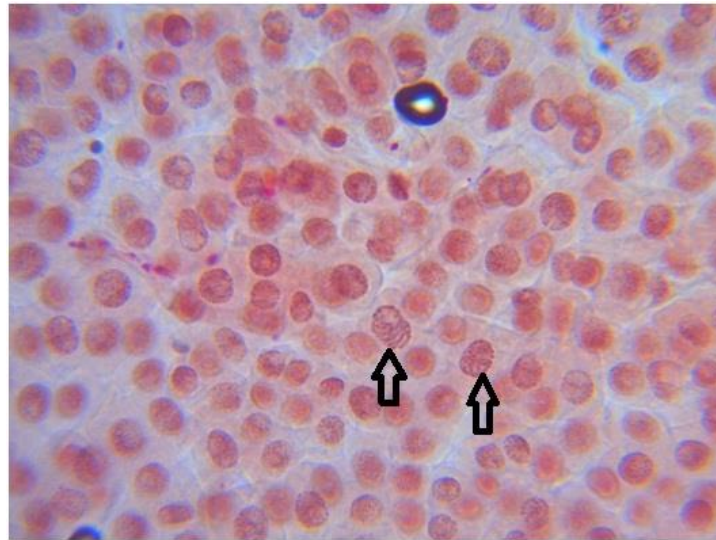


Fig. 11: Microscopic field, 40x lens of cells in division for the Gladstone cultivar with 200 mM salinity (the arrows were used to highlight the cells in division)

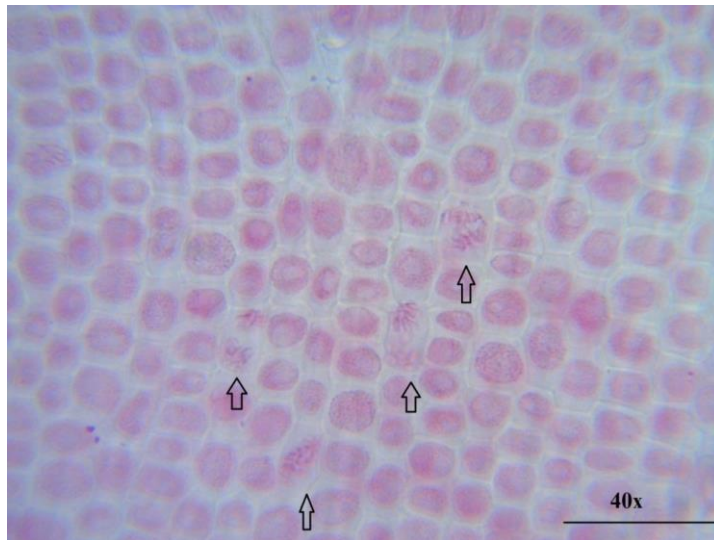


Fig. 12: Microscopic field, 40x lens, of cells in division at the cultivar Golden of Buzau without salt addition. (the arrows are used to highlight cells in division)

Table 2: Mitotic Index Values calculated for the nine batches analyzed

Parameters	No.rootlets	Mitotic Index (MI)		
The cultivar used		Gladstone	Golden of Buzau	Red of Fagaras
Without salt addition	1	6,17	3,22	14,00
	2	9,69	2,00	6,33
	3	8,00	3,39	14,25
	4	5,83	2,86	8,33
	5	8,54		
100mM salinity	1	10,64	2,46	0,00
	2	13,67	2,21	0,00
	3	10,43	4,86	0,00
	4	11,82	5,50	0,00

200mM salinity	5	11,70	1,89	0,00
	6	-	4,57	-
	7	-	5,50	-
	1	9,23	4,83	-
	2	5,60	4,50	0,00
	3	3,80	6,00	0,00
	4	7,57	-	0,00
	5	9,33	-	0,00

The highest values were registered in the Gladstone batch of 100mM salt concentration, with a MI average= 11,65±1,15. The lowest values were registered by the batches of Redof Fagaras watered by salt concentration where no rootlets were generated.

Among the batches with rootlets, the lowest values were registered at the Golden of Buzau cultivar, without salt addition, where the values of the mitotic index MI= 2.87±0.54 were average.

Having in view that a small number of rootlets in the onions to germinate was obtained, a non-parametric test of the samples was used. Thus, the Kruskal-Wallis test was used, resulting in a value $H(\chi^2) = 37,43$ and showing that there is a significant difference between the medians of the batches analyzed, $p = 7,85 \times 10^{-06}$. The Dunn test is a post-hoc one which shows the differences between groups (tab. 3).

Table 3: p Values of post-hoc Dunn test, for the values of the mitotic index analyzed, for the 9 batches. Dunn values are shown in the diagonal below.

	GN	G100	G200	BN	B100	B200	FN	F100	F200
GN									
G100	0,2988								
G200	0,761	0,1792							
BN	0,05907	0,00414	0,1094						
B100	0,0832	0,004307	0,1603	0,6878					
B200	0,3528	0,0674	0,5056	0,4416	0,6265				
FN	0,4959	0,7652	0,3332	0,01482	0,01891	0,1371			
F100	0,001346	2,19E-05	0,00371	0,2564	0,08355	0,0647	0,000213		
F200	0,001346	2,19E-05	0,00371	0,2564	0,08355	0,0647	0,000213	1	

The highest differences between the medians of the batches analyzed were registered by Red Onion of Fagaras, watered by salty water and Goldstone onion batches and that of Red Onion of Fagaras without salt addition. Also, there can be noticed a significant difference $p < 0.05$ in the following pairs: Golden Onion of Buzau with 100 mM and 200mM, being correlated with Red Onion of Fagaras. A linear regression was made to see if the mitotic index is correlated with water salinity (tab. 4).

Table 4: Parameters of linear regression between its concentration and mitotic index (MI). Calculations were made in the Past v 4.01 Program

Parameters of linear regression	Gladstone	Golden of Buzau	Red of Fagaras
r:	-0,08465	0,56882	-0,80253

r^2 :	0,007165	0,32356	0,64406
p (uncorr.):	0,76423	0,033777	0,00168

Therefore, a negative correlation was found $r = -0,80$, $r^2=0,64$ and p (uncorr.)=0,0017 (figure 23) in the Red of Fagaras cultivar, and the mitotic index decreases together with the increase of salinity. No correlation was observed between the other two cultivars. This can be explained by the very small number of samplers, so that our observations are not objective.

Numerous cells and microscopic fields were investigated to form the tables necessary to calculate the mitotic index.

CONCLUSIONS

The nine onion batches of Gladstone, Golden of Buzau and Red of Fagaras cultivars were grown successfully under different salinity conditions, exception for the Red Onion of Fagaras where no rootlets were obtained for the 100 mM salinity concentration and 200 mM respectively.

24.179 of cells were investigated, out of which 1.425 were in division phase. The most cells in division were registered in the Gladstone white onion cultivar without salt addition, and the fewest ones in division were registered in the Red Onion of Fagaras.

The most cells in division were registered in prophase 76,21%, followed by 13,96% cells in anaphase, 5,82% in telophase and the fewest were registered in diakinesis (4%).

The values of the mitotic index varied between $MI=14,25$ in the batch of Red Onion of Fagaras, without salt addition, $MI=2.00$, in the batch of Golden onion of Buzau. This study focused only on the batches where rootlets were obtained.

Significant statistical differences were observed $p < 0.05$ in the following pairs: Golden Onion of Buzau cultivar of 100 mM and 200mM, and Red onion of Fagaras without salt addition and between the batches of Red onion of Fagaras with salt addition and all the Gladstone onion batches.

By carrying out a linear regression there can observe a negative correlation $r = -0,80$, $r^2=0,64$ and p (uncorr.)=0,0017 between the mitotic index in the Red of Fagaras cultivar and salinity as the mitotic index decreases with the salinity increase. No significant correlation was noticed in the other two cultivars. This can be explained by the very small number of samples so that our observations are less objective.

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