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Salinity Influence on Radicle Length of Two Potato Genotypes

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ABSTRACT

Potato is the fourth most important food growth, after three of cereals: rice, wheat and maize. Ii is also one the most important source of food at world level, being an essential economic one in developing countries. In agriculture, salinity stress is one of the main abiotic stress factors affecting potatoes. This kind of stress causes changes in internal and external physiology of potato plants, resulting in a decrease of tubercle quality and production. Moreover, it may cause damages in all developing stages of potato, being extremely devastating in the expansion phase of the tubercle when the nutritional requirements are at maximum for its growth. The paper aims at characterizing the contribution of technological parameters to two of the most grown potato cultivars in Romania: Santé and Roclas, and at investigating the way in which salinity stress can affect their growing rhythm by analyzing its influence on the rate of root division.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most proliferous crops at world level. According to the data provided by FAO, in 2023 there were registered productions reaching almost 375 million of tons harvested in 2022, and China (95.5 million of tons) and India (56 million of tons) being in top among producers (Chauhan *et al.,* 2023; *FAO*, n.d.).

Solanum genus has got a vast source of genetic diversity, but only a small fraction of its diversity has been turned into account in the process of potato development so far. Traditional reproducing methods have had a significant impact upon the development of potato cultivars (Tiwari *et al.,* 2022). The wide expansion of potato cultivation in different regions of the country was possible due to its great economic importance gained by this plant in diverse fields. Potato has not been only an essential food resource, but also as forage, being at the same time an important source of animal feeding. In addition, potato has become a valuable raw material in food and processing industry, thus bringing its contribution to the diversity and development of agricultural economy (Mureşan, T., 1972).

At world level, the human population undergoes a continuous significant change in terms of food, both collectively and individually. Climate changes, food preferences and the difficulties associated with food security assurance have put the potato under different types of stress,

decreasing in this way its quality and quantity. It is well known that a tubercle crop is extremely sensitive to drought due primarily to its superficial roots (Nahirñak *et al*., 2022).

Potato has also a wide range of uses in industry, being used in the manufacture of a variety of processed food products as well as alcohol, animal forage and as substrata used in the production of bio energy, including biofuel.

Especially the waste resulting from potato crops, mainly potato peels, is being used to process all these previously mentioned. Further processing methods such as peel, pulp transformation and of other waste in starch processing factories, their integration into animal feeding formulas or their conversion into ethanol are essential steps towards sustainable waste management. Thus, the potato industry does not only provide essential feed stocks but it also explores new innovating modalities of minimizing its impact upon the environment, contributing thus to the development of circular and long-lasting economy (Chauhan *et al*., 2023).

The potato production is being faced with major difficulties such as the biotic stress, represented mainly by viruses, bacteria, fungi, pests and the abiotic stress represented by drought, floods, salinity, heat and cold as well as the post-harvesting problems (accumulation of residual sugars while being stored and enzymatic browning caused by lesions). Due to the significantly negative impact on tubercles' production and quality, the improvement of resistance against diseases and pests, as well as against abiotic factors, together with quality characteristics, is of a great economic importance (Nahirñak *et al*., 2022).

In Europe, it has brought about considerable contributions to food and agriculture. It has quickly adapted to European climate diversity, becoming a crucial source for Europeans' diet. Therefore, it has become an extraordinary advantegeous and cultivating source, by increasing the farmers' incomes and gains, providing labour force and stabilizing food costs. In Europe, potato was introduced for the first time in the second half of 16thcentury, in the Iberic Peninsula and Spain (Gontariu I., 2022).

Its farming on large plots in Romania has started in the 19th century, as well as on limited areas in the western side of the country, in Transylvania. The farming of tubercles in the Romanian area, has had a remarkable economic importance. Due to the climate changes and economic constraints, farmers together with researchers have been looking for solutions to increase productivity, providing sustenaibility and efficiency (Gontariu I., 2022).

In Romania, once with the decrease in the cultivating surface, the production of tubercles has significantly decreased as well, beginning with the year 2020, along with the pandemic (Fig.1).

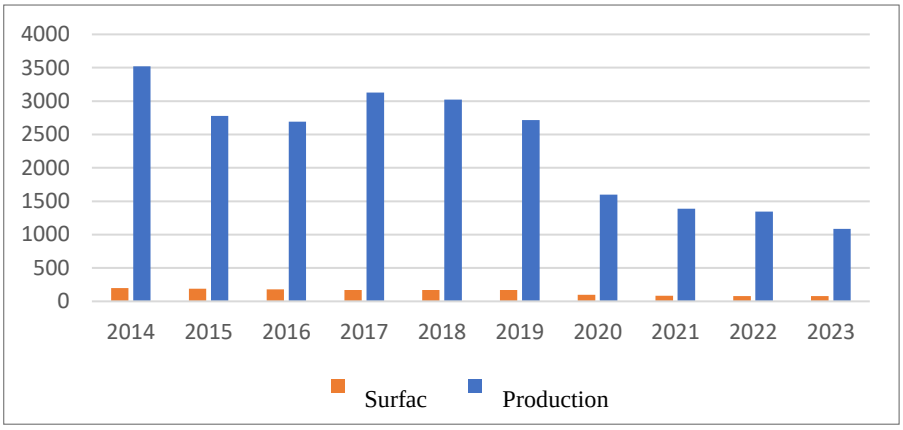


Fig. 1: Potato production in Romania (INS, 2023)

Potato Cytogenetic Studies

Chromosomes are hereditary elements of the whole nuclear genome and discrete units of mutations, being capable of providing very important information on the determining of phylogenetic relationships. They may vary depending on their number, dimension, morphology and coloring properties of taxa. Number of chromosomes is a specific characteristic of species, used at identification, since it is easy to be obtained and known for most plants. Karyotypes provided by chromosomes are important in plants' speciation as the differences given by chromosomes establish postzygotic barriers and they can provide diagnoses for species' systematic and plants' evolution. At *Solanaceae*, chromosomes are essential in delimiting taxa, by means of genetic studies and cultivar selection of economic importance species (Deanna, R., *et al*., 2022).

At potato, the karyotype formula is 2n=24, but there are many varieties which are haploids and polyploids. In the past, ploidy was a significant criterion in the classification of cultivated potatoes (Jansky, S. H., & Spooner, D. M. 2018).

In his studies from 1929 and 1933, Rybin introduced the concept that cultivated potatoes form a polyploid series, including diploid variants (2n = 2x = 24), triploids (3x), tetraploids (4x), and pentaploids (5x). He also identified wild potatoes which are di-, tri- and pentaploids al all these levels, including hexaploid variants (6x) (Sobieh, S. S., *et al*., 2019).

Despite the progress made in the improvement of potatoes, significant efforts and financial resources are necessary because of high demands of big populations and of the long period of time requested. This situation is due to both general challenges in the field of plants' improvement and to the genetic complexity associated with polyploidy, especially with tetraploids, having in view that many developed cultivars of potatoes are tetraploids (Watanabe, K., 2015).

Potato's genetic improvement has occurred by the implementation of some systematic methods of germplasm improvement and by the adopting of some approaches within this paper. The Potato International Center (CIP) has played a significant role in facilitating international collaboration by setting up wide research networks. The germplasm improvement focused on the diploid tubercles of *Solanum* genus, including some diploid

species cultivated and other diploid taxa, has been done by numerous programs of potato improvement (Watanabe, K., 2015).

The fundamental strategy adopted to improve the potato crop is represented by the phenotype of recurrent selection. In a typical interbreeding program, the emphasis is put on the development of new cultivars, underlying the importance of crossing and selection after action testing of multiple generations among reproducing clones. At the same time, this approach provides the crop with adaptability and continuous resistance to stress factors, without making appeal to aggressive methods that might have negative consequences on human health and agricultural ecosystem (Reslow, F., *et al*., 2022).

MATERIAL AND METHODS

At world level, there is an impressive variety of potato cultivars, of different dimensions, shapes, colors, textures, cooking characteristics and flavors. Up to nowadays there have been cultivated more than 10000 potato cultivars, many of them being cultivated at present as well. This diversity stands for the adaptation to the consumers' preferences and local farming conditions (Kondhare K. R., *et al*., 2020).

Despite the fact there is such a great number of cultivars, the production of new cultivars is continuous. The ancient multiplication methods of new tubercle cultivars have not changed significantly over the last decades, and the most visible differences are connected to the amplitude and technologies used in producing new cultivars.(Dobnik, D., *et al.,* 2021).

Roclas Cultivar

Created by the National Institute of Research-Development for Potato and Sugar Beet, Brasov, this cultivar is semi-early, belongs to the genealogy HB8 x GRANDIFOLIA and its tubercles are oval, its peel and pulp are yellow (fig. 2).

Fig. 2: Roclas Cultivar (foto pers.)

It has a well developed scrub, rich in leaves and semi-erectus. It has a medium resistance to manna for leaves and tubercles and to Y virus of potato. It also has resistance to the virus of potato leaf curling and to potato canker. The content in starch is between 16-18%. It is meant for early consumption, but it can be used in industrialization, too. The productive yield is of about 65 tons per hectare. From the organoleptic point of view, Roclas cultivar provides chips of 30.9% yield, medium color at a score of 7.2 (Ianoşi, I. S., 2002).

Santé Cultivar

It is a semi-late cultivar created in the Netherlands, a vegetation period between 100-120 days, meant for summer, autumn-winter consumption and industrialization. The tubercles are round-oval, oval, the peel is yellow, and the pulp is light yellow, with a nice aspect (fig. 3).

Fig. 3: Santé cultivar (foto pers.)

It is considered a well-resisting cultivar to viruses Y, A and X, and medium sensitive to VRFC. Its content in starch is of almost 18% and it can be used fresh or industrialized as flakes, chips and pommes-frites. It has a high productive yield around 43-45 t/ha. From the organoleptic point of view, Santé cultivar provides chips of 27.07% yield, medium color, with a score of 7.8 (Ianoşi, I. S., 2002).

The aim of the paper is to identify the mitotic index of two potato cultivars, *Solanum tuberosum* Santé and *Solanum tuberosum* Roclas, which are under salt stress. The influence of salinity on the development of tubercles and of the whole plant was determined by assessing the division rate of radicular meristems. The division rate was assessed by calculating the *mitotic index*. Five tubercles of each cultivar under three different salinity conditions were cultivated, of the two potato cultivars: Sante and Roclas. The tubercles were grown together in pots and watered with salt solutions of different concentrations: 0 mM, 100 mM and 200 mM. After the radicles got formed, when they reached the size of 1 cm, they were harvested and fixed, and later on the areas of intense divisions of the cap were emphasized by coloring them with acetic carmine.

Stages of Experiment

(a) After being harvested, they were placed in tubes containing a fixing solution of acetic acid (fig. 4): ethanol of 3:1 for 20-24 hours. After sampling, each tube was labeled with the cultivar harvested and the concentration of salt solution and kept in the refrigerator.

(b) After 20-24 hours, the samples were transferred to ethylic alcohol of 70%concentration and kept again in the refrigerator until the cytogenetic plates were made.

(c) Before making the plates, the radicles were measured. The making of preparations starts with the process of hydrolysis, in which the material is placed in a drop of

hydrochloric acid 0.1 N for about 30 seconds on an electric stove, at the temperature of about 60°C.

(d) After the hydrolysis stage, the plate is washed by distilled water and is colored by acetic carmine for 1 minute at a higher temperature, of 100°C, until the color gets permeated and the roots turn dark red.

(e) After waiting time, it is rinsed once again by distilled water in order to remove the dye excess, the plate is placed in and is ground by the Squash method by means of strong and uniform napkin in order to delimit the cell wall.

Fig. 4: Stages of making cytogenetic plates

Further on, cytogenetic preparations made from potato radicles were analyzed. At the same time, the meristematic cells being in division were numbered by calculating the mitotic index. The sampling of meristematic tissues of potato radicles was done after 3-4 hours since sunrise in order to catch the cells at their maximum intensity of divisions. (Ordoñez, B., *et al*., 2014.).

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

Where ***I*** = mitotic index

The number of cells being in different stages of division was also noted: prophase, metaphase, anaphase and telophase. The mitotic index was calculated for at least five radicles of each tubercle of each batch.

Measurement of Radicles' Length

The radicles harvested from each potato batch were measured by means of a ruler and the average of lengths and standard deviation were calculated.

The graphics and statistical data were carried out using the statistics module Microsoft Office Excel (version 2011) and the program Past v. 4.17 (Hammer D.A.T., *et al*., 2001). The asymmetric averages and standard deviation were calculated. The measurements and the mitotic index were compared with the salinity degree using the non-parameter Krustal-Walls test and then the post-hoc Dunn test. These data were used since our sample does not have a normal distribution and there is a small number of samples. The Krustal-Walls test is a statistical non-parameter one which assesses the differences between more groups sampled

independently on a single continuous variable, which is not normally distributed (Hammer D.A.T., *et al*., 2001). In order to see if there is a correlation between the salinity degree and the radicles' length, the mitotic index respectively, a linear regression was made with the variable of length, index and it was carried out also by the program Past v. 4.17 (Hammer D.A.T., *et al*., 2001).

RESULTS AND DISCUSSION

In the case of batches exposed to salt-free water (tab. 1) the radicles' lengths were registered between 11 mm and 30 mm, with an average of 19.32 mm and a SD of 5.54 mm in the Santé cultivar (n=38). In the case of Roclas cultivar belonging to the same batch, values of radicles' lengths were registered between 9 mm and 29 mm, with an average of 15.95 mm and a SD of 4.43 mm (n=36).

Table 1: Values of radicles'lengths, averages and SDs

Concentration	**NaCl-free**		**100 mM NaCl**		**200 mM NaCl**	
Cultivar	Santé	Roclas	Santé	Roclas	Santé	Roclas
N	38	36	32	28	30	32
Minimum	11	9	9	8	8	9
Maximum	30	29	23	23	20	18
Average	19.31579	15.9444444	16.8125	14.57143	13.26667	12.75
SD	5.535081	5.97654882	4.433096	4.73157	3.335999	2.839454

Out of the batches which were exposed to a concentarion of 100 mM NaCl, Santé cultivar registered minimum values of 9 mm and maximum ones of de 23 mm for length, with an average of 16.81 mm, the average being of 14.57 mm and SD of 4.73 mm (n=28).

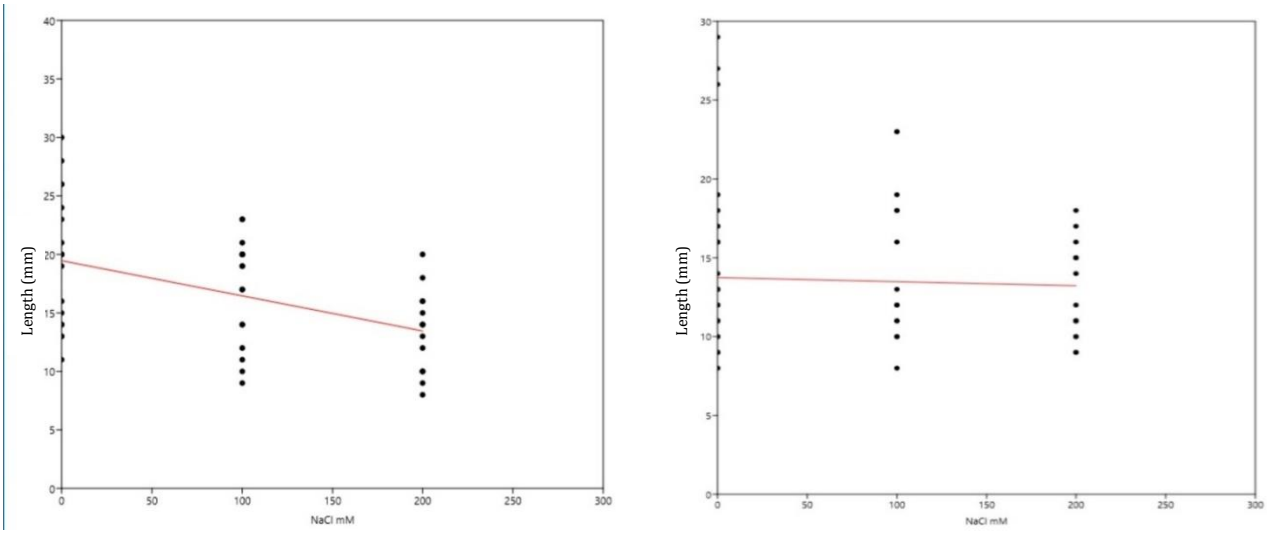
In the case of batches exposed to water, with a concentration of 200 mM, the Santé cultivar registered values of radicles'lengths between 8 mm and 20 mm, with an average of 13.27 mm and SD of 3.34 mm (n=30). Under the same conditions (tab.1), the Roclas cultivar, (values of lengths were between 9 mm and 18 mm, the average being of 12.75 and SD of 2.84 mm (n=32).

After applying the post-hoc Dunn test, the values in table 2 were obtained which actually show the batches between which there are differences. The diagonal below shows the real values of p. Yellow stands for p values with significant differences between medians.

Table 2: Values p of post hoc Dunn test, for radicles' length, calculated for the six batches analyzed.

	0 mM Santé	0 mM Roclas	100 mM Santé	100 mM Roclas	200 mM Santé	200 mM Roclas
0 mM Santé						
0 mM Roclas	6.12E-05					
100 mM Santé	0.3215	0.009011				
100 mM Roclas	0.01479	0.4367	0.1536			
200 mM Santé	0.002301	0.8836	0.04619	0.601		
200 mM Roclas	0.0004748	0.7593	0.01629	0.3713	0.7116	

A significant difference can be seen, with the value $p<0.05$ in the following pairs: Santé without salt addition and all batches of Roclas and Santé 200mM, Roclas without salt addition and Santé 100mM, and Santé 100mM with Roclas 200 mM and Santé 200 mM.



A **B**

Fig. 5: Plot of linear regression between radicles' length and salt concentration A- Santé, B- Roclas

A simple regression was also made, by calculating the correlation coefficient between salinity and radicles' length. Thus, r=-0,47 and p (uncorr)=0,0006 (fig. 5 A şi B). This fact shows that there is a negative correlation between the radicles' length and salinity in the Santé cultivar (fig. 5 A). The Roclas cultivar (fig. 5 B) no correlation between radicles' length and salinity was noticed R= -0.047 and p (non-correlated) significantly = 0,69.

Salinity has influenced negatively the development of radicles, by diminishing the increase in the Santé cultivar, affecting at the same time their number, shortening their length, but also the number of chromosomes decreases with a higher salt concentration. In the case of Roclas cultivar, the hypothesis of non-correlation was significant. The salinity influence of the division process was assessed for the two potato cultivars as follows:

Numerous plates totalizing a number of 9.888 of cells were analyzed, out of which 1.206 were observed in different stages of division.

Out of the total of cells counted one can notice that most of them were in prophase, with a total of 1.124 cells, and then 74 cells were registered in telophase, while in anaphase 8 cells were noticed. The biggest number of prophase were registered by the roots grown in salt-free addition water, and their number decreased with the salt concentration, a lot of pyknotic nuclei being observed (fig. 6, 7).

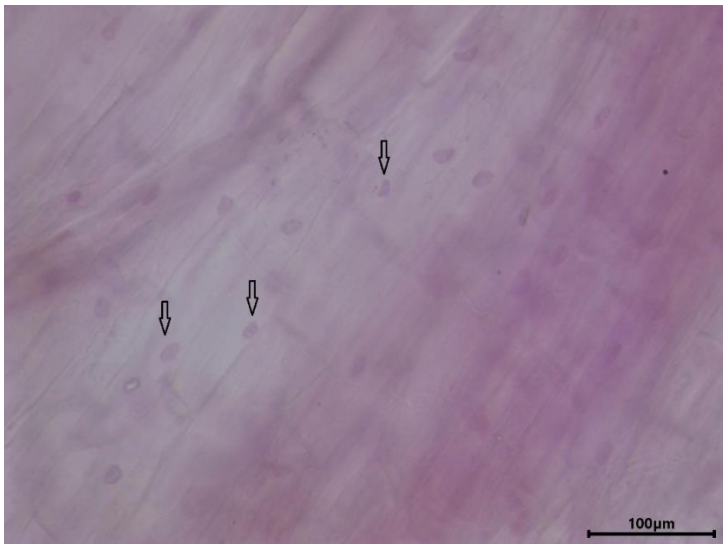


Fig. 6: Pyknotic Nuclei in the Roclas cultivar 200 mM, object glass 40x

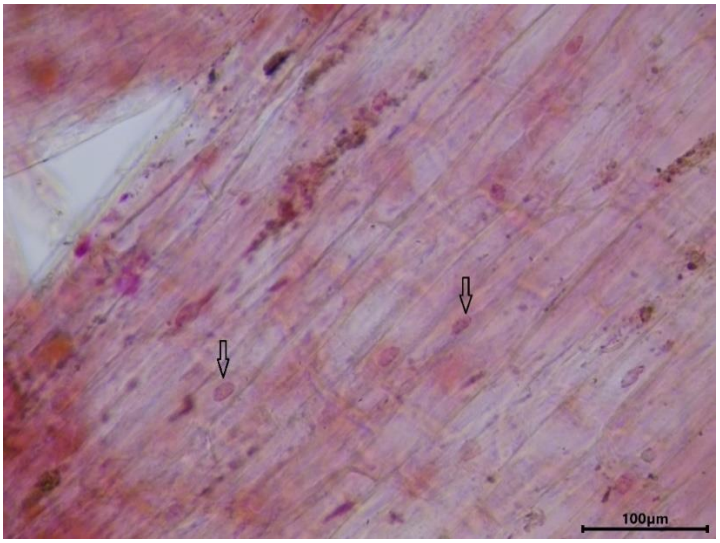


Fig. 7: Pyknotic Nuclei in the Santé cultivar 200 mM, object glass 40x

The Mitotic Index

The increase in salt concentration has led to decrease in the number of cells and their growth. By increasing salinity, the mitotic index decreased. According to the table 3, one can see that standard deviation decreases depending on the number of cells analyzed and the salt concentration used.

Table 3: Average and standard deviation

Cultivar	Santé N	Roclas N	Santé 100	Roclas 100	Santé 200	Roclas 200
N	11	12	7	19	15	20
Average	9.299629	29.87867	105.6436	5.464474	0.723104	1.332791
Standard Deviation	21.55502	19.13221	13.86078	10.83649	1.947556	3.541209

It is obvious that the Roclas cultivar, under normal conditions, has got a much higher mitotic index than Santé bred under the same conditions. Instead, the Santé batch with100 mM registered higher values of mitotic index than the salt-free addition blank batch. The smallest values were registered by the batches watered by an addition of 200mM concentration. By the Kruskal-Wallis test a value of H(chi2) = 32.85 was registered, a significant difference between the samples' medians being noticed (p=6,9 x 10-8). After applying the post-hoc Dunn test, the values of table 2 were obtained which show the batches between there are differences, expressed by the values of table 4.

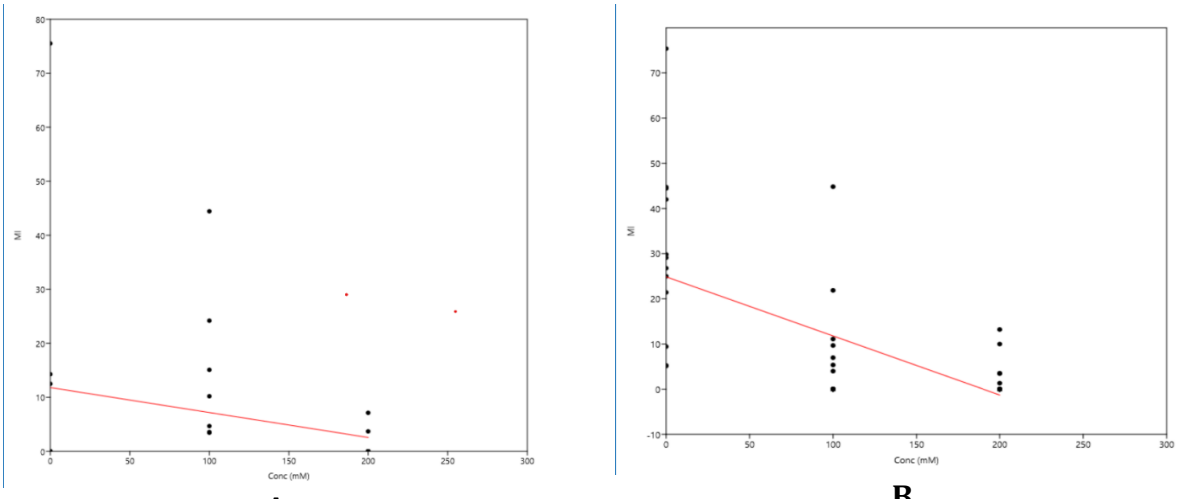
The values p of post-hoc Dunn test, at the mitotic index (I) being calculated for the six batches analyzed, the real values p are shown by the diagonal below.

Table 4: Values p of post-hoc Dunn test, at the mitotic index (I), calculated for the six batches analyzed

	Santé N	Roclas N	Santé 100	Roclas 100	Sant 200	Roclas 200
Sante N						
Roclas N	0,0001647					
Sante 100	0,01511	0,4026				
Roclas 100	0,8443	4.84E-02	0,01283			
Sante 200	0,3074	3.28E-04	0,0005576	0,165		
Roclas 200	0,3441	1.29E-04	0,0004947	0,18	0,8836	

*On the diagonal below there are real values p. The significant P values selected are presented in yellow.

It can be noticed p< 0.05 in the following pairs: Santé without salt addition and Roclas without salt addition; Santé 100 mM and Roclas without salt addition; Roclas 100 mM and Roclas 200 mM; Santé 200 mM, and Santé watered by 100 mM; Roclas 100 mM and Santé and Roclas watered by 200 mM. A test of linear regression was also carried out to see if the mitotic index is correlated with water salinity. Thus, r = -0.27, r^2=0.07 and p (non-correlated) =0.13 (figure 8).



A B

Fig. 8: Plot of linear regression between the mitotic index and salt concentration A- Santé, B- Roclas

This shows that there is a negative correlation between the radicles' length and salinity in the Santé cultivar (figure 8 A). At the Roclas cultivar (figure 8 B) there is a correlation between the radicles' length and salinity, r= -0.6357 and p (non-correlated) insignificantly = 3.13 x 10-7.

CONCLUSIONS

The six potato batches of Santé and Roclas cultivars were bred under conditions of different salinity. As regards the radicles' length, the highest values were registered by the Santé cultivar watered by salt-free addition, having a length of 30 mm, whereas the smallest were registered by the Roclas batch watered by 100 mM and Santé 200 mM, having a length of 8 mm.

The smallest averages were identified in Santé and Roclas watered by a 200 mm concentration, whereas the highest were registered by Santé without salt addition and Santé 100 mM. Consequently, these results show that higher salinity influenced negatively the radicles' growth.

In terms of divisions, the division phases were noticed in cells: prophase, metaphase, anaphase and telophase. The cells with pyknotic nuclei occurred in the Santé and Roclas cultivars bred at the 200 mM concentration.

The mitotic index has registered values between 0.0001647 and 0.8836. The smallest value is the correlation of Santé without addition and Roclas without addition, the highest one being given by the correlation between Santé 200 mM and Roclas 200 mM.

There have been noticed correlations between the mitotic index and salinity, especially between Roclas without salt addition and the other batches, and Santé with 100 mM and the other batches.

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