SALINITY EFFECT ON POTATO (*SOLANUM TUBEROSUM* L.) MICROPROPAGATION

EFECTUL SALINITĂȚII ASUPRA MICROPROPAGĂRII CARTOFULUI (*SOLANUM TUBEROSUM* L.)

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Abstract: *The effect of salinity on plantlets growth was determined under saline medium and non-saline at five cultivars of potato (Christian, Roclas, Marfona, Riviera, Tresor). Plantlets belonging to selected cultivars, were propagated through single nodal culture. To study the effect of salinity (NaCl) on the growth of single nodal explants, they were cultured on MS media with different concentrations of NaCl, including 0, 25, 50, 75 and 100 m mol l*$^{-1}$*. Growth of single nodal explants on the media with NaCl indicated that all the characters differed significantly according to salinity levels. By increasing salinity level the values for all the parameters decreased.*
Key words: *salinity tolerance, in vitro multiplication, concentration of NaCl*

Rezumat: *Efectul salinității asupra creşterii plantulelor a fost determinat în mediu salin și în mediu a cărui concentrație a NaCl a fost 0 m mol l*$^{-1}$*, utilizând cinci cultivare de cartof (Christian, Roclas, Marfona, Riviera, Tresor). Pentru a studia efectul salinității asupra creşterii minibutaşilor, aceştia au fost inoculați pe mediul nutritiv MS cu diferite concentrații ale NaCl (0, 25, 50, 75 și 100 m mol l*$^{-1}$*). Creştere minibutaşilor pe un mediu cu NaCl a indicat că toți parametrii a diferit semnificativ în funcție de concentrația salinității. Prin creşterea nivelului salinității valorile pentru toți parametrii au scăzut.*
Cuvinte cheie: *tolereanța salină, multiplicarea in vitro, concentrații de NaCl*

INTRODUCTION

Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on plant vigour and crop yield (Munns and Tester, 2008).

High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Hasegawa *et at*., 2000; Munns, 2002; Zhu, 2007). These effects reduce plant growth, development and survival. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. The osmotic effects of salinity stress can be observed immediately after salt application and it is posible to continue for the duration of exposure, resulting in inhibited cell expansion and cell division (Flowers, 2004; Munns, 2002). Plant growth reduction is commonly

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correlated either to ion toxicity or to water deficit. Heuer and Nadler (1995) observed a significant decline in leaf water and osmotic potential under intensified salt stress conditions while studying the physiological response of potato plants to soil salinity and water deficit.

Sodium excess and, more importantly, chloride excess have the potential to affect plant enzymes and cause cell swelling, resulting in reduced energy production and other physiological changes (Larcher 1980). Ionic stress results in premature senescence of older leaves and in toxicity symptoms (chlorosis, necrosis) in mature leaves due to high Na which affects plants by disrupting protein synthesis and interfering with enzyme activity (Hasegaw *et al*., 2000; Munns, 2002; Munns and Termaat, 1986).

Salinity stress is a critical environmental constraint to crop productivity especially in arid and semiarid regions. The most of the crop plants is intolerable to high salinity conditions resulting decreased yield. Generally, plants are stressed in next ways in saline soils a) low water potential of the root medium leads water deficit, b) the toxic effects of the Na^+ and Cl^- nutrient imbalance by depression in uptake and/or shoot transport (Munns and Termaat 1986, Chapin 1991, Marschner 1995). Toxic accumulation of Na^+ and Cl^- in leaves has also been correlated with reduction of total chlorophyll content in leaves both of which limit the amount of photosynthetic production (Romero-Aranda and Syvertsen 1996).

Potato (*Solanum tuberosum* L.), a vegetative plant cultivated for its starch-rich tubers, is the fourth most important agricultural crop after rice, wheat, and corn (Byun et al., 2007, Nhut et al., 2006, cited by Aycili and Alikamanoğlu, 2012). Economically, it is the most important tuberous plant, and potato plant cultivars are usually very sensitive to environmental stresses such as temperature changes, drought, and salinity due to their sparse and short root systems. There is significant loss in plant growth and product yields when potato is grown in soil that contains 20-35 mM concentrations of NaCl. When compared to other agricultural plants such as pepper and corn, the potato plant is more resistant to salinity; however, it is less resistant than tomato, rice, soy and barley (Byun *et al*., 2007, Manrique, 2000, cited by Aycili and Alikamanoğlu, 2012).

The selection of crop varieties for greater tolerance to saline environment will allow greater productivity from large saline lands.

In this paper our aim was to follow the growing and development *in vitro* plantlets from different potato varieties and the tolerance to NaCl

MATERIAL AND METHOD

Research was conducted at Laboratory of Vegetal Tissue Culture from National Institute of Research and Development for Potato and Sugar Beet Brasov, to record the effect of various concentration of NaCl on potato varieties Christian, Roclas, Marfona, Riviera, Tresor. Nodal cuttings were used as explants.

A medium MS (1962), with the amount of 20 g sucrose and agar concentrations of 9 g were used, as well as different concentrations of salt were applied. The pH was adjusted at 5.7 with HCl and NaOH. After 30 day, the plantlets were harvested and four vegetative growth parameters were measured. The experience was of type 5*5,

made by combining two experimental factors; the number of studied variants was 25 (Table 1), set in three relicates.

Table 1

Experimental variants according to the NaCl concentration

Variants	Cultivar	NaCl concentration (mmol/l)
V_1	Christian	0
V_2		25
V_3		50
V_4		75
V_5		100
V_1	Roclas	0
V_2		25
V_3		50
V_4		75
V_5		100
V_1	Marfona	0
V_2		25
V_3		50
V_4		75
V_5		100
V_1	Riviera	0
V_2		25
V_3		50
V_4		75
V_5		100
V_1	Tresor	0
V_2		25
V_3		50
V_4		75
V_5		100

- Experimental factor A-cultivar has five graduations:
- a_1- Christian;
- a_2- Roclas;
- a_3- Marfona;
- a_4- Riviera;
- a_5- Tresor.
- Experimental factor B – concentration of NaCl, has five graduations:
- b_1 - MS medium and 0 Mm NaCl (control);
- b_2 - MS medium and 1 Mm NaCl;
- b_3 - MS medium and 2 Mm NaCl;
- b_4 - MS medium and 3 Mm NaCl;
- b_5 - MS medium and 4 Mm NaCl.

The proposed objective of this research is to determine the influence of salinity in the culture medium *in vitro* over different potato cultivars.

The experience was mounted in the laboratory using conditions required by „in vitro" technology; experimental conditions were those specific to growth chamber of plantlets, provided in the working protocol, sterilization of culture vessels was performed in a drying chamber at 180^0C and culture media was sterilized by autoclaving at 121^0C for 20 minutes at pressure of 1.25 atmospheres.

Cultures were transferred to growth chamber under conditions of darkness; after crossing this period light regime is 4000 lux, with a period of 16 hours light and eight hours dark at a temperature of 20^0C.

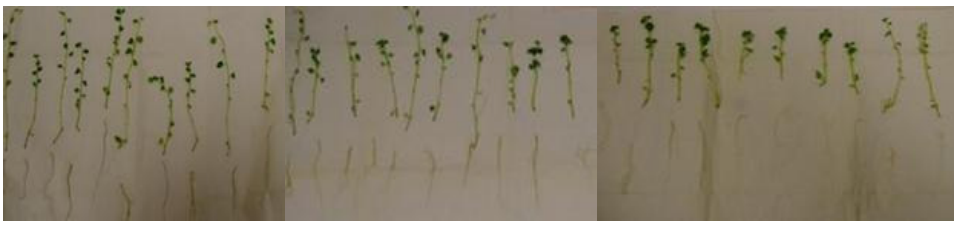
This experience, as shown in Figure 1, included 25 variants. The experimental conditions were the specific in growth room of plantlets.

	a_1					a_2					a_3					a_4					a_5				
r1	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5
	a_1					a_2					a_3					a_4					a_5				
r2	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5
	a_1					a_2					a_3					a_4					a_5				
r3	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5

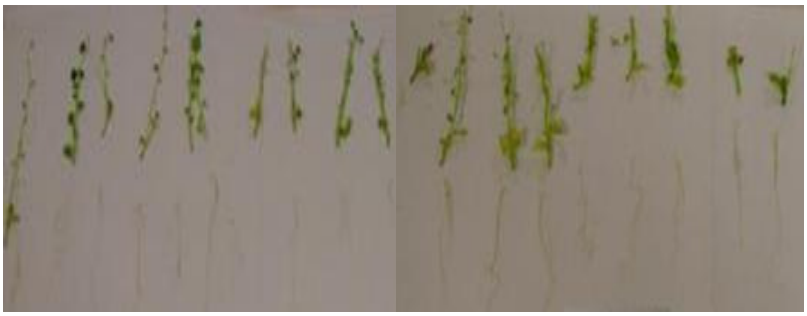
Fig. 1 - Location sketch of the experimental variants made to the five varieties and five NaCl concentration

Legend:
- a – cultivar;
- b – NaCl concentration;
- r – replicates.

RESULTS AND DISCUSSION:



0 m mol/l NaCl 25 m mol/l NaCl 50 m mol/l NaCl



75 m mol/l NaCl 100 m mol/l NaCl

Fig. 2 - Plantlets from control medium and from different NaCl concentrations, with reduced height and reduced number of leaves

Saline stress induces several alterations on growth, cell division and metabolic activities (Wincov, 1993). The results showed that the presence of NaCl in the media affects the growth of single nodal explants (fig. 2). The increase of NaCl concentration in the media is proportional to the decrease of the measured parameters.

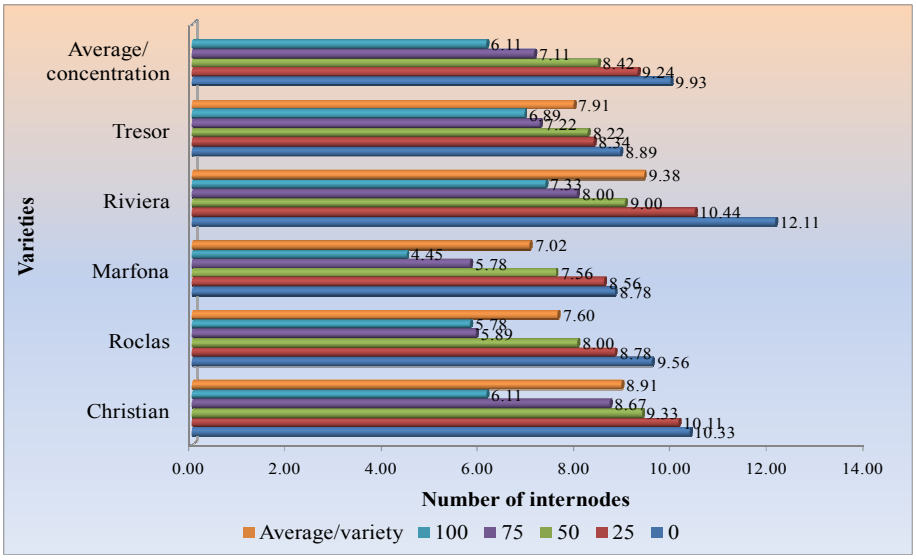


Fig.3 - Variation of number of internodes/plantlets for tested cultivars

If we compare the five cultivars in terms of number of internodes can say that the cultivar Riviera responded the best showing the highest average value (9.38 internodes) and the lowest average value is at Marfona variety (7.02 internodes) (Fig. 3).

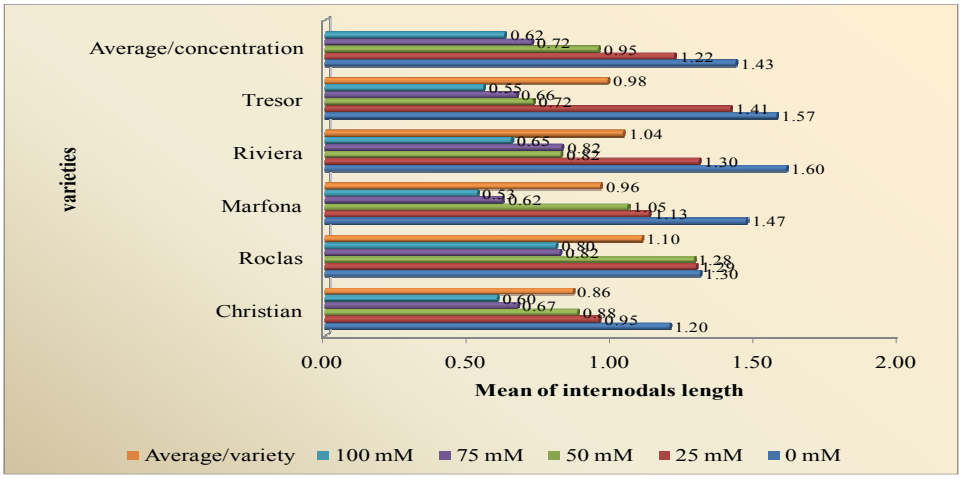


Fig. 4 - Variation of mean of internodes length for tested cultivars

The effect of salinity on mean of internodals length (Fig. 4) was least accentuated for Roclas and Riviera (hey had an average value / concentration 1.10 and 1.04 cm.

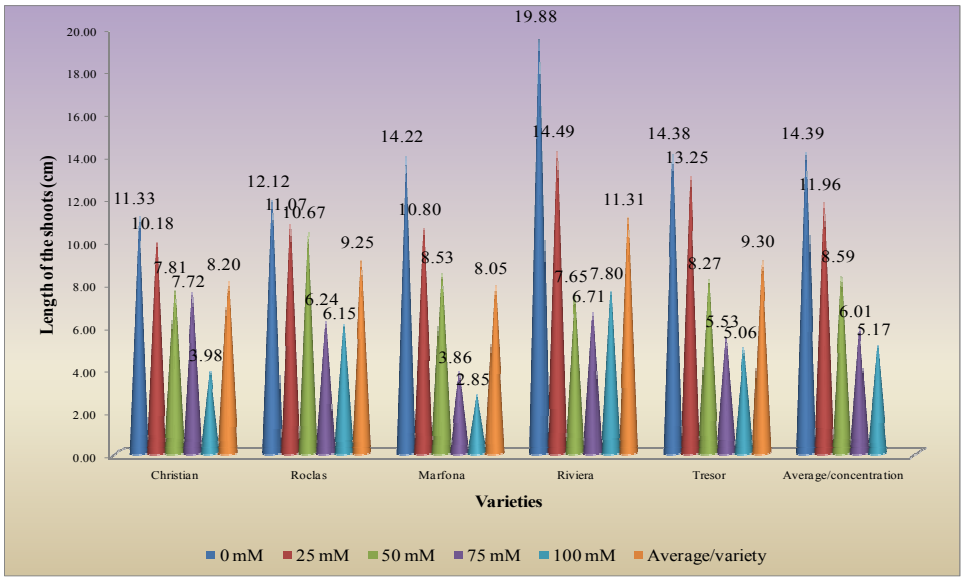


Fig. 5 - Variation of shoot length for tested cultivars

Reffering the shoot length (Figure 5), the cultivar with the least damaged because of salinity level is Riviera that recorded at concentration at 100 mmol/l the higher value (7.80 cm); shoot length decreased with increasing of salt concentration in culture medium.

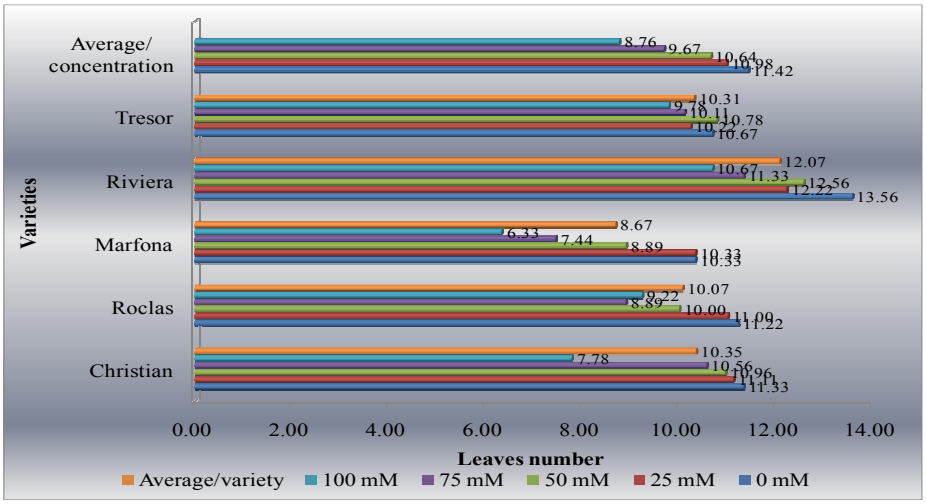


Fig. 6 - Variation of leaves number for tested cultivars

From the point of view the number of leaves, for concentration of 100 mmol/l, Riviera cultivar present the highest value (10.67 leaves/plant) (Fig. 6).

Statistical interpretation:

In table 2 is present the statistical analysis of varieties. The most tolerant variety to salinity stress is Riviera cultivar. On the opposite side is Marfona cultivar which shows for almost all parameters studied (except - mean of internodes length) the lowest values.

Table 2

Salinity effect on the studied potato cultivars

Varieties	Internodes number	Mean of internodes length (cm)	Shoot length (cm)	Leaves number
Christian	8.91a	0.86b	8.20b	10.35b
Roclas	7.96bc	1.1a	9.25ab	10.07b
Marfona	7.02c	0.96ab	8.05b	8.67c
Riviera	9.38a	1.04ab	11.31a	12.07a
Tresor	7.91b	0.98ab	9.30ab	10.31b

Means within same column followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$).

In table 3 is shown the statistical analysis of NaCl concentration. The variant 2 of concentrations (25 m mol/l NaCl) did not lead in significantly different values for the internodes number (compared to the variant 1 - concentration 0 m mol/l NaCl), showing that these are tolerant to salt at this concentration. Additions of 100 m mol/l of NaCl to the medium significantly decreased the internodes number. Shoot length was also negatively affected (inverse-relation) by salinity for concentrations 50, 75, 100 m mol/l.

In this study, the values of leaves number are not significantly different for concentrations 25, 50, 50, 75 m mol/l.

Table 3

Salinity effect on different characters measured

Variants	Salinity concentration (m mol/l)	Internodes number	Mean of internodes length (cm)	Shoot length (cm)	Leaves number
V_1	0	9.93a	1.43a	14.39a	11.42a
V_2	25	9.24ab	1.22a	11.96a	10.96a
V_3	50	8.42bc	0.95b	8.59b	10.66a
V_4	75	7.11cd	0.72c	6.01bc	9.66ab
V_5	100	6.11d	0.62c	5.16c	8.76b

Means within same column followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$).

CONCLUSIONS

The effect of all treatments was very drastic on 100 m mol/l NaCl. The addition of salinity to the culture media decreased the osmotic potential of the

media inducing salinity stress and affected the plants growth of potato cultivars. The results indicate that the "Marfona" cultivar is not salt tolerant, while “Riviera” cultivar present a tolerance to NaCl. On concentration 25 m mol/l, all studied parameters was not affected (the values were not significantly different, compared to concentration 0 mol/l).

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ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

**UNIVERSITATEA DE ŞTIINȚE AGRICOLE
ŞI MEDICINĂ VETERINARĂ
"ION IONESCU DE LA BRAD" IAŞI**

LUCRĂRI ŞTIINȚIFICE

Vol. 57

NR. 1

SERIA HORTICULTURĂ

EDITURA "ION IONESCU DE LA BRAD"

IAŞI 2014

ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

"Ion Ionescu de la Brad" Publishing House Iaşi
ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

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