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Effect of salinity on micropropagation of two potato varieties Desiree and Spunta (*Solanum tuberosum* L.)

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Abstract

Our study has allowed us, to achieve the main objective we set at the beginning; which is the possibility to regenerate *in vitro* an entire plant of two varieties of potato: Desiree and Spunta under saline conditions. This a factorial experiment was planted in MS growth medium with three different concentrations of NaCl (25, 100, 150 Mmol/L) and a control (C_0) by doing ten repetitions. This work was conducted on 80 experimental units within controlled conditions. This study allowed us to know the effect of the salinity on the micro propagation *in vitro* of the two varieties of potato Spunta and Desiree by following the growth and the development of three morphological characters (leaves number, stem length, roots number.) for four weeks. The results show that the response of the plants to salt stress varies with the variety and the salt concentrations. Similarly, it appears from this study that the potato shows a well-differentiated behavior under the high concentrations of NaCl, which proves the sensitivity of the potato to salinity. It also appears that the Desiree variety is more tolerant to high saline concentrations.

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Introduction

The potato (*Solanum tuberosum* L.) is a tuberous herbaceous plant native to Latin America. In agricultural practice, the production cycle of the potato is mainly vegetative; the tubers produced constitute both an asexual reproductive organ and the alimentary part of the plant.

The potato is a strategic agriculture because of its position in the world, where it ranks fourth after wheat, rice and maize. The world production was evaluated in 2013 at more than 368 million tones on 19.4 million hectares (Rolot and Vanderhofstadt, 2014).

In Algeria, land erosion, salinization and desertification threaten 3.2 million hectares of land, formed by forest cover in the northern region. This is also the case for one million hectares in the steppe areas, 400,000 hectares in the western region, and 100.000 hectares in the southern part of the country where potato cultivation is practised in the pivot of Oued Souf (Ministry of Agriculture, 2005). This salinization of the arid or semi-arid zones is mainly due to the strong evaporation of the waters which favors the concentration of the total salts in the water, and their rise to the surface of the grounds.

In this context, plant biotechnology techniques and more specifically *in vitro* tissue culture can play important roles in the selection of new cultivars in addition to conventional field breeding. For this, different biotechnological pathways are used to increase the diversity and the ability of plants to tolerate saline stress, knowing that in a natural population it is the whole plant that faces the selective pressures where the screening is carried out at a global level. On the other hand, in vitro, it is the cell, freed from the stresses encountered in the whole plant, which is the target of the selective pressures in order to arrive at a range of variants thus generated, which differ from those resulting from the classical selection (Sibi, 1996).

The objective of our work is therefore to look for the effect of salinity *in vitro* on 2 different varieties of potato: Spunta and Desiree, which are the two most cultivated varieties at present.

Materials and methods

Materials

Plant material

The explants used in this study consist of two varieties namely: Desirre and Spunta of the species *Solanum tuberosum* L.

Proposed treatments

The treatments applied on this experiment are different concentrations of NaCl:

CO: 00 M mol NaCl.

C1: 25 M mol NaCl.

C2: 100 M mol NaCl.

C3: 150 M mol NaCl.

Each treatment was repeated ten times in this experiment.

Culture medium

The culture medium used in our work is the MS Basic Medium (Murashige and Skoog, 1962).

Composition of the culture medium

The culture medium is prepared in 1 liter beaker with continuous stirring. It consists in putting 500 ml of distilled water. Then we add in order the following elements:

50 ml of Macro-elements

10 ml of micro-elements

10 ml of iron

10 ml of vitamins

The pH of the medium is adjusted to 5.7 ± 0.1 with NaOH (base) or HCl (acid). The medium is then supplemented with distilled water to 1 liter with continuous stirring. Then, 30 g of saccharose and 7 g of agar are added to solidify the culture medium. The mixture is then boiled until all the agar particles have dissolved. Finally, the medium thus prepared, is transferred into tubes of 25 x 75 mm using a vending machine at the rate of 10 ml per tube while sealing the tubes with stoppers.

Methods

Micro-propagation

Under the horizontal laminar flow hood, each vitro plant is removed from the bocale by using a sterile forceps and placed on the sterile blotting paper and then fragmented into as many sections as nodes whose sections are 0.5 cm to 1 cm. (removing however the basal bud or the root part) using a scalpel passed to the flame in order to obtain homogeneous material or microbouture.

Measurements taken

During our experience, the parameters selected are:
Number of leaves
Number of roots
Length of the stems
Root length
Branching of the stem
These measurements are made weekly for 28 days.

Statistical analysis of the results

In order to define and highlight the effect of salinity on the potato and to identify the best variety and the most determining parameter against salinity, we decided to perform a statistical analysis using a specified program (EXEL Stat, 2014).

Results and discussion

The results recorded about the growth and development of the aerial part, negatively correlated with the NaCl concentration, but good growth is observed with the concentration of 25 Mmol/l relative to the control. Taleisnik and Grunberg (1994)have also shown that low concentrations of NaCl, less than 3 $g.l^{-1}$, can stimulate the growth of aerial parts of some tomato cultivars (Marmande and Edkawi variety).

This phenomenon is interpreted as being the result of improved water relations, attributed to an accumulation of mineral ions (Cuartero *et al.*, 1992, Munns *et al.*, 1986).

Salinity affects the growth of young seedling stems, according to the values of the figure, it is noted that when the growth medium is highly saline (100, 150 Mmol), vitroplants undergo an aggressive decrease of stem growth compared to the control (Fig. 1.)

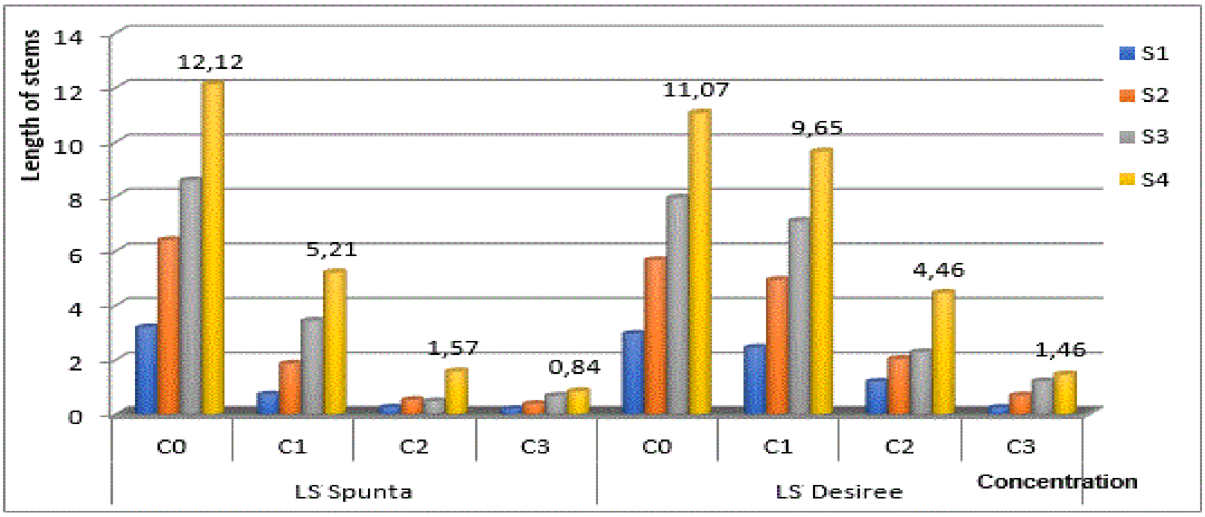

Fig. 1. Evolution of the average number of leaves formed during the growing season in both Spunta and Desiree varieties.

According to Benmahioul *et al*, (2009), the higher saline concentrations cause a cellular ionic imbalance and toxicity in the plants; this may affect some vital metabolic processes such as growth reduction and necrosis of sensitive callus. Mwai *et al.* (2004) show that the reduction of plant height due to the effect of salt, which delays the processes of cell division and extension that are the basis of growth. However, the application of ANOVA indicates that a slightly saline medium (25 M mol) has a significant influence on height growth, according to the work of (Bouraoui *et al*, 1998).

The analysis of data obtained during regeneration of vitro plants shows that the different concentrations of NaCl have a significant influence on the number of the formed leaves. The best values are obtained in the MS medium with an NaCl concentration of 25 Mmol. However, the high concentrations of NaCl cause a remarkable decrease in the number of leaves, particularly the concentration of 150 Mmol (Fig. 2.).

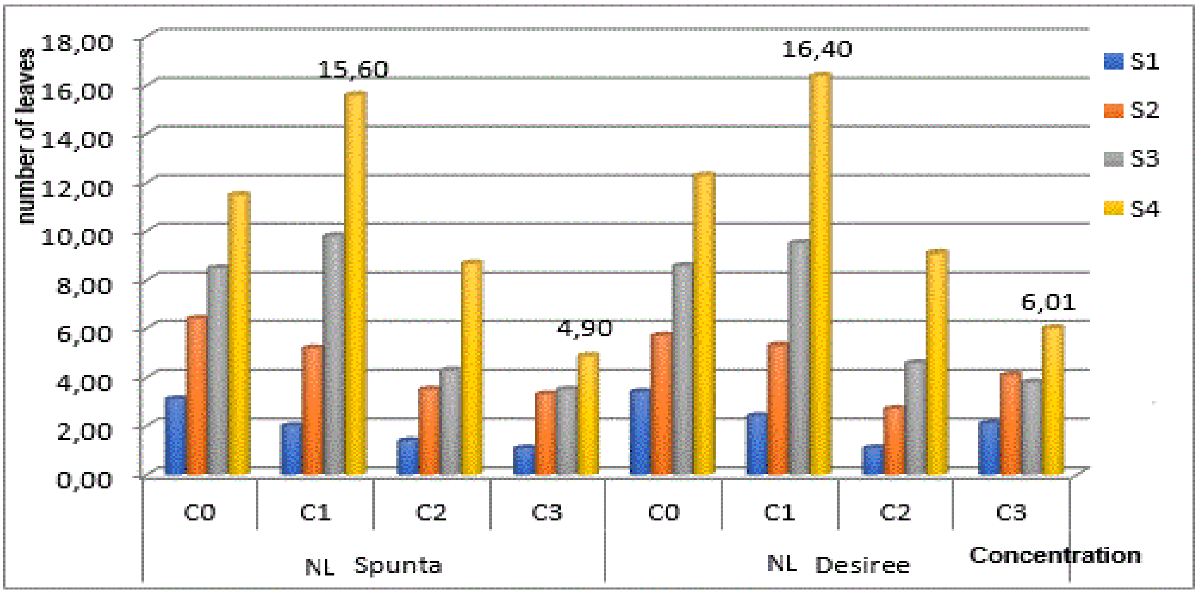

Fig. 2. Evolution of the average stem length during the growing season in both Spunta and Desiree varieties.

Our results are in agreement with the results obtained by Yeo *et al.*, Neumann, (1994) and Ben yahmed (2013), during these studies, the authors showed that the primary effect of salt stress in many plant species is the osmotic phase that inhibits the growth of young leaves, rate of onset and appearance of the leaf, and overall shoot development.

As researchers Munns and Tester, (2008) show that the photosynthetic activity will be unable to meet the carbohydrate needs of the young leaves, which are reduced in growth. It is therefore generally considered that the decrease in vegetative growth, expressed either as a reduction in the number of leaves or as leaf area, is generally the first response to genotypes exposed to salt stress (Benmahioul *et al.*, 2009).

The results of the study of the root system is illustrated which shows that, in the condition of moderate salt stress in 25 Mmol NaCl concentration, all seedlings are significantly affected. This particular behavior would be a form of tolerance to moderate salt stress (Fig. 3.).

This same behavior was reported by Mallek *et al.* (1998) during the study of some cereal varieties.

For both concentrations of 100 and 150 Mmol of NaCl, all the plants respond negatively, in the same way until complete absence of rhizogenesis.

A study conducted by Bouaouina *et al.* (2000) has shown that the impact of salinity is perceived primarily at the root level. For these authors, the root part would be more affected by salinity than the aerial part. To adapt to salt stress, the plant would first of all reduce the development of its root system in order to preserve the aerial part.

Suhayda *et al.* (1992) note that in barley, a decrease in the elongation of the root system was observed at high concentrations of 100 to 200 Mmol NaCl. Guerrier, (1996) show that the first response of glycophytes exposed to salinity is a slowdown in their development with root growth often less affected than leaf growth.

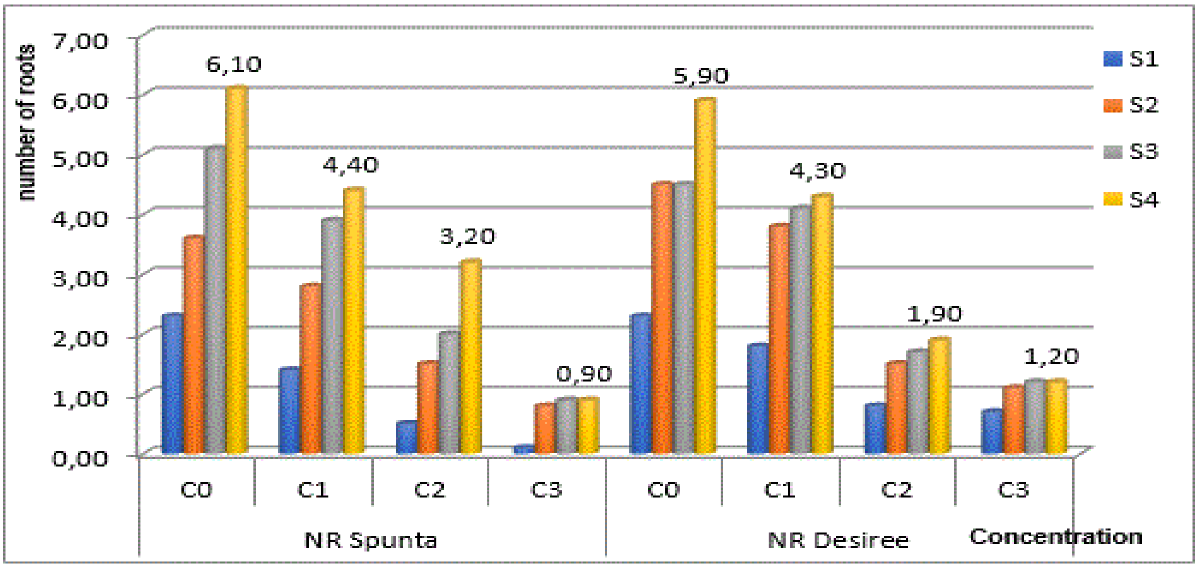

Fig. 3. Evolution of the average number of roots formed during the cultivation period in the two varieties Spunta and Desiree.

Netondo, (1999) in Mwai *et al.* (2004) showed that the reduction in root growth could be attributed to the reduction of the rate of cell division and prolongation, and thus the decrease in the diameter of the root.

Conclusion

This work aims to study in vitro the influence of NaCl and the effect of the genotype (Spunta and Desiree) on the micropropagation of the potato Solanum tuberosum. To achieve our goal, we have studied the following morphological parameters: number of leaves, stem length, number of roots . These parameters were evaluated in order to characterize the level of tolerance of these two varieties with respect to salt stress. In the present work, we have used different saline concentrations (0, 25, 100 and 150 Mmol / L of NaCl) with stress duration of 7 to 28 days.

The results obtained show that the control medium C0 has a high regeneration rate, after 28 days, compared to the other concentration studied (C25, C100 and C150). Although the presence of NaCl in the growth medium at low concentrations does not have a significant influence on the growth and development of vitro plants, the results obtained with the C25 medium are comparable with those of the control medium.

However, we found a weak development of the two varieties studied in the C100 and C150 media.

It is also apparent from our study that the Desiree variety seems more tolerant towards saline environments. This behavior can be explained by the difference of their genotypes.

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