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Assessment of the Cytological and Chemical Changes of Some Varieties of Potato Tissues *(Solnum tubersum* L.*)* under Salt Stress

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A B S T R A C T

In vitro culture can provide a controlled and uniform system for studying the morphological and chemical effects of salt stress at the tissue development level. Explants cultured under a biotic stresses such as salinity has been examined through morphological and chemical analyses. This has led to much information through studies of plants subjected to salt treatments. The aim of the work reported in this study, was to evaluate two varieties of Potato (Spunta and Agria) for their competence for Sodium Chloride (NaCl) Within the (0, 10, 20, 40, 60, 80 mmol) tolerance and tissues developments. Data reported in this study are summarized as follows:

Increase salinity levels in MS medium to a decrease the number of leaves, number of branches and plant length of each explant. Plantlets established from two varieties potato and developed on MS medium supplemented with NaCl proved to be tolerant to and 80 mmol NaCl, in addition to the control treatment.

1 Introduction

Plant tissue culture

Plant tissue culture technique to produce plant by using plant tissue, considered as one of the most important methods being used in plant propagation, while the produced plant often identical to the mother plant which being taken to establish the primary tissue cultures (AL-Baher *et al*., 1999). Plant tissue culture was defined as a science containing several methods used to grow plant tissue or cells on artificial media under controlled growth conditions (Abughnia *et al.*, 2013).

Using plant tissue culture rather than conventional agriculture the produced plants number will be largely increased with homogeneity in the physiological composition and usually resulted free- disease plants through plant micro propagation seedlings which will be available throughout the year specially those plants in their dormancy period **(Arias, 1992)**. Recently significant development has been found in the field of plant propagation using plant tissue culture method which called plant Micropropagation while this technique has become one of the most important applications on both scientific and economic levels (**Albaher *et al*., 1999).**

Plant potato (*Solnum tubersum* L.)

Taxonomic classification.

Potato in the rank fourth as strategic and economic crop after rice, wheat and corn **(Bowen, 2003).** Furthermore potato is daily food for more than 75-90% world population **(Santamaria and Elia, 1997).** The target plant in this study is potato plant, which is known in some Arab countries as the potato and the scientific name is (*Solnum tubersum* L.) follows the Solanaceae family which include about 90 species and 2000 variety, while the formations of tubers are only from specie which potato belong to S. *tubersum* and seven other cultivated species as well as 154 wild species on the other hand potato considered as one of the most

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important and most used vegetable crop, moreover potato crops tops the list of tuberous **(Hassan, 1999).** Solanaceae family includes 24 species resistant to salinity **(David and Nilsen, 2000).** while potatoes are generally moderately sensitive to temperate salts, **(Maas and Hoffman,1976; Katerji*etal.*,2000).**

Status of potato plant in Libya

At the local level potato crop is an important vegetable crop in terms of agriculture and consumption and there are two verity which are Spunta and Arinda the most important cultivars cultivated in Libya.

The imported potato cultivars are planted in Libya in two period during the year, the first one cultivated in autumn time which starts in September and second one cultivated in spring time which growing in January and February on the other hand potato production depends on seeds breeding the tuber must be imported tubers from abroad every year. Potato seeds are imported during July and August to be ready for agriculture in autumn while farmers reserve part of the production of the autumn season to grow it in spring season, planted in spring season usually decreased in both quality and quantity due to that potato plant is propagated by vegetative way which let the tuber infected by bacterial and viral disease **(Abughnia *et al*., 2013).** As well-known potato *S. tuberosum* propagated by traditional methods but on the other hand many attempts have been made to develop methods of propagation specially by plant tissue culture to provide disease-free seeds in a relatively short time compared to traditional methods **(Miller and 1984; Wang and Hu, 1985; Dodds *et al*., 1992).** Moreover, the easiest and fastest method to propagate potato which provide free-disease plants and tubers specially virus disease is plant tissue culture method **(Khosrarifar*et al*., 2008).**

Soil salinity

Soil salinity considered as one of the historical problems facing agricultural production for many years ago and even now days because it has an effect on plant growth through its effect on water and essential nutrients uptake by plants **(Zubaidi, 1989).** Furthermore salinity is one of the most important major stress affecting plant species and their productivity, particularly in arid and semi-arid areas, while salinity significantly reduces the production of many plant species also due to salinity problems world loses about 10 million hectares of arable land annually and lands with salinity problems has reached 594 million hectares **(Munns, 2010).** There fore researchers studied effect of salinity on plant growth and development, thus the study the effect of salinity on the plant depends on putting plants in different levels of salt concentrations by controlling salt quantity knowing that effect of salinity on plant depends on stress severity time it occurs, length exposure period and stage of plant growth (Sinhabab and Kumar, 2003).

Plants require the presence of some salts in the root zone for their growth and development but the optimal concentration of these salts is usually low for many plant species 10^3 mmol or less, The higher concentrations (100-150 mmol) even for the salt necessary, causes a state of salt stress which can reduce the growth, development and productivity of plants moreover effective of salinity on plants depending on the type of salts, their concentration, plant species, variety and growth stage of the plant**(Chapman and Nieman, 2000).**

Salt stress happens as a result of high concentration of sodium and potassium hydroxide, which negatively effects the rate of the water absorption by plant roots in addition salinity affects most aspects of plant growth and development also involves morphological changes **(Flowers, 2001; Sheldon *et al*., 2004).**

The main aim of this study is to determine the sensitively of the embryos to sodium chloride toxicity for two potato variety Spunta and Agria. The study was carried out at the plant tissue culture laboratory which belong to Biotechnology Research Center located in Tajoura region to determine the best level of tolerance of the potato tuber buds for sodium chloride salt.

2 Materials and Methods

Agria variety

Agria variety measured as Semi-late maturing, Its tubers are very large, oval longitudinal, outer color yellow and interior dark yellow, soft, superficial eyes, high in dry matter content, suitable for the manufacture of chips, thick stem standing, color crimson light, large leaves, big white flower, resistant to virus A, immune against virus X and resistant to gold nematodes,. **(Khosrarifar*et al*,2008).**

Spunta variety

Spunta is Dutch variety characterized by medium early maturing, very low in dry matter ratio, drought

resistance, resistance to virus Y, immune to virus A, many stems spread over side with crimson color at base and leaf hubs. The leaves are relatively small and drooping, flowers are white and small while the tubers are large, long, slightly curved, somewhat pointed of its top, soft, its outer color is pale yellow, its interiorcolor is light yellow and the buds are very superficial. Agria is among the best five varieties in terms of high productivity. **(Khosrarifar *et al*., 2008).**

Sample collection

Tubers of Spunta and Agria varieties have been taken after were imported from Netherlands during the season 2015 by Libyan agricultural marketing company.

First stage

1.Buds induction

After the tubers were cleaned with soap and water then dried on filter paper at the room temperature, placed in a dark place to induce them to produce buds (Figure.1)

Figure. (1) Potato tubers placed in a dark place to induce them to produce buds

After growing buds and reaching 1-2 cm during 4-6 weeks (Figure .2) the selected shoots were taken and surface sterilization was performed.

Figure. (2): Tuber buds of Spunta and Agria varieties.

Surface sterilization of potato tubers buds.

The vegetative parts (potato tubers buds) were collected from the plant and cut into parts of suitable lengths then placed in under running water for 30 minutes to remove surface contaminants from soil and insect residues ,after wards samples were transferred to the laminar airflow cabinet for sterilization by using ethanol with 70% concentration for two minutes then samples were sterilized by sodium hypochlorite (Clorox) with concentrations 2%, 2.5% and 3% for 20 minutes with keeping stirring of the samples time to time for sterilization from bacteria and fungus. finally samples were washed by using sterilized distill water for three times for five minutes each time to remove the toxic effect of sodium hypochlorite.

Media preparation

prepared MS media for the purpose of obtaining culture media (Murashige and Skoog, 1962), which manly containing, 3% sucrose and 0.7% ager before adjusting the pH on level of 5.7 to 5.8 the prepared cutter media MS was placed in sterilized 250ml with value of 25ml in each jar and prepared media sterilized in autoclave at 121C and air pressure 1.02bar for 15min.

Buds development *in vitro* culture

The buds of the potato samples were sterilized while the culture operation started by planted the selected buds in containers supplemented with MS media however one bud placed in each container for the purpose of obtaining tissue cultures free of pathogens (Fig.3) afterwards samples were incubated in growth chamber at 16 hour light /day and 8 hour dark/day while the light intensity was400µmol^{-1}m^{-2}sec^{-1} photon flux density and, temperature 25±2C$^{\circ}$ and 40% humidity, while all the conditions were under control and the samples incubated for four weeks.

MS media under the seam conditions for another four weeks. **Figure. (3)**

Second stage Subculture process

After four weeks of growth and a whole plants being obtained through tissue culture technique Fig. (4). Subculture stage started by transfer the single nodes on MS media under the scam condition for another four weeks (Fig. 5)

Figure. (4) plants obtained through tissue culture

Figure. (5) plant single nodes.

While sodium chloride was added to the culture media with different concentrations determined according to several studies (**Hatami *et al*., 2010; Upreti and Murti, 2010**). Whereas, the used treatments in this study were prepared by using MS Media supplemented with sodium chloride as follows (0, 10, 20, 40, 60, 80 mmol) for purpose of study the effect of salinity on used potato verities in the study, while each treatment contains ten replicates and only two plant was planted in each jar then all plant samples incubated in the growth chamber under the same pressure conditions.

Experimental design.

The experiment was designed by using completely randomized design (CRD) system, the LSD were calculated at significant differences at 5%. The main factors were measured and recorded of this experiment, explains after passed two of time under NaCl stress plant length, number of branches, number of roots, number of leaves, Na^+ accumulation and osmolality.

3 Results and discussion

Surface sterilization process

one of objectives in this experiment is to determine the best concentration of sodium hypochlorite (Clorox) used to sterilize the plant samples a plants during the experiment. The results showed that the best concentration of sodium hypochlorite was 3% which led to an increase in the percentage of contamination-free explants to 92% and 89% for Spunta and Agria respectively, however the concentration of 2.5% showed a proportion of explants free of contamination 82% and 70% for spunta and Agria respectively while the use of Clorox 2% showed the lowest proportion of contamination- free plants which was 65% and 58% for Spunta and Agria (Fig. 6). These results are consistent with the result of (Abughnia *et al*., 2013) when the researchers found that the best concentration of Clorox is 3% for 20 minutes.

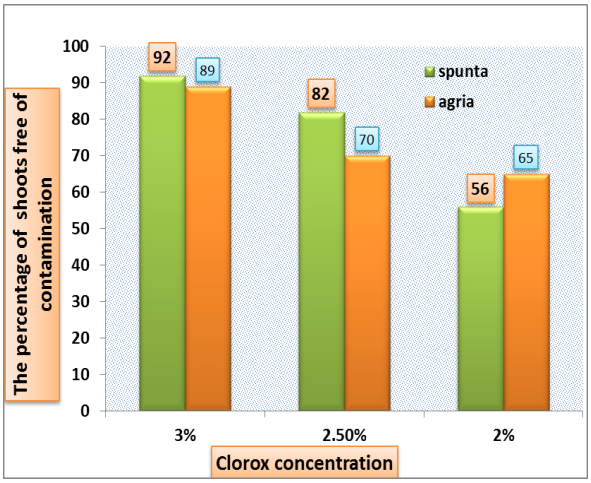

Figure. (6) Percentage of the uncontaminated a shoots under different concentrations of Clorox for spunta and Agria varieties.

Effect of adding sodium chloride on plant improveme Spunta variety

After obtaining uncontaminated explants through using of plant tissue culture technique from Spunta variety. The obtained planted from tissue culture were replanted in MS media supplemented with different concentration of sodium chloride which are (0, 10, 20, 40, 60 and 80 mmol). The results (Fig. 7). showed that the number of leaves for obtained planets there was no significant difference between all treatments after 30 days of planting, while the treatment of control characterized among the other treatments after 75 days of planting exceeded the average of 24 leaves followed by the treatment of 10 mmol which exceeded the rest of the remaining treatments and recorded an average of 22.6 leaves, whatever from these results the number of leaves non significantly affected by the toxicity of sodium chloride salt until arriving to 75 days since day of culture or after which mean that the number of leaves poorly estimate these results were agree with **(Wang *et al* 2007; Hasegawa *et al*, 2000; Munns, 2010).** the effect of sodium chloride due to that there was no clear different between the treatments.

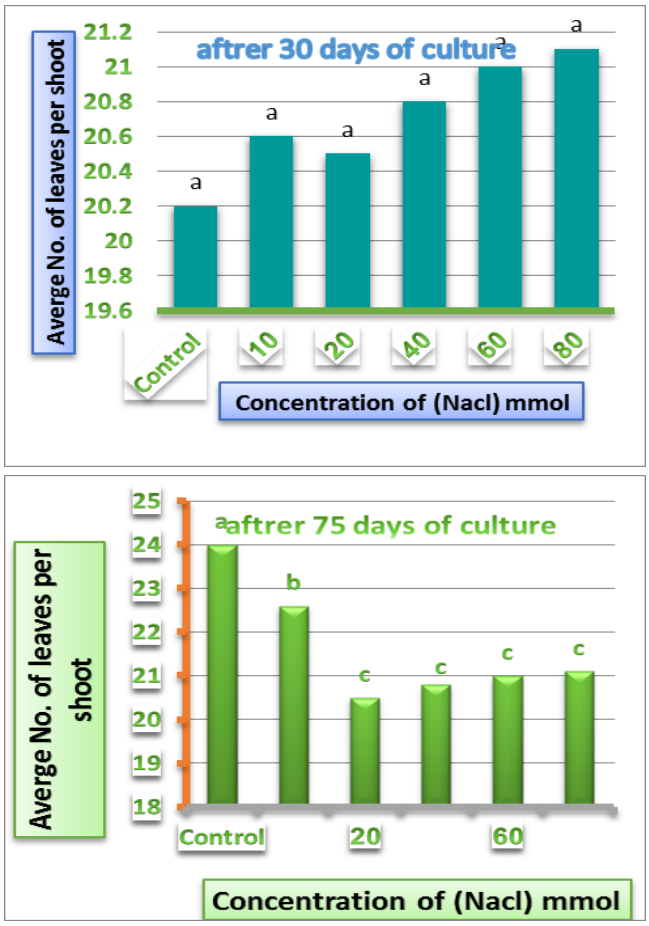

Figure. (7): the effect of adding (NaCl mM) to MS medium on leaves per shoot of potato (spunta) produced by single nodes after 30 and 75 days of culture.

Either by number of nodes shown in (Fig. 8), while culture of plant single nodes in MS media supplemented with different concentrations of sodium chloride salt was directly affected and the results showed that the control treatment gave the highest significant number of nodes compare with the other treatments and it was superiority on the other treatments after 30 days of planting, while the average of the number of nodes in control treatment was 4.5 branches while no more than 3.8 branches in the rest of the other treatments, in a related when reading the results after 75 days continued to exceed the treatment of control on the rest of the treatments recording an average of 5.2 branches followed by the treatment of 10 mmol of NaCl which recorded an average of 4.2 nodes while the other treatments did not pass the average of 3.8 nodes. These results were agree with **(Wang *et al* 2007; Hasegawa *et al* , 2000; Munns, 2010).**

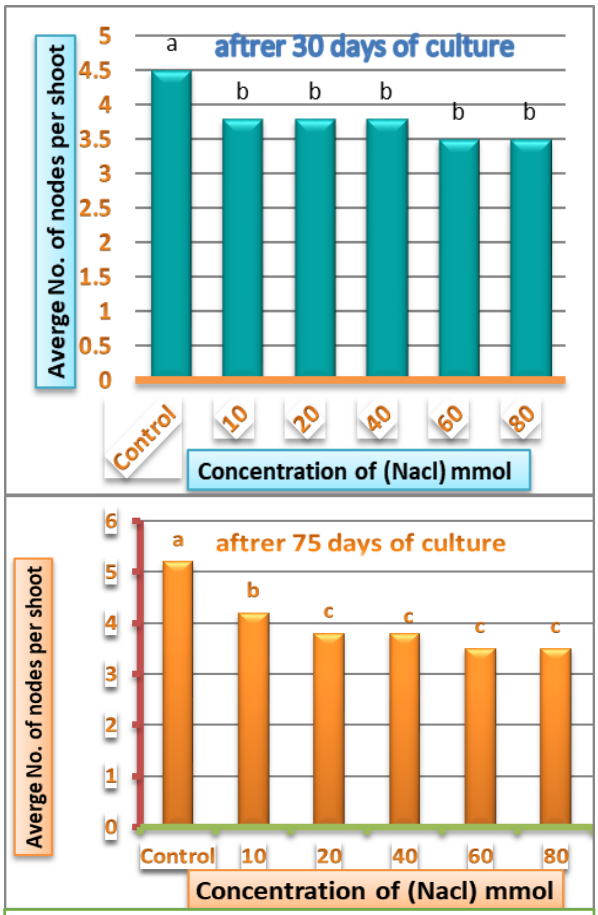

Figure. (8) Effect of adding (NaCl) to MS medium on nodes per shoot potato (spunta) produced by single nodes after 30 and 75 days of culture. Columns with the same letter are not significantly different, by Duncan multiple test at (0.05).

Results (Fig. 9) that there was non significant different of 10mmol Nacl which main the planted did not affect by salt stress get also the was no different among treatments of (10,20mmol) Nacl and treatments of (20mmol) Nacl and treatments of (40,60,80mmol) Nacl finally we observe that the planted plants clearly affected by the in increasing of salt concentration. **(Wang *et al.*, 2007; Hasegawa *et al.*, 2000; Munns, 2010).**

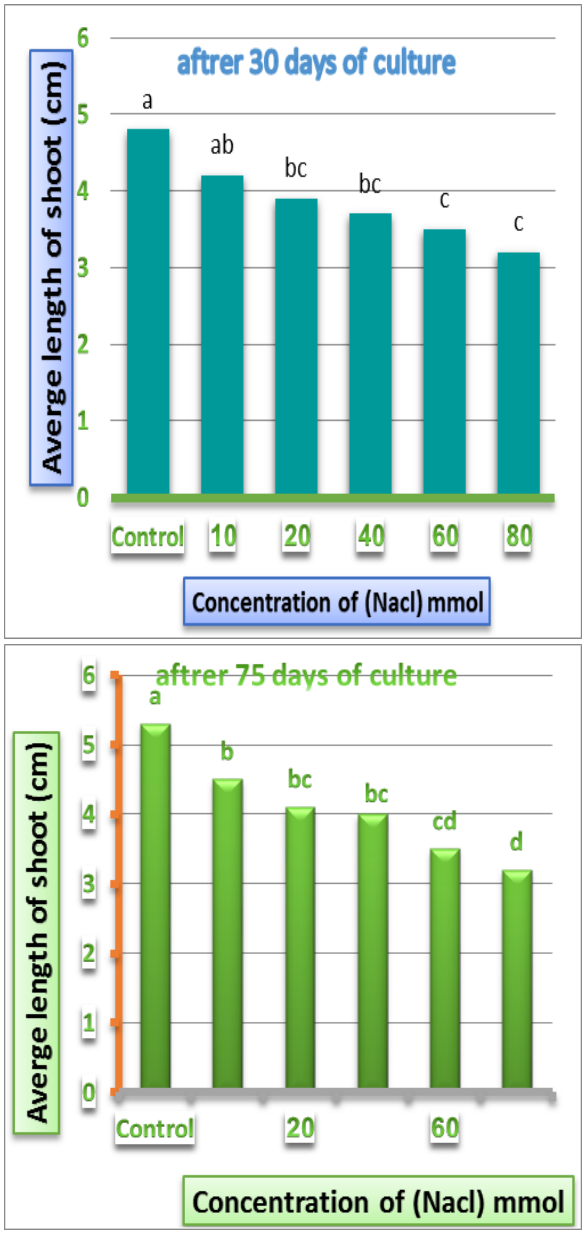

Figure. (9) Effect of adding (NaCl) to MS medium on shoot length of potato (spunta) produced by single nodes after 30 and 75 days of culture. Columns with the same letter are not significantly different, by Duncan multiple test at (0.05).

while for the number of roots the results showed that there were no significant differences between all the treatments (Fig. 10) even after 30 days of planting there was no significant effect of NaCl on the number of roots but after observation the results at or after 75 days there are significant effect in control and 10mmol Nacl from other treatments and the data changed and significant differences were found among the treatments, while the treatments of (20, 40, 60 and 80 mmol) were distinguished on the other rest treatments and recorded average of (5.2, 5.2, 5.4 and 5.6) respectively, moreover the results indicated that increased salt concentration increase number of roots and exceeded them even on the treatment of control . these results were agree with **(Jorge and Susana, 2007).**

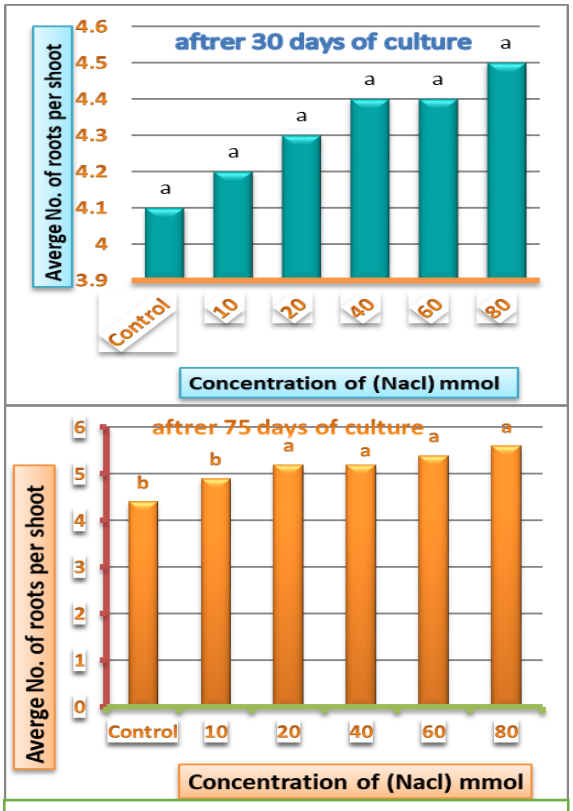

Figure. (10) Effect of adding (NaCl) to MS medium on roots per shoot of potato (spunta) produced by single nodes after 30 and 75 days of culture. Columns with the same letter are not significantly different, by Duncan multiple test at (0.05).

In general results of these experiment explained that increase concentration of sodium chloride led to decrease the number of leaves, number of branches and plant length and these results were agree with **(Wang *et al.*, 2007; Hasegawa *et al.*, 2000; Munns, 2010).** The results also showed that increase concentration of sodium chloride led to increase number of roots which in combine with results as (**Jorge and Susana,2007).**

Figure. (11) Effect of adding (NaCl) to MS medium on roots per shoot of potato (spunta) produced by single nodes after 75 days of culture.

Agria variety

After obtaining non-contaminated tissue cultures the obtained plants were replanting in MS media supplemented with several concentrations of sodium chloride salt (0, 10, 20, 40, 60, 80 mmol), while the results for this variety described in (Fig.12) . Beginning from plant length factor the results showed that plant length in treatments (0, 10, 20 mmol) was recorded significantly higher than the other treatments which are (40, 60, 80mmol) which main that plant length significant superiority of treatments (0, 10 and 20 mmol) on the other treatments and their recording of the mean (4.9, 4.6 and 4.4cm) respectively after 30 days of planting and this superiority continued even after 75 day of planting followed by the treatment of 40Mm which recorded an average of 5.2 cm, furthermore the toxicity effect of sodium chloride salt there was non-significant different among on treatments of (60, 80 Mm). NaCl specially during period of 75 days of planting for the plant length factor these results were agree with **(Bsharaa *et al.*, 2013),**

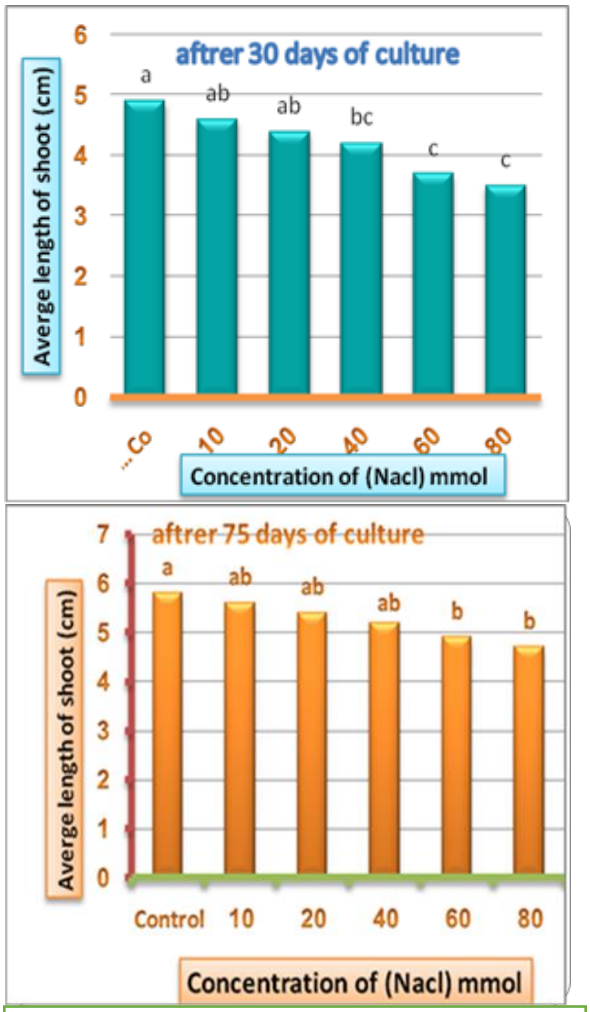

Figure. (12) Effect of adding (mmol NaCl) to MS medium on shoot length of potato (Agria) produced by single nodes after 30 and 75 days of culture. Columns with the same letter are not significantly different, by Duncan multiple test at (0.05)

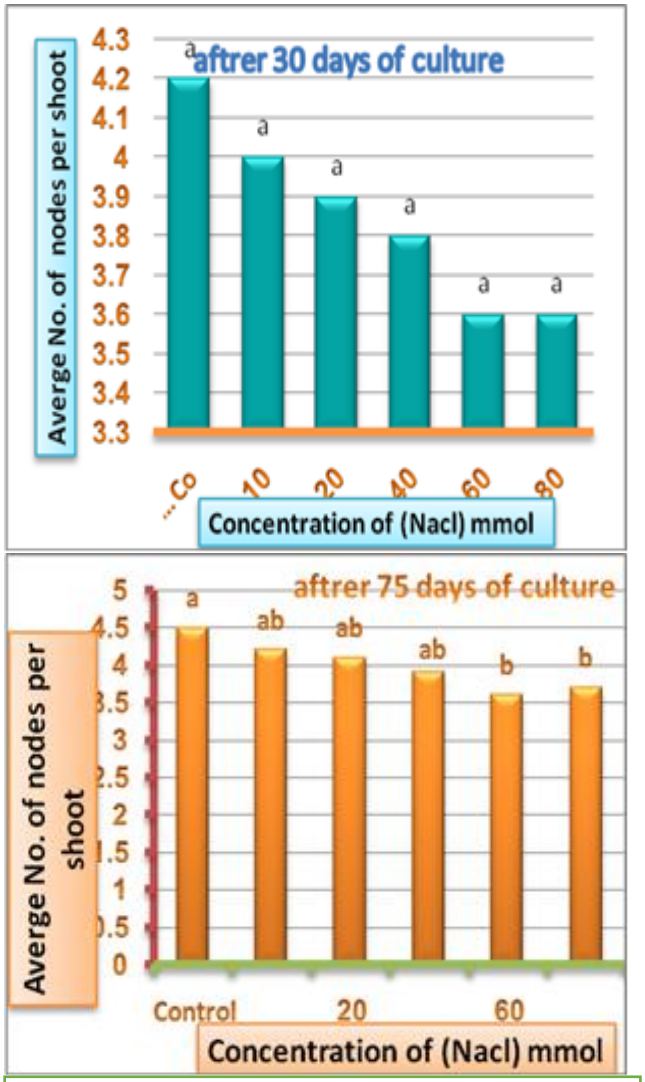

Figure. (13) Effect of adding (mmol NaCl)to MS medium on nodes per shoot potato (Agria) produced by single nodes after 30 and 75 days of culture. Columns with the same letter are not significantly different, by Duncan multiple test at (0.05)

Either by number of branches the results described in (Fig. 13), while planting single nodes in MS media supplemented with different concentration of sodium chloride salt grown plants were not affected by toxicity of NaCl after 30 days of grown even after 75 days of culture all the treatments were not affected except for the treatment of 60 and 80mmol with an average of 3.6 and 3.7 branches respectively these two treatments directly affected by added NaCl which led the other treatments to had significant superiority in compare with treatments of 60, 80 mmol NaCl. these results were agree with **(Munns, 2010),**

Followed by number of leaves factor the results explained in (Fig. 14) in which the treatments of 0 and 10Mm exceeded the other treatments by recording the averages (19.7 and 19.2 leaves) respectively. While the effect of sodium chloride salt toxicity being occurred on the other treatments after 30 days of planting and exceeded of 0and 10 mmol Nacl treatments continued even after 75 days of grown. The toxicity effect of sodium chloride salt continued on the other treatments started from treatment of 20mmol which exceeded the average of 19.9 leaves until reaching an average of 19.1 leaves for the treatment of 40mmol NaCl these results were agree with **(Munns, 2010).**

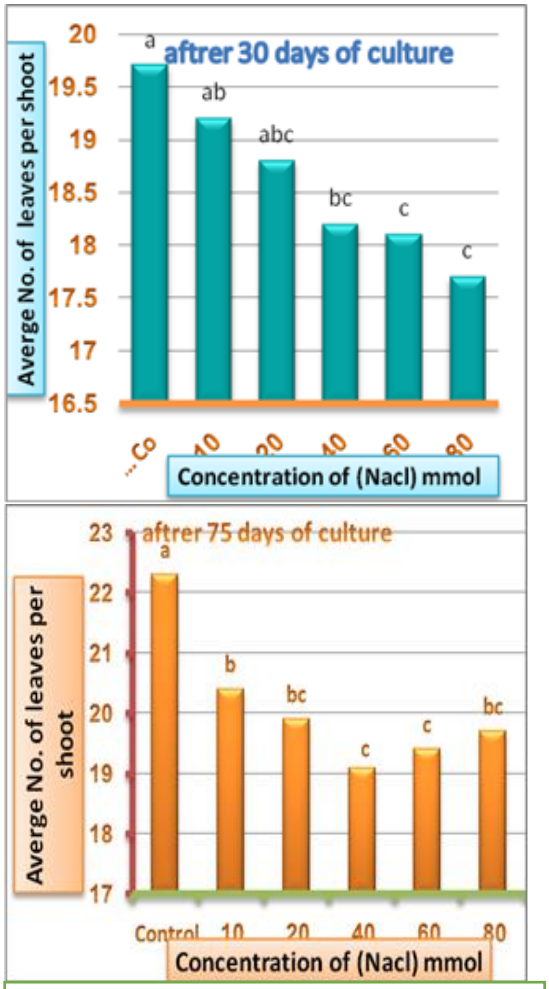

Figure. (14) Effect of adding (mmol NaCl) to MS medium on leaves per shoot of potato (Agria) produced by single nodes after 30 and 75 days of culture. Columns with the same letter are not significantly different, by Duncan multiple test at (0.05)

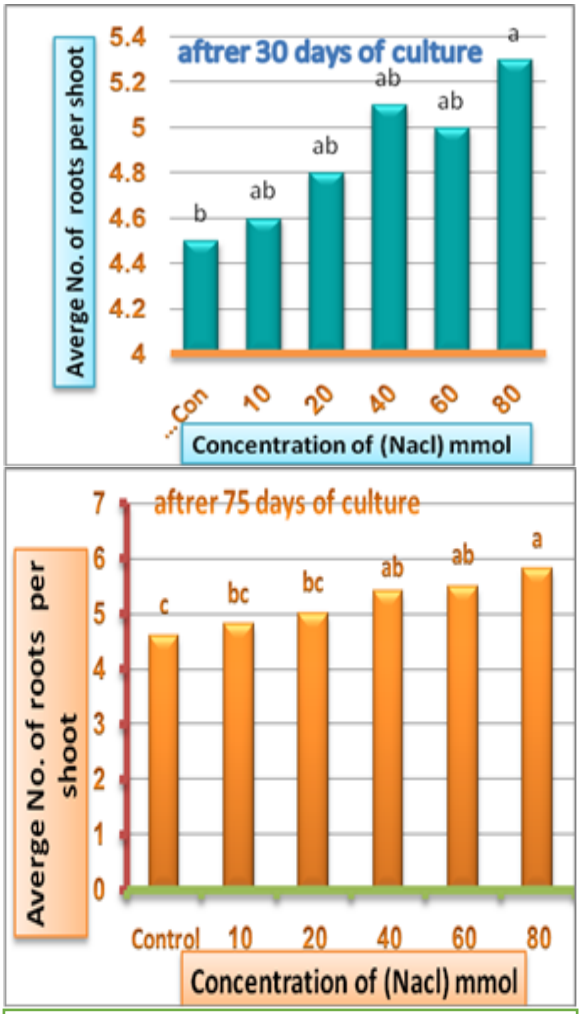

Figure. (15) Effect of adding (mmol NaCl) to MS medium on roots per shoot of potato (Agria) produced by single nodes after 30 and 75 days of culture. Columns with the same letter are not significantly different, by Duncan multiple test at (0.05

As for number of roots character the results which shown in(**Fig. 15**) the results showed that the increase sodium chloride salt concentration increase the number of roots, this was proved in the treatment of 80 mmol which recorded an average of 5.3root after 30 days of planting and this prove continued even after 75 days of culture while treatment of 80mmol recorded an average of 5.8 root, although it did not significantly exceed the other treatments except treatments of 0, 10 and 20 which recorded an average of (4.6, 4.8 and 5 root) respectively. (**Jorge and Susana, 2007)**.

Increasing the concentration of sodium chloride salt leads to a decrease in the leave area which represented in the number of leaves, number of branches and length of the plant and these results were agree with (Munns, 2010), also increase the salt concentration leads to decrease the plant length and this result corresponds to what was fond by **(Bsharaa *et al*., 2013)**.

In general through the results the ability to tolerate salt stress by plant depends on the ability of the variety to maintain the intracellular effort of fullness in the plant cell, also related to the efficiency of the variety to absorption control , this is consistent with it fond by

(Khrerdiev, 2000). Whatever the case it has been proven that Agria variety was one of the best varieties to tolerate high levels of salinity when compared to Spunta variety while Spunta variety considered as one of best varieties capable to tolerating high levels of salinity **(Bsharaa *et al*., 2013).**

Figure. (16) Effect of adding (mmol NaCl) to MS medium on roots per shoot of potato (Agria) produced by single nodes after 75 days of culture

4 Conclusion

The best method to sterilize the surface of potato explants use commercial Clorox solution at a concentration of 3% for 20 minutes, which gave the highest proportion of tissue cultural free of contamination.

Increase salinity levels leads to a decrease in the number of leaves and number of nodes. Plant length for each of the two potato varieties. Increase the number of roots for a specific period of time .Possibility of plant tissue.

growth for the two varieties Agria and Spunta without any effect of the concentration of salinity stress which reached a maximum concentration of 80 mmol NaCl after 30 days of planting. The significant impact of potato tissues after 75 days of explants were cultured under stress at the concentration of 80mmol NaCl.

The ability of salt tolerance depends, on the ability of the variety of maintain the osmotic adjustment in the cell. Agria variety considered as one of the best varieties to tolerate of high levels of salinity when compared with Spunta variety.

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