J. Agric. Sci. Mansoura Univ., 31 (9): 5921 - 5927, 2006

MICROTUBERIZATION OF POTATO (*Solanum tuberosum* L.):- 2- *IN VITRO* PRODUCTION OF MICROTUBER UNDER SALT STRESS.

Aboshama, H. M. S.*; S. T. El-Afifi; Omaima M. Abdel-kafie** and Hala E. Abd Allah**

*** Minufiya University, Genetic Engineering and Biotechnology Research Institute, Plant Biotechnology Department, Egypt.**

**** Mansoura University, Faculty of Agriculture, Veg. and Flor. Dept.**

ABSTRACT

This study was conducted in the tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, Mansoura University during the period from 2002 to 2005, aiming to investigate the shoot multiplication and production of microtubers in *in vitro* of *Solanum tuberosum* L. cv spunta and evaluated this microtubers under salinity conditions.

Effect of different concentrations of NaCl (0.0, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 g/L) was recorded on shoot proliferation and microtuberization. All parameters under this study (shoot length, number of both shoots and leaves / explant and cluster fresh weight) were significantly decreased at the highest levels of salinity (6.0 and 8.0 g/L NaCl). However, number of shoots/explant was significantly increased by 4.0 g/L NaCl. It is a matter of interest to mention that multiplication rate was not significantly affected by salinity up to 4.0g/L NaCl. Survival percentage and number of shoots per explant at 4.0 g/L NaCl were 81.10% and 6.85, respectively. However at 8.g/L NaCl these parameters were 47.50% and 2.14, respectively.

Microtuber characteristics (number, weight, length and diameter) were recorded under saline condition. All these parameters were decreased by increasing NaCl concentration in the culture media and the decrease was significant at 6.0 and 8.0 g/L NaCl. The number of microtubers per shoot was not significantly decreased by increasing salinity level up to 4.0 g/L NaCl (2.55) , while at 6.0 and 8.0 g/L NaCl was significantly decreased (2.14 and 1.53, respectively).

INTRODUCTION

Salinity is a major factor limiting and depressing the growth and productivity of many crops. Potato is considered as a moderately salt-sensitive plant, since a soil salinity level of 2.0dsm reduces plant growth and tuber yield up to 50% (Maas and Hoffman, 1977). Sodium toxicity is an important problem in arid regions because of the gradual build up of salt (NaCl) in soils where irrigation is employed extensively (Maliga, 1984). So, the identification and evaluation of salt tolerant cultivars could help in solving salinity problem.

Tissue culture technique is recently used for the evaluation of cultivars towards environmental stresses. This technique offers the control of environmental, pathogenic and nutritional factors. So, the evaluation of stress tolerance would be more valuable and reliable. *In vitro* culture, besides its use as a tool for obtaining salt tolerant plants, may offer potential for quick evaluation of germplasm against salt stress. (Emilio *et al.*, 1998).

The aim of this investigation was to production of microtubers *in vitro* of spunta cultivar under salt stress conditions in order to be a source of salt tolerance clones.

MATERIALS AND METHODS

The present investigation was carried out in the tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, Mansoura University during the period from December 2002 to December 2005 in order to study the responses of spunta proliferation of shoots (multiplication) and microtuberization to salt stress under *in vitro* conditions, as well as evaluate microtuberization for salt stress.

Multiplication stage under salt stress.

The aim of these experiments was performed to study the possibility of *in vitro* shoot multiplication under NaCl condition. Aseptic single nodes were excited from multiplicated shoots from first part of this study. Due to the results of the first part of this study, it cleared that the best shoot multiplication medium was the MS medium (Murashige and skoog, 1962) at full strength supplemented with 30 g/L sucrose, 0.2 mg/L BAP combined with 0.1 mg/L NAA. Solidification of this medium achieved by 7.0 g/L agar. For salt stress evaluation, this medium was supplemented with NaCl at different levels (0.0, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 g/L NaCl). The pH of this medium (under salt conditions) was adjusted to 5.8 prior to the addition of agar. The medium was distributed into culture jars (250 ml) each jar contained 30 ml. The jars were capped with polypropylene closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressures for 15 min. Three single nodes were cultured in jar. All cultures were incubated at 22 °C ± 2 and photoperiod of 16 hours light using florescent lamps (about 2000 lux) and 8 hours darkness. The following data were collected after 4 weeks from culture initiation:

1) Shoot length (cm).
2) Numbers offshoots/explant
3) Numbers of Leaves/explant.
5) Fresh weight of shoot (mg).
4) Survival percentage

Survival percentage of explants was calculated. In other words, the explant is considered survive if:-

- It multiplicated.
- More than 50% of it was still green and healthy.

Microtuberization under salt stress

This experiment was carried out on proliferated shoots of the spunta derived from *in vitro* shoot multiplication under NaCl stress. These multiplicated shoots were considered the source of explants (each one included 4 nodes) for *in vitro* tuberization under NaCl stress condition. Due to the results of microtuberization experiments (in the first part of this study), it was cleared that the best microtuberization medium was MS supplemented with 80 g/L sucrose and 2.0 mg/L BAP. Solidification of this medium was achieved by 7.0 g/L agar. For salt tolerance evaluation, this medium was supplemented with NaCl at different levels (0.0, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0

g/L NaCl). The pH of this medium (under salt conditions) was adjusted to 5.8 prior to the addition of agar. The medium was distributed into culture jars (325 ml) where each jar contained 40 ml. The jars were capped with polypropylene closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressures for 15 min. Cuttings prepared from in vitro-formed shoots were harvested from shoot clusters (in multiplication stage) of each NaCl concentrations. Three stem cuttings were cultured in one jar. All cultures were incubated at 22±2°C under dark conditions. The following data were collected after two months from culture initiation:

- Number of Microtubers/explant.
- Microtuber diameter (mm).
- Microtuber length (mm).
- Microtuber fresh weight (mg).

Statistical Analysis

Experiments were set up in completely randomized block design with five replicates; each replicate consisted of one jar containing three explants. All experiments were repeated two times. The results were analyzed by analysis of variance (ANOVA) according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Effect of different concentrations of NaCl on shoot proliferation.

This experiment showed the proliferated shoots (multiplication) of potato under salt stress.

Data presented in Table (1) and Fig. (1) indicate that the survival percentage of potato explant was gradually decreased by increasing NaCl concentrations in the media. It is the matter of interest to mention that survival percentage was 80.1% at 4.0 g/L NaCl and 47.50% at 8.0 g/L NaCl. Data in the same Table show that salinity levels up to 2.0 g/L NaCl did not affect length and number of shoots per explant, while at 4.0 g/L NaCl, a significant increase in the number of shoots per explant was obtained (6.85). The highest levels of salinity (6.0 and 8.0 g/L NaCl) significantly decreased these parameters (2.28 and 1.31, respectively for shoot length and 4.17 and 2.14, respectively for shoots number per explant). These results may be attributed to the specific effects of salinity at certain concentrations on the meristems activity. This was inagree with Winicov (1993) who reported that Saline stress induces several alterations on growth, cell division and enzymatic activities, among others. Salinity tolerance by plants depends primarily on the genotype that determines alterations on processes such as uptake and transport of salts by roots, together with metabolic and physiological events occurring at cellular level. In addition it could be related to the growth inhibition caused by high salinity levels, where chloride ions interfere in the metabolic process causing a reduction in the plant growth (Boyer, 1965). Moreover, Imbalance of water relation of the plant cell caused by rapid uptake of specific ions under salinity conditions may also be responsible for the depression of the shoot length. Reduction of plant growth under salt stress is usually attributed to osmotic stress due to a lowering of external water potential (Maas and Nieman, 1978). Sivritepe and Eris (1999) mentioned that shoot proliferation

and growth of explants were decreased due to an increase in NaCl concentration in the culture medium.

Table (1): Effect of different concentrations of NaCl on survival percentage and multiplication rate of potato under salinity after 4 weeks from culture.

Characters / NaCl g/L	Survival percentage	Length of shoots cm	Number of shoots	Number of leaves	Fresh weight mg
0.0	100.0	5.78	5.85	18.0	553
0.5	96.80	5.57	5.71	18.13	545
1.0	93.50	5.28	6.14	18.42	583
2.0	90.30	5.14	6.42	18.71	610
4.0	81.10	4.42	6.85	16.28	531
6.0	62.70	2.28	4.17	13.70	436
8.0	47.50	1.31	2.14	7.15	355
L.S.D at 5%	-	0.90	0.89	1.92	146

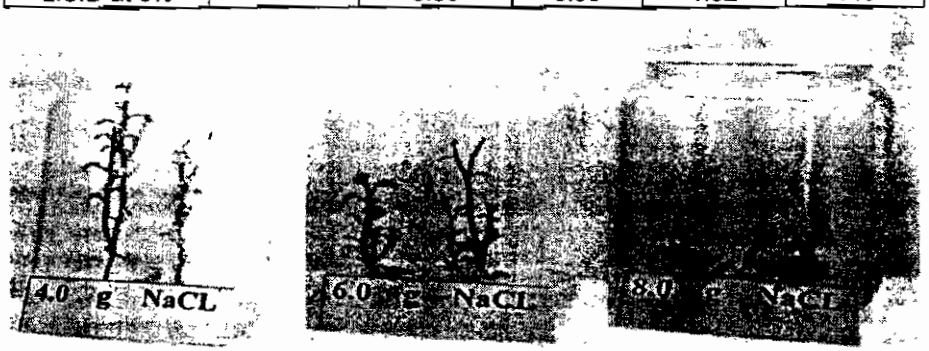

Fig (1): Effect of NaCl on multiplication of potato after 4 weeks from culturing.

In addition number of leaves per explant and cluster fresh weight were not significantly affected by salinity up to 4.0 g/L NaCl. However, increasing NaCl concentration from 4.0 to 8.0 g/L decreased these characters significantly, especially at 8.0 g/L NaCl (7.15 and 355, respectively). The heaviest fresh weight (610) was obtained when NaCl was added at 2.0 g/L to MS medium. It was a matter of interest to notice that there was moderate increment in fresh weight by using NaCl at 1.0 and 2.0 g/L in this study. Similar result was obtained by Sasikala and Prasad, (1993) who reported that, a lower concentration of salt (0.2%) was beneficial and increased shoot weight in most potato verities.

It could be concluded that the reduction in cluster fresh weight was expected in this study due to the decrease in shoot length and number of shoots and leaves per explant under the high level s of salinity. These results are in harmony with those reported by Khrais *et al.*, (1998). Injury or growth depression of plants may be referred to a toxic effect of the salt on the ion uptake or metabolism of essential nutrients, which cause direct damage to the plant (Richards, 1954).

Effect of different concentrations of NaCl on microtuberization of potato.

This experiment show the effect of different levels of NaCl on microtuberization of potato. Nodal cutting with four nodes used as explants from multiplication experiment (under salinity conditions) and cultured on MS medium supplemented with 80 g/L sucrose and 1.0 mg/L 2,4-D and jars were incubated in complete darkness. Data were recorded after two months of culturing.

Data of explants grown in MS medium including different concentrations of NaCl were shown in Table (2) and Fig. (2). It clear that the highest number of microtubers per shoot (2.90) was obtained in the absence of NaCl. In case of adding 0.5, 1.0, 2.0 and 4.0 g/L NaCl, the number of microtubers was slightly decreased. Low salinity levels stimulated microtuber yield in some potato cultivars (Zhang and Donnelly, 1997). However, the highest level of NaCl (6.0 and 8.0 g/L) significantly decrease this parameter (2.14 and 1.53, respectively). Concerning the effect of NaCl on microtuber weight, it was found that all NaCl levels reduced the microtuber weight than the control. However adding NaCl to the media at 8.0 g/L significantly reduced the microtuber weight.

As for length and diameter of microtuber results in the same Table show that all NaCl levels reduced these parameters than the control. Adding 0.5, 1.0 and 2.0 g/L NaCl reduced the length and diameter of microtuber, but the decrease was not significant when compared with control. However, there was significant decrement at 4.0, 6.0 and 8.0 g/L NaCl than the control. Supporting results were reported by Morpurgo (1988) and Silva *et al.*, (2001).

Table (2): Effect of different concentrations of NaCl on microtuber characteristics of potato under salinity after two months from culturing.

Characters / NaCl g/L	No.per shoot	weight mg	Length mm	Diameter mm
0.0	2.90	115	7.7	4.9
0.5	2.85	111	7.2	4.7
1.0	2.77	107	6.5	4.4
2.0	2.71	97	5.9	3.8
4.0	2.55	78	5.5	3.4
6.0	2.14	54	4.9	3.1
8.0	1.53	32	4.2	3.1
L.S.D at 5%	0.58	61	2.1	1.2

The negative effects of salinity on microtuberization of *S. tuberosum* were probably a consequence of the reduction in osmotic potential due to increasing salt levels in cells of both stolon and microtuber tissues. Salinization possibly caused a reduction of water content and nutrient uptake in microtubers. In this concern, Kramer and Boyer (1995) mentioned that reduction in growth rate might be correlated to metabolic effects due to salt accumulation in cells, resulting in a decrease in water absorption.

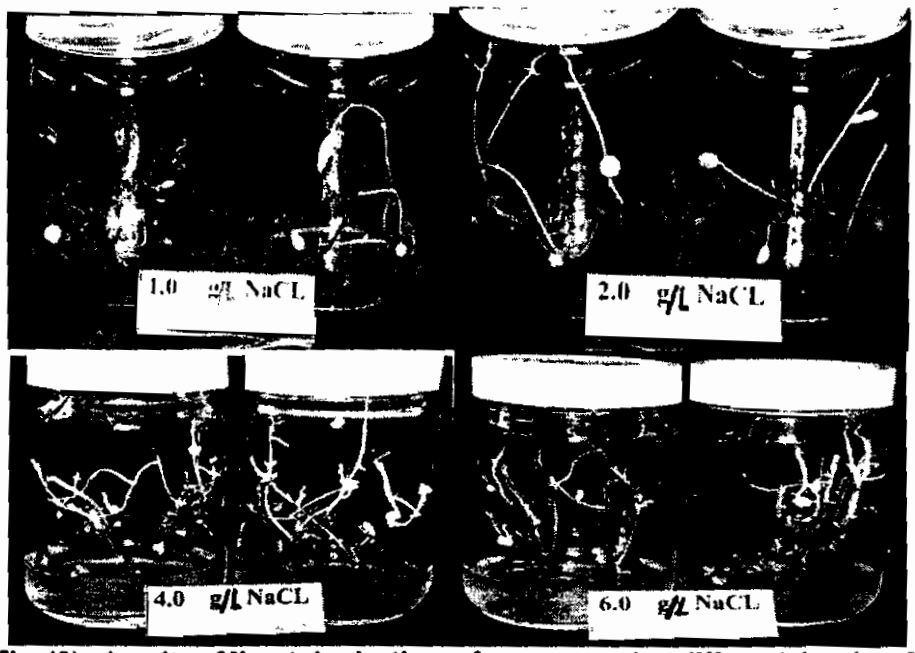

Fig (2): *In vitro* Microtuberization of potato under different levels of NaCl

REFERENCES

Boyer, J. S. (1965): Effect of osmotic stress on metabolic rates of cotton plants. Plant Physiol. 40: 229-234.

Emilio A. C.; F. Perez-Alfocea, V. Moreno. M. Caro and Maria C. Bolarin (1998). Evaluation of salt tolerance in cultivated and wild tomato spicies through *in vitro* shoot apex culture. Plant cell,tiss. and organ Culture 53: 19-26.

Gomez, K. A. and A. A. Gomez (1984): Statistical Procedures for the Agricultural Research. John Wiley & Sons ; Int. Rice Res. Inst. Book 2nd Ed.

Khrais, T. ; Y. Leclerc and D. J. Donnelly (1998): Relative salinity tolerance of potato cultivars assessed by *in vitro* screening. Amer. J. Potato Res., 75 (5): 207-210.

Kramer, P. J. and J. S. Boyer (1995): Water Relations of Plant and Soils. Academic Press, San Diego, CA. Li, P.H., 1985. Potato Physiol. Academic Press, New York.

Mass, E. V. and G. J. Hoffman.(1977). Crop salt tolerance- current assessment. J. Irr. Rain age 103: 115-134.

Maas, E. V. and R. H. Nieman (1978): Physiology of plant tolerance to salinity. In G.A. Yung. (ed.). Crop Tolerance to Suboptimal Land Conditions. American Society of Agronomy Special Publication. Crop Science Society of America, Madison, WI, pp. 277-299.

Maliga. P. (1984). Isolation and characterization of mutants in plant cell cultures. Ann. Rev. Plant Physiol. 35: 519-552.
Morpurgo, R. (1988): Correlations between *in vivo* and *in vitro* potato clones grown under salt stress conditions. Genetica - Agraria, 42 (1): 85-86.
Murashige, T. and F. Skoog (1962): A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, 15: 473-497.
Richards, L. A. (1954): Diagnosis and improvement of saline and alkali soils. US. Dept. Agric. Handbook No. 60.
Sasikala, D. P. P. and Prasad, P. V. D (1993): Influnce of salinity on axillary bud cultures of six lowland tropical varieties of potato (*Solanum tuberosum*) plant. Cell. Tissue and Organ Culture 32: 185-191.
Silva, J. A. B. ; W. C. Otoni ; C. A. Martinez ; L. M. Dias and M. A. P. Silva (2001). Microtuberization of Andean potato species (*Solanum spp.*) as affected by salinity. Scientia Hort. 89 (2): 91-101.
Sivritepe, N. and A. Eris (1999). Determination of salt tolerance in some grape vine cultivars (*Vitis vinifera* L.) under *in vitro* conditions. Turkish journal of Biology, 23 (4): 473-485.
Winicov, I. (1993). Gene expression in relation to salt tolerance. In: Basra, A.S. (Ed.), Stress-induced Gene Expression in Plants. Hardwood Academic Publishers, Switzerland, pp 61-130.
Zhang, Y. and D. J. Donnelly (1997). *In vitro* ranking for salinity tolerance of potato cultivars. Potato Res. 40: 285-295.

تكوين درينات البطاطس معمليا:

٢- انتاج درينات معمليا تحت مستويات مختلفه من الملوحه.

هارون محمد صالح ابوشامه* ، سمير طه العفيفى ، اميمه محمد عبد الكافى** و هاله عبدالله**

* جامعه المنوفيه - معهد الهندسه الوراثيه والتكنولوجيا الحيويه - قسم البيوتكنولوجيا النباتيه

** جامعه المنصوره - كليه الزراعه- قسم الخضر والزينه

أجريت هذه الدراسة فى معمل زراعة الأنسجة النباتية بكلية الزراعة جامعة المنصورة خلال الفترة من ديسمبر ٢٠٠٢ حتي ديسمبر ٢٠٠٥، بهدف انتاج درينات البطاطس صنف اسبونتا باستخدام تكنيك زراعة الأنسجة تحت تاثير ملح كلوريد الصوديوم ودراسه معدل التضاعف الافرع وتكوين الدرينات بالمعمل في مستويات مختلفه من كلوريد الصوديوم(صفر ، ٠,٥ ، ١,٠ ، ٢,٠ ، ٤,٠ ، ٦,٠ ، ٨,٠ جرام/لتر).

انخفضت جميع الصفات تحت الدراسه (نسبة البقاء ، طول الأفرع ، عدد الأفرع ، عدد الأوراق و الوزن الطازج) معنويا عند ٦ و ٨ جم/ لتر كلوريد صوديوم. بينما ادى التركيز ٤ جم/لتر الى زياده عدد الافرع المتكونه معنويا. من الاهميه بمكان الاشاره الى قدره النبات على تحمل الملوحه بدون تاثير معنوى حتى تركيز ٤ جم/لتر كلوريد صوديوم حيث بلغت نسبه البقاء ٨١,١٠ ٪ ووصل عدد الافرع المتكونه ٦,٨٥ فرعا عند هذا التركيز. بينما عند التركيز ٨ جم/ لتر كلوريد صوديوم لم تتعدى نسبه البقاء ٤٧,٥٠ ٪ وعدد الافرع ٢,١٤ فرعا.

اوضحت النتائج انه يمكن انتاج الدرينات تحت جميع مستويات الملوحه المختبره. ولم تتاثر انتاج الدرينات معنويا حتى ٤ جم/ لتر كلوريد صوديوم حيث كان عدد الدرينات (٢,٥٥ درينه /فرع) وكان متوسط وزن الدرينه (٧٨ ملليجرام).بينما عند ٨ جم/لتر كلوريد صوديوم انخفض عدد الدرينات معنويا حيث كان ١,٥٣ وكذلك انخفض الوزن الطازج ٣٢ ملليجرام.