Salt tolerance in tissue culture of onion (*Allium cepa* L.)

(Received: 10 .05 .2006; Accepted: 05 .06 .2006)

S.A. Bekheet ; H.S.Taha and M.E. Solliman
Plant Biotechnology Dept., National Research Centre, Cairo,Egypt .

ABSTRACT

The response of onion tissue cultures to salinity stress was investigated. Callus initiated from aseptic seedlings was exposed to different levles of salt mixture. Fresh weight and growth value of callus inoculms were decreased as salt mixture increased in culture medium. However, dry weight and dry matter increased as salt level increased up to 4000 ppm and then decreased. Total proteins of callus enhanced as salt mixture increased in culture medium. For selection under salt stress, regenerated shoot buds derived from tolerant callus cultures were exposed to the different levels of salts mixture. The number of proliferated shoot buds and their fresh weight and growth value were depressed upon increasing of salts in medium. The best results of salt tolerance ratio were reached at 2000 ppm salts. Although protein content took similar trend of callus, it was relatively higher than in callus cultures at the same salt levels. Esterase patterns showed new band with the tolersnce lines. This band had different mobility and more intensity with the shoot bud lines exposed to 2000 ppm of salt mixture. The tolerant shoot buds were in vitro rooted and successfully adapted to free-living conditions.

***Key words**: Onion, tissue culture, salt stresss, isozyme.*

INTRODUCTION

Crop production is hindered or restricted in several areas of the world by naturally saline soil. Salinity inhibits the growth of plants by affecting both water absorption and biochemical processes, such as nitrogen assimilation and protein biosynthesis (Dubey, 1994). Under saline conditions, the plants fail to maintain the required balance of organic constituents leading to suppressed growth and yield. In developing countries, the limited supply of good quality water in many arid and semi-arid regions necessitates the use of saline water where available for crop production. This, in turn, requires the screening of crop plant varieties for their tolerance to salinity. Screening for improved salt tolerance is difficult in the field because of lateral, vertical and temporal variability in salt distribution within the soil profile. In addition, plant salt tolerance varies with ontogeny, the growth parameter measured and environmental factors.

Tissue culture techniques have been applied to the plant species in an attempt to produce new clones and cultivars with improved characteristics. In this respect, numbers of researchers have suggested that cultured tissues and cells may prove useful both in selections of the salt-tolerant plants and in studies of the physiological basis for salinity tolerance (Chen *et al.*, 1980; Umiel *et al.*, 1980). Easy manipulation of salt mixture concentrations in media, especially in suspension culture, also permits uniform and direct treatment on cell growth with a given salt stress level. Most efforts at selecting salt–

resistant cell lines have involved direct selection for capacity to grow on otherwise inhibitory levels of NaCl (Dix, 1985; Tal, 1983).

The onion crop is of great importance in Egypt and is one of the main exported fresh vegetables. In cultivated onion, the development of new and improved crop genotypes is of vital importance for its growing in various ecological areas. The techniques of plant tissue culture may play a key role in the development of new cultivars. Aseptic culture technique offers a potential for selection of salt-tolerant lines of onion. The present work was planned to study the influence of different salt levels on growth and chemical contents of onion tissue cultures and *in vitro* selection for salt stress tolerant genotypes.

MATERIALS AND METHODS

Establishment of aseptic culture

Seeds of *Allium cepa* (L.) cv. Giza 6 were surface sterilized under aseptic conditions by 70 % ethanol for 1 min, followed by 30% commerical Clorox (contained 5.25 % sodium hypochlorite) for 20 min. Seeds were rinsed several times with sterile distilled water and then cultured on basal MS medium plus (per liter) 30 g sucrose and 7 g agar for *in vitro* germination. Four-week-old seedlings were used as a source of explants for callus induction and regeneration. Leaf segments (5 mm in length) were plated on MS medium supplemented with different combinations of growth regulators.

Effect of salt stress on callus cultures

Equal inoculms (250 mg) of proliferated callus were subcultured on callus growth medium (MS+2 mg/l 2,4-D + 1 mg/l BA) supplemented with 0, 2000, 4000 and 6000 ppm of salt mixture [3 NaCl:1(3 MgCl : 1$CaCl_2$)] described by Ibrahim and El-Kobbia (1986). After five weeks of culturing, fresh and dry weights were recorded. Dry matter, growth value and salt tolerance ratio were calculated as follow:

$$\text{-Dry matter (\%)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

$$\text{-Growth value} = \frac{\text{Final fresh weight} - \text{Initial fresh weight}}{\text{Initial fresh weight}}$$

$$\text{-Salt tolerance ratio} = \frac{\text{Fresh weight on salt medium}}{\text{Fresh weight on salt-free medium}}$$

Effect of salt stress on rgenerated shoot bud cultures

Calli growing on the three levels of salinity were taken and subcultured on regeneration medium (MS + 2 mg/l 2,4-D + 0.5 mg/ kin) without salts. Shoot buds proliferation was obtained from calli exposed to 2000 and 4000 ppm of salt mixture. Shoot buds were taken in uniform length (about 1 cm) and weight (about 0.5 g) and cultured on shoot bud multiplication medium (MS + 2 mg/l BA +0.5 mg/l NAA) in addition of 0, 2000, 4000 and 6000 ppm salt mixture. After five weeks of culturing, the number of proliferated shoot buds (per explant), the average of shoot bud length and fresh weight were scored. Also, growth value and salt tolerance ratio were calculated as mentioned before.

Survival shoot bud cultures grown on the different levels of salt stress were subcultured on rooting medium (MS+ 1 mg/l IBA). The rooted plantlets taken and washed with current tap water, then disinfected by immersion in Benlate solution (1 g/l) for 20 min. Then, plantlets were transplanted into plastic pots (10 × 5 cm) containing perlite and peat moss (1:1). The pots were covered with transparent polyethelene bags to maintain a high relative humidity around the plantlets, which were

sprayed with water in three-day intervals. The relative humidity was reduced after two weeks and the bags were completely removed after four weeks of transplanting.

Culture conditions and statistical analysis

Culture media were adjusted to pH 5.8 before autoclaving at 126 ºC and 1.5 Ib/M^2 for 20 min. Cultures were incubated in growth chamber at 25± 2ºC under 16 hr light (2000 Lux) and 8 hr dark. Experiments were designed in completely randomized design and data were statistically analyzed using standard error (SE) (Snedecor and Cochran, 1967).

Determination of total protein

Fresh samples (callus and shoot bud cultures) exposed to different concentrations of salinity were homogenized in sodium phosphate buffer (pH 6.8) and centrifuged at 1400 xg for 10 min. Then, total soluble proteins in the supernatant were assayed by method of Bradford (1976).

Isozyme analysis

Isozyme analysis, i.e., esterase patterns, was carried out for biochemical characterrization of the regenerated salt-tolerant lines of onion. Leaf tissue samples of shoot buds exposed to 0, 2000 , 4000 and 6000 ppm of salt mixture in addition to non-selected shoot bud culture were taken and cruched in extraction buffer consisting of 15 % (w/v) sucrose, 5 mM tris-glycine buffer, pH 8.3. Samples were electrophoresed for 3 hr at constant current (3 mA per well), using mini vertical electrophoresis unit of Bio-Rad.

RESULTS AND DISCUSSION

Effect of growth regulators on morphogenesis

Frequencies of callus induction, and regeneration capacity as well as mean fresh weight of initiated callus leaf explants of onion as affected by exposure to different combinations of growth regulators are shown in Table (1). Results indicated that incorporation of culture medium with 2,4-D obviously enhanced the callus formation and subsequently the *in vitro* regeneration. The highest frequency of callus induction was observed with medium contained 2 mg/l 2, 4-D + 1 mg/l BA. However, the highest percentage of embryonic callus and regeneration capacity as well as culture fresh weight of the leaves of explants were scored with medium contained 2 mg/l 2,4-D + 1 mg/l kin, followed by 1 mg/l 2,4-D + 1 mg/l kin-containing medium. Data of explant (leaf) differentiation in this experiment seem to be much more promising for the advanced breeding methods of onion. The present results are in agreement with those obtained by Zheng *et al.* (1998). They reported that 2,4-D is the most important determining factor for callus production and later plant regeneration in *Allium cepa*. Moreover, Luthar and Bohanec (1999) in their study on onion mentioned that direct organogenesis structures induced mature flowers or ovaries when cultured on medium containing 2 mg/l 2,4-D. On the other hand, NAA and picloram have been reported as having benefical effects on callus induction or differentiation in onion (Hansen *et al.*, 1995; Roy, 1995).

Influence of salt stress on callus cultures

Callus growth

Growth dynamics of onion callus grown on three concentrations of salt mixture, i.e., 2000, 4000 and 6000 ppm in addition of its growth parameters on salt-free medium are presented in Table (2) and Fig. (1-A). The results showed that fresh weight and growth value decreased as salt mixture increased in culture medium. However, increasing of salt mixture caused marked increase in dry weight and dry matter till 4000 ppm and then depressed. The lack of inhibition of dry matter

accumulation at these salt levels show that onion cells have been adapted to or tolerant to salt stress at such levels. The best result of salt tolerance ratio was noticed with 2000 ppm of salt mixture. The reduction of callus fresh weight of onion as a result of salinity stress is in line with the results obtained by Bekheet *et al.*(2000) and El-Bahr *et al.* (2001) in their studies on *Asparagus officinalis* and Globe artichoke, respectively. They reported that growth paramaters of callus gradually depressed as salt level increased in culture medium. In this respect, Greenway and Munns (1980) mentioned that the reduced growth of nonhalophytes in saline culture could be due to water loss or solute accumulation.

Protein content

Under stress conditions, some proteins that specifically respond to stress are induced in many plants. Although both the expression and function of such protein is unclear, it is suggested that there is a relationship between some forms of plant adaptation and tolerance to stresses and the expression of stress induced proteins. In this investigation, the influence of salinity resulting from the salt mixture added to culture medium on total protein content of callus cultures of onion was investigated. Data presented in Fig. (3) reveal that total protein gradually enhanced as salt mixture increased in culture medium. The maximum value of protein content was recorded at 6000 ppm of salt mixture. The marked increase in protein content in callus cultures grown on saline media may be due to synthesis of new proteins (osmoprotectant protein) or inactivation of proteolytic enzymes (Dubey, 1994). The results also are accordance with those reported Bekheet *et al.* (2000) on *Asparagus officinalis*. They found a positive correlation between protein content of callus cultures and salt stress level in culture medium. In this connection, Poljakoff-Mayber (1982) reported that osmotic adaptation under salinity stress may be achieved by ion uptake or by internal synthesis and accumulation of organic solutes.

Influence of salt stress on regenerated shoot bud cultures

Vegetative growth

The effect of salt stress on the growth of selected cultures of onion was studied. Regenerated shoot buds derived from callus exposed to salt mixture (Fig. 1-B) were divided and cultured on medium contained the different level of salt mixture. As shown in Table (3) vegetative growth presented as number of proliferated shoot buds and average of shoot bud length depressed as salt mixture increased in culture medium. Growth dynamics presented as fresh weight (gm) and growth value also decreased as salinity increased. Although shoot buds grown on medium contained 6000 ppm scored the lowest growth parameters, some shoots remained viable and proliferated few new buds. The best result of salt tolerance ratio scored under 2000 ppm salt mixture (Table 2). At this salt level, the shoot buds were healthy and had dark green color (Fig. 1-C). In this context, Mills (1989) reported that *in vitro* shoot production and growth of *Asparagus officinalis* were inhibited at 2% NaCl. Salinity results in a decline in the metabolic activity of plant cells which is reflected in an inhibition of their growth. Moreover, Asparagus exihibits different degrees of salt tolerance depending on the level of organization. Plantlets consisting of several shoots and rhizomes were found to be very tolerant to salinity. One shoot segments was less tolerant than plantlets. Callus was most sensitive tissue to salinity compared with shoots and rhizomes.

Protein content

The influence of salt stress on total protein content of shoot bud cultures of onion was studied. Data of Fig. (3) generally reveal that there is a positive correlation between protein content and salt level. The highest value of protein was recorded at 6000 ppm. It is important to mention that the value of protein content in shoot bud cultures of onion were relatively higher than in callus cultures when exposed to the same salt levels. It may be attributed to the difference between differentiated cells (shoot buds) and undifferentiated cells (callus) in their tolerance mechanisms. In this respect, Dubey (1994) reported that one of the salt tolerance mechanisms in plant cells is the avoidance of dehydration by increasing the content of solutes in the cells following its rehydration, a process called osmoregulation. The solutes may be salt ions or organic substances such as protein. Moreover, there are many reports about plant membrane proteins that change in expression or activity in response to salt stress: i.e., H+ -ATPase (Low *et al.*, 1996; Niu *et al.*, 1993), Na+/H+ antiporter (Barkla and Blumwald, 1991). On the other hand, some researchers reported that, under stress conditions, only proteins that specifically respond to stress (stress-induced proteins) are induced in many plants (Ben-Hayyim *et al.*, 1989; Ferguson *et al.*, 1994).

Isozyme anaysis

Esterase patterns was carried out to characterize the *in vitro* selected lines of onion. The esterase zymogram patterns (Fig. 3) showed that the four selected lines exhibited two active bands; one fast and the other relatively slow. The fast one is not present in control (non-selected line). These results was confirmed with growth tolerance data. Moreover, shoot buds exposed to 2000 ppm of salt mixture which gave the most active and more intense esterase in the gel showed the highest value of salt tolerance ratio. The salt tolerance may result from adaptation to the salinity stress or due to genetic variability. As the enzymes are genes products, the genetic makeup of a given lines can be established on information gained from isozyme analysis. The correlation between the increasing level of salt tolerance and the expression level suggests that the altered expression of tolerance genes may be functionally involved in the ability of cells to survive and grow in salt containing medium. In this respect, biochemical markers have been reported to distinguish cell lines of different regenerants produced *in vitro* (Ultrika *et al.*, 1993; El-Kazzaz and Taha, 2002).

Rooting and acclamatization of plantlets

For some species, this stage may also include a shoot development or elongation phase, whereby the multiplied shoots are very short. In the present work, the roots were induced on elongated tolerant shoots within four weeks of culturing on MS-medium supplemented with 1 mg/l IBA (Fig. 1-D). Then the complete plantlets were succufully transplanted into free-living conditions within four weeks of acclamatization period using pots contained equal volume of perlite and peat moss (Fig. 1-E). In this respect, it is worthy to mention that the transfer of rooted plantlets from aseptic tissue culture conditions to growth in an external environment had always to be done carefuly or significant numbers of plants may be lost. Usually *in vitro* roots frequently lack root hairs. Moreover, the internal anatomy and ultrastructure of shoots propagated *in vitro* was often different to that of greenhouse or field grown plants (Dunstan and Sutter, 1982; Wetzatein and Sommer, 1982).

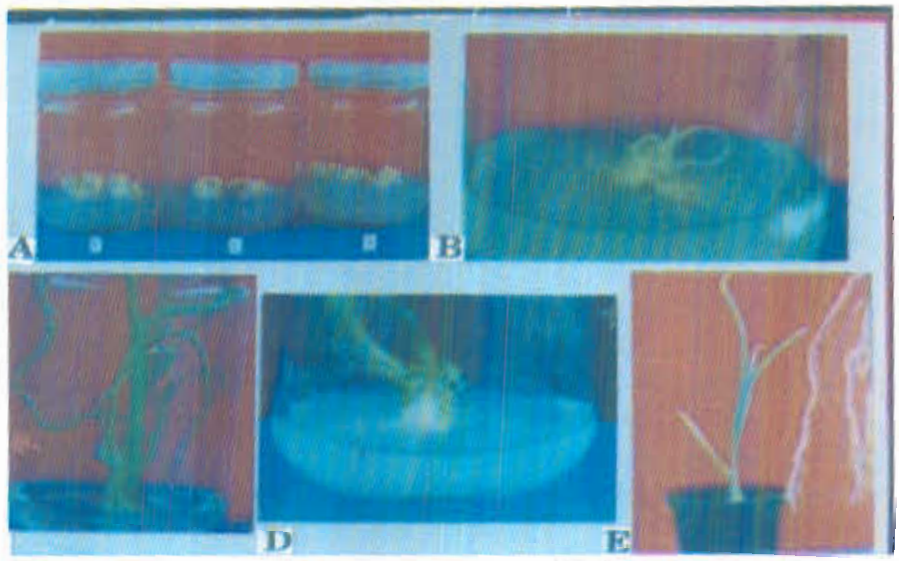

Fig. (1): A-Callus cultures of onion growing on medium contained 2000(1), 4000(2) and 6000ppm (3) of salts.
B-Regenerated shoot buds derived from callus exposed to 4000ppm of salts.
C-Tolerant shoot buds growing on medium contained 2000 ppm of salts.
D-Rooted plantlets cultured on MS+1 mg/l IBA.
E-Four week's adapted plantlets of onion.

Table (1): Effect of different combinations of growth regulators on in vitro morphogenesis of onion tissue cultures.

Growth regulators (%)	Callus frequency (%)	Fresh weight (g)	Embryonic callus (%)	Regeneration capacity (%)
1 mg/l 2,4-D +1 mg/l BA	50	0.68 ± 0.03	20	20
2 mg/l 2,4-D +1 mg/l BA	75	0.80 ± 0.08	35	30
1 mg/l 2,4-D +1 mg/l Kin	56	1.10 ± 0.06	40	30
2 mg/l 2,4-D +1 mg/l Kin	60	1.53 ± 0.05	50	40
1 mg/l NAA +1 mg/l BA	30	0.49 ± 0.05	15	5
2 mg/l NAA +1 mg/l BA	30	0.65 ± 0.02	20	10
1 mg/l NAA +1 mg/l kin	40	0.60 ± 0.05	20	15
2 mg/l NAA +1 mg/l kin	60	0.85 ± 0.05	35	25

± SE =Standard Error. Each treatment is the average of 20 replicates.

Table (2): Effect of different concentrations of salt mixture on growth namics of callus cultures of onion.

Salt level (ppm)	Fresh weight (g)	Dry weight (mg)	Dry matter (%)	Growth value	Salt tolerance ratio
0.0	1.30 ±0.25	20 ±5.00	9.20	4.20	-
2000	1.20 ±0.33	125 ±6.00	10.40	3.80	0.92
4000	1.05 ±0.30	130 ±4.00	12.30	3.20	0.80
6000	0.80 ±0.20	110 ±3.00	11.10	2.20	0.69

± SE = Standard Error. Each treatment is the average of 15 replicates.

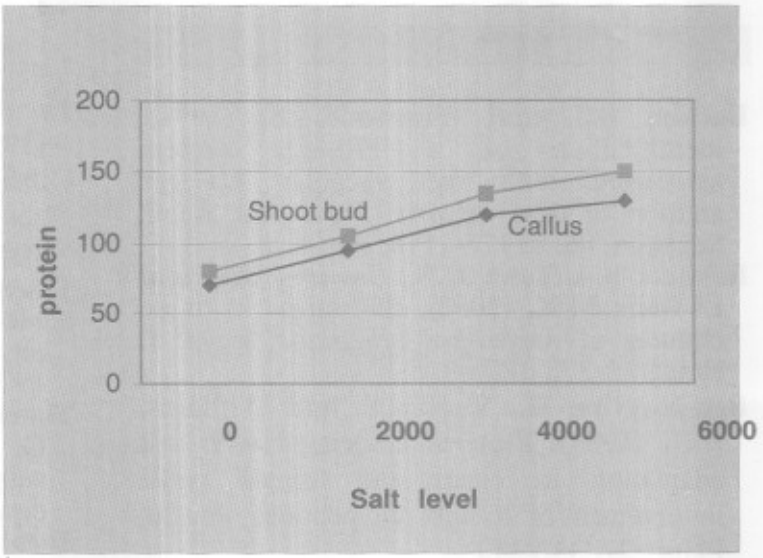

Fig. (2): Effect of salt level (ppm) on total protein (mg/g F.W) of onion tissue cultures.

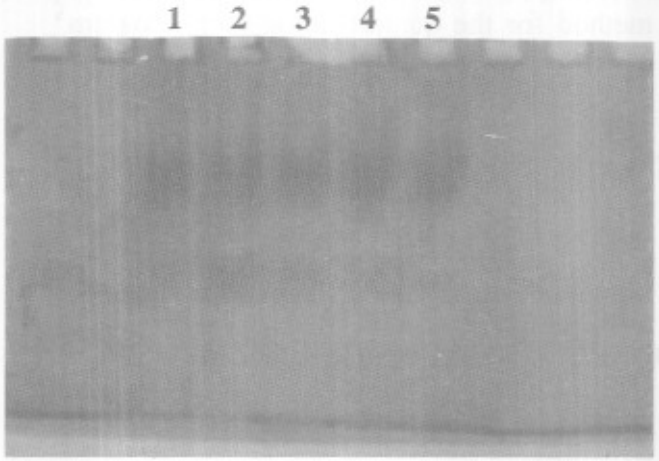

Fig. (3): Esterase enzyme patterens of shoot buds regenrated from salt tolerant lines exposed to 0.0 (1), 2000(2), 4000(3) and 6000 ppm of salt mixture (4) in addition to non-selected shoot bud cultures(5).

Table (3): Effect of different concentrations of salt mixture on growth dynamics of regenerated shoot bud cultures of onion.

Salt level (ppm)	No. of proliferated buds	shoot buds length (cm)	Fresh weight (g)	Growth value	Salt tolerace ratio
0.0	4.60 ± 0.05	4.00 ± 0.02	3.68 ± 0.30	6.36	-
2000	4.10 ± 0.03	3.50 ± 0.03	3.48 ± 0.08	5.96	0.94
4000	3.50 ± 0.04	2.80 ± 0.05	3.00 ± 0.05	5.00	0.81
6000	2.00 ± 0.08	1.90 ± 0.02	2.60 ± 0.04	4.20	0.70

± SE = Standard Error. Each treatment is the average of 15 replicates.

REFERENCES

Barkla, B.J. and Blumwald, E. (1991). Identification of a 170-kDa protein associated with the vacuolar Na+/H+ antiport of *Beta vulgaris*. Proc. Natl. Acad. Sci.USA, 88: 11177-11181.

Bekheet S.A.,Taha H.S., Sawires E.S. and El-Bahr M.K. (2000). Salt stress in tissue cultures of *Asparagus officinalis*. Egyp. J. Hort., 27: 275-187.

Ben-Hayyim, G., Vaadia,Y. and Williams, B.G. (1989). Proteins associated with salt adaptation in citrus and tomato cells: involvement of 26 kDa polypeptides. Physio. Plant., 77: 332-340

Bradford, M.M (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Annals Biochem., 72: 248.

Chen, Y., Zahavi, E., Barek, P. and Umiel, N. (1980). Effect of salinity stresses on tobacco 1.The growth of *Nicotiana tabacum* callus cultures under water,NaCl and Mannitol stress. Z. Pflanzenphysiol., 98: 141-143.

Dix, P.J. (1985). Cell line selection. In: Plant Cell Culture Technology, (M.M.Yeoman,Ed.). Pp.141-199. Oxford,-Blackwell Scientific.

Dubey, R. (1994). Protein synthesis by plants under stressful conditions. In: "Handbook of Plant and Crop Stress", pp:277-299.P.Mohammed(ed.) marcel ,Dekker, New York.

Dunstan, S.K. and Sutter, E. (1982). Effect of greenhouse acclimatization and various light intensities *in vitro* on the anatomy of tissue cultured plants. HortScience, 17: 532.

El-Bahr, M. K., Okasha, Kh.A. and Bekheet, S.A. (2001). Effect of salt stress on callus cultures of Globe artichoke. Arab Univ. J. Agric. Sci.,Ain Shams Univ.Cairo, 9: 783-792.

El-Kazzaz, A.A. and Taha, H.S. (2002). Tissue culture of broccoli and molecular characterization .Bull. NRC, Egypt, 27: 481-490.

Ferguson, I.B. , Lurie, S. and Bowen, J.H. (1994). Protein synthesis and breakdown during heat shok of cultured pear (*Pyrus communis* L.) cells. Plant Physiol., 104:1429-1437.

Greenway, H. and Munns, R. (1980). Mechanisms of salt tolerance in nonhaophytes. Annu. Rev. Plant Physiol., 31:149-190.

Hansen, E., Hubstenberger J. and Phillips G. (1995). Regeneration of shoots from cell suspension-derived protoplasts of *Allium cepa*. Plant Cell Rept., 15:8-11.

Ibrahim, A. and El-Kobbia, T. (1986). Effect of antitranspirants on growth and salt accumulation in the root zone of tomato plant under saline conditions. Symposium on Reclamation on Salinity and Alkalinity Soils in Arab World. Iraq,17-20 March.

Low, R., Rockel, B., Kirech, M., Ratajczak, R., Hdrtensteiner, S., Martinoia, E., Luttge, U. and Rausch, T.(1996). Early salt stress effects on the different expression of vacuolar H+-ATPase genes in roots and leaves of *Mesembyanthemum crystallinum*. Plant Physiol., 110: 259-265.

Luthar, Z. and Bohanec, B. (1999). Induction of direct somatic organogenesis in onion (*Allium cepa* L.) using a two-step flower or ovary culture. Plant Cell Rept., 18: 797-802.

Mills, D. (1989). Differential response of various tissues of *Asparagus officinalis* to sodium chloride. J.Exper.Bot., 40: 485-491.

Niu, X., Narasimhan, M.L., Salzman, R.A. , Bressan. R.A. and Hasegawa, P.M. (1993). NaCl regulation of plasma membrane H+-ATPase gene expression in glycophyte and a halophyte. Plant Physiol., 103: 713-718.

Poljakoff-Mayber, A. (1982). Biochemical and physiological responses of higher plants to salinity sress. In: San Pietro, A. (ed), Biosaline research. A look to the future. pp. 245-270. Plenum Press,New York.

Roy, S. (1995). *In vitro* studies on callus initiation and organogenesis in *Allium cepa* var. Pusa Red. J. Phytol. Res., 8: 57-61.

Snedecor, G.W. and Cochran,W.G.(1967). "Statistical Methods",6th ed.,Iowa Univ. Iowa,USA.

Tal, M. (1983). Selection for stress tolerance. In: Handbook of Plant Cell Culture, Volume 1, (D.A.Evans; W. R.Sharp P.V. Ammirato and Y. Yamada, eds) pp. 481-488. New York, Macmillan.

Umiel, N., Zahavi, E. and Chen,Y.(1980). Effect of salinity sresses on tobacco, 2.Short term kinetics of Na+ and K+ uptke by callus cultures grown on media containing NaCl. Z. Pflanzenphysiol., 100 : 363-367.

Ultrika, E. Lars, H.M. and Sar, V.A. (1993). Extracellular proteins in embryogenic suspension cultures of Norway spruce, *Picea abies* L. Physiol.Plant., 88: 315-321.

Wetzatein, H.Y. and Sommer, H. E. (1982). Leaf anatomy of tissue cultured *Liquidambar styraciflua* (Hamamelidaceae) during acclimatization. Amer.J. Bot., 69: 1579-1586.

Zheng, S., Henken B., Sofiari E , Jacobsen E , Krens A. and Kik C. (1998). Factors influencing induction, propagation and regeneration of mature zygotic embryo-derived callus from *Allium cepa*. Plant Cell, Tissue and Organ Culture, 53: 99-105.

المستخلص العربي

تحمل الملوحة فى مزارع أنسجة البصل

شوقى عبد الحميد بخيت- حسين سيد طه- محيى الدين سليمان
قسم التكنولوجيا الحيوية النباتية- المركز القومى للبحوث- القاهرة- مصر.

تم دراسة استجابة مزارع أنسجة البصل لاجهاد الملوحة. حيث تم تعريض الكالوس المستولد من الشتلات المستنبتة تحت الظروف المعقمة الى مستويات مختلفة من مخلوط الأملاح. أشارت النتائج الى أن الوزن الرطب وقيمة نمو الكالوس قد تناقصا مع زيادة تركيز الأملاح فى بيئة النمو حتى تركيز 4000 جزء/ مليون. محتوى البروتين الكلى فى مزارع الكالوس قد زاد أيضا مع تزايد تركيزات الأملاح. للانتخاب المعملى للملوحة، أخذت البراعم السوقية المستولدة من الكالوس الذى ثبت تحمله لمستويات الملوحة المختلفة وزرعت على بيئات تحتوى على نفس تركيزات الأملاح. تأثر عدد البراعم السوقية المفرخة ووزنها الرطب وكذلك معدل النمو سلبا بزيادة تركيز الملوحة فى بيئة الزراعة. وقد سجل أعلى معدل لتحمل الملوحة عند تركيز 2000 جزء/ مليون من مخلوط الأملاح. على الرغم من أن محتوى البروتين الكلى أخذ نفس اتجاه مزارع الكالوس الا أن مستواه فى مزارع السوق كان أعلى نسبيا عند نفس تركيزات الأملاح. أشارت أنماط التفريد الكهربى لانزيم الاستيريز الى ظهور حزمة جديدة فى السلالات التى أظهرت تحملا للملوحة. هذه الحزمة كانت أسرع فى معدل حركيتها وأكثر كثافة فى السلالة التى نمت على تركيز أملاح 2000 جزء/مليون. البراعم السوقية لنبات البصل المتحملة لتركيزات الأملاح المختلفة تم تجذيرها معمليا و أقلمتها بنجاح للنمو فى الظروف البيئية الحرة(خارج الأنابيب).