Al-Azhar J. Agric. Res., Vol. 39
PP. 27-36, (June), 2004

RESPONSE TO SALT STRESS IN SWEET POTATO CELL CULTURES AND THE CHANGES IN ELECTROPHORETIC PATTERNS OF THE SOLUBLE PROTEIN

A. I. H. Sayed* and A. A. El-Fiki, M. A. M. Moustafa****

*Faculty of Agriculture, Botany Dept., Al-Azhar University, Nasr City, Cairo, Egypt.
**National Center for Radiation Research and Technology, P.O. Box 29, Nasr City, Cairo, Egypt.

ABSTRACT : Two local sweet potato (Ipomoea batatas L. Lam) varieties were selected for this study, Mabruka and Abees. Explants from leaf and shoot were cultured on MS medium supplemented with 0.5 mg^{-1} KIN + 0.5 mg^{-1} 2, 4- D for callus induction. The produced callus were transferred to regeneration medium (MS + 10 mg^{-1} BAP + 20 g^{-1} sucrose or 2 mg^{-1} KIN + 20 g^{-1} sucrose or/and 2 mg^{-1} BAP + 2 mg^{-1} 2, 4- D + 20 g^{-1} sucrose). The calluses were examined on MS medium containing different NaCl concentrations (0, 4000, 6000 and 8000 ppm). Callus growth rate and callus morphology were evaluated. Callus growth rate were decreased with 55-60 %. Calli were survival on different salinity levels transferred to regeneration medium. Examined calli failed to regenerate plants under salt stress. Single nodes pieces were cultured on MS medium supplemented with 1 mg^{-1} KIN + 0.4 mg^{-1} NAA and containing the same NaCl concentrations. The survival, shoot length and some chemical analysis e. g., mineral elements, carbohydrate, phenolic compounds, proline content and protein content were studied. Survival and shoot length were affected with salinity treatments whereas decreased 34-55 %. Chemical analysis some of them were decreased such as: mineral elements (K, Ca & Mg), carbohydrate (total carbohydrate and reducing sugars), and other increasing as: Na, phenolic compounds, proline content and protein content. The effects of salt stress on protein banding patterns (SDS-PAGE) induced qualitative and quantitative changes and were very little. These changes were in appeared new bands, disappeared some bands and in band intensity.

Key words: callus culture, micropropagation, mineral elements, protein, proline, salinity.

INTRODUCTION

Salinity is a major factor limiting the crop productivity in the semi arid area of the world. The difficulty of the irrigated land world wide, approximately 33% is salt affected with Na+ and Cl- as the most common ions (Munns and Termat 1986). Higher salt concentrations decrease crop yields, increase leaching and drainage requirements and increase water management costs, thus the identification of the physiological mechanisms limiting plant growth under salt stress would be a significant step in improving the salt tolerance of cultivated plants (Caines and Shennan, 1999). Salinity inhibits plant growth in three principle ways: by ion toxicity (mainly of Na+ and Cl-), osmotic stress and nutritional disruption (Lauchli, 1986). These include genetic variability between species and among cultivars with-

in species and duration and timing of exposure to salinity (Caines and Shennan 1999). Cushman et al. (1990) reported that salt tolerance of halophytes depends on the constitutive expression of several genes in response to salt stress. There are no reports on systematic breeding attempts for salt tolerance in potato and conventional breeding systems have met with limited success in improving the response of many other crops to salt stress (Epstein et al., 1980). Therefore, the use of unconventional crop improvement methods such as tissue culture selection. Beside its use as a tool for obtaining salt tolerant plants through selection of salt tolerant cell lines (Naik and Widholm, 1993), plant tissue culture techniques may offer a potential for quick evaluation of germplasm against salt stress. If tissue culture methods are to be used it is necessary for the salt tolerance expressed by whole plants also to be manifested at the level of cell or isolated organs such as roots or stem segments. The present paper study the alteration which were induced with salinity on callus culture, bud culture, proline content, carbohydrate, phenolic compounds, mineral elements, total protein and electrophoretic patterns of the soluble protein.

MATERIALS AND METHODS

1. *Source of explants:* Two local varieties selected for this work, Mabruka and Abees were obtained from International Potato Center (CIP), region IV, North Africa and Middle East, Kafr El-Zayat, Egypt. After surface sterilization by soaking for 15 min. in 30% Clorox, they were thoroughly washed to assure that any residues of Clorox had been removed. Stem cuttings isolated from mother plants were used to prepare leaf blade pieces, stem internodes and single nodal piece explants. Explants were cultured on MS medium (Murashige and Skoog, 1962). Depending on the aim of experiments, various plant growth regulators (BAP, GA3, NAA, KIN, IAA, IBA and 2, 4 - D) were added to the basic medium.

2. *Callus induction:* Explants were cultured on MS medium supplemented with $1mg^{-1}$ 2,4- D, 0.5 mg^{-1} 2,4 - D + 0.3 mg^{-1} KIN, 0.5 mg^{-1} 2,4- D + 0.5 mg^{-1} KIN, 0.5 mg^{-1} NAA + 0.5 mg^{-1} KIN, 0.3 mg^{-1} NAA + $1mg^{-1}$ KIN and $1mg^{-1}$ GA_3 + $3mg^{-1}$ BAP.

3. *Effect of salinity:* Calli were produced from different explants tested on different NaCl concentrations (0, 4000, 6000 and 8000 ppm). Single nodal pieces also were examined on the same NaCl concentrations. The response of in vitro plantlets, callus formation and callus growth were investigated.

4. *Multiplication:* Single nodal pieces were cultured on MS medium supplemented with $1mg^{-1}$ KIN + 0.4 mg^{-1} NAA^{-1} after 6-8 weeks when the plantlets 5 cm high.

5. *Plant regeneration:* Calli were obtained from different explants control and tested on salinity stress grew on MS medium supplemented with different combined hormone concentrations e. g., IAA, BAP, KIN, IBA and 2- 4, D Table (2).

6. *Culture condition:* The pH of culture medium was adjusted to 5.8 before autoclaving. The cultures were incubated with a photoperiod 16 h at 24 ± 2°C and 3000 lux light intensity (cool white fluorescent lamp).

7. *Chemical analysis:*

1. Mineral elements (Na, K, Ca and Mg)

a. Na and K values (mg / g dry weight) were determined by using flame photometer according to Knudsen et al. (1982).

b. The determination of Ca and Mg values (mg / g dry weight) were used versinate method according to Kingston and Jassie (1988).

2. Total soluble sugar was determined by using phenol - sulphoric method according to Dubois et al. (1966). Reducing sugar was determined by using dinitrosalicylic acid method according to Miller (1959).

3. Phenolic compounds were determined according to the method by Swain and Hillis (1959).

4. Proline content was determined by using sulphosalicylic acid and ninhydrin spray method according to Bates et al. (1973).

5. Protein analysis

A. Total soluble protein was determined by the method described by Lowery et al. (1951).

B. Separation and quantification of protein, Sodium Dodecyle Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed according to the method described by Laemmli (1970) and modified by Studier (1973).

Densitometric scanning of the gel were made using Epson GT- 8000 scanner, automatic scanning and statistical analyses soft ware ScanPack III, obtained from "Biometra".

2.8. Statistical analysis: Data were analyzed using the ANOVA procedure of the statistical analysis system SAM (V.6.03 SAS Institute Inc).

RESULTS AND DISCUSSION

1. *Callus Culture:*

1.1. *Callus induction*

Leaf and shoot explants were cultured on MS medium supplemented with different hormones and with different concentration. Most of them were able to induce the callus from both explants. The best medium used in callus induction and callus morphology was MS medium supplemented with 0.5mg $^{-1}$ 2, 4- D + 0.5 mg^{-1} KIN.

1.2. *Plant regeneration*

Calli derived from both explants were cultured on MS medium supplemented with different hormone concentrations and sugar concentration. Plant regeneration produced from MS medium supplemented with 10 mg^{-1} BAP + $20g^{-1}$ sucrose, 2.0 mg^{-1} KIN + $20g^{-1}$ sucrose and 2.0 mg^{-1} BAP + 2.0 mg^{-1} 2, 4- D + $20g^{-1}$ sucrose. The addition of BA and sucrose to medium were essential for shoot induction from somatic embryogenesis Roest and Bokelmann, (1976). The organogenesis has been induced in sweet potato through somatic embryogenesis using shoot tips and other

explants but the plant regeneration in sweet potato, the frequencies of regeneration reported are not high (Liu and Cantliffe, 1984).

1.3. *The effect of NaCl on callus culture:*

Calli formed from different explants in both genotypes (Table 1) were tested on MS medium supplemented with 0.5 mg^{-1} KIN + 0.5 mg^{-1} 2, 4- D and containing different NaCl concentrations (0, 4000, 6000 and 8000 ppm). The callus growth rate was decreasing with increasing NaCl concentrations in both genotypes. Root formation also was decreased with increasing NaCl concentration. Calli were grown on saline medium subculture on the same NaCl medium. The survival calli were cultured on MS regeneration medium. This calli failed to plant regeneration in both genotypes. Callus and suspension cultured to various concentrations of NaCl or mannitol were developed from the cultivated potato Solanum tubersom cv. Desire. Growth of the calli was less inhibited by mannitol than by iso-osmotic concentrations of NaCl. Reduction of growth by both NaCl and mannitol was considerably lower in osmotically adapted calli than in non-adapted ones. Salt adapted suspension cultures that grew in the medium to which they been originally adapted had a shorter lag in growth as well as a shorter time required to achieve the maximum growth, as compared with non adapted cells. Suspension cultures adapted to NaCl concentrations higher than 150 mM were obtained only after pre-adaptation to osmotic stress. Adaptation of these cells was found to be stable (Sabbah and Tal, 1990). It is very difficult to regenerate plants from cell culture, and when this possible, usually the trait is not expressed in the plant (Tal, 1993). These results were agreed with Hassan and Wilkins (1988) whereas failed to get regenerate plants from selected cells in Lycopersicon peruvianum.

4. *Tissue Culture:*

4.1. Effect of NaCl on bud survival

Single nodes were cultured on MS medium supplemented with 1mg^{-1} KIN + 0.4mg^{-1} NAA and containing different NaCl concentrations (0, 4000, 6000 and 8000 ppm). Bud survival and shoot length was decreased with increasing saline concentration in both genotypes. Abees genotype was sensitive more than Mabruka (Table 2). The saline treatments were significant on bud survival percentage and shoot length in both genotypes ($P > 0.05$). Salinity is one serious and widespread agricultural problem resulting in losses of yield. The major effect of NaCl is an alteration on cell wall extensibility. The adaptation of tobacco cells involves considerable osmotic adjustment with an altered relationship between turgor and cell expansion. The increase in the ratio of membrane area to volume in small cells can facilitate ion transport and maintenance of ion compartmentation Hasegawa et al. (1986). It has been proved that the cell culture was very effective to obtain salt resistant cell lines in many species, mainly diploid as well as polyploid (Tal, 1989).

4.2. Chemical analysis:

1. Mineral elements (Na, K, Ca and Mg)

The accumulation of Na, K, Ca and Mg ions were estimated in both sweet pota-

to plantlet genotypes. The Na content in both genotypes was increased with increasing NaCl concentrations. The other ion contents (K, Ca and Mg) were decreased with increasing salinity levels in both genotypes Table (2). The results here agree with Sabbah and Tal (1990) on potato, and Cano et al. (1996) on tomato.

2. Carbohydrate :

Total carbohydrate and reducing sugar:

Total carbohydrate and reducing sugar were decreased with increasing NaCl concentration Table (2). The present results are harmony with Potluri and Prasad (1993) on potato and Sawires et al. (1997) on pea and bear.

3. Phenolic compounds:

Data in Table (2) detected that saline media induced increasing in phenolic compounds in both sweet potato plantlets genotypes were grew on them. These results are in agreement with Winston, (1990).

4. *Proline content :*

NaCl concentration induced increasing in proline content in both sweet potato plantlet genotypes. The increasing rate was increased with increasing salinity levels Table (2). Proline has been shown to accumulate in plant cells exposed to salt or water stress (Chandler and Thorpe, 1986). The response to osmotic hallenge, the synthesis of compatible solutes occurs and supports the hypothesis that proline acts as a protective compound during salt stress (Martinez et al. 1996). These results are in agreement with Sangita-Basu et al. (1999) on callus of Orysa sativa.

5. *Total protein:*

The effect of NaCl on total protein in both genotypes Mabruka and Abees were estimated. Data in Table (2) shows increasing in total protein in both genotypes at all salinity level. Protein content in cv. Mabruka was higher than cv. Abees. Protein breakdown and turnover in Vigna sinesis seeds during the germination was delayed by NaCl treatment as compared to the control. This was not to total amount of proteolytic activity that was unchanged by salinity. They suggested that the inhibitory effects of salinity on seed protein reserve mobilization might be due to inhibition of translocation of hydrolysis products that to inhibition of protease activity Gomes-Filho et al. (1983). The results were agreed with (Sawires et al., 1997) whereas the protein content was increased during salinity.

4.3. Biochemical genetic marker SDS-PAGE electrophoresis:

Effect of NaCl concentrations on protein banding patterns in sweet potato plantlet Mabruka and Abees.

The changes in protein banding patterns were studied in sweet potato plantlets grew on different NaCl concentrations (0.0, 4000, 6000 and 8000 ppm). The qualitative and quantitative changes in protein bands were very little. The effect of salinity induced disappeared one new band with molecular weight (1595 KDa). There was a detectable marker in plantlets grew on salinity stress. The induced variations with NaCl concentrations in plantlets were little (2-9%). The variation percentage

induced with NaCl concentrations (4000, 6000 and 8000ppm) were (2, 9 and 2%, respectively). Table (3) represents the salinity effects on banding patterns in plantlet cv. Mabruka. The number of protein bands were (18) unaffected with saline conditions. The effect of 4000 ppm NaCl on protein bands induced appeared three new bands with molecular weights (1225, 201 and 31 KDa, respectively) and protein band intensity (4.4, 1.1 and 1.5%, respectively). Three bands with molecular weights (1595, 113 and 59 KDa, respectively) were absent. The concentration of 6000 ppm NaCl caused present. Three new bands with molecular weights (1095, 770 and 38 KDa, respectively) and band intensity (2.2, 1.8 and 2.4%, respectively). Three bands with molecular weights (1595, 669, 59 KDa, respectively) were disappeared. The plantlets were grown on 8000 ppm NaCl induced appeared to one new band with molecular weight (1875 KDa) and protein band intensity (5.8 %). One band with molecular weight (1595 KDa) was disappeared. On the other hand, Salinity effects on cv. Abees induced appeared one new band with molecular weight (18 KDa) and disappeared Ten bands with molecular weights (1096, 479, 358, 192, 149, 102, 81, 27, 23 and 22 KDa, respectively). There was a detectable protein a marker in cv. Abees grew on salinity stress. The salinity stress (4000 and 6000 ppm NaCl) induced variation in plantlet protein cv. Abees with (41-42%) respectively. Data in Table (4) showed the effect of NaCl concentrations (4000 and 6000 ppm) on protein banding patterns in sweet potato plantlet cv. Abees. The effect of salini-

Table (1): Effect of NaCl concentrations on callus growth rate and No. of roo sweet potato.

Genotype	NaCl conc./ppm	Explants			
		Shoot		Leaf	
		Mean of callus size/cm	No. of root/calus	Mean of callus size/cm	No. of root/callus
Mabruka	0	0.69	8	0.72	2.6
	4000	0.39	5.6	0.19	0
	6000	0.27	3.6	0.07	0
	8000	0.14	0	0.05	0
Abees	0	0.66	3.3	0.6	2.8
	4000	0.38	0.6	0.18	0
	6000	0.21	0	0.12	0
	8000	0.06	0	0.05	0

Table (2): Effect of salinity on bud survival, shoot length, meniral elements, carbohydrate, phenolic compounds, proline content and total protein in sweet potato.

Geno-type	NaCl Conc. /ppm	Survi-val %	Mean of shoot leng-th/cm	Mineral elements				Carbohydrate		Phen-olic comp ounds	Prolin cont-ent	Toral prot-ein
				Na	K	Ca	Mg	Redu-cing sugar	Total Carb.			
Mabruka	0	96	5.4	660.0	661.6	922.3	910.1	147.7	5693.3	1407	1.74	0.107
	4000	82	4.9	888.6	332.1	810.7	882.5	138.8	4733.3	1618	1.88	2.39
	6000	73	4.5	992.0	225.5	796.5	772.0	134.4	3710.0	1681	1.95	0.968
	8000	41	3.9	997.0	223.8	771.2	645.2	90.0	1288.2	1905	1.97	5.11
Abees	0	87	5.0	567.7	552.3	900.1	862.2	118.8	5433.3	681	0.46	1.13
	4000	70	4.6	775.0	227.7	807.9	781.0	101.1	3166.6	1008	0.53	2.23
	6000	53	4.0	886.6	224.0	739.6	689.9	84.4	2783.3	1358	1.02	2.8

Table (3): Comparative analysis of salinity on molecular weight and protein band intensity in sweet potato plantlets cv. Mabruka using SDS-PAGE technique.

Treatment	NaCl Concentration / ppm							
	Cont.		4000		6000		8000	
No. of band	Band %	M.W	Band %	M.W	Band %	M.W	Band %	M.W
1	2.9	1595	4.4	1225	2.2	1095	5.8	1875
2	2.1	669	2.6	628	1.8	770	3.9	669
3	4.7	457	4.9	418	3.0	418	2.0	401
4	3.7	333	3.0	302	2.2	312	5.0	312
5	8.5	277	6.1	248	5.6	255	8.3	248
6	3.6	171	1.1	201	2.5	168	2.9	171
7	3.2	124	4.1	171	1.3	129	2.5	129
8	2.5	113	4.4	124	1.5	111	1.2	113
9	3.0	97	5.0	96	3.1	97	3.8	99
10	3.1	80	3.7	80	2.3	80	3.3	80
11	1.3	65	3.7	65	2.1	65	1.9	65
12	0.7	59	13.2	45	24.9	45	0.9	56
13	11.1	45	3.0	41	3.1	41	13.1	46
14	3.2	42	3.7	33	2.4	38	3.5	42
15	4.7	33	1.5	31	2.2	33	1.7	34
16	7.6	28	9.5	28	5.4	28	2.8	29
17	2.2	24	3.1	24	2.6	25	2.8	25
18	2.8	23	0.5	21	1.4	23	1.8	23
Total No. of band	18		18		18		18	

Table (4): Comparative analysis of salinity on molecular weight and protein band intensity in sweet potato plantlets cv. Abees using SDS-PAGE technique.

Treatment	NaCl concentration / ppm					
	Cont.		4000		6000	
No. of band	Band %	M.W	Band %	M.W	Band %	M.W
1	5.1	1096	0.3	125	1.0	268
2	1.4	479	0.3	106	0.7	170
3	3.2	358	0.4	93	0.8	137
4	4.9	285	0.8	49	0.8	115
5	1.9	192	0.6	46	0.6	91
6	1.5	149	1.0	39	0.5	63
7	1.2	126	3.0	[illegible]	1.0	53
8	1.0	108	6.8	29	2.7	40
9	203	102	22.4	19	3.5	34
10	201	81	2.4	18	5.6	29
11	32.0	47	-	-	24.9	20
12	3.8	41	-	-	9.9	18
13	1.2	38	-	-	-	-
14	2.0	34	-	-	-	-
15	4.2	28	-	-	-	-
16	4.2	27	-	-	-	-
17	2.7	23	-	-	-	-
18	1.7	22	-	-	-	-
Total No. of band	18		10		12	

ty were observed in qualitative and quantitative changes such as appearance of new bands, disappearance of some bands and changes in band intensity. The control showed the highest number of band was (18). The plantlets were grown on 4000 ppm showed the lowest number of band (10). The effect of 4000 ppm NaCl concentration on protein bands caused decreasing on bands eight bands. Four new bands with molecular weights (93, 49, 19 and 18 KDa, respectively) and with band intensity (0.4, 0.8, 22.4 and 2.4%, respectively) were appeared. Twelve bands with molecular weights (1096, 476, 358, 285, 192, 149, 102, 81, 41, 27, 23 and 22 KDa, respectively) were disappeared Fig. (6). The use of 6000 ppm NaCl concentration induced decreasing in protein band six bands. Eight new bands with molecular weights (170, 137, 115, 91, 63, 53, 20 and 18 KDa, respectively) and with band intensity (0.7, 0.8, 0.8, 0.6, 0.5, 1.0, 24.9 and 9.9%, respectively) were presents. Fourteen bands with molecular weights (1096, 476, 358, 192, 149, 126, 108, 102, 81, 47, 38, 27, 23 and 22 KDa, respectively) were absents. These results are in agreement with Ericson and Alfinito, (1984) who found that there are some proteins that are either enhanced in or are unique to culture to tobacco cells growing under salinity stress. Hurkman and Tanaka (1987) demonstrate that the protein patterns for control and salt stressed plants were qualitatively similar, the net synthesis of a number of proteins was quantitative changed.

REFERENCES

Bates, L.S., Waldern, R. P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39:205.

Caines, A. M. and Shennan, C. (1999). Interactive effects of Ca 2+ and NaCl salinity on the growth of two tomato genotypes differing in Ca 2+ use efficiency. Plant. Physiol. Biochem , 47 (718) , 569 ñ 576 .

Cano, E. A., Perez-Alfocea, F., Moreno, V. and Bolarin, M. C. (1996): Responses to NaCl stress of cultivated and wild tomato species and their hybrids in callus cultures. Plant Cell Report, 15: 791-794.

Chandler, S.F. and Thorpe, T. A. (1986). Variation from plant tissue cultures. Biotechnological application to improving salinity tolerance. 4: 117.

Cushman, J.C., DeRocher, E.C. and Bohnert, H.J. (1990). Gene expression during adaptation to salt stress . In "Environmental Injury to plants" (Ed.R. Kattermann) pp.173 ñ 203. Academic press: New York.

Dubois, M., Smith, F., Gilles, K. A., Hamilton, J. K. and Rebers, P.A. (1966). Calorimetric method for determination of sugar and related substances Anal. Chem., 28 (3): 350.

Epstein, E., Norlyn, I. D., Rugh, D. W., Kingsbury, R. W., Kelly, D. B. and Cunningham, G. A. (1980). Saline culture of crops: a genetic approach. Science, 210: 399-404.

Ericson, M.H. AlFinito (1984). Protein produced during salt stress in tobacco cell culture. Plant Physiol. 74: 506-509.

Gomes- Filho, E. and Prisco, J. T. compos, F. D. and Eneas-Filho, J. (1983). Effect of sodium chloride in vivo and in vitro on RNAse activity of Vigna unguiculata cotyledons during germination. Physiol. Plant 59: 1183.

Hasegawa, P.M., Bressan, R.A. and Handa, A.K. (1986). Cellular mechanism of salinity tolerance. Hort. Science, 21: 1324-1356.

Hassan, N. H. and Wilkins, D. A. (1988): In vitro selection for salt tolerant lines in Lycopersicon peruvianum. Plant Cell Report, 7: 463-466.

Hurkman, W. J. and Tanaka, C. K. (1987). The effects of salt on the pattern of protein synthesis in Barley Roots. Plant Physiol. 83, 517-524.

Kingston, H. M. and Jassie, L. (1988). Introduction to microwave sample preparation ACS, washington DC, pp. 126-130.

Knudsen, D., Petrson, G. A. and Pratt. P. E. (1982). Lithium, Sodium and Potassium. In Method of Soil Analysis, Page A. L., Miller, R. H. and Kenney, D. R. (eds.). part 2, 2nd ed., pp. 225-245.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assemble of the head of bacteriophage T4. Nature, 227: 680.

Lauchli, A. (1986). Responses and adaptations of crops to salinity. ActaHort., 190: 243 ñ 246.

Liu, J. P. and Cantliffe, D. J. (1984). Somatic embryogenesis and plant regeneration in tissue culture of sweet potato (Ipomoea batatas Poir). Plant Cell Reports, 3: 112-115.

Lowery, O.N., Rosebrough, A. F. and Ronodall, R. (1951). Protein measurement with Folin reagent. J. Biol. Chem., 193.

Martinez, C. A., Maestri, M. and Lani, E. G. (1996). In vitro salt tolerance and proline accumulation in Potato (Solanum ssp.) differing in frost resistance. Plant science, 116: 177.

Miller, G. I. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31 (3): 624.

Munns, R. and Termat, A. (1986). Whole ñ plant responses to salinity. Aust. J. Plant. Physiol., 13, 143 ñ 160.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and biossay with tobacco tissue cultures. Physiologia Plantarum, 15: 473.

Naik, P. S. and Widholm, J. M. (1993). Comparison of tissue culture and whole plant responses to salinity in potato. Plant Cell, Tissue and Organ Culture, 33: 273-380.

Potluri, S. D. P. and Prasad, P. V. D. (1993). Influence of salinity on auxiliary bud cultivares of six lowland tropical varieties of potato (Solanum tuberosum). Plant Cell Tissue and Organ Culture, 32: 185.

Roest, S. and Bokelmann, G. S. (1976). Vegetative propagation of Solanum tuberosum L. in vitro. Potato Res., 19: 173-178.

Sabbah, S. and Tal, M. (1990): Development of callus and suspension cultures of potato resistant to NaCl and mannitol and their response to stress. Plant Cell, Tissue and Organ Culture, 21: 119-128.

Sangita- Basu, Gaurab Gangopadhyay, Poddar, R., Gupta, S., Mukherjee, B. B., Basu, S., Gangopadhyay, G. and Kishor, P. (1999). Proline and osmotic stress-tolerance in rice (Oryza sativa L.) plant tissue culture and biotechnology, emerging trends. Proceeding of a symposium held at Hyderadad, India 29.

Sawires, E. S., Saker, M. M. and Elbahr, M. M. (1997). Biochemical changes associated with salt stress in pea and bean tissue cultures. Egyptian Journal of Horticulture, 24: 2, 161.

Studier, F. W. (1973). Analysis of bacteriophage T7 early RNA and proteins and gels. J. Mol. Biol., 79: 237.

Swain, T. and Hillis, W. E. (1959). The quantitative analysis of phenolic constituent. J. Sc. Food Agric., 10: 63.

Tal, M. (1989). Somaclonal variation for salt resistance. In : Bajaj, Y.P.S. (ed). Biotechnology in Agriculture and Forestry. Springer VERLAG, Berlin.

Tal, M. (1993): Acta Hortic., 336: 69-78.

Winston, G. W. (1990). Physiochemical basis for radical formation in cells: Production and defenses. In stress responses in plant: Adaptation and Acclimatization Mechanisms. Edited by Alsche, R.G. and Cumming, J. R. pp. 57-86. Wiley, Inc., New York.

استجابة زراعة أنسجة البطاطا للأجهاد الملحى و التغيرات فى الطرز البروتينية للتفريد الكهربائى

عبدالهادى أبراهيم حسن سيد[1] و أيمن عبد المجيد الفقى[2] و محمد عدلى مصطفى[2]
قسم النبات الزراعى - كلية الزراعة – جامعة الأزهر[1] و المركز القومى لبحوث وتكنولوجيا الأشعاع[2]

الملخص العربى

صنفان محليان من البطاطا تم اختيارهم لهذه الدراسة هما ميروكة و أبيس فقد زرعت أجزاء من الورقة و الساق لكلا الصنفين على بيئة موراشيج وأسكوج مضاف اليه (0.5 mg^{-1} KIN + 0.5 mg^{-1} 2, 4- D) لاستحداث الكالوس و الكالوسات الناتجة تم نقلها الى بيئة تكوين النبات الكامل وهى بيئة موراشيج و أسكوج مضاف اليها (MS + 10 mg^{-1} BAP + 20g^{-1} sucrose or 2 mg^{-1} KIN + 20g^{-1} sucrose or/ and 2 mg^{-1} BAP +2 mg 2, 4- D^{-1} + 20g^{-1} sucrose)

هذه الكالوسات تم اختيارها على تركيزات من كلوريد الصوديوم المختلفة وهى (صفر , ٤٠٠٠ , ٦٠٠٠ , ٨٠٠٠ جزىء فى المليون وتم تقييم هذه الكالوسات من حيث معدل النمو و الشكل المورفولوجى وكان معدل النمو يتناقص بنسبة ٥٥ – ٦٠ % و الكالوسات التى نمت على هذه التركيزات المختلفة من الملوحة تم نقلها الى بيئة النبات الكامل وهذه الكالوسات قد فشلت فى تكوين النبات الكامل تحت ظروف الملوحة.

قطع من الساق (العقد) تم زراعتها على بيئة موراشيج وأسكوج مضاف اليها 1 mg^{-1} KIN + 0.4 mg^{-1} NAA ومحتوية على نفس التركيزات من الملوحة وتم دراسة تأثير هذه التركيزات من كلوريد الصوديوم على الحيوية وطول النبات وبعض التحاليل الكيماوية مثل العناصر المعدنية و الكربوهيدرات و المركبات الفينولية و المحتوى من البروتين و البرولين وقد تأثر كل من الحيوية وطول النبات بمعاملة الملوحة حيث تناقصت بنسبة ٣٤ – ٥٥ % أما بالنسبة للتحليل الكيماوى بعضها قد تناقص مثل العناصر المعدنية (البوتاسيوم و والكالسيوم و الماغنيسيوم) و الكربوهيدرات سواء الكربوهيدرات الكلى و السكريات المختزلة و البعض الأخر قد تزايد مثل مثل تركيز الصوديوم و المركبات الفينولية و المحتوى من البروتين و البرولين أما تأثير الملوحة على الطرز البروتينية فقد أدت الى احداث تغيرات قليلة من الناحية الكمية و الوصفية هذه التغيرات ادت الى اختفا طرز بروتينى ذات وزن جزيئى ١٥٩٥ كيلودالتون وهذه يمكن اعتبارها معلم وراثى فى نبات البطاطا النامى تحت ظروف الملوحة.