J. Agric. Sci. Mansoura Univ., 27(3): 1717 - 1726, 2002

PRODUCTION OF SALT-TOLERANT SOUR ORANGE PLANTS (*Citrus aurantium* L.) USING TISSUE CULTURE TECHNIQUE:

II- IN VITRO EVALUATION AND SELECTION OF SALT TOLERANT CALLUS

El-Gazzar, A.M.; M.B. El- Sabrout and N.S. Mustafa
Department of Pomology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

ABSTRACT

This study was conducted through 1996-1999 years in the Department of Pomology, Faculty of Agriculture, Alexandria University. The work involved the evaluation and selection of salt tolerant callus of sour orange under *in vitro* conditions.
The main results can be summarized in the following points:

1-Concerning the effect of exposure to different concentrations of NaCl salt in culture medium on callus survival percentage, the results indicated that the $10gl^{-1}$ NaCl concentration was lethal for most callus with only 20.00% surviving.

2- Regarding the effect of exposure to different concentrations of NaCl salt in culture medium on ion content percentage (on dry weight basis) of salt-sensitive and salt-tolerant calluses of sour orange, the results revealed that at $5gl^{-1}$ NaCl concentration, the NaCl –tolerant callus had higher K^+ content, as compared with the NaCl-sensitive callus. On the contrary, the NaCl-tolerant callus had lower Cl^-, Ca^{2+} and Mg^{2+} contents, as compared with NaCl-sensitive callus, at the same external NaCl concentration ($5gl^{-1}$ NaCl).

3-The obtained results also revealed that proline content increased in salt-tolerant callus as compared to salt-sensitive callus (control). Accumulation of proline in the salt-tolerant callus has been shown to be an adaptive mechanism for resistance to salt stress.

INTRODUCTION

Citrus occupied the top list of fruit crops in Egypt. The total *Citrus* cultivated area in Egypt reached 336822 feddans producing 2150256 tons of fruits according to the statistics of the Ministry of Agriculture, Cairo, 1998.

Salinity is a major problem in irrigated agriculture and its role in reducing the yield of many crops is well documented (Maas and Hoffman, 1977). 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), or to specific ions effect on metabolic processes in the cell (Greenway and Munns, 1980). The two effects are not mutually exclusive. Thus, ion regulation and osmoregulation are subjects of intensive research into possible mechanisms of salt tolerance (Maas and Nieman, 1978).

Citrus plants are among the most sensitive fruit crops to salinity (Furr *et al*., 1963). Therefore, it is of great importance to obtain plants capable to grow with elevated salt levels in the irrigation water (Ben-Hayyim and Kochba, 1983). Therefore, *Citrus* rootstocks adapted to difficult soil situations such as high salt levels are needed (Soost and Roose, 1996).

Objectives of the present study

In vitro evaluation and selection of salt-tolerant callus of sour orange through the characterization of physiological and biochemical effects of salt stress on callus using tissue culture methodology.

MATERIALS AND METHODS

The present investigation was carried out during four successive years (1996-1999), in the Department of Pomology, Faculty of Agriculture, Alexandria University, in order to study the possibility of using tissue culture technique for *in vitro* evaluation and selection of salt-tolerant callus of sour orange (*Citrus aurantium L.*).

This experiment aimed to evaluate the performance of using sour orange callus under different concentrations of NaCl salt added to the nutrient medium. Moreover, the selection of tolerant callus which are capable of surviving and subsequently, regenerating to salt-tolerant plantlets of sour orange.

1. Salt Adaptation Technique

Callus obtained from cotyledon tissues of sour orange was exposed to different concentrations of NaCl salt (1 to $10gl^{-1}$) which increased gradually every one month by one gram added, in a complete medium (Murashige and Tucker, 1969). The callus was transferred to test tubes, each of them filled with 10ml, MT (1969) medium containing 1 gl^{-1} NaCl salt. These tubes were incubated for one month before being subcultured to the next higher salt level ($2gl^{-1}$ NaCl). The surviving callus (according to callus surviving percentage and index) was transplanted to the next higher salt level up to $10gl^{-1}$ NaCl. The experiment was maintained by subculture at one month intervals up to ten months (ten subcultures). The survived callus was subcultured onto fresh medium containing $10gl^{-1}$ NaCl at regular intervals of one month, until they were able to grow on salt medium, as healthy as control on medium NaCl free and considered as selected for salt tolerance. The callus which showed tolerante to $10gl^{-1}$ NaCl was named as the NaCl- tolerant callus, whereas callus unable to grow under this NaCl concentration was referred to as the NaCl-sensitive (control) callus.

The surviving callus was evaluated after one month from culturing date by the use of callus survival percentage and index which calculated as follows:

$$\text{Callus survival percentage} = \frac{\text{No. of cultured tubes with callus surviving}}{\text{Total no. of cultured tubes}} \times 100$$

Callus surviving index = increasing in the fresh weight of callus: $\geq$ 2.5%.

Each treatment (NaCl concentration) consisted of five replicates, three tubes each.

2. Callus Ion Analysis

Callus samples (NaCl- sensitive and NaCl- tolerant) were collected after exposure to different concentrations of NaCl and oven-dried at 85°C for 24 hrs. Dry callus samples of both types, 50mg each, were digested with H_2SO_4 and H_2O_2 for analysis of potassium (K^+) and sodium (Na^+) and with HNO_3 and $HClO_4$ for calcium (Ca^{2+}) and magnesium (Mg^{2+}). Sodium and Potassium were determined with an EEl flame photometer, and Ca^{2+} and Mg^{2+} with a Perkin Elmer Atomic Absorption Spectrophotometer. Chloride (Cl^-) was determined according to Cotlove (1965). Ion content percentage (on dry weight basis) was determined after one month (one subculture) for each NaCl concentration. Mean values of three replications per treatment (NaCl concentration) are given for each type of callus.

3. Callus Proline Determination

Proline was extracted from dried callus samples of NaCl-sensitive and NaCl-tolerant (collected after exposure to different concentrations of Na Cl) and assayed according to the method of Bates *et al.* (1973) using pure proline as standard. Mean values of three replications per treatment (NaCl concentration) are given for each type of callus.

4. Statistical Analysis

All the data of this experiment were arranged in a completely randomized design and the statistical procedures were applied according to Steel and Torrie (1980)

RESULTS AND DISCUSSION

1. Salt Adaptation

Data concerning the effect of exposure to different concentrations of NaCl salt in culture medium on survival callus percentage of sour orange, are shown in Table (1) .

In the, 1st subculture ($1gl^{-1}$ NaCl), the percentage of survival callus was significantly decreased from 100.00% at $0.00gl^{-1}$ NaCl concentration to 26.67% at $1gl^{-1}$ NaCl concentration. Under $1gl^{-1}$ up to $5gl^{-1}$ NaCl, such percentage was gradually increased with significant differences from 26.67% at $1gl^{-1}$ NaCl concentration to 53.33% at $5gl^{-1}$ NaCl concentration. On the contrary, the percentage of survival callus was gradually decreased from 53.33% at $5gl^{-1}$ NaCl concentration to 20.00% at $10\ gl^{-1}$ NaCl concentration (Table 1). The differences among NaCl levels were significant.

Such results indicated that, $10gl^{-1}$ NaCl concentration was lethal for most callus with only 20.00% surviving. Subsequently, the surviving callus was subcultured every 4 weeks onto fresh medium containing $10gl^{-1}$ NaCl for 18 months, until they grew as well on salt medium as control on medium without NaCl.

Table (1): Effect of exposure to ten concentrations of NaCl salt in culture medium on survival percentage of callus induced from cotyledon tissues of sour orange.

Subculture	NaCl concentration (gl^{-1})	Callus survival ^{z} %
0	0	100.00 A*
1st	1	26.67 DE

2ed	2	33.33 CDE
3rd	3	40.00 BCD
4th	4	46.67 BC
5 th	5	53.33 B
6 th	6	46.67 BC
7 th	7	40.00 BCD
8 th	8	33.33 CDE
9 th	9	26.67 DE
10 th	10	20.00 E
L.S.D.$_{0.05}$		18.119

[z] There were 15 calluses (one per culture tube) per treatment (NaCl concentration). The callus survival percentages were determined after one subculture (4 weeks) for each NaCl concentration.

***Values followed by the same letters are not significantly different at the 0.05 level of probability.**

2. Ions Content of Salt -Sensitive and Salt -Tolerant Calluses

2.1. Ions Content of Salt-Sensitive Callus

Data concerning the effect of exposure to different concentrations of NaCl salt in culture medium on ions content percentage (on dry weight basis) of salt-sensitive callus of sour orange, are shown in Table (2).

Table (2): Effect of exposure to five concentrations of NaCl salt in culture medium on ions content percentage (on dry weight basis) of salt- sensitive callus induced from cotyledon tissues of sour orange.

NaCl concentration (gl^{-1})	Salt - sensitive callus (control)				
	Ions content[z] % (on dry weight basis)				
	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}
0	0.12 F*	0.90 A	0.15 F	0.90 A	1.20 A
1	0.20 E	0.78 B	0.26 E	0.86 B	1.12 AB
2	0.28 D	0.62 C	0.39 D	0.83 B	1.08 BC
3	0.37 C	0.56 D	0.50 C	0.79 C	1.00 CD
4	0.41 B	0.53 DE	0.62 B	0.74 D	0.98 D
5	0.48 A	0.51 E	0.75 A	0.70 E	0.96 D
L.S.D. $_{0.05}$	0.018	0.039	0.041	0.035	0.084

[z]Ions content percentage of the NaCl- sensitive callus as a function of NaCl concentration.

At 5 $g1^{-1}$ Na Cl concentration = minimum of callus surviving index.

***Values followed by the same letters are not significantly different at the 0.05 level of probability (in the same column).**

The results represented in Table (2) indicated that, as the NaCl concentration was increased from 0.0gl^{-1} to 5.0gl^{-1} (at the minimum of callus surviving index), the sodium (Na^+) content was significantly increased from 0.12% at 0.0gl^{-1} NaCl concentration to 0.48% at 5gl^{-1}NaCl concentration. On the contrary, potassium (K^+) content was significantly decreased from 0.90% at 0.0gl^{-1}NaCl concentration to 0.51% at 5gl^{-1}NaCl concentration.

As the NaCl concentration was increased from 0.0gl^{-1} to 5gl^{-1}, the chloride (Cl^-) content was significantly increased from 0.15% at 0.0gl^{-1} NaCl concentration to 0.75% at 5gl^{-1}NaCl concentration. In contrast, the calcium (Ca^{2+}) content was significantly decreased from 0.90% at 0.0gl^{-1}NaCl concentration to 0.70% at 5gl^{-1} NaCl concentration.

Similarly, as the NaCl concentration was increased from 0.0gl^{-1} to 5.0gl^{-1}, the magnesium (Mg^{2+}) content was significantly decreased from 1.20% at 0.0gl^{-1} NaCl concentration to 0.96% at 5gl^{-1} NaCl concentration.

2.2 Ions Content of Salt-Tolerant Callus

Data concerning the effect of exposure to different concentrations of NaCl salt in culture medium on ions content percentage (on dry weight basis) of salt-tolerant callus of sour orange, are shown in Table (3).

The results represented in Table (3) indicated that, as the NaCl concentration was increased from 0.0gl^{-1} to 5gl^{-1}, the sodium (Na^+) content was significantly increased from 0.17% at 0.0gl^{-1} NaCl concentration to 0.54% at 5 gl^{-1}NaCl concentration. On the contrary, the potassium (K^+) content was significantly decreased from 1.71% at 0.0gl^{-1} NaCl concentration to 1.58% at 5gl^{-1} NaCl concentration.

As the NaCl concentration was increased from 0.0gl^{-1} to 5gl^{-1}, the chloride (Cl^-) content was significantly increased from 0.22% at 0.0 gl^{-1}NaCl concentration to 0.57% at 5gl^{-1} NaCl concentration. In contrast, the calcium (Ca^{2+}) content was significantly decreased from 0.87% at 0.0 gl^{-1} NaCl concentration to 0.56% at 5 gl^{-1} NaCl concentration.

Similarly, as the NaCl concentration was increased from 0.0gl^{-1} to 5gl^{-1}, the magnesium (Mg^{2+}) content was significantly decreased from 0.92% at 0.0gl^{-1} NaCl concentration to 0.60% at 5gl^{-1} NaCl concentration.

On the other hand, the results represented in Table (3) indicated that, as the NaCl concentration was increased from 0.0gl^{-1} to 10gl^{-1}, the sodium (Na^+) content was significantly increased from 0.17% at 0.0gl^{-1}NaCl concentration to 0.64% at 10gl^{-1}NaCl concentration. On the contrary, the potassium (K^+) content was significantly decreased from 1.71% at 0.0 gl^{-1}NaCl concentration to 0.57% at 10gl^{-1}NaCl concentration.

As the NaCl concentration was increased from 0.0gl^{-1} to 10gl^{-1}, the chloride (Cl^-) content was significantly increased from 0.22% at 0.0gl^{-1}NaCl concentration to 1.04% at 10gl^{-1}NaCl concentration. In contrast, the calcium (Ca^{2+}) content was significantly decreased from 0.87% at 0.0 gl^{-1}NaCl concentration to 0.30% at 10gl^{-1}NaCl concentration.

Similarly, as the NaCl concentration was increased from 0.0gl^{-1} to 10gl^{-1}, the magnesium (Mg^{2+}) content was significantly decreased from 0.92% at 0.0 gl^{-1}NaCl concentration to 0.25% at 10 gl^{-1}NaCl oncentration.

Table (3): Effect of exposure to ten concentrations of NaCl salt in culture medium on ions content percentage (on dry weight basis) of salt – tolerant callus induced from cotyledon tissues of sour orange.

NaCl concentration (gl^{-1})	Salt- tolerant callus				
	Ions content^z % (on dry weight basis)				
	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}

0	0.17 I*	1.71 A	0.22 K	0.87 A	0.92 A
1	0.23 H	1.68 AB	0.26 J	0.80 B	0.89 A
2	0.32 G	1.65 ABC	0.31 I	0.71 C	0.83 B
3	0.39 F	1.62 ABC	0.38 H	0.65 D	0.79 B
4	0.47 E	1.60 BC	0.45 G	0.60 E	0.72 C
5	0.54 D	1.58 BC	0.57 F	0.56 E	0.60 D
6	0.58 C	1.57 C	0.70 E	0.51 F	0.53 E
7	0.60 BC	1.32 D	0.75 D	0.43 G	0.47 F
8	0.62 AB	1.00 E	0.82 C	0.40 G	0.40 G
9	0.63 A	0.60 F	0.90 B	0.34 H	0.34 H
10	0.64 A	0.57 F	1.04 A	0.30 H	0.25 I
L.S.D. $_{0.05}$	0.026	0.107	0.039	0.048	0.041

[z]Ions content percentage of the NaCl- tolerant callus as a function of NaCl concentration.

***Values followed by the same letters are not significantly different at the 0.05 level of probability (in the same column).**

2.3 Comparison between Salt -Sensitive and Salt-Tolerant Calluses concerning Ions Content

The comparison of ions content between NaCl- tolerant callus and NaCl –sensitive callus can be shown in Tables (2 and 3). From these Tables it was cleared that, at 5gl^{-1} NaCl concentration, the NaCl –tolerant callus had higher K^+ content, as compared with the NaCl-sensitive one. However, there was a marked difference between the two callus types in the extent of reduction of internal K^+ as a function of increasing external NaCl concentrations. On the contrary, the NaCl-tolerant callus had lower Cl^-, Ca^{2+} and Mg^{2+} contents, as compared with NaCl-sensitive one, at the same external NaCl concentration (5gl^{-1} NaCl). Consequently, in the NaCl – tolerant callus, the Ca^{2+} and Mg^{2+} contents were lower than in NaCl – sensitive callus.

No considerable difference was found among the NaCl-tolerant and NaCl- sensitive calluses in Na^+ uptake.

The above findings agreed with those obtained by Piqueras *et al.* (1996) and Ben-Hayyim *et al.* (1985). The latter investigators found that at the same external NaCl concentrations, the NaCl- sensitive callus cell line of sour orange lost much more K^+ than the NaCl – tolerant callus cell line did. In other words, they reported that exposure of callus cells of NaCl – sensitive and NaCl – tolerant lines of sour orange to equal external concentrations of NaCl, resulted in a greater loss of K^+ from the NaCl – sensitive callus cell line. Also, they suggested that, there was an important role for internal K^+ concentrations in the mechanism of salt tolerance for sour orange callus cells. In the meantime, they mentioned that, in some plant systems, such as sour orange, salt tolerance can be attributed to a reduced loss of internal K^+.

Such results agreed with those reported by Ben-Hayyim and Kochba (1982), who mentioned that the NaCl – selected “Shamouti” orange callus cell line (R-10) took up considerably less Cl^- compared to non- selected callus cell line. These results suggest that R-10 is a true genetic variant. In addition, Ben-Hayyim and Kochba (1983) reported that Cl^- uptake is considerably lower in NaCl – tolerant callus cell line of “Shamouti” orange (R-10) than in salt – sensitive callus cell line (L-5) at a given external NaCl concentration.

Referring to the obtained results it can be suggested that the toxic effect in the NaCl- sensitive callus cells is due to the accumulated Cl^-, which was lethal for all cells of NaCl-sensitive callus. This suggestion also agreed with those reported by Ben-Hayyim and Kochba (1983).

Furthermore, Bouharmont *et al.* (1993) found that the NaCl-selected callus cell lines of citrange and Poncirus rootstocks were characterized by their ability to maintain a normal content of K^+ and Ca^{2+} in spite of increasing concentrations of Na^+ in the medium.

The results concerned with Na^+ and Cl^- ions in the present study did not agree with those reported by Piqueras *et al.* (1996) with the selected callus cultures of lemon (*Citrus limon* cv. Verna) for resistance to salt stress by exposure to 170 mM NaCl for 6 weeks. They found from inorganic analysis that selected callus accumulated more Na^+ and Cl^- ions than the non-selected one.

In conclusion, the NaCl-tolerant callus cells selected from cotyledon callus have a similar degree of salt tolerance as well as very similar behavior in changing their internal ions content in response to various concentrations of NaCl salt. All the experiments described in this study were designed to find whether a correlation between tolerance to NaCl and internal concentration of major ions exists.

3. Proline Content of Salt-Sensitive and Salt-Tolerant Calluses

Data concerning the effect of exposure to different concentrations of NaCl salt in culture medium on proline content (μg/g dried callus tissue) of salt-sensitive and salt-tolerant calluses of sour orange, are shown in Table (4).

The results represented in Table (4) indicated that in both salt-tolerant and salt-sensitive calluses proline content was significantly increased as NaCl externally increased from 0.0 $g1^{-1}$ to $10g1^{-1}$ for the former callus or from 0.0 $g1^{-1}$ to 5.0 $g1^{-1}$ for the latter one. The increasing values of salt-tolerant callus were from 114.69 μg/g at $0.0g1^{-1}$ to 224.16 μg/g dried callus tissue at $10g1^{-1}$ NaCl. The corresponding values for salt-sensitive callus were 90.00 μg/g at 0.0 $g1^{-1}$ to 101.22 μg/g dried callus tissue at 5.0 $g1^{-1}$ NaCl.

The tabulated results for the two tested callus types at the same external NaCl concentrations (from $0.0g1^{-1}$ to 5.0 $g1^{-1}$) revealed that both callus types in spite of they showed a similar trend but they differed in the values of proline content. The proline values in case of salt-tolerant callus were higher than those of salt-sensitive callus.

These findings greatly confirmed by those reported by Piqueras *et al.* (1996), who found that proline concentration increased in salt-tolerant callus cells of lemon (*Citrus limon* cv. Verna) as compared to control callus cells.

Accordingly it can be suggested that accumulation of proline in the salt-tolerant callus cells has been shown to be an adaptive mechanism for resistance to salt stress.

Table (4): Effect of exposure to ten concentrations of NaCl salt in culture medium on proline content (μg /g dried callus tissue)

of salt- sensitive and salt – tolerant calluses induced from cotyledon tissues of sour orange.

NaCl concentration (gl^{-1})	Proline content[z] (μg/g dried callus tissue)	
	Salt- sensitive callus	Salt –tolerant callus
0	90.00 E*	114.69 K
1	93.04 D	121.25 J
2	95.60 C	129.72 I
3	96.22 C	140.00 H
4	98.00 B	152.11 G
5	101.22 A	160.32 F
6	------	173.60 E
7	------	181.91 D
8	------	192.00 C
9	------	210.12 B
10	------	224.16 A
L.S.D. $_{0.05}$	1.721	2.500

[z]Proline content of the NaCl- sensitive and NaCl- tolerant calluses as a function of NaCl concentration. Callus proline content (on dry weight basis) was determined after one month for each NaCl concentration.

***Values followed by the same letters are not significantly different at the 0.05 level of probability (in the same column).**

One of the initial reports dealing with *in vitro* selection for salt stress in *Citrus* trees was by kochba *et al.* (1982). Several tolerant and sensitive callus cell lines of “Shamouti” orange (*Citrus sinensis* L. Osbeck) and one cell line of sour orange (*C. aurantium* L.) were subcultured on basal media containing 0.0 to 10 gl^{-1} NaCl. Also, Ben-Hayyim and Kochba (1983) used tolerant cell line selected from callus of “Shamouti” orange (C*itrus sinensis* L. Osbeck) which was routinely kept for over one year on the MT (1969) medium containing 0.2M NaCl. They appeared that tolerant callus cell line was stable and tolerated NaCl by its partial avoidance.

Further studies on salt tolerant callus cell line of C*itrus* species, have generally used single salt, with the majority employing NaCl as a selective agent (Ben- Hayyim and Kochba, 1982 and 1983; Ben-Hayyim *et al.,* 1985; Spiegel-Roy and Saad, 1986; Ben-Hayyim *et al.,* 1987; Piqueras and Hellin, 1992; Bouharmont *et al.,* 1993; Piqueras *et al .,* 1994 and 1996).

REFERENCES

Bates, L. S; R. P. Waldren and I. D. Teare (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Ben- Hayyim, G. and J. Kochba (1982). Growth characteristics and stability of tolerance of *Citrus* callus cells subjected to NaCl stress. Plant Sci. lett., 27: 87- 94.

Ben- Hayyim, G. and J. Kochba (1983). Aspects of salt tolerance in a NaCl-selected stable cell line of *Citrus sinensis.* Plant Physiol., 72: 685- 690.

Ben-Hayyim, G.; P. Spiegel-Roy and H. Neumann (1985). Relation between ion accumulation of salt sensitive and isolated stable salt tolerant cell lines of *Citrus aurantium.* Plant Physiol., 78: 144 -148.

Ben-Hayyim, G.; U. Kafkafi and R.G. Neumann (1987). Role of internal potassium in maintaining growth of cultured *Citrus* cells on increasing NaCl and $CaCl_2$ concentrations. Plant Physiol., 85: 434 - 439.

Bouharmont, J.; N. Beloualy; J. V. Sint; S. J. V. Van and H. Lieth (1993). Improvement of salt tolerance in plants by *in vitro* selection at the cellular level. Proceedings of the ASWAS conference, 8-15 December1990, Al Ain, United Arab Emirates.1993, 83-88.

Cotlove, E. (1965). Determination of Cl^- in biological materials. In D. Glick (ed.) Methods of Biochemical Analysis. Vol. 12. Interscience Publishers, New York , 227-392.

Furr, J. R.; J. B. Carpenter and A. A. Hewitt (1963). Breeding new varieties of *Citrus* fruits and rootstocks for the southwest. J. Rio. Grande Val. Hort. Sci., 17: 90-107.

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

Kochba, J.; G. Ben-Hayyim; P. Spiegel–Roy; S. Saad and H. Neumann (1982). Selection of stable salt– tolerant callus cell lines and embryos in *Citrus sinensis* and *Citrus aurantium.* Z. Pflanzenphysiol, 106: 111-118. [C. F. Hort. Abst. 52 (9) 6361].

Maas, E. V. and G. J. Hoffman (1977). Crop salt tolerance-current assessment. J. Irrig. Drain. Div. ASCE, 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.

Murashige, T. and D.P.H. Tucker (1969). Growth factor requirements of *Citrus* tissue culture. In : Chapman, H. D. (Ed.). Proceedings of the First International *Citrus* Symposium. Vol. III. Univ. of California, Riverside, 1155-1161.

Piqueras, A. and E. Hellin (1992). Selection and characterization of an NaCl tolerant cell line of *Citrus limonum.* Suelo y planta, 2 (4): 629- 640.

Piqueras, A.; E. Olmos and E. Hellin (1994). Cytological changes related with salt tolerance in embryogenic callus of *Citrus limon.* Plant Cell, Tissue and Organ Culture, 39: 13-18.

Piqueras, A.; J. A. Hernandezy; E. Olmos; E. Hellin and F. Sevilla (1996). Changes in antioxidant enzymes and organic solutes associated with adaptation of *Citrus* cells to salt stress. Plant Cell, Tissue and Organ Culture, 45: 53-60.

Soost, R.K. and M. L. Roose (1996). *Citrus*. In: Janick, J. and Moore, J. N. (eds.). Fruit Breeding, Vol. 1: Tree and Tropical Fruits. John Wiley and Sons, Inc., 257-323.

Spiegel-Roy, P. and S. Saad (1986). Effect of carbohydrates and inhibitors of GA_3 biosynthesis on embryogenic potential of salt tolerant and non-tolerant callus lines of orange (*Citrus sinensis* Osbeck). Plant Science, Irish Republic, 47(3): 215-220.

Steel, R. G. and J. H. Torrie (1980). Principles and procedures of statistics. 2nd Ed. Mc Graw Hill Book Company, New York. USA.

إنتاج نباتات نارنج متحملة للملوحة باستخدام تقنية زراعة الأنسجة"
٢- تقييم وانتخاب كالس متحمل للملوحة معمليا

عبد العظيم محمود الجزار ومحمد بدر الصبروت و نبيل ثابت مصطفى
قسم الفاكهة – كلية الزراعة- جامعة الإسكندرية -الإسكندرية – مصر

أجريت هذه الدراسة خلال الفترة من بداية عام ١٩٩٦ وحتى نهاية عام ١٩٩٩ بقسم الفاكهة كلية الزراعة جامعة الإسكندرية وكان الهدف من إجراء هذه الدراسة هو تقييم وإنتخاب كالس متحمل للملوحة معمليا على بيئة صناعية.

ويمكن تلخيص النتائج الرئيسية لهذه الدراسة فى النقاط التالية:-

1- نتائج تأثير التعرض لتركيزات مختلفة من ملح كلوريد الصوديوم فى بيئة الزراعة على النسبة المئوية لبقاء الكالس فى صورة حية تدل على أن تركيز كلوريد الصوديوم ١٠ جرام فى اللتر كان مميت لمعظم الكالس فيما عدا نسبة ٢٠% فقط هى عبارة عن نسبة الكالس الذى ظل فى صورة حية.

2- فيما يختص بتأثير التعرض لتركيزات مختلفة من ملح كلوريد الصوديوم فى بيئة الزراعة على النسبة المئوية للمحتوى المعدنى (على أساس الوزن الجاف) للكالس المتحمل للملوحة والكالس الحساس للملوحة. فإن النتائج أوضحت أنه عند تركيز كلوريد الصوديوم ٥ جرام فى اللتر فإن الكالس المتحمل لكلوريد الصوديوم كان يحتوى على تركيز عالى من البوتاسيوم مقارنة بالكالس الحساس لكلوريد الصوديوم. وعلى العكس من ذلك فإن الكالس المتحمل لكلوريد الصوديوم كان منخفض فى محتواه من الكلوريد والكالسيوم والمغنسيوم مقارنة بالكالس الحساس لكلوريد الصوديوم وذلك عند نفس التركيز الخارجى لكلوريد الصوديوم (٥ جرام فى اللتر).

3- من ناحية أخرى أوضحت النتائج أن تركيز البرولين قد إزداد فى الكالس المتحمل للملوحة مقارنة بالكالس الحساس للملوحة (الكنترول). ويعتبر تراكم البرولين داخل خلايا الكالس المتحمل للملوحة ميكانيكية للتأقلم على مقاومة الإجهاد الملحى.