J. Agric. Sci. Mansoura Univ., 29 (6): 3495 - 3514 , 2004

BEHAVIOUR OF EXPLANTS AND PLANTLETS OF DATE PALM UNDER *IN VITRO* AND *IN VIVO* SALINE CONDITIONS

Kamal, Hemat M.*; M.H. Abd El-Zaher* and A.S. Al Adawe**
* Pomology Dep.,Fac. Agric, Cairo Univ.
** Agricultural Research Institute.

ABSTRACT

In vitro and *in vivo* studies and proline analysis were carried out during 2002 – 2003 seasons on two Egyptian cultivars of date palm (Zaghloul and Samani). The aims of these studies were tested the tolerant to *in vitro* and *in vivo* saline conditions in the two cultivars. Also,study the relationship between the proline content and the tolerant to saline conditions in these cultivars.

Data revealed that all treatments of mercuric chloride gave the lowest contamination percentages (0.0% in both cultivars) whilst the treatment of Clorox at 10% for 15 min, gave the highest contamination percentages (70% in Zaghloul and 80% in Samani). The lowest survival percentages in both cultivars were obtained by the treatment of mercuric chloride at 0.1% for 10 min. The treatment of Clorox at 10% for 15 min. gave the highest survival percentage in both cultivars (100% in Zaghloul and Samani), while the treatment of mercuric chloride at 0.1% for 5 min. gave the highest survival disinfected explant percentage (60% in Zaghloul and 70% in Samani). Zaghloul cultivar was superior to Samani cultivar in averages of weight and diameter of callus ; number and length of the roots from *in vitro* shoots and the growth parameters in the acclimatization stage. On the other hand,Samani cultivar was superior to Zaghloul cultivar in averages of number and length of *in vitro* shoots and growth parameters of *in vitro* embryos. The treatment of 7000 p.p.m. salts gave the lowest weight and diameter of the callus , the lowest number and length of *in vitro* shoots ; the lowest number and length of *in vitro* roots from *in vitro* shoots and the lowest growth of *in vitro* embryos compared with untreat. *In vivo* study showed that irrigation with solutions of 8000,9000 and 10000 ppm. salts gave the worest results (growth parameters) in the acclimatization stage for the two studied cultivars compared with the irrigation with water (control or untreat). There were reversely proportional between concentration of the salts and the growth parameters of the two studied cultivars in either *in vitro* or *in vivo* conditions. Zaghloul cultivar had more proline content than Samani cultivar and exhibited more tolerant to either *in vitro* or *in vivo* saline conditions which reflected positive relationship between the proline contents and the tolerant to saline conditions in the two cultivars.

INTRODUCTION

Date palm shoot tips surface sterilized in 0.1% HgCl2 for 5 - 7 min (Bhansali and Kaul, 1991).Mercuric chloride(0.19%) gave the fewest contaminated buds of *Phoenix dactylifera* and highest survival percentage (91%), followed by sodium hypochloritc at 2.65%. Rooting of *in vitro* - produced shoots achieved on MS medium supplemented with NAA at 0.1 mg / litre (70% of shoots rooted). (Shatnawi *et. al.*,1997).

Callus was proliferated onto MS medium supplemented with 10mg 2,4 - D + 3g activated charcoal (AC) / litre. Shoot proliferation after a phase of callus formation was on medium containing 0.1 mg NAA + 3g. AC/litre (Saker *et.al.*,1998). Callus of dates enhanced by increasing auxin

concentration, being highest (55%) on medium with 10mg 2,4-D/litre Somatic embryos were formed and plantelets were regenerated on medium without growth regulators. Plantelets were transferred to soil in the green house when they were 10 – 15 cm tall. (Shakib *et al* ., 1994).

Explants taken from selected off shoots of 2 Egyptian date cultivars (Zaghloul and Samani) were cultured on MS media supplemented with IAA (0.5 mg / litre) + NAA (0.5 mg / litre), and BAP (10 mg litre). Shoot initiation was best with IAA, NAA, and 10mg BAP/litre. In general, Zaghloul had a higher shoot and root length than Samani , but Samani produced a higher number of axillary shoots than Zaghloul. A limited number of plantelets was produced due to low multiplication rates and slow growth and development Rooting was observed in the presence of 3 mg NAA and 0.5 mg Kinetin / litre (Belal and El - Deeb , 1997).

Shoot tip of dates grown on MS media containing high levels of 2,4 - D or NAA formed nodular embryogenic callus (asexual pro-embryos). When the nodules were transferred to media with 0.1 mg/litre of NAA, they grew into platelets. Vigorous callus from Shoot tip of dates cultivars Barhee and hallw occurred on MS medium with NAA at 10 mg/ litre. A low-auxin media produce organs only, a medium containing NAA gave roots only. The plantelets obtained (10- 15 cm after 2 – 3 months) were successfully transplanted to soil (Mater, 1986).

Somatic embryo nodles of dates formed in callus when transfer to MS medium with 0.1 –10 mg GA_3 and 0.1 - 2.0 mg 2 ip / litre and plantlets developed directly from embryoids. Roots were induced on MS medium containing 0.1 mg NAA/litre. Survival ex vitro was 70 - 80% when well-rooted plantlets were 8 - 12 cm in length. (Quraishi *et al.*,1997). The somatic embryos were developed into platelets in hormone - free medium. The plants were acclimatized in the glass house and finally transferred to the field (Rao *et al.*,2001).

Organogenesis responses such as root formation and shoot development from shoot tips produced on media containing 10 mg/l NAA (Zaid and Tisserat , 1983). Plantelets were successfully transferred to pots containing a mixture (1 : 1) of vermiculite and peat moss. (Sharon *et al*; 1998)

Volkamer lemon showed greater salt tolerance up to 4000 ppm and after that the stem, leaf growth , root size, number of lateral roots and D.W. of the roots were significantly reduced (El-Desouky and Atawia, 1998). NaCl and $CaCl_2$ reduced growth in tolerant Celopatra mandarin seedlings (Romero-Aranda *et al.*, 1998). Growth of Celopatra Mandarin, Sour Orange and Rough lemon rootstocks was depressed by increasing salinity up to 11000 p.p.m. of NaCl and $CaCl_2$ (Taher, 1983). Sour-Orange and Cleopatra Mandarin growth reduced due to the osmotic effects and the inhibitory effects of CL- and Na^+ (Ruiz *et. al.*,1997). Cleopatra Mandarin considered more tolerant to NaCL than Troyer Citrange attributed to a greater capacity to exclude chloride ions and Ca+ increased CL- accumulation in the basal stem and roots(toxicity) (Banuls *et al.*,1991).

Increasing osmotic stress in rice *in vitro* culture, induced proline accumulation and the highest concentration of proline. (Al-Khayri and Al-Bahrany, 2002). Saline irrigation water (6000ppm.) significantly increased the

average proline contents of Manzanillo olive seedling leaves as compared with tap water (Ahmed ,1999).

The aims of this investigation were to illustrate effects of the *in vitro* and *in vivo* saline conditions on the characteristics of the studied cultivars and hence test tolerance of them to salinity and give some idea about the relationship between the proline content and the saline treatments.

MATERIALS AND METHODS

This study was carried out in Plant Tissue Culture Laboratory at the Bahteem Research Station belongs to Horticulture Unit, Ministry of Agriculture throughout the period from 2002 to 2003

Plant Material :

Selected Zaghloul and Samani off sets (5 - 7 kg and 50 - 70 cm and about 2-3 years old), cleaned from the old , internal leaves and the roots to obtain the apical grown, which dessicate into small pieces and hence obtain the explant (shoot-tip), which have apical meristem surrounded by its leaf primordia in spherical shapes have about 0.5 cm, palms grown at a farm near Bahteem Research Station. Each treatment has 3 replicates and each replicate contains of 3 jars(it contains one explant).

***In vitro* study:**

Sterilization of plant materials : -

The obtained apical grown washed in running tap water for one hour, then soaked in antioxidant solution (100 mg/L. citric acid 150 mg/L ascorbic acid) for 30 min. to avoid culture browning,after that dissicated into small pieces (about 12cm in diameter)called explants several surface sterilization methods were employed by immersion the explants in 70% ethanol for 1 min. followed by immersion in 0.1% mercuric chloride for 5 or 10 min and commercial bleach Clorox (5.25% w/v sodium hypochlorite at 10% (v/v) for 15 min ; 20% for 15 min or 15% for 10 min.After that,the sterilized explants washed with sterilized distilled water for three times. Treateded explants were incubated on to MS medium into small gars (150 ml.) at the rate of 30 ml. per jar.

Data have taken as survival percentage, contamination percentage and recovering percentage (uncontaminated and survival explant percentage).

Callus and Somatic emobryos induction :

Slices of sterilized shoot-tips were initially cultured on MS medium supply with 40 mg/L adenine - suflate, 2 mg/L. 2ip, 1 mg/l 2,4 - D, 30g/ L surcorse, 2 g/L activated charcoal and 6.69/L agar (Shakib *et al*, 1994 and Shaker *et al*, 1998). Different concentrations of CaCl + $NaCl_2$ mix solution (1000, 3000, 5000 and 7000 ppm) were added to the previous medium, sterilized sections (explants) were cultured on the previous media.

Cultures jars were incubated at total darkness under $27\pm1^{\circ}C$ for growth with subculturing to fresh medium every 5-6 weeks to produce embryogenic callus. Date have taken as weight and diameter of callus mass.

Embryogenesis Stage :

Embryogenic callus (White Friable Nodular Callus) about 1 g in weight and 2 - 3 mm in diameter) was transferred to MS fresh medium containing 2mg / L NAA, 5 mg/L BA, 2 mg / L Kin, 200 mg / L glutamine and 4mg / L thiamine - HCL, (Quraishi *et. al.*,1997). Different previous concentration of salts were added to MS media as follow: control or untreated was MS medium without salts and media were incubated under 27±2°C at light intensity of 1000 - 1500 lux for 16 hrs. to produce somatic embryogenesis.Data were recorded after 6 weeks from the culture as averages number of the embryos /one piece of callus , the total length of embryo and root length of the enbryo.

Organogenesis Stage : -

Shoot proliferation and multiplication MS basal medium with adding of 2 mg/L. 2ip, 1.5 mg/L Kin and 2 mg/L NAA in the all cases of organogenesis media (Shakib *et. al.*,1994 and Shaker *et al.*, 1998) and without any hormonal additions, was used as control medium, the same previous concentrations of salts were used. The explant used in this experiment was piece of callus (about 2 cm in diameter), all cultures jars were incubated in growth room at 27±1°C. under 16 hrs/day and exposure to moderate light intivisty of 3000 lux illumination, three subcultures have done at 6 weeks intervals into corresponding fresh medium. (Belal and El-Deeb,1997 and Shatnawi *et. al.*,1997).

Rooting stage :

In this stage MS basal media were supplemented with 0.1 mg/L NAA + 30 g/L sucrose + 3g / L activated charcoal(Zaid and Tisserat,1983 ,whose described media have NAA for rooting). Media were divided into 5 parts, each part was supplemented with 0, 1000, 3000, 5000 and 7000 ppm of $CaCl_2$ + NaCl mix solution . 3 *in vitro* shoots of about 7 - 10 cm in length with 2 - leaves resulted from organogenesis stage, were used as plant materials and were cultured in jars (350 mL) and were subculatured every 8 weeks on fresh medium in tubes (250 x 28 mm), each tube have one shoot as consider a replicate and the treatment contains of 3 replicates. The incubation was done under 16 hours, illumination of 3000 lux (white florescent lamps). After 6 and 12 weeks in the culture, the following data were recorded: number of roots and root length (cm).

In vivo study:

Acclimatization of plantlets :

The plantelets (10 - 12 cm length, and have distinct tap root and 2 - 3 leaves) produced from the previous stages (embryogenesis and organogensis) were cleaned from agar,washed with distilled water and transferred *in vivo* to mixtures of 1 : 1 peat moss : vermiculite (v/v)treated with fungicide solution in plastic pots(5x18cm3) (Mater,1986; Sharon *et. al.*,1998 and Rao *et. al.*,2001). and watered a quarter strength MS solution with high humidity (90 - 95%) just for two weeks. The pots has embedded in sand bath and covered with a plastic bell (transparent tent for two weeks with exposure the plants to normal conditions in greenhouse).

The plants watered every other day with distilled water and once a week with quarter strength MS solution during the first two weeks of development. After two weeks, the transparent cover was removed and began to watered with water only as control treat and different concentrations of salts solutions ($CaCl_2$ + NaCl) as follows: 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10000 ppm. The watering have done alternatively by normal water at week intervals for 3 months. All steps of the acclimatization have occurred in the greenhouse. 33 plants were using for each cultivars (3 plantlets/ treatment). After 3 months from planting data was recorded as averages of survival percentage, number of roots / plantlet, length of roots(cm), number of leaves / plantlet and length of plantlet of the two cultivars under investigation .

Leaf proline content : -

Proline content was colorimetrically estimated in fresh samples of *in vitro* leaves of plantlets (just before the acclimatization stage)according to method of Batels *et al.*, (1973).

Statistical analysis :

The obtainrd data were satistically analyzed and the means were compared using LSD method at 5% probability as described by Snedecor & Cochran (1980).

RESULTS AND DISCUSSIONS

In vitro study:

1. The sterilization :

As for contaminated explant (%),data in table (1) showed significant differences between all treatments in both cultivars as all mercuric chloride treatments gave the lowest contamination percentages (0.0% in both cultivars) with significant difference and followed by the treatment of Clorox at 20% for 15 min. (15% in both cultivars) followed by the treatment of Clorox at 15% for 10 min (40% in Zaghloul and 50% in samani) , whilst the treatment of Clorox at 10% for 15 min, gave the highest contamination percentages (70% in Zaghloul and 80% in Samani).

Table (1): Effect of mercuric chloride and clorox on the percentages of the contamination , survival and survival disinfected explants of Zaghloul and Samani cultivars (season2002).

Cultivars	Zagloul			Samani		
Characters and trearments	Contamin ated explant %	Survival explant %	Uncontaminat ed & Survival %	Contamina ted explant %	Survival explant %	Uncontamin ated & Survival %
0.1 % Merc Chlo For 5 Min	0	60	60	0	70	70
0.1 % Merc Chlo For 10 Min	0	0	0	0	0	0
Clorox at 20 % for 15 Min	15	30	4.5	15	30	4.5
Clorox at 15 % for 10 Min	40	80	48	50	90	45
Clorox at 10 % for 15 Min	70	100	30	80	100	20
L.S.D(0.05)	14.017	25.839	7.916	12.342	22.881	6.395

Similarly, the survival percentage was also affected by the treatments, since the lowest survival percentages in both cultivars were obtained by the treatment of mercuric chloride at 0.1% for 10 min. with significant difference and followed by the treatment of Clorox at 20% for 15 min (gave 30% survival in both cultivars) with significant difference and followed by treatment of mercuric chloride at 0.1% for 5 min (60% in Zaghloul and 70% in Samani); the treatment of Clorox at 15% for 10 min, came in the following rank (80% in Zaghloul and 90% in Samani) with sgifnicant difference with the treatment of mercuric chloride 0.1% for 5 min, and with insignificant difference with the treatment of Clorox at 10% for 15 min, which gave the highest survival percentage in both cultivars (100% in Zaghloul and Samani). Considering the survival disinfected explant percentage in both cultivars , this percentage was also affected by the sterilization treatments, whereas the lowest percentage, was obtained by the treatment of mercuric chloride at 0.1% for 10 min (gave 0.0% in both cultivars) and followed by the treatments of Clorox at 20% for 15 min (4.5% in both of Zaghloul and Samani), the treatment of Clorox at 10% for 15 min came in the following rank (30% in Zaghloul and 20% in Samani) with significant difference and followed by the treatment of Clorox at 15% for 10 min (48% in Zaghloul and 45% in Samani), while the treatment of mercuric chloride at 0.1% for 5 min gave the highest survival disinfected explant percentage (60% in Zaghloul and 70% in Samani) with significant differences with the other treatments. These results were confirmed with those obtained by Bhansali and Kaul, 1991 and Shatnawi *et al.*, 1997, they reported that o.1% Hg Cl gave the highest survival and the fewest contamination in date palm,followed by sodium hypochlorite.

2. Callus production:

Table (2) and Fig (A) showed that Zaghloul cultivar was superior to Samani cultivar in the most cases as it gave higher weight of Callus (2.09g) than Samani (1.85g); in the same direction, Zaghloul explants gave higher values, in all treatments except control , than Samani explants as shown in table (2) but without significant differences between them.

On the other hand, there are significant differences between the treatments as untreated or control treatment gave the highest significant callus weight (2.78g.) and followed by the treatment of 1000 ppm. salts (2.20g). Treatment of 3000 ppm. salts came in the third rank (2.05 g) with insignificant difference with the treatment of 1000 ppm. salts and followed by the treatment of 5000 ppm, salts (1.78g), whereas the treatment of 7000 ppm, salts gave the significant lowest value (1.06g.).

The effects of interaction between the cultivars and the treatments were insignificant in all cases.

It could be recommended with the explants of Zaghloul cultivar to produce more callus weight than Samani in *in vitro* culture and under salinity conditions as Zaghloul explants appears to be tolerant to salinity conditions whilst Samani was less tolerant than Zaghloul cultivar.

As for the diameter of callus : Zaghloul explants were superior to Samani explants in all cases which indicated that Zaghloul cultivar was more tolerant to salinity than Samani cultivar ,the difference between Zaghloul

callus diameter (2.91 cm) and Samani cullus diameter (2.65 cm) was insignificant.

The effects of interaction between the cultivars and the treatments were insignificant.

In respect of the effect of the treatments, untreated gave the significant highest value (3.38 cm) with insignificant differences and followed by the treatments of 1000 and 3000 ppm. salts (3.22 and 3.10 cm, respectively), the treatment of 5000 ppm. salts came in the fourth rank with significant differences with the previous treatments and followed by the treatment of 7000 ppm slats which gave the significant lowest value (1.71 cm).

Table (2): Effect of some salinity treatments on cullus weight (g) and cullus diameter (cm) of ZagHloul and Samani cultivars (season2002).

characters / treatments		Cullus weight			Cullus diameter		
		Zaghloul	Samani	AverageS	Zaghloul	Samani	Averages
Control		2.66	2.9	2.78	3.55	3.2	3.38
1000 ppm Salts		2.4	2	2.2	3.4	3.03	3.22
3000 ppm Salts		2.2	1.9	2.05	3.2	3	3.10
5000 ppm Salts		1.9	1.6	1.78	2.6	2.46	2.50
7000 ppm Salts		1.3	0.8	1.06	1.8	1.6	1.71
Averages		2.09	1.85		2.9	2.65	
L.S.D (0.05)	Treats	0.138			0.527		
	Cultivars	0.314			Ns		
	Interaction	Ns			Ns		

From the previous results, it could be concluded that Zaghloul explants were more tolerant to salinity in callus formation stage than Samani explant. Also, there are reverse relationship between the concentration of the salts and both of the callus weight and callus diameter and vice versa as increasing in salt concentration resulted in decreasing in the previous parameters in both the two studied cultivars.In some points,these results were confirmed with those of Shakib *et al.*,1994 and Saker *et al.*,1998 whose mentioned that callus proliferated onto MS medium with 10mg 2,4-D+AC

3. Organogenesis :

Table (3) and fig (B and c) Considering the shoot number, Samani explants were superior to Zaghloul explants as Samani explants were gave the highest value (9.72 shoots / explant) with significant difference with Zaghloul explants(7.95 shoots/explant).

The effect of interaction between the cultivars and the treatments was insignificant whereas the differences between the values were insignificant. The effect of treatments was significant since untreated gave the highest value (14.66 shoot/explant) followed by the treatments of 1000, 3000, 5000 and 7000 ppm salts (10.66, 8.2 , 5.66 and 3.83 shoots/explant respectively). All the differences between the treatments were significant.

Table (3): Effect of the Salinity treatments on arerages of the shoot number and shoot length of Zaghloul and samani cultivars (season 2002).

Characters / Treats		Shoot number			Shoot length		
		Zaghloul	Samani	Averages	Zaghloul	Samani	Averages
control		12.66	16.6	14.66	1.6	1.8	2.2
1000 ppm Salts		9.3	12	10.66	2.5	1.8	2.2
3000 ppm Salts		7.3	9	8.2	1.66	1.33	1.5
5000 ppm Salts		4.66	6.66	5.66	1.33	1.33	1.33
7000 ppm Salts		3.33	4.33	3.83	1	1	1
Averages		7.95	9.78		1.79	1.95	
L.S.D (0.05)	Treats	1.363			0.472		
	Cultivars	1.181			Ns		
	Interaction	Ns			Ns		

Considering the shoot length, Samani was gave the highest average of shoot length per explant (1.95 cm) with insignificant difference with Zaghloul cultivar (1.79 cm) as well as the effect of the interaction between the cultivars and the treatments was insignificant as all differences between the values of the interaction were insignificant.

In respect of effect of the treatments, untreated and the treatment of 1000 ppm salts was gave the highest average of the shoot length (2.2 cm for both of them) with significant difference and followed by the treatment of 3000 ppm. Salts, whilst the treatment of 7000 ppm. gave the significant lowest value (1cm).

It could be concluded that the increasing in concentration of the salts lead to the decreasing in averages of the shoot length and shoot number per explant in both the two studied cultivars. Also, Samani explants exhibit more capability of the salinity tolerant than Zaghloul as Samani explant gave more shoot length and the shoot number per explant compared with Zaghloul explant.In parallel with these results, Taher,1983 ; Ruiz *et al.*,1997; El-Desouky and Atawia,1998 and Romero *et al.*,1998 found in Citrus species that the growth was depressed by increasing salinity(NaCl and CaCl2) and the species were varied in their tolerance to saline.Also,Banuls *et*

al.,1991reported that the tolerant to NaCl was attributed to exclude the chloride ions and Ca++ increased Cl- accumulation in the basal stem and roots(toxicity),while Ruiz et al.,1997 found that the growth was reduced due to the osmotic effect of salinity and the inhibitory effects of Cl- and Na+. One or more one from the previous causes may be response for tolerant to salinity in Samani,also the weak tolerant in Zaghloul may be due to one or more of the previous causes.In this respect,Belal and El-Deeb,1997 found that shoot initiated from Samani and Zaghloul explants on MS medium with NAA,IAA and BAB.

4. Embryogenesis :

Table (4) and fig (C)showed that the averag root length of Samani embryos were significantly superior to Zaghloul embryos (0.71 and 0.46 cm. respectively). Also Samani embryos were superior to Zaghloul embryos in all the treatments but with insignificant differences between the values. The differences between the values of the interaction between the cultivars and the treatments were insignificant. Untreat gave the significant highest embryro root length (0.98 cm) with significant difference and followed by the treatment of 1000 ppm salts (0.73cm) while the treatment of 7000 ppm salts gave the significant lowest embryo root length (0.19 cm). There are reverse relationship between concentration of the salts and the embryo root length in both the two cultivars.

Table (4): Effect of different concentrations of the salts of CaCl + NaCl on averages of the root length of the embryo , length of the embryo (cm) and number of the embryos per unite of the Cullus in Zaghloul and Samani (seasons 2002/2003).

characters / Treats		Root length of the embryo(cm)			Total length of the embryo(cm)			Number of the embryos/unite of callus		
		Zaghl -oul	Sama -ni	Avera -ges	Zaghl -oul	Sama -ni	Avera -ges	Zaghlo -ul	Sama -ni	Avera -ges
Control		0.76	1.2	0.98	1.5	2.4	1.95	2.5	3.5	3
1000 ppm Salts		0.70	0.76	0.73	1.66	2	1.83	2.5	3.5	3
3000 ppm Salts		0.5	0.86	0.68	1	1.5	1.25	1.5	2.5	2
5000 ppm Salts		0.23	0.46	0.35	0.5	1	0.75	0.66	1.33	0.99
7000 ppm Salts		0.13	0.26	0.19	0.33	0.5	0.415	0.33	0.66	0.50
Averages		0.46	0.71		0.99	1.4		1.50	2.30	
L.SD (0.05)	Treats	0.199			0.106			0.535		
	Cultivars	0.165			0.285			0.648		
	Interactio n	Ns			Ns			Ns		

As for the embryo length, Samani embryos were significantly superior to Zaghloul embryos (1.4 and 0.99 cm respectively) while there are insignificant differences between the values of the interaction between the cultivars and the treatments. In respect of the treatment, the same previous trend in the case of the embryo root length was found in the embryo length as untreat gave the significant highest embryo length (1.95 cm) while the treatment 0f 7000 ppm. salts was gave the significant lowest embryo length (0.42 cm) and reverse relationship was found between the embryo length and concentration of the salts.

Considering number of the embryos per explant, Samani embryos were significantly superior to Zaghloul embryos (2.30 and 1.50 embryos / explant respectively).

While the differences between the values of the interaction between the treatments and the cultivars , were insignificant. Untreat and the treatment of 1000 p.p.m. salts came in the first rank with insignificant difference as they gave the significant highest number of the embryos per explant (3 embryos / explant) while the treatment of 7000 ppm. salts gave the significant lowest number of the embryos per explant (0.5 embryo / explant). There are reverse relationship between number of the embryos per explant and concentration of the salts as shown in table (4).In the respect of the medium used in this stage, Mater,1986 and Quraishi *et al.*,1997 found that somatic embryo nodles of dates formed in callus when transfer to MS medium with NAA or 2,4-D or GA3 or 2ip. The results of the embryogenesis stage were in parallel with those of obtained by Taher,1983 ; Ruiz *et al.*,1997 ; El-Desouky and Atawia,1998 and Romero *et al.*,1998 whose found in Citrus species that the growth was depressed by increasing salinity(NaCl and CaCl2) and the species were varied in their tolerance to saline. Also,Banuls *et al.*,1991reported that the tolerant to NaCl was attributed to exclude the chloride ions and Ca++ increased Cl- accumulation in the basal stem and roots(toxicity),while Ruiz et al.,1997 found that the growth was reduced due to the osmotic effect of salinity and the inhibitory effects of Cl- and Na+. One or more one from the previous causes may be response for tolerant to salinity in Samani,also the weak tolerant in Zaghloul may be due to one or more of the previous causes.

5. Rooting stage :

Table (5) showed that the root length of Zaghloul explants were superior to Samani explants (20.3 and 13.1 cm) with highly significant difference between them as well as Zaghloul explant gave higher root number per explant than Samani explant in all the treatments.

The effect of the interaction between the cultivars and the treatments was insignificant. As for effect of the treatments, untreat gave the highest root length (32.33 cm) with significant difference and followed by the treatment of 1000 ppm Salts (24.33 cm) while the treatment of 3000 and 5000 ppm salts came in the third and the fourth ranks (16.2 and 6.2 cm respectively).

The treatment of 7000 ppm gave the significant lowest root length (3.5 cm). These results showed reverse relationship between concentration of the salts and averages of the root length (cm).

As for the root number per explant, Zaghloul explants were slightly superior to Samani explants (0.98 and 0.97 root / explant) with insignificant difference, also this trend was found in all the treatments. The effect of the interaction between the cultivars and the treatments was insignificant. On other hand, slightly reverse relationship was found between the root number per explant and concentration of the salts whereas untreat and the treatment of 1000 ppm. were gave the same highest value (1.33 roots / explant) while the treatments of 5000 and 7000 ppm. salts gave the same lowest value (0.5 root / explant) with significant differences with the previous treatments. In the respect of the medium used in this stage, Zaid and Tisserat,1983 and

Quraishi et al.,1997 found that roots of dates formed on MS medium with NAA. The results of the rooting stage were in parallel with those of obtained by Taher,1983; Ruiz *et al.*,1997; El-Desouky and Atawia,1998 and Romero *et al.*,1998 whose found in Citrus species that the growth was depressed by increasing salinity(NaCl and CaCl2) and the species were varied in their tolerance to saline. Also,Banuls *et al.*,1991 reported that the tolerant to NaCl was attributed to exclude the chloride ions and Ca++ increased Cl- accumulation in the basal stem and roots(toxicity),while Ruiz et al.,1997 found that the growth was reduced due to the osmotic effect of salinity and the inhibitory effects of Cl- and Na+. One or more one from the previous causes may be response for tolerant to salinity in Samani,also the weak tolerant in Zaghloul may be due to one or more of the previous causes.

Table (5): Effect of Salinity treatments on averages of the root number/explant and the root length (cm)of Zaghloul and samani cultivars (seasons 2002/2003) .

Characters / Treatments		Root length(cm)			Root number/explant		
		Zaghloul	Sammany	ARS	Zaghloul	Sammany	Ars
Control		36.66	27.66	32.33	1.33	1.33	1.33
1000 ppm Salts		32.66	16.00	24.33	1.33	1.33	1.33
3000 ppm Salts		22.33	10	16.1	1.33	1.0	1.20
5000 ppm Salts		5.66	6.66	6.2	0.33	0.66	0.50
7000 ppm Salts		4	5	6.2	0.33	0.66	0.50
Averages		20.3	13.1		0.98	0.97	
L.S.D (0.05)	Treats	7.193			0.354		
	Cultivars	4.518			Ns		
	Interaction	Ns			Ns		

In vivo study (Acclimatization stage):

Table (6-A) and fig (E) The root number / plantlet of Zaghloul plantlets were significantly superior to Samani plantlets (1.84 and 1.45 roots per plantlet in Zaghloul and Samani plantlets respectively) and the difference was significant. The treatments of 1000, 2000, 3000; 4000 and 5000 ppm salts and untreat gave the signficiant highest number of the roots per plantlet (2.66 roots / plantlet) whilst, the treatments of 8000, 9000 and 10000 ppm salts gave the same significant lowest number of the roots per explant (0.0), the other treatments came between them with significant differences. In respect of effect of the interaction between the cultivars and the treatments, almostly the same trend in the treatments was observed in the interaction.

Table (6-A):Survival percentage of the plantlets in the acclimatization;averages of the root number /plantlet and average of the root length (cm)of the plantlets influenced by some salinity treatments under the acclimatization conditions in Zaghloul and Samani cultivars (season 2003).

Treats		Number of roots/plantlet			Root length(cm)			Survival percentage		
		Zaghl-oul	Sam-ani	Avera-ge	Zaghl-oul	Sam-ani	Aver age	Zagh -loul	Sam-ani	Average
control		2.66	2.66	2.66	26.33	20	23.17	72.66	67.66	70.2
1000 ppm Salts		2.66	2.66	2.66	26.33	19	22.66	9	8.33	8.66
2000 ppm Salts		2.66	2.66	2.66	26.33	18	22.17	6	4.33	5.2
3000 ppm Salts		2.66	2.66	2.66	24.66	17	20.83	4.33	3.33	3.8
4000 ppm Salts		2.66	1.66	2.66	24.33	15	19.66	3.33	2.66	2.99
5000 ppm Salts		2.66	1.66	2.66	24.33	14.66	19.50	3.33	2.33	2.83
6000 ppm Salts		2.66	1.0	1.83	19.66	13	16.33	1.33	1	1.20
7000 ppm Salts		1.66	1.0	1.33	19.33	12	15.66	0.99	0.66	0.83
8000 ppm Salts		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9000 ppm Salts		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10000 ppm Salts		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Averages		1.84	1.45		17.39	11.69		9.17	8.20	
L.SD (0.05)	Treats	0.320			2.227			20.970		
	Cultivars	0.182			1.363			Ns		
	Interact.	0.809			8.036			32.411		

Considering the root length, Zaghloul plantlets were significantly superior to Samani plantlets (17.39 and 11.69 cm respectively) and the difference was significant. The untreat gave the highest root length (23.17 cm) with insignificant and followed by the treatments of 1000, 2000 and 3000 ppm salts while, the treatments of 8000, 9000 and 10000 ppm salts came in the last ranks and gave the significant lowest root length (0.0 cm). in respect of effect of the interaction between the cultivars and the treatments, the treatments of 1000, 2000 ppm salts in Zaghloul plantlets and untreat, gave the same significant highest root length (26.33 cm) while, the treatments of 8000, 9000 and 10000 ppm salts in both the two cultivars were gave the same significant lowest value (0.0 cm) and the other treatments of both the two cultivars came between them in the ranks. As for the success percentage, Zaghloul plantlets was superior to Samani plantlets (9.17 and 8.20 % respectively) with insignificant difference between them. The untreat gave the significant highest success percentage (70.2%) with great significant differences with the other treatments while, the treatments of 8000, 9000 and 10000 ppm salts gave the same significant lowest success percentages (0.0%). In respect of effect of the interaction, untreat of Zaghloul and Samani plantlets gave the significant highest success percentages (72.66 and 67.66% respectively) , while the treatments of 8000, 9000 and 10000 ppm salts in Zaghloul and Samani gave the same significant lowest success percentage (0.0%).

Table (6 – B) could be explained as follows:

Considering the plantelet length , Samani plantlets were significantly superior to Zaghloul plantelets (23.84 and 19.89 cm respectively) and the difference was significant. The treatments of 8000, 9000 and 10000 ppm salts gave the same significant lowest values (0.0 cm) whilst, untreat gave the highest value (37.38 cm) and the other treatments came in the following ranks. Effect of the interaction between the cultivars and the treatments was significant whereas treatments of Samani by 1000, 2000 p.p.m. salts and untreat gave the highly values (38.55, 37.11 and 39.44 cm respectively) with insignificant differences between them whilst treatment of both the two studied cultivars by 8000, 9000 and 10000 ppm slats gave the same significant lowest values (0.0cm).

Table (6-B): **Averagelength of the plantlets(cm) and leave number/plantlet influenced by salinity treatment under the acclimatization conditions in Zaghloul and Samani Cultivars (season 2003).**

Characters / Treatments		Length of the plant(cm)			Number of the leaves/plantlet		
		Zagloul	Samani	Averages	Zagloul	Samani	Averages
Control		35.33	39.44	37.30	3.0	3.0	3.0
1000 ppm Salts		33.44	38.55	35.99	3.0	3.0	3.0
2000ppm. Salts		31.66	37.11	34.38	3.0	3.0	3.0
3000 ppm Salts		27.33	35.66	31.50	3.0	3.0	3.0
4000 ppm Salts		26.33	31.66	28.99	3.0	2.0	2.5
5000 ppm Salts		25.11	31.66	28.38	3.0	2.0	2.5
6000 ppm Salts		24.66	24.66	24.66	2.60	1.30	1.95
7000 ppm Salts		15.0	23.55	19.28	2.0	1.30	1.70
8000 ppm Salts		0.0	0.0	0.0	0.0	0.0	0.0
9000 ppm Salts		0.0	0.0	0.0	0.0	0.0	0.0
10000 ppm Salts		0.0	0.0	0.0	0.0	0.0	0.0
Averages		19.89	23.84		2.10	1.70	
L.S.D (0.05)	Treats	2.846			0.660		
	Cultivars	1.622			Ns		
	Interact.	8.706			1.354		

Considering the leave number, Zaghloul was superior to Samani without significant difference between them (2.50 and 1.69 leaves per plantlet respectively). Untreat, the treatments of 1000, 2000 and 3000 ppm salts gave the same significant highest leave number (3 leaves / plantlet) while the treatments of 8000, 9000 and 10000 ppm salts gave the same significant lowest values (0.0 leave / plantlet). Almostly the same trend in effect of the treatments was found in effect of the interaction between the cultivars and the treatments.

From the previous results in the acclimatization stage, it could be conduded that there were reversely proportional between concentration of the salts and the studied parameters, also the treatments of 8000, 9000, 10000 ppm Salts gave the significant worst results (0.0) as indicated the both the two studied cultivars (Zaghloul and Samani) were not tolerant to salinity at concentration from 8000 - 1000 ppm salts.

The results of the acclimatization stage were in parallel with those of obtained by Taher,1983; Ruiz *et al.*,1997; El-Desouky and Atawia,1998 and Romero *et al.*,1998 whose found in Citrus species that the growth was depressed by increasing salinity(NaCl and CaCl2) and the species were varied in their tolerance to saline. Also,Banuls *et al.*,1991 reported that the tolerant to NaCl was attributed to exclude the chloride ions and Ca++ increased Cl- accumulation in the basal stem and roots(toxicity),while Ruiz et al.,1997 found that the growth was reduced due to the osmotic effect of salinity and the inhibitory effects of Cl- and Na+. One or more one from the previous causes may be response for tolerant to salinity in Samani,also the weak tolerant in Zaghloul may be due to one or more of the previous causes.

Proline content:

Table (7) showed that Zaghloul explant was superior to Samani explant in their contents of the proline and in all of the treatments

In the two cultivars, the increasing in concentration of the salts resulted in the increasing of proline content . This relationship indicated that the proline content was correlated in positive relationship with tolerant to in vitro salinity conditions. Untreat gave the lowest values in the two cultivars (5.8 mg/g F.W in Zaghloul and 5.5 mg / g. F.W. in Samani). The treatment of 7000 p.p.m.. salts resulted in the highest values of the proline content in the two cultivars (7.06 mg/g. F.W. in Zaghloul and 6.80 mg /g. F.w. in Samani). The results of the proline content were harmony with Ahmed(1999) who found that saline irrigation water (6000 p.p.m.) significantly increased the average proline contents of Manzanillo olive seedling leaves as compared with tap water, while Al-Khayri and Al-Bahrany (2002) mentioned that the increasing osmotic stress in rice in vitro culture induced proline accumulation and the highest concentration of proline.

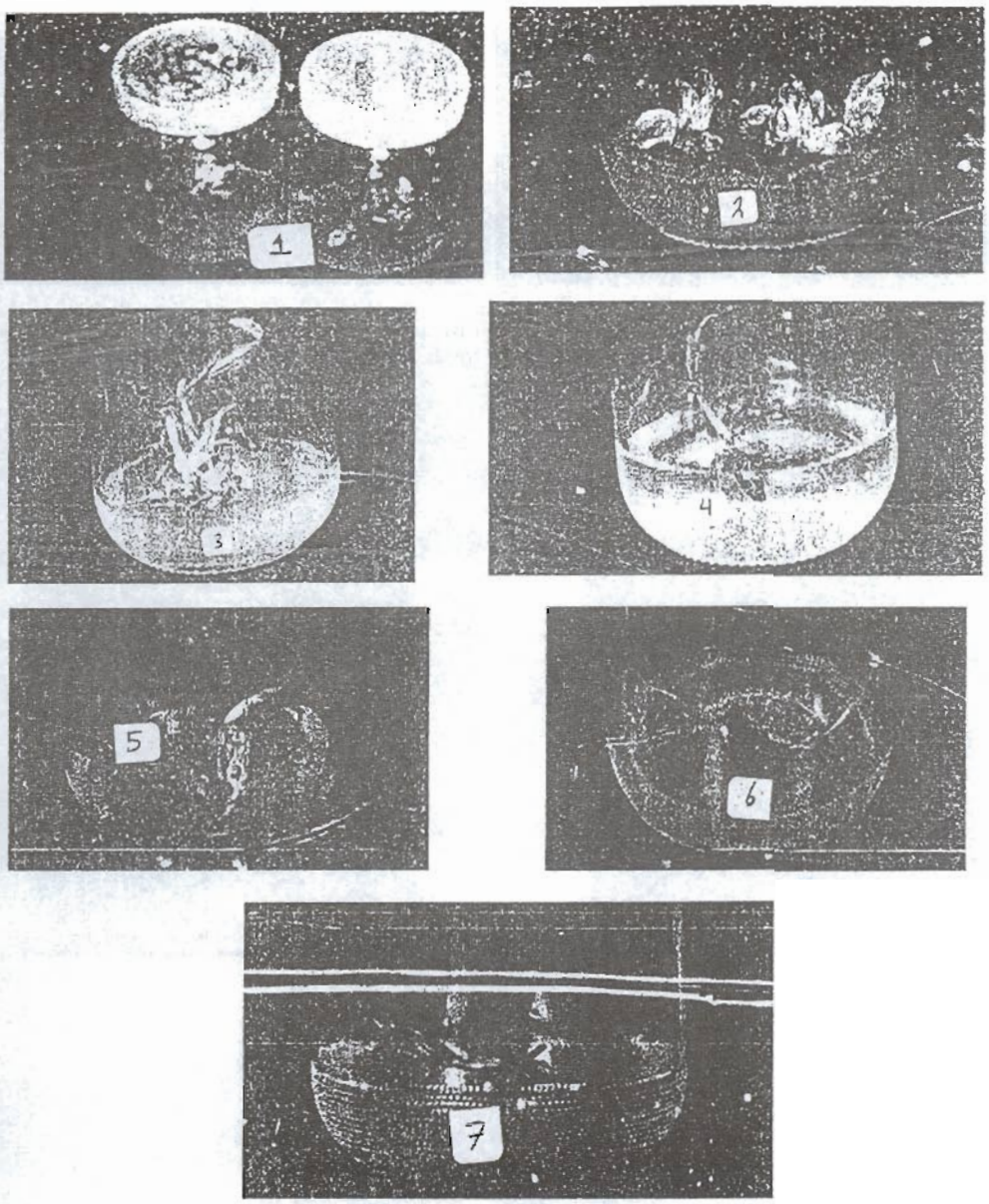

Fig (A) showed the different development stages of both Samani and Zaghloul cultivars in 0.0 p. p.m . salt (control) : picture (1) illustrated that the differentiated callus ; picture (2) showed the developed embryos from callus ; picture (3) showed the growth and elongation of the embryos ; picture (4) showed that the separated and transplanted embryos on fresh medium ; picture (5) showed that initiation of vegetative shoots from callus (organogenesis) ; picture (6) showed that initiation of the roots from callus (rooting stage)and picture (7) showed that the developed plantlet .

Fig (B) showed effect of 3000p.p.m. salt treatment on the growth and development of both Zaghloul (pictur (1)) and Saman' (picture (2)) cultivars .

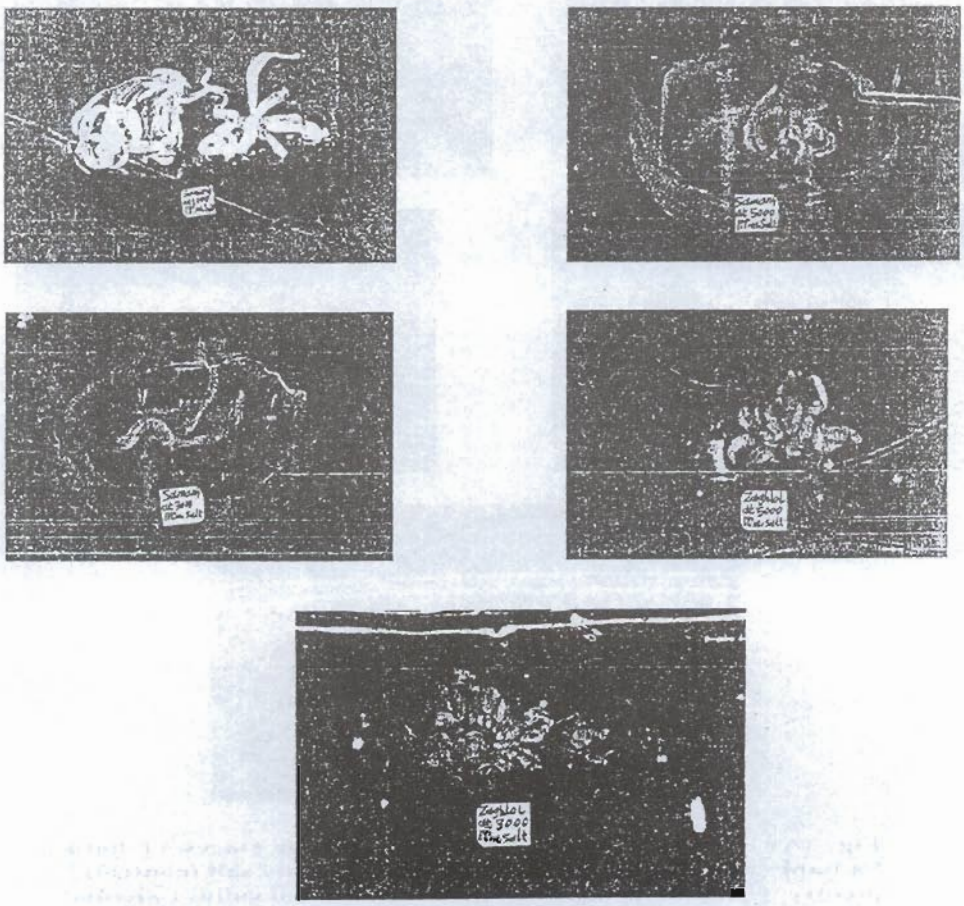

Fig (C) showed effect of some treatments of the salt solutions on the growth of the embryos in both Zaghloul and Saman' cultivars .

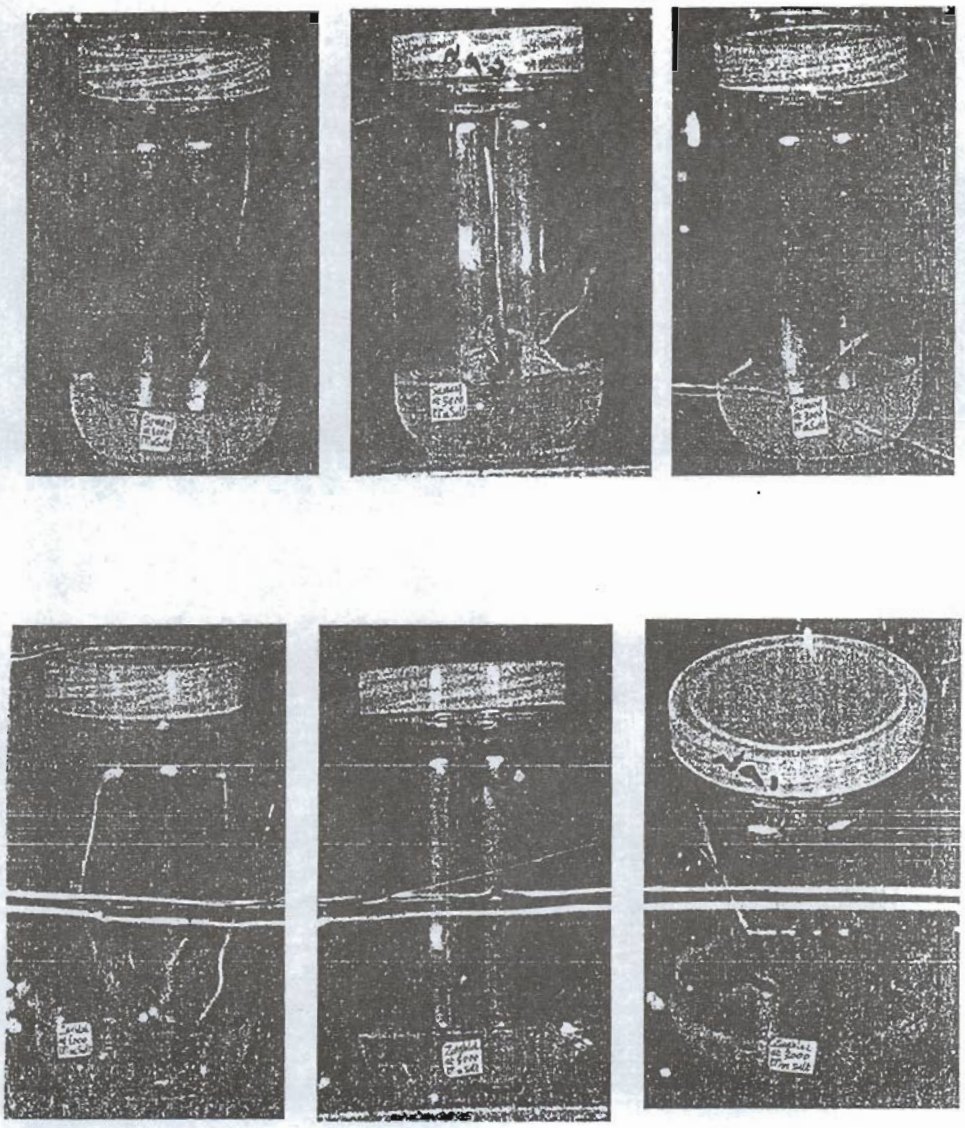

Fig (d) showed effect of salt treatments on the growth of the developed plantlets in both Zaghloul and Samani cultivars.

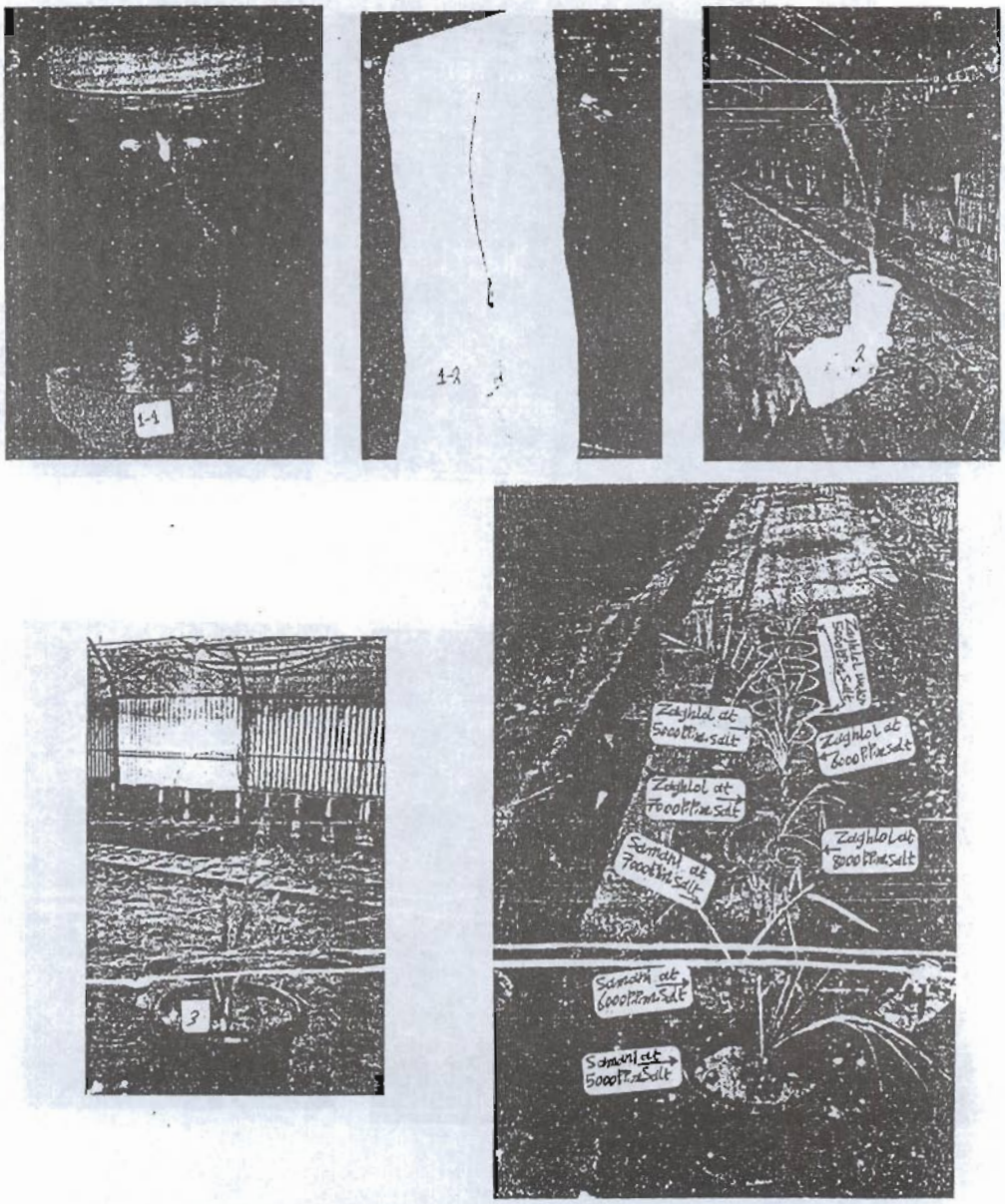

Fig (E) showed the acclimatization stage as follows : picture (1-1) illustrated the *in vitro* plantlet before the acclimatization ; picture (1-2) illustrated the plantlet before put it in plastic cup filled with agriculture soil (picture (2)); picture (3)showed the growing plant in plastic pot at the final step and picture(4)illustrated effect of the irrigation with different concentrations of salt solutions on the growth of both Zaghloul and Samanj cultivars

Table (7): Effect of in vitro saline treatments on the proline content(mg/g. F.W.) in leaves of Zaghloul and Samani cultivars (season 2003).

Treats \ cultivars		Proline content (mg/g F.W. leave)		
		Zaghloul	Samani	Averages
Control		5.5	5.8	5.65
1000 ppm Salts		6	6.4	6.2
3000 ppm Salts		7.1	7.2	7.1
5000 ppm Salts		7.7	7.8	7.7
7000 ppm Salts		7.8	8.1	7.8
Averages		6.8	7.06	6.9
L.S.D (0.05)	Treats	1.043		
	Cultivars	Ns		
	Interact.	Ns		

REFERENCES

Ahmed,W.A. (1999). Effect of some additives on tolerance of olive plants to salinity. M. Sc. Thesis, Fac. Agric. ,Cairo Univ. ,Egypt.

Al-Khayri ,J.M. and Al-Bahrany , A.M. (2002). Callus growth and proline accumulation in response to sorbitol and sucrose-induced osmotic stress in rice. Biologia Plantarum, 45 (4): 609 - 611.

Banuls, J.; F. Legaz, and E. Primo-Millo (1991). Salinity-Calcium interactions on growth and ionic concentration of citrus plants. Plant and Soil , 133(1): 39-46.

Batels, L.S.Waldren, R.P. and Teare,I.D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 939:205-207.

Bhansali , R.R. and Kaul , R.K. (1991). Date through tissue culture. Indian Horticulture, 36 (3):6 - 10.

Belal, A.H. and El-Deeb, M.D. (1997). Direct organogenesis of date palm (*Phoenix dactylifera* L.) *in vitro*. Assiut J. Agric. Sci., 28 (2): 67 - 77.

El-Desouky, S.A. and A.A. Atawia (1998). Growht performance of some citrus rootstocks under saline conditions Alex. J. agric. Res. , 43(3): 231-254.

Mater ,A.A. (1986). *In vitro* propagation of *Phoenix dactylifera* L. Date Palm J., 412: 187 - 152.

Quraishi , A.; Hussain, I.; Ahmad , M.; Rashid , H. and Latif, M. (1997). Sustained multiplication of long term embryogenic cultures of date palm and their field performance. Pakistan J. Bot.,29 (1): 135 - 141.

Rao, A.M.; Reddy , S.K ; Roo, V.R. and Bhalla , J.K. (2001). Clonal propagation of date palm through somatic embryogenesis. Indian Forester. , 127 (4): 463 - 465 .

Romero-Aranda, R.; J.L Moya ; F.R. Tadeo; F. Legaz ; E.primo-Millo and M. Talon (1998). Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. Plant Cell and Environment , 21(12): 1243-1253

Ruiz, D.; V. Martinez and A. Cerda (1997). Citrus response to salinity growth and nutrient uptake. Tree Physiol. 17(3): 141-150.

Saker, M.M.; Moursy, H.A. and Bekheet , S.A. (1998). *In vitro* propagation of Egyptian date palm:morphogenic responses of immature embryos. Bullet. Fac. Agric., Cairo Univ., 49 (2):203 - 214.

Shakib, A.M.; Khoshkam, S. and Majidi, E. (1994). Plant regeneration of date palm variety Estamaran by tissue culture. Seed and Plant , 9 (3 –4), 8 - 11.

Sharon,M. ;Shankar,P. C. and Sharon,M. (1998). Somatic embryogenesis and Plant regeneration from leaf primordia of *Phoenix dactylifera* cv Yakubi. Indian J. Exp. Biol. , 36 (5): 526-529.

Shatnawi ,A.; Hussin ,A. and Quraishi ,A. (1997). *In vitro* response of date palm cv. Dhaki to plant growth regulators. Sarhad J. Agric., 13 (1):45 - 53.

Snedecor , G.W. and Cochran, W.G.(1980). Statistical Methods. 7th Ed. Iowa State Univ. Press, Ames, Iowa, USA.

Taher, H.M. (1983). Effect of salinity on growth and leaf mineral composition of some citrus rootstock seedlings. M.Sc. Thesis, Fac. of Agric. El-Azhar Univ.

Zaid, A. and Tisserat, B. (1983). *In vitro* shoot tip differentiation in *Phoenix dactylifera* L. Date Palm J. 1983, 2 (2): 163 - 182.

سلوك عزلات ونبتات البلح تحت الظروف المعملية والغير معملية

همت محمد كمال * ، محمد حلمى عبد الظاهر * و احمد سيد العدوى **

* قسم الفاكهة . كلية الزراعة . جامعة القاهرة

** معهد البحوث الزراعية

اجريت دراسة معملية وفى خارج المعمل وتحليل للبرولين خلال مواسم ٢٠٠٢ الى٢٠٠٣ على صنفين مصريين من نخيل البلح (الزغلول والسمانى) .

الاهداف من هذه الدراسات كان اختبار تحمل الظروف الملحية فى المعمل وخارج المعمل فى كلا الصنفين وايضا دراسة العلاقة ما بين محتوى البرولين وتحمل ظروف الملوحة فى هذين الصنفين .

اظهرت النتائج ان معاملات كلوريد الزنبق اعطت اقل نسبة مئوية للتلوث (صفر % فى كلا الصنفين) بينما المعاملة بالكلوركس ١٠ % لمدة ١٥ دقيقة اعطت اعلى نسبة مئوية للتلوث (٧٠ % فى الزغلول و ٨٠ % فى السمانى) . اقل نسبة مئوية للعزلات الحية فى كلا الصنفين تم الحصول عليها بالمعاملة بكلوريد الزنبق ٠,١ % لمدة ١٠ دقيقة . المعاملة بالكلوركس تركيز ١٠% لمدة ١٥ دقيقة اعطت اعلى نسبة مئوية للعزلات الحية فى كلا الصنفين (١٠٠%) بينما المعاملة بكلوريد الزنبق ٠,١ % لمدة ٥ دقائق اعطت افضل نسبة مئوية للعزلات الحية الغير ملوثة (المعقمة) (٦٠% فى الزغلول و ٧٠ % فى السمانى) .

صنف الزغلول تفوق على صنف السمانى فى متوسطات وزن وقطر الكالس ، عدد وطول الجذور المتكونة من الفروع المعملية وكذلك قياسات النمو فى مرحلة الاقلمة . من جهة اخرى تفوق صنف السمانى على الزغلول فى متوسطات عدد وطول الفروع المعملية وقياسات النمو للاجنة المعملية . المعاملة بـ ٧٠٠٠ جزء فى المليون املاح فى المعمل اعطت اقل وزن وقطر للكالس واقل عدد وطول للفروع المعملية والجذور المعملية الناتجة من الفروع المعملية واقل نمو للاجنة المعملية مقارنة بالمعاملة بدون ملوحة(الكونترول).

اظهرت الدراسة خارج المعمل ان الرى بمحاليل ملحية تركيز ٨٠٠٠ ، ٩٠٠٠ و ١٠٠٠٠جزء فى المليون اعطت اقل النتائج (قياسات النمو) فى مرحلة الاقلمة لكلا الصنفين مقارنة بالرى بالماء (الكونترول) .

توجد علاقة عكسية بين تركيز الاملاح وقياسات النمو للاصناف المدروسة فى المعمل وخارج المعمل .

كان محتوى صنف الزغلول من البرولين اعلى من صنف السمانى كما اظهر صنف الزغلول تحمل اكثر لظروف الملوحة المعملية وخارج المعمل مما يعكس وجود علاقة موجبة بين تركيز البرولين وتحمل ظروف الملوحة فى كلا الصنفين .