EFFECT OF SALTS STRESS ON GROWTH AND DEVELOPMENT *IN VITRO* CULTURE, ACCLIMATIZATION STAGE ON *PHOENIX DACTYLIFERA* L. CV. SAKUTI IN GREENHOUSE

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Abstract

This investigation was carried out during 2002-2005 to study the effect of salinity. (NaCl +$CaCl_2$) 2:1 by weight on date palm *Phoenix dactylifera* L. cv.Sakuti propagated *in vitro* from shoot tip explants. Different concentrations (0,6000,10000 and 14000 ppm) were added to MS medium+ 0.5 mg/l BA+ 0.1 mg/l NAA +1.5 g/l AC at shooting stage and added to MS medium + 3 mg/l IBA + 1.5 g/l AC at rooting stage. Shootlets and rooted plantlets were recultured on shooting and rooting medium for 18 and 9 weeks respectively (3 weeks intervals). Rooted plantlets resulted from different salinity levels were pre acclimatized by culturing on ¼ MS liquid medium + 3 mg/l IBA without sucrose for 3 weeks and then transferred to greenhouse and planted on plastic bags contain peatmoss: vermiculite : perlite 2:1:1 for 3 weeks. Plantlets were transferred from bags to plastic tunnels until new leaves were grown.

In general increasing the levels of salinity significantly decreased growth parameters (shootlets length cm., number of leaves or roots/plantlet and root length) as compared with control (no salts used), which had the highest value in this respect. The survival percentage was significantly reduced gradually with salinity increased, the highest survival percentage (68.2 %) was obtained by control treatment followed in a descending order by those of 6000 and 10000 ppm treatments (66.6 and 62.5 %, respectively) and the rank last 14000 ppm treatment which produced the lowest value (43.0%).

Key word: date palm, *Phoenix*, salinity, *in vitro*, Na,Ca,Cl

INTRODUCTION

Phoenix dactylifera L.. palms belong to Arecaceae (palmaceae) family, (Pandey, 2005) *Phoenix dactylifera* cv. Sakuti, as a dry cultivar. one of the most important dry cultivars grown at Aswan governorate. Palms can grow under salinity and drought, date palm trees are thought to be more tolerant to salinity than other trees, most of areas in which date palm is grown is subjected to salinity (Hassan and El-Azayem, 1990), Azra *et al.* (1997) on date palm stated that the survival *ex vitro* was 70-80 % when well-rooted plants 8-12 cm in length was used. (Moursy and Saker, 1998) showed that the agronomy importance of date palms (*Phoenix dactylifera* L.) is linked to its high

tolerance to environmental stresses, such as salinity, drought, and high temperature, in addition to its low maintenance and harvesting costs, for these reasons, date palm is an important in plantation of arid regions in the world. Extensive breading programs for the selection of superior clones through traditional methods is slow, due to the long life cycle and heterozygous nature of date palms, introduction of mass *in vitro* propagation will contribute to increase the amount of planting material (offshoots) available, Sharon *et al.* (1998) on date palm indicated that the plantlets were successfully transferred to pots containing a mixture (1: 1) of vermiculite and peat moss. Le *et al.* (1999) on *Phoenix canariensis* used MS medium with 0.45 µM BA and 0.06 µM NAA for root elongation. Wanas *et al.* (1999) on date palm cv. Sewy reported that bud development and shoot proliferation were best on modified medium with 1.0 mg/l 2ip + Kinetin (0,0.5,1.0,2.0 or 5 mg/l) and 1.0 or .05 mg/l NAA while the medium supplemented with Zeatin (1 mg/l) + 0.5mg/l NOA produced well formed shoots with fully expanded leaves, Al-Khayri (2003) on date palm indicated that addition of IBA from 0.2 to 0.4 mg/l to the medium induced higher percentage of complete plantlets with half strength MS . NAA (0, 0.2 ,0.4, 0.6, 0.8 and 1.0 mg/l) enhanced the percentage of embryos that formed only roots irrespective of medium strength, El-Bahr *et al.* (2003) on *Phoenix dactylifera* L. cv. Zaghloul found that the highest survival percentage of plantlets was observed with peat moss + vermiculite + perlite (1:1:1). Wang *et al.* (2003) found that salt stress (150 mM salt) gave significantly inhibition growth of *Populus tremula* plantlets. El-Kazzaz and El-Bahr (2003) on date palm stated that the best medium for developing plantlets was liquid MS medium with perlite as substrate which gave the highest number of roots. Zhang *et al.* (2004) indicated that *Populus euphratica* which is more salt resistant than other poplar cultivars, callus was induced from shoot segments on (MS) medium supplemented with 0.5 mg/l (2.2 micro molle) 6- benzyladenine (BA) and 0.5 mg/l (2.7 micro molle) NAA, the calli were transferred to MS medium supplemented with 0.25 mg/l BA, and 0.5 mg/l NAA, the growth was inhibited with increasing NaCl concentration.

MATERIALS AND METHODS

This study had been carried out at the Department of Ornamental Horticulture, Faculty of Agric., Cairo Univ., and Central Laboratory for Research and Development of Date Palm, Agriculture Research Center (ARC) Giza, during 2002-2005. on *Phoenix dactylifera* cv. Sakuti,

The aim of this study was to investigate the following points:

1- Study the salinity tolerance with different levels ($NaCl+CaCl_2$) *in vitro* of date palm *Phoenix dactylifera* L. cv. Sakuti at shooting and rooting stage.

2- Hardening of plantlets which were tolerant to salinity in the rooting stage.

Starting stage: -

Shoot tip explants of date palm (*Phoenix dactylifera* L. cv. Sakuti) were sterilized and cultured on MS medium (Murashige and Skoog 1962) supplemented with 100 mg/l 2,4-D + 3mg/l 2ip +2 g/l activated charcoal (AC) for 8 months (4 weeks intervals) to produce callus cultures which were subcultured on MS + 10 mg/l 2,4-D + 3 mg/l 2iP + 1.5 g/l AC to produce embryogenic callus for 3 months (4 weeks intervals) and then transferred to growth regulators free medium to produce somatic embryogenesis

Shooting stage:-

The mature embryos resulted from starting stage developed into a cluster, and these clusters produced shoots, these shootlets (8.9-9.5cm/shootlets, 2-3 leaves/shootlets) were cultured in MS medium supplemented with 0.5 mg/l BA+0.1 mg/l NAA +30 g/l sucrose+ 6 g/l phytagar+1.5 g/l (AC) supplemented with three concentrations of NaCl+$CaCl_2$+ by weight (6000,10000 and 14000 ppm NaCl+$CaCl_2$) in addition to control treatment (no salts were used) in three replicates as every replicate contains 3 jars and each jar contain one plantlet, these cultures were transferred into the same fresh medium every 3 weeks. After 18 weeks the following estimations were recorded.

1- Average length of shootlet (cm)

2- Average number of leaves /shootlet.

Rooting stage:-

The plantlets resulted from micropropagation procedure (8-9cm. in length, 2-3 leaves/plantlets, 2-3 root number, 4.5-5.1 cm. root length) were cultured on MS medium supplemented with 3 mg/l IBA+30 g/l sucrose +1.5 g/l AC +6 g/l phytagar) with the concentrations of salts under investigation, in addition to the control treatment. The cultures were transferred into the same fresh medium every 3 weeks, after 9 weeks the following data were recorded.

1- Average survival percentage.

2- Average length of shoots (cm.)

3- Average number of leaves /plantlets.

4- Average number of roots /plantlet

5- Average length of roots (cm)

Pre acclimatization.

Rooted plantlets (28) were cultured in ¼ MS+ vermiculite + 3mg/l IBA for 3 weeks (28 C^0 and 7000 lux).

Hardening stage.

In this stage survival rooted plantlets (9.5 cm. plantlet length, 3 leaves/plantlet, 2-3 roots/plantlet and 4.9 cm. rootlet length) which were tolerant salinity which were

produced from pre acclimatization stage and transferred to greenhouse were planted in plastic pots (18.5cm in length and 5 cm in width) filled with peatmoss+ vermiculite + perlite 2:1:1(v/v/v) and covered with transparent plastic bags for 2 weeks in the greenhouse,_then the plastic bags were punched up 2 cm. from two sides after one week and then another punch for one week and then the plastic bags were removed and irrigated, and then they were put under the plastic tunnels until the new leaf is grown. At the end of hardening the following data were recorded.

1-Survival percentage.

2-Average shoot length (cm)

3- Average leaves number/plantlet.

4- Average root number/plantlet

5- Average root length (cm.).

Data were subjected to the analysis of variances using (randomized complete block design) and L.S.D (0.05) was used for comparison, according to (Snedecor and Cochran 1980).

Results and Discussion

1-1 Shooting stage:-

Results in Table (1) and Photo (1) revealed the reduction effect of different levels of salinity on shootlet length, number of leaves/shootlet, at shooting stage, data show clear differences between four tested treatments, control treatment (no salts were used) gave significantly higher shootlet length (12.30 cm) followed by 6000 and 10000 ppm (11.46 and 10.63 cm, respectively) and the rank last 14000 ppm (9.96 cm).

Regarding number of leaves/shootlet, data show nearly similar trend as observed on shootlet length i.e. control and 6000 ppm produced the highest number of leaves/shootlet with no significant differences between them (4.30 and 4.00) followed by 10000 and 14000 ppm treatments (2.70 and 2.23 leaves/shootlet, respectively). These results were confirmed with Evers *et al.* (1997) on *Populus tremula*. In addition Sidky (2004) on *Phoenix dactylifera* cv. Hayani and Sewi who concluded that the gradul increasing in NaCl and seawater levels (1000-12000 ppm) negatively correlated with all shoot characters (number and length).

2-Rooting stage:-

Data presented in Table (2) and Photo (2) indicated the inhibitory effect of salinity on the averages of survival percentage of rooting stage, the highest survival percentage (100%)was obtained by both of control treatment and 6000 ppm.treatment, followed in a descending order by those of 10000 and 14000 ppm treatments (88.8 and 77.7%, respectively). The above mentioned results were in agreement with Rousseau *et al.* (1999) on *Phoenix canariensis*, Alang and Hisajima(1991)on *Elaeis guineenis*, Chen *et al.* (1998) who found that the growth of

Eucalyptus microcorys was inhibited during salt adaptation from 100 to 150 mmol/l NaCl over 12 month. and Zhang *et al.* (2004) He also found that growth of *Populus euphratica* was depressed with increasing NaCl concentrations (>50 mmol/l).

Concerning growth characteristics, the same Table and photo show that all the different levels of salinity significantly decreased growth parameters (shootlets length , number of leaves or roots/plantlet, and root length) as compared with control treatment which had the highest value in this respect (12.7 cm.,3.8, 4.63 and 7.23cm., respectively), in a descending order by those of 6000,10000 ppm. treatments and the rank last 14000 ppm treatment which produced the lowest value in this respect (9.5 cm., 2.6, 2.4 and 4.96 cm. respectively). Data also reveal that there are no significant differences in number of leaves/plantlet between 6000 and 10000 ppm treatments, it was also found between 10000 and 14000 ppm treatments. These data were in harmony with those of Mahmoud (2002) on *Myrtus communis*, and El-Adawe(2005) on *Phoenix dactylifera* L. cv.Zaghloul and Samany, who stated that length, and number of shoot, and root /plantlet were significantly decreased with high salinity levels (10000ppm.).

3- Acclimatization stage:-

As shown in Table (3) and Photo (3 and 4) it can be seen that the survival percentage was significantly reduced gradually with the different levels of salinity increased, the highest survival percentage (68.2%) obtained by control treatment, followed in a descending order by those of 6000 and10000 ppm (66.6, 62.5 %, respectively) and the rank last 14000 ppm treatment which produced the lowest value in this respect (43.0 %). These results were in harmony with the findings of Tisserat (1984), Bhansali and Kaul (1991), Shakib *et al.* (1994), Azra *et al.* (1997), Sharon *et al.* (1998), and Ahmed (1999) on date palm. Shen *et al.*(1999) found that *Populus maximowiczii* x *P. palntierensis* reduced its adaptability for NaCl concentration at 100 mM. El-Sharabasy (2000), and Sidky (2004) on date palm stated that the survival percentages were depressed with high levels of salinity.

On regard to growth parameters, the same table and photo revealed the negative effect of salinity on shoot length , number of leaves or root/plantlet, and root length. Control treatment (no salts used) recorded highly significant values (14.8 cm. 4.5, 5.3, and 8.4 cm. respectively),the three tested levels of salinity could be descending arranged as follows: 6000 , 10000, 14000 ppm. treatments. The differences between 10000 and 14000 ppm treatments in shoot length, number of leaves/plantlet and root length did not reach the level of significance, it was also found between control and 6000 ppm treatments in number of leaves/plantlet. . The above-mentioned results coincided with that obtained by Wang *et al.* (2003) who stated that salt stress (150 mM NaCl) significantly inhibited the growth of all plantlets of *Populus tremula* and El- Adawe (2005) on *Phoenix dactylifera* L.

Table 1. Effect of salinity levels ($NaCl+CaCl_2$) on average growth characters at shooting stage of date palm (*Phoenix dactylifera* L.)cv. Sakuti

Treatment ($NaCl+CaCl_2$) ppm.	Ave. length of Shootlets (cm).	Ave. number of Leaves / Shootlet
Cont.	12.30	4.30
6000	11.46	4.00
10000	10.63	2.70
14000	9.96	2.23
L.S.D.(0.05)	=0.53	=0.43

Table 2. Effect of salinity levels ($NaCl+CaCl_2$) on average growth characters and survival percentage at rooting stage of date palm (*Phoenix dactylifera* L.) cv. Sakuti

Treatment ($NaCl+CaCl_2$) Ppm.	Survival percentage %	Ave. length of shootlets (cm.)	Ave. number of Leaves / plantlet	Ave. number of roots / plantlet	Ave. length of root (cm.)
Con	100	12.70	3.80	4.63	7.23
6000	100	11.1	3.20	3.96	6.43
10000	88.8	10.30	2.90	2.96	5.50
14000	77.7	9.50	2.60	2.40	4.96
l.s.d. (0.05)		=0.72	=0.54	=0.4	=0.3

Table 3. Effect of salinity levels ($NaCl+CaCl_2$) on average of growth characters at acclimatization stage of (*Phoenix dactylifera* L.) cv. Sakuti after 12 weeks

Treat ($NaCl+CaCl_2$) ppm.	Survival percentage %	Av. shoot length (cm)	Av. leaves number/plantlet	Av. roots number/plantlet	Av. root length (cm).
Cont.	68.2	14.8	4.5	5.3	8.4
6000	66.6	13.9	4.2	4.3	7.5
10000	62.5	13.1	3.1	3.7	6.7
14000.	43.0	12.8	2.7	3.4	6.5
L.S.D.(0.05)		0.8	0.5	0.2	0.34

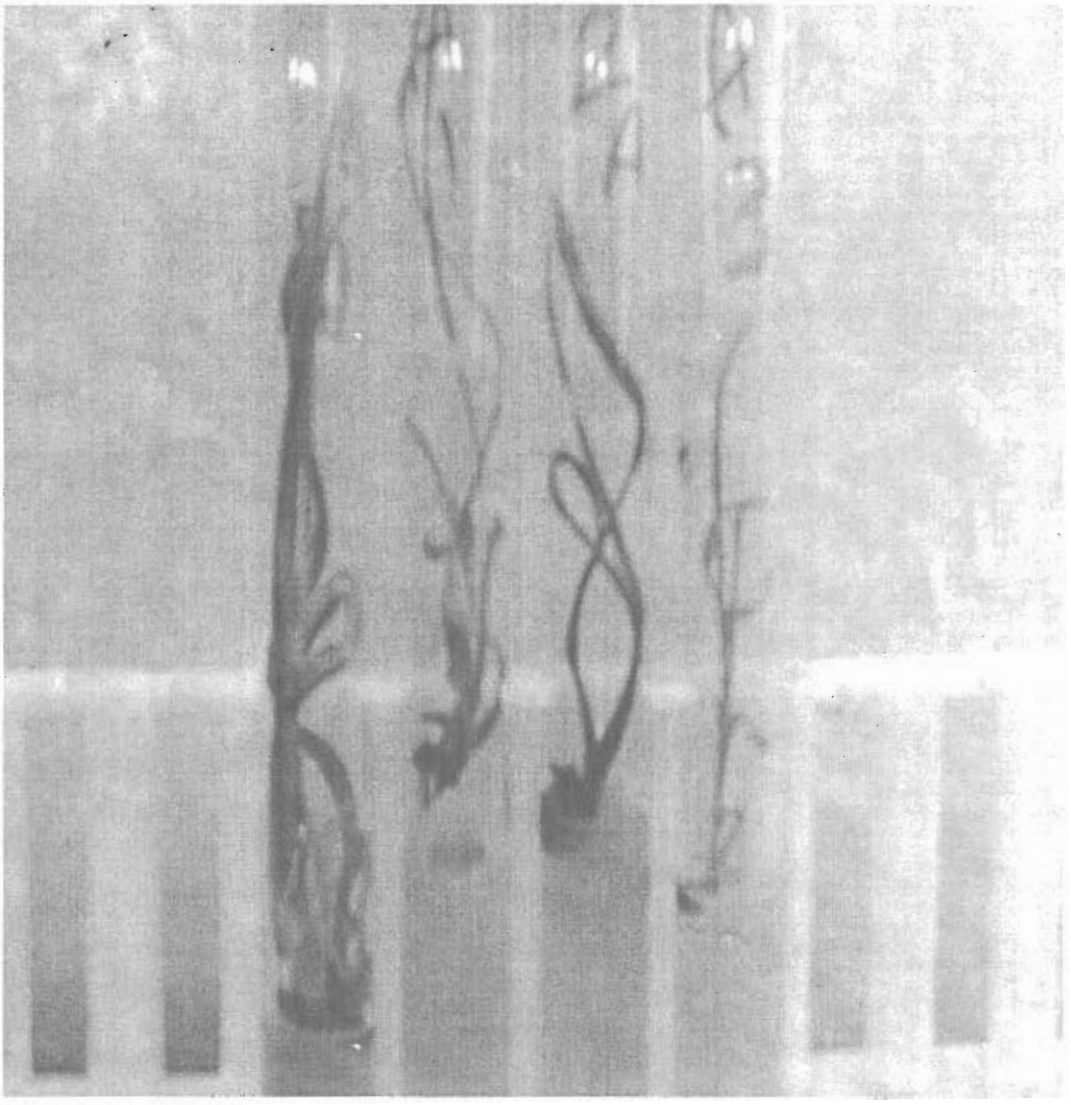


Fig 1. Effect of salinity on shooting stage of *Phoenix dactylifera* cv. Sakuti ,

0=Control treatment
Treat (1) =6000ppm NaCl+$CaCl_2$
Treat (2) =10000ppm NaCl+$CaCl_2$
Treat (3) =14000ppm NaCl+$CaCl_2$

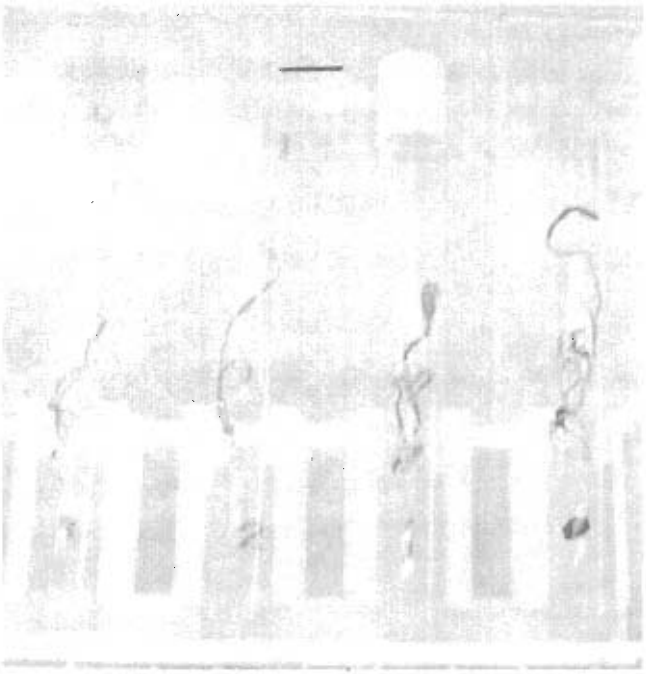


Fig 2. Effect of salinity on rooting stage of *Phoenix dactylifera* cv. Sakuti

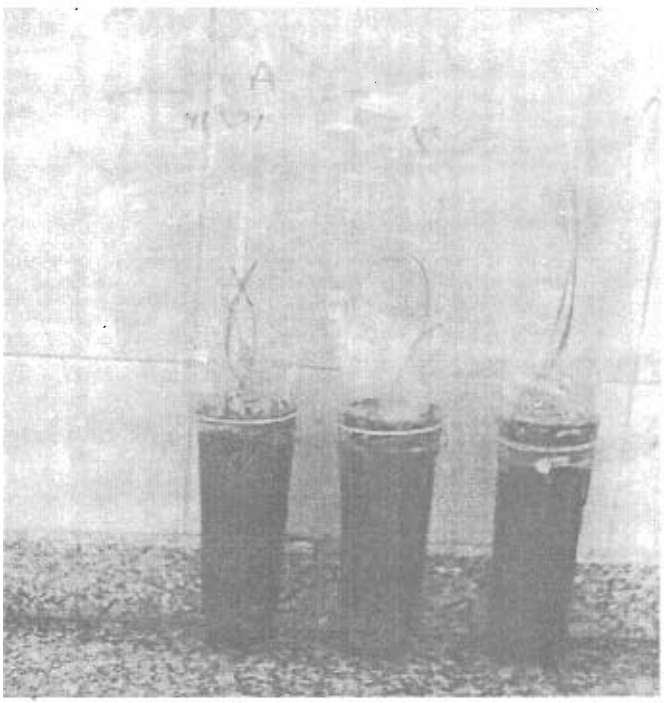
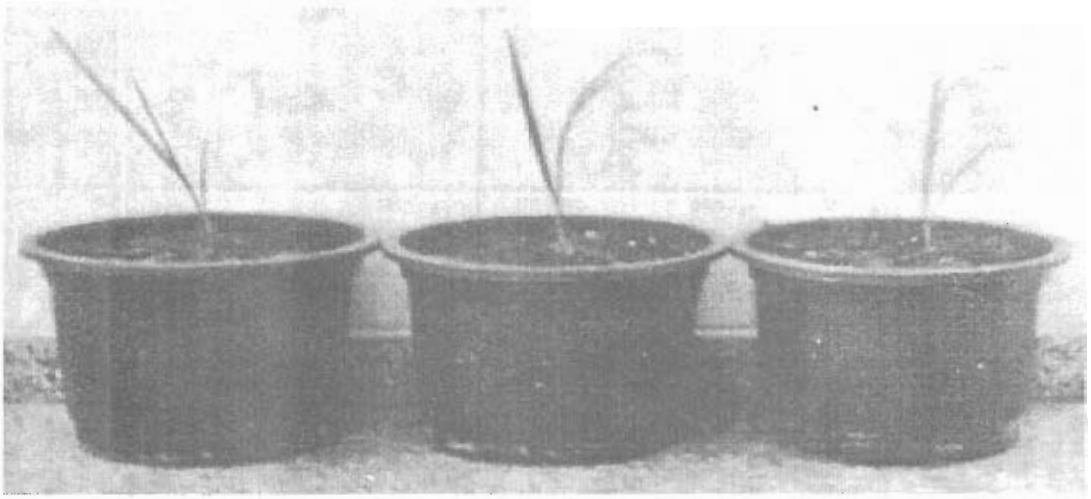
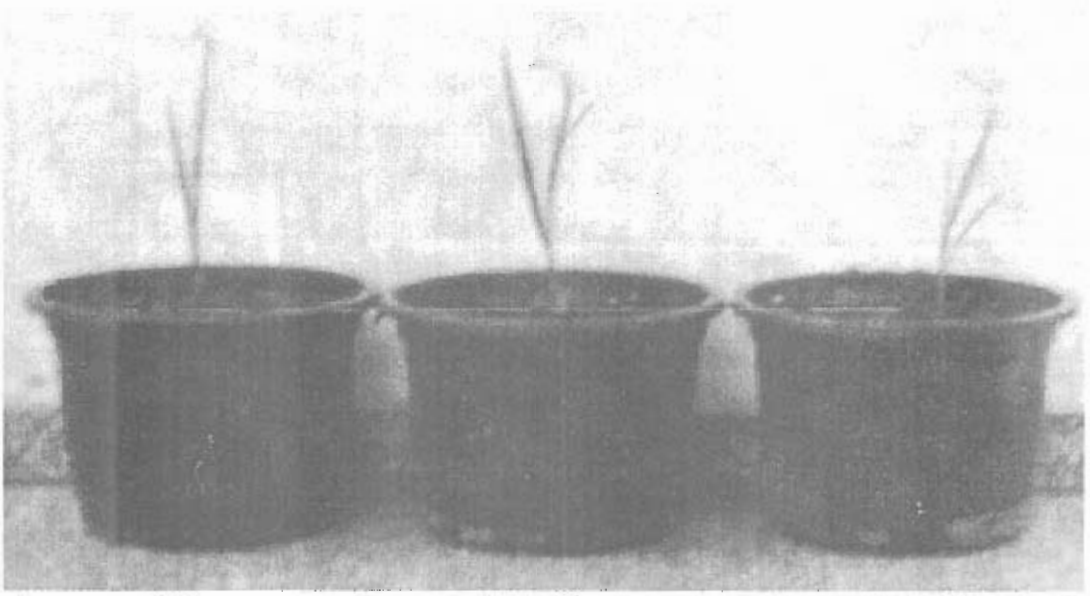


Fig 3. Acclimatization stage of *Phoenix dactylifera* cv. Sakuti first step with plastic bags



a - Con treat (1) treat (2)



b- Con treat (2) treat (3)

a= Control with treat 1+ treat 2
b= Control with treat 2+treat 3
0= Control treatment (no salts)
Treat (1) =6000ppm $NaCl+CaCl_2$
Treat (2) =10000ppm $NaCl+CaCl_2$
Treat (3) =14000ppm $NaCl+CaCl_2$

Fig 4. Acclimatization stage after 12 weeks of *Phoenix dactylifera* cv. Sakuti

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تأثير الاجهاد الملحى على النمو و التطور من خلال زراعة الانسجة و مرحلة الاقلمة على نخيل البلح صنف سكوتى

عبد الله الطنطاوى عبد الله[1] ، عزة محمد سعيد عرفة[1] ، عبد المنعم البنا[2] ، رسمية سيد سيد درويش[2]

١. *كلية الزراعة جامعة القاهرة*

٢. *المعمل المركزى للابحاث و تطوير نخيل البلح*

استهدفت هذا البحث دراسة تأثير ثلاث مستويات من الملوحة (٦٠٠٠ ،١٠٠٠٠ و ١٤٠٠٠ جزء في المليون ١:٢ كلوريد الصوديوم + كلوريد الكالسيوم) على مرحلة التضاعف و التجذير و ايضا مرحلة الاقلمة، حيث أدت التركيزات العالية من الملوحة في مرحلة التضاعف الى أقل القيم من حيث طول الافرع و عدد الاوراق وفى مرحلة التجذير أعطت معاملة الكنترول أعلى القيم من حيث النسبة المئوية للبقاء، طول الافرع، عدد الاوراق، عدد الجذور و كذلك طول الجذور أعطت المعاملة ١٤٠٠٠ جزء في المليون من الملوحة تأثير معنويا مثبطا علي النسبة المئوية للبقاء حيث أعطت ٧٧,٧ % بينما أعطت المعاملة ٦٠٠٠، ١٠٠٠٠ جزء في المليون١٠٠% ، ٨٨,٨% علي التوالي ، أيضا تأثيرا مثبطا علي قيم طول الافرع- عدد الاوراق- عدد الجذور- طول الجذور، أما مرحلة الاقلمة فقد أعطت معاملة الكنترول أعلي نسبة مئوية معنوية للبقاء حيث سجلت ٦٨,٢% بينما أعطت المعاملة ٦٠٠٠ جزء في المليون من الملوحة ٦٦,٦% و أعطت المعاملة ١٠٠٠٠ جزء في المليون ٦٢,٥% أما أقل نسبة مئوية للبقاء فقد كانت من المعاملة ١٤٠٠٠ جزء في المليون حيث أعطت ٤٣% بعد شهر من الزراعة

-اعطت معاملة الكنترول أعلي القيم معنويا من حيث طول الافرع، عدد الاوراق ، عدد الجذور و طول الجذربينما أعطت المعاملة ١٤٠٠٠ جزء في المليون من الملوحة أقل القيم في هذا الصدد .

الكلمات الدالة : نخيل البلح ، كلوريد الصوديوم ، كلوريد الكالسيوم، زراعة الانسجة، الاقلمة