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THE ADVANSE EFFECT OF GA_3 (GIBBERELLIC ACID) ON MEDIUM SALINITY OF *Phoenix dactylifera* L. PLANTLETS *In vitro* ROOTING STAGE

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ABSTRACT

The effect of an application of GA3 to *in vitro* rooting culture medium (MS + 30 g/l sucrose + 6 g/l agar +0.1 mg/l kinetin + 3.0 mg/l IBA + 0.5 g/l AC) of date palm for 6 months (1month interval) to adverse the salinity effects was studied. The plantlets were cultured on a medium with different concentrations of GA3 (0,20,25 and 30 mg/l) combined with various levels of salinity (0,10000,14000 and 16000 ppm NaCl + $CaCl_2$ 2:1 by weight). The plantlets were incubated under controlled laboratory conditions (27 ± 2 C^0 and 6000 lux).These plantlets were transferred to the liquid culture medium as a pre acclimatization treatment (1/4 MS +3.0 mg/l IBA) for two weeks before transferring to the greenhouse. The results showed that salinity has a depressing effect on various growth parameters (shoot and root length, number of leaves and number of roots), in contrast salt stress increased proline which is considered a (compatible osmoticums), Na, Ca, Cl and total sugars contents. GA_3 application had a stimulated effect on the adverse effect of salinity stress on the growth parameters and survival percentage at the acclimatization stage. The advantage of GA_3 application under salinity levels is discussed.

Key words: *GA3 , in vitro, Phoenix dactylifera, proline ,salinity*

1. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) which belongs to Arecaceae family is an important source of income and nutrition in a number of countries. It is considered a salt tolerant plant species (Al Mansoori *et al.*, 2007). Salinity levels (0,50,75 and 100 mM NaCl) *in vitro* culture medium of bitter almond (*Amygdalus communis*) resulted in a reduction in shoot height, rooting percentage, root number and root length (Shibli *et al.*, 2003), When *Populus* x *canescens* micropropagated young trees were exposed to 25 mM and 100 mM in hydropomc culture, the growth rate and biomass were declined after three weeks of exposure to 100 mM NaCl (Bolu and Polle, 2004). Salt stress at 7 dSm had a negative effect on plant height and leaf growth of sugarcane (*Saccharum officinarum* L.) whereas application of 150 ppm GA3 as set treatment mitigated the negative effect of salinity (Gomathi and Thandapani, 2005). Root length, number of roots and shoot of the plantlets of potato treated *in vitro* under NaCl stress at 0-80 mmol were significantly reduced, proline content increased progressively with salinity levels (Li-Huizhen *et al.*, 2006). Under greenhouse conditions the plant height of *Pistachio* nut tree was decreased with gradually increasing salinity levels 0,1200 and 2400 mg/kg soil (Saadatmand *et al.*, 2007). The increasing of salinity (0,50,100 and 150 mole/m^3) affected growth rate of Opuntia (*Ficus indica*) whereas proline, Na and Cl contents were increased with increasing salinity (Franco and Veliz, 2007). Proline was accumulated in leaves of *Suaeda salsa* seedlings with increasing salinity levels 0,0.05,0.1,0.2, 0.3, 0.4, 0.6 mol/l Na, (Duan *et al.*, 2007).

This investigation aimed to study the interactive effects of GA3 and salinity stress on the growth parameters and chemical contents of date palm plantlets grown *in vitro* culture (rooting stage).

2. MATERIALS AND METHODS

This study was carried out at the laboratory for Research and Development of Date palm, Agricultural Research Center (ARC) Giza, during 2007-2008 on *Phoenix dactylifera* L. cv.

Bartomuda plantlets as a dry cultivar of date palm. Three concentrations of GA_3 (0,20,25 and 30 mg/l) in addition to the control in the early rooting stage were used. Moreover three concentrations of salinity (10000,14000 and 16000 ppm mixture of NaCl+$CaCl_2$ 2:1 by weight) in addition to the control were used. Three plantlets (5-7 cm. in length with 2-3 leaves) were used as an explant material in three replicates, for every treatment. The plantlets in different treatments were recultured for 5 months (one month intervals) in rooting medium containing MS (Murashige and Skoog, 1962) + 30 g/l sucrose + 6 g/l agar+ 0.1 mg/l kinetin + 3.0 mg/l IBA + 0.5 g/l AC + 200 mg/l glutamine. After this stage the plantlets were cultured in liquid medium (1/4 MS + 3.0 mg/l IBA) as a pre acclimatization for two weeks. Plantlets in different applications were incubated in the growth room (27 ± 2 C^0 and 6000 lux). The plantlets were transferred to acclimatization in the greenhouse. In this stage the tolerate plantlets were cultured in plastic pots (18.5 cm in length and 5 cm in width) filled in with peatmoss + perlite 2:1 (v/v) under tunnels for 2-3 months at humidity (80-90 %, approximately) and the tunnels were open gradually until new leaves appeared. The data were recorded at the end of the experiment.

-Shoot and root length (cm).
-Number of leaves and roots/plantlet.
-Survival percentage of the plantlets during acclimatization stage
-Chemical contents: proline, total sugars and mineral (Na, Ca and Cl)

2.1. Proline content

As described by Bates *et al.* (1973) proline

$$mg/g = \frac{ppm.x\ ml.\ extract}{2x\ g.samples\ x100}$$

2.2.Total sugars

Total soluable sugars were extracted by hot ethanol and determined by using phenol sulphuric acid as described by Dubois *et al.*(1956).

2.3. Na,Ca and Cl contents

Were determined according to Jakson (1973). Splite plot method was used in statistically analyzed, data were statistically analyzed and means were compared using least significant difference test L.S.D. at 5 % level (Snedecor and Cochran, 1980).

3. RESULTS AND DISCUSSION

The following results indicate an alleviation effect of GA3 on the negative effects of salinity on growth parameters of the plantlets during rooting stage and survival percentage of acclimatization stage of *Phoenix dactylifera* L. cv. Bartomuda.

3.1. Shoot length

It is clear from Table (1) and Fig (1) that the enhancement effect of GA3 on the shoot length of plantlets in the rooting medium, showed the highest significant values of shoot length obtained by 30 mg/l GA_3 (18.0 cm.) compared to the control while the lowest results were from the treatment of 20 mg/l GA_3 (13.7 cm.) All levels of salinity brought about significant shortest shoot length, the treatment of 16000 ppm $NaCl_2$ gave the lowest significant value in this respect (13.1 cm.) The previous results were similar with those of El-Aziz *et al.* (2006) which reported that stem length of *Kaya senegalensis* was depressed with salinity levels (1000,2000 and 3000 ppm.). Zare *et al.* (2007) stated that increasing of GA_3 led to an increase in shoot length of wheat plants under salinity stress, Recently, Iqbal *et al.* (2008) found that plant height of *Cicer arietinum* was increased with GA_3 treatment at 20 mg/l under NaCl at (0,8,12 and 16 dS/m) .

3.2.Root length

Results from Table (1) reveal that root length (cm.) was depressed by all tested levels of salinity (10000, 14000 and 14000 ppm $NaCl_2$) the highest significant reduction of root length (6.7 cm)was produced by 16000 ppm. treatment, whereas the application of GA_3 (20,25 and 30 mg/l)were significantlly enhanced root length compared to the control. These results were supported by Atta (2005) on wheat plants who stated that GA_3 at 25,50 and 100 mg/l stimulated root length under salinity stress (0,3000,6000 or 8000 ppm NaCl). In addition Patel and Pandey (2007) on *Cassia montana* stated that root elongation was depressed by salinity levels (0.3,3.9,6.0,7.9,10.0,12.1 and 13.9 dSm). Similarly Jaleel *et al.* (2007) on *Catharanthus roseus* reported that root length was affected by salinity levels (15,30,45 and 60 mM).

3.3.Number of leaves

Regarding the effect of GA_3 the results from Table (2) indicate that the application of GA_3 (20,25 and 30 mg/l) mitigated the negative effect of salinity levels on the number of leaves, 20 mg/l GA_3 resulted in the significant high value (3.6 leaves/plant). While the salinity treatments had the significant depressive effect on the number of leaves, the highest depression was found at 16000 ppm salts. In this respect, Barhoumi *et al.* (2007) on *Aeluropus littoralis* revealed that salinity levels (0- 800 Mm NaCl) decreased total plant growth.

3.4.Number of roots

It was noticed from the results in Table (2) a gradual negative effect of different levels of salinity (10000,14000 and 16000 ppm $NaCl_2$ + CaCl) on the number of roots, the lowest depressive effect was obtained by level 10000 ppm(3.2), the highest significant reduction was noticed with 16000ppm (2.6). The different concentrations of GA3 (20,25 and 30 mg/l) had significant adverse effect on root number (3.7,3.4 and 2.9), respectively compared to the control. The present results are in agreement with those published by Dashtakian and Bahrani (2007) on *Rubia tinctoria* also stated that the number of roots/plant was decreased with different salinity (0,4.5,9.0,13.5,18.0 and 22.5 dS/m). The previous results showed that the increasing effect of GA3 on for date palm planted was due to increasing cell elongation of subapical meristems.

3.5.Survival percentage of plantlets at acclimatization stage

Concerning the effect of GA3 (20,25 and 30 mg/l) as alleviated the effect of salinity stress, data in Fig (2 and 3) exhibit that the treatment of 30 mg/l GA3 produced the best results (63.3%). On the other hand, all tested levels of salinity (10000,14000 and 16000 ppm) depressed the survival percentage during acclimatization of the plantlets (60.5,49.5 and 46.8 %, respectively). The present findings are in agreement with those reported by (Bolu and Polle, 2004) and (El-Tantawy *et al.*, 2006) on date palm who found that all levels of salinity (6000, 10000 and 14000 ppm $NaCl+CaCl_2$) decreased survival percentage of acclimatization stage of the plantlets.

3.6.Chemical contents

3.6.1. Proline content

The proline which accumulated naturally under salt stress conditions may help to sustain salt effects (Prasad and Madhurendra, 2005). It seemed that Na + and proline accumulation in shoot were effective mechanisms for osmotic pressure adjustment and plant tolerance to salinity (Pakniyat and Armion, 2007). It is clear from the data in Table (3) that proline content rose with increasing salinity stress. the application of 16000 ppm. gave the highest significant accumulation of proline in the leaves in spite of the presence of GA_3 . In this respect. treatment with GA_3 seems to nullify the harmful effects of salinity by increasing synthesis of different metabolites such as proline and enhancing the biochemical and physiological processes involved in salt tolerance (El- Yazal and Matter 2001).Stress induced proline accumulation under water deficit stress. A It acts as a component of an anti oxidative defense system rather than as an osmotic adjustment mediator (Molinari *et al.*, 2007). The application of GA_3 counteracted some of the adverse effects of NaCl+ $Cacl_2$ salinity with accumulation of proline which maintained membrane permeability and increased macro and micronutrient levels (Tuna *et al.* 2008).

3.6.2. Total sugars

Table (3) exhibits that the same tendency of total sugars with increasing of salinity levels, treatment with 16000 ppm NaCl+ $CaCl_2$ produced significant results compared to the control which gave the lowest values of total sugars. The current findings are similar to the results of Pouresmaeil *et al.* (2005) who found that salinity levels 0,100,200,300,400, and 500 mM NaCl increased soluble sugars in *Suaeda fruticosa*. Uma *et al.* (2005) showed that total sugars increased progressively with salinity levels 0,4 and 8 dS/m on *Vigna mungo*. Choubisa and Vimal (2006) indicated that salinity 1% NaCl and 10 ppm GA3 increased total sugars in wheat plants. In addition, El- Aziz *et al.* (2006) revealed that total sugars increased with increasing of salinity levels (1000,2000 and 3000 ppm) on *Kaya senegalensis*. The above mentioned results.

3.6.3.Na, Ca and Cl content

Regarding the effect of salinity levels on mineral contents, Table (4) demonstrates the progressively increase of Na, Ca and Cl content with increasing of salinity levels. The lowest significant values occurred in the control levels, whereas the treatment of 16000 ppm was produced the significant highest values of these mineral contents. These results are confirmed by (Ottow *et al.*, 2005) who showed that increasing of Na^+ concentrations were required for adjustments of the osmotic pressure of leaves, which were achieved by accumulation of Na^+ and compensatory decreases in calcium and soluble carbohydrates. Nedjimi *et al.*, (2006) on *Atriplex halimus* stated that Ca and Cl were increased with increasing of salinity (0,4,8,12,16,and 20 g/l $CaCl_2$). In addition, Pakniyat and Armion (2007) reported that Na accumulation in shoots of sugar beet was an effective mechanism for osmotic pressure adjustment and plant tolerance to salinity stress.

Table (1) Effect of GA_3 and salinity (ppm.)($NaCl+CaCl_2$) on shoot and root length (cm) of plantlets of *Phoenix dactylifera* L. cv. Bartomuda.

SalinityB / A GA3	Shoot length (cm.)					Root length (cm.)				
	Con.	10000	14000	16000	mean	Con.	10000	14000	16000	mean
Control	10.8	10.0	9.8	9.3	10.0	7.3	6.8	6.3	5.9	6.6
20	14.9	14.5	13.3	12.1	13.7	9.0	8.4	7.9	7.4	8.2
25	17.0	17.0	15.9	14.6	16.1	8.5	8.1	7.3	6.9	7.7
30	19.4	18.5	17.7	16.4	18.0	7.7	7.6	6.5	6.5	7.1
mean	15.5	15.0	14.2	13.1		8.1	7.7	7.0	6.7	

l.s.d. (0.05) A = 0.8
l.s.d.(0.05) B = 0.8
l.s.d. (0.05) AB = 1.1

l s d. (0.05) A =0.4
l s.d.(0.05) B = 0.3
l s d (0.05) AB= 1.1

Table (2) Effect of GA_3 and salinity (ppm.)($NaCl+CaCl_2$) on number of leaves and roots / plantlet of *Phoenix dactylifera* L. cv. Bartomuda.

SalinityB / A GA3	Number of leaves /plantlets					Number of roots / plantlets				
	Con.	10000	14000	16000	mean	Con.	10000	14000	16000	mean
Control	3.0	2.7	2.4	1.7	2.5	3	2.5	2.2	1.7	2.4
20	4.5	3.7	3.4	2.6	3.6	4.2	4.0	3.5	3.2	3.7
25	4.2	3.4	3.0	3.0	3.4	3.9	3.4	3.2	3.1	3.4
30	4	3.1	2.9	2.7	3.2	3.3	3.0	2.9	2 5	2.9
mean	3.9	3.2	2.9	2.5		3.6	3.2	3.0	2.6	

l.s d. (0.05) A= 0.1
l s.d. (0 05) B = 0.2
l s.d.(0.05) AB = 0.3

l s.d. (0.05) A = 0 1
l.s.d. (0 05) B = 0.2
l s d (0.05) AB

Table (3) Effect of GA_3 and salinity (ppm.)($NaCl+CaCl_2$) on proline content (mg/g d.w.) and total sugars (%)of *Phoenix dactylifera* L. cv. Bartomuda.

SalinityB / A GA3	Proline (mg/g d.w.)					Total sugars (%)				
	Con.	10000	14000	16000	mean	Con.	10000	14000	16000	mean
Control	0.8	1.0	1.04	1.1	1.0	65.4	66.0	66.1	66.2	65.9
20	0.82	1.0	1.1	1.2	1.03	65.3	66.2	66.6	67.0	66.3
25	0.84	1.1	1.2	1.4	1.1	65.6	67.2	67.5	67.9	67.1
30	0.84	1.2	1.4	1.5	1.2	65.9	68.7	70.1	70.8	68.9
mean	0.83	1.1	1.2	1.3		65.6	67.0	67.6	68.0	

l.s.d. (0.05) A = 0.04
l.s.d. (0.05) B = 0.05
l.s.d. (0.05) AB = 0.1

Table (4) Effect of GA_3 and salinity (ppm.)($NaCl+CaCl_2$) on content of Na, Ca and Cl of *Phoenix dactylifera* L. cv. Bartomuda.

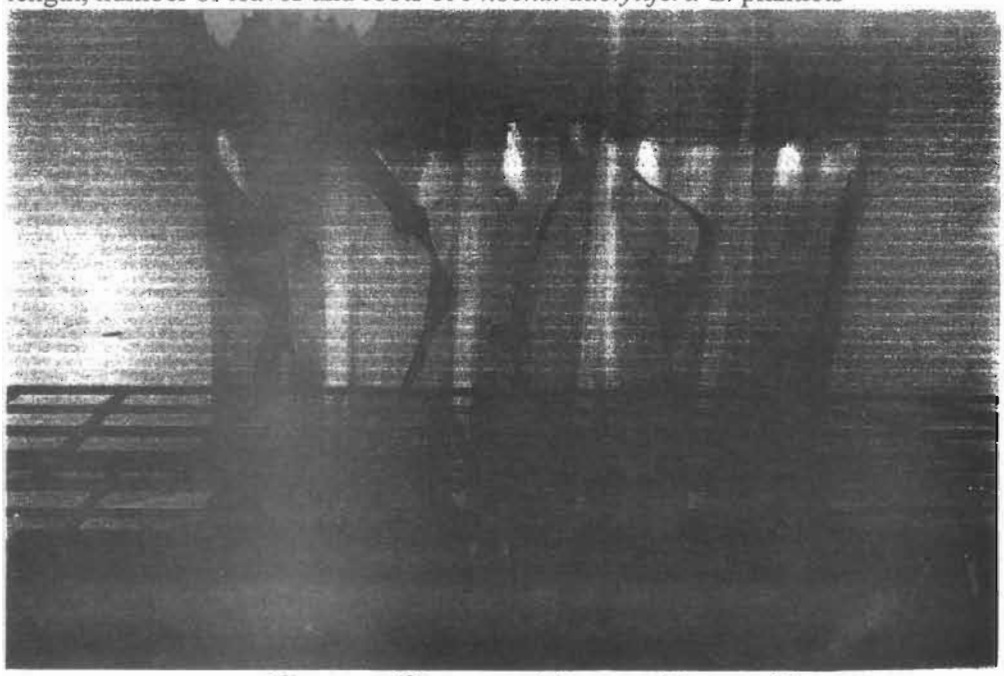
Salinity B / A GA3	Na mg/g d.w					Ca mg/g d.w.					Cl mg/100g d.w.				
	con	10000	14000	16000	mean	con	10000	14000	16000	mean	con	10000	14000	16000	mean
Con	3 2	4.2	7 3	9 2	5 9	2 5	2.8	4.6	6.5	4.1	1 7	2.3	2.6	2.9	2.4
20	2 9	5 5	6.9	8.9	6.1	2 1	3.1	5 1	6.7	4 3	1 6	2.1	2 7	3.0	2.4
25	2.8	7.3	7 5	8 8	6 6	2	3.6	5.8	7 5	4.7	1.5	2 6	2 7	3 5	2.6
30	2.7	7.6	8 7	9 3	7 1	1 9	3 9	6 8	7.8	5 1	1 4	2.8	2.9	3.8	2.7
mean	2 9	6 2	7.6	9 1		2 1	3 4	5.6	7 1		1.6	2.5	2.7	3.3	

l s.d. (0 05) A = 0.2
l.s.d.(0.05) B= 1.0
l.s d (0 05) AB= 2 4

l s.d (0.05) A= 0.4
l.s d (0.05) B= 0 3
l.s d (0.05) AB= 0.7

l.s d. (0.05) A= 0.1
l.s.d.(0.05) B= 0.2
l s d.(0.05) AB =0.5

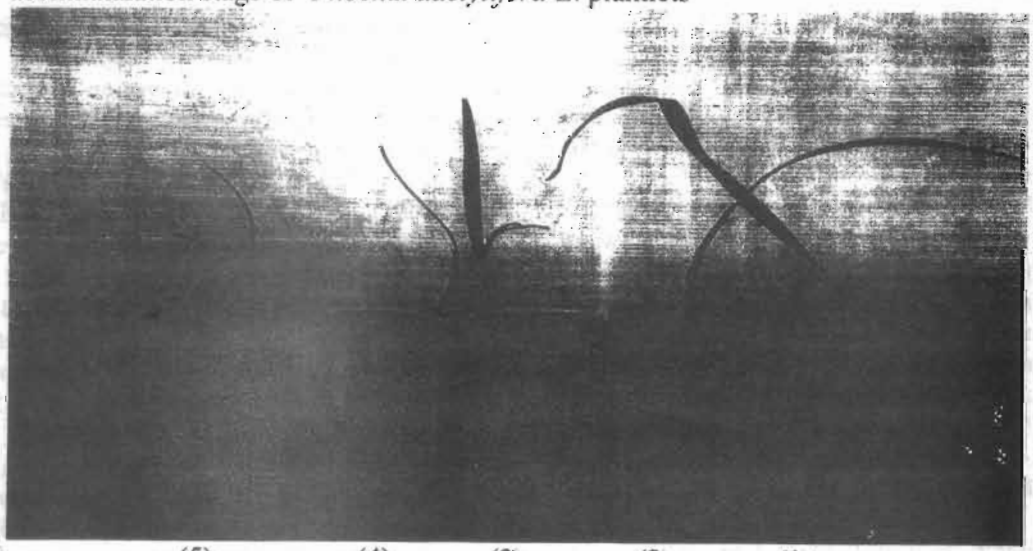
Fig (1): Effect of GA3(mg/l) and salinity ppm. ($NaCl + CaCl_2$) shoot and root length, number of leaves and roots of *Phoenix dactylifera* L. plantlets



(1) (2) (3) (4) (5)

(1) Control GA3 (2) Control salinity (3) Treat 1 (10000 ppm salinity)
(4) Treat 2 (14000 ppm salinity) (5) Treat 3 (16000 ppm salinity)

Fig (2) Effect of GA3(mg/l) and salinity ppm ($NaCl + CaCl_2$) on acclimatization stage of *Phoenix dactylifera* L. plantlets



(5) (4) (3) (2) (1)

(1) Control GA3 (2) Control salinity (3) Treat (1) 10000 ppm salinity
(4) Treat (2) 14000 ppm salinity (5) Treat (3) 16000 ppm salinity

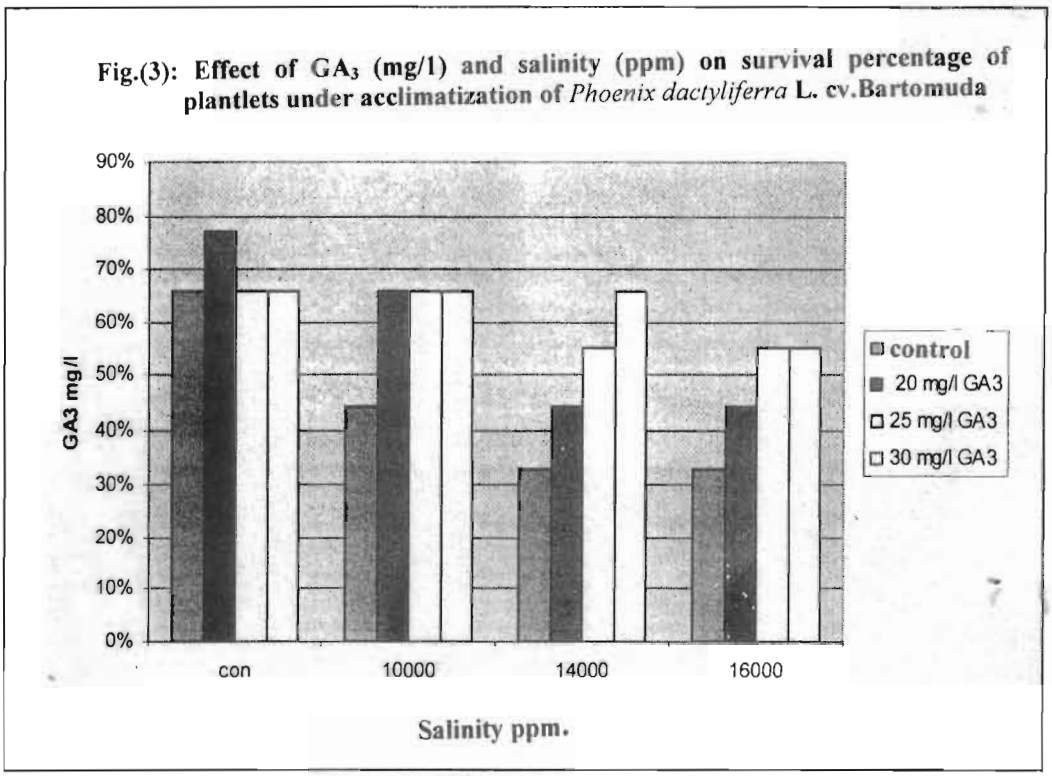


Fig.(3): Effect of GA_3 (mg/l) and salinity (ppm) on survival percentage of plantlets under acclimatization of *Phoenix dactyliferra* L. cv.Bartomuda

Similar results were reported by Barhoumi *et al.* (2007). They reported that, Na and Cl ions content in shoots of *Aeluropus littoralis* increased with salinity levels (0-800 mM NaCl).

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التأثير العكسى للجيبرالين على الملوحة فى بيئة نبيتات نخيل البلح خلال مرحلة التجذير بزراعة الأنسجة

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مركز البحوث الزراعية – الجيزة – مصر

ملخص

أجريت هذه التجربة لدراسة المعاملة بالجبريللين فى بيئة مرحلة التجذير بزراعة الأنسجة .

عوملت نبيتات نخيل البلح بـ (MS+30 g/L Sucrose+6 g/L Agar+0.1 mg/L Kinetin+3.0 mg/L IBA + 0.5 g/L AC) لمدة ستة أشهر (شهر فاصل بين كل نقلة وأخرى) لدراسة التأثير العكس للجيبرالين على التركيـــزات المختلفـــة للملوحة. أخذت النبيتات فى بداية مرحلة التجذير وتمت زراعتها على بيئة التجذير بتركيـــزات مختلفـــة مـــن الجيبـــرالين (صفر/٢٠/٢٥ و ٣٠ ملجم/لتر) بالإضافة إلى تركيزات الملوحة (صفر /١٠٠٠٠ / ١٤٠٠٠ / و١٦٠٠٠ جزء فى المليون كلوريد صوديوم + كلوريد كالسيوم ٢:١بالوزن) وتم تحضين النبيتات بالمعاملات المختلفة تحت التحكم فى المعمل (٢٧ ± ٢ درجة مئوية و ٦٠٠٠ شمعة إضاءة). تم بعد هذه الفترة نقل النبيتات للبيئة السائلة كمرحلة ما قبل الأقلمة (1/4 MS + 3.0 mg/L IBA)

نقلت بعد ذلك النبيتات لمرحلة الأقلمة فى الصوبة الخاصة بذلك. وأوضحت النتـــائج التـــأثير الســـلبى المعنـــوى للتركيزات المختلفة للملوحة على القياسات الخضرية مثل (طول الأفرع والجذور وعدد الأوراق والجذور) وعلـــى العكـــس إرتفع محتوى النبات معنوياً من العناصر المختلفة مثل الصوديوم والكالسيوم والكلوريد وكذلك البرولين و السكريات الكليـــة بزيادة تركيزات الملوحة تدريجيا, أما المعاملة بالجبريللين مع الملوحة فقد كان لها التأثير العكسى الإيجابى المعنوى وذلـــك بتحسين القياسات الخضرية السابقة وايضا النسبة المئوية للاقلمة .

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٦٠) العدد الأول (يناير ٢٠٠٩):١٠٦-١١٣.