Effect of sucrose and abscisic acid on *in vitro* growth and development of date palm during rooting stage

(Received: 01.03.2008; Accepted: 15.03.2008)

M. M. Hassan ; E. G. Gadalla and A .H.Abd-El Kareim
Central Laboratory for Date Palm Research and Development, Agricultural Research Center, Giza, Egypt

ABSTRACT

A typical somatic embryogenesis protocol for date palm involves a series of consecutive stages beginning with somatic embryo induction, maturation, germination and ending with rooting stage. One of the main problems of in vitro propagation is acclimatization of derived plantlets. Rooting and subsequently acclimatization stages were considered the most important stages in the commercial scale. This study was carried out to overcome the problems through rooting stage. Shootlets (5-7 cm in length) of date palm cv. Bartamuda were cultured on rooting MS medium (three quarters or half strength) supplemented with ABA (0.0, 0.25, 0.50, and 1.0mg /l), sucrose (15, 30 and 45 g/l) to study their effects on root number, root length, length of plantlets, growth vigor, plantlet and root thickness. This study showed that, sucrose at the highest concentration (45g/l) and ABA at 0.25 mg/l increased significantly root formation during rooting stage. Using ¾ MS salt strength medium supplemented with ABA at 0.25 mg/l and sucrose at 45 g/l increased significantly the number of roots, plantlet length, growth vigor, plantlet and root thickness, while control MS medium (ABA free medium) recorded the highest value of plantlet lengt ‹ ½ MS medium increased significantly the root length.

***Key words**: Date palm, micropropagation, rooting; sucrose, abscisic acid.*

INTRODUCTION

Bartamuda is one of the most famous dry date palm cultivars grown in Egypt. Aswan is one of the important areas in Egypt for producing dry cultivars. The dried dates have a high percentage of sucrose (up to 80%) of the flesh date, whereas the soft dates are rich in glucose and fructose. There is a small amount of fiber, protein, fat, vitamins and minerals (Hussein *et al.*, 1979).

Date palm plantlets from callus have poor root system due to the lack of adventitious roots. However, the initial primary root was unnecessary for further plantlet growth in date and other palms, and should be trimmed to 1-2 cm in length to facilitate easier transfer *ex vitro* (Tisserat, 1981 and 1982).

Robbins *et al.*, (1996) showed that although ABA stimulated growth in *Lotus corniculatus*, tannin content decreased. Similarly, Vanhala *et al.*, (1998) demonstrated that exogenous application of ABA inhibited hyoscyamine accumulation in hairy root culture of *H. muticus* with adverse effect on biomass. Root initiation is a critical stage in date palm micropropagation, as it governs the subsequent success of production of free living date palm plants (Shaheen, 1990). The concentration of inorganic salts plays an important role in root induction (Ibrahim, 1999) who reported that reduction of MS salts strength to ¾ of the original concentration

stimulated root formation in date palm tissue culture. In *Quercus suber,* sucrose and glucose were the best carbon sources during proliferation and rooting phases, respectively (Romano *et al.*, 1995). Rizogenesis *in vitro* is an energy-demanding process where glucose being a faster and effective metabolizer as compared to sucrose; may induce high mitotic activity (Rolland *et al.*, 2006).

The continuous formation of lateral roots is a vital part of establishing a root system and enables plants to react with developmental plasticity to changing soil conditions. Evidence is accumulating that abscisic acid (ABA), which is known to be involved in stress responses, has an important role in lateral root formation. Interestingly, ABA seems to have distinct roles at different stages in lateral root development. The emerging role of ABA in lateral root development fits well with its general functional properties as a stress hormone, including its role in dormancy (Ive, De Smet *et al.*, 2006).

The present study aimed to investigate the effect of various concentrations of ABA (0.0, 0.25, 0.50 and 1.0 mg /l), sucrose (15, 30 and 45 g/l) and MS salt strength on *in vitro* rooting of date palm cv.Bartamuda.

MATERIALS AND METHODS

The present study was performed throughout the period from 2007 to 2008 at the Central Laboratory of Date Palm Researches and Development, Giza, Egypt.

Plant material

Shootlets of date palm *(Phoenix dactylifera* L.) cv. Bartamuda at 5-7 cm in length and with 2-3 leaves resulted from direct somatic embryogenesis as described by Hassan (2007) were used as a source for plantlet formation.

Experiment design and culture medium

Shootlets of cv.Bartamuda were cultured *in vitro* using the treatments presented in Table (1). The following effects were studied *in vitro* on growth during rooting stage:

1- Effect of salt strength (¾ or half strength) of MS (Murashig & Skoog, 1962) medium.

2- Effect of abscisic acid at concetration (0.0, 0.25, 0.5 and 1.0 mg/l).

3- Effect of sucrose concentrations (15, 30 or 45 gm/l).

All culture media were supplemented with 0.1 mg/l NAA (naphthalene acetic acid) + 2 mg/l glycine+ 5 mg/l thiamine HCl+ 1.0 mg/l biotin

Each treatment contained 12 jars (replicates), which were incubated in a growth room at 27±2°C in 16 hr illumination of 4000 - 6000 lux (white fluorescent lamps). Subculturing the explants was done onto the same medium every four weeks and three subcultures were done on each treatment.

Statistical analysis

Data obtained were subjected to the analysis of variances of completely randomized design as recommended by Steel and Torrie (1980). LSD at 5% level of probability was used to compare means for number of roots, root length, plantlets length and plantlet vigor plantlet and root thickness were scored visually as following, according to (Pottino, 1981):

1=Thin thickness. 2=Below average thickness. 3=Average thickness. 4=Good thickness. 5=Very good thickness.

RESULTS

Effect of sucrose, ABA and MS salt strength on the number of roots and root length of date palm cv. Bartamuda after 12 weeks during root formation stage are presented in Tables (1 and 2).

Regarding the effect of ABA data in Table (1) clearly indicated that, using ABA at 0.25 mg/l increased the number of roots to (2.5) compared with control medium (2.4). However, data showed that there was a gradually significant decrease the root length with increasing the concentration of ABA from 0.0 to 1.0 mg/l (5.9 cm to 4.7 cm), respectively (Table2).

Concerning the effect of sucrose, data indicated that the average number of roots and root length increased with increasing the concentration of sucrose from 15 g/l which recorded (2.3 roots/explant of 4.8 cm length) to 45mg/l (2.8 roots/explant of 6.1cm length).

In general data indicated that using ¾ MS medium which gave (3.0 roots/explant of 4.9 cm root length) was the preferred medium as compared with ½ MS medium which gave (1.8 roots/explant of 5.8 cm length) with significant differences between them, regardless sucrose and ABA concentration.

Table (1): Effect of sucrose, ABA and MS salt strength on the number of roots of date palm cv. Bartamuda after 12 weeks during root formation stage.

MS strength (C)	Sucrose concen. (g/l)(B)	ABA concentration (mg/l) (A) 0.0	0.25	0.50	1.0	Mean (C)	Mean (B)
½ MS	15	1.0	1.3	2.0	2.2	1.8	2.3
	30	2.0	3.0	2.5	1.5		
	45	1.7	1.3	1.7	2.3		2.1
¾ MS	15	3.0	4.1	2.0	3.0	3.0	
	30	3.0	2.0	2.0	1.5		2.8
	45	4.0	4.0	4.0	4.0		
Mean(A)		2.4	2.5	2.3	2.4		

L.S.D (A) =0.11 L.S.D (B) =0.10 L.S.D (AB) =0.20 L.S.D (C) =0.82
L.S.D (AC) =0.16 L.S.D (BC) =0.14 L.S.D (ABC) =0.28

Regarding the interaction between ABA and sucrose concentrations, data indicated that the highest number of roots/explant was obtained by using ABA at 1.0 mg/l and 45 g/l sucrose, while the longest root was obtained by using 0.25 mg/l ABA+ 45 g/l sucrose followed by using 0.5 mg/l ABA+ 45 g/l sucrose or medium without ABA+15 g/l sucrose. The interaction between ABA and MS strength indicated that the highest number of roots was obtained by using ¾ MS medium (with 0.25 mg/l ABA or without) with no significant difference between them and the longest root was obtained by using ½ MS medium without ABA.

The interaction between sucrose concentrations and MS strength, data indicated that the highest significant mean number of roots was obtained by using ¾ MS medium+ 45g/l sucrose. Whereas, the highest significant mean root length was obtained by using ½ MS medium+ 45g/l sucrose followed by ¾ MS medium+ 45g/l sucrose with a significant difference between them Data in Table (3) revealed that, the mean plantlet length (cm) as affected by different concentrations of ABA, sucrose and MS salt strength.

With respect of the effect of ABA concentrations, data showed that the highest significant mean of plantlet length (cm) was obtained by using control medium ABA-free medium) as the value was 12.97cm, followed by adding 0.25 and 0.50 mg/l .

Table (2): Effect of sucrose, ABA and MS salt strength on root length (cm) of date palm cv. Bartamuda after 12 weeks during root formation stage.

MS strength (C)	Sucrose concen. (g/l)(B)	ABA concentration (mg/l) (A) 0.0	0.25	0.50	1.0	Mean (C)	Mean (B)
½ MS	15	8.0	4.7	5.2	3.0	5.8	4.8
	30	6.2	6.0	3.7	5.7		
	45	7.5	6.5	7.2	5.7		5.1
¾ MS	15	5.0	6.0	3.5	3.0	4.9	
	30	4.0	4.0	4.5	7.0		6.1
	45	5.0	7.0	6.0	4.0		
Mean(A)		5.9	5.7	5.0	4.7		

L.S.D (A) =0.17 L.S.D (B) =0.15 L.S.D (AB) =0.30 L.S.D (C) =0.12
L.S.D (AC) =0.24 L.S.D (BC) =0.21 L.S.D (ABC) =0.42

ABA to culture medium (11.85 and 11.51 cm, respectively), with no significant difference between them. While the lowest value was obtained by adding 1.0 mg/l ABA to culture media as the value was 10.38 cm.

Regarding the effect of sucrose concentration , data clearly showed that the highest significant mean of plantlet length (cm) was observed when adding 45 g/l sucrose to culture medium, followed by adding 30 g/l sucrose with a significant difference between them, as the values were (12.72 and 11.94 cm, respectively), while the lowest significant mean (10.36 cm) was obtained by adding 15 g/l sucrose to culture medium.

The interaction between different ABA and sucrose concentrations showed that, ABA free medium supplemented with 45 g/l sucrose produced the highest significant mean of plantlet length, followed by adding 0.25 mg/l ABA with 45 g/l sucrose and ABA free medium with 30 g/l sucrose with no significant differences between them, while the lowest mean was observed by using the medium of adding 1.0 mg/l ABA with 15 g/l sucrose.

Table (3): Effect of sucrose, ABA and MS media strength on mean plantlet length (cm) of date palm cv. Bartamuda after 12 weeks during root formation stage.

MS strength (C)	Sucrose concen. (g/l)(B)	ABA concentration (mg/l) (A) 0.0	0.25	0.50	1.0	Mean (C)	Mean (B)
½ MS	15	11.5	10.5	10.5	9.20	11.30	10.36
	30	12.0	11.0	11.25	11.0		
	45	13.0	12.5	12.5	10.7		11.94
¾ MS	15	11.8	10.0	10.4	9.0	12.50	
	30	14.0	12.6	12.0	11.7		12.72
	45	15.5	14.5	12.4	10.7		
Mean(A)		12.97	11.85	11.51	10.38		

L.S.D (A) =0.4169 L.S.D (B) =0.3610 L.S.D (AB) =0.7220 L.S.D (C) =0.2948
L.S.D (AC) =0.5895 L.S.D (BC) =0.5106 L.S.D (ABC) = 1.021

Concerning the effect of MS salt strength on plantlet length (cm), data in Table (3) showed that the highest significant mean (12.05) of plantlet length was observed when culturing explants on ¾ strength MS medium as compared with ½ MS which recorded the lowest significant mean 11.30 cm).

The interaction between ABA concentrations and MS salt strength showed that ¾ MS without ABA produced the highest significant mean of plantlet length, followed by ¾ MS + 0.25 mg/l ABA and ½ MS without ABA, with no significant differences between the, while the lowest mean were observed by using ¾ MS or ½ MS+ 1 mg/l ABA with no significant differences between them. The interaction between sucrose concentrations and MS salt strength showed that, ¾ MS + 45 g/l sucrose produced the highest significant mean plantlet length, followed by ¾ MS + 30g/l sucrose and ½ MS+ 45 g/l sucrose with no significant differences between them, while the lowest mean were observed by using ¾ MS or½ MS+ 15 g/l sucrose with no significant differences between them.

Data in Table (4) and Fig.(1) represented the thickness of plantlet as affected by different concentrations of ABA, sucrose and MS salt strength.With respect of the effect of ABA concentrations, data showed that the highest significant mean of plantlet thickness (4.13) was obtained by adding 0.25 mg/l ABA to culture medium followed by adding 0.50 mg/l ABA 4.0, with significant differences between them. While the lowest mean (3.61 and 3.54) were obtained by adding 1.0 mg/l ABA to culture medium and using control medium (ABA free medium) without significant differences in between.

Regarding the effect of sucrose concentrations, data clearly showed that the highest significant values of plantlet thickness (4.03 and 3.98), was observed when adding 30 and 45 g/l sucrose to culture medium, without significant differences between them, while the lowest significant value (3.46) was noticed by adding 15g /l sucrose to culture medium.

The interaction between ABA and sucrose concentrations showed that adding ABA at 0.25 mg/l and 30 or 45 g/l sucrose to culture medium produced the highest significant values of plantlet thickness without significant differences between them, while the lowest values were observed by using media containing 1.0 mg/l ABA with 45 or 15 g/l sucrose, with a significant difference between them.

With respect of the effect of MS salt strength on plantlet thickness, data in Table (4) showed that the highest significant mean of plantlet thickness (3.92) was observed when culturing explants on ¾ MS as compared with ½ MS 3.73.

The interaction between ABA concentration and MS salt strength showed that the addition of ABA at 0.25 mg/l to ½ MS produced the highest significant mean of plantlet thickness, followed by ¾ MS without ABA; 0.25 mg/l ABA or ¾ MS + 0.50 mg/l ABA and ½ MS+ 0.5mg/l ABA which produced the same mean, while the lowest value was observed with ½ MS free of ABA .

The interaction between sucrose concentrations and MS salt strength, showed that ¾ MS + 30 or 45g/l sucrose produced the same highest significant value of plantlet thickness; this value was reduced significantly when using ½ MS+ 30 or 45 g/l sucrose respectively, while the lowest significant values were observed by ½or ¾ MS with no significant differences between them.

Data in Table (5) revealed that the mean thickness of roots as affected by different concentrations of ABA, sucrose and MS salt strength.

Concerning the effect of ABA concentration, data showed that the highest

significant mean of root thickness (2.82) was obtained by adding 0.25 mg/l ABA to culture medium followed by adding 0.50 and 1.0 mg/l ABA (2.69 and 2.33, respectively) with significant differences among them. The lowest value (2.07) was obtained by using ABA -free medium.

Regarding the effect of sucrose concentrations, data clearly showed that the highest significant mean of root thickness (2.74) was observed when adding 45g/l sucrose to culture medium, followed by adding 30g/l sucrose to culture medium (2.63) with significant differences between them, while the lowest significant mean (2.06) was noticed by adding 15g/l sucrose to culture medium.

Table (4): Effect of sucrose, ABA and MS salt strength on plantlet thickness (score) of date palm cv. Bartamuda after 12 weeks during root formation stage.

MS strength (C)	Sucrose concen. (g/l)(B)	ABA concentration (mg/l) (A)				Mean (C)	Mean (B)
		0.0	0.25	0.50	1.0		
½ MS	15	2.00	4.50	4.00	3.20	3.73	3.46
	30	3.75	4.00	4.00	4.00		
	45	3.50	4.00	4.00	3.50		
¾ MS	15	3.50	3.50	4.00	3.00	3.91	4.03
	30	4.00	4.50	4.00	4.00		
	45	4.50	4.00	4.00	4.00		3.89
Mean(A)		3.54	4.13	4.00	3.61		

L.S.D (A) =0.084 L.S.D (B) =0.073 L.S.D (AB) =0.147 L.S.D (C) =0.060
L.S.D (AC) =0.120 L.S.D (BC) =0.103 L.S.D (ABC) =0.207

Table (5): Effect of sucrose, ABA and MS salt strength on the root thickness (score) of date palm cv. Bartamuda after 12weeks during root formation stage.

MS strength (C)	Sucrose concen. (g/l)(B)	ABA concentration (mg/l) (A)				Mean (C)	Mean (B)
		0.0	0.25	0.50	1.0		
½ MS	15	1.50	3.25	2.50	1.80	2.46	٢,٠٦
	30	2.00	2.67	2.67	2.25		
	45	2.25	3.00	3.00	2.67		٢,٦٣
¾ MS	15	1.20	2.00	2.00	2.00	2.49	
	30	3.00	3.00	3.00	2.50		٢,٧٤
	45	2.50	3.00	3.00	2.50		
Mean(A)		2.07	2.82	2.69	2.32		

L.S.D (A) =0.079 L.S.D (B) =0.068 L.S.D (AB) =0.137 L.S.D (C) =0.056
L.S.D (AC) =0.112 L.S.D (BC) =0.097 L.S.D (ABC) =0.194

The interaction between ABA concentration and sucrose concentration showed that adding ABA at 0.25mg/l with 30 and 45 g/l sucrose to culture medium produced the highest significant values of root thickness without significant differences between them,

while the lowest mean was observed by the medium of adding 1.0 mg/l ABA + 15 g/l sucrose (Table5).

With respect of the effect of MS salt strength on root thickness, data in Table (5) showed that no significant difference could be observed when culturing explants on medium containing ¾ or ½ MS salt strength.

The interaction between ABA concentration and MS salt strength showed that adding ABA at 0.25 mg/l to ½ MS produced the highest significant mean of root thickness, while the lowest significant mean was observed on ½ MS without ABA.

The interaction between sucrose concentration and MS salt strength revealed that adding 30 g/l sucrose to ¾ MS produced the highest significant mean of root thickness, followed by adding 45 g/l sucrose to ½ MS or ¾ MS salt strength without significant differences between them, while the lowest mean was observed by using ¾ MS + 15g/l sucrose.

Table (6): Effect of sucrose, ABA and MS salt strength on plantlet growth vigor of date palm cv. Bartamuda after 12 weeks during root formation stage.

MS strength (C)	Sucrose concen. (g/l)(B)	ABA concentration (mg/l) (A)				Mean (C)	Mean (B)
		0.0	0.25	0.50	1.0		
½ MS	15	2.50	4.00	4.25	3.00	3.87	3.63
	30	4.25	4.00	4.00	3.75		
	45	4.00	4.50	4.25	4.00		
¾ MS	15	3.60	4.00	4.00	3.60	4.14	4.08
	30	4.00	4.30	4.40	4.20		
	45	4.50	5.00	4.00	4.00		4.31
Mean(A)		3.81	4.30	4.19	3.72		

L.S.D (A) =0.1602 L.S.D (B) =0.1387 L.S.D (AB) =0.2775 L.S.D (C) =0.1133
L.S.D (AC) =0.2265 L.S.D (BC) =0.1962 L.S.D (ABC) =0.3924

Data in Table (6) revealed that the plantlet growth vigor as affected by different concentrations of ABA, sucrose and MS salt strength.

With respect of the effect of ABA concentrations, data showed that the highest significant values of plantlet growth vigor (4.30 and 4.19) were obtained by adding 0.25 or 0.50 mg/l ABA to culture medium respectively, without significant differences between them. While the lowest values (3.81 and 3.72) were obtained by using control medium (ABA free medium) and by adding 1.0 mg/l ABA to culture medium respectively, without significant differences between them.

Date concerning the effect of sucrose indicated that the plantlet growth vigor increased with increasing the concentration of sucrose from 15 g/l (3.62) to 45g/l (4.31).

The interaction between ABA concentration and sucrose concentrations showed that adding ABA at 0.25 mg/l+ 45 g/l sucrose to culture medium produced the highest significant mean of growth vigor followed by medium without ABA + 45 g/l sucrose and 0.50 mg/l ABA with 45 and 30 g/l sucrose, while the lowest mean were observed by using medium supplemented with 1.0 mg/l ABA + 15 g/l sucrose and with 0.0 mg/l ABA + 15 g/l sucrose.

Concerning the effect of MS strength on growth vigor, data in Table (6) showed that the highest significant mean of growth vigor was observed when culturing explants on ¾ MS salt

as compared with ½ MS (4.14 vs. 3.87 respectively).

The interaction between ABA concentration and MS salt strength showed that adding ABA at 0.25 or 0.50 mg/l to ¾ MS produced the highest significant mean of growth vigor without significant differences between them, while the lowest significant mean were observed on ½ MS without ABA and 1.0 mg/l ABA.

Finally, the interaction between sucrose concentration and MS salt strength revealed that adding 45 g/l sucrose to ¾ MS produced the highest significant mean of plantlet growth vigor, followed by adding 45g/l sucrose to½ MS and 30g/l sucrose to ¾ MS or ½ MS salt strength without significant differences among them, while the lowest mean was observed by ½ MS+ 15g/l sucrose. In general thick plantlet which have a thick roots were successfully transferred to soil in greenhouse with a high survival percentage.

DISCUSSION

Root initiation is a critical stage in date palm micropropagation, as it governs the subsequent success of production of free living date palm plants (Shaheen, 1990). ABA, sucrose and MS salts affect the formation of roots in date palm and subsequent successful transfer to soil.

Effect of ABA

There are some interesting studies suggesting that plant hormones can play important roles in altering root growth/ morphology and secondary metabolism. For example: abscisic acid (ABA) can inhibit secondary product accumulation, but not hairy root growth (Ive,De Smet *et al.,* 2006) Results under discussion showed that, ABA at 0.25mg/l in the presence of NAA in root culture medium increased the number of roots, plantlet growth vigor, plantlet and root thickness .These results are on line with those reported by Ive,De Smet *et al.,* (2006) who found that ABA acts on root branching by mediating stress responses and by functioning as an endogenous developmental signal. The continuous formation of lateral roots is a vital part of establishing a root system and enables plants to react with developmental plasticity to changing soil conditions. Evidence is accumulating that ABA, which is known to be involved in stress responses, has an important role in lateral root formation. Interestingly, ABA seems to have distinct roles at different stages in lateral root development.

The emerging role of ABA in lateral root development fits well with its general functional properties as a stress hormone, including its role in dormancy. ABA acts as a root-to-shoot signal that controls closure of stomata and briefly stimulates elongation of the main root in response to drought (Hose, 2002 and Davies and Bacon, 2003). ABA is considered to be a 'stress hormone' that has a role in response to biotic and abiotic stresses (MacRobbie, 1998and Verslues and Zhu, 2005), but is also important in non-stress-related regulatory functions (Cheng, 2002 and Sharp, 2002). ABA is recruited as an endogenous signal in many plant develop-mental processes, including seed maturation and dormancy (Koornneef, 2002 and Gubler (2005), heterophylly (leaves of different shapes on the same plant) (Hsu, 2001) and root growth (Sharp and LeNoble, 2002). The effect of ABA is difficult because of the enormous complexity of the signalling steps involved, the inability to make a distinction between primary and secondary effects and the apparent post-transcriptional control (Himmelbach, 2003), there is no strong evidence of a direct relationship between ABA and lateral root initiation. However, some examples suggest interplay between ABA and auxin during lateral root initiation, with ABA as a

repressing agent and auxin as a promoting agent.

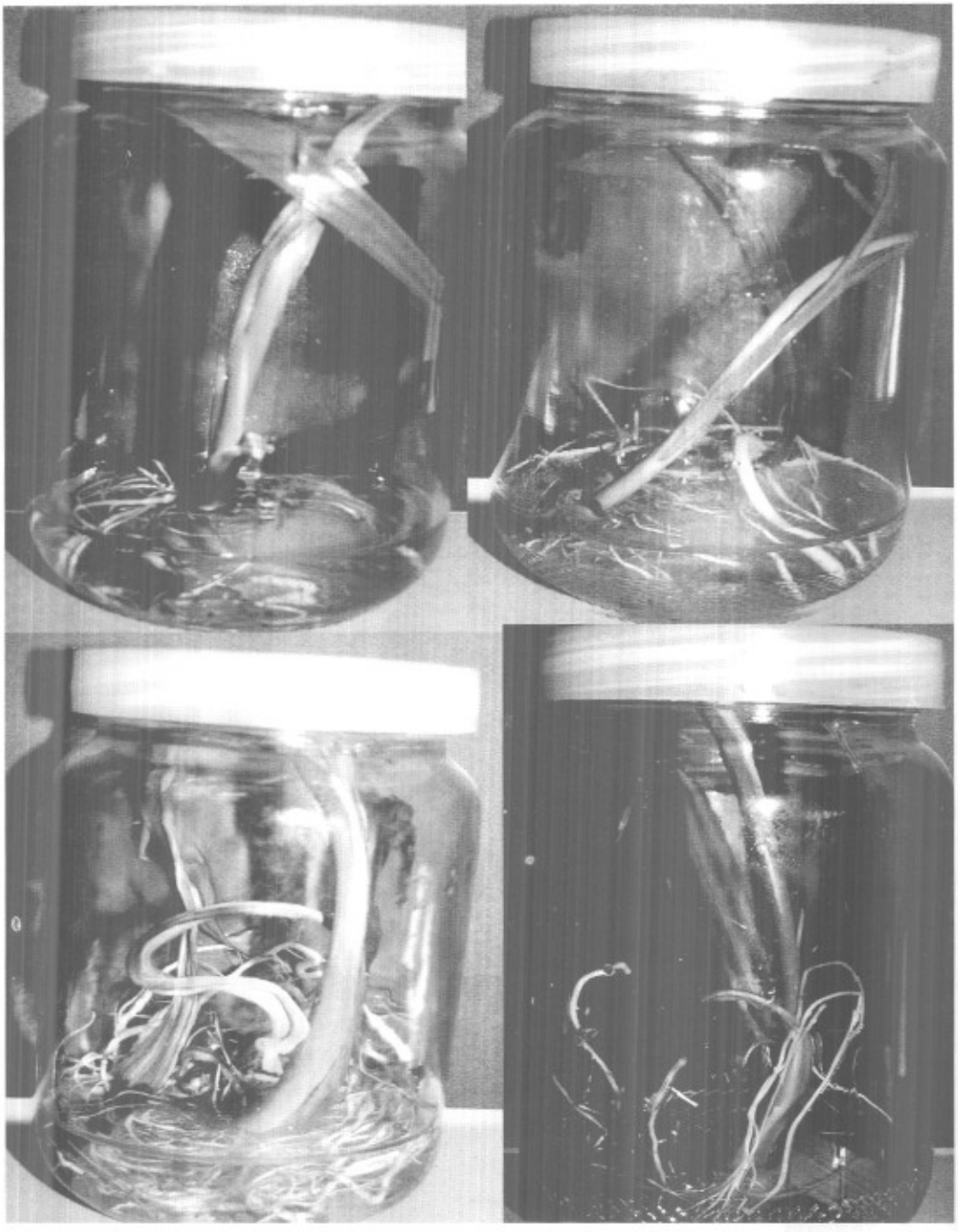

Fig. (1): Well rooted and thickness of plantlet of date palm on 3/4MS medium containing 45g sucrose +0.25mg/l ABA+0.1mg/l NAA.

Effect of sucrose

Carbohydrate source plays an important role as a source of energy and for maintaining osmoticum (Cuenca and Vieitez, 2000). Dey and Harborne, (1997) showed that sucrose has a role in controlling the expression of several important enzymes and other proteins. For example, sucrose acts as an inhibitor for proteinase to stimulate plant growth and high level of sucrose and invertase are often characteristic of growing and differentiating tissues. Data about the effect of sucrose on date palm rooting showed that increasing concentration of sucrose in culture medium to 45 g/l increased the number of roots, root length, plantlet length, plantlet growth vigor, plantlet and root thickness. These results are on line with those reported by Abdel Satar, (2005), who stated that on date palm, the number of roots as well as root and stem length of plantlets induced from shootlets were significantly high when cultured on MS medium supplemented with 0.1mg/l NAA, 50g/l sucrose. Abo-El-Souad *et al.*, (2002) showed that in date palm mass propagation, plantlets produced from rooting medium supplemented with 50g/l sucrose under light intensity of 9000 lux had achieved the best survival percentage in greenhouse. In date palm micropropagation, sucrose plays an important role in the iniation of roots. The addition of sucrose to culture medium caused an increase in the number and length of adventitious roots per shoot as compared with either glucose or fructose. Concentration of 30 or 40g/l sucrose gave the highest average number of adventitious roots per shoot (Al-Dawayati, 2000). In *Quercus suber,* sucrose and glucose were the best carbon sources during proliferation and rooting phases respectively (Romano *et al.*, 1995).Shoots of Rosa grown in media containing high sucrose concentrations (146.07–262.93 m*M*) produced more and longer roots than those grown in media containing 0–87.64 m*M* sucrose (Hyndman *et al.*, 1981). Data also showed that, the highest number of roots was noticed with medium containing 45g/l sucrose +1mg/l ABA and the highest root length was recorded by medium containing 45g/l sucrose+0.25 mg/l ABA. In this respect, relationship between ABA and sugars is crucial for the root system (Ive,De Smet *et al.*, 2006).

Effect of MS salt strength

Data about MS salt strength also showed that ¾ MS salt strength increased the number of roots, plantlet growth vigor, plantlet length and plantlet thickness. These results are in agreement with those reported by Ibrahim (1999) who found that, the concentration of inorganic salts plays an important role in rooting induction, and that reduction of MS strength to ¾ of the original concentration stimulated root formation in date palm tissue culture. While using ½ MS increased significantly root length, in this respect. Faisal *et al.* (2005) on *Tyophora indica* reported that, the *in vitro*-regenerated shoot induced roots when transferred to full- and half strength MS medium. Half-strength growth regulator-free medium was found superior to full-strength MS medium for root development. The number and length of roots increased when the shoots of Rosa were grown in media with the nitrogen concentration of the Murashige-Skoog (MS) salt formulation reduced. (Hyndman *et al.*, 1981). On the other hand, Zong *et al.* (2005) showed that ½ or full strength MS medium inhibited root elongation of *Incarvillea sinensis* and shoot turned brown, but shoot browning was greatly decreased on ¼ strength MS medium.

REFERENCES

Abdel-Sattar, M.M. (2005). Advanced physiological studies on date palm. Ph.D.Thesis, Pomology Dept., Fac. Agric., Cairo Univ., Egypt.

Abo-El-Soud, A, A.; Zaid, Z.E; Salah, A. and Sidky, R. (2002). Tissue culture of date palm (*Phoenix dactylifera*). The Third Scientific Agricultural Sciences Conference. Faculty of Agriculture,Assiut Univ.,Egypt.

AL-Dawayti, M. M. (2000). Physiological studies on date palm micropropagation. M.Sc. Thesis, Pomology Dept., Fac. Agric., Cairo Univ., Egypt.

Cheng, W.H. (2002). A unique short-chain dehydrogenase/ reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 14:2723–2743.

Cuenca, B. and Vieitez, A.M. (2000). Influence of carbon source on shoot multiplication and adventitious bud regeneration in *in vitro* beech cultures. Plant Growth Regul., 32, 1-12.

Davies,W.J. and Bacon,M.A. (2003). Adaptation of roots to drought. In Root Ecology (Ecological Studies) (Vol. 168) (de Kroon, H. and Visser, E.J.W., eds), pp. 173–192, Springer-Verlag

Dey, P.M. and Harborne, J.B. (1997). Plant biochemistry. Academic Press. New York, pp.409-421.

Faisal, M; Singh, S. and Anis, M. (2005). *In vitro* regeneration and plant establishment of *Tylophora indica* (Bur..F.) Merrill:Petiole callus culture. *In vitro* Cell.Dev.Biol. Plant, 41:511-515.

Gubler, F. (2005). Dormancy release, ABA and pre-harvest sprouting. Curr. Opin. Plant Biol., 8: 183–187

Hassan, M. M. (2007). Induction of direct somatic embryogenesis of date palm (*Phoenix dactylifera* L.) using of ABA. Egyptian Journal of Genetics and Cytology (36) 1: 93-104.

Himmelbach, A. (2003). Relay and control of abscisic acid signaling. Curr. Opin. Plant Biol., 6: 470– 479

Hose, E. (2002). Abscisic acid in roots–biochemistry and physiology, In Plant Roots: The Hidden Half (3rd ed) (Waisel, Y. *et al.*, eds), pp. 435–448, Marcel Dekker.

Hsu, T.C. (2001). Early genes responsive to abscisic acid during heterophyllous induction in *Marsilea quadrifolia*. Plant Mol. Biol., 47: 703–715

Hussein, F.; El-Khatany, M.S. and Wally, Y.A. (1979). Date palm growing and date production in the Arab and Islamic world. Ain-Shams Press (in Arabic), Egypt.

Hyndman, Scott E. Paul M. Hasegawa and Ray A. Bressan (1981). The role of sucrose and nitrogen in adventitious root formation on cultured rose shoots. Plant Cell, Tiss. Organ Cult., 1 (1): 229-238.

Ibrahim, A.I. (1999). Somaclonal variation during micropropagation of date palm *via* embryogenesis. The first International Conference, in Egypt, on Plant Tissue Culture and Its Application pp.189-199.

Ive, De Smet, Hanma Zhang; Dirk Inze´ and Tom Beeckman (2006). A novel role for abscisic acid emerges from underground. Trends Plant Sci., 11(9) : 434-439.

Koornneef, M. (2002). Seed dormancy and germination. Curr. Opin. Plant Biol., 5: 33–36.

MacRobbie, E.A.C. (1998). Signal transduction and ion channels in guard cells. Philos. Trans. R. Soc. Lond. Biol. Sci., 353: 1475– 1488.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant,15: 473-497.

Pottino, B.G. (1981). Methods in plant tissue culture. Dept. of Hort., Agric. College,

Maryland. Univ., College Park, Maryland, USA, pp. 8-29.

Robbins, M.P.; Evans,T.E and Morris, P.(1996). The effect of plant growth regulators on growth, morphology and condensed tannin accumulation in transformed root cultures of *Lotus corniculatus*. Plant Cell Tiss. Organ Cult.,44:219-227.

Rolland, F., Gonzalez, E.B., Sheen, J. (2006). Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu.Rev.Plant Biol. ,57:675-709.

Romano,A; Noronha ,C. and Martins-Loução M. A. (1995). Role of carbohydrates in micropropagation of cork oak. Plant Cell Tiss. Organ Cult., 40(2) :159-167.

Shaheen, M.A. (1990). Propagation of date palm through tissue culture: A review and an interpretation. Annals Agric. Sci., Ain-Shams Univ., Cairo, Egypt., 35:895-909.

Sharp, R.E. (2002). Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. Plant Cell Environ., (25) 211–222

Sharp, R.E. and LeNoble, M.E. (2002). ABA, ethylene and the control of shoot and root growth under water stress. J. Exp. Bot., 53: 33–37

Steel, R.G.D and Torrie, S.H. (1980). Principles and Procedures of Statistics.2nd Ed.McGraw-Hill, New York.

Tisserat, B. (1981). Production of free-living date palms through tissue culture. Date Palm J., 1:43-54.

Tisserat, B. (1982). Factors involved in the production of plantlets from date palm callus cultures. Euphytica 31(1):201-214.

Vanhala, L.; Eeva, M.; Lapinjoki, S.; Hiltunen, R. and Oksman-Caldentey,K. (1998). Effect of growth regulators on transformed root cultures of *Hyoscyamus muticus*. J.Plant Physiol., 153:475-481.

Verslues, P.E. and Zhu, J.K. (2005). Before and beyond ABA: upstream sensing and internal signals that determine ABA accumulation and response under abiotic stress. Biochem. Soc. Trans., 33: 375–379

Zhong, H.U; Wei, L.I and Guang-Qin, G.U.O. (2005). High efficiency *in vitro* plant regeneration from cotyledon explants of *Incarvillea sinensis*. *In vitro* Cell.Dev.Biol. Plant, 41:662-665.

الملخص العربي

تاثير السكروز وحمض الابسيسك علي نمو وتطور نخيل البلح خلال مرحله التجذير معمليا

منى محمد حسن؛ عز الدين جاد الله؛ عبير هاشم إبراهيم عبد الكريم

المعمل المركزي للأبحاث وتطوير نخيل البلح– مركز البحوث الزراعية – الجيزة – مصر .

اجري هذا البحث في معمل بحوث النخيل خلال الفتره من ٢٠٠٧ -٢٠٠٨ حيث تم دراسة تاثير كل من السكروز وحمض الابسيسك وقوة الاملاح و علي التجذير وعلى صفات عدد الجذور وطول الجذور وطول النبات وقوة النمو وسمك النبات وسمك الجذور لصنف البرتمودا فى المعمل .واظهرت النتائج ان استخدام التركيزات العالية من السكر واضافة حمض الابسيسك ادت الي زيادة تكوين الجذور بصورة معنوية في مرحلة التجذير, وان استخدام الاملاح بقوة ٣/٤ MS وحمض الابسيسك بتركيز ٠,٢٥ ملجم / لتر واستخدام السكروز بتركيز ٤٥ جم /لتر اعطى افضل النتائج في معظم الصفات المدروسة (زيادة عدد الجذور وطول النبات وسمك النبات وسمك الجذور وقوة النمو) بينما ادي استخدام بيئة خالية من حمض الابسيسك الى زيادة طول النباتات مقارنة بالتركيزات الاخري التى ادت الي قصره. كما ادي استخدام الاملاح بقوة 1/2 MS الي زيادة طول الجذور .