14th Conf. Agric. Develop. Res., Fac. of Agric., Ain Shams Univ., March, 2019, Cairo, Egypt
Special Issue, 27(1), 737 - 747, 2019
Website: http://strategy-plan.asu.edu.eg/AUJASCI/

DIRECT EMBRYOGENESIS AND INDIRECT ORGANOGENESIS OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CV. SEWI USING IMMATURE FEMALE INFLORESCENCES

[68]

Mervat M.H. Malhat[1*], El-Wakeel H.[2], Abd El-Hamid A.[2], Khalil S.M.[1] and Mona M. Hassan[3]

1. Genetic Engineering Research Institute, Agric., Res. Center, Giza, Egypt
2. Horticulture Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadyek Shoubra11241, Cairo, Egypt
3. Central Laboratory for Date Palm Researches Development, Agric., Res-Center, Giza, Egypt

*Corresponding author: mervatT83@yahoo.com

Received 25 November, 2018, Accepted 18 December, 2018

ABSTRACT

This study was achieved at the Tissue Culture Laboratory of the Agricultural Genetic Engineering Research Institute, Giza, Egypt during the period from 2013 to 2017, Direct embryo initiation and callus formation of date palm cv. Sewi from immature female inflorescences have been achieved on modified MS medium supplemented with 4 mg l^{-1} Picloram plus 3 mg l^{-1} 2 iP and 2 g l^{-1} PVP. Results also showed that BA at 0.5 mg l^{-1} produce the highest number of differentiated embryos/culture, while kinetin at 0.25 mg l^{-1} significantly increased the average number of adventitious shoots/culture. NAA at 1.0 mg l^{-1} induced the highest rooting percentage and micro-shoot length. On the other hand the best survival percentage during the acclimatization stage was observed with plantlets produced from IBA at 0.5 mg l^{-1} during the rooting stage.

Key words: In vitro, *Phoenix dactylifera L.,* cv. Sewi, Immature Inflorescence, Direct embryogenesis, Indirect organogenesis, callus formation, Embryo formation

INTRODUCTION

Phoenix dactylifera L. is a monocotyledonous and dioecious plant. It is considered as one of the important strategic commodities and the cheapest source of energy in the advanced and developing countries producing their raw and manufactured materials. It is the most important member of the family Arecaceae for proper maturation of fruits; Date palm requires a prolonged summer heat without rain or high humidity during the ripening **(Badawy et al 2005).** The date fruit contains numerous chemical components that possess high nutritional and medicinal values **(Al-Khayri and Naik, 2017).** Tissue culture propagation method is the most promising technique for production of efficient plant materials with high quality **(Sane et al 2006).** Somatic embryogenesis is considered as the most efficient regeneration process for date palm micropropagation **(Fki et al 2003).** It is reported to be a quick and efficient method for large scale propagation of date palm and could also be highly useful for breeding programs. Embryogenic callus induction in date palm is influenced by different factor such as genotype, explant type, induction period and plant growth regulators. The inflorescence explants proved promising alternative explants for micropropagation of the elite cultivars and rare male and female individuals of date palm (**Feki and Drira, 2007**). Tissue, somatic embryo and callus were induced, in many plant species, by the addition of exogenous auxins such as 2,4-D, IBA and NAA to the culture medium. Most tissue culture studies of palms have focused on the effects of different auxin types such as IBA, 2,4-D, NAA, NOA, IAA and their concentrations on various explants cultures **(Eke et al 2005).** Moreover, **El-Hammady et al (1999)** indicated that in the rooting medium of date palm cv Sewi., the addition

of NAA at concentration of 0.5 mg/l resulting in higher rooting percentage**.**

Therefore, this study aimed to find propagation protocol for large scale production of date palm cv. Sewi plantlet through direct and indirect embryogenesis using immature female inflorescences.

MATERIALS AND METHODS

This study was achieved at the Tissue Culture Laboratory of Agricultural Genetic Engineering Research Institute, Agric. Res. Center, Giza, Egypt, during the period from 2013 to 2017.

Plant Material.

Date palm cv. Sewi (*Phoenix dactylifera* L.) was used throughout this study. The used explants were immature female inflorescence (5-7 cm) taken on late January to early February from mature palm of 15 year old **(Fig. 1a)**.

1- Sterilization procedure.

The immature female spathes were collected from date palm cv. Sewi at the beginning of the spathe appearance and then transferred to the lab. The spathes were rinsed under tap water for about two hours then, surface sterilization was performed by mercuric chloride solution at 0.1% for 10 min. Then rinsed by sterile distilled water for three times.

2- Initiation stage

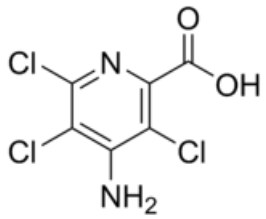
Spiklets were divided into small pieces (1-2 cm in length). They were cultured on MS medium (**Murashige and Skoog, 1962**) supplemented with 100 mg/l glutamine, 100 mg/l myo- inositol, 1 mg/l biotin, 5 mg/l thiamin HCl, 40 g/l sucrose, and 6 g/l agar .The pH was adjusted to 5.7-5.8 before adding agar to the media. The media were dispensed into jars, where each jar contained 20 ml medium. Sterilization of the medium was achieved by autoclaving at 1.1 kg/cm^2 and 121º C for 20 minutes.

3- Incubation condition

All cultured explants, for all different experiments, were incubated under dark conditions at 27±2^{0}C at the initiation stage. For direct embryogenesis and indirect organogenesis, cultures were incubated under 16 hours light (cool white fluorescent lamps). For the rooting experiments, all cultured tubes were incubated under light intensity of 3000 lux 16 hours light for 12 weeks.

In this stage, the effect of three antioxidants (1 g/l activated charcoal, 2 g/l PVP, or 2 g/l PVP + 1g/l activated charcoal) with three auxin types (2, 4 -D, Picloram or NOA) at three concentrations (1, 2 or 4 mg/l) for each were investigated.The 27 treatments [(3antioxidants × (3 auxin types × 3 concentrations)] were distributed in a factorial experiment. Each treatment had three replicates with three explants for each. All media during the initiation stage ware supplemented with 3 mg l^{-1} 2iP. Callus formation percentage and embryo formation percentage were recorded at the end of sixth subculture.

4-amino-3,5,6 trichloro-2-pyridinecarboxylic acid



4- Picloram

5- Differentiation stage.

In this stage, clusters of direct somatic embryos (3-4 embryos) were used as explant materials. Clusters were cultured for three subcultures (one month interval) on 3/4 MS strength supplemented with 40 g/l sucrose, 0.1 mg/l NAA, 100 mg/l glutamin, 100 mg/l myo- inositol, 1 mg/l biotin, 5 mg/l thiamin HCl, 1 g/l activated charcoal and 6 g/l agar and the media were supplemented with one of the following cytokinins .

1- Control (without cytokinin)
2- 0.25 mg /l BA
3- 0.5 mg /l BA
4- 0.25 mg /l kinetin
5- 0.5 mg /l kinetin
6- 0.25 mg/l 2 ip
7- 0.5 mg/l 2 ip

This experiment contained 3 cytokinin types × 3 concentrations = 9 treatments in a factorial experiment. Each treatment contained 3 replicates, and each replicate contained three jars, each jar contained one cluster. After three months, embryo number/ culture, number of adventitious shoots / culture, shoot length were recorded.

5- Rooting stage

During this stage, shoots of 5-7cm in length resulting from the differentiation stage were cultured on 1/2 MS media containing 40 g/l sucrose, 6 g/l agar. The auxins of NAA , IBA, or IAA each at 0.0, 0.5 or 1.0 mg/l were added to the rooting culture media. The prepared media were dispensed into glass tubes (2.5 × 15 cm) at rate of 20 ml. Each treatment had three replicates and each replicate contained three tubes and each tube contained one shoot. There were 3 auxin types × 3 concentrations =9 treatments in a factorial experiment. After three month, the rooting percentages, root number /shoot, root length (cm) and plantlet length were recorded.

***In- vivo* acclimatization stage**

The effect of in vitro rooting treatments on survival percentages, plant length, number of leaves per plant during the acclimatization stage. Plantlets from different auxin treatments of NAA, IBA or IAA each at 0.0, 0.5 or 1 mg/l were transplanted and grown under the ambient temperature 27C ± 2, humidity at 80-90% and natural light under plastic tunnel inside saran house. Each treatment had three replicates which contained three torpedo (each torpedo 5×18 cm) had one plantlet with 2-3 leaves, 2-3 roots and 7-9 cm length. Perlite + Peatmoss (1:2 v/v) mixture was used as planting medium for all treatments. Survival percentage and Plantlet length were recorded after 12 weeks.

Experimental design and statistical analysis of data

The experiments were subjected to a completely randomized design. Analysis of variance (ANOVA) and Duncan's multiple range test (**Duncan, 1955**), as modified by **Snedecorand Cochran (1982),** were performed to analyze the obtained data. The differences among means of the recorded parameters for all treatments were tested for significance at 5% level. Means followed by the same letter are not significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

1. Callus formation percentage

Data in **Table (1.a and Fig. 1b)** show the effect of antioxidant type and showed that the highest significant callus formation percentage was obtained with AC 1 g l^{-1} + PVP 2 g l^{-1} (18.87%) but the lowest significant was obtained with AC 1 g l^{-1} (10.03%).

Data in **Table (1.b)** show the effect of auxin type and showed that the higher significant callus formation percentages were achieved by 2,4-D or Picloram (19.53or 19.40%) than NOA (4.57%).

Data in **Table (1.c)** show the effect of auxin concentration results which showed that the highest significant callus formation percentage was recorded by 4 mg l^{-1} (21.50%) but the lowest significant percentage found by 1 mg l^{-1} (7.66%).

Data in **Table (1.d)** show the effect of antioxidant type and auxin type results which showed that the highest significant callus formation percentage was recorded by PVP at 2 g l^{-1} with Picloram (26.36%) but AC 1 g l^{-1} with NOA had the lowest significant callus formation percentage (2.61%).

Data in **Table (1.e)** show the effect of antioxidant type and auxin concentration results which showed that PVP at 2 g l^{-1}or AC at 1 g l^{-1} +PVP at 2 g l^{-1} antioxidant type with 4 mg l^{-1} of auxin concentration treatment gave the highest significant callus formation percentages (24.50 & 25.14 %) respectively but the lowest significant callus formation percentage found by AC 1 g l^{-1} or PVP 2 g l^{-1} with 1 mg l^{-1} of auxin concentration (5.94 &5.58 %), respectively.

Data in **Table (1.f)** show the effect of auxin type and concentration results which which showed that the highest significant callus formation percentage was found by Picloram at 4 mg l^{-1} concentration (31.52 %) but NOA at 1 mg l^{-1} had the lowest significant one (1.48%).

Data in **Table (1.g)** show the effect of antioxidant type, auxin type and concentration results which showed that the highest significant value was recorded by PVP 2 g l^{-1} with Picloram at 4 mg l^{-1} concentration (50.13%) but AC 1 g l^{-} with NOA at 1 mg l^{-1} did not give callus**.**

Table 1. Effect of antioxidant type, auxin type and concentration on callus formation percentage of date palm cv. Sewi using female immature inflorescence explants during the initiation stage.

1.a. Effect of antioxidant type.

AC 1 g l^{-1}	10.03 C
PVP 2 g l^{-1}	14.59 B
AC 1 g l^{-1} + PVP 2 g l^{-1}	18.87 A

Means having the same letter (s) in each column, are insignificantly different at 5% level.

AC : activated charcool

PVP : polyvinyl pyrrolidone

1. b - Effect of auxin type.

2,4-D	19.53 A
Picloram	19.40 A
NOA	4.57 B

Means having the same letter (s) in each column, are insignificantly different at 5% level.
AC : activated charcool PVP : polyvinyl pyrrolidone

1.c - Effect of auxin concentration.

1 mg l^{-1}	7.66 C
2 mg l^{-1}	14.34 B
4 mg l^{-1}	21.50 A

Means having the same letter (s) in each column, are insignificantly different at 5% level.
AC : activated charcool PVP : polyvinyl pyrrolidone

1.d - Effect of antioxidant type and auxin type.

Antioxidant	Auxin type		
	2,4-D	**Picloram**	**NOA**
AC 1 g l^{-1}	21.18 c	6.32 e	2.61g
PVP 2 g l^{-1}	12.61 d	26.36a	4.81f
AC1 g l^{-1}+ PVP2 g l^{-1}	24.81 b	25.53ab	6.28e

Means having the same letter (s) interaction are insignificantly different at 5% level
AC : activated charcool PVP : polyvinyl pyrrolidone

1.e- Effect of antioxidant type and auxin concentration.

Antioxidant	Auxin concentration		
	1 mg l^{-1}	**2 mg l^{-1}**	**4 mg l^{-1}**
AC 1 g l^{-1}	5.94 g	9.29 f	14.87 c
PVP 2 g l^{-1}	5.58 g	13.70 d	24.50 a
AC 1 g l^{-1}+PVP 2 g l^{-1}	11.47 e	20.01 b	25.14 a

Means having the same letter (s) interaction are insignificantly different at 5% level.

1.f - Effect of auxin type and concentration.

	Auxin concentration		
Auxin type	**1 mg l^{-1}**	**2 mg l^{-1}**	**4 mg l^{-1}**
2,4-D	13.70 d	19.67 c	25.23 b
Picloram	7.79 e	18.89 c	31.52 a
NOA	1.48 g	4.45 f	7.76 e

Means having the same letter (s) interaction are insignificantly different at 5% level.
AC : activated charcool PVP : polyvinyl pyrrolidone

1.g -Effect of antioxidant type, auxin type and concentration.

	2,4-D			**Picloram**			**NOA**		
	1mg l^{-1}	**2 mg l^{-1}**	**4 mg l^{-1}**	**1 mg l^{-1}**	**2 mg l^{-1}**	**4 mg l^{-1}**	**1mg l^{-1}**	**2 mg l^{-1}**	**4 mg l^{-1}**
AC 1 g l^{-1}	15.57 g	21.20 f	26.77d	2.25 n	4.47 m	12.23 h	0.00 o	2.22 n	5.60 lm
PVP 2 g l^{-1}	8.90 ij	13.33 h	15.60g	5.60lm	23.33e	50.13 a	2.23 n	4.43 m	7.77 jk
AC 1 gl^{-1}+ PVP 2 g l^{-1}	16.63 g	24.47 e	33.33b	15.53g	28.87 c	32.20 b	2.23 n	6.70 kl	9.90 i

Means having the same letter (s) interaction are insignificantly different at 5% level.
AC : activated charcool PVP : polyvinyl pyrrolidone

Effect of antioxidant type, auxin type and concentration on embryo formation percentage of date palm cv. Sewi using immature female inflorescence explants during the initiation stage.

Data in **Table (2.a and Fig. 1c)** show the effect of antioxidant type and showed that the highest significant embryo formation percentage with PVP at 2 g l^{-1} (22.83%) but the lowest significant one was obtained with AC at 1 g l^{-1} + PVP at 2 g l^{-1} (7.64%).

Data in **Table (2.b)** show the effect of auxin type and showed that the highest significant embryo formation percentage with was obtained 2,4-D (16.00%) but the lowest significant one was acheived with NOA (12.58%).

Data in **Table (2.c)** show the effect of auxin concentration results which showed that the highest significant embryo formation percentage was noticed with 2 mg l^{-1} of auxin concentration (18.38%) but the lowest significant one was shown with 1 mg l^{-1} (7.40%).

Data in **Table (2.d)** show the effect of antioxidant type and auxin type results which showed that the highest significant embryo formation percentage with PVP at 2 g l^{-1} and Picloram (26. 67 %) but the lowest significant one was observed with AC 1 g l^{-1} with NOA (4.06%).

Data in **Table (2.e)** show the effect of antioxidant type and auxin concentration results which showed that the highest significant embryo formation percentage was obtained with AC 1 g l^{-1} with 2 mg l^{-1} of auxin concentration (21.82%) but the lowest significant one was recorded with AC at 1 g l^{-1}+ PVP at 2 g l^{-1} with 1 mg l^{-1} of auxin concentration (6.29%) compared to the other treatments.

Data in **Table (2.f)** show the effect of auxin type and concentration results which showed that the highest significant embryo formation percentage was showed with Picloram at 4 mg l^{-1}

(30.03%) but the lowest significant one was noticed with NOA at 1 mg l^{-1} (2.58%).

Data in **Table (2.g)** show the effect of antioxidant type, auxin type and concentration results which showed that the highest significant value was found by PVP 2 g l^{-1} with Picloram at 4 mg l^{-1} (42.23%) but the lowest significant values recorded by AC 1g l^{-} NOA 1 mg/l (0.0 %) or PVP 2 gl^{-1} with 2,4-D 4 mg l^{-1} (1.11%).

Table 2. Effect of antioxidant type, auxin type and concentration on embryo formation percentage of date palm cv. Sewi using immature female inflorescence explants during the initiation stage.

2. a - Effect of antioxidant type.

AC 1 g l^{-1}	12.56 B
PVP 2 g l^{-1}	22.83 A
AC 1 g l^{-1}+ PVP 2 g l^{-1}	7.64 C

Means having the same letter (s) in each column, are insignificantly different at 5% level.

2.b - Effect of auxin type.

2,4-D	16.00 A
Picloram	14.44 B
NOA	12.58 C

Means having the same letter (s) interaction are insignificantly different at 5% level.

2. c - Effect of auxin concentration.

1 mg l^{-1}	7.40 C
2 mg l^{-1}	18.38 A
4 mg l^{-1}	17.25 B

Means having the same letter (s) in each column , line or interaction are insignificantly different at 5% level.

2.d - Effect of antioxidant type and auxin type.

Antioxidant type.	**Auxin type**		
	2,4-D	**Picloram**	**NOA**
AC 1 g l^{-1}	19.89 c	24.06 b	4.06 i
PVP 2 g l^{-1}	5.55 h	26.67 a	11.11f
AC 1 g l^{-1} + PVP 2 g l^{-1}	12.23 e	17.76 d	7.76 g

Means having the same letter (s) interaction are insignificantly different at 5% level.

2. e - Effect of antioxidant type and auxin concentration.

Antioxidant type.	**Auxin concentration**		
	1 mg l^{-1}	**2 mg l^{-1}**	**4 mg l^{-1}**
AC 1 g l^{-1}	8.51 f	21.82 a	17.67 c
PVP 2 g l^{-1}	7.40 g	19.62 b	16.30 d
AC 1g l^{-1} + PVP 2 g l^{-1}	6.29 h	13.69 e	17.77 c

Means having the same letter (s) interaction are insignificantly different at 5% level.

2. f - Effect of auxin type and concentration.

Auxin type	**Concentration**		
	1 mg l^{-1}	**2 mg l^{-1}**	**4 mg l^{-1}**
2,4-D	6.69 g	17.80 c	13.18 d
Picloram	12.93 d	25.51 b	30.03 a
NOA	2.58 h	11.82 e	8.52 f

Means having the same letter (s) interaction are insignificantly different at 5% level.

2. g- Effect of antioxidant type, auxin type and concentration.

	2,4-D			**Picloram**			**NOA**		
	1 mg l^{-1}	**2 mg l^{-1}**	**4 mg l^{-1}**	**1 mg l^{-1}**	**2 mg l^{-1}**	**4 mg l^{-}**	**1 mg l^{-1}**	**2 mg l^{-1}**	**4 mg l^{-1}**
AC 1 g l^{-1}	8.87 j	27.80c	23.00d	16.67g	34.33b	21.17e	0.00 o	3.33 n	8.83 j
PVP 2 gl^{-1}	4.43mn	11.10 i	1.11o	14.43h	23.33d	42.23a	3.33 n	24.43d	5.57l m
AC 1 g l^{-1} + PVP 2 g l^{-1}	6.77 kl	14.50h	15.43gh	7.70j k	18.87f	26.70c	4.40 mn	7.70 jk	11.17 i

Means having the same letter (s) interaction are insignificantly different at 5% level.
AC : activated charcool PVP : polyvinyl pyrrolidone

From the previous results the highest, callus formation and embryo formation percentages were recorded by PVP 2 g l^{-1} + Picloram 4 mg l^{-1}. These results are in a harmony with those found by **Steinmache et al (2007)** using picloram , dicamba and 2,4-D with peach palm inflorescence. They found that picloram enhanced the embryogenic induction rate more than 2,4-D and dicamba, picloram at 300 µM enhanced the induction percentage. Both the type and level of auxins had the greatest influence on *in vitro* responses. **Kysely and Jacobsen (1990)** found that somatic embryogenesis was dependent on the type and concentration of auxin in the medium. Somatic embryos were never observed in the absence of exogenous auxin. Picloram was found to be the most suitable

callogenic agent for both types of explants, leaf and inflorescence of arecanut (*Areca catechu* L.) as well as for the varieties studied **(Karun et al 2004). Zayed (2011)** found that callus formation induced from spike explants of spindle palm was significantly affected by the addition of different picloram concentrations to culture media.

Differentiation stage.

Data in **Table (3 and Fig. 1d)** show the effect of different cytokinin type, concentration and their interaction on embryo number of date palm cv. Sewi cultured on the differentiation media for 12 weeks. Results showed that BA had the highest significant embryo number/culture while, 2iP had the lowest significant value. Regarding the cytokinin concentration, 0.5 mg l^{-1} had higher significant value than both other concentrations. The interaction between the studied factors, showed that the highest significant values were gained by BA or kinetin at 0.5 mg l^{-1}, while the lowest significant value was found 2iP at 0.25 mgl^{1}

Table 3. Effect of different cytokinin type, concentration and their interaction on embryo number/culture of date palm cv. Sewi cultured on differentiation media for 12 weeks.

Cytokinin type	Cytokinin concentration (mg l^{-1})			
	0.00	**0.25**	**0.50**	**Mean**
BA	32.70d	36.04c	45.02a	**37.93A**
Kin	32.70d	33.00d	45.00a	**36.90 B**
2 iP	32.70d	31.00e	42.00b	**35.23 C**
Mean	**32.72B**\	**33.35B**\	**44.01A**\	

Means in each column, row and interaction with the same letter (s) are not significantly different at 5% level.

Data in **Table (4)** show the effect of different cytokinin type, concentration and their interaction on number of adventitious shoots/culture of date palm cv. Sewi cultured on the differentiation media for 12 weeks. Results showed that Kin and 2iP had the highest significant number of shoots (7.26 & 6.92) while BA had the lowest significant value (6.25). Regarding the cytokinin concentration, 0.25 mg l^{-1} had the highest significant value (10.34) but the control had the lowest significant value concentrations (0.75).As for the interaction between the studied factors, the highest significant values were gained by kinetin at 0.25 mg l^{-1} ,while the lowest significant value was found with BA at 0.0 concentration.

Table 4. Effect of different cytokinin type, concentration and their interaction on number of adventitious shoots /culture of date palm cv. Sewi cultured on differentiation media for 12 weeks.

cytokinin type	cytokinin concentration (mg l^{-1})			
	0.00	**0.25**	**0.50**	**Mean**
BA	0.75 f	10.00c	8.00 d	**6.25 B**
Kin	0.75 f	14.01a	7.01 e	**7.26 A**
2iP	0.75 f	7.01 e	13.02b	**6.92 A**
Mean	**0.75 C**\	**10.34 A**\	**9.34 B**\	

Means in each column, row and interaction with the same letter (s) are not significantly different at 5% level.

Data in **Table (5)** Show the effect of different cytokinin type, concentrations and their interaction on average shoot length of date palm cv. Sewi cultured on the differentiation media for 12 weeks . Concerning cytokinin type, Kintein had higher significant shoot length than the other cytokinin types , Regarding the cytokinin concentration, (0.25 or 0.50 mg l^{-1}) had higher significant values (3.35 & 4.04) respectively than the control. As for the interaction between the studied factors, showed that the the highest significant values were gained by kinetin at (0.25 & 0.50 mg l^{-1}),

Table 5. Effect of different cytokinin type, concentrations and their interaction on average shoot length of date palm cv. Sewi cultured on differentiation media for 12 weeks

Cytokinin type	cytokinin concentration mg l^{-1}			
	0.00	**0.25**	**0.50**	**Mean**
BA	2.50c	2.01c	3.00bc	**2.50 B**
Kin	2.52c	5.01a	5.04a	**4.19 A**
2IP	2.50c	3.05bc	4.07ab	**3.20 B**
Mean	**2.51B**\	**3.35A**\	**4.04A**	

Means in each column, row and interaction with the same letter (s) are not significantly different at 5% level.

From the previous results, BA had the highest significant embryo number with 0.50 mg l^{-1} These results are in a harmony with those found by **Karun et al (2004)** they reported that . BA was found to be the best cytokinin form the germination

of somatic embryos. Different levels of BA (0, 5, 10, 20, 30 and 40µM) were studied for somatic embryos derived from inflorescence explants of arecappnut (*Areca catechu* L.). MS medium supplemented with 20 µM BA was found to be the best. Furthermore, Kin and 2iP had the highest significant number of shoots. These results are in a harmony with those found by **Al-Khateeb (2006)** who he observed that low hormone concentrations promoted the formation of buds and shoots while high hormone concentrations inhibited their formation.

Rooting stage.

Data in **Table (6 and Fig. 1e)** show the effect of different auxin types, concentrations and their interaction on rooting percentage of date palm cv. Sewi shoots during *in-vitro* rooting stage. Concerning auxin type, the highest significant rooting percentage was found by NAA (63.01%) but IAA had the lowest significant value (55.58%). Regarding the auxin concentration, 1.00 mg l^{-1} gave the highest significant value (77.80 %) than other auxin concentrations. As for the interaction between the studied, factors, the highest significant values were gained by NAA at 1 mg l^{-1} (88.92%) while the lowest significant value was found by control treatment with all auxin type (33.33%) .

Table 6. Effect of different auxin types, concentrations and their interaction on rooting percentage of date palm cv. Sewi shoots during *in-vitro* rooting stage.

Auxin type	Auxin concentration (mg l^{-1})			
	0.0	0.5	1.0	Mean
NAA	33.33 d	66.80 c	88.92 a	**63.01 A**
IBA	33.33 d	77.80 b	66.70 c	**59.27 B**
IAA	33.33 d	55.60 d	77.80 b	**55.58 BC**
Mean	**33.33 C**\	**66.73B**\	**77.80A**\	

Means in each column, row and interaction with the same letter (s) are not significantly different at 5% level.

Data in **Table (7)** show the effect of different auxin type, concentrations and their interaction on average number of roots of date palm cv. Sewi during *in vitro* rooting stage. Concerning auxin type, the highest significant average number of roots value was found by IBA (2.24) but NAA or IAA had lowest significant values . Regarding the auxin concentration, 1.00 mg l^{-1} had higher significant value (2.78) than both other auxin concentrations. The interaction between the studied factors showed that the highest significant values were gained by IBA at 1 mg l^{-1} (3.31), meanwhile, the lowest significant value was found by control treatment with all auxin type (1.01).

Table 7. Effect of different auxin type, concentrations and their interaction on average number of roots of date palm cv. Sewi during *in vitro* rooting stage.

Auxin type	Auxin concentration (mg l^{-1})			
	0.0	0.5	1.0	Mean
NAA	1.03 d	2.05 bcd	2.67 ab	**1.92 AB**
IBA	1.04 d	2.37 abc	3.31 a	**2.24 A**
IAA	1.01 d	1.35 cd	2.35 abc	**1.57 B**
Mean	**1.02 C**\	**1.92 B**\	**2.78 A**\	

Means in each column, row and interaction with the same letter (s) are not significantly different at 5% level.

Data in **Table (8)** show the effect of different auxin type, concentrations and their interaction on average root length of date palm cv. Sewi .Auxin type had insignificant effect on average root length. Regarding the auxin concentration, 0.50 mg l^{-1} had the highest significant value (1.03) than other auxin concentrations . As for the interaction between the studied factors, showed that the highest significant values were gained by IBA at 1.40 mg l^{-1} ,however, the lowest significant value was found by IBA at 1.00 mg l^{-1}(0.36).

Table 8. Effect of different auxin type, concentrations and their interaction on average root length (cm) of date palm cv. Sewi during *in-vitro* rooting stage.

Auxin type	Auxin concentration (mg l^{-1})			
	0.0	0.5	1.0	Mean
NAA	0.51 bc	0.76bc	0.53 bc	**0.60 A**
IBA	0.51 bc	1.40a	0.36 c	**0.76 A**
IAA	0.51 bc	0.94ab	0.76 bc	**0.74 A**
Mean	**0.51B**\	**1.03 A**\	**0.55 B**\	

Means in each column, row and interaction with the same letter (s) are not significantly different at 5% level.

Data in **Table (9)** show the effect of different auxin type, concentrations and their interaction on plantlet length of date palm cv. Sewi during *in vitro* rooting stage. As for the auxin type, NAA or IBA had higher significant plantlet length values (16.52 &16.03) respectively than IAA. Regarding the aux-

in concentration, 1.00 mg l^{-1} had the highest significant value (18.14) than both other auxin concentrations. Concerning the interaction between the studied factors, showed that the highest significant values were obtained by NAA at 1.00mg l^{-1}(19.37), while the lowest significant value was found by the control treatment (13.00).

Table 9. Effect of different auxin type, concentrations and their interaction on microshoot length (cm) of date palm cv. Sewi during *in-vitro* rooting stage.

Auxin type	Auxin concentration (mg l^{-1})			
	0.0	0.5	1.0	Mean
NAA	13.07d	17.13bc	19.37a	**16.52A**
IBA	13.10d	16.60c	18.40ab	**16.03A**
IAA	13.00d	13.07d	16.67c	**14.24B**
Mean	**13.06 C|**	**15.60 B|**	**18.14 A|**	

Means in each column, row and interaction with the same letter (s) are not significantly different at 5% level.

From the previous results, the highest rooting percentage found by NAA with the concentration of 1 mg/l. These results are in a harmony with those **El Hammady et al (1999) and Khierallah & Bader (2007)** who indicated that in rooting medium of date palm cv Sewi. the addition of NAA led to an increase in rooting percentage, with the concentration 0.5 mg/l which result in the best rooting percentage. Many researchers have mentioned the importance of NAA in the rooting of date palm shoots *in vitro* **(Al-Maari and Al-Gamdi, 1997).** Usually *in vitro* grown micro- shoots are rooted in an auxin -enriched medium to give rise to plantlets. **(Bekheet, 2013).**

Acclimatization stage

Data in **Table (10 and Fig. 1f)** showed the effect of pre acclimatization *in vitro* rooting tretmentes on survival percentage of date palm cv. Sewi after three month of the acclimatization stag . Concerning the auxin type, the highest significant survival percentage was found by IBA (51.84%) but IAA had the lowest significant value (40.74%). Regarding the auxin concentration, 1.00 mg l^{-1} gave the highest significant value (55.56 %) than both other auxin concentrations . As for the interactions between the studied factors, showed that the highest significant values were recorded by IBA at 0.5 mg l^{-1} (66.67%) while the lowest significant value was found by control treatment (33.33%).

Table 10. Effect of pre acclimatization *in-vitro* rooting tretmentes on survival percentage of date palm cv. Sewi after three months of acclimatization stage .

	Auxin concentration (mg l^{-1})			
Auxin type	0.0	0.5	1.0	Mean
NAA	33.33d	44.40 c	55.56 b	**44.43 B**
IBA	33.30d	66.67 a	55.56 b	**51.84 A**
IAA	33.33d	33.33 d	55.57 b	**40.74 C**
Mean	**33.32 C|**	**48.13 B|**	**55.56 A|**	

Means in each column, row and interaction with the same letter (s) are not significantly different at 5% level.

Data in **Table (11)** show the effect of pre acclimatization *in vitro* rooting tretmentes on plantlet length of date palm cv. Sewi after three month of the acclimatization stage . Results showed that the highest significant plantlet length found by NAA (18.97) but IAA gave the lowest significant value (17.15) .Regarding the auxin concentration, 1.00 mg l^{-1} had higher significant value (20.61) than both other auxin concentrations. As for the interaction between the studied factors, showed that the highest significant values were gained by NAA. at 1 mg l^{-1} (22.60), whereas the lowest significant value was found by control treatment (15.00).

Table 11. Effect of pre acclimatization *in-vitro* rooting tretmentes on plantlet length (cm) of date palm cv. Sewi after three months of acclimatization stage.

	Auxin concentration mg l^{-1}			
Auxin type	0.0	0.5	1.0	Mean
NAA	15.00e	19.30c	22.60a	**18.97A**
IBA	15.10e	18.65c	20.30b	**18.02B**
IAA	15.21e	17.33d	18.92c	**17.15C**
Mean	**15.10C**	**18.43 B**	**20.61A**	

Means in each column row, and interaction with the same letter (s) are not significantly different at 5% level.

Results concerning the acclimatization stage showed that micro-shoots obtained from 0.5 mg l^{-1} IBA treatment exhibited the highest survival percentage after 3 months *in vivo*. This result was in an agreemen with those obtained by **Al-Mana et al (1996)** who reported that rooting medium was an

important factor in determining the extent of root formation of off shoots. The highest rooting percentages were obtained using the following media, perlite: peat moss (3:1) medium. **Ibrahim et al (2017)** reported that root formation of date.

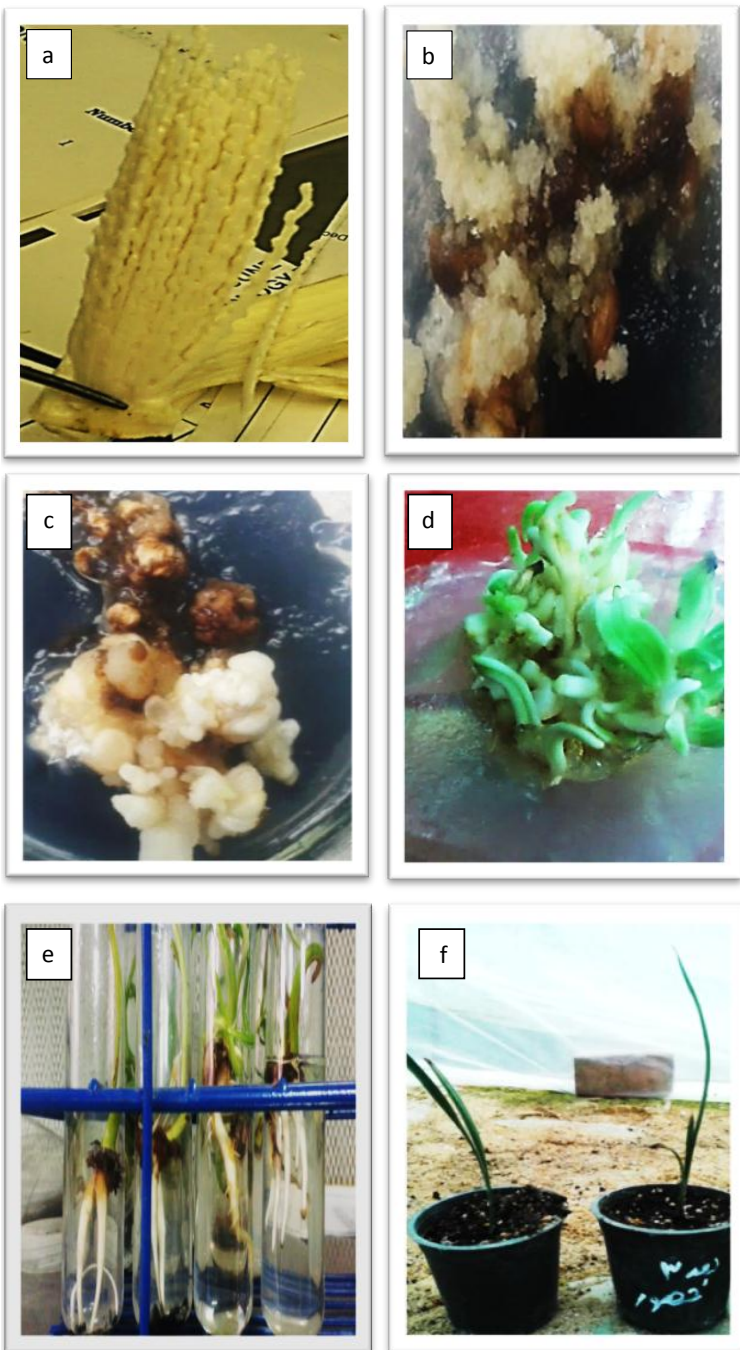


Fig. 1. *In vitro* propagation of date palm cv. Sewi (a) Immature female inflorescences (b) Callus formation (c) Direct embryo formation on 4 mg l^{-1} Picloram + 3 mg l^{-1} 2iP and 2 g l^{-1} PVP. (d) Differentiated embryos obtained on medium supplemented with BA at 0.5 mg l^{-1} (e) Rooting of microshoots on half strength MS. medium containing NAA at 1.0 mg l^{-1} (f) Acclimatizated plantlets produced from IBA at 0.5 mg l^{-1} treatment

palm "Medjool and Khalas" shoots enhanced on MS medium supplemented with 1mg l^{-1} IBA *in vitro*.

REFERENCES

Al-Khateeb, A.A., 2006. Role of cytokinen and auxin on the multiplication stage of date palm (*phoenix dactylifera* L.) cv. Sukry. **Biotechnology, 5(3), 349-352 .**

Al-Khayri, J.M. and Naik P.M. 2017. Date palm micropropagation : Advances and applications. **Ciencie Agrotecnologia, 41, 347-358.**

Al-Maari, K.W. and Al-Ghamdi, A.S. 1997. Micropropagation of five date palm cultivars through in vitro axillary buds proliferation. **Du. J. Agri. Sci., 13: 55-71 (Cited after Bekheet, 2013).**

Al-Mana, M.A., El-Hamad M.A.Y., Bacba A.A. and Ab-Delrabman F.A., 1996. Improving root development on ground and aerial date Palm off shoots. **Res. Bult. Agric. Res. Center, King Saud Univ., pp. 5-19.**

Badawy, E.M., Afaf M.A. Habib, El-Bana A. and Gehan M., Yosri, **2005.** Propagation of date palm (*Phoenix dactylifera*). Plants by using tissue culture technique. **Arab J. Biotech., 8(2), 343-353.**

Bekheet, S., 2013. Direct organogenesis of date palm (*Phoenix dactylifera* L.) for Propagation of True to Type Plants. **Sci. Agri., 4 (3), 85-92.**

Duncan, D.B., 1955. Multiple range and multiple f tests. **Biometrics, 11, 1-42**

Eke, C.R., Akomeah P. and Asemota O., 2005. Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) from apical meristem tissues from 'zebia' and 'loko' landraces. **African Journal of Biotechnology, 4(3), 244-246.**

El-Hammady, A.M., Wafaa, H. Wanas, Abo Rawash M. and Awad A.A., 1999. *In-vitro* propagation of date palm 2 factors affecting rooting and acclimatization of *in-vitro* proliferated shoots of date palm "Sewy" cv. **Annals Agric. Sci., Fac. Agric., Ain Shams Univ., pp. 112-114.**

Feki, L. and Drira N., 2007. Development of an efficient plant regeneration system for date palm. **Fourth Symposium on Date Palm. 5-8 May, King Faisal University, Al-Hassa, Saudi Arabia.**

Fki, L., Masmoudi R., Drira N. and Rival A. 2003. An optimized protocol for plant regeneration from embryogenic suspension cultures of date palm, *Phoenix dactylifera L*., cv. **Deglet Nour. Plant Cell Reports, 21, 517-524.**

Ibrahim, I.A., Badr-Elden A.M., Emara H.A., Gabr M.F., Nower A.A. and Abd Elaziem T.M., 2017. Improving In Vitro Somatic Embryos Production of Medjool and Khalas Date Palm Cultivars via Modification of Ammonium and Potassium Nitrate. **Global Journal of Botanical Science, 5, 16-24.**

Karun, A., Siril E.A., Radha E. and Parthasarathy V.A., 2004. Somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants of arecanut (*Areca catechu* L.). **Current Science, 86, 12- 25.**

Khierallah, H.S.M. and Bader S.M., 2007. Micropropagation of date palm (*Phoenix dactylifera* L.) Var. Maktoom through direct organogenesis. **ISHS Acta Hort., III International Date Palm Conference , 736, 213–224.**

Kysely, W. and Jacobsen H., 1990. Somatic embryogenesis from pea embryos and shoot apices. **Plant Cell Tissue and Organ Culture, 20 (1), 7-14.**

Murashige, T. and Skoog F., 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. **Physiologia Plantarum, 15(3), 73-97.**

Sane, D., Aberience-Bertossi F., Gassama-Dia Y., Sagna M., Trousiot M., Duval Y. and Borgel A., 2006. Histocytological analysis of callogensis and somatic embryogenesis from cell suspensions of date palm (*Phoenix dactylifera L.).* **Annals of Botany, 98, 301-308.**

Snedecor, G.W. and Cochran W.G., 1982. Statistical Methods. 7th Ed. Iowa State Univ. Press Ames, Iowa, USA. **297 p..**

Steinmacher, D.A., Clement C.R. and Guerra M.P., 2007. Somatic embryogenesis from immature peach palm inflorescence explants: towards development of an efficient protocol. **Plant Cell Tissue Organ Culture, 89, 15-22.**

Zayed, E.M.M., 2011. Propagation of *Phoenix Dactylifera* L., *Chamaerops Humilis* L. and *Hyophorbe Verschaffeltii* L. Palms by Using Tissue Culture Technique. **Ph.D. Thesis, Department of Ornamental Horticulture, Fac. Agric. Cairo University, Egypt. 169p.**

المؤتمر الرابع عشر لبحوث التنمية الزراعية،
كلية الزراعة، جامعة عين شمس، مارس 2019، القاهرة، مصر
مجلد(27)، عدد (1)، عدد خاص مارس، 737-747، 2019
Website: http://strategy-plan.asu.edu.eg/AUJASCI/

تكوين الأجنة المباشر وتكوين الأعضاء غير المباشر لنخيل البلح صنف السبوى با ستخدام النورات الزهرية المؤنثة غير الناضجة

[68]

مرفت حسن محمد ملهط[1*]– حسن الوكيل[2]– أحمد عبدالحميد[2]– سعيد محمد خليل[1]– منى محمد حسن[3]

1. **معهد بحوث الهندسة الوراثية– مركز البحوث الزراعية– الجيزة– مصر**
2. **قسم البساتين– كلية الزراعة– جامعة عين شمس– ص.ب 68– حدائق شبرا 11241– القاهرة– مصر**
3. **المعمل المركزي لأبحاث وتطوير النخيل– مركز البحوث الزراعية– الجيزة– مصر**

[*]Corresponding author: mervatT83@yahoo.com

Received 25 November, 2018, Accepted 18 December, 2018

الموجـــــز

أجريت هذه الدراسة بمعمل زراعة الأنسجة النباتية بمعهد الهندسة الوراثية الزراعية بمركز البحوث الزراعية بالجيزة خلال الفترة من 2013–2017 . وقد أوضحت النتائج أن إضافة 4 ملجم/لترمن بيكلورام وايزو بنتيل ادينين بتركيز 3 مللجم / لتر بالاضافة إلى 2 جم / لتر بولى فينيل بيروليدون أدى لتكوين الأجنة الجسدية المباشرة وأيضا دفع النورات الزهرية لتكوين الكالس. كما أوضحت النتائج ان البنزيل أدنين من أفضل منظمات النمو بتركيز 0.5 mg/l على عدد الاجنة المتكشفة لكل مزرعة مقارنة بالتركيزات الاخرى، بينما أدى أستخدام الكينتين بتركيز 0.25 mg/l إلى زيادة عددالأفرع العرضية لكل مزرعة .أوضحت النتائج ان إستخدام نفثالين حمض الخليك بتركيز 1مللجم /لتر سجل أعلى نسبة مئوية للتجذير، بينما أدى إستخدام إندول بيوتريك اسيد بتركيز 1.00 mg/l للحصول على أعلى نسبة بقاء أثناء مرحلة الأقلمة .

الكلمات الدالة: نخيل البلح، صنف السيوي ,النورات الزهرية غير الناضجة، التكوين الجنيني المباشر التكوين العضوي غير المباشر.

تحكيم: ا.د صبري موسى
ا.د أيمن شعبـــان