Micropropagation of some Sewa Oasis Date Palm (*Phoenix dactylifera* L.) Cultivars Grown in Egypt

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Abstract: Shoot tip and leaf primordial explants of *Phoenix dactylifera* L. cvs. (Sewi and Oshkingbeel) were cultured in modified MS medium supplemented with various combinations between different growth regulators cytokinins (2iP or BA) and Auxins (2, 4-D or NAA). Friable callus was produced from shoot tip and leaf primordial explants. The highest percentage of callus initiation was achieved on modified MS medium supplemented with 100 mg/L 2,4-D and 3 mg/L 2iP for shoot tip and leaf primordial explants. The highest percentage of embryogenic callus was achieved on modified MS medium supplemented with 10 mg/L 2,4-D, 5 mg/L NAA, 3 mg/L 2iP and 3.0 g/L activated charcoal which gave 87.5% and 75% for Sewi and Oshkingbeel cvs., respectively. Somatic embryos formation were obvious on modified MS medium plus 0.1 mg/L NAA which gave the highest average number (5.16, 4.94) for Sewi and Oshkingbeel, respectively after three subcultures. Greatest embryonic elongation resulted at 0.5 mg/L 2iP + 0.5 mg/L kinetin + 40 g/L sucrose in solid full strength MS medium. Also, the highest value of leaves number was shown on the same medium. The complete plantlets developed from individual embryos (5-10 cm) were transferred to rooting MS medium supplemented with 1 mg/L NAA with good growth in solid and liquid media. Rooted date palm plantlets from both cultivars were transferred to plastic pots (5cm diameter) containing peat-moss, sand and vermiculite at equal volume. Acclimatization percentage decreased gradually during four months in the first acclimatization stage. Date palm plantlets produced from the first acclimatization stage were transferred to plastic pots (20 cm diameter) containing peatmoss, washed sand and vermiculite at equal volume for more growth and development to be ready to cultivate in the field. Although survival percentage was low during the first acclimatization stage, it was very high in the second acclimatization stage and date palm survival percentage became constant after another six months with good growth.

Keywords: Date palm, Embryogenic callus, Somatic embryogenesis.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is the sole member of the tribe phoenicaea of the monocotyledonous family palmae (Areaceae). It is adapted to areas with very hot summers and with abundant underground water. These conditions are found in Oasis (Siwa, The Siwa Oasis is an oasis in Egypt, between the Qattara Depression and the Egyptian Sand Sea in the Western Desert, nearly 50 km (30 mi) east of the Libyan border, and 560 km (348 mi) from Cairo) and Nile valley is the arid subtropical desert of the Middle East. Moreover, the importance of date palm tree lies in its high tolerance to environmental stresses such as salinity, drought and high temperature. In Egypt, date palm is one of the most important fruits and widely distributed in different districts. There are three main types of dates based on fruit moisture content, i.e., soft, semi- dry and dry cultivars [1]. Date palm is vegetatively propagated through offshoots. The availability of offshoots is limited because the number produced by each palm tree is low, very erratic and can not be successfully controlled. Date palm plantlets may be produced by tissue culture both by organogenesis or by somatic embryogenesis either directly from the mother explants tissue or through callus [2-4]. Callus initiation was obtained from various date palm tissue that included; shoot tip, lateral buds, leaf primordial, inflorescence, petiole, rachis, roots and zygotic embryos [5, 6]. However, different morphogenic responses including callus, root and shoot formation were recorded for the different explants and varieties. Also, several authors claimed that the highest rate of callus formation was obtained from shoot tip explants [6, 7] and leaf primordial explants [8]. Currently, the process of somatic embryogenesis seems to be more attractive for an industrial production but the date palm derived from somatic embryos must be true-to type. Obviously, the successful utilization of *in vitro* propagation of date palm will be dependent on the genetic stability of the cultures, physical growth factors, nutrient medium and plant growth regulators [9-12].

The purpose of this study was to investigate *in vitro* propagation system of date palm grown in Siwa oasis through indirect somatic embryogenesis by obtaining embryonic callus from shoot tip and leaf primorida explants and germination of somatic embryos from embryonic callus. The study included the effect of plant growth regulators on callus formation and

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differentiation and MS salt strength (liquid and solid) on *in vitro* growth during multiplication stage of both cultivars (Sewi and Oshkingbeel) and subsequently rooting and acclimatization.

MATERIAL AND METHODS

The current study was carried out in Plant Tissue Culture Unit, Plant Genetic Resources, Desert Research Centre (DRC).The offshoots 25- 30 cm in diameter, 50-80 cm in height and 5-7 kg. in weight were carefully separated from adult date palm Sewi and Oshkingbeel (semi-dry cultivars) grown at Sewa Oasis, Matroh governorate, Egypt and used as explants source. Surface sterilization were conducted according to Ibrahim and Hegazi, 1999) Shoot tip and leaf primordial were cultured on MS [13] medium supplemented with 170 mg/L $NaH_2PO_4.2H_2O$; 200 mg/L glutamine; 40 mg/L adenine sulfate; 0.4 mg/L Thiamine-HCl, 0.01 mg/L biotin, 3 g/L activated charcoal (AC), 30 g/L sucrose; 1.5 g/L gelrtie and various combinations between different growth regulators, iso pentenyl adenine (2iP) or benzyl adenine (BA) at 3 or 5 mg/l and 2,4 dichloro phenoxy acetic (2,4-D) or naphthalene acetic acid (NAA) at 100 mg/L and combined with 2,4-D at 10 mg/L plus NAA at 5 mg/L plus 2iP at 3 mg/L (Table **1**). The pH of culture medium used in these experiments was adjusted at 5.7 $\pm$ 0.1 prior to addition of gelrite. The culture medium was dispensed into small jars (170 ml) contained 30 ml of prepared medium, the jars were autoclaved at 121°C and 1.1 kg/ cm^2 for 20 min. The cultures were incubated in complete darkness under temperature of 27°C $\pm$ 2°C for five months or until the formation of callus. Cultures were transferred to corresponding fresh medium every eight weeks. The friable calli were transferred to the MS medium supplemented with various concentrations of 2,4-D alone or/ and NAA and 2iP at 3 mg/L with activated charcoal 3.0 g/l. All cultures were transferred to fresh medium every four weeks. And somatic embryogenic callus percentage were recorded.

Approximately 0.1 g embryonic callus were cultured on MS medium included NAA at 0.1 mg/L alone or/ and 3 types of cytokinins (Kinetin, 2iP and BA) as show in Table **1**. All cultures were incubated in growth room at 27 $\pm$ 2°C and complete darkness. Developed embryogenic callus were subcultured every four weeks on fresh medium of the same composition for at least three subcultures. At the end of each subculture, data on average number of embryos per culture for two cultivars were recorded. For multiplications stage, a small cluster containing 3-4 embryos were used as the explant materials in this stage. Clusters of both date palm cultivar were cultured on different combinations of modified MS salt strength ($^1/_4$, ½ and full strength) and two physical condition (liquid and solid). Liquid cultures were on shaker at 75 rpm and the other solidified with 2 g /l gelrite. Both nutrient media supplemented with 40 g/L sucrose, in addition to 2iP and Kinetin each at 0.5 mg/l. All cultures were incubated in 27 $\pm$ 2°C for 6 weeks in growth room under 16 hrs, illumination of 3000 Lux, white florescent lamps. Each treatment contained seven replicates and each replicate contained one cluster. After four weeks in culture, growth vigor and leaf number were recorded at the end of each subculture (three subcultures). Individual shoots of about 7-10 cm in length 2-3 leaves were cultured on MS nutrient medium supplemented with 1.0 mg/L NAA (Rooting medium) according to the methods described by [14].

Table 1: Plant Growth Regulators (Cytokinins and Auxins) and its Concentration Used in Date Palm Propagation via Tissue Culture Techniques

Micropropagation stage	Plant growth regulators mg/l				
	BA	2IP	KIN.	NAA	2,4-D
Starting Stage	0.00	3.00	0.00	100	0.00
	5.00	0.00	0.00	100	0.00
	0.00	3.00	0.00	0.00	100.00
	5.00	0.00	0.00	0.00	100.00
	0.00	3.00	0.00	5.00	10.00
Somatic embryogenesis and shoot formation	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.10	0.00
	0.10	0.00	0.00	0.00	0.00
	0.10	0.00	0.00	0.10	0.00
	0.00	0.10	0.10	0.00	0.00
	0.00	0.10	0.00	0.10	0.00
	0.00	0.00	0.10	0.00	0.00
	0.00	0.00	0.10	0.10	0.00

The pH of culture medium used in these experiments was adjusted at 5.7 ± 0.1 prior to addition of gelrite. The culture media were dispensed into small jars (170 ml), contained 30 ml of prepared medium in callus and embryos formation, and big jars (300 ml contained 50 ml nutrient media) in shoot and root formation. The jars were autoclaved at 121°C and 1.1 kg/ cm^2 for 20 min. Callus initiation percentage and number of embryos were recorded after three subcultures for both callus and embryos formation stages while growth vigour (G.V.) and number of leaves were recorded after three subcultures too during multiplication stage. Growth vigor was determined according to the rate of Scalling by [15], which included: No Growth (- Slight growth (+) = (2) Average growth (++) =(3),Above average growth (+++) = (4) Excellent growth(++++)=5.

All the experiments were arranged in a completely randomized design. Duncan's range test was employed for means comparisons according to [16].

Rooted plantlets were carefully transplanted in plastic pots (5cm diameter) filled with moistened mixture of peat-moss, vermiculite and washed sand at equal volume. The plantlets were covered with transparent polyethylene bags for one month to raise the relative humidity around the plantlets. The plants were sprayed with fungicide solution 0.2 % (w/v) and irrigated with $^1/_{10}$ strength MS inorganic medium. Survival percentage of plantlets, were recorded monthly (four months) and the experiments were repeated twice. Each treatment consisted of 15 replicates in one plantlet per each. Survival percentages were recorded monthly for two cultivar for four months.

RESULTS AND DISCUSSION

Effect of Plant Growth Regulators on

Callus Initiation

Data presented in Table **2** and Photo **1** show the effect of auxins (2,4- D and NAA) and cytokinins (2iP and BA) on callus initiation derived from shoot tip and leaf primordial (Table **3** and Photo **1**) for both date palm cultivars (Sewi and Oshkingbeel). The best medium for callus initiation was MS medium supplemented with

Table 2: Effect of Plant Growth Regulators on the Callus Initiation Percentage on Shoot Tip Cultures of Date Palm Sewi and Oshkingbeel Cultivars

Growth regulators mg/l	Cultivars		Mean
	Sewi	Oshkingbeel	
Control	00.0e	00.0e	00.0E
100 2,4-D+ 3 2iP	91.7a	83.3a	87.5 A
100 2,4-D+ 5 BA	75.0c	58.3c	66.7 C
100 NAA+ 3 2iP	50.0d	50.0d	50.0D
100 NAA+ 5 BA	50.0d	50.0d	50.0D
10 2,4-D + 5 NAA + 3 2iP	83.3b	75.0b	79.2 B
Mean	58.3A\	52.8B\	

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

Table 3: Effect of Plant Growth Regulators on the Callus Initiation Percentage on Leaf Primordial Cultures of Date Palm Sewi and Oshkingbeel, Cultivars

Growth regulators mg/l	Cultivars		Mean
	Siwy	Oshkingbeel	
Control	00.0e	00.0d	00.0 E
100 2,4-D+ 3 2iP	83.3a	75.0a	79.2 A
100 2,4-D+ 5 BA	66.7c	66.7b	66.7 C
100 NAA+ 3 2iP	50.0d	50.0c	50.0 D
100 NAA+ 5 BA	50.0d	50.0c	50.0 D
10 2,4-D + 5 NAA + 3 2iP	75.0b	66.7b	70.9 B
Mean	54.2A\	51.4C\	

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

100 mg/l 2,4-D + 3.0 mg/L 2iP which gave the highest callus initiation percentage for shoot tip explants (91.7 and 83.3%) and leaf primordia explants (83.3 and 75.0%) of date palm Sewi and Oshkingbeel, cvs. respectively. Sewi cultivar produced the highest significant value of callus initiation percentage (58.3, 54.2) compared with Oshkingbeel cv (52.8, 51.4) for shoot tip and leaf primordial respectively.

Photo 1: *In vitro* growth of shoot tip explant in starting stage of date palm *Phoenix dactylifera*cv. Sewi (1) and Oshkgnbeel (2).

Data also show that MS medium supplemented with 10 mg/l 2,4-D+ 5 mg/l NAA + 3mg/l 2iP recorded good results for callus initiation as presented in Table **2** and **3**. These results are in harmony with those of [6, 17]. Increasing the levels of auxins caused increased callus production. Shoot tip explants were preferred more than other explants because of its high callus production (83.67%). As well as [18] mentioned that, shoot tips were superior to any other tissues in the offshoots to produce callus.

Somatic Embryogenesis

Data presented in Tables **4**, **5** and Photos **2** and **3** show the effect of various concentrations of Kin., 2iP and BA and 0.1 mg/l NAA on somatic embryos formation for Sewi and Oshkingbeel cultivars. Full MS medium supplemented with 0.1 mg/l NAA and control treatment gave the highest number of embryos (5.16 and 4.89 embryos / explant) of Sewi cv. and (4.94 and 4.52 embryos / explant) of Oshkingbeel cv. for both two media, respectively. Increasing the culture period (subcultures) of embryogenic callus stimulate somatic embryos formation of both cultivar. Observation about interaction between treatments and subculture, data show that the highest significant value of embryos number was observed when explants cultured on MS basal medium supplemented with 0.1 mg/L NAA and control treatments (7.83 and 7.33 embryos/ explant), of Sewi cv. and (7.17 and 6.83 embryos/ explant), of Oshkingbeel cv., respectively.

From previously data in Table **5**, it could be concluded that, the MS medium supplemented with 0.1 mg/l NAA was suitable for embryos formation and gave the best results for embryos number (5.16 and 4.94 embryos/explant) of date palm Sewi and Oshkingbeel cultivars, respectively. Also, it could be concluded that, with increasing the number of subcultures, the average number and length of embryos increased (irreversible relation). These result are in agreement with those found by [8, 14, 18] who reported that embryos differentiation and plantlets growth was best on medium containing law level (0.1 mg/L) NAA. However, somatic embryogenesis was induced by transferring the callus produced on the medium without hormones. The embryonic callus developed from white friable embryonic nodular callus obtained of Sewi, Bent-Esha and Hiane cultivars, and abnormal shape percentage of somatic embryos increased by increasing the subculture number [14, 19].

Effect of MS slat strengths (solid or liquid medium) on the *in vitro* growth and development of date palm Sewi and Oshkingbeel cultivars during shoot formation stage.

Data in Table **5** show the effect of MS salt strengths (¼, ½ and full strength) on growth value and average number of leaves. These factors are estimated as indicators for growth development of two date palm cultivars. The highest significant of growth value (G.V) was occurred in response to solidified full strength MS medium (4.2) followed by half strength MS medium, where G.V. was (3.7), compared to the quarter MS salt strength (2.9) with significant difference in between.

Table 4: Effect of Different Plant Growth Regulators Added to Modified MS Medium on Number of Somatic Embryos Formation of Date Palm Sewi and Oshkingbeel Cultivars during Three Subcultures

Treatments		Average number of embryos/explant cultures							
		Sewi				Oshkingbeel			
		subcultures				subcultures			
cytokinin (mg/l)	Auxin (NAA) (mg/l)	1	2	3	Mean	1	2	3	Mean
0.0	0.0	2.83a	4.50b	7.33b	4.89B	2.40b	4.33a	6.83b	4.52B
0.0	0.1	2.83a	4.83a	7.83a	5.16A	2.83a	4.83a	7.17a	4.94A
0.1 Kin.	0.1	1.17e	2.5g	4.67h	2.78I	1.00d	2.50d	4.83e	2.77E
0.2 Kin.	0.1	1.67d	2.83f	5.67f	3.39G	1.00d	2.50d	4.83e	2.77E
0.1 2iP	0.1	2.17d	4.33c	6.83c	4.44CD	2.00c	4.00b	6.00c	4.00C
0.2 2iP	0.1	2.00c	4.17d	6.67c	4.28DE	2.00c	3.83c	5.83d	3.89D
0.1 BA	0.1	2.00c	4.00e	6.00e	4.00F	1.00d	2.50d	4.83e	2.77E
0.2 BA	0.1	1.67d	2.83f	5.17g	3.22H	1.00d	2.33e	4.33f	2.55F
Mean		2.04C	3.75B	6.267A		1.65C	3.76B	5.50D	

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

Table 5: Effect of MS Salt Strength and Physical Condition (Type of Medium,Solid or Liquid) on the *In Vitro* Growth and Development of Date Palm Sewi and Oshkingbeel Cultivars (Multiplication Stage)

Parameters	MS salt strength	Solid			Liquid		
		S	O	Mean	S	O	Mean
Degree of growth (vigor)	Quarter	3.4c	2.4c	2.9C	2.1c	1.6c	1.9C
	Half	3.9b	3.4b	3.7B	2.3b	2.0b	2.1B
	Full	4.7a	3.7a	4.2A	2.9a	2.3a	2.6A
	Mean	4.0A\	3.2C\		2.4A\	2.0B\	
Number of leaves	Quarter	2.6c	2.3b	2.5B	1.3c	0.9c	1.1C
	Half	2.9b	2.3b	2.6B	2.1b	1.7b	1.9B
	Full	3.7a	2.7a	3.2A	3.1a	2.1a	2.6A
	Mean	3.1 A\	2.4 C\		2.2A\	1.6C\	

Means having the same letter(s) in each column or line are insignificantly different at 5% level.
Degree of growth: It was determined according to the rate of Scalling by Pottino (1981), which included: No Growth = (1) Slight growth = (2) Average growth =(3) Above average growth = (4) High growth = (5).

Regarding the use of liquid medium, the high significant growth value was obtained on full strength MS (2.6) compared to half strength MS (2.1) while, quarter strength MS gave the lower significant value (1.9). Concerning to the effect of cultivars, data in Table **5** indicate that the solidified medium gave the highest significant value of growth degree (4.0 and 3.2) of Sewi and Oshkingbeel cvs., respectively. While, liquid medium was significantly higher growth value (2.4) for Sewi cv. compared to Oshkingbeel cv., which gave 2.0, with significant difference in between.

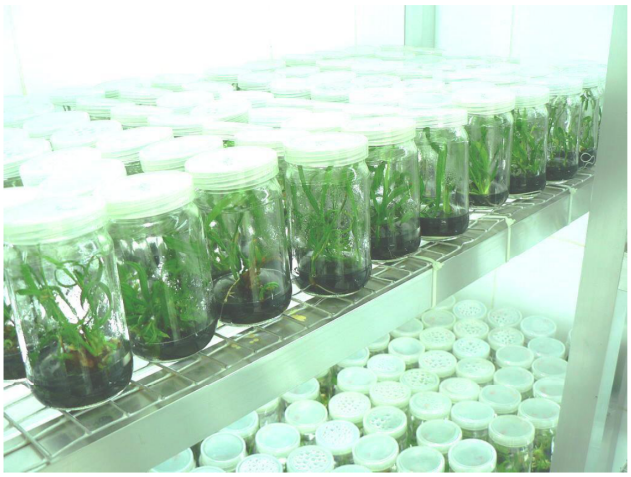
Interaction between salt strength of MS medium and type of cultures, the highest significant growth value was observed by using full strength, as the values were (4.7 and 3.7) in solid medium compared to (2.9 and 2.3) in liquid medium of Sewi and Oshkingbeel cvs., respectively. While, the lowest significant value was observed when explant of Sewi and Oshkingbeel cvs., cultured on quarter strength medium either solid or liquid, as gave (3.4 and 2.4) in solid and (2.1 and 1.6) in liquid medium, respectively.

Data in Table **5** revealed that the highest significant number of leaves was 3.2 leaves/ shoot in solidified full strength followed by 2.6 and 2.5 leaves/ shoot in half and quarter strengths MS medium, respectively without significant difference in between. In regard to liquid

Photo 2: Somatic embryos of date palm cv. Sewi grown on modified MS nutrient medium.

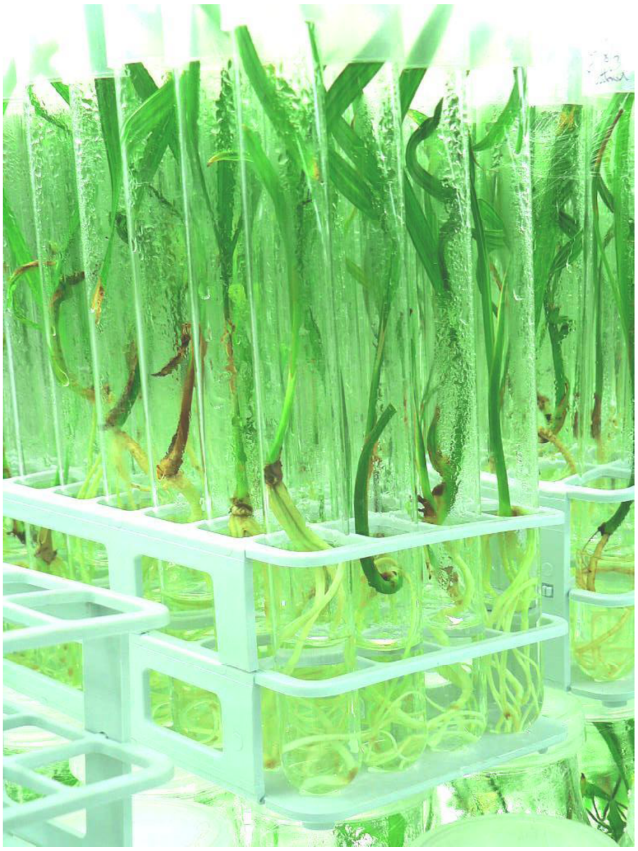
Photo 3: Somatic embryo formation of date palm cv. Oshkingbeelgrown on modified MS nutrient medium.

medium, the highest significant number of leaves was obtained on full strength (2.6) compared to the half strength (1.9) while, quarter strength MS medium gave the lowest significant value (1.1). The cultivars affect cleared a significant in leaves number of Sewi and Oshkingbeel in solid medium (3.2 and 2.4 leaves/shoot), respectively when compared to in the liquid medium which gave 2.2 and 1.6 leaves/shoot, respectively. With significant differences in the mean number of leaves in either solid or liquid medium, respectively. The highest significant number of leaves was observed by using MS at full salt strength, as the values were (3.7 and 2.7 leaves/ shoots) in solid medium compared to (3.1 and 2.1 leaves/ shoots)) in liquid medium of Sewi and Oshkingbeel cvs., respectively. While, the lowest significant value was observed when explant of Sewi and Oshkingbeel cvs., cultured on quarter strength medium either solid or liquid, as gave (2.6 and 2.3 leaves/ shoots) in solid and (1.3 and 0.9 leaves/shoots) in liquid, respectively. It is generally evident that full strength medium is more effect in promoting growth and development of date palm cultivars comparing to the other media strengths. Furthermore, both solid and liquid medium were necessary to achieve both growth value and number of leaves/shoot for the different tested cultivars. Thus, rooting was influenced by genotype, salt strengths and physical form of the medium comparing to the other media strengths either solid or liquid (Photos **4** and **5**). These results are in harmony with those found by [20] who showed that, using of full strength of MS salt in the culture medium during repetitive embryos of Zaghloul and Barhi cultivars had significantly higher values of embryos multiplication and growth rate which was accompanied by lower degrees of both browning and hyperhydricity comparing with the other studied MS strengths, i.e. ¾, ½ and ¼ MS salt strength.



ROOTING STAGE NO.1

Photo 4: *In vitro* date palm plants *(Phoenix dacylifera* cv. Sewi) in the first rooting stage.



ROOTING STAGE NO.2

Photo 5: The second *in vitro* rooting stage of date palm (cv. Oshknbeel).

Acclimatization Stage

Data presented in Table **6** clearly show the growth of date palm plants cv. Sewi and Oshkngbeel during acclimatization stage through four months estimated as survival percentage acclimatization percentage of date palm cultivars (Sewi and Oshkengbeel) as affected by acclimatization period through four months decreased gradually from the first month to the fourth month and this percentage was significant at 5% level. The effect of planting media containing peat-moss, washed sand and vermiculite mixtures at equal volume on survival percentage of date palm Sewi and Oshkingbeel cvs. during acclimatization stage through four months were shown in Table **6** and illustrated in Photo **6**. Rooted plantlets were transferred to plastic pots containing peat-moss, sand and vermiculite at equal volume for two cultivars. The survival percentage was 46 and 20 for Sewi and Oshkingbeel respectively after four months as indicated in Table **6**. Survival percentage of date palm plantlets cultivars was decreased with aging through 4 months of the *Ex Vitro* acclimatization stage. These results are in harmony with those found by [2, 3]. Survived date palm plants cv. Sewi (46% Survival) and Oshkingbeel (20% Survival) produced from the first acclimatization stage were transferred to plastic pots (20 cm diameter) containing the same planting media for more growth in the second acclimatization stage. The survival percentage was 100% after another four months and date palm plants grow well as illustrated in Photo **6**.

Table 6: Effect of Acclimatization Period (Months) on Survival Percentage of Date Palm Sewi and Oshkingbeel cvs. during Acclimatization Stage. Rooted Date Palm Plantlets Cultured on Planting Medium Containing Peatmoss, Washed Sand and Vermiculite at Equal Volume

Cultivars	Acclimatization period (months)				Mean
	1	2	3	4	
Sewi	86.67a	73.33b	66.67c	46.67d	68.34A
Oshkingbeel	66.67c	46.67d	40.00e	20.00f	43.34B
Mean	76.7A\	60.0B\	53.3C\	33.3D\	

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

Photo 6: Date palm plants produced through tissue culture techniques in the nursery after six months from the first acclimatization.

The acclimatization percentage of date palm cultivars namely Oshkingbeel and Sewi are still low and it may be due to some factors affecting during *in vitro* growth especially genetic make-up, physical growth factors and nutrient medium during pre-acclimatization stage as well as the components of planting nutrient medium (peatmoss, vermiculite and washed sand at equal volume). The greenhouse conditions are other factors affecting the survival percentage of date palm like humidity, temperature and light intensity. It needs more scientific research for *in vitro* growth and in the

greenhouses for date palm plantlets produced through tissue culture technique. Results under discussions are in line with [11, 12].

REFERENCES

[1] Moustafa A. Studies on pollination of date palms. Proceedings the First International Conference on date palm. AL-Ain. United Arab Emirates, March 8-10, 1998; pp. 39-48.

[2] Ibrahim IA, Hegazy AE. Micropropagation of date palm *(Phoenix dactylifera* Z, cv. Samany) via direct organogenesis. The First International Conferenence in Egypt on Plant Tissue Culture and its Application 1999; pp. 201-207.

[3] Hassan MM, Ibrahim IA, Fathy NM, Ebrahim MK, Komor E. Protocol for Micropropagated Date Palm Acclimatization: Effect of Micropropagated Plantlet Type, Soil Composition, and Acclimatization Season. International Journal of Fruit Science 2014; 14: 225-233. https://doi.org/10.1080/15538362.2013.819219

[4] Ibrahim IA, Hassen MM, Seif A, Maksoud AI. Propagation of Semi Dry Date Palm *(Phoenix dactylifera* L.) grown in New Valley Using Tissue Culture Techniques. 1st International Conference of Genetic Engineering and Biotechnology" Integrative Science and Biotechnology". Sharm El-Sheikh-Egypt April 26-29-2016.

[5] Gabr MF, Tisserate B. Propagation palms *in vitro* with special emphasis on the date palm *(Phoenix dactylifera* L.). Scientia Horticulture 1985; 25(3): 255-263. https://doi.org/10.1016/0304-4238(85)90123-2

[6] Eke CR, Akomeah P, Asemota O. Somatic embryogenesis date palm *(Phoenix dactylifera* L.) from apical meristem tissues from " Zebia" and "Loko" landraces. Afr J Biotechnol 2005; 4(3): 244-246.

[7] Eslam M. High frequency somatic embryo in date palm by shoot tip. Agricultural Biotechnology Research Institute of Iran 2006; 3:19-21.

[8] Khattab MM, Ibrahim IA, Gadalla EG. *In vitro* propagation of Egyptian dry date palm. 1.Effect of explant type and date of culture on browning phenomenon & callus formation and plant growth regulators containing medium on embryogenic callus formation.Bulletin of Faculty of Agriculture, Cairo, University 2003; 54: 4, 555-568.

[9] Pierik RL. *In vitro* Culture of Higher Plants. Martinus Nijhoff Publishers,Dordecht / Boston / Lancaster 1987.

[10] El-Taras AE, Hossni YA, El-Banna A, Aly AS. Date palm (*Phoenix dactylifera* L.) Cultivars Identification Using RAPD Technique. Egypt J Hort 2002; 29(2): 217-228.

[11] Ibrahim IA, Hassn M, Darwesh RSS. Pre-acclimatization stage of date palm (*Phoenix dactylifera*) plantlets cv. Barhee as affected by potassium nitrate and sucrose. Journal of Biological Chemistry & Environmental Sciences 2012; 7(3): 1-20.

[12] Ibrahim IA, Hemeida AA, Abdelkader S, Girguis AA, Abou-El-Enin H. Genetic variance between some Egyptian date palm cultivars using PCR – based markers with emphasize on the prevalence of Al wijam disease. Archieve of Phytopathology and Plant Protection 2011; 44: 732-742. https://doi.org/10.1080/03235400802246929

[13] Murashige T, Skoog FA. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 1962; 15: 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

[14] Hassen MM, Ibrahim IA, Ebrahim MK, Komor E. Improvement of somatic embryogenesis and plant regeneration of seven date palm (*Phoenix dactilifera* L.) cultivars: Effect of cytokinins and activated charcoal. J Applied Horticulture 2013; 15(1): 26-31.

[15] Bottino BG. Methods in Plant Tissue Culture. Dept. of Hort., Agric., Maryland Univ., College Park, Maryland, USA, 1981; pp. 8-29.

[16] Snedecor: Statistical methods 6th ed. The Iowa state Univ. Press, Ames. Iowa, USA 1980.

[17] El-Dawayati MMA. Physiological studies on date palm micropropagation M.Sc. Thesis, Faculty of Agriculture, Cairo University. Egypt 2003.

[18] Mater A. *In vitro* propagation of date palm *(Phoenix dactylifera* L.) Date Palm J 1986; 4(2): 138-152.

[19] Zaid ZE, Gomaa AH, Ibrahim IA. Somatic embryogenesis production of palm *(phoenix dactylijera L.)* by application of abscisic acid (ABA). Proceed. International Conference. Genet. Eng. & Appl., Sharm EL-Sheikh, South Sinai, Egypt (April, 8-11, 2004); 467-481.

[20] Hegazy AE, Nesiem MRA, Ibrahim IA, EL-Ghamrawy NK. Direct Somatic Embryos of Date Palm I- Induction and Development The Third International Date Palm, Abu Dhabi, United Arab Emirates 19-21 February 2006; p. 38.

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