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EFFICIENT MICROPROPAGATION VIA SOMATIC EMBRYOGENESIS OF POTENTIAL CULTIVAR SAGAI OF *PHOENIX DACTYLIFERA* L.

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Abstract

The plantlets of date palm were regenerated using somatic embryogenesis. The combination of phytohormones, 5 mg BA/L and 80 mg 2,4-D/L gave the highest percentage of callus induction (88%) as compared to the other combinations. Moreover, significant differences were found between the explants (shoot tip and leaf primordia) in the induction of callus. The first initial callus emergence was observed in a shortest period (25.5 days) with above combination. The highest mean values index for the formation of embryogenic callus was 4.75 on MS medium containing 5 mg NAA/L and 2.5 mg BA/L. The best result on somatic embryogenesis was observed on 1/3 of MS medium, and 13 weeks of culture duration. The highest percent germination of somatic embryos was found 100% on MS media with full salt strength supplemented with 0.7 mg IBA/L or MS media having half salt strength supplemented with 0.5 mg IBA/L. The highest number of roots with length were observed on MS medium supplemented with 60 g/L of sucrose or ¼ strength of MS medium**.** At the acclimatization stage, the combination of peat moss and perlite (2:1 ratio) gave the maximum percentage of survival of plants (86.66%) with significant difference.

Key words: MS medium, Tissue culture, Phytohormones, Plant regeneration.

Introduction

Date palm (*Phoenix dactylifera* L.) (2n=2; X =36) is one of the most ancient fruit trees in the world. It is known as the "tree of life" because of its long-term productivity, resilience, low water requirements, and various uses of its products. Date palm is the most important species in the palm family (Arecaceae), which consists of about 200 genera with more than 2,500 species (Jain *et al.*, 2011). Date palm is considered the main source of food and income for the native population in the North Africa and Middle East. It has played important roles in the economy, society, and environment. It is used in the treatment of several diseases and palm parts are also used for different purposes (Chao & Krueger, 2007).

Date palm cultivation facing numerous constraints mainly growth in the harsh desert conditions viz., the lack of water, high temperature, and pathogenic diseases such as Bayoud disease caused by the fungus, *Fusarium oxysporum* f. sp. *Albedinis* (El-Juhany, 2010). Furthermore, this disease is the most destructive for the date palm where it destroyed more than 12 million of the date palm trees in Algeria, Morocco and North Africa (Jain, 2012). Moreover, red palm weevil (*Rhynchophorus ferrugineus*) is becoming the most serious pest on the date palm (Gomez & Ferry, 1999). It is difficult to detect the early infection with red weevil, which grows within the tissues of palm trees, and does not appear on the surface, thus causing tissue rot, and wilting then yellowing of leaves (Ferry & Gomez, 2002). One of abiotic factor i.e. desertification is the most hazardous factor which serves to limit the spread of palm groves. So, there is a need to renew the palm farms to replace the deteriorating orchards, and therefore use every means available to date palm propagation to meet the growing demand for palm (Abahmane, 2011).

Propagation of date palm by seeds gives heterogeneous progeny i.e., half of them become male and the other half female with reduced field performance and lower fruit quality (Naik & Al-Khayri, 2016). Genetic integrity of cultivars is maintained by offshoots propagation which is safe method for the conservation of genetic stock. However, limited number of offshoots are produced (20-30 per plant in whole life) by date palm plant in early life (10 to 15 years from the date of its planting) and it also depends on cultivar type (Naik & Al-Khayri, 2016). In addition, some cultivars do not produce offshoots (Heselmans, 1997). Moreover, rooting problem was observed in offshoot planting (Asemota *et al*., 2007; Eke *et al*., 2005). The success of offshoots planting was found up to 60% (Saaidi *et al*., 1979). However, there might be more chances of diseases transmission (Bayoud disease, or red palm weevil) for such type of propagation (Abahmane, 2011).

Several researchers have used this technique using different explant sources (El Modafar, 2010; Fki *et al*., 2011; Shareef *et al*., 2016). The plantlets of four cultivars viz., Ajwa, Khodary, Ruthana and Sukary were regenerated from apical meristematic tissues taken from shoot (Al-Qurainy *et al.*, 2017). Somatic embryogenesis is an important technique for plant regeneration. This technique has been used for date palm plant regeneration from embryogenic culture (Abohatem *et al.*, 2017; Alwael *et al.*, 2017). In the light of above information, the use of biotechnology is necessary for the date palm propagation, breeding, and its germplasm conservation. Despite being many works performed on date palm micropropagation, research is still required to optimize culture conditions for the recalcitrant cultivars and newly selected genotypes. In this study, we aim to standardize the different stages of *In vitro* propagation of date palm (*Phoenix dactylifera* L.) cultivar Sagai by tissue culture technique that could be used for production of date palms. However, to our knowledge, this is the first study about Sagai cultivar.

Materials and Methods

This study was carried out at Tissue Culture and Molecular Biology Laboratories, Department of Botany and Microbiology, Faculty of Science, King Saud University, Riyadh, Saudi Arabia.

Plant material: Offshoots were collected from Al-Rajhi farm (Al-Qassim) and Agricultural Research and experimental station- Dirab (Riyadh) in Saudi Arabia. Young offshoots (2–3 years old) of cultivar Sagai were chosen and separated from healthy mother plant after making sure that offshoot connected with mother plant not produced from seed.

The outer leaves and fibrous tissues surrounding the base of offshoot were removed very carefully until to get shoot tip. The shoot tip was cut into small sizes having height 10 cm, 4 cm diameter, and in a cylindrical shape. The shoot tips were washed with tap water for 10 min to remove any debris. To reduce browning, the shoot tips were put into a chilled antioxidant solution (100 mg/L of citric acid and 150 mg/L of ascorbic acid) for 24 hours at 4°C in a refrigerator (Fig. 1A). After taking out from the antioxidant solution, the shoot tips were washed with distilled water then placed in a plastic magenta box containing 70% ethanol for 1 min. The shoot tips were surface sterilized by sodium hypochlorite solution at concentration 1.6% (30% v/v Clorox, commercial bleach), supplemented with two drops of Tween 20 per 100 ml of sterilization solution for 20 minutes shaken time to time. Then shoot tips were washed with sterile distilled water three times for 15 minutes with shaking to remove any trace of sterilization solution. The leaf primordial was cut into two pieces and the apical meristem into 4-8 pieces used as explants (Fig. 1B).

MS medium (Murashige & Skoog, 1962) was used with sucrose 30 g/L, 120 mg/L myo-inositol, 2 mg/L glycine, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine HCI, 0.1 mg/L thiamine HCL, sodium dihydrogen phosphate (NaH_2PO_4.H2O) 170 mg/L, adenine sulfate 40 mg/L, glutamine 200 mg/L and plant growth regulators according to the propagation stage. The pH was adjusted to 5.6 with 0.1 N of NaOH or 0.1N of HCl. The optimum concentration of Agar (6-7 g/L) was added to MS medium and autoclaved at 121°C for 30 min and 1x105 Pa (1.1 kg cm-2). This medium was used in all stages of the propagation with few modification, especially through the manipulation of the hormones concentrations and MS salts according to the need of micro-propagation stages.

Callus induction using shoot tip and leaf primordial: The modified MS media was used with different combination of phytohormones viz. 3 mg/L of 2iP and 30 mg/L of NAA, 3 mg/L of 2iP and 10 mg/L of 2,4 D, 3 mg/L of BA and 30 mg/L of NAA, 5 mg/L of BA and 80 mg/L of 2,4 D and MS free of plant growth regulators (PGRs). The shoot tip and leaf primordial were used for callus induction. The cultures were incubated at 27±2°C, in the dark for nine weeks with sub-culturing every three weeks interval on the same media. The response of explants was assessed by daily monitoring of cultures and number of days required to form a callus was calculated as well as the percentage of the explants forming callus. Thus, the callus was subcultured on callus proliferation medium.

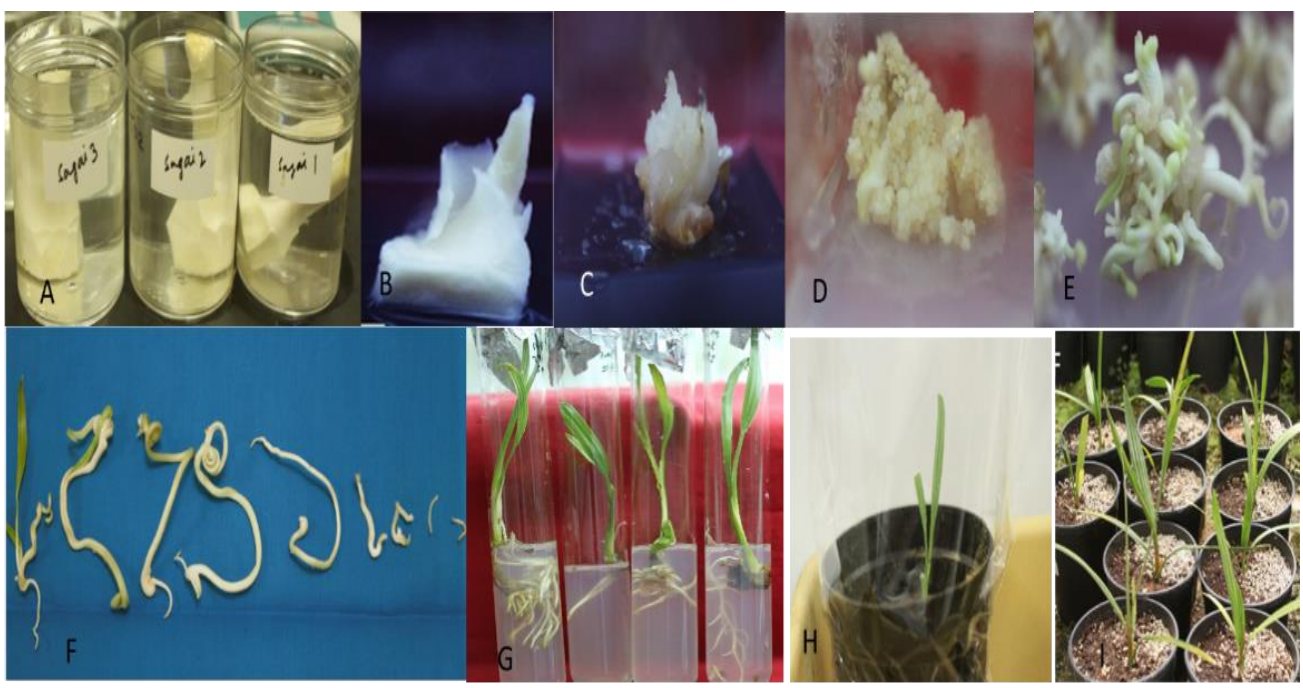

Fig. 1. Regeneration stages for cultivar Sagai; (A) Shoot tip in antioxidant solution, (B) Cultured explant on MS basal medium, (C) Induction of callus from shoot tip explant on modified MS basal medium supplemented with 3 mg 2iP/L + 30 mg NAA/L (D) Formation of embryogenic callus on modified MS basal medium supplemented with 5 mg NAA/L + 2.5 mg BA/L, (E) Mature embryos observed after 9 weeks on full and 1/3 of MS medium, (F) Different stages of embryogenesis and germination, (G) Rooted plantlets obtained from somatic embryos, (H) Acclimatization stage as plantlets covered with transparent plastic bag to keep the humidity, (I) Acclimatized plants in greenhouse (8 months old).

Embryogenic callus production: The modified MS media used in this study was supplemented with different concentrations of auxin NAA (0, 1.5, 5, 7.5 mg/L) and cytokinin BA (0, 1, 2.5, 5 mg/L), individually or combination. 100 mg of initial callus was cultured on the modified MS medium (eight replicates per treatment) for six weeks with subculture every three weeks in the same media. The degree of embryonic callus formation was estimated visually as scores, using the method described by Pottino (1981).

Somatic embryogenesis: The modified MS media used in this study were supplemented with different MS salt strengths (full MS, ½ MS, 1/3 MS and ¼ MS). The optimal MS salt strengths and duration were determined to stimulate somatic embryogenesis of embryogenic callus. 100 mg of initial embryogenic callus was cultured on the modified MS media in magenta boxes (8 replicates per treatment) for 6, 9 and 13 weeks with sub-culturing every after three weeks in the same media. The cultures were monitored and evaluated daily to see the response of somatic embryogenesis, and number of somatic embryos formed were calculated.

Somatic embryos germination: In this study we used full and half salt strengths of MS media with different concentrations of IBA (0, 0.1, 0.3 0.5 0.7 mg/L). The optimal concentration was determined for the germination of embryogenic calli. Mature somatic embryos were cultured in culture tubes (one embryo in each tube). Somatic embryos were selected according to size, shape, and age as much as possible. After 6 weeks of culture, the type and the percentage of germination were recorded.

The modified MS media with different salt strengths (full MS, ½ MS and ¼ MS) or supplemented with different sucrose concentrations including 30, 40, 60 and 90 g/L to determine the optimal concentration of sucrose and/or MS salt strengths to stimulate rooting in plantlets. The shoots which were derived from somatic embryos were selected based on size, shape, and age as much as possible, with the length of 2 cm, and containing two leaves. Shoots were cultured in magenta vessels in replicates. After three weeks of culture, individual shoots were cultured in 150 x 25 mm culture tubes contain 15 ml per tube of the same medium (the second subculture). After six weeks of culture, the number of roots and root length were recorded.

The plantlets were selected for acclimatization according to different hight, leaves and root. The sterilized mixtures of soil were used as a media as mentioned earlier. The produced plantlets were rinsed with sterile distilled water to remove adherent medium under aseptic conditions and put them in antifungal solution (0.25% Tebuconazole) for 10 min. Plantlets were cultured in pots containing soil as peat moss alone and Perlite (2:1) in twenty replicates. The cultures were incubated at 27±2°C, and a photoperiod of 8h dark/ 16 h light under a high light intensity (9,000 lm $m^{-}2$). Irrigation of plantlets with distilled water was conducted every after 3-4 days as needed and depending on the level of soil moisture; plantlets were sprinkled with pesticide fungal once every week. After three weeks, the covers of plastic transparent were removed and the plantlets cultured in pots shifted to the nursery. After two months, the percentage of plants that have been adapted were recorded.

Statistical analysis: Statistical analysis was carried out for data using SPSS software and the significant differences among the treatment means were calculated using the Duncan's Multiple Range Test (Crawley, 2005).

Results and Discussion

Induction of callus *In vitro* depends on concentration and type of PGRs added to the basal medium (Ikeuchi *et al*., 2013). It also depends on type of cultivars or explant and PGRs interaction. Our study showed that the combination of 5 mg BA/L and 80 mg 2,4-D/L gave the highest percentage of callus induction (88%) with a significant difference for the rest of the combinations that were used in this study (Regardless of the effect of explants) (Table 1, Fig. 1C). This result is contrast with Jasim *et al*., (2009) who found that the medium supplemented with 50 mg/L of NAA and 3 mg/L of 2iP gave the highest percentage of primary callus induced in all cultivars (cvs. Khsab, Um Al-Dihin, Sheraify, and Auwaidy).

The explant cultured on media without PGRs did not give any response. However, it has been reported that PGRs plays a key role in the induction of callus. Barbier-Brygoo *et al*., (1989) reported the role of auxin in the induction of callus. The low percent of callus induction (48.5%) was observed under the combination of 3 mg 2iP/L and 10 mg 2,4-D/L), whereas intermediate values (70% and 53%) were obtained on application of PGRs (3 mg BA/L + 30 mg NAA/L and 3 mg 2iP/L + 30 mg NAA/L). Callus induction depends on explant genotypes and concentration of PGRs as reported by Eshraghi *et al*., (2005) that the callus induction in palm (cv. Khanizi) was initiated when they used MS medium with (100 mg/L of 2,4-D + 3 mg/L of 2ip + 2.5 mg/L of ABA). Moreover, shoot tip (cv. Zaghlool) was used to produce callus on MS medium supplemented with 10 mg/L of 2,4-D and 3 mg/L 2ip (Bekheet *et al*., (2001). Furthermore, Badawy *et at.*, (2005) stated that in date palm (cv. Sewei) the MS medium containing 100 mg 2, 4-D/L and 3 mg 2ip/L gave a significant effect on callus formation than that obtained on media supplemented with (100 mg 2,4-D/L + 5 mg BA/L) or (10 mg 2, 4-D/L + 2 mg kinetin/L).

The significant differences were obtained between shoot tip and leaf primordia regarding callus induction and higher percentage (70%) of callus induction was obtained in the shoot tip culture (Table 1). This might be due to the explant selection at the time of cutting or due to the difference of internal content of auxin in explants (Jasim, 1999). The concentration and type of auxin have a potential role in the induction of callus and increase levels of auxin lead to a formation of callus (Tisserat, 1979; Mohamed *et al*., 2001).

The callus initiation needs optimum phytohormones concentrations and incubation period as 5 mg BA/L and 80 mg 2,4-D/L induced the initial callus formation in a shortest period (25.5 days (Table 2) whereas, the combination of 2iP/L (3 mg) and 2,4-D/L (10 mg) induced the initial callus production in longest period (46 days). Emergence of initial callus also depends on type of explants as shortest period for the emergence of the initial callus was obtained in the shoot tips (31.75 days), whereas the longest period was taken with leaf primordia (37.5 days). It is evident from this study that the balance between auxin and cytokinin is very important in callus induction. However, the response of explants to induction of callus has been associated positively with the increase in the level of auxin.

Table 1. Effect of different treatments of plant growth regulators in the percentage of callus induction from different explants of cultivars Sagai of date palm.

MS Media (B)	Explants of cv. Sagai		Average (B)
	Leaf primordia (A)	Shoot tip (A)	
3 mg 2iP/L + 30 mg NAA/L	47 c	95 f	53 bc
3 mg 2iP/L + 10 mg 2,4-D/L	31 b	66 d	48.5 c
3 mg BA/L + 30 mg NAA/L	51 cd	89 e	70 c
5 mg BA/L + 80 mg 2,4-D/L	76 de	100 j	88 d
MS free of PGRs	0.0 a	0.0 a	0.0 a
Average (A)	41a	70 b	

*Data with the same letters are not significantly different according to Duncan's test at level (p<0.05)

Table 2. Effect of different treatments of plant growth regulators on time required for first appearance of initial callus of various explants of date palm (in days).

PGRs in MS Media (B)	Explants of cv. Sagai (C)		Average (B)
	Leaf primordia (A)	Shoot tips (A)	
3 mg 2iP/L + 30 mg NAA/L	34 c	28 b	31 a
3 mg 2iP/L + 10 mg 2,4-D/L	48 f	44 e	46 b
3 mg BA/L + 30 mg NAA/L	39 d	33 c	36 c
5 mg BA/L + 80 mg 2,4-D/L	29 b	22 a	25.5 a
Average (A)	37.5 b	31.75 a	

*Data with the same letters are not significantly different according to Duncan's test at level (p<0.05)

These results are in line with Jasim, (1999) and Yadav *et al*., (2001), who stated that the induction of callus from shoot tip was faster than other explants used in the study. However, Jasim *et al*., (2009) reported the first emergence of the initial callus was 36 days in shoot tip and leaf primordia of cv. Khsab, while in cv. Um Al-Dihin was 54 and 53 days in shoot tip and leaf primordia, respectively. The difference between our findings and previously reported might be due to the difference in cultivars types, as well as type and concentration of PGRs which were used in two studies. However, the current results are in contrast with the results reported by Eshraghi *et al*., (2005), who stated that callus induction from shoot tips was observed after five months of cultured on MS medium supplemented with (100 mg/L 2,4-D + 3mg/L 2ip + 2.5 mg/L AB) in cv. Khanizi and (150 mg/L 2,4-D + 3 mg/L 2ip) in cv. Mordarsing.

The embryogenic callus formation was observed visually and counted using the method described by Pottino, (1981). The embryogenic callus was formed with NAA (5 mg/L) and BA (2.5 mg/L) alone or in combination (Table 3, Fig. 1D**)**. The result was found significant with this concentration as compared to the others. The media strength also plays important role in embryogenic callus formation as high strength of the MS medium (full or 3/4 strength) gave a high score of embryogenic callus formation than the MS medium contained low strengths 1/2 or 1/4 strength (Badawy *et al*., 2005). Whereas, Al-Khayri, (2001) reported that the optimum growth of embryogenic callus was found in treatment consisted of 0.5 mg/L thiamine and 2 mg/L biotin compared to the other combinations of biotin at 0, 0.1, 1, or 2 mg/L with thiamine at 0.1, 0.5, 2, or 5 mg/L.

The media salt strength and culture duration are very important for somatic embryogenesis. The MS medium containing 1/3 MS salt strength gave the highest somatic embryos (23.41 embryos) (Fig. 1E). This might be attributed due to the presence of optimal nutrients in the media. Whereas, the lowest somatic embryos (6.79 embryos/culture) was recorded on medium containing 1/4 MS salt strength, however, the somatic embryos produced from this treatment were weak, and most of them were in the globular stage. This may be due to the insufficient nutrients concentration. These findings are in line with Al-Khayri, (2011), who reported that the number of somatic embryos production was influenced by medium formulation. The current study showed that different culture mineral formulation affects growth and morphogenesis as demonstrated in a number of plant species. However, Tripathy & Reddy, (2002) found that the MS medium was the most suitable for callus induction and plant regeneration in Indian cotton cultivars among the different basal media, including MS, B5, LS.

Table 3. Effect of different concentrations of BA and NAA on friable callus formation during *In vitro* propagation.

Conc. (BA)(B)	Embryogenic callus formation (mean ± SE)**				
	Conc. NAA (A)				
	0.0 mg/L	1.5 mg/L	5 mg/L	7.5 mg/L	Average B
0.0 mg/L	2.47 ± 0.35	2.75 ± 0.43	4.37 ± 0.23	2.00 ± 0.35	2.90 a
1 mg/L	2.25 ± 0.47	2.87 ± 0.42	3.75 ± 0.62	2.75 ± 0.25	2.90 a
2.5 mg/L	4.12 ± 0.42	3.62 ± 0.68	4.75 ± 0.25	3.00 ± 0.57	3.87 b
5 mg/L	2.75 ± 0.25	2.75 ± 0.25	2.37 ± 0.37	2.25 ± 0.25	2.53 a
Average A	2.90 a	3.00 a	3.81 b	2.50 a	

*Data with the same letters are not significantly different according to Duncan's test at level (p<0.05)

**Negative results = 1, Below average results = 2, Average results = 3, Good results = 4, excellent = 5

Table 4. Effect of MS salt strength and culture duration on somatic embryogenesis during *In vitro* propagation.

MS strength (A)	Number of embryos (Mean ± SE)			Mean (A)
	Culture duration (B)			
	6 Weeks	9 Weeks	13 Weeks	
Full MS	13.75 ± 0.161 b	16.75 ± 0.868 bcd	21.75 ± 0.564 ef	17.41 b
½ MS	14.62 ± 0.460 bc	18 ± 0.870 cde	21.00 ± 0.677 ef	17.87 b
1/3 MS	18.5 ± 0.560 de	22.75 ± 0.493 f	29.0 ± 0.779 g	23.41 c
¼ MS	1.375 ± 0.182 a	4.5 ± 0.645 a	14.50 ± 1.04 bc	6.79 a
Mean (B)	12.06 a*	15.50 b	21.56 c	

*Data with the same letters are not significantly different according to Duncan's test at level (p<0.05)

Table 5. Effect of different concentrations of IBA and MS salt strength on the percent germination of somatic embryos.

Type of germinations (A)	Germination (%) full MS medium (C)					Germination (%) half MS medium (C)					Average (A)
	Con. of IBA mg/L (B)					Con. of IBA mg/L (B)					
	0.0	0.1	0.3	0.5	0.7	0.0	0.1	0.3	0.5	0.7	
Shooting	40	50	40	30	40	30	40	30	20	20	34 b
Rooting	30	20	10	20	10	20	0	20	20	20	17 a
Plantlets	10	20	30	40	50	20	30	30	60	50	34 b
Total germination	80	90	80	90	100	70	70	80	100	90	85
Average (B)*	75 a**	80 a	80 a	95 b	95 b						
Average (C)*	**88 a**					**82 a**					

**Data with the same letters are not significantly different according to Duncan's test at level (p<0.05)
*Average for total germination

Culture duration also plays important role in somatic embryos formation. The prolong culture duration caused a steady increase in the number of somatic embryos, where incubation for the longest period (13 weeks) gave higher number of somatic embryos (21.56 embryos/culture) significantly than incubation for 6 or 9 weeks (Table 4). It may be the accumulation of carbohydrates, protein, lipids which have a positive effect on the maturation of somatic embryos. Our results are line with the work reported by Badawy *et al*., (2005) as culture duration caused a steady increase in the number of somatic embryos. However, the high number of embryos per explant was observed when MS medium devoid of growth regulators after three months from culture (Bekheet *et al*., 2001). Additionally, Vermendi & Navarro., (1996) reported that after three months of callus culture on MS medium without hormone, the somatic embryos were formed. While, Zaid & Tisserat., (1983) indicated that the somatic embryos were produced per culture after two months of culturing on solid MS-medium. This result is in line with Badawy *et al*., (2005) who reported that the highest number of somatic embryos was observed when culture duration was the longest (24 weeks) in *Phoenix dactylifera* L. cv. Sewei.

The effect of different concentrations of IBA and MS salt strength (full and a half) was investigated on germination of somatic embryos. The MS salts at full strength gave the highest percentage of germination of somatic embryos (88%) as compared to using the half strength of MS salts (82%), non-significantly (Table 5, Fig. 1F). Contrary to these findings, Jasim *et al.,* (2009) reported that half strength of MS medium enhanced the germination of most embryos in cv. khsab. Moreover, Al-Khayri, (2003) stated that the optimum treatment for somatic embryos germination and complete plant formation (86%) was on half-strength MS medium containing 0.2 to 0.4 mg/L of IBA.

The different concentrations of IBA (0.0, 0.1, 0.3, 0.5 and 0.7 mg/L) enhanced germination of somatic embryos significantly (Table 5). The highest percent germination of somatic embryos was produced at concentrations 0.5 and 0.7 mg/L of IBA (95%). Whereas the lowest percent germination of somatic embryos was produced at concentrations 0.0 mg/L of IBA (75%). The results of this study are in contrast as reported by Zouine & El Hadrami, (2007) when somatic embryos were transferred to solid MS medium containing 0.05 mg/L BAP, 0.1 mg/L IBA and 0.1 mg/L NAA, where about 14.2% of them were germinated. However, embryos cultured on MS medium free of PGRs often produce shoots only and require another step for root and shoot formation and addition of IBA to the culture medium induced complete plantlets formation (Al-Khayri, 2003). The highest percent germination of somatic embryos was 34% in shooting and plantlets formation (Table 5). On the other side, the lowest percent germination of somatic embryos was 17% as for the root formation. The somatic embryos germinated in shoot only were rooted in rooting medium. Whereas the somatic embryos germinated roots only, they considered unviable. The findings of this study are in contrast with Othmani *et al*., (2009), who replaced ABA in MS medium with 1 mg/L of NAA which gave 81% germination of the somatic embryos with normal roots and shoots. However, in the present study, the complete plant was formed in one step from somatic embryo using the IBA at concentration 0.7 mg/L, and thus reducing the culture duration (Table 5).

Rooting is an important stage in date palm micropropagation (Shaheen, 1990). The root initiation and plant growth need high energy and it can be obtained using metabolic substrates as carbohydrates (Thorpe, 1982). The sucrose acts as a source of energy and plays an important role in maintaining osmoticum (Cuenca &

Vieitez, 2000). Increasing the concentration of sucrose from 30-60 g/L led to a gradual increase in the number of roots and roots length. The highest number of roots (4 roots) at 30-40 g/L and the longest root length (4.37 cm) was observed at 60 g/L of sucrose (Table 6, Fig. 1G). However, the concentration of sucrose 90 g/L gave a significant reduction in both number of roots and roots length. Our results are agreement with Al-Khalifa *et al*., (2009) who stated that at application of sucrose (60 g/L) increased root elongation (6.8 cm) and led to higher number of roots 3.6 roots/plantlet.

However, sucrose at concentration 45 g/L and 30-40 g/L led to significant increase in rooting percentage and root number (AL-Meer *et al*., 2008; Al Khateeb & Alturki, 2014; Al-Dawayti, 2000). Our study showed that as decreasing salt strength of MS media led to gradual increase in the number of roots (0.75, 2.25 and 4 roots) and root length (1.65, 4.37 and 5.50 cm) significantly. Perhaps this is due to the low concentration of media components which enhance the growth and elongation of roots in order to provide nutrients for the plantlets. The concentration of inorganic salts at 2/3 MS medium strength helped in root induction in date palm (Ibrahim, 1999). The shootlets were rooted on half-strength of MS salts supplemented with 5.0 µM NAA and (45 g/L) sucrose in cultivars Barhi and Maktoom (Bader *et al*., 2007). AlKhateeb and Alturki (2014) stated that the using ½ MS medium salt supplemented with 0.25 mg/L of ABA and 45 g/L of sucrose increased significantly of the root length, while 3/4 MS medium salt strength gave increased number of roots significantly in their study on date palm cv. Bartamuda. On the other hand, the ½ or ¼ strength of MS medium inhibited root elongation of *Incarvillea sinensis* and shoot turned brown (Hu *et al*., 2005). This differences in root elongation and root number might be due to different incubation period, plant species or cultivars that have been investigated. Different plant species require various concentration of sucrose for their growth development. The highest rate of root growth estimated as root length and root number was found in *Ficus carica* at 5 % sucrose concentration (Elazab & Shaaban, 2015). The highest bud formation rate and root number was found in *Cypripedium macranthos* at 10 g/L sucrose concentration (Huh *et al*., 2016).

Table 6. Effect of different sucrose concentrations and MS salt strength on the number and length of roots formed during *In vitro* propagation.

Sucrose concentration (A)	Number of roots/ plantlet cv. Sagai	Root length (cm) cv. Sagai	Strength MS (A)	Number of roots/ plantlet cv. Sagai	Root length (cm) cv. Sagai
MS 30 g/L	0.75 ±.250 a*	1.65 ± .232	MS full 30 g/L	0.75 ±.250 a*	1.65 ± .232 a
MS 40 g/L	2.25 ±.478 abc	2.95 ± .932	0.5 MS 30 g/L	2.25 ±.853 b	4.37 ± .943 b
MS 60 g/L	4 ±.818 cd	4.37 ± .746	0.25 MS 30 g/L	4 ±1.436 c	5.50 ± .645 bc
MS 90 g/L	2.25 ±.478 abc	4.20 ± .606	Average (B)	2.33	3.83
Average (B)	2.31	3.29			

*Data with the same letters are not significantly different according to Duncan's test at level (p<0.05)

Table 7. Effect of agricultural soil on percent acclimatization of date palm plantlets.

Treatment (A)	Acclimatized % (B)
Peat moss	66.66 a
Peat moss and Perlite (2:1)	86.66 b
Average (B)	76.66 a

*Data with the same letters are not significantly different according to Duncan's test at level (p<0.05)

The higher percentage of acclimatized plantlets was 86.66% in the medium contained peat moss and Perlite (2:1 ratio) (Table 7, Fig. 1H). It might be due to the perlite soil which has good porocity for growth and spreading roots as well as being an excellent medium to drain the spare of the water and prevents suffocation of plantlets. Also, the presence of peat moss in the medium has good ventilation as perlite has good and suitable for the growth of plantlets (Sharma *et al*., 1991). The low rate of acclimation (66.66%) was in the medium which contained only peat moss and it may be due to lack of sufficient ventilation (Table 7). Further, plantlets were transferred in the field for proper growth and development. The all plants were survived well and no phenotypic characteristics were observed among *In vitro* propagated individuals and the donor plants (Fig. 1I).

The results of the current study are in line with Khierallah & Bader, (2006) who reported that the survival percentage of plantlets was 85% after 3 months when the plants were transferred to pots containing a mixture of peat moss and perlite (2:1). Moreover, Al-Taha *et al*., (2011) found that the sand and peat moss medium (2:1) gave high survival percentage of acclimatized plantlets (*Phoenix dactylifera* L.) which reached to 85%. However, Al-meer *et al*., (2008) stated that the sand and peat moss medium (3:1) showed a high efficiency on the acclimatized plantlets percentage (*Phoenix dactylifera* L.cv. Barhee) which reached 80%. The modification of components of MS medium and PGRs combination improved the plantlet regeneration. Thus, micropropagation of date palm cultivar "Sagai" was successful *In vitro* and could be used for raising more plants for plantation in the date palm garden as the cultivar is more demanding due to its good quality of fruit.

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