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In Vitro Propagation of Date Palm Seedling Strain (Shaba) Grown Under Aswan Conditions

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Abstract: *The presentinvestigation is one of the first works on micropropagation of the seedling strain (Shabah) dry date palm (Phoenix dactylifera L.) grown at Aswan Governorate. Explants (shoot tips and leaf primordia) were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of casein hydrolysate (CH). The highest swelling and callus initiation percentage were achieved at 2 g/L of CH. Results indicated that the shoot tips gave the highest swelling and callus initiation percentage. The percentage of embryogenic callus formation obtained from shoot tip and leaf primordia explants reached the highest value, when cultured on MS medium with 3 mg/L 6-benzyl adenine (BA) + 10 mg/Lnaphthalene acetic acid (NAA) + 10 mg/L 2,4-dichlorphenoxy acetic acid (2,4-D), compared with other tested treatments. In addition, the same medium recorded the highest fresh weight of embryogenic callus. Addition of salicylic acid (SA) as a supplement to the MS medium led to a significant increase in number and percentage of germinated somatic embryos. The culture medium containing 50 mg/L SA gave the highest number and germination percentage of embryos for somatic embryogenesis derived from the shoot tip and leaf primordia explants. Polyethylene glycol (PEG) treatment at different concentrations significantly increased root number and reduced average root length by increasing PEG concentration as compared with plantlet produced in culture medium without PEG. MS medium supplemented with 4 g/L PEG resulted in the highest significant number of adventitious roots.Percentage of survival plantlets, which treatedwith PEG during rooting stage reached the highest value of survival percentage in acclimatization (80%) on MS medium supplemented with 10 g/L PEG.*

Keywords*: In vitro,embryo formation, soot tip, leaf primordial, casein hydrolysate, salicylic acid, polyethylene glycol*

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I. Introduction

Date palm, *Phoenix dactylfera* L. (Family: Palmaceae) is a monocotyledonous and dioecious plant. It is considered the most important fruit tree in many Arab countries, such as Saudi Arabia and Iraq (Badawy *et al*., 2005). Date palm is a fruit tree resilient to a diverse climatic conditions predominating in hot arid regions of the Middle East and North Africa. The date fruit contains numerous chemical components that possess high nutritional and medicinal values (Al-Khayri and Naik, 2017). Phenolic compounds present in dates, have beneficial effects on human health and act against cancer and cardiovascular diseases (Vayalil, 2012). The annual international market value (including import andexport) of the date crops reached nearly 1.9 billion USD (FAOSTAT, 2013), from which a country can build a strong economic platform.

Expansion of date palm plantations in different areas of Egypt and replanting trees to compensate loss due to diseases or human factors are restricted due to the lack of adequate planting materials. Furthermore, improvement of Egyptian date palm cultivars using traditional breeding programmers has been faced with certain limitations due to the long generation period and heterozygosis of date palm cultivars. In recent years, the conventional methods used for propagation, conservation, and genetic improvement of date palm have been supplemented by rapid developments in plant biotechnology (Bekheet, 2013). The date palm could play important role in the ecology of various desert and semi–desert environments. Date palm, which is an irreplaceable tree protection to under–crops from the harshness of the climate (heat, wind and even cold weather), reduces damage caused by sand storms and wind erosion(Badawy *et al*., 2005).More than 2000 date varieties with differences in color, valve, shape, size and ripening timeare present (Al-Farsi and Lee, 2008).Date palm cultivars are grouped into three classes; soft, semidry or dry, based on upon the texture of the fruit under normal ripening conditions. Top 10 producing countries of dates are Egypt, Saudi Arabia, Iran, United Arab Emirates, Pakistan, Algeria, Sudan, Oman, Libya, and Tunisia (Kader and Hussein, 2009).

Tissue culture propagation method is the most promising technique for production of efficient plant materials with high quality (Sane *et al*., 2006). Because of inherent limitations associated with conventional date

palm propagation using offshoots (Al-Khayri, 2011). Somatic embryogenesis is considered 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 breeding programs. Embryogenic callus induction in date palm is influenced by different parameters such as genotype, explant type, induction period and plant growth regulators (PGRs). In the case of either shoot tips or leaf primordia, high levels of auxins have been used to induce embryogenic callus. The most effective auxin is2,4-dichlorphenoxy acetic acid (2,4-D) thataimed atembryonic callus induction in date palm, and it has been used mainly at the concentration of 100 mg/L (Behnas*et al*., 2014). Embryogenic callus induction using 100 mg/L2,4-D was reported in many date palm cultivars such as Khanizi and Mordarizing (Eshraghi *et al*.,2005a), Khasab and Nabout (Al-Khayri, 2005) and Nabout Saif (Al-Khayri,2011). However, Fki *et al*. (2011) mentioned that high doses of 2,4-D may induce somaclonal variation. Therefore, other researchers used lower 2,4-D concentrations or other auxins in order to induce somatic embryogenesis. *In vitro* production of date palm via indirect organogenesis or somatic embryogenesis requires the application of relatively high concentrations of 2,4-D or naphthalene acetic acid (NAA) for the initiation process. However, these auxins are known to be associated with genetic instability in plants (Behnas *et al*., 2014). The use of natural compounds instead of plant growth regulators in culture medium may reduce or omit the possibility of genetic instablility in plants.

Organic additives, such as casein hydrolysate and coconut water have been used to increase embryogenic callus growth and somatic embrogenesis in several plant species and date palm as well. Salicylic acid (SA)is an onohydroxybenzoic acid, a type of phenolic acid and a beta hydroxyl acid. It is widely used in organic synthesis and its function is as a plant hormone. It appears to have a role in systemic acquired resistance to pathogens and is able to induce various pathogen resistance proteins (Goerge *et al*., 2008a). SA act as a potential non-enzymative antioxidant as plant growth regulator, playing an important role in regulating a number of plant physiological processes (Fariduddin *et al*., 2003). Exogenously supplied SA was shown to affect a large variety of processes in plants, including stomatal closure, seed germination, fruit yield and glycolysis (Cutt *et al*., 1992). Polyethylene glycol (PEG) molecules are inert, non-ionic, virtually impermeable to cell membranes and can induce uniform water stress without causing direct physiological damage (Kulkarni and Deshpande, 2007).Al-Khateeb and Ghazzawy (2015) reported that addition of PEG to the culture media is able to promote the embryo formation and mutation in different plants. Increased medium osmolality and hence the frequency of somatic embryogenesis was also obtained by the addition of PEG (Brown *et al*., 1989). In panax ginseng, Langhansova *et al*. (2004) stated that shoot regeneration of non-treated somatic embryos was 31%, while that for somatic embryos treated with PEG 4000 and ABA was 70%.

Some of the elite palms could be promoted asnew cultivars in the future, but others could serve as avaluable genetic reserve for future crop improvement.Seedlings of date palms are reported to be cultivated for two major reasons: (1) breeding and germplasm conservation and (2) conserve seedling dates with desirable traits for farming (Johnson, 2010 and Johnson*et al*., 2013)

Plant tissue culture techniques offer several tools to plant breeders based on anunderstanding of genetic principles. For instants, embryo rescue, *in vitro*fertilization, *in vitro* flowering, somatic hybridization. The objective of this study was selecting the elite seedling strain (Shabah) of dry date palm grown at Aswan Governorate, andestablishing a micropropagation protocol for large scale production of true to type elite seedling of this dry date palm strain. This strain could be promoted as a new cultivar in Egypt.

II. Materials and Methods

1. Plant material

Plant materials of the elite seedlings strain (Shabah) date palm (*Phoenix dactylifera*) were collected from Aswan, where they grown at Al-Aakab Bahri region, 20 km from Aswan Governorate (Fig. 1a). The off-shoots (3-5 years old) were about 15 kg.They were prepared *in vivo* by removing the older leaves and root system, until the off-shoots were about 20-30 cm long and 15-20 cm in diameter. Then were wiped with wet paper and transferred in ice box to the lab of the Desert Research Center in Cairo, where they were prepared for culture. Shoot tips and leaf primordia of offshoots were used as source of explants. The shoot tip explants were prepared by removing the undesirable outgrowth and leaves were stripped away, except for the apical dome surrounded by 10-15 of the youngest leaf primordia. To obtain a segment of 5-7 cm long and 4-6 cm in diameter (Fig. 1b). Explants were washed several times with sterile distilled water, following by soaking in sterile antioxidant solution of citric acid (150mg/L) and ascorbic acid (100mg/L), then placed in the refrigerator for 24 hours. The isolation of explants were done under complete aseptic conditions. The leaf primordia were again stripped away to become 5-8 leaf primordia surrounding the apical dome. The explants were surface disinfected in 70% ethanol for 3 minutes, then rinsed once with sterile distilled water and followed by immersion in 3% sodium hypochlorite containing afew drops of Tween-20 with continuous stirring for 20 minutes. The explants were rinsed six times with sterile distilled water to remove residual disinfection before planting.

2. The basic nutrient media

The medium used in the initiation stage was Murashige and Skoog (MS) (1962) salts in addition to1.0 mg/Lthiamin-HCl, 1.0 mg/Lpyridoxine-HCl, 80 mg/Ladenine sulfate, 100 mg/Lmyo-inositol, 200 mg/Lglutamine, 30 g/Lsucrose, 3 g/Lactivated charcoal,1.0 mg/Lnicotinic acid, and 7 g/L agar agar. Before adding agar to the media, the pH was adjusted to 5.7-5.8 with adding few drops of either 0.1 NaOH or 0.1 HCl. The media were dispensed into jars, where each jar contained 50 ml media. Sterilization of the medium was achieved by autoclaving at 1.1 kg/cm^2 at 121˚C for 20 minutes.

3. Callus induction

All surface sterilized explants (shoot tips and leaf primordia) were cultured in full-strength basal MS medium supplemented with 3 mg/L BA + 100mg/L 2,4-D different concentrations of casein hydrolysate (CH);0.0,1.0, 2.0, 3.0 and 4 g/L. All cultures were incubated in a culture room at 27$\pm$2^{o}C under complete darkness with regular transfer to fresh medium of the same composition every four weeks. Swelling% and callus initiation% were recorded after 4 months.

4. Embryognic callus induction

For embryogenic callus induction, callus initiated from explants were subcultured on MS medium supplemented with 3mg/L BA and different concentrations of NAA and 2,4-D (5mg/L NAA $_+$5mg/L 2,4-D, 10mg/L NAA, 10 mg/L2,4-D and 10mg/L NAA + 10 mg/L 2,4-D). Cultures were insulated in darkness and recaptured onto the same fresh medium every four weeks, thereafter; the percentage of embryogenic callus formation and fresh weight of embryogenic callus were recorded after four months.

5. Somatic embryos formation and germination

Embryogenic callus obtained from the previous stage was segmented (0.5g) and cultured on MS medium supplemented with 0.1mg/L NAA and different concentrations of salicylic acid (SA) (0.0, 25, 50, 75 and 100 mg/L). The cultures were incubated at 27+^{o}C with 16-hours photoperiod under cool white florescent tubes. After eight weeks, number of somatic embryos and percentage of germination of somatic embryo were recorded.

6. Rooting and acclimation of plantlets

For rooting, individual shoots (5-7 cm) were cultured on MS medium supplemented with 1mg/Lindolebutyric acid (IBA) with different concentrations of PEG (0.0, 2.0, 4.0, 6.0, 8.0 and 10g/L)for eight weeks.Thereafter, percentage of rooting, average root number formed per shoots and average root length (cm) were recorded. Plantlets were transplanted intoplastic pots containing autoclaved mixture of peat moss and sand (1:1v/v), thencovered with polyethylene bag, which were tightly closed to maintain high humidity. The plantletswere watered with 0.25 strengthHoaglandsolution once a week for eight weeks.Thereafter, the polyethylene bags were removed and plantlets were maintained under nursery conditions. Percentage of plantlets survival were determined after eight weeks. The plants produced fromtissue culture were cultivated under Siwa Oasis conditions in the Experimental Research Station of Desert Research Center, Khamisa Farm Siwa Oasis.

7. Experimental Design and Statistical Analysis of Data

The experiments were subjected to 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 averages of the recorded parameters for all treatments were tested for significance at 5% level. Averages followed by the same letter are not significantly different at $p\leq$ 0.05.

III. Results and Discussion

1. Callus induction

Addition of CH to the culture medium affected the swelling and callus initiation percentage of date palm Shabah strain. Results shown in Table (1)indicate that the addition of CH increased both swelling and callus initiation for the shoot tip and leaf primordia explants. Swelling and callus initiation percentages were affected differently to the used CH treatments according to explants type response. The highest swelling and callus initiation percentage were achieved at 2 g/L of CH for both explants (Fig. 1c and d). Results indicated that shoot tips achieved the highest swelling and callus initiation (91.66 and 96.97%, respectively), while leaf primordia recorded swelling and callus initiation percentagesof 63.33 and 74.03%, respectively, while the lowest value of swelling and callus initiation percentage was noticed on medium free from CH (control). CH

can be a source of calcium, micronutrients, vitamins and most importantly a mixture of up to 18 amino acids. Several investigators have concluded that CH itself is more effective for land cultures than the addition of the major amino acids, which its provide. This has led to the speculation that CH might contain some unknown growth promoting factor, which promoted callus growth (Khaleda and Al-Forkan, 2006).

Table (1). Effect of different concentrations of casein hydrolysate (CH) on swelling and callus initiation % of Shabah strain dry date palm (*Phoenixdactyllfera*).

CH conc. (g/L)	Swelling (%)		Callus initiation(%)	
	Explant type		Explants type	
	Shoot tip	Leaf primordia	Shoot tip	Leaf primordia
0.0	66.66 b	41.66 b	44.44 c	33.33 a
1.0	75.00 ab	50.00 ab	73.89 b	60.00 a
2.0	91.66 a	63.33 a	96.97 a	74.03 a
3.0	83.33 ab	61.10 a	89.47 a	72.33 a
4.0	75.00 ab	49.99 ab	74.26 b	61.27 a

Hussam *et al*. (2013) reported that supplementation of organic additives such as CH improved callus growth and somatic embryogenesis in date palm tissue culture. Also, significant differences betweenpercentages of callus initiation were recorded in *Oryza sativa*among some tested media, CH tremendously increased callus production. In CH free MS medium, the frequency of callus formation wasabout 35%, which were comparatively lower than on MS supplemented with CH, where the percentage of callus producton was about 83% (Khaleda and Al-Forkan, 2006). The addition of natural products such as CH to the *in vitro* medium could meet the needs of growth and development of excised tissues and promote cell growth (Zhao and Li, 2000). CH contains a mixture of various organic compounds and amino acid. It increased cell growth of *Panax quinquefolium* (Tang and Wu, 1994). They provide an inexpensive natural source nutrients and growth enhancement. Commonly used complex organic additive include CH, and is added to supplement the culture medium with mainly sugar, amino acids, vitamins, plant growth regulators and secondary plant metabolites (Goerge*et al*., 2008b). In date palm, only a few studies have investigated the effect of complex organic additives. Sharma *et al*. (1980) found that adding coconut water (CW) and malt extract (ME) did not support callus induction, however, CH was stimulatory. Abdel-Rahim *et al*. (1998) confirmed the promoting activity of CH on callus induction. Hegazy *et al*. (2009) found that CH resulted in the highest repetitive somatic embryo multiplication rate in date palm cv. Malakaby compared to other complex organic additives tested including ME, YE, peptone, trypton and pineapple extract. Similarly, CH was found to stimulate *in vitro* response of different plant species. For example, callus growth induction in mulberry *Morinda citrifolia* was enhanced when the culture medium was supplemented with 4g/L CH (Zenk *et al*., 1975). Cardi and Monti (1990) found that, the addition of 2g/L CH was important for callus production in pea (*Pisum sativum*). To enhance callus induction and proliferation from seeds of milestone, *Arceuthobium tsugense*, Deeks *et al*. (2002) added 0.5 g/L CH to the culture medium. Similarly, Chaturved *et al*. (2003) obtained triploid plants of neem, *Azadiracta indica*, from immature endosperm cultured on a medium fortified with 0.5 g/L CH. In peach palm, *Bactris gasipaes*, somatic embryogenesis was best achieved by transferring the callus to MS medium containing 0.5g/L CH (Steinmacher *et al*., 2007). Das and Mandal (2010) working with leaf explant from the medicinal plant sweetleaf, *Stevia rebaudiana*,and have reported that adding 0.1g/L CH produced greenish, healthy nodular callus with more embryogenic capacity and less necrotic in comparison to the control.

CH has been the principal source of nitrogen utilized in tissue culture and the growth of date palm callus tissue was significantly stimulated by the addition of amino acid, specifically CH. This stimulation suggested that organic nitrogen was a growth limiting factor in date palm culture (Sara and Khatd, 2011).

2. Embryognic callus formation

Data in Table(2) clearly show that the percentage of embryogenic callus formation obtained of date palm Shabah strain from shoot tip and leaf primordia explants reached the highest value (93.33-73.33%, respectively), whencultured on MS medium supplemented with 3 mg/L BA + 10 mg/L NAA + 10mg/L 2,4-D compared with other treatments. In addition, the same medium recorded the highest fresh weight of embryogenic callus (4.81 and 3.73g/explant, respectively) (Fig. 1e). On the other hand, the medium with3 mg/L BA +5 mg/L NAA gave lower percentage of embryogenic callus (40 and 20.13% for shoot tip and leaf primordia explants, respectively), also the same treatment gave the lowestfresh weight of embryogenic callus (1.77 and 1.31 g/explant, respectively). It was found that percentage of embryogenic callus formation and fresh weight of embryogenic callus were significantly higher with shoot tip explants as compared with leaf primordia. Zaid *et al*. (2006) found that all types of explants (shoot tip, sub-shoot tips and leaf primordia) of date palm produced callus, but the shoot tip explants were superior in increasing the number of explants, which were able to produce callus compared with other explants.

Regarding the effect of auxin type, it was found that the most effective auxins was 2,4-D, which recorded higher percentage of embryogenic callus formation and fresh weight of embryogenic callus for both shoot tip and leaf primordia explants.

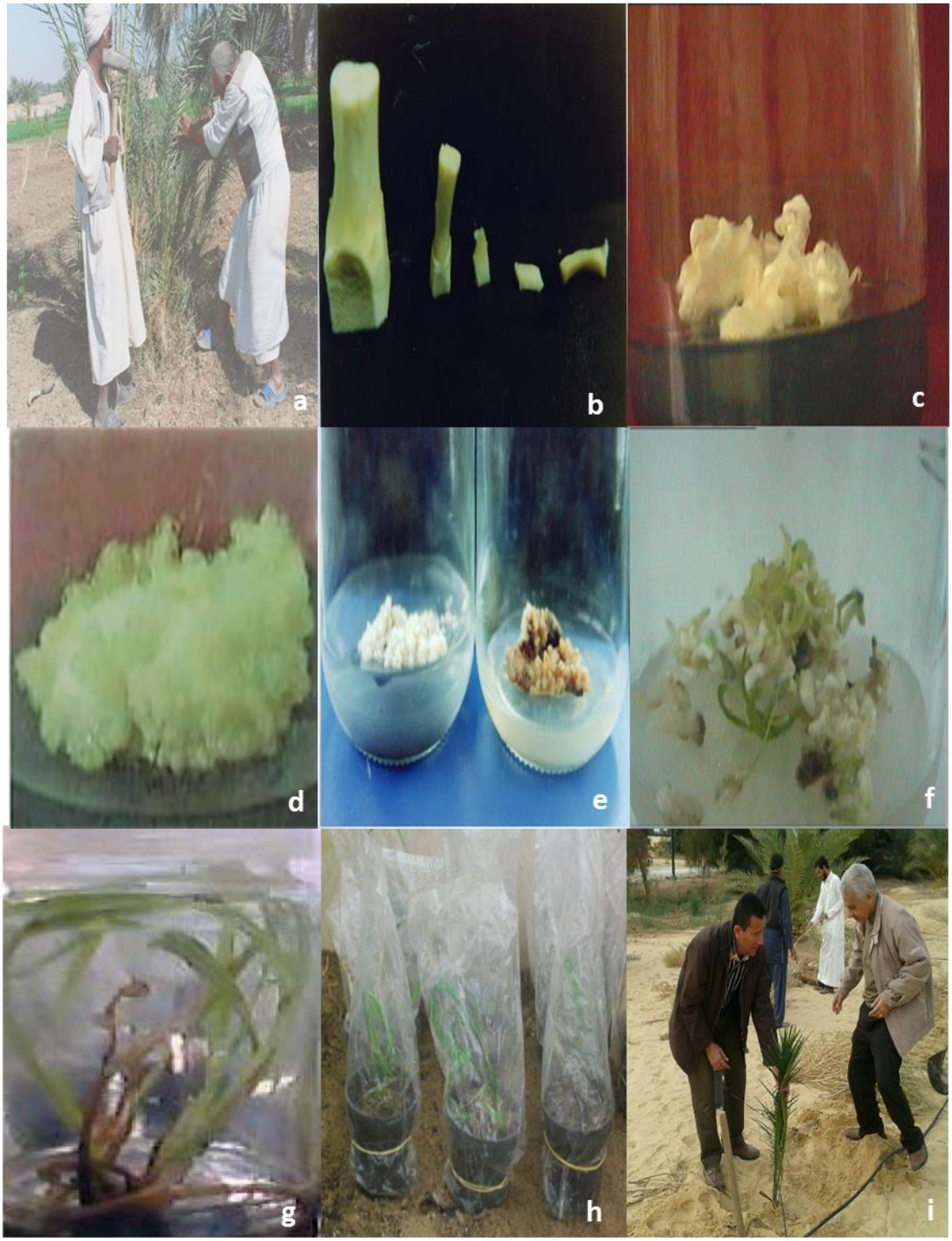

Fig. (1). In vitro propagation of date palm seedling strain (Shabah) grown under Aswan conditions*;* isolation of offshoots from mother plant, (b) explant types, (c) Swelling, (d) Callus formation, (e) Somatic embryo, (f) germination of somatic embryos, (g) Root formation, (h) plantlets acclimatization, (i) plants cultured in Siwa Oasis (Khemisa farm).

Table (2). Effect of different concentrations of growth regulators on callus formation of Shabah strain dry date palm (*Phoenix dactyllfera*).

PGRs combination	Explant type			
	Shoot tip		Leaf primordia	
	Embryogenic callus formation (%)	Fresh weight (g/explant)	Embryogenic callus formation (%)	Fresh weight (g/explant)
3 mg/LBA+5 mg/LNAA	40.00 d	1.77 d	20.13 c	1.31 e
3 mg/LBA +5 mg/L2,4-D	66.66 b	3.06 c	53.33 ab	2.36 c
3 mg/LBA+10 mg/LNAA	53.33 c	2.76 c	46.66 b	2.09 d
3 mg/LBA +10 mg/L2,4-D	86.66 a	3.77 b	66.66 ab	2.93 b
3 mg/LBA + 10 mg/L NAA +10 mg/L2,4-D	93.33 a	4.81 a	73.33 a	3.73 a

Eshraghi, (2005b) reported that in order to induce embryogenesis callus in Mordarsing cultivars, there must be a high concentration of 2,4-D hormone. These results are in line those obtained by several researchers Hervan *et al.*(1991) cultured five different explants from date palm varieties on MS basal medium with different concentration of PGRs, 2,4-D stimulated callus formation in these explants and the highest rate of callus formation was obtained with shoot tip. Behnas*et al*. (2014) showed that using 2,4-D resulted in producing the highest weight of embryogenic callus. Embryogenic callus induction in date palm is influenced by different parameters such as genotype, explant type, induction period and PGRs. In the case of either shoot tips or leaf primordia, high levels of auxins have been used to induce embryogenic callus and 2,4-D is reported as the most effective auxin for embryogenic callus induction in date palm. However, Fki *etal*. (2011) mentioned that high doses of 2,4-D may induce somaclonal variation. Therefore, other researchers used lower 2,4-D concentrations or other auxins in order to induce somatic embryogenesis (Lamp and Dixon, 1997).

3. Somatic embryos formation and germination

Salicylic acid is a type of phenolic acids. It is widely used in organic synthesis and it is function as a plant hormone. It appears to have a role in systemic acquired resistance to pathogens and is able to induce various pathogen resistance proteins (Goerge*et al*., 2008b). Data presented in Table (3) show that somatic embryogenesis derived from the shoot tip explants recorded high number and percentage of germinated somatic embryos as compared with leaf primordia explants. Addition of SA as a supplement to the MS medium led to a significant increase in number and percentage of germination of somatic embryos. Concentrating on the effect of different concentrations of SA, it was shown that culture medium containing 50 mg/L SA gave the highest number and germination percentage of somatic embryos (18, 15.7% and 63.5, 55.1% forshoot tip and leaf primordia explants,respectively) (Fig. 1f). While, MS medium without SA (control) recorded the lowest number and germinationpercentage of embryos.

Table (3). Effect of different concentrations of salicylic acid (SA) on somatic embryo number and germination % of Shabah strain dry date palm (*Phoenixdactyllfera*).

Treatments of SA (mg/L)	**Somatic embryosno./explant**		**Embryo germination (%)**	
	Shoot tip	**Leaf primordia**	**Shoot tip**	**Leaf primordia**
0	9 d	8.0 b	31.90 d	29.00 b
25	12 cd	11.0 b	41.70 cd	36.36 b
50	18 a	15.7 a	63.50 a	55.10 a
75	16 ab	12.0 ab	56.30 ab	50.00 a
100	13 bc	10.0 b	49.12 bc	38.20 b

SA is an important signaling molecule, not only involved in defense responses but also in somatic embryogenesis of many plant species. SA is a mobile molecule, which is capable of acting as a cell and might help in programming towards embryogenesis duringcloning. SA is involved (together with nitrogen oxide, hydrogen peroxide and other metabolites) in the function of several signal systems, unifying them into an intricate network of regulatory interactions. Perhaps embryo differentiation may sharing some of the intermediates in the salicylate signal bathway. SA is a natural substance, which is often associated with a role in plant responses to physical and biological aggressions (Goerge *et al*., 2008a).Some studies have found that the development of somatic embryos could be promoted by adding exogenous SA in embryogenic cultures (Hutchinson and Saxena, 1996). Since, SA has been proposed to be a new inhibitor of ethylene synthesis. The promoting effect of SA on somatic embryogenesis could be related to the inhibition of ethylene synthesis (Roustan *etal*., 1995). Inhibitions of ethylene synthesis and ethylene action have been applied to stimulating somatic embryogenesis in different plants (Kuklin, 1995and Liang*et al*., 1997).Also, in recent year SA has received particular attention because its accumulation is essential for expression of multiple modes of plant disease resistance.

During transfer of date palm plantlets from lab to the greenhouse a high percentage of plantlets may be lost, because the micropropagated plantlets are not well adapted to the *in vitro* climate. Therefore, the well rooted plantlets were subjected to PEG to study its effects on plantlets response during acclimatization.

4. Rooting and acclimation of plantlets

Data presented in Table (4) show that, PEG treatment significantly increased root numbers and significantly reduced average root length by increasing PEGconcentration, as compared with plantlets produced in culture medium without PEG. As for the effect of different media on root number, data reveal that MS medium supplemented with 4g/LPEG resulted in the highest significant number of adventitious roots (6.46) followed by 6g/LPEG (5.86) (Fig. 1g). On the other hand, the lowest significant number of roots was recorded on MS medium without PEG (2.86) with the highest average root length (6.27 cm).

Table (4).Effect of different of polyethylene glycol (PEG) on rooting %, average root number and average root length (cm) of Shabah strain dry date palm (Phoenixdactyllfera).

PEG conc. (g/L)	Rooting (%)	Root no./explant	Root length (cm)
0.0	100	2.86 d	6.27 a
2.0	100	3.26 d	5.78 b
4.0	100	6.46 a	5.50 b
6.0	100	5.86 ab	4.20 c
8.0	100	5.17 bc	3.67 d
10.0	100	4.51 c	3.32 d

Under the effect of PEG, there was many changes in transcript levels of many genes involved in nitrogen assimilations and utilization, leading to an increase in protein concentration in white spruce embryos (Stasolla *et al*., 2003). Van-Heerden *et al*. (1996) reported that the primary metabolic fate of phenylalanine, following is deamination by activity of PAL enzyme in plant, is conscription of its carbon skeleton for lignin, subrin, flavonoid and related metabolite formation. In *Panax ginseng*, Langhansova *et al*. (2004) stated that shoot regeneration of non-treated somatic embryos was 31%, while that for somatic embryos treated with PEG 4000 and ABA was 70%.

One of the major obstacles concerning the practical application of plant tissue culture to micropropagation has been the difficulty if the successful transfer of plantlets from *in vitro* condition to a soil medium, a loss rate of 50 to 90% of *in vitro* propagated plantlets of many species occur at the time of transfer to soil (Zaid and Hughes, 1995). This is unfortunate because the ultimate success of plant depend on the ability to transfer plantlets out culture, on large scale at low cost and with a high survival rate. Data presented in Table (5) show the percentage of survival plantlets, which treated with PEG during rooting stage reachedthe highest value of survival percentage (80%) on MS medium supplemented with 10g/L PEG (Fig. 1h). On the other hand, plantlets non-treated with PEG recorded the lowest percentage value (45%) of plantlets survival in acclimatization.

Table (4). Effect of PEG at different concentrations in rooting MS medium on plantlets survival percentage through acclimatization stage.

Treatments PEG (g/L)	Plantlets survival (%)
0.0	45
2.0	60
4.0	70
6.0	75
8.0	75
10.0	80

Picoli *et al*. (2001) mentioned that failure of hyperhydric plants to grow when transferred to soil may often be due to malfunctioning of the leaf rather than the poor root ability. Reasons for this leaf malfunctioning are absence of epicuticular wax, stomatal abnormalities and reduced development of palisade tissue. Al-Salih *et al*. (1986) suggested that, success or fail of transferred plantlets to greenhouse are dependent primarily upon the quality and type of materials produced in the previous stages of *in vitro* propagation. Al-Khateeb and Ghazzawy (2015) reported that addition PEG to the culture media are able to promote the embryo formation and maturation in different plant. Increased medium osmolality and hence the frequency of somatic embryogenesis was also obtained by the addition of PEG (Browen *et al*., 1989 and Abdel-Raheem et al., 2007). In*Panaxginseng*, Langhansova *et al*. (2004) stated that shoot regeneration of non-treated somatic embryos was low after PEG and ABA treatment significantly increased.

The present study represented a successful protocol for micropropagation of the elite seedling strain (Shabah) of dry date palm grown at Aswan Governorate, for large scale production of true to type elite seedlings. The produced plantlets were transferred to be grown in the Experimental Research Station of Desert Research Center, Khamisa Farm Siwa Oasis (Fig.1i).

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