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REGULAR ARTICLE

Determination of the date palm cell suspension growth curve, optimum plating efficiency, and influence of liquid medium on somatic embryogenesis

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Abstract

Understanding the behavior of date palm (*Phoenix dactylifera* L.) cell suspension growth and differentiation would foster effective utilization for mass micropropagation and various in vitro investigations. The objectives of this study were to define the growth curve, identify the optimum plating density, and examine the efficiency of somatic embryogenesis of date palm suspension culture on solid and liquid media. Cell suspensions were established from shoot tip-induced callus of cv. Barhee inoculated in MS medium containing 10 mg l^{-1} naphthaleneacetic acid and 1.5 mg l^{-1} 2-isopentenyladenine. Various growth phases including lag, exponential, linear, progressive deceleration, and stationary, along with their specific onsets and durations, were identified based on packed cell volume method. The growth pattern characterizing the exponential phase in date palm cell suspensions commenced 4 weeks after culture initiation which makes this period the most suitable for sub-culturing, assessment of the effects tissue culture factors, and in vitro selection. The effect of cell plating density on cell growth after transferring to solidified medium was also determined. The highest plating efficiency, 14.6%, was obtained at cell density of 10,000 cells ml^{-1}. To stimulate somatic embryogenesis, cell suspension masses were transferred to agar or liquid media devoid of phytohormones. Plant regeneration was marked by the development of globular somatic embryos which progressively matured and germinated. Culturing suspension mass in liquid medium expedited regeneration and resulted in 3.5-fold more somatic embryos than agar medium.

Key words: Cell suspension, Growth curve, Micropropagation, *Phoenix dactylifera*, Plating efficiency, Somatic embryos, Tissue culture

Introduction

Date palm (*Phoenix dactylifera* L.), belongs to the monocotledonous family Arecaceae, and is an economically important tree species predominantly concentrated in arid regions of the Middle East and North Africa (Zaid, 2002). Propagation by seeds produces female trees with inferior fruit quality. Offshoots are preferred for propagation because they produce genetically identical trees. However, offshoots availability is limited. Alternatively, micropropagation provides an efficient means for mass propagation of date palm. Breeding of date palm is hindered by the long generation time and high heterogeneity (El Hadrami and El Hadrami, 2009). This makes biotechnological approaches, such as genetic transformation (Saker et al., 2007a; Habashi et al., 2008; Mousavi et al., 2009; Saker et al., 2009) and in vitro selection (El Hadrami et al., 2005; Al Mansoori et al., 2007; Jain, 2010) indispensable for date palm genetic improvement.

Research in date palm tissue culture has received increasing interest resulting in plant regeneration protocols for numerous commercial date palm cultivars. Several reviews have described research progress of date palm micropropagation (Tisserat, 1991; Omar et al., 1992; Benbadis, 1992; Bhaskaran and Smith, 1995; Al-Khayri, 2005; Al-Khayri, 2007; Singh and Shekhawat, 2009; Abahmane, 2011; Abul-Soad, 2011; Fki et al., 2011; Othmani et al., 2011). The literature indicated that plant regeneration of date palm was achieved via organogenesis and somatic embryogenesis depending upon the genotype and the composition of the culture medium. Date palm somatic embryogenesis was improved using biotin (Al-Khayri, 2001), abscisic acid (ABA) supplement (Sghaier et al., 2009; Sghaier-Hammami et al., 2010), coconut water additive (Al-Khayri, 2010), and suitable basal salt formulation (Al-Khayri, 2011). Moreover, the stimulation of direct somatic

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embryo regeneration from shoot tip explants using N-phenyl N'-1,2,3-thidiazol-5-ylurea (TDZ) was achieved by Sidky and Zaid (2011).

Prompted by successes in numerous plant species; for examples, soybean (Hayashi and Woshida, 1988), orchardgrass (Britain-Loucas et al., 1998), and peach (Schiavone and Wisniewski, 1990), liquid media were eventually utilized in date palm tissue culture. Several researchers successfully obtained in vitro plant regeneration using suspension cultures established from date palm embryogenic callus (Sharma et al., 1986; Bhaskaran and Smith, 1992; Veramendi and Navarro, 1996; Taha et al., 2001; Fki et al., 2003; Zouine and El Hadrami, 2004; Zouine et al., 2005; Sané et al., 2006; Saker et al., 2007b; Zouine and El Hadrami, 2007; Badawy et al., 2009; Othmani et al., 2009a**)**. These studies proved that suspension culture is a prolific source of somatic embryos suitable for mass propagation of several date palm cultivars. In addition, Othmani et al. (2009b) demonstrated the applicability of embryogenic suspension culture for high output of date palm somatic embryos using temporary immersion bioreactor (TIB) system.

Other researchers utilized date palm suspension cultures for in vitro physiological studies related to abiotic stress responses; for example, changes of proline and ions accumulation in response to salinity (Al-Khayri, 2002) and PEG-simulated drought (Al-Khayri and Al-Bahrany, 2004). Moreover, liquid cultures facilitated anatomical and histological studies related to the development of date palm somatic embryos (Bhaskaran and Smith, 1992; Sané et al., 2006; Sghaier et al., 2008). Additionally, suspension culture proved useful in conducting biochemical studies involving the role of protein accumulation in date palm somatic embryogenesis (Zouine and El Hadrami, 2004; Sghaier et al., 2009; Sghaier-Hammami et al., 2010).

Efficient manipulation of cell cultures, however, requires understanding the growth behavior of a given culture system especially identifying the onset and the duration of various culture growth stage phases: lag, exponential or log, linear, progressive deceleration, and stationary. This information can be used to establish a growth curve. Growth curves are essential to assess culture performance and metabolic activities at various growth phases. Furthermore, they provide guidelines for determining the optimal time for sub-culturing of cell suspension cultures.

Cell suspension offers a tremendous opportunity for date palm genetic improvement based on mutagenesis and in vitro selection studies (Jain, 2005, 2007). The success of this technique relies on using actively dividing cells found in the exponential phase, i.e. the most rapid growth. This emphasizes the importance of distinguishing growth phases for mutagenesis and in vitro selection studies. A common approach of in vitro selection involves plating cell suspensions on a solidified medium containing selection agents. The cell density is a determining factor in the growth efficiency of the plated cells, i.e. the plating efficiency (PE). For this reason, it is imperative to identify the optimum plating efficiency to maximize the effectiveness of in vitro selection of date palm biotypes.

The literature lacks information distinguishing the growth phases of date palm cell suspension. Likewise, no data were reported on the effects of the plating density. Moreover, previous studies demonstrated cultivar-dependency in date palm somatic embryogenesis improvement using liquid medium. The objectives of the present study were: to develop a growth curve distinguishing various culture phases of date palm cell suspension, to identify the optimum plating efficiency, and to assess somatic embryogenesis in liquid and agar media.

Materials and Methods

Explant preparation

Date palm cv. Barhee offshoots, 3-4 years old, were removed from mother trees, the outer leaves were removed exposing the shoot tip regions that were excised and immediately placed in a chilled antioxidant solution consisting of ascorbic acid and citric acid, 150 mg l^{-1} each, to prevent browning. The shoot tip tissue, about 8 cm long, was surface sterilized in 70% ethanol for 1 min followed by 15 min in 1.6% w/v sodium hypochlorite (30% v/v Clorox, commercial bleach) containing 3 drops of Tween 20 (Sigma Chem Co, St. Louis, MO) per 100 ml Clorox solution. The tissue was then rinsed with sterile distilled water four times and placed again in sterile antioxidant solution in preparation for explant excision. The tissue surrounding the shoot tips was removed until the leaf primordia were exposed and detached at the base. The shoot tip terminal, about 1 cm long, was sectioned longitudinally into four sections. Each offshoot yielded 10 explants, 6 smallest leaf primordia plus 4 terminal tip sections, which were used for culture initiation. The culture conditions used throughout the study, except where specified, consisted of a 16–h photoperiod of cool-white florescent light (40 μmol m^{-2} s^{-1}) and 23 ± 2°C.

Culture medium

The explants were cultured on a medium consisting of MS salts (Murashige and Skoog, 1962) supplemented with (per liter) 170 mg NaH_2PO_4, 125 mg *myo*-inositol, 200 mg glutamine, 5 mg thiamine-HCl, 1 mg nicotinic acid, 1 mg pyridoxine-HCl, 30 g sucrose, and 7 g agar (purified Agar-agar/Gum agar, Sigma). This basal medium was used throughout the system with modifications made according to each stage. The callus initiation medium contained (per liter) 100 mg 2,4-dichlorophenoxyacetic acid (2,4-D) (452.5 μ*M*), 3 mg 2-isopentenyladenine (2iP) (14.7 μ*M*), and 1.5 g activated charcoal (acid-washed, neutralized, Sigma). These cultures were maintained in the dark for 12 weeks during which they were transferred at 3-week intervals. At the end of this period, resultant callus was separated and transferred to callus proliferation medium that contained (per liter) 10 mg naphthaleneacetic acid (NAA) (53.7 μ*M*), 30 mg 2iP (147 μ*M*), and 1.5 g activated charcoal. These cultures were maintained for an additional 3 weeks. To proliferate embryogenic callus, the callus was transferred to a medium containing (per liter) 10 mg NAA (53.7 μ*M*), 6 mg 2iP (29.6 μ*M*), and 1.5 g activated charcoal. These cultures were maintained for 9 weeks during which they were transferred at 3-week intervals. Embryogenic callus was maintained for 12 weeks on a medium containing (per liter) 10 mg NAA (53.7 μ*M*) and 1.5 mg 2iP (7.4 μ*M*).

Cell suspension establishment

Date palm suspension cultures were initiated by inoculating scalpel-macerated embryogenic callus (1 g per flask) in 150-ml Erlenmeyer flasks containing 50 ml liquid medium. The medium consisted of MS salts with the same supplements described for callus culture medium but without agar and activated charcoal. The suspension cultures were incubated on a rotary shaker set at 150 rpm under a16–h photoperiod of cool-white florescent light (40 μmol m^{-2} s^{-1}) and 23 ± 2°C. After 3 days the suspensions were filtered through a 500 μm-stainless steel sieve and the cell filtrates were brought to a final volume of 50 ml by adding a fresh liquid medium. The cell filtrates were placed in culture flasks and maintained under the same culture conditions for use in the current study. At 2-week intervals, the cell suspension was re-sieved and half of the medium was replaced with fresh medium.

Suspension growth determination

The packed cell volume (PCV) method was used to monitor cell growth. To determine the PCV, 5 ml of cell suspension was placed in a sterile graduated centrifuge tube and centrifuged at 2000 g for 5 min. The packed cell volume was recorded as percentage cell mass of the total centrifuged volume and then the samples were returned to the original cultures. Measurements were taking weekly for 12 weeks. The PCV values were plotted in relation to time, to construct a growth curve reflecting various phases of cellular growth.

Plating efficiency

Concentrated cell suspension was diluted to give initial cell density of 100, 500, 1000, 5000, 10000, 50000, and 100000 cells ml^{-1} with the aid of a hemocytometer. Samples of cell suspensions were mixed with melted agar medium after setting the autoclaved medium to cool down to 30-35°C. The medium used was identical to the suspension medium but contained 7 g l^{-1} agar and dispensed in 15x100 mm Petri dishes, at 20 ml per dish. The cell suspension and molten medium were mixed and evenly distributed in the plate. These were allowed to solidify forming a fixed thin layer of cell. To assess the recovery potential of cell suspension to form cell colonies in relation to the initial cell concentration, colonies formed were counted using an illuminated colony counter. The plating efficiency was determined using the following equation: PE = (final number of colonies per plate / initial number of cellular units per plate) x 100.

Somatic embryogenesis efficiency in liquid and solid media

Cell suspension cultures were induced to undergo somatic embryogenesis by transferring them to a hormone-free medium, either in a liquid or solidified state. The medium consisted of MS salts supplemented with the same additives described above. The liquid cultures were incubated on a rotary shaker at 150 rpm. The cultures were incubated at a 16–h photoperiod of cool-white florescent light (40 μmol m^{-2} s^{-1}) and 23 ± 2°C. The numbers of resultant embryos were counted at bi-weekly intervals for 14 weeks.

Experimental design and statistical analysis

To determine the effect of time on cell suspension growth expressed in PCV, a one-factor randomly designed experiment with the main factor time at 12 levels using 5 replications. To assess PE, plates were inoculated at 7 levels of initial cell concentration in a one-factor randomly designed

experiment with 5 replications. To evaluate the formation of somatic embryos in relation to time and medium solidification status, a two-factor randomly designed experiment was conducted with the main factors being the solidification status at 2 levels and time at 7 levels using 5 replications. The data were subjected to analysis of variance (ANOVA) and the means were separated, where appropriate, with a least significant difference (LSD) at 5% significance level.

Results

Suspension growth determination

The present study resulted in the determination of the growth pattern of date palm in vitro cell suspension which was useful to construct a growth curve identifying the onset and duration of the various growth phases. Based on their characteristic growth pattern, these phases were divided into lag phase, exponential or log phase, linear phase, progressive deceleration phase, and stationary phase.

According to ANOVA (Table 1), growth of date palm cell suspension expressed in PCV, was significantly affected by time (i.e. culture duration). Initially, cell growth progressed slowly as the cells acclimatize to the new environment, but as time passed, growth proceeded relatively rapidly then reached a steady state. The slow growth period, associated with the lag phase, extended from the time of initiating the suspension cultures until week 3 of culture (Figure 1). The exponential phase, where the highest growth rate occurred, was observed within 4-5 weeks of culture initiation. Following this phase, a linear growth pattern commenced and lasted until week 7. Progressive growth deceleration occurred beginning at week 8 followed by a stationary phase pattern starting with week 10. Maximum growth volume (12.1%) was reached 8 weeks after suspension culture initiation.

Table 1. Analysis of variance of the effect of time on growth of date palm cell suspension expressed in packed cell volume (pcv).

Source	df	SS	MS	*F* value	*P* > *F*
Time	11	838.920	76.265	82.264	0.0001
Error	48	44.500	0.927		

P values less than 0.05 are significant. SS, sum of squares. MS, mean of squares.

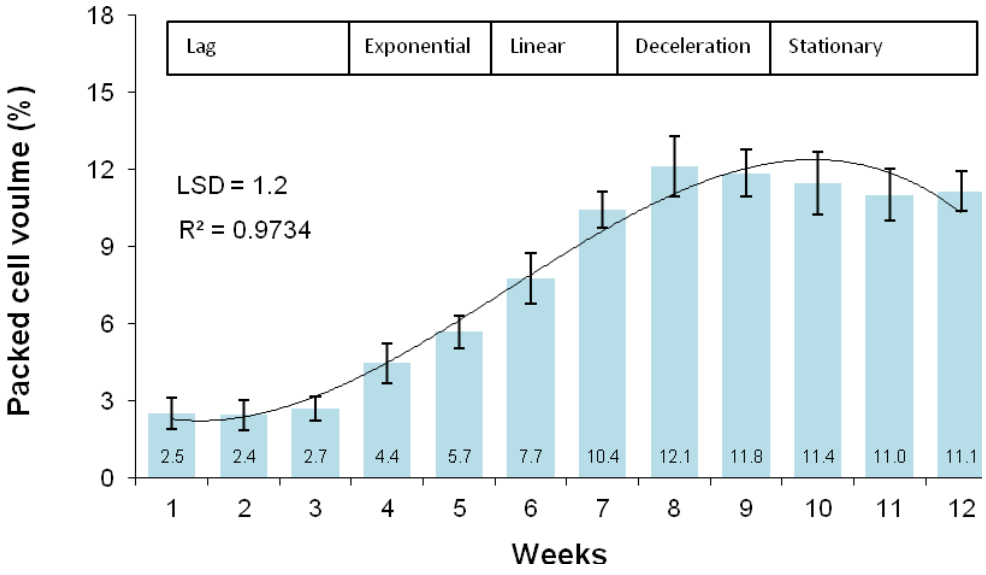

Figure 1. The growth curve of date palm cell suspension culture showing PCV in relation to time as it pertains to each of the growth phases (lag phase, exponential or log phase, linear phase, progressive deceleration phase, and stationary phase).

Table 2. Analysis of variance of the effect of initial cell concentration on re-growth of date palm cell suspension after plating expressed in terms of colony count and plating efficiency.

Source	df	SS	MS	*F* value	*P* > *F*
Colony count					
Cell density	6	54223.486	9037.248	451.540	0.0001
Error	28	560.400	20.014		
Plating efficiency					
Cell density	6	748.686	124.781	23.292	0.0001
Error	28	150.000	5.357		

P values less than 0.05 are significant. SS, sum of squares. MS. mean of squares.

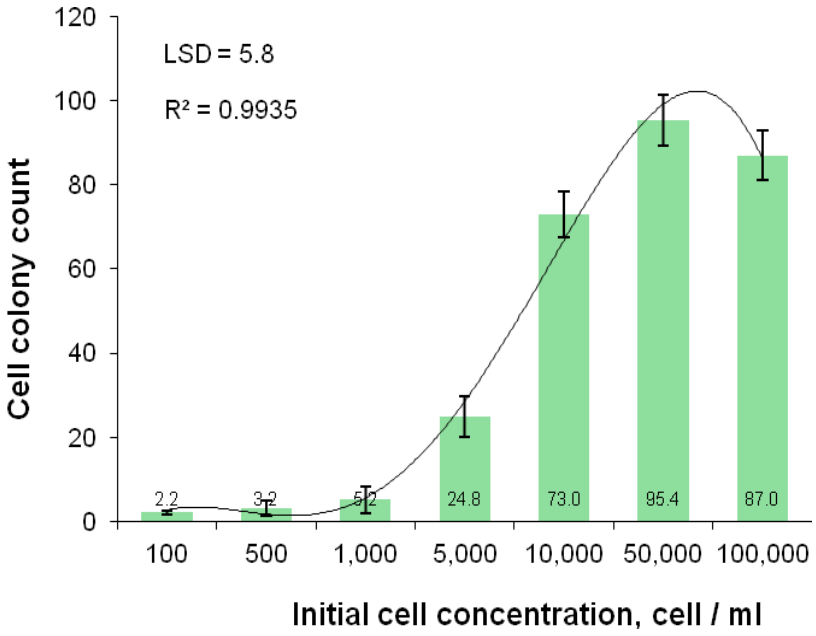

Figure 2. The growth of date palm cell suspension, expressed in number of colonies, recovered after plating on a semi-solid medium at various cell densities.

Cell colony count and plating efficiency

The cell suspension consisted mainly of single cells and small cell aggregates. Plated cells, that were capable of dividing, formed cell colonies that became visible after 3 weeks of plating. Colony count was significantly influenced by the cell density, as revealed by ANOVA (Table 2). Accordingly, when cell count was used to calculate the percentage of cell growth, to express growth in terms of PE, a significant effect due to cell density was also observed (Table 2).

Generally, cell colony count was directly proportional to the initial cell concentration (Figure 2). At low density, 100 to 1000 cells ml^{-1}, cell growth was negligible. The minimum cell density required to obtain detectible cell colony formation was 5,000 cell ml^{-1}. Whereas, the maximum count, 95.4 colonies, was observed at 50,000 cells ml^{-1}. Cell density above this level, appear to impose constrains on growth of date palm plated cells. However, the effects of density ranging from 50,000 to 100,000 cells ml^{-1} were not significantly different (Figure 2).

In terms of PE, which takes into account the percentage of cells that formed colonies in relation to the initial cell population, 10,000 cells ml^{-1} resulted in the optimum PE, 14.6% (Figure 3). Increasing the concentration to 50,000 cells ml^{-1} started to decrease the PE; although, this concentration was associated with the highest colony count. Statistically, however, cell density ranging from 10,000 to 50,000 cells ml^{-1} had similar effect on plating efficiency. At a higher cell concentration, 100,000 cells ml^{-1}, a significant decrease in PE was noted in comparison to the optimum cell densities.

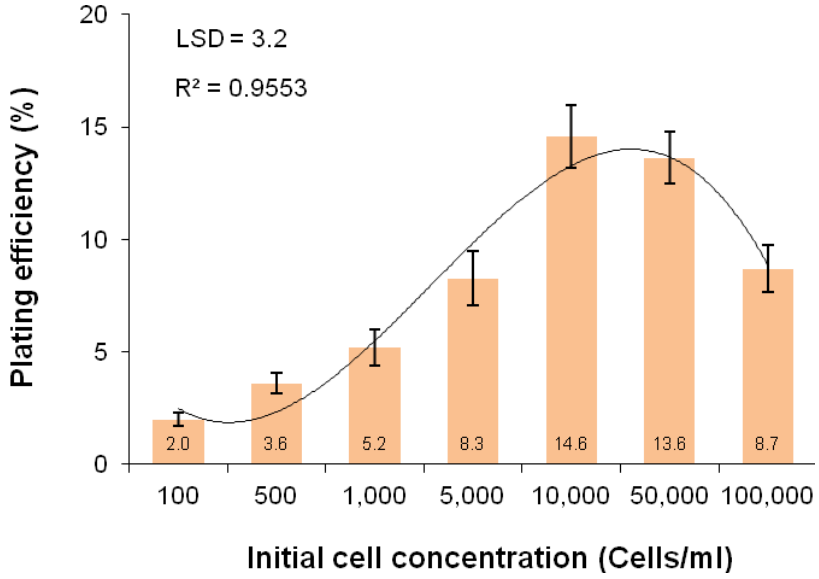

Figure 3. The growth of date palm cell suspension, expressed in plating efficiency, recovered after plating on a semi-solid medium at various cell densities.

Somatic embryogenesis efficiency in liquid and solid media

The culture conditions provided by the liquid medium significantly surpassed that of the solidified medium in supporting somatic embryogenesis of date palm, as indicated by ANOVA (Table 3). Liquid medium did not only increase the number of resultant somatic embryos but also expedited the formation of somatic embryos. This effect was indicated by the significant two-way interaction between medium solidification status (liquid or solid) and the time required for embryo acquisition.

Initially, the cell suspension proliferated callus prior to somatic embryo formation was observed. Within 4 weeks of culturing somatic embryo formation was obvious in the liquid culture medium, although a small percentage of the cultures commenced embryo formation prior to that, within the first 2-3 weeks after introducing to hormone-free medium. At this time the agar solidified cultures exhibited negligible development and it was not until week 6 that somatic embryos developed. At week 8, liquid cultures produced somatic embryos almost 3.5 times as many as that of the agar cultures, respectively, 43 and 12 embryos per culture (Figure 4). In the following weeks, the liquid medium maintained its significant superiority over the solidified medium reaching the end of the process of somatic embryo formation at week 12. Conversely, agar medium cultures continued to produce more embryos in week 14; however this increase was not significant. At the end of the experiment, liquid cultures produced 3.5 times the somatic embryos as compared to agar cultures, respectively, 69 and 20 embryos per culture. The liquid cultures exhibited expedited regeneration process whereby 62% and 85% of the somatic embryos were produced within 8 and 10 weeks, respectively.

Table 3. Analysis of variance of the effect of medium physical status (solid and liquid) on somatic embryogenesis in date palm expressed in term of embryo number produced over time.

Source	df	SS	MS	*F* value	*P* > *F*
Medium status	1	666.514	666.514	65.253	0.0001
Time	6	3075.143	512.524	50.177	0.0001
Medium status X Time	6	226.286	37.714	3.692	0.0004
Error	56	572.000	10.214		

P values less than 0.05 are significant. SS, sum of squares. MS. mean of squares.

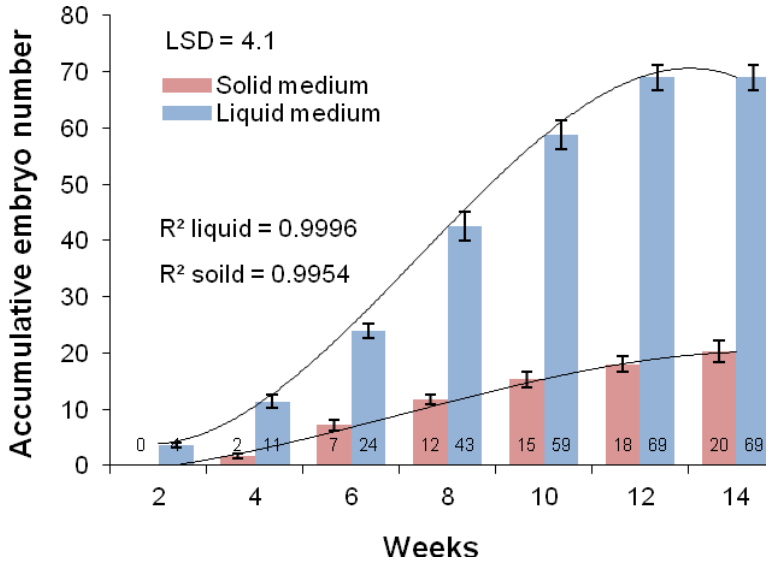

Figure 4. Progress of somatic embryogenesis in date palm cell suspension cultured either in liquid or agar medium in relation to time.

Discussion

Suspension culture establishment

For establishing date palm suspension cultures, different researchers utilized variable sieve pore sizes for filtration. Filter pore size arbitrarily ranged from 380 µm (Othmani et al., 2009a**)** to 500 µm (Fki et al., 2003; Zouine et al., 2005; Saker et al., 2007b). Larger size, 2000 and 1000 µm filter combination, was used by Sané et al. (2006) for the filtration of date palm embryogenic mass. In the current study 500 µm pore size appear to be effective for the establishment of date palm suspension. The optimum filtration pore size for establishing suspension culture merits further investigations related to the influence of cultivar and culture stage. According to a study by Badawy et al. (2009), the optimum filtration pore size during the maturation stage of date palm somatic embryos was 500 µm mesh filter diameter. This resulted in increased globulrization and higher numbers of somatic embryos when 500 µm mesh diameter was used as compared to 100 µm and 200 µM mesh filter diameter.

Suspension growth determination

There are general technical difficulties in defining the growth of plant cell in vitro cultures which pertains to maintaining aseptic conditions while obtaining consecutive samples from culture vessels. In vitro cell growth can be measured in relation to the number of cells, the cell mass, the volume of cells, or as a function of biochemical processes such as sugar dissimilation (Schripsema et al., 1990). Various methods are employed to measure in vitro cell growth, including cell or colony counting, dry weight, and fresh weight, and packed cell volume (Dixon, 1985). Different growth curves are obtained depending upon the methods used (Majerus and Pareilleux, 1986; Yamamoto and Yamada, 1986).

Since cell stretching and volume increase of cells comes after maximum cell division is reached, the maximum fresh weight is reached some time after the maximum dry weight is obtained. Growth patterns of cell number usually parallel that of the dry weight, while the packed cell volume usually parallels that of the fresh weight. Growth curves obtained with different methods vary and may be influenced by the genotype, cell line, and various components of growth medium (Zilkah and Gressel, 1977; Schripsema et al., 1990).

PCV procedure is commonly used to monitor growth of plant cell cultures (Santos-Diaz and Ochoa-Alejo, 1994; Falco et al., 1996). In the present study, this method was used to develop a growth curve for date palm suspension culture

identifying the onset and duration of the various growth phases including lag phase, exponential or log phase, linear phase, progressive deceleration phase, and stationary phase.

Cell colony count and plating efficiency of cell suspension cultures

A major requirement for obtaining growth from in vitro cell cultures after transferring them to agar medium is using suitable initial density of cells for inoculation in the agar culture plates. It is pertinent to use the proper plating densities because lower plating densities reduces the nutrient deficiency effect which can limit growth; whereas, higher densities elicits nutritional competition and limitation to growth. Moreover, accumulation of growth inhibitors like phenolic compounds at higher densities may adversely affect cell growth (Marchant et al., 1997; Yu et al., 2000; Aziz et al., 2006). Usually a high initial cell number, above 1000 cells per ml, is required for plating to obtain development of colonies and subsequent growth to microcalli of appreciable sizes. Using proper initial cell density would facilitate colony isolation and characterization in various in vitro physiological and genetic studies. The optimum cell density that gives the highest percentage of colony growth may differ among plant species. Plating densities of 1,000-100,000 cells per ml fall within the optimum PE range depending on the plant species (Bellincampi et al., 1985).

The current study showed that initial cell density of 10,000 cells ml^{-1} resulted in maximum PE, 14.6%, in date palm. Although this number is acceptable PE may be enhanced by various manipulations. For example, colony formation may be increased by the raft nurse or feeder layer technique (Bellincampi et al., 1985). Nurse culture proved advantageous in date palm protoplast culture (Chabane et al., 2007) and may be beneficial for cell growth as well. Improvement of the PE may also be achieved using conditioning medium (Bellincampi et al., 1985; Astarita and Guerra, 2000). These approaches are worth testing in future studies aimed at improving the PE of date palm suspension cultures. Moreover, the number of microcalli developed from date palm protoplast culture was found to be cultivar dependent (Chabane et al., 2007). Date palm plated cell suspensions are expected to behave similarly; however, more research is required to assess genotypic differences.

Somatic embryogenesis efficiency in liquid and solidified media

Cell suspension cultures offer several distinct advantages over solidified-medium cultures. In liquid medium, calus clusters and regenerated embryos usually disassociate and float freely in the medium; consequently, they are totally submerged and evenly exposed to the medium ingredients and precursors. Ample aeration is achieved by gently agitating the culture vessels on an orbital shaker. These conditions provide an excellent growth environment that allows for more precise manipulation of medium components and control of cells and embryos development leading to plantlets regeneration (Ammirato, 1984). Availability of an efficient cell suspension system offers the necessary tools for large scale cloning of elite cultivars, production of artificial seeds (Onishi et al., 1994; Bekheet et al., 2002), and in vitro selection (Larkin and Scoweroft, 1981). Cell suspension cultures are also convenient for mass production of fine chemicals in bioreactors and offer a simplified model system for the study of plant responses (Schripsema et al., 1995; Mustafa et al., 2011). Studies with different plant species have demonstrated the advantages of liquid media. For instance, Jayasankar et al. (1999) noted a twofold increase of grapevine, *Vitis vinifera*, somatic embryo regeneration when liquid medium was used.

The current study has also shown that date palm somatic embryogenesis efficiency using liquid medium was far superior to solid medium. This confirms previous findings reported in different date palm cultivars. Fki et al. (2003) reported 20-fold increase in cv. Deglet Nour when embryogenic suspensions were used instead of solid cultures. The highest rate obtained in liquid was 200 embryos per month per 100 mg callus, compared to only 10 embryos with agar medium. Embryogenic callus of cvs. Bousthami noir and Jihel placed in liquid medium yielded 72 embryos per 100 ml of culture medium within 2 months, while those placed on solid medium yielded 16 embryos after 4 months (Zouine et al., 2005). This expediting action of liquid medium was also observed in the current study with cv. Barhee. Saker et al. (2007b) reported 2 to 10 times greater numbers of somatic embryos of cv. Sewi cultured in different liquid media as compared to the corresponding solidified media. The highest number recorded was 129 embryos per flask inoculated with 0.5 g callus. Working with date palm cv. Sakkoty, the highest number of somatic embryos obtained by Badawy et al. (2009) was 48 embryos formed from liquid cultures inoculated with 0.2 g callus. In comparison, cv. Barhee tested in the present study produced 69 embryos per flask inoculated with 1 g

callus as compared to 20 embryos on agar medium; i.e. 3.5-fold increase. It is worthy to note that this increase was detected as early as week 6 of culturing on the regeneration medium and retained throughout the culture duration. This implies that data can be accurately collected to assess the effects of various tissue culture parameters on date palm cell suspension within 6 weeks, without the need to continue observations until week 14, thus reducing the experiment duration.

In conclusion, the current investigation has resulted in an efficient protocol for date palm cell suspension culture suitable for monitoring cell growth and differentiation. A growth curve specifying various culture stages of date palm suspension cultures was developed to assess future studies designed to observe changes in cell growth and somatic embryogenesis in response to modifications in culture factors. The growth curve will facilitate identification of the best time for sub-culturing. Based on testing colony growth at various cell densities, the optimal plating efficiency for date palm was determined. This parameter is essential for future in vitro studies related to somaclonal variation selection and protoplast culture. The study has also confirmed that liquid medium was more supportive of somatic embryogenesis than solidified culture medium, not only by giving higher embryo numbers but also by expediting embryo regeneration.

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