

Basal Salt Requirements Differ According to Culture Stage and Cultivar in Date Palm Somatic Embryogenesis

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Abstract: **Problem statement:** Various media formulations differing in basal salt composition are arbitrary selected to provide essential nutrients for plant *in vitro* cultures. Evidence suggests that *in vitro* response to various media formulations varies among genotypes and depends on the culture stage. This study examined the efficacy of five basal salt formulations on callus growth and somatic embryogenesis in date palm *Phoenix dactylifera* L. using three commercial cultivars, Khusab, Berny and Barhee. **Approach:** Callus from shoot tip explants maintained on MS medium was introduced to various media formulations including SH, W, MS, WPM and NN media containing 53.7 μM NAA and 7.4 μM 2iP. To assess the effect on callus growth, fresh callus weight was measured 4 and 8 weeks later. To evaluate embryogenesis response, callus was transferred to hormone-free media corresponding to those during callus growth stage. **Results:** The optimum medium formulation varied according to cultivar and culture stage. Extending callus growth to 8 weeks allowed for greater discernment of differences as compared to 4 weeks because of the inherent slow growth nature of date palm callus. The best callus growth was achieved in cv. Khusab using W and WPM media, cv. Berny using SH and NN medium and cv. Barhee using SH, W and MS media. An optimal medium for callus growth was not necessarily the best for somatic embryogenesis. The highest regeneration percentage in cv. Berny occurred on WPM medium, cv. Khusab on W medium and cv. Barhee using W and WPM media. The highest number of resultant embryos was achieved in cv. Khusab using W and SH media, cv. Berny on WPM and MS media and cv. Barhee using W and SH media. **Conclusion:** This study provides important information to optimize medium formulation in micropropagation protocols of various date palm cultivars, particularly recalcitrant genotypes. It showed that the best basal salt formulation differed among date palm genotypes and suggested culture stage-specific requirement.

Key words: Callus growth, *In vitro*, optimum medium, palm micropropagation, *Phoenix dactylifera*, plant tissue culture, plant regeneration, somatic embryogenesis, commercial cultivars, vegetative propagation

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), a monocotyledonous, dioecious tree species belonging to Arecaceae family, is a major agricultural crop cultivated in the hot, arid regions of the world, mainly in the Middle East and North Africa. It contributes to a sustainable agricultural system in areas where it is grown and plays important social and economic roles (Zohary and Hopf, 2000). Propagation by seeds produce off-type plants and vegetative propagation by offshoots has numerous limitations related to availability and field survivability. Alternatively, micropropagation has gained acceptance for large-scale propagation and commercial production of date palm trees. In addition, it offers controlled conditions

for studying aspects of physiological and genetic improvement.

Micropropagation of date palm has been achieved from several genotypes through organogenesis and somatic embryogenesis using various meristematic explants including zygotic embryos, shoot tips and lateral buds (Omar *et al.*, 1992; Al-Khayri, 2005; 2007). Somatic embryogenesis is considered as the most efficient regeneration process for date palm micropropagation (El Hadrami and Baziz, 1995; Al-Khayri, 2001; Fki *et al.*, 2003). Several studies were conducted to optimize somatic embryogenesis of date palm through the manipulation of culture medium ingredients including amino acids (Abo El-Nil, 1989a; Abdel-Rahim *et al.*, 1998; El-Shiaty *et al.*, 2004; Zouine and El Hadrami, 2007), plant growth regulators

Omar *et al.*, 1992; Eshraghi *et al.*, 2005; Zouine and El Hadrami, 2007), physical status (Veramendi and Navarro, 1996; Taha *et al.*, 2001; Fki *et al.*, 2003), sucrose (Veramendi and Navarro, 1996; Taha *et al.*, 2001; Alkhateeb, 2008), silver nitrate as an inhibitor of ethylene (Al-Khayri and Al-Bahrany, 2001; 2004), biotin and thiamine (Al-Khayri, 2001; El-Shiaty, *et al.* 2004), salt strength (Taha *et al.*, 2001; Al-Khayri, 2003) and coconut water (Al-Khayri, 2010). In addition, Othmani *et al.* (2009) enhanced plant regeneration through partial desiccation of somatic embryos.

These studies have contributed to understanding the effects of a variety of medium components and culture conditions leading to enhanced plant regeneration. It is imperative to investigate other culture medium components to further enhance and expedite regeneration capacity, especially for recalcitrant date palm genotypes. The formation, development and germination of somatic embryos are controlled by basal salt composition among other known tissue culture factors. Protocols of somatic embryogenesis often use only one medium formulation during the entire process, even though this formulation may not be optimal for the various stages of the micropropagation process. A complete nutrient medium contains defined amounts of minerals, in the form of inorganic salts, essential for *in vitro* growth and development. Nevertheless, studies related to mineral nutrients have been limited and often focused on growth responses thus overlooking their role as morphogenic elicitors (Ramage and Williams, 2002). A wide range of formulations of macro-and micro-salt mixtures have been arbitrarily selected as a basal nutrient medium in different plant species. Comparative studies of various culture medium formulations on different *in vitro* culture stages of date palm are scarce. So far, two studies have been carried out on basal salt formulation of date palm tissue culture medium (Sharma *et al.*, 1980; Abo El-Nil, 1989b). Both studies neglected to mention the cultivars used, a highly influential factor controlling *in vitro* morphogenic responses. The former study involved testing callus induction from various explants and the latter addressed also studied the callus induction stage as well as somatic embryogenesis stage. The current study was conducted to assess the effects of a different set of basal salt formulations on callus growth (multiplication) and somatic embryogenesis stages using a different set of basal media. The best media formulation for each of these stages was determined. In addition, this study asserted genotypic differences in response to the various basal salt media using three commercial date palm cultivars, Khusab, Berny and Barhee.

MATERIALS AND METHODS

Explant preparation and culture establishment:

Offshoots derived from 3-year-old date palm commercial cultivars (Khusab, Berny and Barhee) obtained from a local farm were dissected to expose the shoot tip region which was excised, about 8 cm long and 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 per 100 mL solution. The tissue was then rinsed with sterile distilled water four times to remove traces of disinfectant. For preventing tissue browning, place it in a sterile chilled antioxidant solution, consisting of ascorbic acid and citric acid, 150 mg L⁻¹ each. The tissue surrounding the shoot tips was aseptically removed to expose the shoot tip terminal, about 1cm long, which was cut longitudinally into 4-8 segments to serve as explants.

Callus initiation and maintenance: Explants were cultured on a medium consisting of MS salts (Murashige and Skoog, 1962) supplemented with ingredients listed in Table 1. The medium was adjusted to pH 5.7 with 1 N KOH, dispensed in 25×150-mm culture tubes (15 ml per tube) and autoclaved for 15 min at 121°C and 1×10⁵ Pa (1.1 kg cm⁻²). Hormones and activated charcoal were added according to the requirement of each culture stage as listed in Table 2.

Table 1: Constituents of additives supplemented to the basal media formulations

Constituents	Final concentration in culture medium (mg L ⁻¹)
Additional salt	
NaH ₂ PO ₄	170
Vitamins	
Myo-inositol	125
Nicotinic acid	1
Pyridoxine hydrochloride	1
Thiamine hydrochloride	1
Glycine	2
Calcium pantothenate	1
Biotin	1
Antioxidants	
Glutamine	200
Ascorbic acid	100
Citric acid	100
Sugar and solidifying agent	
Sucrose	40,000
Agar	7,000
Hormones and activated charcoal	
2,4-D, NAA, 2-iP, kinetin	
Activated charcoal	According to the culture stage as described Table 2

Table 2: Hormones and activated charcoal supplemented to the culture media in various phases of date palm indirect somatic embryogenesis

Additive	Culture initiation	Callus induction	Embryogenic callus	Callus maintenance	Somatic embryogenesis	Rooting
2,4-Dichlorophenoxyacetic acid (2,4-D)	100 mg L ⁻¹ (453 μM)	-	-	-	-	-
2-Isopentenyladenine (2iP)	3 mg L ⁻¹ (15 μM)	30 mg L ⁻¹ (147 μM)	6 mg L ⁻¹ (30 μM)	1.5 mg L ⁻¹ (7 μM)	-	-
Naphthaleneacetic acid (NAA)	-	10 mg L ⁻¹ (54 μM)	10 mg L ⁻¹ (54 μM)	10 mg L ⁻¹ (54 μM)	-	0.1 mg L ⁻¹ (0.54 μM)
Activated charcoal	1.5 g L ⁻¹	1.5 g L ⁻¹	1.5 g L ⁻¹	-	-	-

Table 3: The chemical composition of the various basal salt media tested in date palm callus multiplication and somatic embryogenesis

Constituents	Concentration in media formulations (mg L ⁻¹)				
	MS	SH	W	NN	WPM
Ca(NO ₃) ₂ ·4H ₂ O	-	-	300	-	556
CaCl ₂ ·2H ₂ O	440	200	-	166	96
CoCl ₂ ·6H ₂ O	0.025	0.1	-	-	-
CuSO ₄ ·5H ₂ O	0.025	0.2	-	0.025	0.25
Fe ₂ (SO ₄) ₃	-	-	2.5	-	-
FeSO ₄ ·7H ₂ O	27.8	15	-	27.8	27.8
H ₃ BO ₃	6.2	5	1.5	10	6.2
KCl	-	-	65	-	-
KH ₂ PO ₄	170	-	-	68	170
KI	0.83	1	0.75	-	-
KNO ₃	1900	2500	80	950	-
K ₂ SO ₄	-	-	-	-	990
MgSO ₄ ·7H ₂ O	370	400	720	185	370
MnSO ₄	16.9	-	7	-	-
MnSO ₄ ·H ₂ O	-	10	-	25	22.3
Na ₂ EDTA·2H ₂ O	37.3	-	-	37.3	37.3
Na ₂ EDTA	-	20	-	-	-
Na ₂ MoO ₄ ·2H ₂ O	0.25	0.1	-	0.25	0.25
Na ₂ SO ₄	-	-	200	-	-
NaH ₂ PO ₄ ·2H ₂ O	170	-	-	-	-
NaH ₂ PO ₄ ·H ₂ O	-	-	16.5	-	-
NaH ₂ PO ₄	-	300	-	-	-
NH ₄ NO ₃	1650	-	-	720	400
ZnSO ₄ ·7H ₂ O	8.6	1	3	10	8.6
Reference	Murashige and Skoog 1962	Schenk and Hildebrandt (1972)	White (1963)	Nitsch and Nitsch (1969)	Lloyd and McCown (1981)

After 12 weak, explants with resultant callus attached were cultured on callus proliferation medium. After 3 weak, callus was separated from original explants and subcultured to embryogenic callus medium. After 9 weak, embryogenic callus was transferred to multiplication medium for callus maintenance. Throughout these stages, cultures were maintained in darkness at 24±3°C and transferred at a 3-week interval. Cultures maintained for 16 weak served as the source of callus for the current comparative study.

Effect of different medium formulations on callus growth: To evaluate callus growth in response to various tissue culture media formulations, five popular media salt formulations were tested including SH medium (Schenk and Hildebrandt, 1972), W medium

(White, 1963), MS medium (Murashige and Skoog, 1962), WPM (Woody Plant Medium; Lloyd and McCown, 1981) and NN medium (Nitsch and Nitsch, 1969). The composition of these basal salts is listed in Table 3. Additives were standardized in all media (Table 1). For callus growth evaluation, these media also contained 10 mg L⁻¹ NAA (53.7 μM) and 1.5 mg L⁻¹ 2iP (7.4 μM). Each cultures tube was inoculated with 0.3 g callus from the maintenance cultures. The cultures were incubated in complete darkness at 24±3°C for 8 weak during which calli were weighed and transferred to fresh media at a 4 weak interval. To determine callus growth, the calli were weighed after 4 and 8 weak.

Effect of different medium formulations on somatic embryogenesis: To evaluate somatic embryogenesis in

response to various tissue culture media formulations, calli maintained on the five media were transferred to the corresponding media formulations devoid of hormones. These cultures were maintained at $24\pm 3^\circ\text{C}$ and a 16-h photoperiod ($50\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) provided from cool-white fluorescent lamps for 8 week during which they were transferred to fresh media every 4 week. After 12 week, the regeneration percentage and number of resultant somatic embryos were recorded.

Embryo germination and plant establishment: The resultant somatic embryos were transferred to a hormone-free MS medium and incubated at $24\pm 3^\circ\text{C}$ and a 16 h photoperiod. After 8 week, the germinated embryos were transferred to MS medium containing $0.1\ \text{mg}\ \text{L}^{-1}$ ($0.54\ \mu\text{M}$) NAA and dispensed at $15\ \text{mL}$ per $25\times 120\ \text{mm}$ culture tubes. For gradual acclimatization, the plantlets were placed vertically in beakers containing a sufficient amount of water to submerge the roots, kept in the culture room covered with transparent plastic bags for 2 week. Plantlets were then transplanted into 5-cm plastic pots containing potting mix (1 soil: 1 peat moss: 1 vermiculite) and watered with $100\ \text{mg}\ \text{L}^{-1}$ Peters (20-20-20) fertilizer (Grace-Sierra). Potted plantlets were covered with plastic bags, watered as needed, uncovered gradually during the next 6 week and then transferred to a greenhouse for further growth.

Experimental design and statistical analysis: To examine callus growth, in terms of fresh weight, in response to various tissue culture media formulations, the experiment was set up as a completely randomized 2-factor factorial design with the main factors being media basal salt formulation at 5 levels and cultivar at 3 levels. Data were based on 10 replications per treatment and proportional data were based on 3 replications consisting of 10 cultures each. Transformation of proportional data was not necessary. Data pertaining to callus weight measured after 4 and 8 week, percentage of cultures exhibiting somatic embryogenesis and numbers of resultant somatic embryos were subjected to Analysis Of Variance (ANOVA). Means were separated, where appropriate, with a Least Significant Difference (LSD) at 5% significance level.

RESULTS

Callus growth: According to an analysis of variance, embryogenic callus growth measured 4 week after treatment was significantly influenced by a two-way interaction between medium formulation and cultivar

(Table 4). This means that individual cultivars responded differently to the dissimilar salt composition of the culture media. Cultivar Berny, which exhibited the lowest callus growth and subsequent plant regeneration, appeared to be unaffected by the change in salt composition (Fig. 1A), while the other two cultivars reacted differently depending upon the formulation. In cv. Khusab, SH and MS media gave the highest callus growth; however, other media used gave closely comparative results. In contrast, cv. Barhee grew best on W and MS media, while using WPM and NN media reduced callus growth.

Extending the culture duration to 8 week resulted in more callus proliferation (Fig. 1B); thus allowing an easier and higher precision comparison of differences in response. This was indicated by a stronger P-value revealed by the analysis of variance of callus growth measured after 8 week of treatment (Table 4). This suggests that although 4 week was sufficient to detect differences, discerning the efficacy of various media formulations, especially difficult to detect small differences, can be better achieved by extending the treatment to 8 week. This finding would facilitate developing a screening method to evaluate the response of the numerous date palm genotypes to various media formulations in future studies.

After 8 week culture duration cv. Berny callus grew better on SH and NN media as compared to the other formulations tested. This difference was undetectable after only 4 week of treatment. In cv. Khusab, suitability of W and WPM media ranked first, SH and MS medium ranked second and NN ranked third. Comparatively, cv. Barhee callus grew best on SH, W and MS media, while MS gave moderate growth and WPM and NN medium appeared to be least suitable. These responses more accurately depict the effect of various culture medium formulations as the differences become larger and more pronounced in comparison to observations made after 4 week of treatment.

Percentage of regeneration: Upon transfer to hormone-free regeneration medium, callus began to redifferentiate and develop somatic embryos. An important indicator of the suitability of a culture medium is the regeneration percentage, i.e., the proportion of cultures that formed somatic embryos. According to an analysis of variance (Table 4), these percentages were affected by a significant two-way interaction between the main factors, medium formulation and cultivar. This means that date palm cultivars responded differently to dissimilar media formulations, as illustrated in Fig. 1C.

Table 4: Analysis of variance of the effect of medium formulation and cultivar on callus growth after 4 and 8 weak expressed in fresh weight, percentage of regeneration and number of resultant somatic embryos. P-values less than 0.05 are significant

Factor	Callus weight after 4 weak			Callus weight after 8 weak		
	DF	MS	P Value	DF	MS	P Value
Medium	4	0.026	0.0122	4	0.091	0.012
Cultivar	2	0.518	0.0001	2	1.056	0.0001
Medium x cultivar	8	0.02	0.011	8	0.076	0.007
Error	135	0.008		135	0.027	
	-----Percentage of regeneration-----			-----Number of somatic embryos-----		
Medium	4	611.111	0.0671	4	831.083	0.0001
Cultivar	2	4862.222	0.0001	2	5188.22	0.0001
Medium x cultivar	8	759.444	0.012	8	762.878	0.0001
Error	30	248.889		135	31.287	

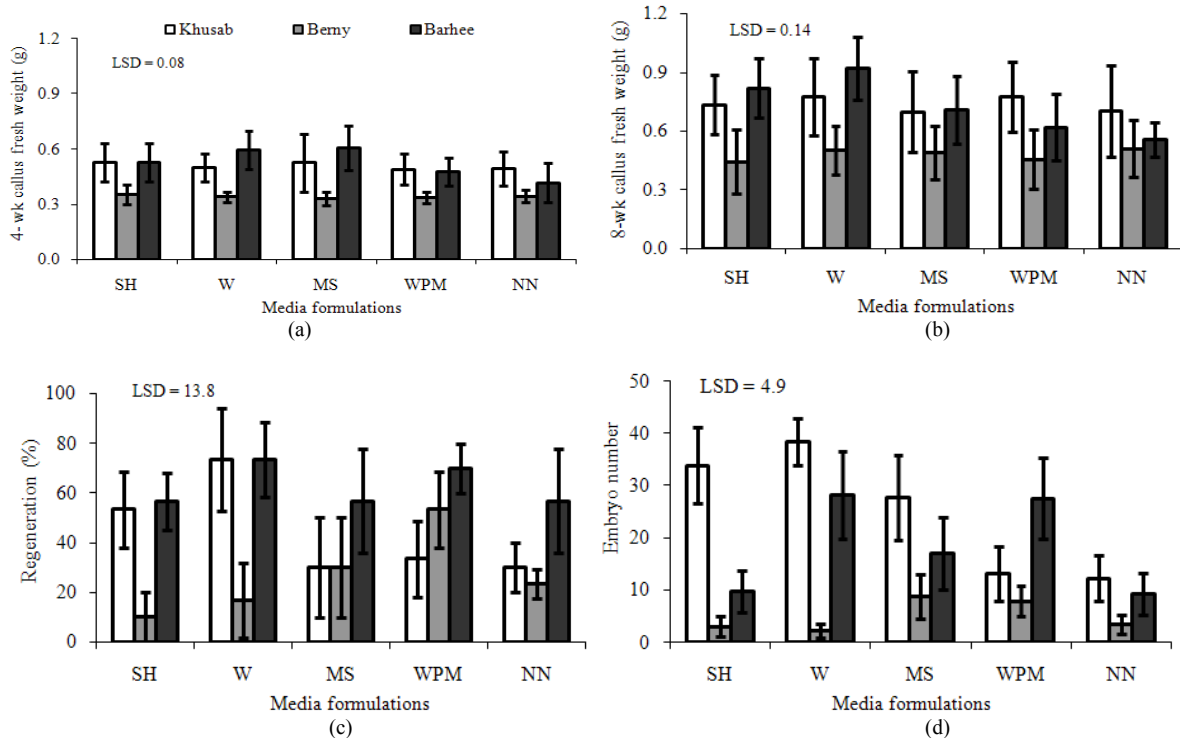


Fig. 1: Date palm cultivars to culture media formulations, SH medium (Schenk and Hildebrandt, 1972), W (White 1963), MS medium (Murashige and Skoog, 1962), WPM (Lloyd and McCown 1981) and NN medium (Nitsch and Nitsch, 1969). (a) Callus growth expressed in fresh weight after 4 weak of treatment; (b) Callus weight after 8 weak; (c) Somatic embryogenesis expressed in percentage of regeneration; (d) Number of resultant somatic embryos

In cv. Khusab, the highest percentage regeneration was achieved on W followed by SH medium, while significantly lower percentages were associated with W, MS and NN media. Conversely, cv. Bery produced highest regeneration on WPM, followed by MS and NN, while least regeneration was associated with SH and W media. Comparatively, maximum percentage of regeneration in cv. Barhee was obtained on W and WPM, followed by SH, MS and NN media. This cultivar seems to be more adaptable to the widest range of media

formulations since it sustained high regeneration percentages in all tested formulations (Fig. 1C).

Number of somatic embryos: As another indicator of the suitability of a given culture medium formulation is the number of resultant somatic embryos as a measure of the potency of somatic embryogenesis. According to an analysis of variance (Table 4), the number of resultant somatic embryos was influenced by a significant two-way interaction between medium formulation and cultivar.

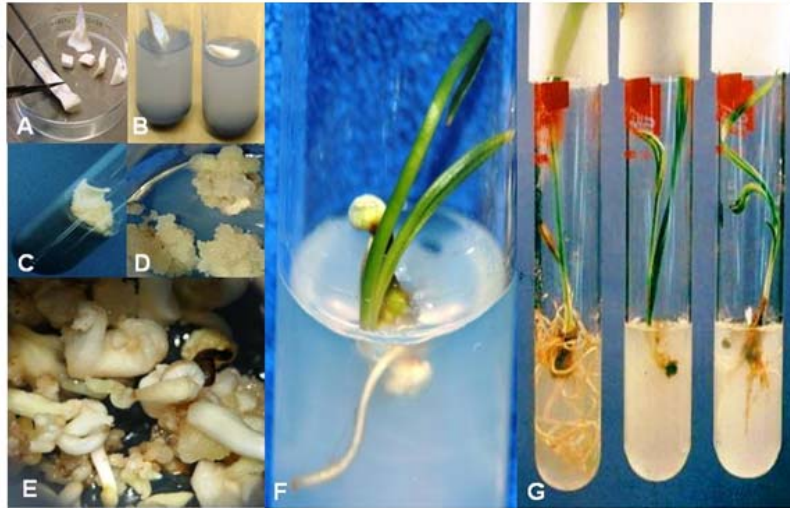


Fig. 2: Consecutive stages of date palm somatic embryogenesis: a Explant isolation from shoot tip; b Explant establishment on culture medium; c Callus induction; d Callus multiplication cultures; e Development of somatic embryos; f Germinated embryo showing shoot and root initiation, g Rooted plantlets ready for transplanting

In cv. Khusab, the highest number of resultant embryos was achieved on W closely followed by SH; MS ranked next and was moderate, while significantly lower numbers were associated with NN and W media (Fig. 1D). In contrast, cv. Berny produced the highest number of embryos on WPM and MS, while the least number of embryos was associated with NN, SH and W. Comparatively, the maximum number of embryos in cv. Barhee was obtained on W and SH media, followed by WPM, MS and NN media. It is noteworthy to mention that in most cases where the medium formulation gave high regeneration percentages, high numbers of somatic embryos also were produced as shown in Fig. 1C and D, respectively.

Embryo germination and plant establishment: The current experiment focused on the effect of various media formulations on both callus multiplication and somatic embryogenesis stages. Thus, these formulations were not tested during preceding or subsequent stages. Further experiments would be required to determine the effect of media formulations on other culture stages such as somatic embryo maturation, germination and rooting. The current study has shown that the resultant somatic embryos germinated and rooted at approximately 60 and 75%, respectively, regardless of the embryo source. The plantlets obtained exhibited phenotypically normal growth in the greenhouse. More research is required to evaluate the effect of media formulations on somatic embryo germination and root

formation. The different stages of date palm somatic embryogenesis starting from explants to plantlets are illustrated in Fig. 2.

DISCUSSION

The current study compared various media formulations to test the response of date palm genotypes during the stages of callus growth and somatic embryogenesis in order to contribute to the improvement of plant regeneration, particularly of recalcitrant date palm genotypes. Recalcitrance can be a major limiting factor for the biotechnological exploitation of economically-important plant species. Overcoming recalcitrance in difficult genotypes can be greatly assisted by optimization of the key medium components, including modifying the basal salt composition (Benson, 2000).

Although, MS medium is the most commonly used for date palm micropropagation, other basal salt formulations are occasionally used. For instance, B5 medium basal salt (Gamborg *et al.*, 1968) was employed by Sharma *et al.* (1986) for establishment of suspension culture and a combination of B5 macerals and MS microsals was used by Abul-Soad *et al.* (2008) for somatic embryo regeneration from inflorescence explants.

Studies comparing the effect of basal salts composition on various stages of date palm micropropagation are rare. A study by Sharma *et al.*

(1980) addressed this effect on the callus initiation stage. They tested explant response to several media formulations, including MS, NN, SH, S (Staritsky, 1970), AB and Y3 (Eeuwens, 1976). Their results showed that the best callus induction was obtained using medium S followed by medium Y3. The other media tested failed to form callus and none of the treatments was evaluated in relation to somatic embryogenesis.

In another study, conducted by Abo El-Nil, (1989a), different basal media including MS, SH, HE, W, ER (Ericksson, 1965) and B5 were assessed to their ability to promote callus induction and somatic embryogenesis date palm. It was concluded that callus induction was best using SH and MS media; whereas, the highest somatic embryo numbers were obtained using SH and ER media. Using SH and ER media was more effective for the induction of somatic embryogenesis as compared to MS and B5 media. Both of the preceding investigations, however, omitted the name of cultivars tested and callus multiplication stage was not assessed.

The present study indicated that the *in vitro* response of date palm to the basal salt formulations was significantly related to the genotype in addition to culture stage. The results obtained clearly show that the best medium for callus growth differed from that best supported somatic embryo formation depending on the cultivar. The best callus growth in cv. Berny occurred on SH and NN, cv. Khusab on W and WPM media and cv. Barhee on SH, W and MS media (Fig. 1B). These differences illustrate cultivar specificity of basal salt requirements. Similarly, (Jain *et al.*, 1989) found that genotype and culture medium are critical factors for the production of embryogenic callus in slash pine (*Pinus elliotii*).

In addition, the data have shown that the best basal medium for callus growth differed from that required for optimum somatic embryogenesis. The highest number of somatic embryos occurred in cv. Khusab using W medium, cv. Berny using WPM and MS and cv. Barhee using W and SH (Fig. 1D). These differences highlight the importance of considering the roles of genotype specificity and culture stage in relation to the basal salt requirements when optimizing plant micropropagation schemes. Because of the well recognized role of the hormonal content in modifying *in vitro* activities, it is relevant to note that the hormonal content used by Abo El-Nil (1989b), 50 μ M NAA for callus induction and 5 μ M NAA for somatic embryogenesis, differed from those used in the current study, 53.7 μ M NAA and 7.4 μ M 2iP for callus multiplication and no hormones for somatic embryogenesis media.

The current study adds to the mounting evidence of the variable influence of different culture mineral formulations on *in vitro* growth and morphogenesis demonstrated in a number of plant species (Fisichella *et al.*, 2000; Nas and Read, 2004; Sul and Korban, 2004; Andreu and Marin, 2005). The best salt formulation varies among plant species. In rice, Khatun *et al.* (2003) found the best callus induction on MS medium as compared to N6 (Chu *et al.*, 1975). The effect of different culture media formulations on the formation of embryo was demonstrated by Bonga (2004) in the European larch tree, *Larix decidua* where the best medium found was AFC medium (Klimaszewska *et al.*, 2001), a medium low in NH_4 , NO_3 , Mg and SO_4 but high in PO_4 . Using nodal explants of rose (*Rosa* sp.), MS medium resulted in somatic embryo production whereas WP medium did not (Estabrooks *et al.*, 2007). Among the different basal media tried, including MS, B5, LS (Linsmair and Skoog, 1965) and W, MS medium, was found the most suitable for callus induction and plant regeneration in Indian cotton cultivars (Tripathy and Reddy, 2002). The basal salt formulation may influence callus quality in relation to plant regeneration capacity. In a study conducted by Rakshit *et al.* (2010), immature embryos of maize (*Zea mays* L.) explants grown on MS medium showed the highest frequency of callusing. However, the best callus quality in term of frequency of organogenic callus was obtained from explants grown on N6 medium.

Although, some tissue culture systems involved different basal salt formulations for different culture stages (Jha *et al.*, 1992), often only one formulation is used for the entire duration of culture systems even though this formulation may be suboptimal for the different stages of growth and development (Ramage and Williams, 2002). In a study to evaluate their ability to support the establishment of callus culture in the medicinal plant *Phyllanthus stipulatus*, Catapan *et al.* (2001) tested several basal salt formulations including MS, B5, AR (Anderson, 1978), W, SH, KM (Kao and Michayluk, 1975) and WPM media. They observed shoot initiation in the various salt formulations tested; however, rhizogenesis was completely inhibited except on MS and WPM; whereas, callus initiation occurred only on the MS formulation. The authors suggested that the poor performance of B5, SH, W and KM may have been due to their low ammonium content as compared to MS. These results suggest that the optimal salt formulation for a particular culture stage may not be the best for the other culture stages. The present study has shown that media formulations that were observed to be most suitable for callus growth of date palm do not necessarily coincide with optimal formulations for

somatic embryogenesis. This illustrates that various culture stages may be affected differently by the formulation of the basal media. This was also demonstrated by Capuana *et al.* (2007) in common ash (*Fraxinus excelsior* L.) where the best proliferation of embryogenic tissue was obtained when the material was subcultured on MS medium; whereas, WPM medium appeared to be more conducive to faster embryo maturation. In a study on the induction potential of somatic embryos in *Eucalyptus globulus* involving MS, B5, WPM and DKW (Driver Kuniyuki Walnut medium; Driver and Kuniyuki, 1984), Pinto *et al.* (2008a) demonstrated that MS and B5 media were the best. In a subsequent study, Pinto *et al.* (2008b) found that MS medium and B5 were equally effective in the formation of secondary globular somatic embryos; however, MS was more efficient for the germination stage as compared to B5 medium. In the same way, Fisichella *et al.* (2000) reported that MS medium appeared to be the most favorable for somatic embryo formation in quince (*Cydonia oblonga* Mill.) as compared to W, WPM, DKW, QL (Quoirin and Lepoivre, 1977), SH, B5 and NN. However, root formation was higher on WPM and SH media.

The current study demonstrated that the response of different genotypes to different formulations varies, indicating an interaction between medium formulation and genotype. The phenomenon of genotype dependency in response to *in vitro* date palm manipulations is well documented in various plant species (Karami, 2008; Shirani *et al.*, 2010) as well as date palm (Al-Khayri and Al-Bahrany, 2004). The results have shown that the effect of basal media formulations can be influenced by various date palm genotypes. Analogous to this finding, several investigations have shown variable responses of different cultivars to different media formulations in a number of plant species (Khanna and Raina, 1998; Perez-Tomero and Burgos, 2000; Khatun *et al.*, 2003). In oil palm (*Elaeis guineensis* Jacq.), the highest embryogenic callus formation from zygotic embryo explants, somatic embryogenesis and germination were achieved using N6 medium as compared to MS medium (Thuzar *et al.*, 2010). However, other cultivars of oil palm exhibited the best responses using Y3 medium as compared to N6 and MS media (Muniran *et al.*, 2008). Rafiq *et al.* (2005) observed interactions between media and different maize (*Zea mays* L.) genotypes in a study involving callus induction and plant regeneration using various media formulations including MS, LS, B5 and N6. Gonzalez *et al.* (2001) demonstrated a strong influence of genotype on callus induction and plant regeneration from immature embryos of durum wheat

Triticum turgidum Desf. Similarly, the influence of genotype and media combination on callus growth, somatic embryo regeneration and root formation was also observed in quince *Cydonia oblonga* Mill. (Fisichella *et al.*, 2000).

CONCLUSION

Although most of the published protocols of date palm micropropagation use the same medium during the entire process, the results reported here emphasize the importance of the salt content of the culture medium on the different somatic embryogenesis stages. Micropropagation systems can be customized to accommodate the stage specificity requirements by switching between optimal basal media according to the culture stage. This study also illustrated the interaction of medium formulation and genotype. It resulted in an effective protocol for assessment of various tissue culture parameters in response to altering medium components, or other culture conditions. The protocol was useful to detect cultivar differences and determine the optimal media formulations suitable for callus and somatic embryogenesis stages of three date palm cultivars. This investigation has shown that accurate assessments of responses to medium manipulation must consider the specific requirement of various culture stages as well as the genotypic-dependent differences. The assessment procedures developed in this study and the resultant information should assist future research aimed at identifying optimum medium formulation for the numerous date palm cultivars, particularly the recalcitrant genotypes. Further studies are necessary to determine the effect of media formulations on the germination of somatic embryo and rooting parameters.

ACKNOWLEDGMENT

This study was funded by a research grant (Project no. SABIC-504) provided by the Deanship of Scientific Research, King Faisal University.

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