ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 11 (6): 318-325, 2012
ISSN 1682-296X / DOI: 10.3923/biotech.2012.318.325

Effect of Abscisic Acid and Polyethylene Glycol on the Synchronization of Somatic Embryo Development in Date Palm (*Phoenix dactylifera* L.)

Jameel M. Al-Khayri and Abdulaziz M. Al-Bahrany
Department of Agricultural Biotechnology, College of Agriculture and Food Sciences,
King Faisal University, P.O. Box 420, Al-Hassa 31982, Saudi Arabia

Abstract: Somatic embryogenesis provides the best method for commercial micropropagation of date palm (*Phoenix dactylifera* L.); however, a current limitation is the lack of synchronization of developing somatic embryos. The objective of this study was to evaluate the effect of Abscisic Acid (ABA) and polyethylene glycol (PEG) combinations on the synchronization of embryo development in date palm cell suspension. Callus maintained on MS medium containing 54 μM Naphthalene Acetic Acid (NAA) and 7 μM 2-isopentenyladenine (2iP) was transferred to regeneration liquid medium supplemented with ABA at 0-100 μM and PEG at 0-15%. Maximum fresh culture weight was obtained with 10% PEG in the absence of ABA. The addition of as low as 1 μM ABA to the suspensions inhibited growth. In the absence of ABA, increasing PEG concentration increased total somatic embryo numbers reaching a maximum number at 10% PEG. Various embryo sizes differing in abundance were associated with different treatments. The highest percentage of medium size embryos, 52%, was obtained at 10 μM ABA; whereas, the highest percentage of small embryos was obtained at 50-100 μM ABA. The small embryos exhibited a state of synchronization. Although, treating suspensions with ABA and PEG affected embryo size distribution, germination was influenced by embryo developmental phase, expressed in size. Germination of 43, 63, 52 and 23% was obtained from the small, medium, large and very large embryos, respectively. Retardation of somatic embryo development caused by ABA can be further exploited to optimize culture synchronization.

Key words: Callus, *in vitro*, micropropagation, somatic embryogenesis, synchronization, tissue culture

INTRODUCTION

The *in vitro* role of plant growth regulators, in particular Abscisic Acid (ABA); a stress plant hormone, is not well understood (Jimenez, 2001). Nonetheless, ABA was found to modify *in vitro* growth and development. It has been suggested that the action of ABA may be exerted through regulation of certain genes involved in desiccation and maturation phases of somatic embryogenesis (Hatzopoulos *et al*., 1990). Senger *et al*. (2001) demonstrated the importance of ABA in the formation of preglobular embryonic structures. Other researchers reported that ABA stimulated somatic embryo maturation and germination (Goebel-Tourand *et al*., 1993), increased the number of somatic embryos (Bell *et al*., 1993; Nishiwaki *et al*., 2000, Fernando *et al*., 2004) and enhanced embryo tolerance to desiccation (Angoshtari *et al*., 2009).

In date palm a few studies were encountered involving the ABA effect on date palm *in vitro* culture. Zouine *et al*. (2005) found that the addition ABA to the culture medium improved the production and maturation of date palm somatic embryos and increased accumulation of sugars and storage proteins. Hassan *et al*. (2008) tested the effect of ABA concentration during the rooting stage of date palm plantlets where they found that ABA significantly increased root formation and growth but reduced shoot length. Adding ABA to the culture medium was found to increase the proliferation rate and protein content of date palm somatic embryos (Sghaier *et al*., 2009; Sghaier-Hammami *et al*., 2010).

Another stress-related compound is Polyethylene Glycol (PEG), a stress-inducing osmoticum occasionally included in culture medium to simulate *in vitro* drought stress, was also observed to exert modifications on somatic embryogenesis in some plant species (Von Arnold *et al*., 1996; Capuana and Debergh, 1997; Denchev *et al*., 1991; Li *et al*., 1997; Viji *et al*., 2012).

In relation to date palm tissue culture, a previous report has demonstrated increased survival during acclimatization by culturing *in vitro* plantlets in liquid medium containing PEG for a week prior to soil transfer

Corresponding Author: Jameel M. Al-Khayri, Department of Agricultural Biotechnology, College of Agriculture and Food Sciences, King Faisal University, P.O. Box 420, Al-Hassa 31982, Saudi Arabia Tel: +966504803962

(Zaid and Hughes, 1995a, b). Other studies have shown that incorporating PEG in the culture medium reduced hyperhydration and enhanced maturation and germination of date palm somatic embryos (Al-Matar *et al*., 1997; Al-Khateeb, 2006). The use of PEG as a selection agent for drought tolerance in date palm *in vitro* cultures was demonstrated by El-Sharabasy *et al*. (2008). Adding PEG to date palm callus cultures elicits increased accumulation of proline as an indication of osmotic stress (Al-Khayri and Al-Bahrany, 2004a). Moreover, PEG is considered an important component of the cryoprotectant solution of date palm tissue *in vitro* cultures (Bekheet *et al*., 2007; Bekheet, 2011).

The use of an osmoticum like PEG along with ABA, both stress-related agents, have been found to influence on *in vitro* growth and differentiation in a number of plant species including pinus (Ishii *et al*., 2008), chestnut (Calic-Dragosavac and Radojevic, 2010), mango (Mishra *et al*., 2010), walnut (Ali *et al*., 2010; Sirmandi *et al*., 2010), avocado (Marquez-Martin *et al*., 2011), grape (Mulwa *et al*., 2010) and tea (Suganthi *et al*., 2012). Although numerous factors relevant to date palm somatic embryogenesis have been investigated (Al-Khayri, 2013), studies related to the effect of ABA in combination with PEG on somatic embryogenesis of date palm have not been reported. The objective of this study was to evaluate the effect of Abscisic Acid (ABA) and Polyethylene Glycol (PEG) combinations on the synchronization of embryo development in date palm cell suspension.

MATERIALS AND METHODS

Plant material preparation: This research project was conducted at King Faisal University during the period February 01, 2011 to August 29, 2012. Shoot tips excised from 3-year-old date palm offshoots, cv. Nabout Saif, were used as a source of explants. To prevent browning, excised shoot tips were placed in a chilled antioxidant solution containing ascorbic acid and citric acid, 150 mg L^{-1} each. Shoot tips, about 8 cm long, were surface disinfected in 70% ethanol for 1 min, followed by 15 min in 1.6% w/v sodium hypochlorite (30% v/v Clorox, commercial bleach) containing 0.1 ml of Tween 20 (Sigma Chem. Co., St. Louis, MO) per 100 mL disinfection solution. The tissues were rinsed with sterile distilled water four times and placed in a sterile antioxidant solution. Explants including 0.5 cm sections of the shoot tip meristematic region and the surrounding leaf primordia were cultured on the surface of solidified culture medium and incubated in complete darkness at 24±3°C.

Culture medium: The culture medium consisted of Murashige and Skoog (1962) basal salt medium containing (per liter) 170 mg NaH_2PO_4, 125 mg myo-inositol, 200 mg glutamine, 1 mg nicotinic acid, 1 mg pyridoxine-HCl, 5 mg thiamine, 30 g sucrose and 7 g agar (purified Agar-agar/Gum agar) (Sigma). Hormonal supplements and activated charcoal were added to the medium according to the culture stages. Culture initiation medium contained 453 μM 2,4-dichlorophenoxyacetic acid (2,4-D), 15 μM 2-isopentenyladenine (2iP) and 1.5 g L^{-1} activated charcoal. After 12 week, during which the entire explants were transferred to a fresh medium at 3 week intervals, resultant callus along with original explants were transferred to callus proliferation medium containing α-naphthaleneacetic acid 54 μM NAA, 147 μM 2iP and 1.5 mg L^{-1} activated charcoal. After an additional 3 week, the callus was separated and transferred to a medium containing 54 μM NAA, 30 μM 2iP and 1.5 mg L^{-1} activated charcoal. After 9 week (three subcultures), embryogenic callus was transferred to maintenance medium containing 54 μM NAA and 7 μM 2iP. Media were adjusted to pH 5.7 with 1 N KOH, dispensed in test tubes (15 mL per tube) or GA-7 Magenta vessels (50 mL per vessel) and autoclaved for 15 min at 121°C and 1.1 kg cm^{-2}.

Treatment with ABA and PEG: Callus obtained from maintenance cultures was segmented and 0.75 g placed in 125 mL culture flasks containing 25 mL liquid MS medium as described above. The medium was supplemented with Abscisic Acid (ABA) at 0, 1, 10, 50 and 100 μM in combinations with polyethylene glycol-8000 (PEG) at 0, 5, 10 and 15% (w/v). These cultures were maintained on a rotary shaker set at 100 rpm and 24±3°C under 16 h photoperiods (50 μmol m^{-2} sec^{-1}) provided by cool-white fluorescent lamps. At 2 week intervals, half of the liquid medium was replaced using a pipette after allowing the suspension to settle to the bottom of the flask. After 6 week, culture fresh weight was determined to assess the effect of ABA and PEG on culture growth. The cultures were maintained for additional 8 week, after which the resultant somatic embryos were counted and measured. Based on the different consecutive developmental stages observed, these immature embryos were categorized into four size groups. The groups were designated small referring to <3 mm globular embryos, medium referring to 3-5.9 mm bipolar embryos, large referring to 6-8 mm elongated bipolar embryos with defined shoot and root poles and very large referring to >8 mm embryos with elongated root pole. To observe the consequential effect of ABA and PEG treatments on germination, the resultant embryos were cultured on hormone-free solid MS medium at 24±3°C and a 16 h photoperiod.

Experiment design and data analysis: The experiment utilized a completely randomized factorial design with 2 main factors, ABA and PEG concentrations, at 5 and 4 levels, respectively. The experiment consisted of 20 treatments replicated 6 times. The data pertaining to fresh weight and total embryo numbers were subjected to analysis of variance (ANOVA) and the means were separated, where appropriate, with a Least Significant Difference (LSD) at 5% significance level. The percentages of embryos that germinated from each size group in response to the ABA and PEG treatments were calculated based on the total embryos regenerated in that particular treatment. This germination test excluded treatments that regenerated a total of less than 10 somatic embryos of a given size group.

RESULTS

Callus growth: A common observation in date palm is that callus continues to proliferate and increases in mass following transfer to regeneration medium and then redifferentiation begins marked by the development of globular somatic embryos. Observations in the present research indicated that this increase in mass was affected by a significant two-way interaction between ABA and PEG as shown by the ANOVA, where p-values less than 0.05 are significant (Table 1). In the absence of ABA, generally growth stimulation was noted in response to increasing PEG concentration up to 10% after which growth began to decline. However, this stimulation was significant only at 10-15% PEG as compared to the control (Fig. 1).

The effect of PEG was modified by the addition of ABA as indicated by a significant two-way interaction between ABA and PEG ($p = 0.007$). As shown in Fig. 1, when ABA was either omitted or added at a low concentration, 1 μM, generally increasing PEG concentration was associated with growth stimulation expressed in increased tissue fresh weight (Fig. 1). Maximum growth was obtained with 10% PEG in the absence of ABA. However, when 1 μM ABA was added, 15% PEG gave the highest fresh weight. As ABA concentration reached 10 μM, an inverse relationship between the growth and PEG concentration was observed. At higher ABA levels, 50-100 μM, increasing PEG concentration had little or no effect on growth modification. Conversely, in the absence of PEG, the addition of 1-100 μM ABA caused similar fresh weight reductions; however, in the presence of PEG, growth inhibition varied depending upon the ABA concentration.

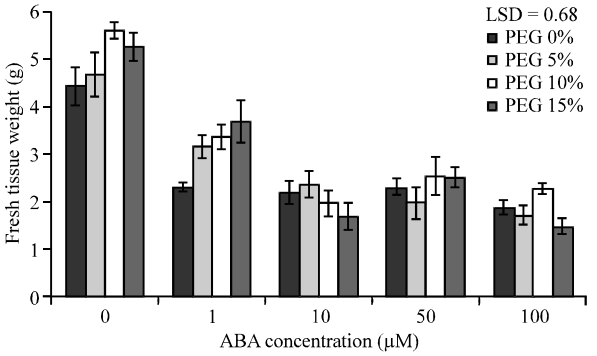

Fig. 1: The effect of ABA and PEG on fresh weight of date palm suspension cultures

Table 1: Analysis of abscisic acid (ABA) and polyethylene glycol (PEG) effect on date palm callus culture growth and somatic embryogenesis

		Culture growth		Somatic embryogenesis	
Source	df	MS	p-value	MS	p-value
ABA concentration	4	40.3935	0.0001*	52835	0.0001*
PEG concentration	3	1.7752	0.0029*	3791	0.0001*
ABA×PEG	12	1.1326	0.0007*	1112	0.0006*
Error	100	0.3558		343	

*Significant at 5%

The extent of growth reduction was a function of ABA concentration. The addition of as low as 1 μM ABA significantly reduced tissue fresh weight. As the concentration of ABA increased to 10 μM, further growth inhibition occurred; however, beyond this concentration growth became invariable (Fig. 1). This suggests that the minimum ABA concentration that elicits response in date palm *in vitro* culture is 1 μM; however, 10 μM is considered the critical concentrations for the purpose of future physiological studies related to stress and selection of somaclonal variants.

Total somatic embryo number: Callus began differentiating into globular embryos, 4 to 6 week after introduction to the regeneration medium. The extent of development of the globular somatic embryos was dependent upon the treatment. The total number of resultant somatic embryos was affected by a significant two-way interaction between ABA and PEG concentrations supplemented to the regeneration medium as indicated by ANOVA (Table 1). The data have shown that in the absence of ABA, increasing PEG concentration was associated with increased development of globular somatic embryos reaching a maximum number at 10% PEG (Fig. 2).

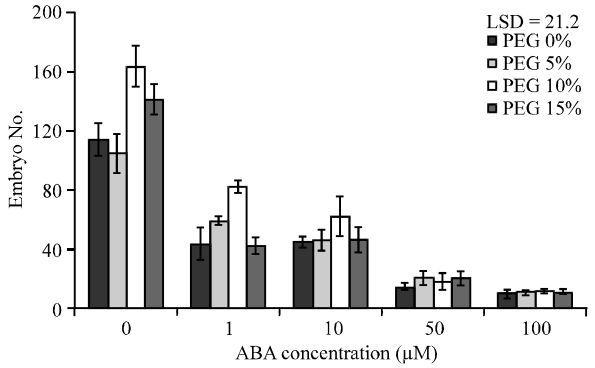

Fig. 2: The effect of adding ABA and PEG to the regeneration medium on the total number of date palm somatic embryos

Somatic embryo size distribution: It is worth noting that in any given culture not all somatic embryos formed concurrently. Therefore, at the end of the incubation period, the embryos exhibited developmental stage resulting in different sizes. The largest somatic embryo size obtained ranged from 9-17 mm which is comparable to the size range of the fully mature zygotic embryos, 10-15 mm. Furthermore, the embryo size was influenced by the ABA and PEG treatments, indicating a possible role for these compounds on the elongation of date palm somatic embryos.

In the absence of ABA, increasing PEG concentration to 5% was associated with higher a percentage of small somatic embryos as compared to the control (Table 2). This PEG concentration was also associated with the highest percentage of small embryos when 1 µM ABA was added to the suspension cultures. At 10 µM ABA, the highest small-size embryos were associated with 15% PEG. The differences in the percentage of small embryos as a result of PEG disappeared when the ABA supplement was increased to 50-100 µM.

Omitting ABA allowed more than 50% of the resultant somatic embryos to further develop and enlarged to medium, large and very large sizes (Table 2). For instance when the embryos were regenerated on a medium free of ABA and PEG, the size distribution of the resultant somatic embryos were 39% small, 22.3% medium, 21.3% large and 17.4% extra large; however, when 100 µM ABA was incorporated into the culture medium this distribution changed to mostly small size (96.2%) with a negligible proportion of the other size classes. This indicates that the majority of somatic embryos that developed in the presence of ABA were arrested at the small globular stage, restricting elongation particularly at high ABA levels. Elongation inhibition was very pronounced as the concentration of ABA reached 50-100 µM where over 90% of embryos remained in the small size category (Table 2). At these high ABA levels, less than 10% of the embryos developed into the medium-size category and none exhibited large or very large sizes. The highest percentage of embryos that developed to a medium size, 52%, was obtained when ABA was 10 µM especially in the absence of PEG; whereas the highest percentage of small embryos was obtained at 50-100 µM ABA.

Table 2: Date palm somatic embryos in each class size response to ABA and PEG concentrations supplemented to the regeneration medium

Treatment		Somatic embryo (%)			
ABA (µM)	PEG (%)	Small (<3 mm)	Medium (3-5.9 mm)	Large (6-8 mm)	Extra large (>8 mm)
0	0	39.0±7.4	22.3±3.9	21.3±7.9	17.4±7.8
0	5	46.6±9.0	22.0±6.6	17.6±4.1	13.8±6.6
0	10	13.6±3.3	38.3±9.1	34.8±8.7	13.3±1.3
0	15	23.3±6.3	26.4±8.6	29.9±6.3	20.3±1.6
1	0	54.9±7.4	39.0±9.1	6.3±2.2	0.0±0.0
1	5	75.1±5.2	17.3±2.7	7.5±4.9	0.0±0.0
1	10	62.0±8.8	34.3±8.5	3.7±1.1	0.0±0.0
1	15	62.4±5.1	33.1±1.3	4.5±2.1	0.0±0.0
10	0	45.3±5.1	52.0±2.2	2.7±0.7	0.0±0.0
10	5	52.9±9.8	47.1±4.9	0.0±0.0	0.0±0.0
10	10	59.1±9.3	40.9±7.9	0.0±0.0	0.0±0.0
10	15	61.5±5.7	38.8±1.8	0.0±0.0	0.0±0.0
50	0	91.9±3.5	8.1±0.6	0.0±0.0	0.0±0.0
50	5	94.3±8.5	5.7±0.6	0.0±0.0	0.0±0.0
50	10	92.2±5.7	7.8±0.7	0.0±0.0	0.0±0.0
50	15	93.2±7.7	6.8±1.5	0.0±0.0	0.0±0.0
100	0	96.2±5.0	4.1±0.5	0.0±0.0	0.0±0.0
100	5	93.7±2.5	5.9±0.3	0.0±0.0	0.0±0.0
100	10	93.8±0.9	5.8±1.9	0.0±0.0	0.0±0.0
100	15	93.1±6.5	6.4±1.8	0.0±0.0	0.0±0.0

Values are Mean±SD

Germination of somatic embryos: Viability of the resultant somatic embryos was assessed based on germination capacity and subsequent plantlet formation on agar medium devoid of ABA and PEG. Treating suspension cultures with ABA and PEG during the embryogenesis was observed to affect embryo size distribution but not the germination capacity. Embryo germination capacity was influenced mainly by the final embryo size (Table 3). The embryos categorized as small exhibited 27.5-52.5% germination (average 43%), whereas medium category germinated at 55-72.5% (average 63%). Large embryos germinated at 42.5-60% (average 52%) while extra large embryos germinated the least ranging from 17.5-27.5% (average 23%).

Table 3: Percentage of date palm somatic embryos germinated in response to ABA and PEG different concentrations

Treatment		Somatic embryo (%)			
ABA (μM)	PEG (%)	Small (<3 mm)	Medium (3-5.9 mm)	Large 6-8 mm	Extra large (>8 mm)
0	0	52.5±15.2	67.5±14.8	42.5±12.3	20.0±11.2
0	5	50.0±29.4	55.0±16.6	57.5±19.2	27.5±10.1
0	10	55.0±25.1	70.0±14.1	60.0±18.7	25.0±12.9
0	15	40.0±23.6	72.5±13.0	47.5±14.3	17.5±14.2
1	0	45.0±19.5	60.0±17.3	-	-
1	5	37.5±18.2	52.5±16.4	-	-
1	10	38.5±15.1	61.5±15.8	-	-
1	15	37.5±12.6	70.0±17.1	-	-
10	0	47.5±14.8	60.0±15.8	-	-
10	5	27.5±17.1	70.0±12.2	-	-
10	10	42.5±13.3	57.5±13.1	-	-
10	15	45.0±16.5	62.5±17.9	-	-
50	0	50.0±29.4	-	-	-
50	5	57.5±20.6	-	-	-
50	10	35.0±17.3	-	-	-
50	15	45.0±16.1	-	-	-
100	0	55.0±18.5	-	-	-
100	5	37.5±14.6	-	-	-
100	10	32.5±14.4	-	-	-
100	15	42.5±13.4	-	-	-

-: These treatments regenerated a total of less than 10 somatic embryos thus excluded from this germination test, Values are Mean±SD

DISCUSSION

In a related study, Al-Khayri and Al-Bahrany (2004a) found that when PEG was added to the callus maintenance medium, instead of the embryo initiation medium as was done in the current study, 10% PEG decreased the relative growth rate and consequently callus fresh weight was reduced. This suggests that PEG effect may vary depending on the culture stage and may be influenced by the hormonal content in the medium. In the same study, it was shown that adding PEG at increasing concentrations to callus maintenance cultures caused increased proline accumulation as an indicator of stress. The observed stimulatory PEG effect on date palm somatic embryogenesis may be attributed to the endogenous accumulation of proline, a major constituent of cell wall glycoprotein and may have a morpho-regulatory function. This is further supported by the fact that exogenous proline has been shown to promote somatic embryogenesis in some plant tissue culture systems (Armstrong and Green, 1985; Ronchi *et al*., 1984; Chowdhury *et al*., 1993; Murch *et al*., 1999). In *Medicago falcata* and *M. sativa*, Denchev *et al*. (1991) found that 2.5% PEG 6000 promoted embryo development from globular up to torpedo stage and stimulated the development of single viable embryos. Similarly, Viji *et al*. (2012) reported enhanced somatic embryogenesis in pigeonpea *Cajanus cajan* in response to incubating explants for 4 h in 4% PEG-containing medium. In the current study, 10% PEG was associated with the highest somatic embryo number obtained especially when combined with 1-10 μM ABA.

In a study, where the effect of ABA on date palm somatic embryos maturation was examined, 1-10 μM ABA was found to improve maturation, germination and *ex vitro* survival (Zouine *et al*., 2005). In *Medicago falcata* and *M. sativa*, Denchev *et al*. (1991) achieved optimal maturation of torpedo stage embryos on 30 μM ABA. In the current study where ABA was incorporated at the beginning of the regeneration stage, i.e., prior to embryo development, the addition of as low as 1-10 μM significantly reduced the total number of regenerated embryos (Fig. 2). Incorporation of 50-100 μM ABA induced further inhibited somatic embryos. In contrast, Fernando and Gamage (2000) found that incorporating 2.5-5 μM ABA in the regeneration medium for the first 5 weeks enhanced the production of somatic embryos of *Cocos nucifera*. A similar enhancement was observed in carrots (Nishiwaki *et al*., 2000; Ogata *et al*., 2005). Liu (2011) added 1 mg L^{-1} ABA to the culture medium to induce the germination of somatic embryos and the regeneration of sweet potato.

The literature indicates that the addition of ABA and PEG, combined or individually, has been shown to either enhance or inhibit somatic embryogenesis depending upon the plant species. Kikuchi *et al*. (2006) suggested that the induction of carrot somatic embryogenesis is caused not only by the presence of ABA but also by physiological processes directly linked by stress. Moreover, they found that somatic embryogenesis was not improved by the application of ABA in the absence of osmotic stress agent. Concurrently, both ABA and PEG were found to exert some influence on date palm somatic embryogenesis. Working on *Camellia* species, Suganthi *et al*. (2012), where 5 mg L^{-1} ABA combined with 3% PEG was used, observed increased numbers of somatic embryos. In line with the current results, Yadollahi *et al*. (2011) concluded that the use of 40 μM ABA in SE induction medium reduced the mean number of somatic embryos of rapeseed (*Brassica napus*), however the percentage of mature embryos increased. Moreover, the combined use of PEG with or without ABA reduced the mean number of somatic embryos but significantly enhanced percentages of mature somatic embryos.

The current study has clearly shown that osmotic stress induced by PEG was involved in promoting somatic embryogenesis of date palm. Furthermore, ABA was found to exert an inhibitory effect on date palm somatic embryogenesis. This inhibition was partially alleviated by the incorporation of the osmotic stress but only when

ABA was used at a low concentration, 1 μM. This suggests an interaction between the roles of ABA and PEG on somatic embryogenesis. Other investigators have noticed this relationship as well (Von Arnold *et al*., 1996; Capuana and Debergh, 1997; Denchev *et al*., 1991; Li *et al*., 1997).

The current study has shown reduction in the number of somatic embryos in response to adding ABA at the beginning of the regeneration stage, before somatic embryo formation commences. However, Zouine *et al*. (2005) has noticed improved date palm somatic embryo maturation and germination when ABA was supplied after somatic embryo development. This suggests that the action of ABA depends on the stage of somatic embryo development.

Although ABA reduced the number of somatic embryos formed, it also exerted control on the developmental progress. In the presence of ABA, the resultant somatic embryos were arrested at the early developmental stage. These cultures were characterized by uniformly small globular embryos that failed to develop further in response to ABA. This unique characteristic can be utilized to enhance synchronization in date palm somatic embryogenesis systems, since current protocols often lack embryo synchronization. Embryo synchronization is of particular importance for commercial micropropagation as well as for synthetic seeds production and physiological *in vitro* studies. Synchronization of somatic embryos was achieved by adding ABA to the regeneration medium in other systems such as celery (Nadel *et al*., 1990; Osuga *et al*., 1999) and sweet potato (Torres *et al*., 2001).

CONCLUSION

The current study has determined the growth response of date palm suspension culture and assessed somatic embryogenesis capacity in relation to ABA and PEG when supplemented to the regeneration medium. The effect of ABA was shown to be inhibitory to somatic embryogenesis. The addition of PEG was found to enhance somatic embryogenesis and modify the inhibitory action of ABA. The incorporation of ABA, however, induced synchronization in embryo size as it arrested the embryo development at the globular stage. This observation merits further investigation to achieve optimum synchronization of embryo development in date palm suspension cultures. Responses to ABA and PEG were found to vary depending upon culture stage and embryo developmental phase. Because date palm genotypes react differently to media modifications (Al-Khayri and Al-Bahrany, 2004b; Al-Khayri, 2010, 2011), further investigations are essential to address these factors in different date palm cultivars.

ACKNOWLEDGMENTS

This study was funded by a research grant (Project No. 120070) provided by the Deanship of Scientific Research, King Faisal University. The authors thank Dr. Dennis V. Johnson, Emeritus Professor, Ohio, USA, for reading and commenting on this manuscript.

REFERENCES

Al-Khateeb, A.A., 2006. Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) cv. Sukary in response to sucrose and polyethylene glycol. Biotechnology, 5: 466-470.

Al-Khayri, J.M., 2010. Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) improved by coconut water. Biotechnology, 9: 477-484.

Al-Khayri, J.M., 2011. Basal salt requirements differ according to culture stage and cultivar in date palm somatic embryogenesis. Am. J. Biochem. Biotechnol., 7: 32-42.

Al-Khayri, J.M., 2013. Factors Affecting Somatic Embryogenesis in Date Palm (*Phoenix Dactylifera* L.). In: Somatic Embryogenesis and Genetic Transformation in Plants, Aslam, J., P.S. Srivastava and M.P. Sharma (Eds.). Narosa Publishing House, New Delhi, pp: 15-38.

Al-Khayri, J.M. and A.M. Al-Bahrany, 2004a. Growth, water content and proline accumulation in drought-stressed callus of date palm. Biol. Plant, 48: 105-108.

Al-Khayri, J.M. and A.M. AL-Bahrany, 2004b. Genotype-dependent *in vitro* response of date palm (*Phoenix dactylifera* L.) cultivars to silver nitrate. Sci. Hortic., 99: 153-162.

Al-Matar, S.M., M.M. Abo El-Nil, J. Al-Khayri and G. Klingaman, 1997. Effect of sucrose and PEG concentration on embryo maturation and hyperhydration in date palm suspension cultures. HortScience, 32: 462-462.

Ali, S.B.G.M., V. Kouroshl, B.S. Hassan, K. Siamak and L. Charles, 2010. Enhancement of maturation and germination of somatic embryos in Persian walnut (*Juglans regia* L.) using osmolites, hormones and cold treatments. Afr. J. Food Sci., 4: 735-743.

Angoshtari, R., R. Tavakkol Afshari, S. Kalantari and M. Omidi, 2009. Effects of abscisic acid on somatic embryogenesis and induction of desiccation tolerance in *Brassica napus*. Asian J. Plant Sci., 8: 276-284.

Armstrong, C.L. and C.E. Green, 1985. Establishment and maintenance of friable embryogenic maize callus and the involvement of L-proline. Planta, 164: 207-214.

Bekheet, S.A., 2011. *In vitro* Conservation of Date Palm Germplasm. In: Date Palm Biotechnology, Jain, S.M., J.M. Al-Khayri and D.V. Johnson (Eds.). Springer, Dordrecht, pp: 337-360.

Bekheet, S.A., H.S. Taha, M.M. Saker and M.E. Solliman, 2007. Application of cryopreservation technique for *in vitro* grown date palm (*Phoenix dactylifera* L.) cultures. J. Applied Sci. Res., 3: 859-866.

Bell, L.M., R.N. Trigiano and B.V. Conger, 1993. Relationship of abscisic acid to somatic embryogenesis in *Dactylis glomerata*. Envir. Exp. Bot., 33: 495-499.

Calic-Dragosavac, D. and L. Radojevic, 2010. Improvement of maturation and conversion of horse chestnut androgenic embryos. Biol. Nyssana, 1: 49-55.

Capuana, M. and P.C. Debergh, 1997. Improvement of the maturation and germination of horse chestnut somatic embryos. Plant Cell Tissue Org. Cult., 48: 23-29.

Chowdhury, C.N., A.K. Tyagi, N. Maheshwari and S.C. Maheshwari, 1993. Effect of L-proline and L-tryptophan on somatic embryogenesis and plantlet regeneration of rice (*Orzya sativa* L. cv. Pusa 169). Plant Cell Tissue Organ Cult., 32: 357-361.

Denchev, P., M. Velcheva and A. Atanassov, 1991. A new approach to direct somatic embryogenesis in Medicago. Plant Cell. Rep., 10: 338-341.

El-Sharabasy, S.F., W.H. Wanas and A.Y. Al-Kerdany, 2008. Date palm cultivars *in vitro* screening to drought tolerance using isozymes. Arab J. Biotech., 11: 263-272.

Fernando S.C., L.K. Weerakoon and T.R. Gunathilake, 2004. Micropropagation of coconut through plumule culture. Cocos, 16: 1-10.

Fernando, S.C. and C.K.A. Gamage, 2000. Abscisic acid induced somatic embryogenesis in immature embryo explants of coconut (Cocos nucifera L.). Plant Sci., 151: 193-198.

Goebel-Tourand, I., M.C. Mauro, L. Sossountzov, E. Miginiac and A. Deloire, 1993. Arrest of somatic embryo development in grapevine, histological characterization and the effect of ABA, BAP and zeatin in stimulating plantlet development. Plant Cell. Tiss. Org. Cult., 33: 91-103.

Hassan, M.M., E.G. Gadalla and A.H. Abd-El Kareim, 2008. Effect of sucrose and abscisic acid on *in vitro* growth and development of date palm during rooting stage. Arab J. Biotech., 11: 281-292.

Hatzopoulos, P., F. Fong and Z.R. Sung, 1990. Abscisic acid regulation of DC8, a carrot embryogenic gene. Plant Physiol., 94: 690-695.

Ishii, K., Y. Hosoi, E. Maruyama and S.I. Kanetani, 2008. Micropropagation of an endangered species *Pinus armandii* var. *armamiana*. Ann. Forest Res., 51: 5-10.

Jimenez, M.V., 2001. Regulation of *in vitro* somatic embryogenesis with emphasis on the role of endogenous hormones. Revista Brasileira Fisiologia Vegetal, 13: 196-223.

Kikuchi, A., N. Sanuki, K. Higashi, T. Koshiba and H. Kamada, 2006. Abscisic acid and stress treatment are essential for the acquisition of embryogenic competence by carrot somatic cells. Planta, 223: 637-645.

Li, X.Y., F.H. Huang and E.E. Gbur Jr., 1997. Polyethylene glycol-promoted development of Somatic embryos in loblolly pine (*Pinus taeda* L.). *In vitro* Cell. Dev. Biol. Plant, 33: 184-189.

Liu, Q., 2011. Sweet potato omics and biotechnology in China. POJ, 4: 295-301.

Marquez-Martin B., R. Sesmero, M.A. Quesada, F. Pliego-Alfaro and C. Sanchez-Romero, 2011. Water relations in culture media influence maturation of avocado somatic embryos. J. Plant Physiol., 168: 2028-2034.

Mishra, M., Y. Shree, R. Pati, S. Seal and N. Shukla *et al*., 2010. Micropropagation of *Mangifera indica* L. cv. kurakkan through somatic embryogenesis. Ind. J. Genetics Plant Breed., 70: 85-90.

Mulwa, R.M.S., M.M.A. Norton and R.M. Skirvin, 2010. Plant regeneration via somatic embryogenesis from leaf and floral explants of Chancellor wine grape. Plant Tiss. Cult. Biotech., 20: 157-170.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Murch, S.J., J.M.R. Victor, S. Krishnaraj and P.K. Saxena, 1999. The role of proline in thidiazuron-induced somatic embryogenesis of peanut. *In vitro* Cell. Dev. Biol. Plant, 35: 102-105.

Nadel, B.L., A. Altman and M. Ziv, 1990. Regulation of somatic embryogenesis in celery cell suspensions. 2. Early detection of embryogenic potential and the induction of synchronized cell cultures. Plant Cell Tiss. Org. Cult., 20: 119-124.

Nishiwaki, M., K. Fujino, Y. Koda, K. Masuda and Y. Kikuta, 2000. Somatic embryogenesis induced by the simple application of abscisic acid to carrot (*Daucus carota* L.) seedlings in culture. Planta, 211: 756-759.

Ogata, Y., M. Iizuka, D. Nakayama, M. Ikeda, H. Kamada and T. Koshiba, 2005. Possible involvement of abscisic acid in the induction of secondary somatic embryogenesis on seed-coat-derived carrot somatic embryos. Planta, 221: 417-423.

Osuga, K., H. Masuda, A. Komamine and J.C. Geesin, 1999. Synchronization of somatic embryogenesis at high frequency using carrot suspension cultures: Model systems and application in plant development. Methods Cell Sci., 21: 129-140.

Ronchi, V.N., M.A. Caligo, M. Nozzolini and G. Luccarini, 1984. Stimulation of carrot somatic embryogenesis by proline and serine. Plant Cell Rep., 3: 210-214.

Senger, S., H.P. Mock, U. Conrad and R. Manteuffel, 2001. Immunomodualtion of ABA function affects early events in somatic embryo development. Plant Cell Rep., 20: 112-120.

Sghaier, B., W. Kriaa, M. Bahloul, J.V. Jorrin Novo and N. Drira, 2009. Effect of ABA, arginine and sucrose on protein content of date palm somatic embryos. Sci. Hortic., 120: 379-385.

Sghaier-Hammami, B., J.V. Jorrin-Novo, R. Gargouri-Bouzid and N. Drira, 2010. Abscisic acid and sucrose increase the protein content in date palm somatic embryos, causing changes in 2-DE profile. Phytochemistry, 71: 1223-1236.

Sirmandi, H.B., K. Vahdati and S. Kalantari, 2010. Enhancement of maturation and germination of somatic embryos of Persian walnut (*Juglans regia* L.) by gellan gum, cold and PGR. Acta Hort., 861: 333-344.

Suganthi, M., S. Arvinth and R.R. Kumar, 2012. Impact of osmotica and abscisic acid on direct somatic embryogenesis in tea. Int. J. Plant Res., 2: 22-27.

Torres, A.C., N.M. Ze and D.J. Cantliffe, 2001. Abscisic acid and osmotic induction of synchronous somatic embryo development of sweet potato. *In vitro* Cell Dev. Biol., 37: 262-267.

Viji, M., P. Maheswari, T. Karuppanapandian and K. Manoharan, 2012. Effect of polyethylene glycol and mannitol on somatic embryogenesis of pigeonpea, *Cajanus cajan* (L.) Millsp. Afr. J. Biotechnol., 11: 10340-10349.

Von Arnold, S., D. Chapham, U. Egerstotter and L.O. Mo, 1996. Somatic embryogenesis in conifers a case study of induction and development of somatic embryos in *Picea abies*. Plant Growth Regul., 20: 3-9.

Yadollahi, A., M.R. Abdollahi, A. Moieni and M. Danaee, 2011. Effects of carbon source, polyethylene glycol and abscisic acid on secondary embryo induction and maturation in rapeseed (*Brassica napus* L.) microspore-derived embryos. Physiol. Plant., 33: 1905-1912.

Zaid, A. and H. Hughes, 1995a. Water loss and polyethylene glycol-mediated acclimatization of *in vitro*-grown seedlings of 5 cultivars of date palm (*Phoenix dactylifera* L.) plantlets. Plant Cell Rep., 14: 385-388.

Zaid, A. and H. Hughes, 1995b. *In vitro* acclimatization of date palm (*Phoenix dactylifera* L.) plantlets: A quantitative comparison of epicuticular leaf wax as a function of polyethylene glycol treatment. Plant Cell Rep., 15: 111-114.

Zouine, J., M. El-Bellaj, A. Meddich, J.L. Verdeil and I. El-Hadrami, 2005. Proliferation and germination of somatic embryos from embryogenic suspension cultures in *Phoenix dactylifera*. Plant Cell Tiss. Org. Culture, 82: 83-92.