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EFFECT OF ABSCISIC ACID, POLYETHYLENE GLYCOL AND THEIR COMBINATIONS ON SOMATIC EMBRYOGENESIS OF DATE PALM

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ABSTRACT

Somatic embryos of date palm *(Phoenix dactylifera* L.) cultivar Sakkoty have a several morphological shapes, normal (individual, repeated and multiple) embryos and abnormal shapes. These asexual somatic embryos have different potential for its conversion into complete plantlets. Adding 1.5 mg/l ABA to the maturation medium had a beneficial effect in an increase the percentages of normal individual, repeated somatic embryos formation, embryo number and decreased the percentage of abnormality. The medium supplemented with 1.0 mg/l ABA increased the percentage of normal multiple somatic embryos formation. Increasing PEG concentration to 15 g/l enhanced the percentages of normal individual and repeated somatic embryos formation, embryo numbers and in the same time decreased the percentage of abnormality. Adding 5 g/l PEG to the medium stimulated the formation of normal multiple somatic embryos .The highest significant value of embryogenic callus fresh weight was recorded in a medium contained 0.5 mg/l ABA plus 5 g/l PEG. The percentages of re-formation of secondary normal repeated and multiple embryos were increased in the MS medium contained 1.5 mg/l ABA or 15 g/l PEG, reverse was true with abnormality. Normal somatic embryos were transferred to the germination medium. The vegetative parameters were taken to investigate the ability of the somatic embryos to convert into complete plantlets. During maturation period using medium containing 1.5 mg/l ABA + 15g/l

PEG and their combinations treatments was the superior in increasing the shoot and root numbers and length.

Key words: *Date palm, Phoenix dactylifera* L., *Somatic Embryogenesis, ABA, PEG and Abnormality*.

INTRODUCTION

Date palm *(Phoenix dactylifera* L.) is considered to be one of the oldest cultivatable crops and an important multipurpose tree . About 105 million date palms are grown in the Africa, Middle and South of America, Spain and Italy (Heselmans, 1997). The most important date palm cultivation zones are in north Africa, where they are a prime source of income for about 10 million people (EL-Hadrami *et al.*, 1998).

The propagation of date palm, a dioecious and monocotyledonous species was traditionally achieved by the seeds and offshoots. However, these methods are not sufficient to rehabilitate the date palm groves and can inadvertently spread diseases such as bayoud (Zouine *et al.*, 2005).

To satisfy increasing demand in international markets, it is necessary to develop alternative methods of vegetative propagation to produce large number of plants from selected genotype. Several attempts have been made to establish micropropagation protocols based on either somatic embryogenesis or organogenesis (Sharma *et al.*, 1990; Tisserat, 1991).

A typical somatic embryogenesis protocol for date palm involves a series of consecutive stages beginning with callus induction, embryogenic callus multiplication, somatic embryo maturation and somatic embryo germination (Zouine *et al.*, 2005).

The normal pathway of development may be diverted (Ammirato, 1985) resulting in a range of structurally aberrant forms. These are epigenetic changes, for normal plants can be grown from them.

However, somatic embryos quality is still the primary barrier to the operational use of somatic embryos as artificial seeds for most species (Merkle, 1995). Although large quantities of somatic embryos can be rapidly produced, normal plants are difficult to obtain from these embryos due to asynchronous maturation of the embryos and subsequent low germination and conversion rates.

ABA promotes normal development of somatic embryos *in vitro by* stimulating reserve substance accumulation and inhibiting precocious germination (Ammirato, 1985). Exogenously supplied of ABA has proved to be an important component of the maturation medium. In the absence of ABA, maturation resulted in poorly developed somatic embryos which often exhibited abnormal morphology, asynchronous development and precocious germination (Lelu *et al*., 1994a) .

Subsequently, these somatic embryos showed the lowest capacities for germination and plantlet development (Lelu *et al* ., 1994b) .

Attree *et al.*,(1993) found that, further improvement of maturation frequencies and germination has been attained for cultures of white spruce by use of a combination of ABA and non- permeating osmoticum such as PEG. The positive effect of osmoticum on embryo maturation has been attributed to increasing levels of endogenous ABA (Wilen *et al.*, 1990).

Zaid (2003) determined the percentage of abnormalities of date palm somatic embryos and the relation between abnormalities percentage and re-culture number. Using ABA to improve the production and development of somatic embryos, to avoid the formation of abnormal shapes of somatic embryos and produce mature somatic embryos that were visually normal and did not germinate precocious. Also the author study the germination and development of different somatic embryos shapes (normal and abnormal shapes). The author put every shape in the optimal pathway of growth, development to determine the potential of each shape to proceed its growth into a complete plantlets.

Hassan *et al.*,(2007) showed that added of PEG to the maturation medium in the presence of 0.5 mg/l ABA increased callus growth and the number of date palm somatic embryo cv. Zaghloul.

Kärkönen (2000) reported that, treatment with abscisic acid (ABA) and polyethylene glycol-4000 during maturation induced the development of somatic cotyledonary embryos of *Tilia cordata* Mill similar to zygotic embryos with respect to morphology and anatomy, as illustrated by the differentiation of the apical meristems and procambium.

The present study aimed to determine factors that would support maturation of somatic embryos in date palm, thus we tested media differing in ABA and osmotic agent PEG at different concentrations.

Improved maturation had a beneficial effect on germination of somatic embryos, resulting in healthy plantlets that were successfully planted outdoors.

MATERIALS AND METHODS

The current investigation was performed during the years from 2006 to 2009 at the Central Laboratory for Date palm Researches and Development, Agricultural Research Center at Giza, Egypt. Callus cultures were obtained from culturing the explants isolated from off-shoots of dry date palm cultivar Sakkoty grown at Aswan governorate , Egypt.

Plant Material and Culture Conditions

The usual procedure to produce friable embryogenic callus is to move the compact callus formed from a highly auxin MS medium (100 mg/l 2,4-D plus 3mg/l 2ip) to a MS medium lacking auxin, containing the same auxin at a lower concentration at 10 mg/l 2,4-D plus 3mg/l 2ip (Tisserat , 1984) , or containing a different auxin at lower concentration, medium supplemented with 0.1 mg/l NAA (Mater, 1986).

Maturation and Realization of Somatic Embryos

White friable embryogenic callus (0.5 gram) was cultured on maturation medium which consists of MS basal medium supplemented with 170 mg/l Na $H_2Po_4.2H_2o$ +100mg/l myo-inisitol + 0.4 mg/l thiamine hydrochloride +200mg/l glutamine +30g/l sucrose + 6g/l agar plus ABA at different concentrations (0.5 , 1.0 and 1.5 mg/l) alone or combined with non-plasmolysing stress, PEG-4000 at different concentrations (5, 10, and 15g/l). Different combinations between them were made for realization of somatic embryos. The cultures were incubated under darkness for 8 weeks .Somatic embryos developed either to normal or to abnormal shapes.

Morphological Growth Parameters

Morphological growth parameters include the percentage of normal somatic embryos and abnormal shapes of somatic embryos, embryogenic callus fresh weight and embryos number.

Fresh weight = (Final fresh weight - initial fresh weight) / initial fresh weight

Normal somatic embryos divided to:

1- Normal individual embryo, small seedling with primary root and shoot (George, 1993).

2- Repeated embryos, clusters of 3-4 embryos arose repetitive, which are usually of normal morphology (Abul-Soad, 1999).

3- Multiple embryo, cluster from 3-4 embryos can occur on the base of the original embryo (George, 1993).

Germination of Mature Somatic Embryos

To induce germination, mature normal somatic cotyledonary embryos were transferred to germination medium (0.1 mg/l NAA plus 0.05 mg/l BA). Germination was recognized by the appearance of a radicle and the first growth leaves, indicating induction of shoot growth, thus vegetative parameters were taken to investigate the ability of the original normal somatic embryos to convert into complete plantlets (shoots number/culture, shoot length (cm)/culture, roots number/culture and root length (cm) / plantlet) .

Also there was secondary somatic embryogenesis in the germination medium. Thus, we also recorded the percentage of re-formation of the secondary somatic embryos as a normal and abnormal shapes, to investigate the ability of the original normal somatic embryos to re-form secondary somatic embryos.

The experiment was conducted in a complete randomized block design with three replicates. The obtained results were subjected to statistical analysis of variance according to method described Snedecor and Cochran (1980) . Using L.S.D test at 5%.

RESULTS AND DISCUSSION

Maturation and Realization of Date Palm Somatic Embryos

Data presented in Table (1) showed the effect of ABA and PEG in the maturation media on the percentage of different shapes of date palm somatic embryos cv. Sakkoty.

Normal Individual Somatic Embryos Formation

The percentage of normal individual somatic embryos formation was enhanced by adding 1.5 mg/l ABA to the maturation medium (21.88 %) followed by using maturation medium supplemented with 1.0 and 0.5 mg/l of ABA (18.75 and 15.63 %) respectively.

Concerning the effect of different PEG concentrations on the percentage of normal individual somatic embryos formation, the percentage was significantly stimulated by added 15 g/l PEG into the maturation medium followed by 5 g/l PEG (25.0 and 20.83 %) respectively. On the other hand using medium without PEG produced the lowest significant percentage of normal individual somatic embryos formation 12.50 %.

Regarding the effect of interaction between different ABA and PEG concentrations, data clearly showed that the percentage of normal individual somatic embryos formation significantly increased (25%) by using maturation medium supplemented with 0.5 mg/l ABA plus 15 g/l PEG, 1.0 mg/l ABA plus 5 , 15 g/l PEG and 1.5 mg/l ABA plus 5 , 10 and 15 g/l PEG .

Normal Repeated Somatic Embryos Formation

The percentage of normal repeated somatic embryos formation was the highest by the culturing of white friable embryogenic callus on maturation medium added with 1.5 mg/l ABA followed by using maturation medium supplemented with 0.5 mg/l ABA (50 and 34.38 %) respectively. While the culture medium with 1.0 mg/l ABA produced the lowest significant percentage of normal repeated somatic embryos formation 31.25 %.

Regarding the effect of different PEG concentrations on the percentage of normal repeated somatic embryos formation, the percentage was significantly increased by added 15 g/l PEG into the maturation medium (45.83 %) followed by using medium without PEG (41.67%). However, medium supplemented with either 5 or 10 g/l of PEG produced the lowest significant percentage of normal repeated somatic embryos formation (33.33 %) for each of them.

The effect of interaction between different ABA and PEG concentrations on the percentage of normal repeated somatic embryos formation, it was noticed that , the percentage was significantly increased (75%) by using maturation medium supplemented with 1.5 mg/l ABA plus 15 g/l PEG.

Normal Multiple Somatic Embryos Formation

The effect of different concentrations of ABA on the percentage of normal multiple somatic embryos formation is shown in Table (1).

Added 1.0 mg/l ABA gave the highest percentage of normal multiple somatic embryos formation (18.75%) followed by using maturation medium incorporated by 1.5mg/l ABA (15.63). Meanwhile culturing in the maturation medium plus 0.5 mg/l ABA produced the lowest significant percentage of normal multiple somatic embryos formation (12.50 %).

The effect of different concentrations of PEG on the percentage of normal multiple somatic embryos formation, added 5 g/l PEG to the maturation medium had a beneficial effect in increment the percentage of normal multiple somatic embryos formation (20.83%) followed by added 15 g/l PEG (16.67%). However, the media supplemented with either 10 g/l of PEG or without it gave the lowest percentage (12.50%) for each of them.

Regarding the effect of interaction between different ABA and PEG concentrations on the percentage of normal multiple somatic embryos formation, data clearly showed that maturation medium supplemented with 1.0 mg/l ABA plus either with 5 or 15 g/l PEG and medium containing 1.5 mg/l ABA plus 5 g/l PEG produced the highest percentage of multiple somatic embryos formation (25%) for each of them.

Abnormal Somatic Embryos Formation

Concentration of ABA that produced the highest significant percentage of abnormality (37.46%) was 1.0 mg/l ABA. While, it was noticed that the best concentration of ABA that produced the lowest significant percentage of abnormal somatic embryos formation (15.63%) was 1.5 mg/l ABA.

The effect of different PEG concentrations on the percentage of abnormal somatic embryos formation, data revealed that 10 g/l PEG gave the highest percentage of abnormality (29.17%). While the lowest percentage (20.83) was given by adding 15 g/l PEG to the maturation medium.

Concerning the effect of interaction between the different ABA and PEG concentrations on the percentage of abnormality, data reflect that, the highest significant percentage of abnormality (50%) was recorded by using media supplemented with 1.0 mg/l ABA plus 10 g/l PEG.

Table (1). Effect of ABA and PEG on the percentage of different shapes of date palm somatic embryos cv. Sakkoty.

ABA (A) mg |l	PEG(B) g| l	Different Shapes Of Somatic Embryos Percentage			
		Individual Embryos	Repeated Embryos	Multiple Embryos	Abnormal Embryos
0.5	0.0	12 .50b	50 .00b	12 .50b	25 .00c
	5	12 .50b	37 .50c	12 .50b	25 .00c
	10	12 .50b	25 .00d	12 .50b	25 .00c
	15	25 .00a	25 .00d	12 .50b	12 .50d
Mean (A)		**15 .63 c**	**34 .38 b**	**12 .50 c**	**21 .88 b**
1.0	0.0	12 .50b	37 .50c	12 .50b	37 .50b
	5	25 .00a	25 .00d	25 .00a	37 .50b
	10	12 .50b	25 .00d	12 .50b	50 .00a
	15	25 .00a	37 .50c	25 .00a	25 .00c
Mean (A)		**18 .75 b**	**31 .25 c**	**18 .75 a**	**37 .46 a**
1.5	0.0	12 .50b	37 .50c	12 .50b	12 .50d
	5	25 .00a	37 .50c	25 .00a	12 .50d
	10	25 .00a	50 .00b	12 .50b	12 .50d
	15	25 .00a	75 .00a	12 .50b	25 .00c
Mean (A)		**21 .88 a**	**50 .00 a**	**15 .63 b**	**15 .63 c**
Mean (B)					
	0.0	12.50d	41.67b	12.50c	25.00b
	5	20.83b	33.33c	20.83a	24.94b
	10	16.67c	33.33c	12.50c	29.17a
	15	25.00a	45.83a	16.67b	20.83c
LSD 5%					
A		0.718	0.524	0.747	0.656
B		0.829	0.605	0.8623	0.757
AB		1.437	1.048	1.494	1.312

Many biochemical and physiological studies have shown that ABA promotes normal development of somatic embryos *in vitro* by stimulating reserve substance accumulation and inhibiting precocious germination (Ammirato, 1985). Both ABA and osmoticum are known to promote synthesis of number of protein in developing embryos.

ABA has been shown to control the expression of genes specific to embryo development and maturation. Thus, using ABA-deficient and ABA-insensitive *Arabidopsis* mutants ABA has been shown to control genes for both LEA (late embryogenesis abundant) and storage proteins (Dodeman *et al.*, 1997).

In addition to ABA, osmoticum plays a role in correct storage protein expression in developing somatic embryos (Finkelstein and Crouch, 1986). The requirement for high osmolarity may reflect changes in osmolarity probably occurring in the environment surrounding the zygotic embryo (Merkle *et al.*, 1995).

Zaid (2003) showed that individual embryos germinated directly to shoot and root without producing secondary embryos, while repeated and multiple somatic embryos were used in multiplication stage of date palm as produced more secondary somatic embryos which differentiated from callus on its body. This process may be useful in date palm micropropagation. Abnormal shapes of date palm somatic embryos can not able to proceed their growth and development to complete plantlets.

Germination of Date Palm Somatic Embryos and Re-formation of Secondary Embryogenesis

Data illustrated in Table (2) show the effect of ABA, PEG and their combinations in maturation media on the average of embryogenic callus fresh weight/culture and average of embryo number, data clearly showed that there was not significant differences between different concentrations of ABA on the average of embryogenic callus fresh weight (g /culture).

As for the effect of PEG concentrations on the average of embryogenic callus fresh weight/culture, it was found that using media supplemented with 10 g/l PEG gave the highest significant value of embryogenic callus fresh weight/culture (2.97 g/culture), while medium without PEG produced the lowest significant value of embryogenic callus fresh weight/culture (1.94 g/ culture) .

Table (2). Effect of ABA, PEG and their combinations in maturation media on the embryogenic callus fresh weight/culture and somatic embryo number/culture of date palm somatic embryos cv. Sakkoty after 8 weeks.

ABA (A) mg\l	PEG (B) g\l									
	Embryogenic callus fresh weight / culture				Mean	Somatic embryos number/ culture				Mean
	0.0	5	10	15	(A)	0.0	5	10	15	(A)
0.5	2.32abc	3.82 a	2.99abc	1.97bc	2.78	4.00cde	3.63cdef	4.88c	4.63c	4.86b
1.0	1.43 c	2.09abc	2.28abc	1.88 c	1.92	2.38 ef	2.25f	2.75def	6.63b	3.05b
1.5	2.07 bc	2.47abc	3.65 ab	2.06abc	2.56	4.25 cd	6.88 b	7.75ab	9.38a	7.07a
Mean (B)	1.94 b	2.79 ab	2.97 a	1.97 ab		3.54 c	4.25 bc	5.13 b	6.88a	
LSD 5%										
A	NS					0.87				
B	1.02					1.01				
AB	1.76					1.74				

Regarding the effect of combination between ABA and PEG , data showed that, the best significant value of embryogenic callus fresh weight (g/culture) was recorded with using medium containing 0.5 mg/l ABA plus 5 g/l PEG (3.82 g/culture) . Using medium supplemented with 1.0 mg/l ABA alone or plus 15 g/l PEG give the lowest significant value of embryogenic callus fresh weight / culture (1.43, 1.88 g/ culture respectively).

Somatic embryos number/culture was the greatest when 1.5 mg /l ABA was added to the culture media (7.07embryos/culture) , while using media containing 0.5 mg/l ABA followed by media containing 1.0 mg/l ABA (4.86 , 3.05 embryos/ culture), respectively .

Concerning the effect of different concentrations of PEG, data showed that added 15 g/l PEG to the culture medium was the superior in increasing the number of somatic embryos/culture (6.88embryo/culture). Using medium without PEG produced the lowest significant value of embryo/culture (3.54 embryo/culture).

Meanwhile, comparing the combinations between ABA and PEG, data clearly showed that average of embryo number/culture was enhanced by adding 1.5 mg/l ABA plus 15 g /l PEG (9.38 embryo/culture), while medium containing 1.0 mg/l ABA plus 5 g /l PEG give the lowest significant value of embryo/culture (2.25 embryos/culture).

Hassan *et al.* (2007) found that using medium supplemented with 0.5 mg /l ABA combined with 20 g/l PEG increased callus growth (2.83g). While using medium incorporated with 0.5 mg/l ABA plus 10 g/l PEG produced the highest significant value of number of mature date palm somatic embryos during maturation stage (6.33 embryo).

Our results are quite similar to those obtained by Kong *et al.* (1998) who found that PEG treatments, increased cotyledonary embryos 2.7 fold per dish or 5.9 fold per g f.wt. tissue after 5 weeks in the maturation media. The same authors showed that non-plasmolysing water stress in the form of PEG-4000 increased endogenous free polyamines (PA_s) levels from day 15-25 from culturing in the maturation media and these increment perhaps corresponded to fast embryo growth .

In *in vitro* cultures, the major role of polyamines (PA_s) has been proposed in cell division and morphogenesis (Minocha *et al.,* 1995). In carrot somatic embryo cultures, polyamines (PA_s) synthesis inhibitor decreased endogenous PA_s levels and reduced somatic embryos maturation (Feirer *et al.,* 1984) .

In white spruce, the number of somatic embryos increased significantly when PEG-4000 was applied in maturation medium (Kong and Young, 1995).

The positive effect of osmoticum on embryo maturation has been attributed to increasing levels of endogenous ABA (Wilen *et al.,* 1990), but it has also been proposed that the effects of ABA and osmoticum are due to different physiological responses and should therefore be considered as additive (George *et al.,* 2008).

The analysis of the sections of somatic embryos of *T. cordata* showed that some epidermal cells were densely cytoplasmic with large nuclei, a prominent feature of embryogenic cells (Sharp *et al.,* 1980). Mitotic activity led to the development of secondary embryos from these merstimatic cells.

Germination of Somatic Embryos Types

In some cases the formation of secondary somatic embryos is significant importance for increasing the yield of the regenerated plants (George *et al.*, 2008).

Data recorded in Table (3) showed the effect of different concentrations of ABA and PEG on the percentage of re-formation of

different shapes of date palm somatic embryos cv. Sakkoty when transfer to the germination medium.

As for the effect of different ABA concentrations, data revealed that, the normal individual somatic embryos formation percentage was enhanced (16.67%) by adding 0.5 mg /l ABA. While the percentages of normal repeated and multiple somatic embryos formation were increased (50.0 and 41.67%) by using media containing 1.5 mg /l ABA respectively . Also the same concentration of ABA (1.5 mg /l) produced the lowest significant percentage of abnormality (33.33%).

Concerning the effect of different concentrations of PEG, data clearly showed that using maturation medium supplemented with 15 g /l PEG gave the highest significant percentages (16.67, 50 and 50 %) of normal individual, repeated and multiple somatic embryos formation, respectively. Meanwhile, adding 15 g /l PEG to the medium had a beneficial effect in reducing the percentage of abnormality (22.22%).

The interaction between different ABA and PEG concentrations, data revealed increasing the normal individual somatic embryos re-formation percentage (33.33%) significantly by using medium supplemented with 0.5 mg/l ABA plus 5g/l PEG. The percentage of normal repeated somatic embryos re-formation was enhanced (66.67%) by adding 1.5 mg/l ABA plus 10 g/l PEG. The percentage of normal multiple somatic embryos re-formation was increased (66.67%) in the case of using maturation medium supplemented with 1.0 mg/l ABA plus 15 g/l PEG. The lowest significant percentage (16.67%) of abnormal somatic embryos re-formation was recorded by added 15 g/l PEG plus either 1.0 or 1.5 mg/l ABA.

It has been suggested that, the role of ABA in somatic embryogenesis is to prevent precocious germination and stimulate the accumulation of storage reserves, such as storage protein (Ammirato, 1988), triglycerides, and lipids (Attree et al., 1991).

In this respect, these data are in agreement with Stasolla et al., (2003) results who reported that, the inclusion of PEG to the culture medium can improve the number and the quality of embryos produced. The same authors analyzed transcript profiles of stage-specific embryos matured without (control) or with (PEG treated) PEG. They found that, several pine genes, increased in expression after PEG treatments. These genes are known to be involved in the formation of the embryo body plan and in the control of the shoot and

root apical meristems. The increased transcript levels of these genes in immature PEG-treated embryos suggest that PEG may improve the quality of spruce somatic embryos by promoting normal differentiation of the embryonic shoot and root. Changes in the transcript levels of many genes involved in sucrose catabolism and nitrogen assimilation and utilization were also observed between control and PEG-treated embryos.

Table (3). Effect of ABA and PEG on the percentage of re-formation of different shapes of date palm somatic embryos cv. Sakkoty in the germination media.

ABA(A) mg ‖	PEG(B) g ‖	Different Shapes Of Somatic Embryos Percentage			
		Individual Embryos	Repeated Embryos	Multiple Embryos	Abnormal Embryos
0.5	0.0	16.67b	50.00b	16.67d	50.00b
	5	33.33a	16.67d	16.67d	50.00b
	10	0.0c	33.33c	33.33c	50.00b
	15	16.67b	50.00b	33.33c	33.33c
Mean (A)		**16.67 a**	**37.50 b**	**25.00c**	**45.83 a**
1.0	0.0	0.0c	33.33c	16.67d	66.67a
	5	0.0c	16.67d	50.00b	50.00b
	10	0.0c	33.33c	16.67d	50.00b
	15	16.67b	50.00b	66.67a	16.67d
Mean (A)		**4.17 c**	**33.33 c**	**37.50b**	**45.83 a**
1.5	0.0	0.0c	50.00b	16.67d	50.00b
	5	0.0c	33.33c	50.00b	33.33c
	10	16.67b	66.67a	50.00b	33.33c
	15	16.67b	50.00b	50.00b	16.67d
Mean (A)		**8.34 b**	**50.0 a**	**41.67 a**	**33.33 b**
Mean (B)					
	0.0	5.56c	44.44b	16.67d	55.56a
	5	11.11b	22.22c	38.89b	44.44b
	10	5.56c	44.44b	33.33c	44.44b
	15	16.67a	50.00a	50.00a	22.22c
LSD 5%					
A		0.481	0.847	0.574	0.555
B		0.521	0.978	0.662	0.640
AB		0.902	1.693	1.147	1.109

Attree and Fowke, (1993) appeared that the post-embryonic performance of somatic embryos was strictly dependent upon their maturation conditions, has led to a tremendous effort towards the optimization of new protocols for the production of embryos with superior quality and improved germination (radicle emergence) and conversion (radicle emergence and production of new leaf primordia).

Data presented in Table (4) showed the effect of ABA, PEG and their combinations in the maturation media on the germination (shoot number, shoot length (cm), root number and root length (cm)/culture). Shoot number/culture was enhanced (24.75, 18.12 and 16.21) by culturing in medium incorporated with 1.5 mg/l ABA followed by using media containing 1.0 and 0.5 mg/l ABA, respectively. Shoot length (cm)/culture was the best (12.55) in medium supplemented with 1.5 mg/l ABA.

Normal somatic embryos matured on medium containing 15g /l PEG gave the best results in increment (24.28) the shoot number/culture when transfer into germination medium,while using medium incorporated with 10 g/l or without PEG produced the same shoot number/culture (16.78) .

The highest significant values of shoot and root numbers /culture (31.50 and 25.67) and shoot and root lengths (cm)/culture (15.6 and 6.8) were in media containing 1.5 mg/l ABA plus 15 g/l PEG.

Data presented in Table (4) revealed that the root number/culture and root length\plant were enhanced (18.83 root/culture) when somatic embryos were matured in medium containing 1.5 mg/l ABA and 0.5 mg/l ABA (6.2 cm/ plant), respectively.

Using medium containing 15g/l PEG produced the highest significant values of root number/culture and length/ plant (18.56 and length 6.78 cm).

A remarkable change occurring during the maturation period is that the developmental programme switches from pattern formation to storage product accumulation in order to prepare the young sporophyte for dormancy and postembryonic development. The rate of synthesis and deposition of storage proteins, lipids and starch increases and results in cell expansion in both cotyledons and axis. Cell vacuoles exhibit a specialized behavior during maturation in that they split up and dehydrate to give rise to protein bodies and aleurone grains (Dodeman *et al.*, 1997). An essential regulator of the process is ABA (George *et al.*, 2008).

Table (4). Effect of ABA, PEG and their combinations in the maturation media on the conversion (shoot number/culture and length (cm), root number/culture and length (cm) of date palm somatic embryos on germination medium .

ABA (A) mg |l	PEG (B) g | l	Shoot number/ culture	Shoot length cm / culture	Root number/ culture	Root length cm/culture
0.5	0.0	19.33c	8.50e	17.67c	5.80ab
	5	17.50d	10.30d	15.33d	6.00a
	10	12.33f	10.50d	10.67f	6.30a
	15	15.67e	11.30cd	12.67e	6.70a
Mean (A)		**16.21c**	**10.15c**	**14.09b**	**6.20**
1.0	0.0	15.67e	8.33e	10.33f	5.50ab
	5	19.82c	11.80bcd	15.33d	5.70ab
	10	11.33f	12.50bc	10.67f	5.30ab
	15	25.67b	13.50b	17.33c	6.80a
Mean (A)		**18.12b**	**11.41b**	**13.41b**	**5.80**
1.5	0.0	15.33e	10.60d	10.67f	4.30b
	5	25.50b	11.30cd	18.67bc	5.30ab
	10	26.67b	12.70bc	20.33b	5.70ab
	15	31.50a	15.60a	25.67a	6.80a
Mean (A)		**24.75a**	**12.55a**	**18.83a**	**5.50**
Mean (B)					
	0.0	16.78c	9.14c	12.89c	5.20b
	5	20.94b	11.13b	16.44b	5.70b
	10	16.78c	11.73b	13.89c	5.80b
	15	24.28a	13.47a	18.56a	6.78a
LSD 5%					
A		0.89	0.865	0.87	NS
B		1.03	0.998	1.01	0.92
AB		1.78	1.729	1.75	1.51

Hydration of the seed leads to its germination (Kermode, 1990) .It is possible to induce quiescence in somatic embryos by dehydration treatment. Numerous attempts to improve the quality of somatic embryos have shown the stimulatory role of low osmotic potential in the maturation medium in embryo development, in angiosperms (Attree and Fowke, 1993). Different osmotic agents, including low (e.g., inorganic salts, amino acids and sugars) and high molecular mass compounds (e.g., polyethylene glycols (PEG) and dextrans) can provide low osmotic potential medium.

Although the addition of PEG to the maturation medium in many cases has been shown to stimulate maturation, there are also reports showing adverse effects of PEG on embryo germination (Bozhkov and Von-Arnold, 1998). In date palm growth was completely inhibited at higher concentration of PEG (Hassan *et al.*, 2007).

However, Al- Khayri and Abu-Ali (2006) found that added ABA alone to the callus growth medium of date palm was inhibitory, but the degree of inhibition was modified by adding PEG combined with ABA. The low osmotic potential is important for slow development of the embryos, necessary for regulating the pattern of histo-differentiation (Yeung , 1995).

In many of the cases where ABA has been shown to be inhibitory this is almost certainly due to the use of un-physiological concentrations (George *et al.* ,2008).

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تأثير حامض الأبسيسيك و البولى إثيلين جليكول وتداخلا تهم على تكوين الأجنة الجسمية لنخيل البلح

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أظهرت الأجنة الجسدية لنخيل البلح صنف السكوتى صور عديدة من الأشكال المورفولوجية الطبيعية و الغير طبيعية (المشوهة) و التى أظهرت أستجابات مختلفة أثناء الأنبات و التحول الى نبيتات كاملة. و قد أظهرت النتائج أن أستخدم بيئة موراشيجى و سكوج المحتوية على 1.5 ملجم / لتر حامض الأبسيسك قد أعطت أعلى نسبة مئوية من الأجنة الجسدية الفردية و المتكررة الطبيعية، فى حين أنها قد أدت لأنخفاض النسبة المئوية لتكون الأجنة الجسدية الغير طبيعية. وسجلت أعلى زيادة لتكون الأجنة الجسدية المتضاعفة عند أستخدام بيئة غذائية محتوية على1.0 ملجم / لتر حامض الأبسيسيك . أدت أضافة 15 جم / لتر البولى إثيلين جليكول إلى بيئة الزراعة أعلى نسبة مئوية من الأجنة الجسدية الفردية و المتكررة الطبيعية و إلى أنخفاض النسبة المئوية لتكون الأجنة الجسدية الغير طبيعية . أعطت بيئة موراشيجى و سكوج المضاف لها 5جم / لتر البولى إثيلين جليكول أعلى زيادة فى وزن الكالوس الجنينى .زادت النسبة المئوية لتكون الأجنة الجسدية المتكررة و المتضاعفة عند أستخدام بيئة الزراعة المحتوية على 1.5 ملجم / لتر حامض الأبسيسيك أو15جم / لتر بولى إثيلين جليكول و العكس كان صحيح مع الأجنة المشوهة .

تم أخذ العديد من القياسات الخضرية لأختبار قدرة الأجنة على الأنبات و التحول لنبيتات كاملة . كانت أعلى نسبة معنوية لعدد وطول الأفرع الخضرية و الجذور للأجنة الناضجة على بيئة موراشيجى و سكوج المحتوية على 1.5 ملج / لتر حامض الأبسيسك و15جم / لتر البولى إثيلين جليكول و التداخل بينهم .