SOME FACTORS AFFECTING MATURATION AND GERMINATION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) SOMATIC EMBRYOS

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Abstract

Callus culture of date palm (Phoenix dactylifera) was initiated from shoot tip explants cultured on Murashige and Skoog medium supplemented with 10mg /l 2,4-D, 3mg /l 2iP and 1.5g/l activated charcoal for 32 weeks with regular reculture every 8 weeks. To determine the effects of GA3, ABA, poly ethyleneglycol (PEG) in the presence of ABA and gelling agents (agar & gelrite) on maturation and germination stages, piece of callus of approximately 1×1cm and clusters of mature somatic embryos were transferred to solidified MS medium containing 0.1 mg /l NAA (maturation stage) and containing 0.1 mg /l NAA &0.05 mg /l BA for germination stage and reculture three times with regular transferring to fresh medium containing the same composition medium enriched with different treatments every 4weeks. MS nutrient medium containing GA3 at 1mg/l or ABA at 0.5 mg/l or PEG at 10 g/l in the presence of 0.5 mg /l ABA and agar at 7 g/l increased significantly the number of mature embryos during maturation stage. The addition of GA3 or PEG at different concentrations or ABA at 0.25 or 0.5 mg/l to germination medium enhanced the conversion frequencies percentage of matured somatic embryos to plantlets compared with control medium. Dissection caused by raising agar to highest concentration produced the highest conversion frequency percentage.

Keywords: In vitro, Date palm, GA3, ABA, PEG, Gelling agent, Conversion Frequency

INTRODUCTION

The date palm (*Phoenix dactylifera* L) is an important multipurpose 'tree. (Heselmans, 1997). The most important date palm cultivation zones are in North Africa, where they are a prime sucrose of income for about 10 million people (El Hadrami *et al.*, 1998). The application of *in vitro* systems based on somatic embryos for plant regeneration is determined not only by a high efficiency of somatic embryo formation, but also frequently depends on capacity of the embryos for plant development. The process of developmental changes, which a somatic embryo undergoes, was called conversion, and it involves the formation of primary roots, a shoot meristem with a leaf primordium and greening of hypocotyls and cotyledons (Redenbaugh *et al.* 1986). In numerous systems, in spite of the high number of

somatic embryos produced, problems with a lack or a low frequency of embryo conversion into plants occurred. To stimulate embryo conversion, and to improve the efficiency of plant regeneration, a number of different strategies were tested. Giberellic acid (GA_3) is frequently employed in media used for somatic embryo conversion (Kim *et al.* 1997), and a significant stimulatory effect of this phytohormone was proved in cultures of *Sesamum indicum* (Xu *et al.* 1997) and *P. ginseng* (Yang and Choi 2000). Rarely, GA_3 inhibited development of somatic embryos (Hutchinson *et al.* 1997). It is believed that GA_3 is especially necessary in cultures of somatic embryos, which undergo dormancy (Choi *et al.*, 1999. Kim *et al.*, 1999 and Klimaszewska *et al*, 2001) reported that the culture medium constituents particularly osmoticum, maturation promoter and the type of gelling agent have a marked effect on the maturation of somatic embryos. Also, the attempt to increase the quality of somatic embryos by using the height molecular mass osmoticum, PEG 4000, and ABA was accomplished by insertion of a maturation phase of culture between multiplication (maintenance) and regeneration phase. The combined application of ABA and PEG has become a routine method for stimulation of somatic embryo maturation in some genera of coniferales (Bozhkov and von Arnold 1998) and selected tree species such as *H.braziliensis* (Linossier *et al*, 1997).

The aim of this work was to improve development (maturation and germination) of date palm cv. Zaghloul somatic embryos. The effect of different media components GA_3, ABA, non penetrating osmotic PEG in the presence of ABA and different gelling agent concentrations were investigated.

MATERIALS AND METHODS

This study was carried out in the Central Laboratory of Development of Date Palm Research at Giza, Egypt during the period from 2003– 2006.

Plant material and initiation of callus cultures (Establishment stage):

The propagation process was started with the selection of healthy offshoots from mother date palm trees of soft cultivar Zaghloul, was obtained from palms grown at Rashaid. The young offshoots were of 2 - 4 years, ranging in weight from 5 - 7 kg and about 50 - 80 cm in length. The selected young offshoots were carefully transferred to the laboratory after separation from mother tree and then prepared by removing the adventitious roots, fibrous sheath and leaves by knife. Removing leaves from offshoots were continued until the white soft leaves nearer the apical meristem had appeared. The apical meristem plus few leaf primordia was used as explant material. Explants were soaked in running tap water for 1-2 hrs, and soaked in sterile anti-oxidant solution of ascorbic (100 mg/l) and citric acid (150 mg/l) for 25-30min to

avoid culture browning. Explants were surface sterilized under aseptic conditions by using ethyl alcohol (70%) for 1min followed by immersion in (0.5g/l) mercuric chloride ($HgCl_2$) for 5min and then rinsed one-time with sterile distilled water and transferred to double surface sterilization by commercial Clorox (5.25%) sodium hypochlorite (NaOCl) supplemented with two drops of Tween-20 per 100 ml solution, the first one by 40% Clorox for 15 min and thoroughly washed with sterilized distilled water for one time and the second one by 60% Clorox for 25 min and then washed with sterilized distilled water for three times. Under aseptic conditions, outer soft leaves were removed to obtain a shoot-tip. Shoot-tip 5 - 10 mm in length, composed of the apical meristem and (4–6) leaf primordial, cut longitudinally into 4 sections and inoculated onto culture medium. Shoot-tip sections were cultured individually on sterilized Murashige and Skoog (1962) (MS) basal nutrient medium supplemented with 10mg/l dichloro-phenoxyacetic acid (2,4-D), 3mg/l N6-(2-iso-pentenyl adenine) 2iP, 40 mg/l adenine sulphate $2H_2O$ (Tisserat, 1979) + 170mg/l $NaH_2PO_4.2H_2O$ +200 mg/l glutamine +1.5 g/l activated charcoal + 40 g/l sucrose and 5 g/l agar. The cultures were raised in 4cm x 10cm glass culture jar containing 30 ml of the medium. Cultured jars were incubated at 27±2°C in total darkness through 32 weeks. Cultures were transferred to fresh medium every 4-6 weeks to form callus cultures. The effects of GA_3, ABA and PEG in the presence of ABA and gelling agent (agar or gelrite) were studied at the following stage.

Maturation stage:

The effects of GA_3 (0.0, 0.5, 1.0 and 1.5mg/l), ABA at (0.0, 0.25, 0.50 and 1.0mg/l), PEG at 0.0, 5, 10 and 20 g/l in the presence of ABA at 0.5 mg/l and finally gelling agent, agar at (5, 7 and 9 g/l) or gelrite at (2.5, 3.5 and 4.5 g/l) on callus growth and number of embryos were studied. ABA and GA_3 was filter sterilized and added to sterilized culture medium cooled to 40°C. Basal medium consisted of MS medium with 30 g/l sucrose+ 100 mg/l glutamine + 170 mg/l NaH_2PO_4 + 1g /l activated charcoal + 0.1 mg/l NAA and solidified with 5 g/l agar except in the case of gelling agent study. Embryogeneic callli resulted from initiation stage were divided into pieces of approximately (1x1cm) and cultured on previous different factors for 12 weeks (4 weeks interval). Each treatment included 3 replicates (small jars). Each jar contained about (1x1cm) embryogenic callus (friable callus). All cultures were incubated in 500-1000 Lux at 16 hrs, 27±2°C.

The following data were recorded after 12 weeks:

(1) Callus growth {estimated visually according to (Pottino 1981)}.

(2) Number of formed embryos.

Germination stage:

Small cluster containing 3-4 embryos, were used as the explants materials in this stage. Cluster explants individually were cultured on MS basal nutrient media containing 0.1 mg/l NAA + 0.05 mg/l BA + 170 mg/l NaH_2PO_4 +30 g/l sucrose +100 mg/l glutamine and supplemented with different concentrations of GA_3 (0.0, 0.5, 1.0 and 1.5mg/l), ABA at (0.0, 0.25, 0.50 and 1.0 mg/l), PEG in the presence of 0.5 mg/l ABA at (0.0, 5, 10 and 20 g/l), gelling agent agar at (5, 7 and 9 g/l) & gelrite at (2.5, 3.5 and 4.5 g/l). All cultured jars were incubated at 27±2°C for 12 weeks in light provided by white fluorescent tubes giving of about 1500-2000 lux for 16 hrs per day to study its effects on germination stage. Developed embryos explants were reculture after 4 weeks on fresh medium. Each treatment contained 3 replicates and each replicate contained 3 clusters.

The following data were recorded after 12 weeks:

1- Number of secondary embryos.

2- Number of converted embryos

3- Conversion frequency percentage = $\frac{\text{Number of converted embryos} \times 100}{\text{Total number of secondary embryos}}$

Statistical analysis:

Data obtained were subjected to the analysis of variances of randomized complete design as recommended by (Sendecor and Cochran 1980). LSD at 5% level of significance was used to compare between means according to (Steel 1960).

RESULTS

Maturation stage:

Effect of GA_3:

The addition of GA_3 at 1.5 mg/l increased callus growth followed by concentration at 1.0 mg/l without significant difference in between while addition of GA_3 at 0.5 mg/l did not differ significantly with control culture medium as shown in Table (1). According to the effect of GA_3 concentration on number of embryo matured in this stage, data in the same Table and Fig (1) recorded that the highest value of embryo number was obtained when GA_3 was added to culture medium at 1.0 mg/l followed by control medium without significant difference in between.

Effect of ABA:

Data in Table (2) reflected that ABA at different concentrations under investigation increased callus growth compared with medium free-ABA. 0.25 and 1.0 mg/l ABA produced the highest same value (3.83) which reduced to 2.83 without

significant difference with 0.5 mg/l ABA while number of embryo matured under this condition was highest in the presence of 0.5 mg/l ABA (6.0 embryo/culture) follow by (4.0 embryo/culture) produced when 0.25 mg/l was used. Matured embryo produced with control medium did not differ significantly with those obtained when 1.0 mg/l ABA was used.

Effect of PEG in the presence of ABA:

Data in Table (3) and clarified that all concentration of PEG added to maturation media increased callus growth. The highest value were obtained by the addition of 20, 10 and 5 gm/l without significant difference as the results were (4.0, 4.0 and 3.0) while medium lacking PEG produced the lowest value. However number of matured embryos was significantly highest on control medium (without PEG). While PEG at 10 g/l produced the highest number of matured embryos compared with 5 and 20 gm without significant difference among them.

Effect of Gelling agent:

Data in Table (4) has shown that gelling agent had stimulatory effect on callus growth of date palm cv. Zaghloul. However the degree of stimulation was dependent upon the concentration of agar and gelrite used.

Callus growth was significantly promoted in response to adding 4.5, 2.5 gelrite and 7.0 g/l agar as the values of growth were (4.67, 4.33 and 4.33) respectively. Using agar at 7.0 g/l produced the highest significant value of matured embryo (5.67 embryo) while the lowest value of matured embryo number was observed by using the lowest concentration of agar (5.0g/l). However no significant difference could be observed between other treatments under investigation.

Germination stage:

Effect of GA_3:

Data in Table (5) show that noticeable differences were found between GA_3 concentrations and control medium on secondary somatic embryos production. The addition of 0.5 mg/l GA_3 to germination medium of date palm somatic embryos increased significantly the secondary somatic embryos number (31) followed by the addition of 1.0 mg/l GA_3 and control medium with significant difference among them. While the lowest value of secondary somatic embryos was noticed with higher concentration of GA_3.

Numbers of converted embryos as affected by GA_3 were significantly highest by using GA_3 at 1.0 and 0.5 mg/l. While control medium and medium of adding 1.5 mg/l GA_3 produced the nearest lowest value. Conversion frequencies as shown in this Table reflected that increasing the concentration of GA_3 from (0.5, 1.0 to 1.5 mg/l) in the germination medium increased the conversion frequency of somatic embryos to

plantlets as the percentages were (54.83, 66.66 and 76.90 %) respectively while control medium produced the lowest value.

Effect of ABA:

Data in Table (6) has shown that different ABA concentrations added to germination medium decreased the secondary somatic embryos number compared with control medium. Also increasing the concentration of ABA in culture medium from 0.25 to 1.0 mg/l decreased significantly secondary somatic embryos number from 32 to 18 embryos.

While the number of germinated (converted) embryos was higher by using ABA at the concentration 0.25 mg/l followed by control medium and that of adding 0.5 mg/l ABA without significant differences among them. The addition of ABA at 1.0 mg/l produced the lowest significant value in this respect. Conversion frequencies in the same Table clarified that the addition of 0.25 and 0.50mg/l ABA to germination medium achieved the highest conversion frequencies (56.3 and 50.0 %) respectively. While control medium and medium with 1.0 mg/l ABA produced the nearest lowest values.

Effect of PEG in the presence of ABA:

Data in Table (7) has shown that, the incorporation of PEG at 5.0 g/l in germination medium produced the highest significant value of secondary somatic embryos (23) which decreased significantly to (19 embryo) with the addition of 20 g/l while control medium and that of adding 10 g/l produced the same number of secondary somatic embryos (16 embryos). PEG concentrations significantly enhanced the number of germinated (converted) embryos with optimum results obtained in the presence of 5 gm/l PEG. No significant differences could be observed between all concentrations of PEG under investigation. On the other hand medium lacking PEG produced the lowest significant number of converted embryos. Conversion frequencies ranged from 62.5, to 57.9 and 52.0 % with 10, 20 and 5 g/l PEG respectively while control medium produced the lowest percentage of conversion.

Effect of Gelling agent:

Gelling agent as shown in Table (8) affected secondary somatic embryos number and their conversion into plantlets (shoot and root) solidified the culture germination medium with agar at 7, 5 g/l produced the highest significant number of secondary somatic embryos in accordance to other concentrations of agar and gelrite, followed by solidification with 2.5 g/l gelrite. While raising the concentration of gelrite to 4.5 g/l and agar to 9 g/l reduced significantly the number of embryos to the lowest value. Converted embryos were improved significantly to (22 and 20 converted embryos) with medium solidified by agar at 5, 7g/l respectively without significant differences. These values reduced significantly to 15, 14 and 13 converted embryos

with media solidified with 9g/l agar, 2.5 and 3.5g/l gelrite respectively. The lowest significant number of converted embryo was noticed with medium solidified by higher concentration of gelrite (4.5g/l). Table (8) shows how the germination (conversion) percentages vary with gelling agent at different concentrations. It is clear that the germination percentage increased to 83.3 % by using higher concentration of agar (9 g/l) reduced to 60 % with 5 g/l agar. From all somatic embryos formed under solidified treatments, only 56.5 and 54.1 % were converted by using 3.5 g/l gelrite and 7 g/l agar. The lowest conversion percentages were obtained by medium solidified by 2.5, 4.5 g/l gelrite.

DISCUSSION

In numerous systems, in spite of the high number of somatic embryos produced, problems with a lack or a low frequency of embryo conversion into plants occurred. To stimulate embryo conversion, and to improve the efficiency of plant regeneration, a number of different strategies were tested.

Gibberellic acid (GA_3):

Data under investigation showed that GA_3 added to culture medium at 1 mg/l during maturation stage produced the highest significant number of embryos while 1.5 mg/l produced the lowest value and when added to germination medium at different concentrations enhanced the conversion frequencies to shoot and roots. These results are associated with those reported by (Kim *et al.* 1997) whose recorded that gibberellic acid (GA_3) is frequently employed in media used for somatic embryo conversion, and a significant stimulatory effect of this phytohormone was proved in cultures of Sesamum indicum (Xu *et al.* 1997) and P. ginseng (Yang and Choi 2000). Rarely, GA_3 inhibited development of somatic embryos (Hutchinson *et al.* 1997). It is believed that GA_3 is especially necessary in cultures of somatic embryos, which undergo dormancy (Choi *et al,*. 1999. Sunandakumari *et al.*, 2005) reported that maturation of somatic embryos of *Euphorbia nivulia* Buch-HAM at different stage, especially at the early stage, took place in the presence of GA_3. Half strength MS medium enriched with 2-89 µM GA_3 facilitated maturation of 78 % embryos, whose cotyledons became dark green. The promoting effect of GA_3 on embryo maturation may be due to the synthesis of new gene products required for the completion of embryo development. Gibberellins (GA_3) are generally responsible for plant cell expansion and elongation, but their role in embryo development is not well understood. Gibberellins have been reported to both increase and decrease SE in

plants depending on plant species (Rademacher 2000, Rudus *et al*, 2000). Contributing to variability in response to GA_3 are differences in biological effects on SE at different steps in the process, such as embryogenic tissue induction or embryo development and maturation.

Abscissic acid (ABA):

ABA at different concentrations enhanced callus growth compared with control medium and using 0.25 and 0.5 mg/l ABA produced the highest value of embryos formed in this stage. Also, 0.25 and 0.5 mg/l ABA added to germination medium produced the highest conversion frequencies compared to control medium. On the other hand, ABA in germination medium decreased the secondary embryo production especially at higher concentrations comparable with control medium. In this respect, (Zouine *et al.*, 2005) found that embryogenic callus placed in liquid medium with 10^{-5} M ABA yielded an average of 72 embryos per 100 ml of culture medium within 2 months. Protein and sugar accumulation by somatic embryos in liquid culture medium increased linearly as the ABA concentration in the medium increased from 10^{-7} to 10^{-5}. Thus, the accomplishment of further maturation stages of date palm somatic embryos seems to be more closely dependent on exogenous ABA. (Hong and Debergh 1995) found that, addition of ABA to the medium of garden leek gave rise to a new type of callus: white, compact and with a lot of pro-embryonic structures developing from the original nodular callus. Different stages of embryo development could be observed in this compact embryogenic callus. (Teixeira *et al.*, 1994) found that, callus formed from immature inflorescence oil palm were made onto medium containing NAA (15 µM), ABA (2 µM) and no activated charcoal, globular structures developed into somatic embryos. Abscisic acid concentrations in the range 10-50 µM have been used to stimulate somatic embryo maturation. Generally, 1month treatment is considered to be adequate, although prolonged exposures increase the number of mature embryos form. (Peran-Quesada *et al.,* 2004) in avocado, 4-12 weeks exposures to concentrations in the range 1-1000 µM had no clear effect on the formation rate of w-o embryos, although an increase in average number of these structures was obtained in ABA-treated culture as compared to the control. (Branton and Blake 1983) noted that pearly-white globular, somatic embryos produced in cultures derived from immature inflorescences of oil palm were similar to those observed in callus lines derived from immature inflorescences of coconut. These structures developed into mature embryos when transferred onto a regeneration medium containing ABA. (Mauri and Manzanera 2004) found that, when immature somatic embryos were cultured on medium with ABA for 11 monthly subcultures, recurrent embryogensis was significantly reduced and maturation increased with continuous culture on medium

with ABA compared to control. (Onay *et al.* 2000) reported that all of the tested maturation treatments with ABA promoted germination of mature somatic embryos of Pistachion. After 4, 5 and 6 weeks of maturation stage on media containing different levels of ABA and sucrose. Lower concentrations of ABA gave the highest proportion of germination. (Dunstan *et al.*, 1995) stated that ABA plays an important role in both somatic and zygotic embryo maturation. ABA promotes embryo maturation supports the accumulation of storage proteins, lipids and starch, suppresses the formation of aberrant embryo structures and finally, prevents the maturing embryos from germinating precociously. The morphological changes accompanying ABA-promoted somatic embryo development in white pine are associated with gene expression, proteins (Dong *et al.*, 1997). The use of ABA for improved somatic embryo formation and their maturation in mature zygotic embryo- derived callus of coconut has been reported by (Samosir *et al.*, 1997). They showed that application of ABA increased the somatic embryo formation and plantlet regeneration in coconut immature zygotic embryo- derived. In addition, (Huong *et al.*, 1999) declared that proliferation and maintenance of embryogenic callus of *Phoenix canariensis* was on MS basal medium with 2.26 µM 2,4-D, 0.833µM kinetin and 2 µM abscisic acid (ABA), with a regular subculture every 3-4 weeks. Somatic embryo development was promoted by two months of culture on MS liquid medium enriched with 2µM ABA, for torpedo stage development. (Hassan 2002) recorded that the addition of ABA at 1.6 mg/l to culture medium was not effective on stimulating the production of secondary somatic embryos of date palm more than that produced from (ABA-free medium).

PEG in the presence of ABA:

All concentrations of PEG added to maturation medium in the presence of 0.5 mg/l ABA increased callus growth and the highest number of embryo formed in this stage was noticed by using 10 g/l PEG. On germination stage all concentrations of PEG increased conversion frequencies. However, higher concentration of PEG decreased significantly the number of embryo formed in maturation stage and also decreased the conversion frequencies compared with other concentration. In this respect (Al-Khayri and Al-Bahrany 2004) found that callus growth of date palm and water content were shown to be negatively related to water stress induced by increasing PEG concentrations. Osmotic adjustment through the accumulation of proline was positively related to PEG concentration. Growth was completely inhibited at higher concentrations of PEG. (Al-Khayri and Abu-Ali 2006) found that the addition of ABA to callus growth medium of date palm was inhibitory, but the degree of inhibition was modified by the concentration of PEG. In the absence of ABA,. 5%PEG stimulated culture growth. The number of resulting embryos was almost completely inhibited in

response to the highest concentration of embryogenesis occurred when 5% PEG was used. Also polyethylene glycol 4000 (PEG 4000) was reported to improve germination frequencies (root and shoot emergence) with limiting embryo histodifferentiation in soybean somatic embryo (Walker and Parrott 2001). Likewise in spruce, it was reported that PEG might improve the quality of somatic embryos by promoting normal differentiation of the embryonic shoot and root (Stasolla *et al.* 2003). Non-penetrating osmotic cannot penetrate into the plant cells, but restrict water uptake and provide a stimulated drought stress during embryo development. A combination of ABA and a non-penetrating osmotic as a supplement in the culture medium can help prevent precocious germination (Attree *et al.* 1991) and allow embryo development to precede (Linossier *et al.* 1997). An anatomical study performed on embryogenic cultures of *Panax ginseng* after 4 weeks of cultivation on maturation medium containing ABA and PEG 4000 revealed that the culture were not more synchronized compared to non-treated cultured at the multiplication stage and contained somatic embryos in different development stages appearing simultaneously. The somatic embryos started their development at the stage of meristematic centers through globular and heart-shape to torpedo-shape embryos. In spite of the fact that typical fine distinct inner histological differentiation of torpedo-shape embryos was not present, the PEG- and ABA-treated embryos were structurally more developed than untreated embryos. Treated somatic embryos were formed mainly of compact cells of meristematic character, of a small shape with dense cytoplasm, and an obviously as well as cotyledons and distinct root pole. Thus, ABA- and PEG-treated embryos of *Panax ginseng* exhibited improved histological differentiation, improved meristematic development of embryoids, and finally better regeneration and rooting of plantlets (Langhansova *et al.,* 2004). On the other hand, (Onay 1996) reported that the type of osmoticum used in conjunction with ABA affected development of conifer somatic embryos. In *Panax vera*, they found that sucrose and glucose were most favorable for embryo maturation and germination and that sorbitol, mannitol and PEG were completly ineffective in promoting proliferation, maturation or germination of somatic embryos. (Agarwal *et al* 2004) found that Polyethylene glycol (PEG) a potent osmoticum induces stress conditions equivalents to drought. When added to the medium the cells adapt to PEG accumulate additional sugars and amino acid. The PEG at15 $g1^{-1}$ with 6% sucrose was most responsive and also produced 20 % cotyledonary embryos. The high sucrose level also promotes osmoregulation by depositing sugars and proteins. Maturation of *Acacia nilotica* (Garg *et al.*, 1996) and *Aesculus hippocasranum* (Capuana and Debergh 1997) somatic embryos were improved by PEG treatment either alone or in combination with activated charcoal or ABA.

Gelling agent:

Callus growth of date palm was increased to highest value with 4.5gm/l gelrite used as a gelling agent compared with agar and other gelrit concentrations, while number of produced embryo in maturation stage was noticed with agar at 7 gm/l compared with other concentrations .Also increased the agar concentration to 9 gm/l and increasing gelrite to 3.5 gm /l increasing the conversion frequencies. In this respect (Peran *et al.*, 2004) reported that water relations between the embryo and its environment, *in vivo* or *in vitro*, play an important role in embryo development and particularly during maturation phase. To decrease water availability of the medium without the interference of osmotically active substances as sucrose, the concentration of gellan gum was increased and noticeable increase in number of w-o somatic embryos was observed. Increased concentration of gelling agent probably caused the conversion of translucent to w-o embryos, as suggested by (Monsalud *et al.*, 1995) in mango. Moreover, Avocado, w-o somatic embryos obtained in medium with high concentrations of gelling agent showed a higher germination capacity than those obtained in presence of high sucrose. In *Pinus strobes*, increasing gellan gum concentration also increased the number of w-o embryos as well as their conversion into plants (Klimaszewska and smith, 1997). The use of gelling agent Gellan gum (Phytagel) in the initiation and maintenance of embryogenic cultures instead of agar has also improved the development of embryonal suspensor masses. One of the gel characteristics that were influenced by the level of gelling agent in the medium was the strength. The use of higher concentration of Gellan gum in the initiation medium (2g/l) and 4g/l in the maintenance medium resulted in gels of higher strength. As a result of higher gel strength concomitant with reduced water availability stimulated a shift in the development program of the culture, from proliferation of embryogenic cells with cleavage polyembryony to the development of proembryos in the maintenance medium (Klimaszewska *et al.*, 2000). The higher concentration of maltose, Gellan gum and reduced concentration of growth regulators improved the development of proembryos on the maintenance medium (II) due to the reduced water availability of the medium. The embryogenic cultures with proembryos were maintained for 2-3 weeks on the maintenance medium. The current results confirmed earlier observations (Klimaszewska and Smith, 1997, Klimaszewska *et al.*, 2000) that reduced water availability resulting from high GelIan gum concentration promotes somatic embryo maturation of *Pinus strobus*, and that it does so for several embryogenic lines. The development of somatic embryos by partial- desiccation treatment caused by higher concentration of gelling agent in callus medium might be due to the factor that desiccation affected the endogenous ABA level (Rance *et al.*,

1994) found that partial desiccation of mature embryo derived calli led to differences in the soluble protein pattern as early as 1 day after the desiccation of the calli. They also suggested that the desiccation might trigger the rapid biochemical changes in the calli. Specific enzymes or polypeptides may appear in callus cultures in response to water stress. Stress induced proteins might be playing a key role in water stress tolerance, and improvement of maturation.

Table 1. Effect of GA_3 on callus growth and number of mature embryos during maturation stage.

GA_3 Concentration (mg/l)	Callus growth	No. of embryo
0.0	1.66	4.72
0.5	1.83	2.83
1.0	2.00	6.33
1.5	2.83	0.67
L.S.D at 0.05	0.880	2.311

Table 2. Effect of ABA on callus growth and number of mature embryos during maturation stage.

ABA concentrations (mg/l)	Callus growth	No. of embryos
0.0	2.00	2.33
0.25	3.83	4.00
0.50	2.83	6.00
1.00	3.83	2.17
L.S.D at 0.05	1.153	2.189

Table 3. Effect of PEG in the presence of 0.5 mg/l ABA on callus growth and number of mature embryos during maturation stage.

PEG concentration (gm/l)	Callus growth	No. of embryos
0.0	1.66	4.72
5	1.83	2.83
10	2.00	6.33
20	2.83	0.67
L.S.D at 0.05	1.189	1.891

Table 4. Effect of gelling agent (gelrite and agar) on callus growth and number of mature embryos during maturation stage.

Gelling agent concentration (gm/l)	Callus growth	No. of embryos
2.5 gelrite	4.33	2.67
3.5 gelrite	2.83	2.33
4.5 gelrite	4.67	2.33
5 agar	3.17	1.33
7 agar	4.33	5.67
9 agar	3.83	2.17
L.S.D at 0.05	0.774	1.458

Table 5. Effect of GA_3 on embryo formation (number of embryos, number of converted embryos) and conversion frequency during germination stage.

GA_3 concentrations (mg/l)	Secondry somatic embryo	Converted embryos	Conversion frequency (%)
0.0	17	8	47.00
0.5	31	17	54.83
1.0	27	18	66.66
1.5	13	10	76.90
L.S.D at 0.05	3.106	3.106	

Table 6. Effect of ABA on embryo formation (number of embryos, number of converted embryos) and conversion frequency during germination stage.

ABA concentration (mg/l)	Secondary somatic embryo	Converted embryo	Conversion frequency (%)
0.00	38	16	42.10
0.25	32	18	56.30
0.50	26	13	50.00
1.00	18	8	44.00
L.S.D at 0.05	4.791	6.021	

Table 7. Effect of PEG in the presence of ABA on embryo formation (number of embryos, number of converted embryos) and conversion frequency during germination stage.

PEG concentrations (g/l)	Secondary somatic embryo	Converted embryo	Conversion frequency (%)
0.0	16	7	46.70
5	23	12	52.00
10	16	10	62.50
20	19	11	57.90
L.S.D at 0.05	2.883	3.826	

Table 8. Effect of gelling agent (agar and gelrite) on embryo formation (number of secondry somatic embryos, number of converted embryos) and conversion frequency during germination stage.

Gelling agent concentration (g/l)	Secondary somatic embryo	Converted embryo	Conversion frequency (%)
2.5 gelrite	29	14	48.3
3.5 gelrite	23	13	56.6
4.5 gelrite	17	8	47.1
5 agar	35	22	60.0
7 agar	37	20	54.1
9 agar	18	15	83.3
L.S.D at 0.05	2.799	3.238	

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بعض العوامل المؤثرة على نضج وانبات الأجنة الجسمية لنخيل البلح

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تم الحصول على زراعات الكالوس لنخيل البلح بزراعة القمة النامية فى بيئة مواشيجى وسـكوج (١٩٦٢) مضافا إليها ١٠ مجم ثنائى كلورو حامض الخليك 2.4 – D ، ٣مجم/لتر ايزوبنتايل 2 i P ادنين بالإضافة لوجود ١,٥ مجم/لتر فحم نشط لمدة ٣٢ اسبوع مع نقل الزراعات بانتظـام كـل ٨ أسابيع. وقد تم دراسة تأثير كل من حامض الجبريليك، حامض الأبسيسك، بولى اثيلين جليكول فـى وجود حامض الأبسيسك وكذلك تم دراسة تأثير عوامل التصلب فى البيئة (أجار وجيلريت). وقد تم دراسة هذه العوامل وإضافتها إلى البيئة لمدة ١٢ اسبوع مع النقل المنتظم إلى بيئة طازجـة تحتـوى على نفس المعاملات كل ٤ أسابيع وذلك في مرحلتي النضج والإنبات وقـد أدى إضـافة حـامض الجبريليك بتركيز ١مجم/لترأو إضافة حامض الأبسيسك بتركيز ٠,٥ مجم/لتـر أو البـولى اثيلـين جليكول بتركيز ١٠جم/لتر فى وجود ٠,٥جم/لتر حامض الأبسيسك وكذلك إضافة الأجار بتركيـز٧ جم/لتر إلى الزيادة المعنوية لعدد الأجنة فى مرحلة النضج. وقدأدى إضافة حامض الجبريليك والبولى ايثلين جليكول بكل التركيزات تحت الدراسة وكذلك إضافة حامض الأبسيسـك بتركيـز ٠,٢٥، ٠,٥ مجم/لتر للبيئة إلى زيادة نسبة تحول الأجنة إلى نبيتات مقارنة بالمعاملة الكنترول. وقـد أدى زيـادة تركيز الاجار كمادة مصلبة للبيئة إلى أقصى تركيز (٩ جم/لتر) إلى زيادة نسبة تحول الأجنـة إلـى نبيتات.