Somatic embryogenesis in *Phoenix dactylifera*: maturation, germination and reduction of hyperhydricity during embryogenic cell suspension culture

Sidky R.A. and Gadalla E.G.
The Central Laboratory for Date Palm Research and Development,
Agricultural Research Center, Egypt. sidky1234rehab@hotmail.com

ABSTRACT

This work describes an improved and efficient method for optimum maturation and normal embryo growth to produce healthy plantlets through somatic embryogenesis using suspension cultures for date palm cv. Sakkoty. Cell suspensions were supplemented with low amounts of dichlorophenoxyacetic acid (0.5mg/1) and different concentrations of abscise acid (ABA) (0.1, 0.3 and 0.5 mg/l) and polyethylene glycol (PEG 4000) (1, 3 and 5g/l). Factors affecting embryogenic callus maturation and germination embryos were investigated. Somatic embryos and callus fresh weight increased at 3g/l of PEG 4000. Treated somatic embryos with 5g/l PEG reduced hyperhydricity. Transferring them on the germination medium (G1) supplied with 3 g/l PEG improved the embryo growth. Secondary embryos were produced at 0.5 mg/l ABA .Shoot proliferation and development of plantlets occurred on medium containing 0.1 NAA mg/l, 0.2 mg/l BA and 0.2 mg/l kin (G2). The highest accumulation of proteins was obtained with 0.5 or 0.3 mg/l ABA. The plantlets were transferred successfully to greenhouse.

Keywords: *In vitro,* Date palm, ABA, PEG, Somatic embryogenesis, Maturation, Desiccation, Germination and proteins.

INTRODUCTION

Date palm, *Phoenix dactylifera* L., is one of the oldest fruit trees in the world and is mentioned in the Holy Qur'an and Bible. Date palm is one of the most important fruit trees in the Middle East and in the Saharan and Sub-Saharan regions of Africa. In some areas, this is the only tree which provides food, shelter and fuel to the communities. Dates are not only a staple food but are also an important export cash crop (Zaid and Hegarty, 2006). Embryogenic suspension culture is defined as single cells or small cell aggregates in agitated liquid media (Preil, 2005).

The use of large-scale liquid cultures and automation have been well documented, and benefit have been shown both for resolving the manual handling of various stages of micropropagation, decreasing production cost signiafantly and for better plant performance by allowing a direct contact of the medium throughout the plant material (Zobayed and Saxena, 2003).

A typical somatic embryogenesis protocol for date palm involves a series of consecutive stages beginning with callus induction, embryogenesis callus multiplication, somatic embryo maturation and somatic embryo germination (El Hadrami, 1995 and El Bellaj, 2000). In most cases, embryogenic calluses were induced on medium containing growth regulators, especially 2,4-D (El Hadrami, 1995; ELBellaj, 2000; Fki *et al.,* 2003 and Gadalla, 2007)). Maturity of somatic embryos may be induced by the application of exogenous ABA (El Bellaj, 2000 and Corredoira *et al.,* 2003).

Label and Lelu (2000) indicated that ABA plays an important role in both somatic and zygotic embryo maturation. These same authors indicated that ABA promotes embryo maturation, supports the accumulation of storage proteins, lipids and starch; it suppresses the formation of aberrant embryo structures and, finally prevents the mature embryo from germinating precociously. Choi *et al*, (1999); Kim *et al.,* (1999) and Klimaszewska *et al.,* (2001) reported that the culture medium constituents particularly osmoticum, has a marked effect on somatic embryos. Also, the attempt to increase the quality of somatic embryos by using the high 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 gerera of coniferales (Bozhkov and Von Arnold 1998) and selected tree species such as *H. braziliensis* (Linossier *et al.,* 1997).

This paper describes an improved and efficient method for optimum maturation and normal growth of embryo to produce healthy plantlets without hyperhydricity through somatic embryogenesis using suspension cultures for date palm cv. Sakkoty; the most common dry cultivar in Upper Egypt.

MATERIAL AND METHODS

Plant material and culture conditions

Embryogenic cultures were induced from shoot tips of *Phoenix dactylifer*e L. (cv.Sakkoty)cultured on solid medium containing MS salt and vitamins (Murashige and Skoog, 1962), 10mg/l 2,4-D, 3mg/l 2ip, 40 g/l sucrose, 200 mg/l glutamine, 40 mg/l adenine sulfate and 1.5 g/l activated charcoal (AC) solidified with 6 g/l Agar-Agar. Prepared medium was adjusted to pH 5.7 ± 0.1 and distributed into small jars (200 ml), each one contains 50ml of prepared medium and then autoclaved at 121 °C and 1.5 cm /ins2 for 20 min. Cultures were kept in darkness at 28 ± 2 °C and re-cultured every 6 weeks until the initiation of embryogenic callus.

Establishment of embryogenic suspensions

Five hundred milligrams of friable callus was chopped into small pieces and transferred aseptically into 50 ml liquid medium in 250 ml Erlenmeyer flasks containing half salts and vitamins (Murashige and Skoog, 1962) except Fe-EDTA which was full strength, 0.5 mg/l 2.4 –D, 200 mg/l KH2PO4, 40 gm sucrose, 100 mg/l myoinsitol and 100 mg/l Arginin, 100 mg-1 glutamine, 0.3 g activated charcoal (AC) (Gadalla, 2007), in addition to different concentrations of ABA (0.1, 0.3 and 0.5 mg/l), polyethylene glycol 4000 (1,3 and 5 g/l) . Cultures were maintained on a rotary shaker at 120 rpm at 25 ± 2 °C under darkness. Suspensions were subcultured every two weeks by decanting off the old medium and replacing it with fresh medium of the same composition for maturation. Embryogenic cell clumps were filtered through a 1 mm sieve to determine number of somatic embryos and callus fresh weight.

Partial desiccation and reducing Hyperhydricty

The developed of embryos occurred after sieving (mesh size= 1 mm) were put in sterile empty Petri dishes containing two sterile Whatman filter paper disks and kept in the dark for 2 h desiccation. The partial desiccation embryos were transferred to solid medium. After desiccation, three somatic embryos / jar from every concentration of ABA and PEG was cultured on MS solid medium supplemented with the same composition, each treatment consisted of three replicate . Data were taken on the hyperhydricy percentage (vitrified embryos/ total embryos*100), number of secondary embryos and germination percentage (G1) after one subcultures (4 weeks).

Germination and shoot proliferation of somatic embryos

Advanced somatic embryos produced were cultured on MS solid medium supplemented with 0.1 NAA mg/l + 0.2 mg/l BA+0.2 mg/l kin, 200 mg/l KH2PO4, 40 g sucrose, 100 mg/l myoinsitol, 100 mg/l glutamine and 0.3 g AC. The medium was distributed to small jars (200ml), each one contains 40 ml. Cultures were kept in light (2000 lux) at 25 ± 2 °C. Data were taken on the germination percentage (G2) (embryos with single shoot and root / total embryos x 100) and number of shoots after two subcultures (8 weeks). Advanced somatic embryos were considered germinated as soon as radical emergence occurred with plantlet based on shoot greening and elongation.

Rooting stage

Plantlets were cultured on *1/2* MS liquid medium supplemented with 1.0 NAA mg/l, 200 mg/l KH2PO4, 40 g sucrose, 100 mg/l myoinsitol and 1g/AC, and incubated under 6000 lux light (Fig.3c).

Plant acclimatization

Healthy regenerated plantlets were individually removed from tubes, agar was rinsed off, and plants were cultivated in plastic pots filled with peat moss and perlite (1:1 ratio) in a greenhouse (Fig.3d).

Extraction and analysis of soluble proteins

The protein was extracted according to the method described by Lecouteux *et al* (1994). Fresh callus (250 mg) was ground with 2 ml of 0.25 M phosphate buffer (pH 7.2) and centrifuged for 3 min at 7000 rpm.

Supernatant was used as the crude extract. The level of total proteins was measured by spectrophotometry at 595 nm according to Bradford (1976) method.

Statistical analysis

All data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1980). LSD at 5% level of significance was used to compare between means according to Steel and Torrie (1980).

RESULTS

Number of somatic embryos and callus fresh weight

Embryogenic callus placed in liquid culture medium with 0.5 mg/ l 2, 4-D in order to examine the influence of ABA and PEG concentration on somatic embryos number and growth of fresh weight Table 1. Both substances were used separately. The highest production of number embryos was obtained with PEG 3g/ l (22.33 embryo/jar), followed by the addition of 0.3 mg/l ABA to culture medium (16.33 embryo/ jar) without significant difference in between while addition of PEG at 5.0 g/l decrease number of embryos significantly (3.66 embryo/jar). Data in the same Table clarified that all concentrations of ABA and PEG added to media increased callus growth. The highest significant value of callus growth was obtained by the addition of 3.0 gm/l PEG (4.413).while the lowest value of callus growth was observed by using PEG at 5.0 gm/l (2.550). However no significant difference could be observed between other treatments under investigation Fig.2.PEG was beneficial to somatic embryo proliferation and increases the quality of mature somatic embryos.

Hyperhydricty percentage, number of secondary embryos and germination percentage (G1)

The duration of the culture period in liquid medium was found to be very important for the balanced germination of somatic embryo, Culture of mature embryos in liquid medium for longer than 1 month led to hyperhydration phenomena (Fki *et al.,* 2003). Interestingly, the partial desiccation of somatic embryos, corresponding to a reduction of hyperhydracty (Fig.1), then transfer embryos to the germination medium (G1) supplied with ABA and PEG concentrations reduced hyperhydricy with more mature somatic embryos formation. The hyperhydricty percentage of somatic embryos (Table 2) was significantly reduced in the presence of PEG; the lowest percentage of hyperhydricty resulted at 5g/l PEG (21.67 %) compared to 0.1mg/l ABA which was 63.33%. The germination rate (G1) of somatic embryos was low when cultured into medium supplemented with ABA and PEG concentration (Fig.3a). The highest germination percentage of somatic embryos was record at 3 g/l and 1 g/l PEG (88.87 and 66.63% respectively) without hyperhydricty compared to the concentration of ABA 0.3 and 0.5 mg/l (11.10%). Data revealed that adding 0.5 mg/l ABA, 3 g/l PEG or 0.3 mg/l ABA to the culture medium was superior in increasing the number of secondary embryos (6.33, 5.66 and 5.33, respectively).

Transferring advanced embryo to medium supplemented with 0.1 NAA mg/l + 0.2 mg/l BA+0.2 mg/l kin proliferated normal shoots. In Table (3), the highest germination percentage (100%) was observed in embryos that had been cultured on media containing 3, 1 g/l PEG or 0.1mg/l ABA. After 8 weeks on proliferation medium, shoot number was determined (Table 3 and Fig.3b). The highest significant value of shoots was obtained with 3g/l PEG (6.33 shoots), followed by 0.1 or 0.3 mg/l ABA resulted in the same value (4.66 shoots). Increasing concentration of ABA or PEG decreased significantly values of shoots (3.00 and 2.66 respectively).

Total protein content

The addition of ABA and PEG had an important effect on date palm protein content. As illustrated in Fig.4, the amount of total proteins increased significantly at 0.5 and 0.3 mg/l ABA (1.591, 1.584 mg/g DW, respectively) followed by 1 g/l PEG (1.504 mg/g DW) in embryogenic callus.

DISCUSSION

Maturation stage

Using 2,4-D at 0.5 mg/l and different concentrations of ABA (0.1,0.3, and 0.5 mg/l) and PEG (1.0,3.0 and 5.0 g/l) enhanced callus fresh weight and number of mature embryos of date palm. These results are in line with those reported by Gadalla (2007) who found that in date palm Khalas cv., liquid culture medium with half strength of MS formula containing 0.5 mg/l 2, 4-D influenced fresh weight of embryogenic callus (5 to 6 fold approximately/month), number of pro-embryo (globular and juvenile) and number of mature embryos). Zouine and El Hadrami (2007) reported that the liquid medium containing 0.1 mg /l of 2,4-D was beneficial for somatic embryo production. Zouine *et al.,* (2005) found that embryogenic callus placed in liquid medium with 10-5 M ABA, 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. Othmani *et al. (*2009) found that callus transferred to medium supplemented with 1mg/l ABA+ 0.1 g/l AC proliferated normally with an average of 51 somatic embryos observed after about 7 weeks per 0.5 g FW of embryogenic callus. Fki *et al.,* (2003) found that in date palm cultivar (Deglet Nour), the subculture of embryogenic

suspension in a fresh medium with low amounts of 2,4-D (1 mg/l) resulted in the differentiation of a large number of somatic embryos (from 10 to 200 embryos per month per 100 mg fresh weight of embryogenic calli). Fernando and Gamage (2000) concluded the possibility of using ABA to enhance somatic embryogenesis and plant regeneration of coconut. 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. (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 and suppresses the formation of aberrant embryo structures. According to Langhansova *et al*. (2004), a maturation stage was accomplished by insertion of PEG 4000 and ABA between multiplication and regeneration phase. Adding of PEG to maturation medium in many cases has been shown to stimulate maturation (Bozhkov and Von Arnold, 1988).

In our case, the quality of fresh weight was observed in presence of PEG 4000 and number of mature somatic embryos was increased; these data are in agreement with Kong and Yeung, (1995) who found that the number of mature somatic embryos increased significantly when PEG 4000 was applied in maturation medium. The positive effect of osmoticum on embryo maturation has been attributed to increasing levels of endogenous ABA (Wilen *et al*. 1990). Stasolla *et al.,* (2003) reported that, the inclusion of PEG to the culture medium can improve the number and the quality of the embryos produced. 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.

Germination stage

PEG at 5.0 and 3.0 g/l and desiccation treatment caused reduction of hyperhydricty phenomena of date palm. Increasing the concentration of ABA reduced the germination (shoot number) but increased number of secondary embryos. However, using ABA at 0.5, 0.3 mg/l increased protein content. These results are in line with those reported by Zouine *et al. (*2005) reported that the quality of somatic embryos was markedly lowered in the absence of exogenous ABA and a number of hyperhydricty somatic embryos were observed. According to Reidiboym-Talleux *et al*. (1999) hyperhydricty may also be explained by a lack of desiccation period and low endogenous ABA level. Fine chopping and partial desiccation (6 and 12 h) of embryogenic calli with proembryos prior to transfer to MS medium supplemented with 1 mg l−1 ABA stimulated the rapid maturation of somatic embryos. Othmani *et al. (*2009), and Fki *et al*. (2003) reported that, chopping the callus into small pieces favorite the formation of Pro-embryonic masses and the partial desiccation of mature somatic embryos (corresponding to a decrease in water content from 90 to 75%) significantly improved germination rates (from 25 to 80%).

Dunstan *et al*. (1995) stated that ABA prevents the maturing embryos from germinating precociously. Adding of PEG to 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 1988). Fernando *et al. (*2003) revealed that abscisic acid induced plant regeneration through somatic embryogenesis of oil palm. 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 found that PEG might improve the quality of somatic embryos by promoting normal differentiation of the embryonic shoot and root (Stasolla *et al*. 2003). Sghaier *et a*l. (2009) reported that ABA plays an important regulatory role in ZE maturation and its action was mainly observed on the synthesis of storage proteins by blocking germination and anabolism. This hormone also favors the transport of storage compounds from plant to seeds. A very similar study has been performed in oil palm by Morcillo *et al.* (1999), in which ABA displayed a noticeable role in the accumulation of the total quantity of soluble protein content and especially of the storage protein (Globuline 7S). Similarly, Preeti *et al.* (2004) showed that ABA treatment (5mgl-1) for 14 days significantly increased the starch level and total protein content of *Camellia sinensis* SE. The embryo induction medium supplemented with ABA increased the total storage protein content (to about 29% of embryo DW) in alfalfa SE (Sreedhar and Bewley, 1998). Pliego-Alfaro *et al*. (1996) suggested that ABA is an important PGR for the accumulation of storage reserves, lipids, proteins, and carbohydrates that have a positive effect in the maturation of somatic embryos.

References

Bozhkov, P. V. and Von Arnold, S. (1998). Polyethylene glycol promotes maturation but inhibits further development of *Picea abies* somatic embryos. Physiol. Plant., 104: 211-224.

Bradford, M.M (1976):. A rapid and sensitive method for the quantification of micrograms quantities of protein utilization the principle of protein – dye binding. Anal. Bioch., 72: 248-254.

Capuana, M. and P.C. Debergh (1997). Improvement of the maturation and germination of horse chestnut somatic embryos. Plant physiol., 89:768-775.

Choi Y.E.; Yang D.C.; Yoon E.S. and Choi K.T. (1999). High efficiency plant production via direct somatic single embryogenesis from preplasmolysed cotyledon of Panax ginseng and possible dormancy of somatic embryos. Plant Cell Rep., 18:493-499.

Corredoira, E., Ballester, A. and Vieitez, A.M., (2003). Proliferation, maturation and germination of *Castanea sativa* Mill. Somatic embryos originated from leaf explants. Ann. Bot., 92, 129–136.

Dunstan, D.I, Tautorus, T.E. and Thorpe, T.A. (1995). Somatic embryogenesis in woody plants. In: Thorpe T.A (ED.) *in vitro* embryogenesis in plant. Dordrecht: Kluwer Academic Publishers, Pp. 471-538.

El Bellaj, M., (2000).Etude de quelques parame`tres biochimiques en relation avec l'acquisition des potentialite´s embryoge`nes et la maturation des embryons somatiques chez le Palmier dattier (*Phoenix dactylifera* L.). The`se de Doctorat. Universite´ Cadi Ayyad, Faculte´ des Sciences-Semlalia, Marrakech.

El Hadrami, I., (1995). L'embryogene`se somatique chez *Phoenix dactylifera* L.: quelques facteurs limitants et marqueurs biochimiques. The`se de Doctorat. Universite´ Cadi Ayyad, Faculte´ des Sciences-Semlalia, Marrakech, 227 pp

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

Fernando, S. C.; Verdeil, J.L.; Hocher, V.; Weerakoon, L.K. and Hirimburegama, k. (2003). Histological analysis of plant regeneration from plumula explants of *Cocos nuifera*. Plant Cell Tissue and Organ Culture, 72(3): 281-284.

Fki, L., Masmoudi, R., Drira, N and Rival, A. (2003). An optimised protocol for plant regeneration from embryogenic suspension cultures of date palm, *Phoenix dactylifera* L., cv. Deglet Nour. Plant Cell Rep. 21: 517–524.

Gadalla, E. G. (2007). High frequency somatic embryo production and maturation into plantlets in date palm (*Phoenix dactylifera* L.) through suspension culture. Egypt.J.Agric.Res., 85(B): 349-365.

Garg, L. Bhandari, N.N. Rani, V. and Bhojwani, S.S. (1996). Somatic embryogenesis and regeneration of triploid plants in endosperm cultures of *Acacia nilotica* .Plant Cell Rep.,15: 855-858.

Huong, L. T., Baiocco, M., Huy, B. P., Mezzetti, B., Santilocchi, R. and Rosati P. (1999). Somatic embryogensis in Canary Island date palm. Plant Cell Tissue and Org. Cult. 56: 7.

Kim, Y.W., Youn J., Noh, E. R. and Kim, J.C. (1999): Somatic embryogenesis and plant regeneration from imature zygotic embryos of japanes larch (*Larix leptolepis*). Plant Cell Tissue and Organ Culture, 55; 95-101.

Klimaszewska, K., Park, Y. S., Overton, C.; Ian, N. and Bonga, M. J. (2001). Optimized somatic embryogencis in *Pinus strubus* L. *In vitro* Cell Dev. Biol. Plant. 37: 392-399.

Kong, L. and Yeung, E. C. (1995). Effects of silver nitrate and polyethylene glycol on white spruce (*Picea glauca*) somatic embryo development: enhancing cotyledonary embryo formation and endogenous ABA content. Physiol. Plant., 93: 298-304.

Label, P. and Lelu, M.A. (2000). Exogenous abscisic acid fate during maturation of hybridlarch (*Larix leptoeuropaea*) somatic embryos. Physiol. Plant, 109: 456–462.

Langhansova, L.; Konradova, H. and Vanek, T. (2004). Polyethylene glycol and abscisic acid improve maturation and regeneration of *Panax ginseng* somatic embryos. Plant Cell Rep., 22: 725-730.

Lecouteux, C.G., Lai, F.M., Bryan, D., Mc Kresie, B.D., 1994. Maturation of Alfalfa (Medicago sativa L.) somatic embryos by abscisic acid, sucrose and chilling stress. Physiol. Plant. 94, 207–213.

Linossier, L.; Veisseire, P.; Cailloux, F. and Coudret, A. (1997). Effect of abscisic acid and high concentration of PEG on *Hevea brasiliensis* somatic embryos development. Plant Sci. 124; 183-191.

Morcillo, F.; Aberlenc-Bertossi, F; Noirot, M.; Hamon, S and Duval, Y. (1999). Differential effects of glutamine and arginine on globulin accumulation during the maturation of oil palm somatic embryos. Plant Cell. Rep., 18, 868–872.

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

Othmani, A., Bayoudh, C. and Drira, N. (2009). Somatic embryogenesis and plant regeneration in date palm *Phœnix dactylifera* L., cv. Boufeggous is significantly improved by fine chopping and partial desiccation of embryogenic callus. Plant Cell Tissue Organ Culture 97, 71–79.

Pliego-Alfaro, F., Monsalud, M.J.R, Litz, R.E., Gray, D.J. and Moon, P.A. (1996). Effect of abscisic acid osmolarity and

partial desiccation on the development of recalcitrant mango somatic embryos. Plant Cell Tissue Organ Cult., 44:63–70.

Preeti, S., Pandey, S., Bhattacharya, A., Nagar, P.K and Ahuja, P.S. (2004). ABA associated biochemical changes during somatic embryo development in *Camellia sinensis* (L.) Kuntze O. Plant Physiol., 161: 1269–1276.

Preil, W. (2005). General introduction: a personal reflection on the use of liquid media for *in vitro* culture. In: Hvoslef-Eide AK, Pril W (eds.) liquid culture systems for in vitro propagation. Spriger, Netherlands, pp 1-18.

Reidiboym-Talleux, L.; Diemer, F.; Sourdioux, M.; Chapelain, K. and Grenier-De March, G. (1999). Improvement of somatic embryogencis in wild cherry *(Prunus avium)*. Effect of maltose and ABA supplements. Plant Cell Tissue Organ Culture 55, 199–209.

Sghaier, B., Kriaa, W., Bahloul, M., Jorrín-Novo, J. V. and N. Drira.(2009). Effect of ABA, arginine and sucrose on protein content of date palm somatic embryos.120, 379–385.

Snedecor, G. W. and W. G. Cochran (1980). "Statistical Methods", 7th edition, the Lowa State Univ. press. Amer., PP: 365-372.

Sreedhar, L. and Bewley, J.D. (1998). Nitrogen- and sulphur containing compounds enhance the synthesis of storage reserves in developing somatic embryos of alfalfa (*Medicago sativa* L.). Plant Sci., 134: 31–44.

Stasolla, C., L., Van Zyl, U., Egertsdotter, D., Craig, Liu, W. and Sederoff, R.R (2003). The effect of polyethylene glycol on gene expression of developing white spruce somatic spruce somatic embryos. Plant Physiol 131: 49-60.

Steel, R.G.O and J. H. Torrie (1980). Principles and procedures of statistics. A biometric approach 2 nd Ed McGrauf – Hill Book Co., New York NY.

Walker, D.R. and Parrott, W.A. (2001). Effect of polyethylene glycol and sugar alcohols on soybean somatic embryo germination and conversion. Plant Cell Tiss. Org. Cult. 64: 55-62.

Wilen, R. M., Mandel, R. M., Pharis, R. P., Holbrook, L. A. and Moloney, M. M. (1990). Effect of ABA and high osmoticum on storage protein gene expression in microspore embryos in *Brassica napus*. Plant physiol., 94: 875-881.

Zaid, A and Hegarty, V. (2006). Focus on: Producing date palm trees with improved fruit yield, short height, and resistance to Bayoud disease. "The Third International Conference on Date Palm", 20-22 Feb. 2006. Emirates Palace Hotel, Abu Dhabi, UAE.

Zobayed, S.M.A and Saxena, P.K (2003). *in vitro*- grown roots: a superior explant for prolific shoots regeneration of St. John,s wort (*Hypericum perforatum* L. cv., New Stem,) in a temporary immersion bioreactor. Plant Sci 165:463-470.

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

Zouine, J. and El-Hadrami, I. (2007). Effect of 2,4-D, glutamine and BAP on embryogenic suspension culture of date palm (*Phoenix dactylifera* L.). Sci. Hort., 112:221-226.

Tables

Table (1): Number of somatic embryos and callus fresh weight produced at different concentrations of abscisic acid and polyethylene glycol after 2 months.

Treatments	No. of somatic embryos/ jar	Callus Fresh weight (FW) g/l
0.1 mg/l ABA	6.00	3.387
0.3 mg/l ABA	16.33	2.990
0.5 mg/l ABA	9.33	4.223
1g PEG	14.00	3.887
3g PEG	22.33	4.413
5g PEG	3.66	2.550
LSD 0.05%	3.86	1.700

Table (2): Effect of various concentrations of ABA and PEG on hyperhydricty percentage and number of secondary embryos after 4 weeks.

Treatments	Hyperhydricty Percentage (%)	Germination Percentage (%) (G1)	No. of secondary embryos
0.1 mg/ lABA	63.33	22.20	4.00 B
0.3 mg/l ABA	56.67	11.10	5.33 A
0.5 mg/l ABA	50.00	11.10	6.33 A
1g/l PEG	46.67	66.63	3.33 B

Treatments	Hyperhydricty Percentage (%)	Germination Percentage (%) (G1)	No. of secondary embryos
3g/l PEG	30.00	88.87	5.66 A
5g/l PEG	21.67	55.50	3.66 B
LSD 0.05%			1.229

All treatments were exposed to two hours under laminar flow (Desiccation treatment)

Table (3): Effect of proliferation medium on germination percentage and shoot number after 8 weeks.

Treatments	Germination Percentage (%)(G2)	No. of shoots
0.1 mg/l ABA	100	4.66
0.3 mg/l ABA	88.87	4.66
0.5 mg/l ABA	77.73	3.00
1g/l PEG	100	3.33
3g/l PEG	100	6.33
5g/l PEG	88.87	2.66
LSD 0.05%		1.473

Figures:

Fig.(1): Desiccation of embryos from suspension culture.

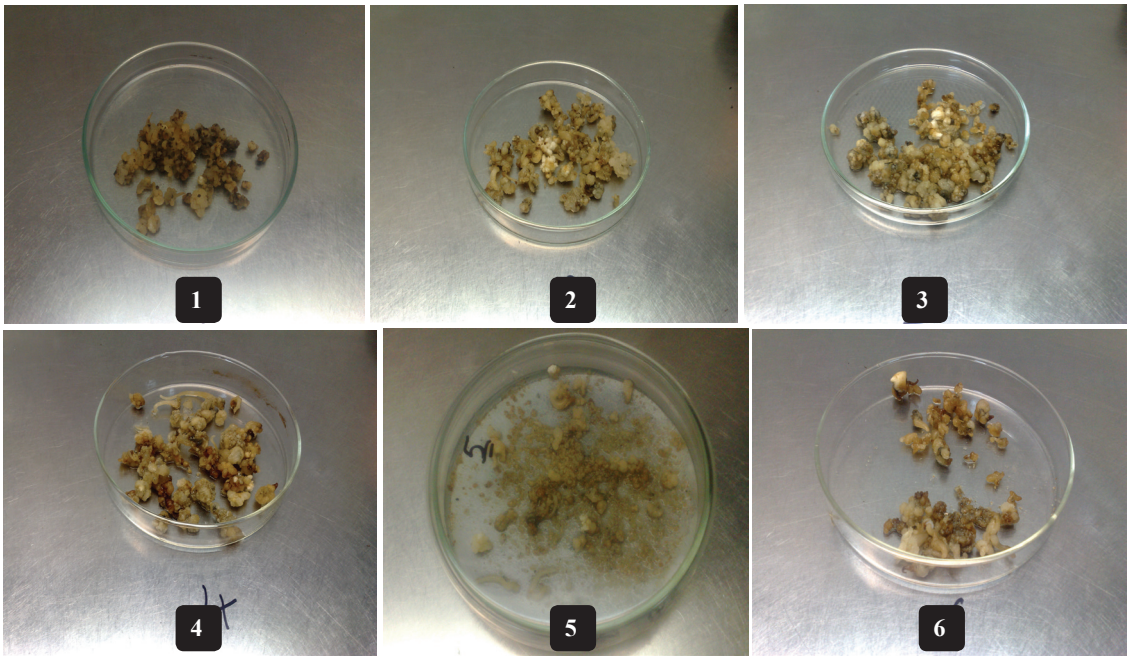

Fig.(2): Production of pro-embryos in suspension culture of date palm using different concentrations of ABA (1) 0.1 mg/l,(2) 0.3 mg/l, (3) 0.5 mg/l and PEG(4) 1 g, (5) 3gm, (6) 5g respectively.

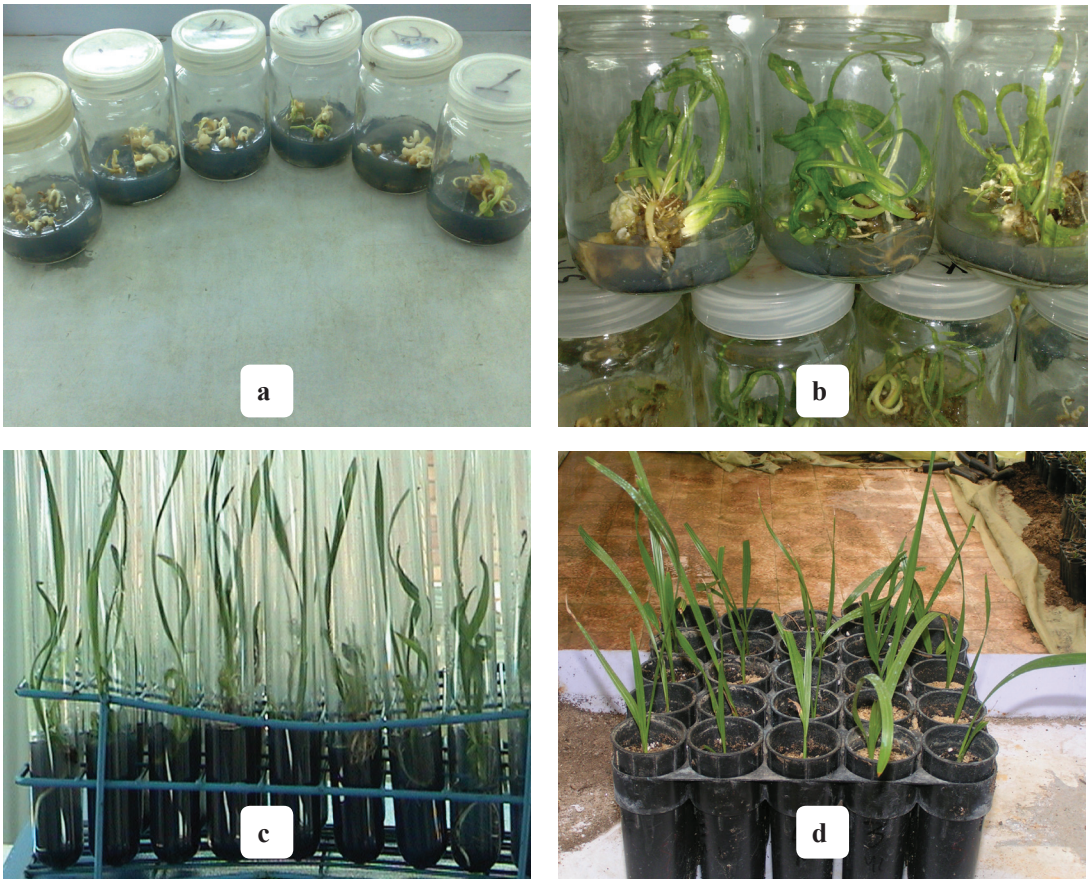

Fig.(3): (a) Different concentration of ABA and PEG on somatic embryos after 4 weeks (b) Shoot proliferation (c) plantlet derived from embryogenic suspension and d) Acclimatization of plantlets.

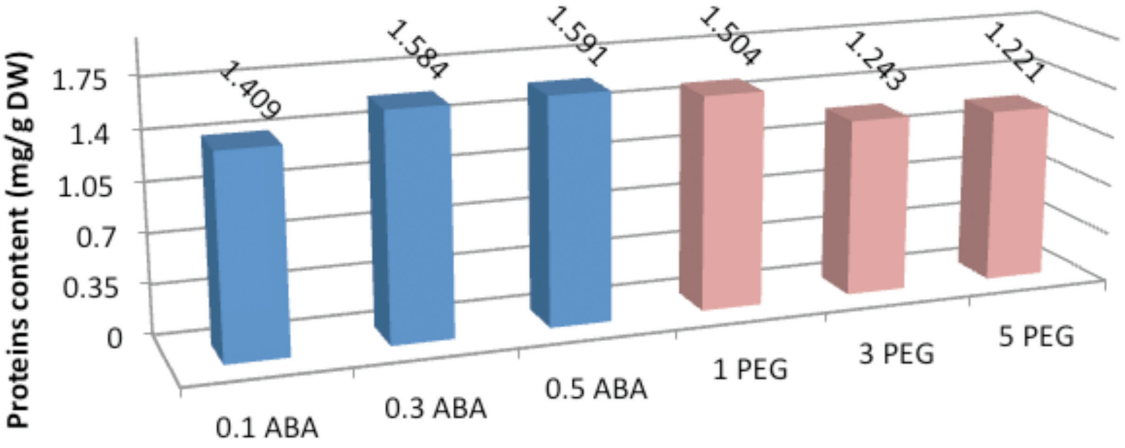

Fig.(4): Protein content of date palm embryogenic callus.