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Research Paper

INFLUENCE OF POLYETHYLENE GLYCOL AND GIBBERELLIN ON SOMATIC EMBRYOGENESIS OF DATE PALM (*Phoenix dactylifera*) CV SUKKARY

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Abstract

The objective of this research was to study the effect of polyethylene glycol (PEG) at different concentration i.e. 2, 4, 6% and gibberellin (GA_3) at 0.2, 0.3, 0.6 mg/l on somatic embryogenesis of date palm cv. Sukkary. The results shown that PEG at low concentration (2%) recorded the highest number of both total embryos and germination while the higher concentration (6%) gave the highest value in fresh and dry weights. Gibberellin at 0.3 mg/l was improved production of embryogenesis and germination. The interaction between 4% PEG and 0.6 mg/l GA_3 produced the highest number of embryos and germinating embryos. Moreover, the interaction 2%+0.3 mg/l came in the second rank without significant effect. Finally, it can be concluded that addition PEG at 2% and GA_3 at 0.3 mg l^{-1} produced the highest number of somatic embryogenesis and germination whereas the high concentration of PEG improved the shoot length, fresh and dry weight of Sukkary cv.

Key words: Polyethylene glycol, gibberellin, tissue culture, *Phoenix dactylifera,* somatic embryogenesis.

INTRODUCTION

Date palm is a major and dominant fruit crop in the Kingdom of Saudi Arabia. The Kingdom is considered as one of the major countries for date palm production. It is well known that date palm is propagated sexually through seeds and vegetatively by offshoot. Seed propagation is not suitable because of heterozygosity and dioecious nature of the date palm which may resulted in producing offtype plant. Offshoot as vegetative propagation is slow and the mortality of offshoot is low. Therefore, the use of plant tissue culture technique for propagation and large scale production is very important. Somatic embryogenesis is the development of embryos from somatic cells. This is achieved via a series of developmental stages, most of which are similar to zygotic embryogenesis (Alkhateeb and Ali-Dinar, 2002).

The increasing demand for date palms requires massive multiplication of selected plants. This could be achieved through tissue culture. Somatic embryogenesis is the development of embryos from somatic cells. The medium supplied with 3.75% PEG proved to be the most

beneficial for embryo development of the studied genotype of Norway spruce (Hana et al., 1998).

Its well known that adding PEG to the culture media are able to promote the embryos formation and maturation in different plant.

Increased medium osmolarity and hence the frequency of somatic embryogenesis was also obtained by the addition of polyethylene glycol (Brown et al., 1989; Kavi, 1989; Ramarosandratana et al., 2001). Ramarosandratana et al. (1999) reported that addition of polyethylene glycol (PEG) 4000 at 6% greatly enhanced the production of mature embryos by 2 to 4 times. The best results were obtained with polyethylene glycol 4000, a non-plasmolyzing osmoticum which cannot penetrate into the plant cell but can induce water stress. Azpeitia et al. (2003) found that in the induction of somatic embryos of Cocus nucifera the best treatment was 90 µM ABA+15 g PEG litre-1 with the formation of 10.6 somatic embryos per embryogenic callus at 150 days of culture. In Panax ginseng, Langhansova et al. (2004) stated that shoot regeneration of non-treated somatic embryos was 31% while that for somatic embryos treated with PEG 4000 and ABA was 70%.

The capacity of GA3 to improve plant growth by cell elongation and enlargement is well documented in the literature. The exact concentration of the hormone to be used for proper improvement of shoot, however, needs to be determined since plant species and stage of shoot growth differ in their hormonal requirements.

Azra et al. (1997) found that somatic embryo nodules formed in callus of date palm when transferred to MS medium with 0.1-1.0 mg l-1 GA3. Komai and Masuda (2002) reported that the regeneration regime required an exogenous supply of GA3; the addition of GA3 seemed to be crucial not for the development of embryo proper but for producing embryogenic calluses in Spinacia oleracea.

The aims of this investigation were to determine the effects of PEG and GA3 at different concentrations on somatic embryogenesis of date palm (Phoenix dactylifera L.) cv. Suckary in vitro.

MATERIALS AND METHODS

Offshoots taken from adult date palm (Phoenix dactylifera L.) cv. Sukkary were used as plant material. The shoot tip and lateral buds were sectioned into approximately 1 cm explants which were used for in culture organogenesis media as described by Alkhateeb and Ali-Dinar (2002). 1–2 buds resulted from direct organogenesis were transferred to 100 ml flasks filled with 50 ml of modified MS salts media (Murashige and Skoog 1962) supplemented with 170 mg-1 NaH2PO4.2H2O, Inositol 125 mg-1; Glutamine 200 mg-1; Thiamine 5 mg-1; Pyridoxine HCl 1 mg-1; Nicotinic acid 1 mg-1; Glycine 2 mg-1 ; Sucrose 30 g-1; Activated charcoal 1.5 g-1 and Purified agar 6.5 g-1. In order to form embryogenic callus culture were transfered in 3 media described below:

1. MS media supplemented with 100 mg-1 2,4-D and 3 mg-1 2ip for culture initiation medium. Culture were incubated at 25±2°C in darkness for 9 weeks.
2. MS media supplemented with 10 mg-1 NAA and 30 mg-1 2ip for culture swelling medium. Culture were incubated at 25±2°C in a 16h photo period provided from cool white florescent lamps for 3 weeks only.
3. MS media supplemented with 10 mg-1 NAA and 6 mg-1 2ip for embryogenic callus formation. Culture were incubated at 25±2°C in a 16h photo period provided from cool white florescent lamps for 16 weeks only.

Media pH was adjusted to 5.7 and sterilized by autoclaving for 15 min at 121°C. Cultures were transferred to fresh media at 4 week intervals. The result out callus used for the experiment. The experimental was designed as a factorial in randomized complete with two factors. The first factor was polyethylene glycol (PEG) MW 8000 with three levels (2%, 4% and 6%), and the second factor was gibberellen (GA3) included three levels (0.2, 0.3 and 0.6 mg l-1). Treatments were replicated 10 times and the cultures were incubated at 25±2°C in 16 h of light daily supplied by 65/80 Warm White Weisse 3500 fluorescent tubes.

Data were taken after 5 weeks of culture as followed:
- Total number of somatic embryos.
- Total number of germinating embryos.
- Length of embryo (cm).
- Fresh and dry weights of culture (g).

Data subjected to statistical analysis as a factorial in randomized complete were design according to Gomez and Gomez (1984). The treatment means were compared using least significant difference (LSD) at 5 % level of probability according to Waller and Duncan (1969). All statistical analysis was performed using the facility of computer and SAS software package (SAS, 2001).

RESULTS AND DISCUSSION

Data in Table 1 show that the number of somatic embryos and its germinated were significantly affected by polyethylene glycol (PEG) at 2% (79.9 and 4.6, respectively) compared to other concentration. PEG at 4% came in the second rank in this respect. These results are the same line with this obtained by Ramarosandratana *et al*. (2001). On the other hand, higher concentration of polyethylene glycol (6%) was produced the longest shoot (1.08 cm) and heaviest values in fresh (2.060 g) and dry weights (0.2431 g) compared with the others.

The results in Table 1 revealed that gibberellin (GA_3) at 0.3 mg l^{-1} increased significantly number of somatic embryos and number of embryos germinated (76.7 and 4.0, respectively) compared to 0.2 and 0.6 mg l^{-1}. Whereas, there is no significant effect between other characters in these study since, GA_3 at 0.6 mg l^{-1} gave the highest values of both fresh and dry weights.

The interaction between PEG and GA_3, was highly significant in the all characteristics. The interaction of 4% PEG + 0.6 mg l^{-1} GA_3 produced the highest values for each number of somatic embryos and its germinated with no significant different with treatment 2% PEG + 0.3 mg l^{-1} GA_3. Regarding to shoot length, the interaction between higher concentration of the two factors under this study (had significant effects on shoot length (1.22 cm) as shown in (1-C). The results in (2-A) stated that the interaction 2% PEG + 0.2 mg l^{-1} GA_3 recorded heaviest values of fresh weight (2.295 g). While, the highest dry weight (0.2570 g) was obtained from the interaction 6% PEG + 0.3 mg l^{-1} GA_3 followed by 6% PEG + 0.6 mg l^{-1} GA_3 (0.2533) with no significant difference.

Table 1. Number of embryos, no. of embryos germinated, shoot length, fresh weight and dry weights of date palm cv Sukkary cultured *in vitro* as affected by polyethylene glycol and gibberellic acid.

Characters **Treatments**	**No. of embryos**	**No. of embryos germinated**	**Shoot length (cm)**	**Fresh weight (g)**	**Dry weight (g)**
A. PEG:					
2%	**79.9**	**4.6**	**0.74**	**1.780**	**0.1799**
4%	**74.0**	**3.5**	**0.83**	**1.709**	**0.1767**
6%	**54.2**	**3.2**	**1.08**	**2.060**	**0.2431**
LSD 5%	**4.1**	**0.39**	**0.07**	**0.166**	**0.0250**
B. GA_3 (mg l^{-1}):					
0.2	**59.3**	**3.8**	**0.92**	**1.834**	**0.1906**
0.3	**76.7**	**4.0**	**0.86**	**1.816**	**0.2032**
0.6	**72.1**	**3.5**	**0.87**	**1.898**	**0.2059**
LSD 5%	**4.1**	**0.39**	**NS**	**NS**	**NS**

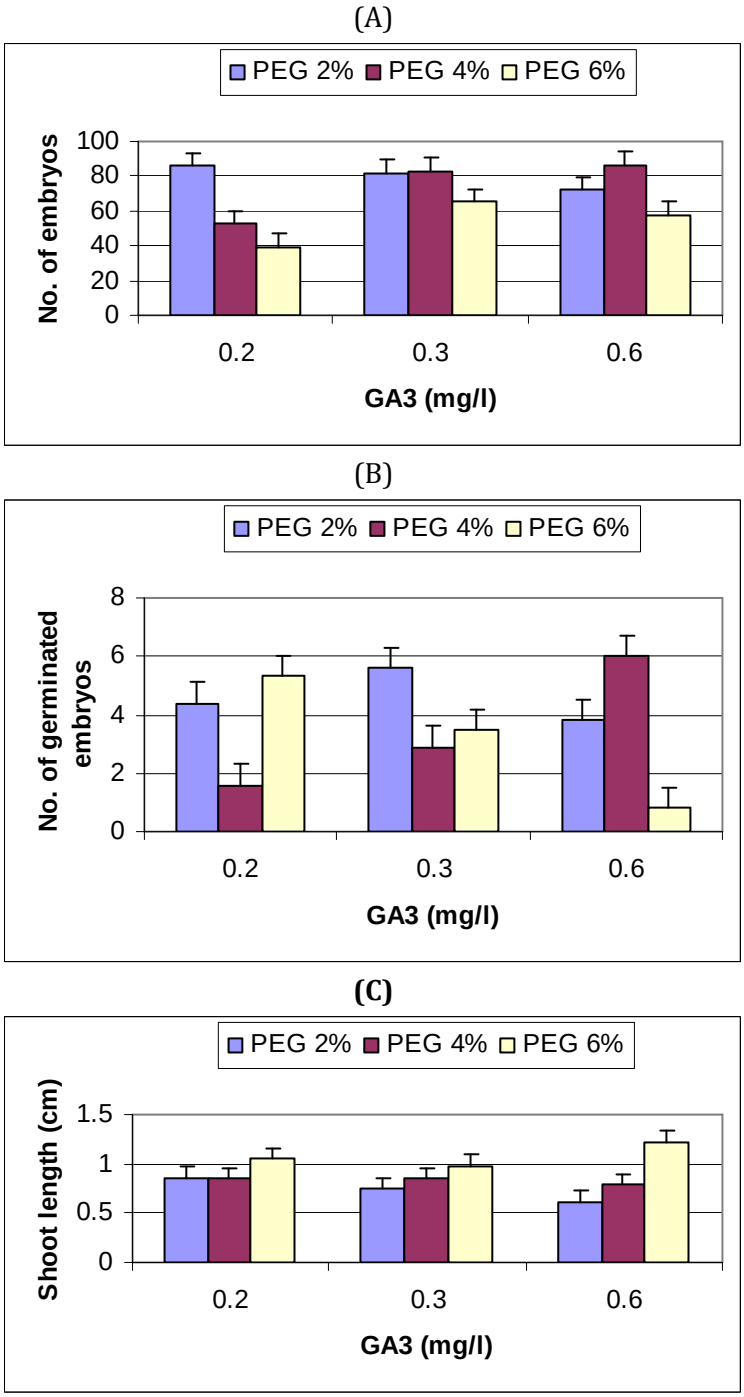

Fig. 1: No. of somatic embryos (A), No. of embryos germinated (B) and shoot length (C) of date palm cv Sukkary cultured *in vitro* as affected by polyethylene glycol and gibberellic acid.

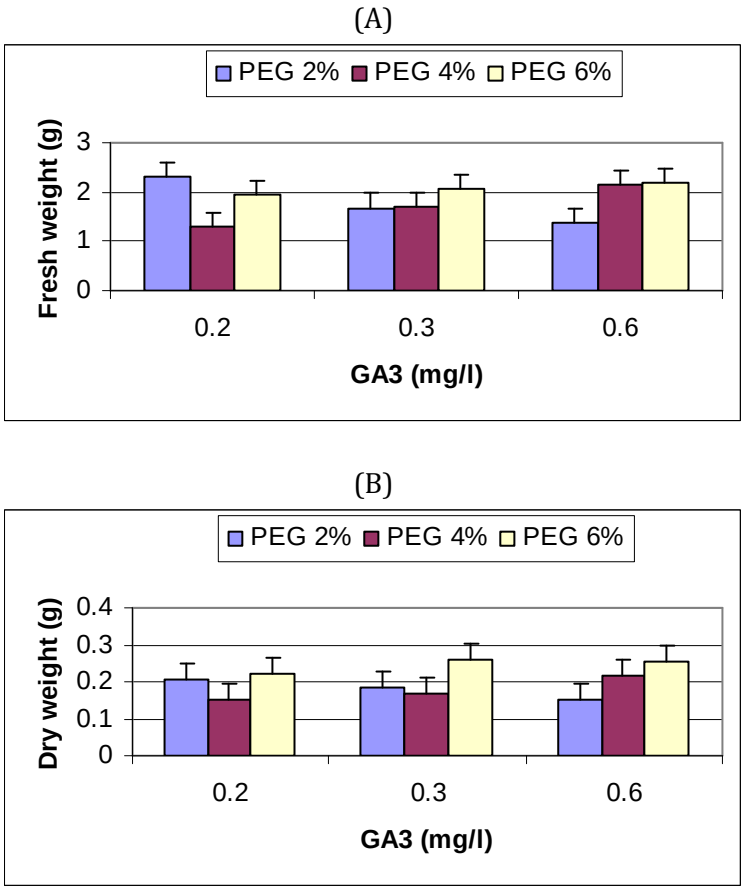

Fig. 2: Fresh weight (A) and dry weight (B) of date palm cv Sukkary cultured in vitro as affected by polyethylene glycol and gibberellic acid.

From obvious results, the improved number of somatic embryogenesis and no. of embryos germination due to polyethylene glycol may be to the playing role somatically stress to formation and induction the somatic embryos. Also, the effects of PEG application was positively correlated with the total free amino acids, proline, total soluble phenols, sugars as well as Na and Cl concentration (Ebrahim *et al*., 2004; Harb *et al*., 2005). Moreover, the good effects of GA_3 may be due to the role of gibberllins in cell elongation and developed embryos (Komai and Masuda, 2002).

Stress-induced somatic embryogenesis has already been reported in carrot (Harada *et al*., 1990) and *Arabidopsis thaliana* (Miho *et al*., 2003) in which it was shown that somatic embryos could be induced by osmotic stress (sucrose, mannitol). In this study, number of embryos and germinated embryos was enhanced 2% PEG. However, further increase of PEG concentration up to 6%, number of embryos and germinated was reduced. The kind of reduction could be attributed to osmotic stress generated by such high concentration. Furthermore, it is known that plant cells which actually experience desiccation to the point of turgor loss must regain turgor through osmotic adjustment in order to resume growth. Therefore, the ability of cells to generate osmoticum, and thereby sufficient water were detrimental the rate of cell enlargement. It seems that, the plant culture on the medium supplemented with 6% PEG didn't reach to the osmotic adjustment in order to resume the vegetative growth.

Based on all of the obtained results we concluded that, addition PEG at 2% and GA_3 at 0.3 mg l^{-1} produced the highest number of somatic embryogenesis and germination whereas the high concentration of PEG improved the shoot length, fresh and dry weight of Sukkary cv.

REFERENCES

[1] Alkhateeb, A.A. and Ali-Dinar, H.M., 2002, Date palm in Kingdom of Saudi Arabia: Cultivation, Production and Processing. Translation, Authorship and Publishing Center, King Faisal University, Kingdom of Saudi Arabia. pp. 188.

[2] Azora, Q.; Huassain I.; Mushtaq, A.; Hamid, R. and Muhammad, L. 1997. Sustained multiplication of long term embrogenic culture of date palm and their field performance. Pakistan J. Bot. 29: 135-141.

[3] Azpeitia Morales, A., L. Saenz Carbonell, J. L. Chan, and C. Oropeza Salin, 2003. Induction of somatic embryos in plumule explants of coconut by abscisic acid and polyethylene glycol. Revista Fitotecnia Mexicana, 26 (4): 309-317.

[4] Brown, C.; F.J. Brooks; D. Pearson and R. J. Mathias, 1989. Control of embryogenesis and organogenesis in immature wheat embryo callus using increased medium osmolarity and abscisic acid. J. Plant Physiol. 133: 727- 733.

[5] Ebrahim, M. K. H.; Ibrahim, I. A. and Emara, H. A. 2004. *In vitro* selection of drought-tolerant banana cultivar: effect of polyethylene glycol-induced water deficit, and medium liquification. Egyptian Journal of Horticulture 29: 525-538.

[6] Gomez, K. A. and Gomez A. A. 1984. Statistical procedures for Agricultural research. 2nd Ed. John Wally & Sons

[7] Harada, H., Kiyosue, T., Kamada, H., Kobayashi, T., 1990. Stress-induced carrot somatic embryogenesis and its application to synthetic seed. In The Impact of Biotechnology in Agriculture (Sangwan, R.S. and Sangwan-Norreel, B.S., eds). The Netherlands: Kluwer Academ. Publishers pp. 129-157.

[8] Harb, E.M.Z.; El-Shihy, O.M.; Ahmed, A.H.H. and Bayerly, R.M.S. 2005. Effect of gamma irradiation on increasing water stress tolerance of micropropagation banana plants. Bulletin of Fac. Agric. Cairo Univ. 56: 17-53.

[9] Kavi Kishor, P.B., 1987. Energy and osmotic requirement for high frequency regeneration of rice plants from long-term cultures. Plant Sci., 48: 189-194.

[10] Komai, F. and Masuda, K. 2002. Somatic embryogenesis in *Spinacia oleracea* L. Current Topics in Plant Biology 3: 187-197.

[11] Langhansova, L.; Konradova, H. and Vanek, T. 2004. Polyethylene glycol and abscisic acid improve maturation and regerenation of *Panax ginseng* somatic embryos. Plant Cell Reports 22: 725-730.

[12] Miho, I.I., Umehara, M., Satoh, S., Kamada, H., 2003. Stress-induced somatic embryogenesis in vegetative tissues of *Arabidopsis thaliana*. Plant J. 34, 107-114.

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

[14] Ramarosandratana, A., L., Harvengt, A., Bouvet, R., Galvayrac, M., Paques, 2001. Effects of carbohydrate source PEG and gellan fum concentration on embryonal-suspensor mass (ESM) proliferation and maturation of maritime pine somatic embryos. In Vitro Cell. Dev. Biol.-Plant 37: 29-34.

[15] Ramarosandratana, A., L., Harvengt, E. Garin, F. Canlet, M. Paques and R. Calvayrac, 1999. Importance of carbohydrate source and PEG on maturation of *Pinus pinaster* somatic embryos: Yield, morphology and conversion rate. Advances in Tissue Culture and Transformation (Forest Biotechnology Conference).

[16] SAS Institute 2001. SAS for Windows, SAS user's guide: Statistics. Version 8.0 e. SAS Inst., Inc., Cary, North Carolina.

[17] Svobodova, H.; Albrechtova, J.; Kumstyrova, L.; Lipavska, H.; Vagner, M. and Vondrakova, Z. 1998. Somatic embryogenesis in Norway spruce: Anatomical study of embryo development and influence of polyethylene glycol on maturation process. Plant Physiol. Biochem. 37: 209-221.

[18] Waller, R.A. and Duncan, D. P. 1969. A bays rule for symmetric multiple comparison problem. Amer. Stat. Assoc. J. December: 1485- 1503.