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REGULATION OF GROWTH AND DEVELOPMENT OF DATE PALM SOMATIC EMBRYOGENSIS BY EXOGENOUS L-PROLINE

Mona M. Hassan , Abeer H. Abd El-Kareim and Ezz G. Gadalla

Central Laboratory for Date Palm Research and Development, Agricultural Research Center, Giza, Egypt.

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ABSTRACT: ***The effect of L-proline at different concentrations 0, 25, 50, 100 and 200 mg/l on callus growth, callus globulrization degree, embryo formation and embryo germination of date palm (Phoenix dactylifera L.) cultivars (Zaghloul, Amry and Sakkoty) was studied. Friable callus of different cultivars was obtained when shoot tip explants were cultured on MS medium supplemented with 10 mg/l 2,4-D +3mg/l 2iP +1.5g/l activated charcoal (AC) for thirty two weeks with regular transformation to fresh medium every four weeks. Pieces of friable calli (1×1cm) were cultured on MS basal nutrient medium supplemented with 0.1 mg/l NAA +30 g/l sucrose +6 g/l agar and different concentrations of L-proline for eight weeks. Different concentrations of L-proline were added to MS medium supplemented with 0.5mg/l BA +0.5mg/l kinetin, 1g/l A C+40 g/l sucrose to study their effects on growth and development of somatic embryos resulted from maturation stage. The present study shows that, L-proline has several beneficial effects on tissue culture system of date palm cvs during maturation stage. The highest significant value of callus growth and globulrization of callus cultures were observed when 200 mg/l L-proline was added to culture medium followed by the addition of 100 mg/l L-proline. The highest number of embryo was observed when L-proline was added to the culture medium at the concentration of 200 mg/l. All concentrations of L-proline added to germination medium increased significantly the number of germinated embryos compared to control medium. The lowest values of all studied parameters were obtained when culture medium devoid of L-proline. Germinated embryos were rooted well on half strength MS medium + 0.1 mg/l NAA + 0.05mg/l BA.***

Key words: ***Phoenix dactylifera, L-proline, maturation, globulrization, germination.***

INTRODUCTION

Date palm, *Phoenix dactylifera* L., belongs to Order: Palmales, Family: Palmae (Areaceae) is one of the oldest fruit trees in the world and is mentioned in the Qur'an and Bible. The genus *Phoenix* is named due to the

purple colour of the dates that is similar to the purple dye the Phoenicians were renowned for making. *Dactylifera* is Greek for 'finger-bearing' and alludes to the shape of the dates. Areaceae family is contains over 200 genera and over 2500 species (Corner, 1966 and Tomlinson, 1961).

L-proline synthesis is implicated as a mechanism of alleviating cytoplasmic acidosis, and may maintain $NADP^+$/NADPH ratios at values compatible with metabolism (Hare and Cress, 1997). Rapid catabolism of L-proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress-induced damage (Hare and Cress, 1997; Hare *et al*, 1998). L-proline is a major amino acid; the physiological and biochemical role of L-proline in plant tissue culture is unclear. In intact plants, L-proline is thought to have several protective functions against stress since plant tissue subjected to stress accumulates endogenous L-proline. The exact role of proline accumulation in intact plants is also unclear but functions proposed includes osmoprotection (Delauney and Verma, 1993), enzymatic regulator functions (Stewart and Boggess, 1977) or the storage of nutritional nitrogen and carbon reserves to be used during recovery from stress (Jager and Meyer, 1997). Many studied stated that proline functions in intracellular osmotic adjustment between cytoplasm and vacuole (Sharp *et al*., 1990). Other hypothesis suggest that proline is a protective agent of enzymes (Bandurska,1993) and intracellular structures, a free radical scavenger or a storage compound of carbon and nitrogen for lipid recovery from stress. Other investigators suggested that proline overproduction in stressed conditions is an attempt to regulate cytosolic pH (Binzel *et al*., 1989).

Several workers have been published describing some culture media for organogenesis or somatic embryogenesis of date palm (Al-Khayri and Al-Bahrany 2004) & (Al –khayri and Abu-Ali 2006). The present study aimed to investigate the effects of various concentrations of exogenous L-proline on callus growth, callus globulrization, number of formed embryos during maturation stage and somatic embryos growth and development during germination stage of date palm genotype.

MATERIALS AND METHODS

This study was carried out during 2005-2007 at The Central Laboratory for Research and Development of Date Palm at Giza- Egypt.

Plant material:

The propagation process started with the selection of healthy offshoots from mother date palm (*Phoenix dactylifera* L.) of the three cultivars

Zaghloul, Amry grown in Rashid city and Sakkoty grown in Aswan, Egypt. Selected young offshoots of 5-7 kg in weight and about 50-70 cm in height were carefully separated from adult date palm.

Sterilization of plant material:

Explants were surface sterilized under aseptic conditions. At first, they were immersed in 70% ethanol for 30 sec. then immersed in 0.5 g/l mercuric chloride ($HgCl_2$) for 5 min. and thoroughly washed with sterilized distilled water. After that, additional outer leaves primordial were removed from the sterilized explants. These explants were then exposed to double surface sterilization by commercial Clorox (5.25 % Sodium hypochlorite) first one by 50% Clorox for 25 min then thoroughly washed with sterilized water. The second one was by 50% Clorox for 25 min and then washed with sterilized distilled water for three times. Some surrounding leaf primordial were carefully removed and shoot tip explants with 4-6 leaf primordial were sliced longitudinally into 4 sections and inoculated onto culture medium

Culture medium:

Murashige and Skoog medium (MS 1962) was used in this investigation. Culture medium was supplemented with 10mg/l 2,4-D, 3mg /l 2iP, 40g/l sucrose, 100 mg/l glutamine and 1.5 g/l activated charcoal (AC) solidified with 2.5 g/l phytagel. Prepared medium was adjusted to pH 5.7 ± 0.1 and distributed into small jars (200 ml); each one contains 35 ml of prepared medium and then autoclaved at 121 ºC and 1.5cm /ins^2 for 25 min. Sterilized shoot tip slices were cultured on previous medium for thirty two weeks with regular transfer to fresh culture medium of the same composition every four weeks to form callus (establishment stage). Cultures were incubated at 25 ±2 ºC under complete darkness. This experiment was conducted to investigate the effect of L-proline at concentrations of 25, 50,100 and 200mg/l in addition to control medium (amino acid-free medium) on maturation and germination stages.

Maturation stage:

Friable callus resulted from establishment stage was divided into pieces (1cm x1cm) and cultured on three–quarter MS medium supplemented with 0.1mg/l NAA (Mater 1986) in addition of activated charcoal and different concentrations of L-proline. Jars were incubated at 25± 2ºC and light intensity 1000 lux for eight weeks (four weeks interval). Callus growth, globularization of callus and number of mature embryos were recorded.

Callus growth and globularization of callus (embryogeneic callus formation) were estimated visually according to Pottino (1981) as follows:

Negative result (-) =1 **Below average results (+) = 2**

Average results (++) = 3 **Good results (+++) = 4**

Germination stage (embryo growth and development):

In this stage, somatic embryos formed on previous stage was cultured on 3/4 MS medium supplemented with 0.5mg/l BA +0.5mg/l kinetin, 1g/l AC+40 g/l sucrose (Hassan, 2002) in addition to different concentrations of L-proline for eight weeks (four weeks interval) and incubated at 25±2 ºC and light intensity 2000 lux. After eight weeks the following data were recorded:

1-number of embryos **2- number of germinated embryos**

The effect of L-prolin on previous stages was studied using 3 different genotypes of date palm Zaghloul cv. as a soft cultivar, Amry cv. as a semi-dry cultivar and Sakkoty cv. as a dry cultivar.

Statistical analysis:

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

RESULTS

L-proline, at all concentrations used in this study, had an overall beneficial effect on the date palm different cultivars. It promote the callus growth, globulrization, increase number of embryos formation and number of germinated embryos.

Effect of L- proline on callus growth of date palm different cultivars

Results in Table (1) indicated that mean of callus growth was significantly increased by increasing concentration of L-proline in culture medium. The highest significant value of callus growth was observed when 200 mg/l L-proline was added to culture medium followed by the addition of 100 mg/l L-proline (3.66 and 3.39 respectively) Fig.(1). No significant difference was observed between the addition of 25 mg or 50 mg/l L-proline. While the lowest significant value of callus growth was noticed by using medium without L- proline. The effect of different cultivars of date palm reflected that Zaghloul cv. produced the highest significant value of callus growth mean (3.40), this value was reduced significantly to 2.93 or 2.87 with Amry and Sakkoty cvs respectively. Interaction between the proline concentrations and cvs of date palm had no significant differences. However the highest same values (3.83) were achieved when Zaghloul cv cultured on medium with (50 or 100 mg/l) L-proline and Amry cultured on 200 mg/l L-proline.

Table (1): Effect of L-proline on callus growth of date palm different cultivars

L-proline con. (mg/l) A	Date palm cultivars (B)			
	Zaghloul	Amry	Sakkoty	Mean
Control(0)	2.50	2.33	2.17	2.33 d
25	3.17	2.67	2.50	2.78 c
50	3.83	2.67	3.00	3.17 c
100	3.83	3.17	3.17	3.39 ab
200	3.66	3.83	3.00	3.66 a
Mean	3.40 a	2.93 b	2.87 b	

L.S.D (A) at 0.05 = 0.42

L.S.D (B) at 0.05 = 0.33

L.S.D (AB) at 0.05 = NS

Effect of L-proline on globularization of callus culture

The addition of L-proline significantly affected globularization of callus cultures of date palm cultivars at different concentrations compared with control medium. Table (2) shows that, when high concentrations of exogenous L-proline (200 or 100 mg/l) were applied to culture medium caused an increase in globularization degree (3.56 or 3.28 respectively) Fig. (1). Also the addition of 50 mg/l L-proline to culture medium improved significantly globularization degree compared with the addition of 25 mg/l as the values were (2.67 and 2.05 respectively). In respect to the cultivar response to L-proline data in Table (2) shows that no significant differences were noticed among different cultivars. However Zaghloul and Amry cvs. produced the highest same value (2.80), while Sakkoty cv. produced the lowest value of globularization degree. Interaction between L-proline and different cultivars had no significant effect in this respect.

Table (2): Effect of L-proline on globularization of date palm callus

L-proline con. (mg/l) A	Date palm cultivars (B)			
	Zaghloul	Amry	Sakkoty	Mean
Control(0)	1.83	1.67	1.67	1.72 d
25	2.16	2.00	2.00	2.05 cd
50	2.83	2.50	2.67	2.67 b
100	3.50	3.33	3.00	3.28 a
200	3.67	3.50	3.50	3.56 a
Mean	2.80	2.80	2.57	

L.S.D T(A) at 0.05 = 0.501

L.S.D CV(B) at 0.05 = NS

L.S.D (AB) at 0.05 = NS

Effect of L- proline on somatic embryos formation during maturation stage.

Data in Table (3) and Fig.(2) revealed that, number of embryos formation in maturation stage was affected significantly by the addition of L-proline to culture medium. Increasing the concentration of L-proline in culture medium form 25 to 200 mg/l significantly increased the mean of formed embryos compared with control medium. The highest mean of embryos (7.67embryo/explant) was observed when L-proline added to the culture media at 200 mg/l. While the lowest significant value in this respect was noticed (1.0 embryo/explant) when culture medium devoided of L-proline (control medium). Variations in number of formed embryos as an indicator of different tested date palm cultivars are clearly pronounced in Table (3).It appeared that Zaghoul cv. over exceded Amry and Sakkoty cultivars .Furthermore proline concentrations and different cultivars affected significantly the number of formed embryo during maturation stage pronounced in this Table.

Table (3): Effect of L-proline on number of embryos formation of date palm cultivar during maturation stage

L-Proline con. (mg/l) A	Date palm cultivars(B)			
	Zaghloul	Amry	Sakkoty	Mean
Control(0)	2.00	1.00	0.00	1.00 e
25	3.00	3.00	2.00	2.67 d
50	6.00	3.00	3.00	4.00c
100	8.00	6.00	3.00	5.67b
200	9.00	7.00	7.00	7.67a
Mean	5.60 A	4.00 B	3.00 C	

L.S.D (A) at 0.05 = 0.95

L.S.D (B) at 0.05 = 0.74

L.S.D (AB) at 0.05 = 1.64

Effect of L-proline on growth and development of date palm somatic embryos during germination stage:

Number of embryos formation:

Data in Table (4) shows the number of embryos that appeared during germination stage as affected by L-proline and cvs. of date palm. L-proline concentrations improved significantly the mean of formed embryos during germination stage. Furthermore increasing the concentration of L-proline in culture medium from 0.0 to 100 mg/l significantly increased the mean of formed embryos from 20 to32 embryos/explant. However increasing the concentration of L-proline from 100mg/l to 200 mg/l decreased significantly the mean of formed embryos from 32 to 24.67 embryos/explant. L-proline free medium produced the lowest significant value.

From the same Table data shows that the mean of formed embryos/explant was affected by date palm genotype significant values 25.2 and 25.4 embryos/explant respectively compared with Sakkoty cv. which produced the lowest value. Interaction between L-proline concentrations and date palm cultivars appeared to affect significantly the mean of formed embryos. The highest significant values were obtained when either Amry or Zaghloul were cultured on medium supplemented with 100 mg/l L-proline as the values were 35 or 34 embryos/explant respectively.

Table (4): Effect of L-proline concentration on date palm secondary somatic embryos number during germination stage

L-Proline con. (mg/l) (A)	Date palm cultivars (B)			
	Zaghloul	Amry	Sakkoty	Mean
Control(0)	14.00	21.00	20.00	20.00 e
25	24.00	26.00	26.00	25.33 c
50	28.00	22.00	24.00	26.33 b
100	34.00	35.00	27.00	32.00 a
200	31.00	32.00	11.00	24.67 d
Mean	25.20 a	25.40 a	20.60 b	

L.S.D (A) at 0.05 = 0.99
L.S.D (B) at 0.05 = 0.76
L.S.D (AB) at 0.05 = 1.72

Number of germinated embryos

Data in Table (5) revealed the number of germinated embryos of date palm different cultivars as affected by L-proline concentrations. Results show that all concentrations of L-proline used improved significantly the number of germinated embryos/explant compared with control medium. Medium containing 100 mg/l L- proline produced the highest significant value of germinated embryos/explant followed by medium containing either 200 mg/l or 50 mg/l L-proline (21.0, 17.67 and 12.0 germinated embryos/explant respectively). No significant difference could be observed between media containing 50 or 25 mg/l L-proline. The lowest significant value was obtained with medium lacking L- proline.

Table (5): Effect of L-proline on number of germinated embryos of date palm cultivar during germination stage

L-Proline con. (mg/l) A	Date palm cultivars (B)			
	Zaghloul	Amry	Sakkoty	Mean
Control(0)	9.00	5.00	8.00	7.33 e
25	12.00	9.00	13.00	11.33 cd
50	15.00	13.00	9.00	12.00 c
100	20.00	28.00	15.00	21.00 a
200	18.00	26.00	9.00	17.67 b
Mean	14.80 a	16.20 a	10.80 b	

L.S.D (A) at 0.05 = 3.13

L.S.D (B) at 0.05 = 1.54

L.S.D (AB) at 0.05 = 3.49

In regard to the effect of date palm genotype Table (5) showed that Zaghoul and Amry cvs produced significant value of germinated embryo means compared with Sakkoty cv. which produced the lowest significant one as the mean values of germinated embryos/explants were (14.8, 16.2 and 10.8 respectively). Interaction between L-proline concentrations and date palm genotype had a significant effect on mean of germinated embryos/explant. Amry cv. cultured on media supplemented with 100 mg/l or 200 mg/l L-proline produced the highest significant value of germinated embryos/explant (12.0 and 17.67 germinated embryos respectively) without significant difference in between. While the same lowest significant value 9.0 germinated embryos/explant was observed when Zaghoul cv. cultured on control medium or Amry cv. cultured on medium with 25 mg/l L-proline and finally when Sakkoty cv. was cultured on media supplemented with 50 or 200 mg/l L-proline. Generally germinated embryos were rooted well on half strength MS medium + 0.1 mg/l NAA + 0.05 mg/l BA (Hassan 2007) as shown in Fig. (4).

DISCUSSION

The present study shows that L-proline has several beneficial effects on the tissue culture system of tested date palm (*Phoenix dactylifera* L.) cultivars. During maturation stage of date palm somatic embryos protocol, the highest significant values of callus growth and globulrization degree of callus cultures were observed when 200 mg/l L-proline was added to culture medium, followed by the addition of 100 mg/l L-proline without significant difference in between. Also the highest number of embryos was observed when L- proline added to the culture medium at 200 mg/l. The lowest significant value in this respect was noticed when culture medium devoiced of L- proline.

Also data in this investigation showed the beneficial effect of L-proline on growth and development of date palm somatic embryos during germination stage . L-proline at different concentrations improved number of somatic embryos and also enhanced the number of germinated embryos compared with control medium which produced the lowest significant values.

These results are in agreement with Brisibe *et al*., (1994) which stated that, on sugarcanes the addition of 300 mM proline to the MS callus induction and suspension media supported the formation of embryogenic callus and the proliferation of suspension aggregates with very high plant regeneration capacities. This may be due to proline at 300 mM decreased water potential of the media considerably, indicating that the cells might be osmotically stressed. Osmotic stress mediated increase in plant regeneration has been observed in cultures of several species and was found to be associated with an increased accumulation of storage reserves that present in cells of

zygotic embryos. Ehsanpour and Fatahian, (2003) stated that, on *Medicago sativa*, the addition of exogenous proline to the culture medium increased the dry weight and free proline content of callus. In suspension cultures of orchard grass, somatic embryogenesis was only stimulated by the addition of proline in combination with serine (Trigiano and Conger, 1987). In alfalfa, L-proline was found to have little effect on somatic embryogenesis without the inclusion of ammonium in the medium (Stuart and Strickland, 1984). In fact increment of embryogenic callus cultures due to Prolin presence have been decommented in different plants as maize and sugarcane (Vasil and Vasil, 1986; Fitch and Moore, 1993). These results reflect that proline might act on growth as an additional nitrogen supply. Britikov *et al*., (1970) applied radioactive labeled proline to leaves and inflorescences of plants and obtained evidence that proline is an important nitrogen source in plant metabolism. Holme *et al*., (1997)stated that ,on *Miscanthus x ogiformis* Honda Giganteus the addition of L-proline at the concentrations 0, 12.5, 25, 50, 100 or 300 mM to the callus induction and suspension culture media (containing MS or N6)for shoot apices and leaves from *in vitro*-propagated shoots, affected the formation of embryogenic callus and the growth of suspension cultures. Improvements depended on the L-proline concentration and the basal salts of the medium. Addition of 12.5 to 50 mM proline to callus induction medium with MS salts increased embryogenic callus compared with N6 salts. Increased growth with increasing L-proline concentration was obtained in suspension aggregates growth in medium with N6 salts, whereas L-proline only increased growth of suspension aggregates grown in medium with MS salts at concentrations of 12.5 or 25 mM. A stimulating effect of L-proline on plant regeneration was observed in short-term cultures of callus as well as in long-term cultures of suspension aggregates. An optimum L-proline concentration for plant regeneration was found at 12.5 mM. This concentration is similar to the optimum L-proline concentration found for plant regeneration in rice and barley (Chowdhry *et al*., 1993; Rengel and Jelaska, 1986). However, at these concentrations, L-proline seemed to stimulate the growth of larger amounts of soft non-embryogenic callus within the embryogenic callus resulting in fewer regenerated plants per callus. The development of soft callus within the embryogenic callus has been observed in other culture systems and is usually preceded by active growth of embryogenic callus (Chandler and Vasil, 1984) indicating that fast proliferation and growth are not necessarily beneficial for the attainment of an optimal embryogenic response.

Fig. (1): Effect of higher concentrations of L-Proline on callus growth and globularization during maturation stage.

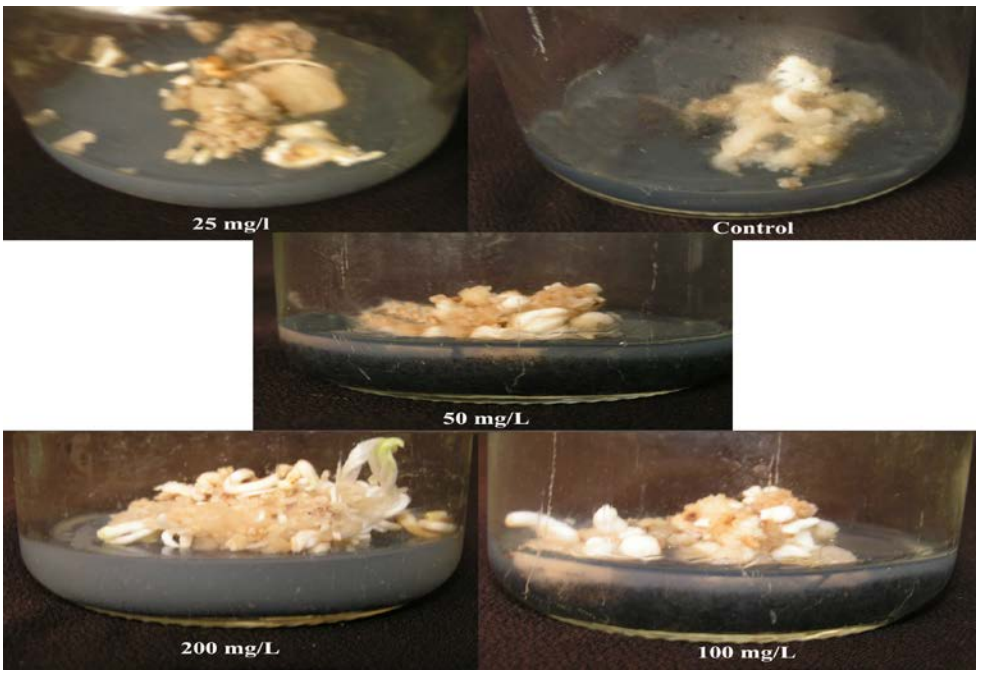

Fig. (2): Effect of L-Proline concentrations on embryos formation during maturation stage.

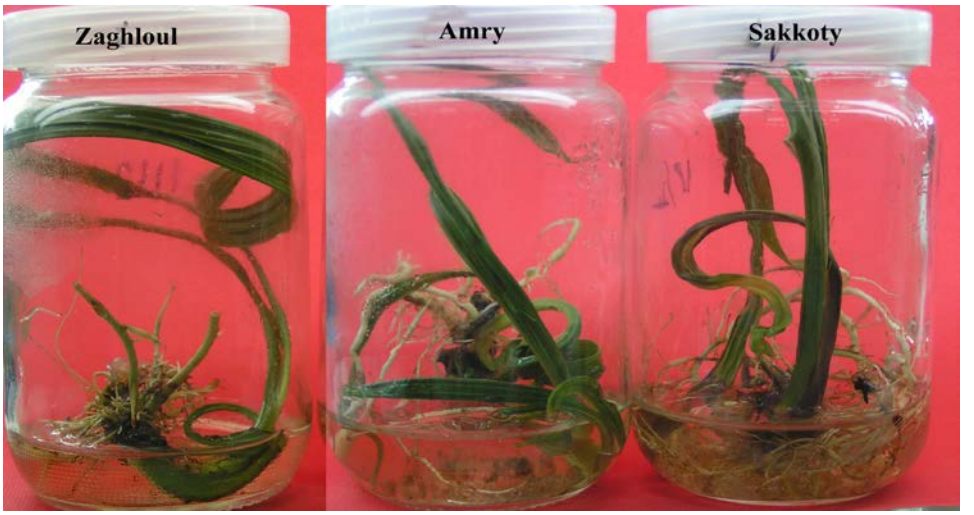

Fig. (3): Root formation of germinated embryos of date palm cultivars (Zaghloul, Amry and Sakkoty) resulting form L-Proline treatments.

REFERENCES

Al-Khayri, J. M. and A. L. Bahrany (2004). Growth, water content, and proline accumulation in drought-stressed callus of date palm. Biologia plantarum 48(1): 105-108.

Al-Khayri, J. M. and M. A. Abu-Ali (2006). *In vitro* response of date palm to Abscisic acid and poly ethylene glycol (Third 3rd International date palm conference; February 19th-21st 2006, Abu Dhabi.

Bandurska, H. (1993). *In vitro* and *in vivo* effect of proline on nitrate reductase activity under osmotic stress in barley .Acta Physiol. Plant, 15:83-88.

Binzel, M. L., Dana F. Hess, R. A. Bressan, and P. M. Hasegawa (1989). Mechanism of adaptation to salinity in cultured glycophyte cells. In: Cherry J.H (ed) Environmental Stress in Plants

Brisibe, E. A., H. Miyake, T. Taniguchi and E. Maeda (1994). Abscisic acid and high osmoticum regulation of development and storage reserve accumulation in sugarcane somatic embryos. Jnp.J.Crop.Sci.63:689-698.

Britikov, E. A., J. Schrauwen and H. F. Linskens (1970). Proline as a source of nitrogen in plant metabolism. Acta Bot. Neerl. 19:15-520.

Chandler, S. F. and I. K. Vasil (1984). Optimization of plant regeneration from long term embryogenic callus cultures of *Pennisetum purpureum* Schum. (Napier grass) J. Plant Physiol. 117:147-16.

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

Corner, D. J. H. (1966). The natural history of palm. Univcal Press, Berkeley.

Delauney, A. J. and D. P. S. Verma (1993). Proline biosynthesis and osmoregulation in plants. Plant J. 4:215-223.

Ehsanpour, A. A. and N. Fatahian (2003). Effects of salt and proline on *Medicago sativa* callus. Plant Cell, Tissue and Organ Culture 73:53-56.

Fitch, M. M. M. and P. H. Moore (1993). Long-term culture of embryogenic sugarcane callus.Plant Cell Tiss.Org.Cult. 32:335-343.

Hare, PD, WA. Cress (1997). Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regulation 21: 79-102.

Hare, P. D., W. A. Cress, J. Van Staden (1998). Dissecting the roles of osmolyte accumulation during stress. Plant Cell Envir. 21: 535-553.

Hassan, M. M. (2002).*In vitro* studies on somatic embryogenesis conservation of date palm. Ph.D. Thesis, Faculty of Agriculture, Cairo University.

Hassan, M. M. (2007). Induction of direct somatic embryogenesis of date palm (*Phoenix dactylifera* L) using ABA .Egyptian Journal of Genetics and Cytology, 36(1):93-104

Holme, I. B., P. Krogstrup and J. Hansen (1997). Embrygenic callus formation, growth and regeneration in callus and suspension cultures of *Miscanthus x ogiformis* Honda Giganteus as affected by proline. Plant Cell Tissue and Organ Culture 50:203-210.

Jager, H. J. and H. R. Meyer (1997). Effect of water stress on growth and proline metabolism of *Phaseolus vulgaris* L. Oecologia 30:3-96.

Mater, A. A. (1986). *In vitro* propagation of *Phoenix dactylifera* L. Date Palm J., 4(2):137-152.

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

Pottino, B. G. (1981). Methods in plant tissue culture. Dept. of Hort., Agric. College, Maryland Univ., College Park, Maryland, USA, pp. 8-29.

Rengel, Z and S. Jelaska (1986). The effect of L-proline on somatic embryogenesis in long-term callus culture of *Hordeum vulagare*. Acta Bot.Croat. 45:71-75.

Sendecor, G. W. and W.G. Cochran (1980). "Statistical Methods". Oxford and J.B.H. Publishing Co., 6th Edition.

Sharp, R. E., T. C. Hsiao and W. K. Silk (1990). Growth of maize primary root at low water potential's. Plant Physiol. 93:1337-1346.

Stell, R. G. O. (1960). Principles and procedures of statistics. New York, 481.

Stewart, C. R. and S. F. Boggess (1977). The effect of wilting on the conversion of arginine, ornithine, and glutamate to proline in bean leaves. Plant.Sci.Lett:147-153.

Stuart, D. A. and Strickland S. G. (1984). Somatic embryogenesis from cell cultures of *Medicago sativa* L.II.The interaction of amino acids with ammonium. Plant.Sci.Lett. 34:175-181.

Tomlinson, P. B. (1961). Anatomy of the monocotyledons. II- Palmae, Clorendon Press, Oxford.

Trigiano, R. N. and B. V. Conger (1987). Regulation of growth and somatic embryogenic by proline and serine in suspension cultures of *Dactylis glomerata*. J.Plant Physiol. 130:49-55.

Vasil, V. and IK. Vasil (1986). Plant regeneration from friable embryogenic callus and cell suspension cultures of *Zea mays* L. J. Plant Physiol. 124:399-408.

تأثير حمض البرولين على نمو وتطورالأجنة الجسمية لنخيل البلح

منى محمد حسن ؛ عبير هاشم ابراهيم عبدالكريم ، عز الدين جاد الله

المعمل المركزي للأبحاث وتطوير نخيل البلح – مركز البحوث الزراعية – الجيزة – مصر

الملخص العربي

أجرى هذا البحث بهدف دراسة تأثير التركيزات المختلفه للحامض الأمينى L-proline (صفر؛ ٢٥؛ ٥٠؛ ١٠٠؛٢٠٠ مجم/لتر) على مرحلتى النضج والانبات لأصناف نخيل البلح زغلول (كصنف رطب) وعمرى (كصنف نصف جاف) وسكوتى (كصنف جاف).

تم الحصول على الكالوس من الأصناف المختلفه قيد الدراسة بزراعة أجزاء القمة النامية على بيئة MS المحتوية على ١٠ مجم/لتر 2,4-D ؛٣ مجم/لتر 2iP ؛١.٥جم/لتر فحم نشط لمدة ٣٢ أسبوع مع النقل المنتظم للأجزاء النباتية كل ٤أسابيع. اثناء مرحلة النضج تم زراعة أجزاء من الكالوس(١×١سم)والناتج من المرحله السابقه على بيئة تحتوى على أملاح MS + ٠.١ مجم/لتر NAA مضافا اليها التركيزات المختلفة للحامض الأمينى L-proline وتم الحصول على أعلى قيم لنمو الكالوس وتحول الكالوس لكالوس جنينى وكذلك عدد الأجنة المتكونه فى هذه المرحلة باضافة ١٠٠ أو ٢٠٠ مجم/لتر L-proline لبيئة الزراعة.

كما أدى اضافة الحامض الأمينى L-proline بكل التركيزات قيد الدراسة لبيئة الانبات لزيادة عدد الأجنة النابتة زياده معنوية مقارنة ببيئة الكنترول. وقد تم تجذير الاجنه النابته بنجاح علي بيئة٢/١ MS مضافا اليها ٠.١ملجم /اللترNAA +٠.٠٥ملجم/ اللتر BA.