# *IN VITRO* MICROPROPAGATION OF 'KHALAS' DATE PALM (*Phoenix dactylifera* L.), AN IMPORTANT FRUIT PLANT

Junaid Aslam[1]* and Saeed Ahmed Khan[2]

[1]Plant Tissue Culture Laboratory and [2]Department of Pharmaceutical and Medicinal Chemistry, Dubai Pharmacy College, Al Muhaisanah 1, Al Mizhar, P.O. Box 19099 UNITED ARAB EMIRATES (U.A.E.)
*Corresponding author: e-mail: Junaidg1@gmail.com

(Received October 10, 2008/Accepted January 5, 2009)

A B S T R A C T

A protocol has been developed for an efficient tissue culture cycle (callus induction, callus growth, plant regeneration and root induction) for date palm 'Khalas' using various plant growth regulators (PGR) in both liquid and solidified MS medium. The shoot explants showed high callus induction potential on MS medium supplemented with 45.24 µM 2,4-dichlorophenoxyacetic acid (2,4-D) followed by 54.21 µM 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The highest frequency of shoot regeneration (85%) and number of shoots per an explant (5.6) were obtained on solid MS medium supplemented with 7.84 µM $N^6$-benzylaminopurine (BAP). Rooting (87.34%) was high on solid MS medium supplemented with 24.6 µM indole-3-butyric acid (IBA). However, the root length was higher in MS liquid medium. This protocol will be useful for rapid, large-scale propagation of 'Khalas' date palm.

**Key words**: *Phoenix dactylifera* L., tissue culture, plant regeneration, shoot multiplication, rooting

## INTRODUCTION

Date palm (*Phoenix dactylifera* L.), a monocotyledonous and dioecious species belonging to the Arecaceae family, is widely cultivated in arid regions of the Middle East and North Africa (Al-Khayri, 2001). It is considered as one of the most important cash crops in the Middle East as about 90% of the total world production is produced in this region. It is propagated sexually through seeds and vegetatively by offshoot

(Bonga, 1982). However, vegetatively propagated plants accumulate several bacterial, fungal, viral and mycoplasmal diseases from air, soil and insect-vectors, which results in a decline of their productivity (Anonymous, 1969). The availability of the offshoots is also limited because their number produced by each palm tree is low (Popenoe, 1973). Propagation through seeds has many limitations as well, like seed dormancy, low rate of germination and progeny variation (Venkataramaiah et al., 1980; Chand and Singh, 2004). To overcome these problems and fulfil the demand for planting material, it is necessary to develop the method of date palm propagation with the use of plant tissue culture (Mujib et al., 2004; Bhattacharjee, 2006). This technique also provides a rapid system for production of large number of genetically uniform and disease free plantlets for agriculture and forestry. Although there have been previous reports on date palm micropropagation through the organogenesis and somatic embryogenesis (Rhiss et al., 1979; Tisserat, 1979, Beauchesne, 1983; Sharma et al., 1984, Daquin and Letouze, 1988), the protocols needs to be improved. The objective of the work presented was to develop reliable method of organogenesis for 'Khalas' date palm.

## MATERIAL AND METHODS

### Plant Material

The experimental material used for the study was 'Khalas' date palm (*Phoenix dactylifera* L.), a well known cultivar throughout the U.A.E. The offshoots were collected from the residential premises of the Chairman, Dubai Pharmacy College. The selected offshoots were 4-5-year-old, each weighting approximately 6-8 kg.

### Cleaning of explants

To remove the attached soil and other debris, the offshoots were washed with the tap water and the outer large leaves and fibres were carefully removed with the sharp knife until the shoot tip zone was exposed. Shoot tips were then trimmed to approximately 6-7 cm in length and 4-6 cm in width.

### Disinfection and antioxidant treatment

The excised shoot tips were washed three-four times with double distilled water. Thereafter, the cleaned shoot tips were subjected to two steps of disinfection: a) the washed shoot tips were dipped for 20 minutes in a fungicide (Benlate, 5 g $l^{-1}$) solution; b) the shoot tips were dipped in 33% commercial Clorox solution for 25-30 minutes. The explants were then rinsed three times with autoclaved distilled water in a laminar flow hood. The disinfected explants were then soaked in an antioxidant solution to minimize oxidation of phenolic compounds (responsible for the browning of tissues), and to protect them from desiccation. The antioxidant solution consisted of 2 g $l^{-1}$ polyvinylpyrrolydone (PVP, Mw = 40,000), 200 mg $l^{-1}$ anhydrous caffeine and 100 mg $l^{-1}$ sodium diethyldithio-

carbonate AR. The shoot tips were kept in this solution for 20 minutes and finally washed with double distilled water.

**Explant preparation**

After the proper disinfection and antioxidant treatments, the shoot tips were cut into 1-1.5 cm pieces under the laminar flow hood.

**Callus induction**

Two – four shoot tip pieces were placed on MS medium (Murashige and Skoog, 1962) solidified with 3% agar-agar and supplemented various auxins types and concentrations, viz.: 0.0-54.28 µM 2,4-dichlorophenoxy-acetic acid (2,4-D), 0.0-46.96 µM 2,4-5-trichlorophenoxyacetic acids (2,4,5-T), 0.0-64.31 µM chlorophenoxy-acetic acid (CPA). 0.3 g $l^{-1}$ of activated charcoal was added to the medium in order to remove the phenolic compounds.

**Regeneration and shoot multiplication**

Callus (80-90 mg) was cultured on MS liquid and solid medium supplemented with beznzylamino-purine (BAP) at concentrations 0.0-8.96 µM and kinetin (KN) at concentrations 0.0-9.28 µM. Data were recorded on percentage of calli producing shoots (%), shoot number per a callus and shoot length.

**Rooting**

After 14 weeks in the regenerating medium, shoots were separated and placed on MS liquid and solid medium supplemented with various concentrations of IBA (0.0-29.52 µM), IAA (0.0-34.24 µM) and NAA (0.0-32.22 µM). Data were recorded on percentage of shoots producing roots, root number per a shoot and root length.

**Culture conditions**

The pH of all the media were adjusted to 5.6-5.8 before autoclaving. The media were sterilized in an autoclave for 15 min at 121°C. Cultures were incubated at 25°C (±2°C) under 16-h photoperiod with cool white fluorescent light (100 µmol $m^{-2}$ $s^{-1}$ PFD).

**Statistical analysis**

The data were analyzed by one-way analyses of variance (ANOVAs) and the mean values were separated using Duncan's multiple range test (DMRT) at $p \leq 0.05$. Values presented on tables and figures are means of five replicates from two experiments.

## RESULTS

**Callus induction**

Two – four pieces of excised shoot tips were placed on MS medium supplemented with various auxins types and concentrations viz., 2,4-D (0.0-54.28 µM), CPA (0.0-64.31 µM) and 2,4,5-T (0.0-46.96 µM). A significant variation in callus induction percentage and biomass was noticed with increasing auxins concentrations. Amongst the tested auxins, 2,4-D at concentration 45.24 µM proved to be the most effective (Tab. 1).

Table 1. Callus formation by date palm shoot explants cultured on MS medium supplemented with various auxins types and concentrations

| Plant growth regulator type and concentration | | | Percentage of callus-producing explants |
|---|---|---|---|
| 2,4-D [µM] | CPA [µM] | 2,4,5-T [µM] | |
| 0.00 | | | 7.25 l* |
| 9.04 | | | 19.26 j |
| 18.09 | | | 32.74 h |
| 27.14 | | | 51.35 d |
| 36.19 | | | 75.24 b |
| 45.24 | | | 86.72 a |
| 54.28 | | | 60.25 c |
| | 0.00 | | 0.00 n |
| | 10.71 | | 2.34 m |
| | 21.43 | | 11.74 k |
| | 32.15 | | 22.15 j |
| | 42.87 | | 46.25 e |
| | 53.59 | | 42.87 f |
| | 64.31 | | 37.25 g |
| | | 0.00 | 0.00 n |
| | | 7.82 | 5.32 m |
| | | 15.65 | 10.25 k |
| | | 23.48 | 29.74 i |
| | | 31.32 | 38.65 g |
| | | 39.14 | 54.21 d |
| | | 46.96 | 42.73 f |

*Values are means of 5 replicates from 2 experiments. Means marked with the same letter are not significantly different at $p \leq 0.05$, according to Duncan's multiple range test

Table 2. Callus growth on MS medium with optimized auxins concentration

| Optimized concentration of auxins [µM] | 5 weeks old callus | | 7 weeks old callus | | 9 weeks old callus | |
|---|---|---|---|---|---|---|
| | fresh wt. [g] | dry wt. [g] | fresh wt. [g] | dry wt. [g] | fresh wt. [g] | dry wt. [g] |
| 2,4-D (45.24) | 0.88 a* | 0.32 a | 2.25 a | 1.35 a | 3.69 a | 1.63 a |
| CPA (42.87) | 0.62 c | 0.21 c | 1.71 c | 1.23 b | 1.85 c | 1.40 c |
| 2,4,5-T (54.21) | 0.76 b | 0.27 b | 1.88 b | 1.29 b | 3.00 b | 1.49 b |

*Explanations, see in Table 1

Table 3. Shoot regeneration in MS solid and liquid medium supplemented with BAP

| BAP [µM] | Shoot number per callus clump | | Shoot length [cm] | |
|---|---|---|---|---|
| | solid | liquid | solid | liquid |
| 0.00 | 0.0 d* | 0.0 d | 0.0 e | 0.0 d |
| 2.24 | 2.8 c | 1.0 c | 2.1 d | 1.2 c |
| 4.48 | 2.9 c | 1.2 c | 4.4 b | 2.2 b |
| 6.72 | 5.3 ab | 2.1 b | 7.5 a | 3.8 a |
| 7.84 | 5.7 a | 2.9 a | 7.3 a | 3.5 a |
| 8.96 | 5.0 b | 3.2 a | 3.9 c | 2.4 b |

*Explanations, see in Table 1

Table 4. Shoot regeneration in MS liquid and solid medium supplemented with KN

| KN [µM] | Shoot number per callus clump | | Shoot length [cm] | |
|---|---|---|---|---|
| | solid medium | liquid medium | solid medium | liquid medium |
| 0.00 | 0.0 e* | 0.0 e | 0.0 e | 0.0 e |
| 2.32 | 1.8 d | 1.1 d | 1.2 d | 0.7 d |
| 4.64 | 2.3 c | 1.8 c | 2.5 c | 1.6 c |
| 6.96 | 2.8 b | 2.1 b | 4.4 a | 2.1 b |
| 8.12 | 2.9 ab | 2.4 ab | 4.0 b | 2.8 a |
| 9.28 | 3.3 a | 2.8 a | 1.5 d | 0.8 d |

*Explanations, see in Table 1

Induced calli were compact and creamy in colour. Initially, the calli appeared watery but they grew compact and globular and their colour became milky with time, usually after two subcultures of the initial callus. However, at higher 2,4-D concentrations it become black and necrotic after the 9$^{th}$ week of culture. Data presented in Table 2 show the effect of different auxins at optimized concentration on callus biomass (fresh and dry weight.). Highly effective in stimulating high callus biomass production was 2,4-D at concentration 45.24 µM.

**Regeneration and shoot multiplication**

Undifferentiated masses of callus (80-90 mg) were cultured on MS liquid and solid medium supplemented with different concentrations of BAP (0.0-8.96 µM) and KN (0.0-9.28 µM). A significant difference in shoot multiplication was found with increasing cytokinin concentration. Besides, the growth regulators, effect of solid and liquid medium was also evaluated in order to optimize medium. Comparative analysis of the results on the various cytokinins used indicated that proliferation of shoot was more effective in most of the BAP concentrations. The percentage of explants showing proliferation and the number of shoots per culture increased gradually with an increase of cytokinins concentration. Maximum shoot regeneration response (in terms of shoot development percentage, shoot number and shoot length) was noticed on medium supplemented with 7.84 µM BAP (Fig. 3 A). KN at concentration 9.28 µM proved to be highly effective in inducing shoot regeneration, but in general this cytokinin was less effective compared to BAP. When the concentration of BAP and KN increased to above 7.84 µM and 9.28 µM, shoot regeneration rate decreased. At the highest level of BAP and KN, the explants produced minimum number of shoots. Both of the cytokinins tested were more effective in solid medium that in the liquid medium. However, the leaves in liquid medium were bigger in size than those developed in solid MS medium. Tables 3, 4 and Figure 1 A, B show a comparative effect of the different concentrations of BAP, KN in liquid and solid medium on shoot regeneration.

**Rooting**

Root induction was carried out on *in vitro* regenerated, well elongated shoots by culturing them on MS solid and liquid medium supplemented with IAA, IBA and NAA at concentrations 0.0-29.52 µM; 0.0-34.24 µM and 0.0-32.22 µM, respectively. A significant difference in root induction, root number per a shoot and root length was found in all the tested growth regulators at different concentrations. The maximum rooting was found in cultures supplemented with IBA at 24.60 µM, followed by IAA at 28.54 µM and NAA at 26.85 µM (Fig. 3 B). When the concentration of the auxins increased to above 24.60 µM IBA, 28.54 µM, IAA and 26.85 µM NAA, the rooting was hampered and the shoots became black at the ends. In addition, a remarkable difference in root induction on solid and liquid medium was noticed. In all the three tested auxins, root number per shoots were higher in solid medium (Fig. 2 A, B, C), whereas liquid medium supplemented with IBA proved to be highly effective for stimulating root length. Table 5 shows a comparative effect of the growth regulators on *in vitro* root initiation on liquid and solid medium.

## DISCUSSION

*In vitro* multiplication is useful for date palm because of the dioecious nature of the palm, which puts

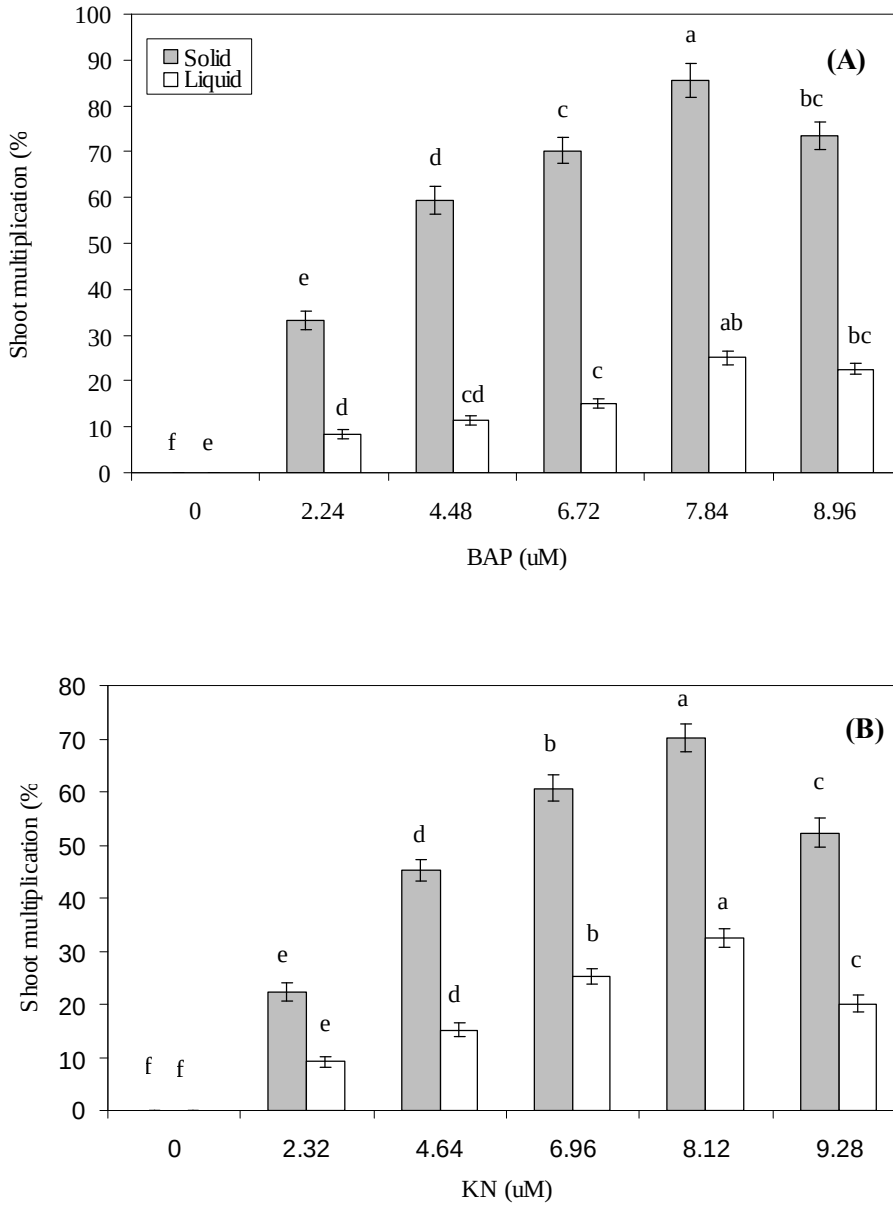


**Figure 1**. Shoot multiplication rate (%) on MS liquid and solid medium supplemented with various concentrations of BAP (**A**) and KN (**B**). Values are means of 5 replicates from 2 experiments. Means marked with the same letter are not significantly different at $p \leq 0.05$, according to Duncan's multiple range test

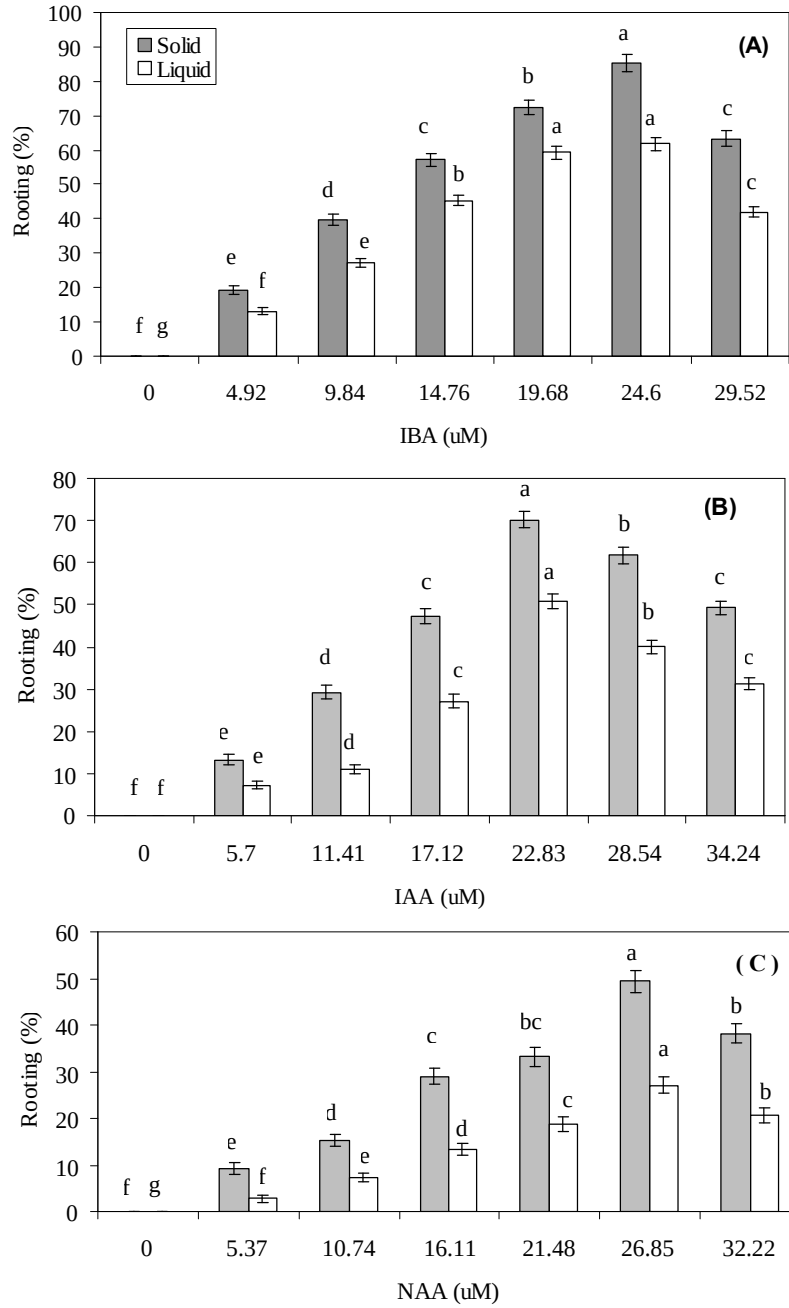


**Figure 2**. Root induction (%) on MS liquid and solid medium supplemented with various concentrations of BA (**A**) IAA (**B**) and NAA **(C**). Values are means of 5 replicates from 2 experiments. Means marked with the same letter are not significantly different at $p \leq 0.05$, according to Duncan's multiple range test

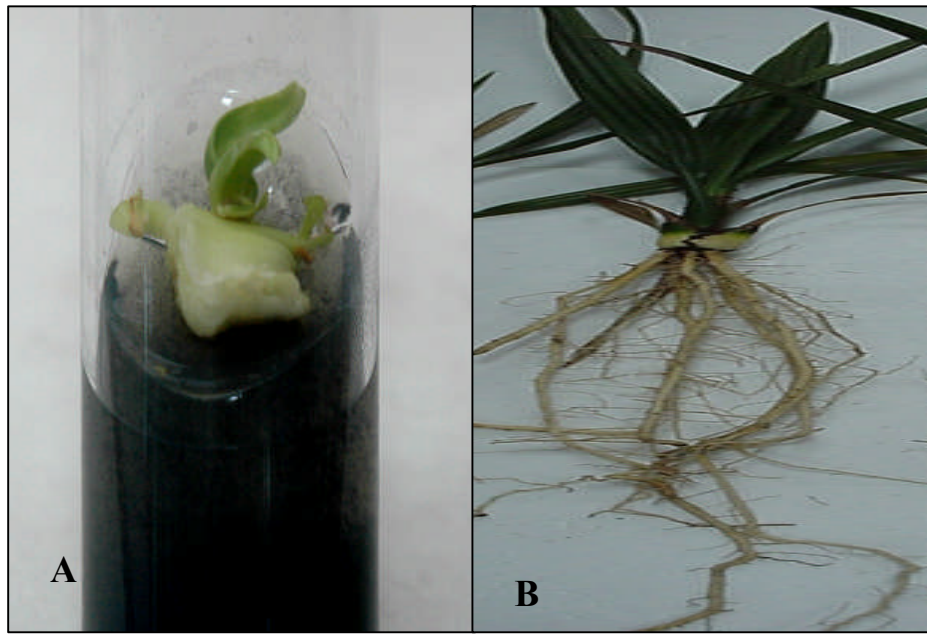


**Figure 3**. *In vitro* callus induction and plantlet regeneration of 'Khalas' date palm (*Phoenix dactylifera* L.). **A**) Shoot regeneration and multiplication on MS medium fortified with 7.84 µM BAP. **B**) Rooting on MS medium supplemented with 24.60 µM IBA

limitations on seed propagation for the production of planting materials. On the other hand, the date palm usually does not produce branches and thus has only one apical meristem. It does produce only a few suckers early in its life time, thus the number of offshoots and consequently, the number of meristems available as sources of explants from a single palm is usually low. The methods used for micro-propagation have been successfully used in many countries, although improvements in protocols are still being made to overcome some growth and multiplication problems. In the study presented, a tissue culture cycle has been refined using different plant growth regulators and medium types (liquid and solid). Shoots showed maximum callusing and high biomass on MS medium supplemented with 2,4-D. A similar promoting effect of 2,4-D on callusing was earlier reported in other date palm cultivars (Rashid and Quraishi, 1994; Hussain et al., 1995; Quraishi et al., 1997; 1999; Hassan and Roy, 2005). The stimulating effect of the 2,4-D on callus formation was also noticed in many other plant tissue cultures (Khan et al., 2002).

Of the two cytokinins tested, BAP was more active than KN during multiple shoot formation. The highest frequency of shoot regeneration (85%) and the number of shoots produced per

Table 5. Effect of various concentrations of IBA, IAA and NAA in solid and liquid MS medium on root induction

| Root length [cm] | | Number of roots per a shoot | | PGR [µM] | | |
|---|---|---|---|---|---|---|
| liquid medium | solid medium | liquid medium | solid medium | NAA | IAA | IBA |
| 0.0 g | 0.0 f | 0.0 h | 0.0 j* | | | 0.00 |
| 1.5 d | 1.2 de | 0.9 f | 1.2 g | | | 4.92 |
| 1.5 d | 1.3 cd | 1.1 f | 2.9 e | | | 9.84 |
| 1.9 cd | 1.6 cd | 2.9 c | 4.7 c | | | 14.76 |
| 2.7 b | 2.1 b | 3.7 a | 6.6 a | | | 19.68 |
| 3.9 a | 2.7 a | 3.0 c | 5.9 b | | | 24.60 |
| 2.8 b | 1.7 c | 2.2 d | 4.0 d | | | 29.52 |
| 0.0 g | 0.0 f | 0.0 h | 0.0 j | | 0.00 | |
| 0.0 g | 0.0 f | 0.0 h | 0.0 j | | 5.70 | |
| 0.8 e | 1.1 de | 1.0 f | 1.4 f g | | 11.41 | |
| 1.2 de | 1.5 cd | 3.0 c | 3.3 e | | 17.12 | |
| 1.7 d | 2.0 b | 3.7 a | 4.9 c | | 22.83 | |
| 2.2 c | 2.5 a | 3.2 bc | 4.2 d | | 28.54 | |
| 1.6 d | 1.6 cd | 2.9 c | 3.0 e | | 34.24 | |
| 0.0 g | 0.0 f | 0.0 h | 0.0 j | 0.00 | | |
| 0.3 f | 0.3 e | 0.4 g | 0.5 i | 5.37 | | |
| 0.6 ef | 0.5 e | 0.7 g | 0.7 hi | 10.74 | | |
| 0.5 ef | 0.5 e | 1.0 f | 1.1 gh | 16.11 | | |
| 0.6 ef | 0.8 de | 1.6 e | 1.7 f | 21.48 | | |
| 1.1 de | 1.4 cd | 3.1 c | 3.2 e | 26.85 | | |
| 1.0 e | 1.1 de | 3.0 c | 2.9 e | 32.22 | | |

*Explanations, see in Table 1

an explant (5.6) were obtained on solid MS medium supplemented with 7.84 µM BAP. The superiority of BAP over KN for multiple shoot formation was also demonstrated in other plants like *Jetropha integerrima* (Sujatha and Dhingra, 1993), *Sapium sebiferum* (Siril and Dhar, 1997), and *Bombax ceiba* (Chand and Singh, 1999, 2004). However, shoot multiplication rate decreased with increasing BAP concentrations. Similar effect was also noticed in many other plant tissue cultures (Ben Jouira et al., 1998; Biroscikova et al., 2004; Junaid et al., 2007).

Solid medium proved to be more effective in inducing rhisogenesis than liquid medium. The highest rooting frequency was noticed on medium supplemented with 24.6 µM IBA. These results agreed with the previous reports (El-Hennawy and Wally, 1978; Zaid and Tisserat, 1983; Gaber and Tisserat, 1985; Nasir et al., 1994; Al-Kharyi and Al-Maarri, 1997).

## CONCLUSION

Efficient regeneration protocol for date palm (*Phoenix dactylifera* L.) propagation, which required ten to twelve month from culture initiation to plant regeneration, was established. Callusing was high on MS medium supplemented with 45.24 µM 2,4-D. 7.84 µM BAP proved to be highly effective in stimulating shoot multiplication. Maximum rooting was observed in cultures supplemented with 24.6 µM IBA. Solid medium proved to be more effective for shoot multiplication and root initiation; however, root length was higher in liquid medium. This protocol will be useful for rapid, large scale propagation, which will enable meeting the high demand for the 'Khalas' date palm.

**Acknowledgement**: We are very thankful to the Chairman (Al Haj Saeed Bin Ahmed Al Lootah), Board of Trustees of Dubai Pharmacy College for providing all the facilities to carry out the research, and to Prof. M.P. Sharma (Department of Botany, Hamdard University, New Delhi INDIA) for their reading and suggestion during the preparation of present communication.

## REFERENCES

Al-Kharyi J.M., Al-Maarri K.W. 1997. Effect of seasonal variation on the regeneration capacity of date palm. IN VITRO 33: 22-26.

Al-Khayri J.M. 2001. Optimization of biotin and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera* L.). IN VITRO CELL. DEV. BIOL. PLANT 37: 453-456.

Anonymous 1969. The wealth of India: a dictionary of Indian raw materials and industrial products. Vol. VIII. New Delhi: Council of Scientific and Industrial Research, pp. 303-305.

Beauchesne G. 1983. Vegetative propagation of date palm (*Phoenix dactylifera* L.) by *in vitro* culture. In: Proceedings of first symposium on date palm, King Faisal University, Hofuf, Saudi Arabia, pp. 698-699.

Ben Jouira H., Hassairi A., Bigot C., Dorion N. 1998. Adventitious shoot production from strips of stem in the Dutch elm hybrid 'Commelin': plantlet regeneration and neomycin sensitivity. PLANT CELL TISSUE ORG. CULT. 53: 153-160.

Bhattacharjee S.K. 2006. Advances in Ornamental Horticulture. Pointer, Jaipur 6, 2065, ISBN 81-7132-432-0.

Biroscikova M., Spisakova K., Liptak S., Pichler V. 2004. Micropropagation of mature wych elm (*Ulmus glabra* Huds.). PLANT CELL REP. 22: 640-644.

Bonga J.M. 1982. Clonal propagation of mature trees: problems and possible solutions. In: Bonga J.M. (ed.), Tissue culture in forestry. Dordrecht, Martinus Nijhoff Publishers, pp. 249-271.

Chand S., Singh A.J. 2004. *In vitro* shoot regeneration from cotyledonary node explants of a multipurpose leguminous tree, *Pterocarpus marsupium* roxb. IN VITRO CELL. DEV. BIOL. PLANT. 40: 167-170.

Chand S., Singh A.K. 1999. *In vitro* propagation of *Bombex ceiba* L. (Silkcotton). Silvae. GENETICS 48: 313-317.

Daquin F., Letouze R. 1988. Regeneration of date palm (*Phoenix dactylifera* L.) by somatic embryogenesis, improved effectiveness by dipping in a stirred liquid medium. FRUITS 43: 191-194.

El-Hennawy, H.M., Wally Y.A. 1978. Date palm (*phoenix dactylifera* L.) bud differentiation *in vitro*. EGYPT. J. HORT. 5: 81-85.

Gaber M.F., Tisserat B. 1985. Propagation palms *in vitro* with special emphasis on the date palm (*Phoenix dactylifera* L.). SCI. HORT. 25: 255-262.

Hassan A.K.M.S., Roy S.K. 2005. Micropropagation of *Gloriosa superba* L. through high frequency shoot proliferation. PLANT TISSUE CULT. 15: 67-74.

Hussain I., Mushtaq A., Quraishi A. 1995. Effect of explant source on *in vitro* regeneration of plants through tissue culture proliferation in (*Phoenic dactylifera* L.) "c.v. Fusli". PAKISTAN J. BOTANY 27(1): 101-104.

Junaid A., Mujib A., Bhat M.A., Sharma M.P., Samaj J. 2007. Somatic embryogenesis and plant regeneration in *Catharanthus roseus*. BIOL. PLANT. 51: 641-646.

Khan M.R., Rashid H., Quraishi A. 2002. Effect of various growth regulators on callus formation and regeneration in *Brassica napus* cv. Oscar. PAKISTAN J. BIOL. SCI. 5: 693-695.

Mujib A., Cho M.J., Predieri S., Banerjee S. 2004. *In Vitro* Application in Crop Improvement. Oxford IBH Publi. Co. Pvt. Ltd, New Delhi.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. PHYSIOL. PLANT. 15: 473-495.

Nasir I.A., Khan M.A., Butt S.J. 1994. *In vitro* culture of date palm (*Phoenix dactylifera* L.) through excised embryo. SARHJAD J. AGRIC. 10: 633-637.

Popenoe P. 1973. The date palm. Field Research Projects, Coconut Grove, Miami, pp. 274.

Quraishi A., Hussain I., Ahmed M., Latif M. 1997. Sustained multiplication of long term embryogenic cultures of date palm and their field performance:.PAKISTAN J. BOT. 19: 135-141.

Quraishi A., Hussain I., Ahmed M., Latif M., Rashid H. 1999. Micropropagation of date palm through tissue culture. ASIA PACIFIC ASSOCIATION FOR MICROPROPAGATORS 3(1): 22-28.

Rashid H., Quraishi A. 1994. Micropropagation of date palm (*Phoenix dactylifera* L.) cv. Dhakki through tissue culture. PAKISTAN J. AGRIC. 15: 1-7.

Rhiss A., Poulain C., Beauchesne G. 1979. La culture *in vitro* appliqué ala multiplication vegetative du palmier dattier (*Phoenix dactylifera* L.). FRUITS 34: 551-554.

Sharma D.R., Sunita D., Chowdhury J.R. 1984. Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L.) cv. 437 "Khadravi" through tissue culture. INDIAN J. EXP. BIOL. 22: 596-598.

Siril E.A., Dhar U. 1997. Micropropagation of mature Chinese tallow tree (*Sapium sebiferum* Roxb.). PLANT CELL REP. 16: 637-640.

Sujatha M, Dhingra M. 1993. Rapid plant regeneration from various explants of

*Jetropha integerrima*. PLANT CELL TISSUE ORG. CULT. 35: 293-296.

Tisserat B. 1979. Propagation of date palm (*Phoenix dactylifera* L.) *in vitro*. J. EXP. BOT. 30: 1275-1283.

Venkataramaiah V., Prasad S.V., Rajeswara R.G., Swamy P.M. 1980. Levels of phenolic acids in *Pterocarpus santalinus* L. INDIAN J. EXP. BIOL. 18: 887-889.

Zaid A., Tisserat B. 1983. *In vitro* shoot tip differentiation in (*Phoenix dactylifera* L). DATE PALM J. 2: 163-182.

---

# ROZMNAŻANIE *IN VITRO* PALMY DAKTYLOWEJ (*Phoenix dactylifera* L.) 'KHALAS', WAŻNEJ ROŚLINY ZE WZGLĘDU NA OWOCE

Junaid Aslam i Saeed Ahmed Khan

S T R E S Z C Z E N I E

Opracowano skuteczną metodę rozmnażania w kulturach tkankowych palmy daktylowej (indukcja kalusa, wzrost kalusa, regeneracja pędu i korzeni) stosując regulatory wzrostu (PGR) zarówno w pożywce MS płynnej, jak i zestalonej. Eksplantaty pędów wykazywały wysoki potencjał indukcji kalusa na pożywce MS uzupełnionej kwasem 2,4-dwuchlorofenoksyoctowym (2,4-D) w stężeniu 45,24 µM i kwasem 2,4,5-trójchlorofenoksyoctowym (2,4,5-T) w stężeniu 54,24 µM. Najwyższą regenerację pędów (85%) i liczbę pędów na eksplantacie (5,6) otrzymano na zestalonej pożywce MS uzupełnionej $N^6$-benzyloaminopuryną (BAP) w stężeniu 7,84 µM. Ukorzenianie (87,34%) było wysokie na zestalonej pożywce MS zawierającej kwas indolilomasłowy (IBA) w stężeniu 24,6 µM. Korzenie były najdłuższe na płynnej pożywce MS. Metoda ta jest użyteczna dla szybkiego rozmnażania palmy daktylowej 'Khalas' w dużych ilościach.

**Słowa kluczowe**: *Phoenix dactylifera* L., regeneracja rośliny, namnażanie pędów, ukorzenianie