Micro Propagation of *Phoenix dactylifera* L. var karama

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Abstract: *Phoenix dactylifera* L.var karama was micropropagated via somatic embryogenesis. The embryogenic callus of *Phoenix dactylifera* L.var Karama was initialed on Murashige and skoog medium containing 100mg 2,4-D/L and 3.0 mg 2iP/L with presence of activated charcoal and was solidified with 2.5g phytagel /L. Healthy embryos were formed on media containing 0.1mg NAA/L and 0.2mg 2IP/L. Best multiplication was on medium contained 0.5mg 2iP+0.5mgBA and 0.5mg/LNAA. Embryos were transferred to elongation media supplemented with 1.0mg BA/L and 1.0 mg 2IP/L with 0.1mg/L NAA which rooted on this media. The acclimatization process was well in the presence of PEG in media at 4.0g/L or 8.0g/L which was effective to enhance root hardening. Roots were strong and vigor. The pH value at 5.7 in the acclimatization process produced 72.72 percent of plantlets.
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1. 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). Date palm is one of the most important cash crops in the Middle East. Most of the total world production is produced in the Arab Region. Date palm is propagated sexually through seeds and vegetatively by offshoots (Bonga, 1982). The availability of the offshoots is also limited because their number produced by each palm tree is limited. Propagation through seeds has many limitations as well, like seed dormancy,high percent of male plants, low rate of germination and progeny variation.To overcome these problems and fulfill the demand for planting material, it is necessary to develope the method of date palm propagation with the use of plant tissue culture (Mujib *et al*., 2004; and Eshraghi *et al*,2005).

Tissue culture technique has been used to propagate a wide range of important palms (coconut, Oil palm and date palm) Tisserat (1982).Tissue culture techniques are the most promising ways for rapid clonal propagation of date palm (Zaid and Tisserat, 1983). *In vitro* production is applied through two main ways, one of them is the somatic embryogenesis and the second is via meristim apexes or buds in the axil bottom of the leaves, Beauchesne 1982, Bekheet *et al* 2001, Eke *et al* 2005, Sharma *et al* 1984, and Zaid, 1986.

Somatic embryos can be obtained from the meristimic regions in the leaves or from shoot tips by callus formation (Beauchesne, 1982). Callus formation occurred on medium containing 100mg 2,4-D/L and 3mg 2iP/L with 3g activated charcoal/L Shaheen (1990) and Aslam and Khan(2009) .

Direct somatic embryos has been obtained on media supplemented by 100mg 2,4-D and 3mg 2iP/L (Daguin and Letouze 1988, Eshraghi *et al* 2005;Taha *et al* 2003;Tisserat 1982, Zaid 2003, and Zaid and Tisserat 1983). The regenerated shoots rooted in the media contained 0.1 NAA /L Eke *et al* (2005).Tisserat(1984) proliferated shoot buds on medium supplemented with NAA and BA, however Eshraghi *et al*,(2005) found that, embryogenic callus developed into plantlets with shoot and root when were transferred into medium supplemented with NAA and 2iP.

Date palm (*Phoenix dactylifera*L.) var. Karama is a good semi dry date with good quality grows in Siwa Oasis. This variety is suffering from some problems, the decrease in the number of young offshoots and the ageing of the adult plants, which are affected by high level of the ground saline water. For these reasons this variety, karama, could be threatened. To prevent that and keep this genetic resource, it was necessary to use tissue culture for its propagation. we concentrated in this study on propagation of *Phoenix dactylifera*L. var. Karama and production of whale plantlets through somatic embryogenesis .

2. Materials and methods

This study was carried out in Tissue Culture Laboratory, Genetic Resources Department, Desert Research Center, Cairo, Egypt through out the years 2002-2005.

Plant material and explants were obtained

from offshoots (10-15cm diameter 2-4 kg in weight from Siwa Oasis and were detached from the adult female *Phoenix dactylifera* L. Karama plants during Spring, Summer, Autumn and Winter. The outer leaves were removed with the hard bottom and fibrous sheaths belled off. The shoot tips were soaked under running tap water overnight. These shoot tips were washed after that with distilled sterilized water and immersed in sterilized anti-oxidant (150mg ascorbic acid and 100mg citric acid/L) solution and stored at 4ºC overnight to stop phenolic compounds formation. These shoot tips were about 3-5cm in diameter which were treated gentelly during disinfection.

For disinfection, shoot tips were immersed in 50% of sodium hypochlorite solution (5.2%active matter) for 30min and then explants were rinsed well with sterilized antioxidant (100mg Citric acid+150mg Ascorbic acid/L) for several times to remove disinfecting solution and stored in antioxidants until culture. Murashige and Skoog, 1962 supplemented with vitamins, 100mg *Myo*-inositol and 30.0 g sucrose/L was prepared. The pH value was adjusted at 5.6- 5.7 before adding 2.5g phytagel /L. Media was distributed onto jars and autoclaved at 121°C for 20 min .The sterilized shoot tips were cultured on the previously prepared establishment medium supplemented with 100 mg 2,4-Dichlorophenoxy acetic acid (2,4-D) + 3.0 mg Isopentenyl adenine 2ip/L + 3.0g activated charcoal/L and incubated in dark for three months to initiate callus. The initiated callus was maintained for another three months on the same composition of nutrient medium to increase callus formation. The maintained callus was cultured on regeneration media as follow:

Embryogenesis media: 0.1mg NAA/L combined with 0.0, 0.2, 0.5 and 1.0mg of 2iP/L.

Multiplication media: 0.5mg NAA/L combined with 0.0, 0.5 and 1.0mg each of Benzyl aminopurine (BA) and Isopentenyl adenine (2iP).

BA mg/L	2ip mg /L	NAA mg/L
0.0	0.0	0.0
0.5	0.0	0.0
0.5	0.5	0.0
0.5	0.5	0.5
1.0	1.0	0.5

For acclimatization polyethylene glycol (PEG) was added to control MS basal media at concentrations (0.0, 2.0 4.0 and 8.0 g/L) before acclimatization in green house.

Culture in green house:

Mixture of peat, vermiculite and sand at equal volumes was prepared and rinsed with water and divided into two parts, pH of the first part of this mixture was 8.5.The pH of the other part was adjusted with 1N HCl or 1N NaOH at 5.6 to 5.7 as the *in vitro* plantlets were grown.

Statistical analysis:

The design of each experiment was completely randomized with three replicates. The recorded data were number of forming embryos, length of embryos, plantlet length, root length and root thickness. All data obtained from the experiment were subjected to the proper statistical analysis of variance of the completely randomized design according to the procedure outlined by Snedecor and Cochran (1969). Mean values of treatments were differentiated by using L.S.D at 5% level as mentioned by Steel (1960).

3. Results and Discussion:

The sterilized shoot tips were cultured on the establishment medium previously mentioned to select contamination free explants which were used subsequently for callus induction.

Contaminations were noticed during culture some were fungi like *Asparagillus niger* and others were types of bacteria. Fungi was observed after few days of culture while bacteria was noticed after ten days on explants which were disinfected again by mercuric chloride and recultured on the same media. Because of the internal contamination in date palm tissues, there were some contaminations appearing under explants after one or two months during subcultures on medium.

The initiated callus was maintained on the initiation media (100mg 2,4-D +3 mg 2iP/L+plus 3g Activated charcoal/L) to get considerable amount of callus. The induced calli were compact, globular and creamy in colour. However at that concentration of 2,4-D ,callus became black with time, but has the ability to regeneration. The produced callus was subsequently transferred on callus growth media (100mg 2,4-D+3 mg 2iP/L+ 3.0 g Activated charcoal/L) to produce embryogenic callus. The same results were obtained by Beauchesne, 1982, Daguin and Letoutze (1988), Tisserat(1982), Zaid(2003),and Zaid and Tisserat1(982). Similar results were noticed by Bekheet *et al* 2001 , Eke *et al* 2005; and Sharma *et al* 1984.

The embryogenic callus was cultured on the differentiation media to differentiate embryos on media to discuss the effect of auxin and cytokinin on embryogenic callus after 6 weeks of culture.

Data in table (1) showed the effect of NAA at 0.1mg/L combind with different concentrations of

2iP, the highest number of embryos (14) was observed with the media containing 0.1mg NAA/L + 0.2mg 2iP/L followed by 2 embryos/ culture at media with 0.1mg NAA + 0.5mg 2iP/L. However, in medium containing 0.1 mg/L NAA and 1.0mg 2ip the least number of formed embryos (2.0) was obtained and there were high significant effect between media in the number of embryos. The length of these embryos was highly significant (5cm) with the concentration 0.1mg NAA + 0.5mg 2iP/L and the other treatments. The lowest mean length was 1.5 cm with 0.1 NAA + 1.0mg 2iP/L. The embryos were normal in their shapes and no significant observations were noticed.

The produced embryos showed a high potency for multiplication in the successive subcultures as regenerative embryos which gave the potentiality to get several thousands of plantlets from one offshoot or small amount of callus (embryogenic callus) Fig.(1).

Table (1). The effect of different combinations of NAA and 2ip on embryogenesis of *Phoenix dactylifera* callus after 60 days.

Media		Mean No of proliferated Embryos	Mean length of growing embryo cm	abnormal
NAA mg/l	**2iP mg/l**			
0.1	**0.0**	**4**	**2.25**	**0.0**
0.1	**0.2**	**14**	**3.0**	**0.0**
0.1	**0.5**	**6**	**5.0**	**0.0**
0.1	**1.0**	**2**	**1.5**	**0.0**
L.S.D. at 5%		**2.36**	**0.85**	**N.S**

Fig (1) Establishment of *Phoenix datyliferea* explants var.Karama on starting and callus formation media.

Data in Table (2) showed the multiplication rate of the mean No embryos / culture. The highest rate of multiplication was 4.46 embryos followed by 3.9 and then 3.78 embryos / embryo in media containing 0.5mg BA +0.5mg 2iP+0.5mg NAA/L and 0.5mg BA +0.5mg 2iP/L followed by1.0mg BA+1.0mg 2iP/L +0.5mgNAA respectively and there were no significant differences between them. While the length of the growing embryos was highest 3.0 cm/embryo in media which produced the highest multiplication rate followed by 3.0cm/embryo when cultured on medium with 1.0mg BA + 1.0mg 2iP + 0.5mg NAA/L. The produced embryos in this media (0.5mg/L of each of BA, 2iP and NAA) were long and thin than those produced by other treatments, the multiplication ability on this media was higher than which contained (1.0mg BA + 1.0mg 2iP/L + 0.5mg NAA) or 0.5mg BA + 0.5mg 2iP/L. These media showed high significant differences with other treatments. This means that presence of NAA improved the quality of the embryos growing on this media which have good chance for germination and grew well to produce complete plantlets. Auxins increase cell division, cell enlargement and synthesize of nucleic acids and proteins. The other treatments produce small embryos which subsequently produce small embryos and also plantlets. These results are in agreement with that obtained with Eshraghi *et al*,(2005) who found that, embryogenic callus developed into plantlets with shoots and roots when were transferred into medium supplemented with NAA and 2iP. However, Tisserat (1984) used medium supplemented with NAA and BA for shoot formation.

These results are also in agreement with those of Zaid, (1986), and Beauchesne, (1982), they propagated date palm *in vitro* via somatic embryogenesis from apex merestim and buds in the

bottom of leaves. The calli were initiated and maintained on the initiation media (100mg 2,4-D+3 mg 2iP/L+3g Activated charcoal/l) to get considerable amount of callus. The same results were obtained by Aslam and Khan (2009); Daguin and Letoutze,(1988);Eke *et al* (2005), Eshraghi *et al*.(2005), Tisserat (1982), Zaid,(2003), and Zaid and Tisserat,(1983).

Table (2). The effect of media on the multiplication of date palm somatic embryos.

Media			Embryo/culture mean	Length of embryo (cm)	Vigor of growth
BA	2iP	NAA			
0.0	0.0	0.0	2.16	0.6 cm	+
0.5	0.0	0.0	2.75	0.9 cm	+
0.5	0.5	0.0	3.9	1.8 cm	+2
0.5	0.5	0.5	4.46	3.0 cm	+3
1.0	1.0	0.5	3.78	3.0 cm	+3
L.S.D. at 5%			0.71	0.44	

\+ Embryo has ability to multiply again
+2&+3 embryo has ability to germination and also give multiplication

Fig.(2) The formation of somatic embryos from the embryogenic callus of *Phoenix dactylifera*L. var. karama.

Table (3). Elongation of the vigourous somatic embryos *of Phoenix dactylifera*.

Media			Length of plantlet Mean (cm)	Root no/plantlet	Root length mean cm
BA	2iP	NAA			
0.5	0.5	0.1	8.0 cm	5	10
1.0	0.5	0.1	9.2 cm	5	15
1.0	1.0	0.1	19.0 cm	5	15
0.5	1.0	0.1	13 .0cm	5	15
L.S.D. at 5%			2.32	N.S	2.05

Data in table (3) illustrated the effect of media components on the elongation of the somatic embryos which were developed on the medium and showed significant differences as in table (3). Plantlet length was affected by the presence of cytokinins at concentration twice or more than which was found in multiplication stage, but NAA was in the opposite, it was less than the previous stage (0.1mg/L).

The highest length of the plantlets was 19cm with media containing equal concentration of BA and 2ip (1mg/L) and 0.1mg NAA/L. followed by 13.0 cm on the media with 0.5mg BA + 1.0mg 2ip + 0.1mg NAA/L. Plantlets rooted on these media. Roots were grading in length and thickness from media to other. Number of roots/plant (5 roots) was insignificant in all treatments but the root was thick with the short roots and some thin with the long roots which were containing sub roots. Root length was insignificant except for the first media. The formed somatic embryos which were cultured on the best multiplication media (0.5mg/l BA+ 0.5 mg 2iP/l +0.5mg NAA/l)and subsequently transferred to the best elongation and rooting media (1.0mgBA+1.0mg 2iP+0.1mgNAA/ L) to produce plantlets . Roots of the proliferated plantlets from somatic embryos were weak, thin, and rarely survive when transferred into the soil. To solve this problem, plantlets were transferred to hardening process on the previous

media in addition of 0.0,2.0,4.0 and 8g polyethylene glycol (MW20000)/L before green house to enhance formation of strong and vigour roots in order to increase water and salts uptake .

Table (4) Effect of polyethylene glycol on the pre-adaptation of *Phoenix dactylifera* plantlets

Treatment PEG(20000) G/L	Survival plants		
	%	Root thickness mm	Root length mean cm
0.0	25	1.0	10
2.0	40	1.21	15
4.0	80	2.18	15
8.0	80	2.14	15
L.S.D. at 5%		0.20	2.05

Fig. (3) Preacclimatization of *Phoenix dactylifera* var. karama.*in vitro* and in farm.

The process between rooting and acclimatization is a very important step to complete propagation process. In this stage plantlets are grown in optimum conditions (moisture, salts, sucrose and water). Therefore, plantlets leaves don't have cuticle layer and the transpiration is high. The presence of PEG in media increases the cuticle formation on leaves, decreases transpiration and also root thickness (Table 4), which will make a balance between transpiration and salts uptake from media and polypropylene caps of the jars were removed gradually. High frequencies of survival rates were obtained after five weeks of transplanting the plantlets (with well developed root system). The successful transplanting may be due to the strong and the healthy root system formed during the pre-adaptation stage.

Plantlets were transferred to green house for acclimatization. As it appears from table (5) the percent of survival was high in the mixture at pH value 5.7 than 2d mixture 72.7% and 22.2%, respectively. This value indicates that the pH value (5.7) has a great effect on the acclimatization process because plantlets were growing in an optimum condition in media (moisture, temperature, light, minerals and growth regulators). At this pH value (5.7) minerals are in a soluble state and easier for the uptake by plantlet roots.

Table (5). Effect of pH value on acclimatization of date palm plantlets.

Treatment	plantlets NO	Surviveal	
		No.	%
pH 5.7	22	18	72.72
pH 8.5	18	4	22.22

From the results obtained we can come to the conclusion that thousands of the micropropagated date palm (*Phoenix dactylifera)* plantlets via somatic embryogenesis are produced but the survival percent of these plantlets were low due to acclimatization stage. The survival percent increased when plantlets were hardened and the pH was similar to that of the growing media (5.7).The cuticle layer of these plantlets is often poorly developed and extra water loss through evaporation when plantlets were transferred to soil in which the relative humidity is lower. Leaves of the in vitro plantlets are thin, soft, undeveloped plastids, the mesophyll air space and their stomata are not photosynthetically active and subsequently not adapted for in vivo conditions.

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4. References:

1- Abd Alla M.M.,(2000): Biotechnological studies on the stability of the tissue cultured date palm plants .Genetic Depart. Faculty of Agri. Ain Shams Uni.
2- Al-khairi J.M.,(2001).Optimization of biotine and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera L.*) . In Vitro Cell. Dev. Biol. Plant 37: 453-456.
3- Aslam j.,and Khan S.A.;(2009);In vitro micropropagation of Khalas date palm (*Phoenix dactylifera L.*) as important fruit plant . Journal fruit and ornamental plant research. Vol.17 (1):15-27.
4- Bhattacharjee S.K.2006. Advanced in ornamental horticulture. Pointer Jaipur 6,2065,ISBN81-7132-432-0.
5- Beauchesne,G.(1982).The vegetative propagation of date palm through tissue culture.In:1st symp.date palm, King Faisal Univ., Al-Hassa, Saudi Arabia.
6- Bekheet, S.A., Saker, M.M., Taha, H.S. and Moursy, H.A. (2001). Plant regeneration via somatic embryogenesis in date palm (Phoenix dactylifera L.). Arab J. Biotech.Vol 4(1): 111-118.
7- Daguin,F. andR. Letouze(1988), Regeneration of date palm (*Phoenix dactylifera L.*) by somatic embryogenesis improved effectiveness by dipping in a stirred liquid medium. Fruits,43:191-194
8- El-Kazzaz, A.A. and El-Bahr, M.K. (2001).A method for in vitro propagation of Egyptian date palm cultivar Samany. Arab. J.Biotech. Vol. 4 No.(2) 285-292.
9- Eke C.R., Peter A.and Omorefe A.(2005);Somatic embryogenesis in date palm (Phoenix dactylifera L.) from apical meristem tissues from ‘zebia’ and ‘loko’ landracesAfrican Journal of Biotechnology Vol. 4 (3), pp. 244-246.
10- Eshraghi P., Zarghami R., and Mirabdulbghi M., 2005. Somatic embryogenesis in two Iranian palm cultivars. African journal of biotechnology vol.4 (11), pp.b1309-1312.
11- Kumar, U. (1999). Somoclonol and somaclonol variant in; Methods in plant tissue culture; (ed.) U. Kumar. Agrobios, India, 218-228.
12- Mater, A.A. (1986). *In vitro* propagation of *Phoenix dactyliferal* L. Date Palm Journal, 4(2): 137-152.
13- Mujib A., Cho M.J., Predieri S., Banerjee S.,(2004). In Vitro Application in crop improvement. Exford IBH Publi. Co. Pvt. Ltd, New Delhi.
14- Murashing, T. and F.A. Skoag (1962). A revised media for rapid growth and bioassays with tobaeco tissue culture. Physio. Plant 15: 473-475.
15- Shaheen,M.A.(1990)propagation f date palm through tissue culture., A review and an interpretation. Annals Agriculture sci., Ain shams Univ., Cairo Egypt, 53:985-909.
16- Sharma, D.R.,S. Dawrara, and J.B.Chowdury (1984).Somatic embryogenesis and regeneration in date palm (*Phoenix dactyliferL)*cv. Khadravi through tissue culture. indian journal of experimental Biology,22,596:598.
17- Snedecor, G.W. and W.G. Cochran. (1969). Statistical methods. 6th ed. Iowa State Univ., Press, Ames, Iowa, U.S.A.
18- Steel, G.D.R. (1960). Principles and procedures of statistics. New York McGraw-Hill Book Co., pp. 481.
19- Taha H.S.; Bekheet S.A. and El-Bahr M.K.(2003) Alternative approach for micropropagation of the date palm c.v. Zaghlool Arab J.Biotech.,Vol.6,No.(1)Jan.):103-112.
20- Tisserat, B. (1982). “Factors involved in the production of plantlets from date palm callus cultures” Euphytica, 31(1): 201-214.
21- Tisserat, B. (1984). “Propagation of date palm by shoot tip cultures” Hort. Science 19:230-231.
22- Zaid,Z(2003)comparative studies on the production of date palm cultivars via tissue culture technique ph.D Dissertation, Department of pomology of agriculture, Cairo Univ., Egypt, pp.65
23- Zaid,A and B.Tisserat,(1983)In Vitro shoot tip differentiation in *Phoenix dactylifer* L ,Date palm j.,2(2):163-182.

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