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MICROPROPAGATION OF SOME ORNAMENTAL PALMS

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ABSTRACT: ***Callus formation and plant regeneration from different explants of various ornamental palms is described. Leaf base, shoot tip, roots, seeds or inflorescence of Washingtonia, Canary Island palm, Royal palm, Doum palm and Chamaedorea were cultured on callus induction media. The medium was composed of Murashige and Skoog medium (MS) supplemented with different combinations of 2,4 dichlorophenoxyacetic acid (2,4-D); 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram) and naphthaleneacetic acid (NAA). Produced callus was transferred into MS supplemented with 0.2 mg/l 2,4-D and 0.5 mg/l kinetin for further proliferation and development or into MS supplemented with 0.2 mg/l benzyladenine (BA) and1 mg/l kinetin for shoot induction. Shoot primordial and bud clusters were transferred into MS containing 1.5 g/l activated charcoal (AC) and supplemented with 2 mg/l BA, 2mg/l kinetin and 1 mg/l NAA. Callus was produced from different explants of the various palms; nevertheless, shoot regeneration was affected greatly by genotype and type of explant. Produced shoots were then transferred into root-induction medium composed of MS containing 0.1mg/l NAA. In some instances, rooted shoots were transferred into MS supplemented with 3 mg/l paclobutrazol prior transfer to soil. Rooted plantlets were acclimatized in soil and normal phenotypic plants were established in the field.***

Key Words: ***Arecaceae, leaf base, ornamental palms, somatic embryogenesis, tissue culture***

INTRODUCTION

Ornamental palms are becoming increasingly popular for cultivation as indoor and container plants, likewise in streets, parks and gardens. They are especially popular in tropical and subtropical areas and constitute an essential part of many nurseries (Johnson, 1995). Palm is cultivated nowadays in northern European towns, where they do not grow naturally, since they are progressively admired (Scariot and Larcher, 2004).

Washingtonia (*Washigtonia flifera* L.), Canary Island plam (*Phoenix canariensis*) and Royal palm, (*Roystonea regia*), represent an outstanding elements in the landscape. Doum palm (*Hyphaene thebaica*) is considered a botanical curiosity due to its branching habit (Jones, 1955). It is sometimes used for landscape and for its edible fruits which are employed in folk medicine for treating high blood pressure and hypoglycemia (Khattab *et al.*,

1998). *Chamaedorea elegans* is a very popular palm which is used for indoor decoration. Unfortunately this palm does not produce seeds in Egypt and seeds are imported.

Production of ornamental palms faces a lot of economical losses since many ornamental palms are good host plants for several insects, pests and bacterial, fungal, and viral diseases which hinder commercial propagation and breeding programs (Fletchman and Etienne, 2004; Riolo *et al.*, 2004; Broschat and Elliott, 2005). In addition, hundreds of ornamental palm species are threatened due to deforestation which is the major threat to palms *in situ* and habitat preservation is needed to avoid species extinction (Johnson, 1995; Britt *et al.*, 2004). Tissue culture has the potential to establish an efficient system for mass propagation, germplasm preservation and improving plant characteristics (Tisserat, 1991). An efficient method for plant regeneration from Washingtonia palm from immature inflorescence and immature zygotic embryo was reported previously (Mohamed-Yasseen *et al.*, 2005). The aim of this work is the establishment of an efficient system for plant regeneration of several ornamental palms using different explants.

MATERIALS AND METHODS

Abbreviations: AC: activated charcoal; BA: benzyladenine; MS: Murashige & Skoog,s (1962) medium; NAA: naphthaleneacetic acid; Paclobutrazol: 1-(4-chlorophenyl) - 4, 4 – dimethyl – 2 - (1H - 1, 2, 4 triazol – 1 - ly) pentan – 3 - ol; Picloram: 4 - amino- 3, 5, 6 – trichloro – 2 - pyridinecarboxylic acid; 2, 4 - D: 2, 4 dichlorophenoxyacetic acid.

Source of plant material.

Mature seeds of *Washingtonia filifera, Phoenix canariensis, Roystonea regia, Hyphaene thebaica* L. were collected from certain palms growing at Orman Garden, Ministry of Agriculture. Female inflorescence of *Chamaedorea elegans* were harvested at the primordial stage from plants growing in the greenhouse. All experiments were conducted from 2004 to 2006 at the Genetic Engineering and Biotechnology Research Institute at Sadat City, University of Minufiya.

Preparation of seeds.

Mature seeds of Washingtonia, Canary Island plam and Royal palm were extracted from fruits and washed thoroughly with sand and running water. Seeds were subjected to chemical-scarification treatment to remove dormancy caused by hard seed-coat. Seeds were incubated on concentrated solution of KOH (approximately 10 N) for 60-90 min, with agitation. They were then rinsed several times and incubated in distilled water for 30 min to remove excess of KOH. Seeds of Doum palm were extracted from fruits, cut transversally and the embryo along with a small portion of the endosperm (approximately 5 mm around the embryo) was used during this study.

Surface sterilization of seeds and inflorescences.

All seeds were surface sterilized with 0.1% $HgCl_2$ for 10 min and rinsed three times in sterile distilled water. Seeds and female inflorescences of Chamaedorea were subsequently sterilized with 2.5 % (v/v) sodium hypochlorite, with two drops of Tween x100, for 30 min then rinsed five times in sterile distilled water and placed on culture medium. Seeds were cultured on germination medium composed of Murashige and Skoog medium (MS) containing 30 g/l sucrose and 2 g/l gelrite and cultures were maintained in the dark at 32 ± 2°C (Broschat and Donselman, 1988).

Preparation of explants from seedlings and inflorescences.

Seedlings of Washingtonia, Canary Island plam, Royal palm and Doum, previously germinated in the dark on MS medium, were used as a source of leaf base, shoot tip and root explants. Explants were excised from seedlings reaching 10-14 cm in length. Leaf base (approximately 4-10 mm in length), shoot tip (approximately 4-8 mm in length) and root explants (approximately 15-30 mm in length) were excised from seedling and cultured on callus-induction medium. Female inflorescences of Chamaedorea were cut into small segments approximately, 15- 20 mm in length and rachis with florets were cultured on callus induction medium.

Callus induction and proliferation media.

Explants were cultured on MS containing 30 g/l sucrose, 2 g/l gelrite, 170 mg/l NaH_2PO_4, 300 mg/l glutamine, 500 mg/l casine hydrolysate and 50 mg/l adenine sulfate. Media were supplemented with different combinations of growth regulators: (0.2, 0.5, 0.8, 1, 1.5, 2, 2.5, 3 mg/l) 2,4-D, (0.5, 1.5, 3, 5, 7 mg/l) picloram and (1, 5, 10, 12mg/l) NAA. Cultures were maintained in the dark for twelve weeks. Produced callus was then transferred into callus proliferation medium or into shoot induction medium. Callus proliferation medium was composed of MS containing 30 g/l sucrose, 2 g/l gelrite, 170 mg/l NaH_2PO_4, 300 mg/l glutamine, 500 mg/l casine hydrolysate, 50 mg/l adenine sulfate and supplemented with 0.2 mg/l 2,4-D and 0.5 mg/l kinetin, for eight weeks, for further proliferation and development.

Shoot induction from callus.

Shoot-induction medium was composed of MS containing 30 g/l sucrose, 2g/l gelrite and supplemented with 0.2 mg/l BA and 1mg/l kinetin.

Shoot proliferation and elongation.

Produced shoots primordial and bud clusters were transferred from shoot induction medium into MS containing 1.5 g/l activated charcoal (AC) and supplemented with 2mg/l BA, 2mg/l kinetin and 1 mg/l NAA.

Rooting and transfer to soil.

Produced shoots were separated and cultured for eight weeks on root-induction medium composed of MS containing 30 g/l sucrose, 2g/l gelrite with 0.1 mg/l NAA. Rooted shoots were transferred into medium composed of MS containing 30 g/l sucrose, 2g/l gelrite with 3 mg/l paclobutrazol. Plantlets with good root system were planted in Magenta jars filled with autoclaved commercial potting soil (Agro Mix No. 2; Conard Fafard, Springfield, MA) for twelve weeks . Plants were then transferred into 165 cm^3 plastic pots filled with autoclaved commercial potting soil and covered with a transparent polyethylene bag for acclimatization in a greenhouse prior transfer to the field.

Media and culture conditions.

Media pH was adjusted to 5.7 with (1N) KOH after adding growth regulators but before adding gelling agent. Growth regulators were added before sterilization in an autoclave at 121°C and 98 KPa for 20 min. Germination, shoot elongation and rooting media were dispensed on 300-ml jars while callus culture media were dispensed on 150-ml jars. Jars were sealed with clear plastic polypropylene lids. Unless other wise stated, cultures were maintained at 26 ± 2°C. Cultures were maintained in the dark during seed germination, callus induction and proliferation. Cultures were thereafter, transferred into an 18 hr photoperiod (cool white fluorescent light, 40 $\mu mol.m^{-2}.s^{-1}$) for shoot induction, proliferation, elongation and rooting.

Experimental design.

All experiments were conducted using a completely randomized design. Twenty replicates were used in each treatment and each experiment was repeated three times. Data were evaluated by analysis of variance and means between treatments were compared according to (Duncan, 1955).

RESULTS AND DISCUSSION

Morphogenesis from leaf base explants.

Leaf base explants of Washingtonia, Canary Island plam and Royal palm produced callus after eight weeks on almost all media containing different concentrations of 2,4-D, NAA and picloram (Tables 1, 2 and 3). Percentage of callus formation and callus weight of explants of Washingtonia (Table 1) was high on media containing low concentrations of 2,4-D and picloram and decreased with increasing concentration. The best results were obtained on media containing 0.2, 0.5 and 0.8 mg/l 2,4-D as well as 1.5 and 3 mg/l picloram. Nevertheless, the high concentration of NAA (10 mg/l NAA) was more effective on callus formation than the low ones (5 mg/l).

Similar results were obtained with leaf base explants of Canary Island plam and Royal palm (Tables 2 and 3). It seems that high concentrations of

Table (1). Effect of different concentrations of 2,4-D, NAA and picloram on callus induction from leaf-base, shoot tip and root explants of Washingtonia.

Auxin	Callus formation %	Callus weight (mg/explant) induction	proliferation	Morphogenesis
Leaf base explants				
0.5 mg/l 2,4-D	52 b	536 d	NA	globular friable callus
0.8 mg/l 2,4-D	44 bc	780 c	1233 de	globular friable callus
1.5 mg/l 2,4-D	10 d	312 f	NA	globular friable small callus
3 mg/l 2,4-D	6 d	83 h	NA	globular friable small callus
3 mg/l picloram	100 a	767 c	1472 d	globular friable callus
5 mg/l NAA	0.0 e	0.0 i	NA	root formation on leaf base
10 mg/l NAA	53 b	1816 a	2123 b	Profuse roots with friable callus
0.2 mg/l 2,4-D + 1 mg/l NAA	68 b	1525 a	1994 bc	globular friable callus with some roots
0.5 mg/l 2,4-D + 5 mg/l NAA	57 b	1725 a	2897 a	globular friable callus
1mg/l 2,4-D + 1mg/l NAA	8 d	455 e	NA	globular friable callus
1mg/l 2,4-D + 5 mg/l NAA	12 d	252 g	NA	globular friable callus
0.5 mg/l picloram + 1 mg/l NAA	38 c	860 b	1085 f	globular callus
1.5 mg/l picloram + 1 mg/l NAA	3 bc	943 b	1613 d	globular callus
Shoot tip explants				
0.8 mg/l 2,4-D	34 b	800 d	1350 d	globular callus
5 mg/l NAA	0.0 c	0.0 e	NA	root formation on shoot tip
10 mg/l NAA	64 a	970 bc	1763 c	profuse roots with friable callus
0.2 mg/l 2,4-D + 1 mg/l NAA	76 a	1935a	2652 a	globular callus with some roots
0.5 mg/l 2,4-D + 5 mg/l NAA	63 a	1650ab	2 423ab	globular callus
0.5 mg/l picloram + 1 mg/l NAA	28 b	942 cd	1785 c	globular callus
1.5 mg/l picloram + 1 mg/l NAA	72 a	1143 c	2122 b	globular callus
Root explants				
0.5 mg/l 2,4-D	93 a[z]	3289a	5432 a	cluster of friable and globular callus
0.8 mg/l 2,4-D	87 bc	1786 d	3124 d	friable callus
1.5 mg/l 2,4-D	40 d	0457 e	765 e	necrotic roots with small callus
3 mg/l 2,4-D	0.0 e	0.0 f	NA	brown dark roots without growth
5 mg/l NAA	80 c	3012a	NA	few callus with new root growth
10 mg/l NAA	100 a	3285a	6862 a	friable callus with new root growth
0.2 mg/l 2,4-D + 1 mg/l NAA	100 a	2753b	4238 b	friable callus
0.5 mg/l 2,4-D + 5 mg/l NAA	100 a	2863b	4768 b	friable callus
3 mg/l picloram	90 b	2133 c	3647 c	yellowish friable callus
0.5 mg/l picloram + 1 mg/l NAA	94 a	1650 d	2875d	friable callus
1.5 mg/l picloram + 1 mg/l NAA	95 a	2250 c	3823c	friable callus

[z]Mean separation within columns for each type of explant by Duncan's multiple range test at P = 0.05.

[y]Callus weight for root explants was determined as mg callus per mg root explants.

NA: not applicable

Table (2). Effect of different concentrations of 2,4-D, NAA and picloram on callus induction from leaf base, shoot tip and root explants of Canary Island plam.

Auxin	Callus formation %	Callus weight (mg/explant)	Morphogenesis
Leaf base explant			
0.5 mg/l 2,4-D	32 d[z]	620 c	small friable callus
0.8 mg/l 2,4-D	28 d	835 b	small friable callus
1.5 mg/l 2,4-D	45 c	1020 a	friable callus
2.5 mg/l 2,4-D	30 d	430 d	small friable callus
12 mg/l NAA	40 c	765 b	friable callus with some roots
0.2 mg/l 2,4-D + 1 mg/l NAA	57 b	640 c	friable callus
0.5 mg/l 2,4-D + 5 mg/l NAA	75 a	800 b	friable callus
2 mg/l 2,4-D + 1 mg/l NAA	30 d	770 b	friable callus
2 mg/l 2,4-D +1 mg/l NAA + $AgNO_3$	25 d	730 b	friable callus
1.5 mg/l picloram	25 d	610 c	yellowish friable callus
3 mg/l picloram	22 d	540 cd	yellowish friable callus
7 mg/l picloram	10 e	180 e	dark brown callus
Shoot tip explant			
0.5 mg/l 2,4-D	80 a	550 c	friable callus
0.8 mg/l 2,4-D	63 b	980a	small friable callus
1.5 mg/l 2,4-D	65 b	960a	friable callus
2.5 mg/l 2,4-D	35 e	250d	brownish callus
12 mg/l NAA	40 d	765 b	friable callus with some roots
0.2 mg/l 2,4-D + 1 mg/l NAA	50 c	530 c	friable callus
0.5 mg/l 2,4-D + 5 mg/l NAA	80 a	850 b	friable callus
2 mg/l 2,4-D + 1 mg/l NAA	70 b	1030a	friable callus
2 mg/l 2,4-D +1 mg/l NAA + $AgNO_3$	65 b	980a	friable callus
1.5 mg/l picloram	50 c	620bc	yellowish friable callus
3 mg/l picloram	60 b	750b	yellowish friable callus
7 mg/l picloram	30 e	250d	brownish callus
Root explant[y]			
1.5 mg/l 2,4-D	67 b	1830d	necrotic roots with small callus
3 mg/l 2,4-D	45 c	1480 e	necrotic roots with brownish callus
12 mg/l NAA	100 a	2186 c	friable callus
0.5 mg/l 2,4-D + 5 mg/l NAA	100 a	3760 a	friable callus
2 mg/l 2,4-D + 1 mg/l NAA	100 a	2470 b	small friable callus
2 mg/l 2,4-D +1 mg/l NAA + $AgNO_3$	100 a	1780 d	small friable callus
3 mg/l picloram	100 a	1980 d	yellowish friable callus

[z]Mean separation within columns foe each type of explant by Duncan's multiple range test at P = 0.05.

[y]Callus weight for root explants was determined as mg callus per mg root explants.

Table (3). Effect of different conecntrations of 2,4-D, NAA and picloram on callus induction from leaf base, shoot tip and root explants of Royal palm.

Auxin	Callus formation %	Callus weight (mg/explant)	Morphogenesis
Leaf base explants			
1.5 mg/l 2,4-D	60a[z]	260a	friable callus
3 mg/l 2,4-D	25	125	necrotic brownish callus
12 mg/l NAA	55	240	small callus with roots
0.5 mg/l 2,4-D + 5 mg/l NAA	68a	190	friable callus
2 mg/l 2,4-D + 1 mg/l NAA	43	233	friable callus
2 mg/l 2,4-D +1 mg/l NAA + $AgNO_3$	47	260	friable callus
3 mg/l picloram	33	145	yellowish friable callus
Shoot tip explants			
1.5 mg/l 2,4-D	40	127	friable callus
3 mg/l 2,4-D	15	80	friable callus
12 mg/l NAA	35	140	friable callus with some roots
0.2 mg/l 2,4-D + 1 mg/l NAA	35	120	friable callus
0.5 mg/l 2,4-D + 5 mg/l NAA	15	80	friable callus
2 mg/l 2,4-D + 1 mg/l NAA	20	85	friable callus
2 mg/l 2,4-D +1 mg/l NAA + $AgNO_3$	18	90	friable callus
1.5 mg/l picloram	20	65	yellowish friable callus
3 mg/l picloram	25	85	yellowish friable callus
7 mg/l picloram	25	70	yellowish friable callus
Root explants[y]			
1.5 mg/l 2,4-D	25	40	small brownish callus
12 mg/l NAA	100	257	small friable callus
0.2 mg/l 2,4-D + 1 mg/l NAA	100	300	small friable callus
0.5 mg/l 2,4-D + 5 mg/l NAA	100	234	small friable callus
2 mg/l 2,4-D + 1 mg/l NAA	30	65	small brownish callus
2 mg/l 2,4-D +1 mg/l NAA + $AgNO_3$	25	70	small brownish callus
1.5 mg/l picloram	100	256	yellowish friable callus
3 mg/l picloram	100	193	small brownish callus

[z]Mean separation within columns for each type of explant by Duncan's multiple range test at P = 0.05.
[y]Callus weight for root explants was determined as mg callus per mg root explants.

2,4-D reduced percentage of callus and callus weight suggesting that leaf-base explants require low concentrations of 2,4-D, picloram and high concentrations of NAA for callus formation. Produced callus was globular and friable (Fig. 1a and b). Callus was not produced on medium containing 5mg/l NAA, but the addition of 0.5mg/l 2,4-D to 5 mg/l NAA improved callus formation. Percentage of callus formation and callus weight from leaf-base

of Canary Island plam and Royal palm increased with increasing the concentration of 2,4-D up to 1.5 mg/l 2,4-D then decreased with the higher concentration. Callus induction on media containing picloram was less than callus formation on media containing 2,4-D.

Callus formation from leaf base of Washingtonia was much better than callus formation from leaf base of Canary Island plam and reached its lowest level with Royal palm. Adding silver nitrate to callus induction media did improve neither callus formation nor morphogenesis of callus in either, Canary Island plam or Royal palm.

Callus weight of leaf base explants of Washingtonia was increased upon incubation on callus-proliferation medium. It was evident to observe the effect of composition of pervious culture media. Explants cultured on low concentrations of 2,4-D, on callus induction media, produced better callus upon transfer to callus proliferation medium.

The potential of leaf base explants of date palm for callus formation and plant regeneration was investigated by several researchers using different combinations of auxins and cytokinins (Drira, 1983; Zaid and Tisserat. 1983; Daikh and Demarly,1987; Madhuri and Shankar, 1998; Omar,1988; Anjarne and Zaid, 1993; Veramendi and Navarro, 1997; Wanas *et al*., 1999). This study described the first protocol for callus formation and plant regeneration from leaf base of some ornamental palm. Establishment of a reliable and an efficient system for plant regeneration from leaf base promises a great success for possibility of using leaf primordia from elite ornamental palms for propagation. Moreover, the use of leaf primordial bases is prerequisite for the propagation of individual elite genotypes, when the availability of explants is very limited.

Morphogenesis from shoot tip explants.

Shoot tip explants of Washingtonia, Canary and Royal palms produced globular callus (Fig. 1c) on media containing 2,4-D, picloram or high concentrations of NAA (Tables 1, 2 and 3). Percentage of shoot tip explants producing callus and callus weight were high on media containing 0.2, 0.5, 8, 1.5 mg/l 2,4-D; 1.5 and 3 mg/l picloram and 10 and 12 mg/l NAA. Roots were produced from shoot explants cultured on media containing NAA, nevertheless the presence of 2,4-D or picloram inhibited or reduced root formation. Shoot tip explants cultured on media containing 10 mg/l NAA produced callus and roots from their base and some roots were also produced from leaves which were adjacent to culture medium. Callus formation from shoot tip of Washingtonia was better than callus formation from shoot tip of Canary Island plam and reached its lowest level with Royal palm. It is worthy to note that in occasional cases some shoot tip explants of Washingtonia produced from 2-3 shoots/explant. These shoots were produced directly from axillary buds and not from callus.

Morphogenesis of root explants:

Root explants produced callus on all media containing 2,4-D, picloram or NAA (Tables 1, 2 and 3). Increasing concentrations of 2,4-D reduced percentage of explants producing callus and callus weight. Callus formation from root explants required high concentrations of NAA. Roots cultured on media containing 5 and10 mg/l NAA produced orthotropic roots and small callus. Picloram levels at 1.5 and 3 mg/l were effective for callus induction. Produced callus was globular (Fig.1d). Callus formation from root explants of both Washingtonia and Canary Island plam was better than callus formation from Royal palm.

It is interesting to note that root explants were responsive for callus formation and shoot initiation (Fig. 1e) in all ornamental palms used in this study. The advantage of using root segments for callus formation is that roots can be harvested every 4-6 weeks from plantlets established in culture without harming the mother plants.

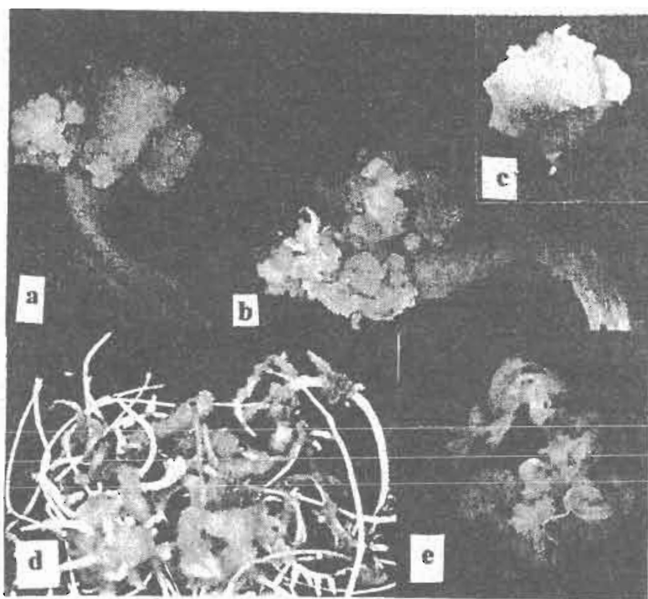

Figure (1). Callus formation and shoot initiation from different explant of ornamental palms. (a). friable and globular callus formation on Washingtonia leaf bases. (b). shoot initiation from callus produced on Washingtonia leaf base. (c). friable callus produced on Canary Island plam shoot tip. (d). friable callus on Royal palm root explants. (e). shoot initiation from Canary Island plam callus derived from root.

Morphogenesis from Chamaedorea inflorescence:

Inflorescence of Chamaedorea palm produced dark brown exudates after culture initiation. Explants were regularly transferred into fresh media, every four weeks to alleviate the harmful effects of the phenolic exudates. Callus was produced from ovary, sepals and petals of florets. Inflorescence of Chamaedorea palm produced white globular callus Table (4). Multiple shoots were produced from this globular callus upon transfer into shoot-induction medium (Fig. 2a). Plant regeneration from immature inflorescence of palms was previously described on date palm, raphis and Washingtonia (Drira and Benbadis, 1985, Daquinta *et al.*, 1997; Loutfi and Chlyah,1998, Mohamed-Yasseen *et al.*, 2005). The technique of shoot regeneration from florets offers a great promise for rapid propagation since numerous plants could be produced from inflorescence of Chamaedorea which do not produce seeds in Egypt.

Shoot regeneration, proliferation and elongation.

Produced callus was transferred into shoot-induction medium containing 0.2 mg/l BA and 1mg/l kinetin. Produced shoots primordia and bud clusters were transferred to shoot elongation and proliferation medium containing AC. Shoots elongated on this medium and root primordia appeared on shoots. Shoots were separated and cultured on root-induction medium for further elongation and root growth.

Shoot regeneration from callus produced on media containing 2,4-D was generally higher than from either NAA or picloram derived callus. Leaf-base explants and roots showed high potential for callus formation however, production of plantlets from callus was greatly affected by genotype. Callus resulted from Washingtonia leaf base was regenerable and produced multiple shoots upon transfer into shoot-induction medium. Shoot regeneration from leaf base of Canary Island plam and Royal palm was sporadic. Number of produced shoots increased upon transfer into shoot proliferation media. The best medium used for callus induction for all types of explant was MS supplemented with 0.5 mg/l2,4-D and 5 mg/l NAA.

Number of produced shoots was estimated in different explants cultured on this medium. The number of shoots regenerated from leaf base of washintonia was higher (average of 6.82 (± 2.4 SD) shoots/explant) than number of shoots regenerated from shoot tip (average of 3.22 (± 1.5 SD) shoots/explant) or root explants (average of 4.82 (± 1.8 SD) shoots/explant). The number of shoots regenerated from chamadeorea inflorescence explant on this medium gave average of 3.8 (± 1.2 SD) shoots/explant. The use of stepwise decrease of 2.4-D and addition of kinetin in callus-proliferation medium was suggested to stimulate shoot regeneration and somatic embryogenesis (Fei *et al.*, 2002). However, this method was not useful for the

rest of genotypes since callus lost its viability and required transfer into auxin-free medium.

Leaf base, shoot tip and root explants of Doum seedlings produced small non-regenrable callus, however, it was possible to obtain rapid, consistent and uniform germination *in vitro*. Culture of dissected embryo with small section of endosperm resulted in rapid and uniform germination on MS medium after one week from culture.

Rooting and transfer to soil.

Produced shoots from all type of explants formed roots and elongated in MS medium supplemented with 1mg/l NAA. All shoots easily produced multiple roots in root-induction medium. Attempts were made to improve percentage of survival during acclimatization using paclobutrazol. Smith *et al.*, (1991) suggested that several growth retardants can be used in rooting medium to reduce loses due to wilting during acclimatization. In the other hand rooted plantlets were cultured on Magenta Jars filled with soil (Fig. 2b) since roots produced in gelling agents are few, had thick root hairs and had underdeveloped vascular system compared to those formed in soil (Preece and Sutter, 1991; Hazarika, 2003). These treatments were conducted in order to improve root growth and hence percentage of plant survival during acclimatization. Nevertheless percentage of plants established in the field did not exceed twenty five percent. Virtually all plants established in soil showed normal phenotypic growth (Fig. 2c).

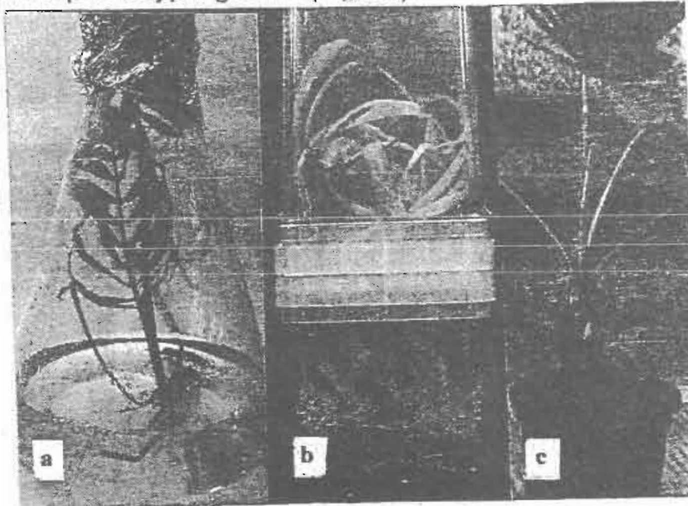

Figure (2). Plant formation, rooting and transfer to soil. (a). typical *Chamaedorea elegans* plant produced *in vitro*. (b). Washingtonia plantlet after transfer to soil *in vitro*. (c). typical plant established in soil in the greenhouse.

Table (4). Effect of different concentrations of 2,4-D and NAA on callus induction from inflorescence of *Chamaedorea elegams*.

Auxin	Callus formation %	Callus weight (mg/explant)	Morphogenesis
1.5 mg/l 2,4-D	30b	432b	globular friable small callus
3 mg/l 2,4-D	10c	135c	brownish globular small callus
0.2 mg/l 2,4-D + 1 mg/l NAA	78a	1525a	globular friable callus with some roots
0.5 mg/l 2,4-D + 5 mg/l NAA	85a	1672a	globular friable callus

[z] Mean separation within columns by Duncan's multiple range test at P = 0.05.

Conclusion.

This paper describes protocol for tissue culture of some ornamental palms. It represents callus-induction and plant regeneration from different explants of various ornamental palms. The protocol presented herein, offered the basis for the purposes of mass propagation of economically important ornamental palms as well as elite and threatened palms.

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الإكثار الدقيق لبعض أنواع نخيل الزينة

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يتناول هذا البحث استخدام تقنية زراعة الأنسجة في إنتاج نباتات من الكالوس المتكون من قواعد الأوراق والأفرع والجذور والبذور والأزهار لنخيل الواشنجتونيا ونخيل جزر الكنارى والنخيل الملوكى ونخيل الدوم ونخيل الشمادوريا وقد تم زراعة الأجزاء النباتية على بيئة ميراشيج وسكوج المحتوية على 2,4-D أو بيكلورام أو نفثالين حمض الخليك وقد تم نقل الكالوس الى بيئة ميراشيج وسكوج المحتوية على 2,4-D لإكثار الكالوس المتكون من الأجزاء الناتية المختلفة وتم الحصول على العديد من النباتات بعد عدة أسابيع من الزراعة على بيئة ميراشيج وسكوج المحتوية على ٠,٢ مليجرام/لتر بنزيل ادينين و١ مليجرام/لتر كينيتين ثم نقلت هذه النباتات الى بيئة ميراشيج وسكوج المحتوية على ٢ مليجرام/لتر بنزيل ادينين و٢ مليجرام/لتر كينيتين و١مليجرام/لتر نفثالين حمض الخليك و ١،٥ جرام/لتر من الفحم النشط و ذلك لتشجيع استطالة الأفرع. تم نقل الأفرع المتكونة الى بيئة ميراشيج وسكوج المحتويةعلى ٠,٢ مليجرام/لتر من نفثالين حمض الخليك لتكوين الجذور وفى بعض الحالات أضيف باكلوبيوترازول الى البيئة بمعدل ٣ مليجرام/لتر وتم أقلمة النباتات وزراعتها في الصوبة. وقد وجد ان قدرة الكالوس على تكوين نباتات تعتمد بشكل واضح على نوع النخيل وكذلك طبيعة الجزء النباتى المنزرع.