J. Agric. Sci. Mansoura Univ., 32 (12): 9999 - 10018, 2007

EFFECTS OF SOME GROWTH FACTORS ON STRUCTURE AND GROWTH BEHAVIOUR OF STEM SHOOT TIP CULTURES OF DATE PALM (*Phoenix dactylifera, L.*)

Helali, M.N. and Hanan R. El-Hosieny
Agric. Bot. Dep., Faculty of Agric., Mansoura Univ., Egypt

ABSTRACT

Some aspects of micropropagation of date palm (*Phoenix dactylifera* , L) Zaghloul cv . as affected by certain growth factors including type of explants ,media and some growth substance were examined . indirect somatic embryogenesis by initation callus from shoot apices , axillary buds,and leaf primordia explants and germination of somatic empryoes from callus were used.

Shoot tips explants were superior for surviving and swelling valus fllowed by leaf primordia and axillary buds. The callus obtained in all culture media were yellowish and aggregated

Callus formation /explant was positive correlated with levels of auxins used (2,4-D and NAA) especially in the presence of 2 ip and friable callus growth/explant was increased with culturing period.

The presence of auxins and cytokinins in compinations gave the best results for date palm empryogenic callus formation at the stage of callus formation ,callus differentiation and the balances between these substances are important at these stages .However ,MS alone gave the lowest results in this respect and the exogenous supply of growth substances is recommended.For the Mature somatic embryoes production, Average number of embryos per explant was found to be dependent on the explant used as well as the exogenous growth substans.

As for Shooting multiplication , the data show that shoot number formed from different sources of the explants used as well as average number of the secondary mature somatic embryoes which developed and produced on the basal part of the developed shoot explants in all treatments were increased with an increase in culturing period up to the 3rd one . Therefore,repeated subculture explants on a fresh medium stimulated shoot formation /explant .

All auxin treatments increased number of adventitious roots and root length of the plantlets formed from the different developed shoots. NAA gave higher values than IBA and the increase was a concentration dependent

.

INTRODUCTION

Schroeder (1970) used various tissues excised from offshoots (axillary shoots at the base of a mature tree) of the date palm and produce a few variable cultures. He obtained callus in one instance and maintained the tissue *in vitro* for three years. Roots formation was reported in the callus culture. Tisserat (1982) mentioned that shoot tips were proliferated through axillary buds outgrowths on liquid medium without charcoal but containing 0.1 mg/l NAA and 10 mg/l BA. Anjarne and Zaid (1993) found that early rooting of date palm tissues *in vitro* culture reduces bud multiplication and occasionally inhibits. Leaf base explants from the young leaves of date palm cv. Bouski offshoots were cultured on MS medium supplemented with various concentrations of auxins and cytokinins. These roots showed varied growth when subcultured on a medium containing low auxin concentration. The best

tissue growth and least organogenesis (i.e. root, shoot or leaf formation) occurred on media with low auxin concentration.

For adventitious root formation, Nasir *et al .* (1994) found that MS basal nutrient media supplemented with 0.05, 1.0, 3.0 mg/l NAA should be used. The length of adventitious roots was also greater from subcultures. They longest roots being formed on MS media containing 3.5 mg NAA/liter. Kintin appeared to have little effect on root growth. In conclusion, NAA (10 mg/l) and A.C. (0.3%) in the culture MS medium induced the formation of strong root system (Saker et al ., 1998).

The present investigation aimed to study the effects of certain growth factors via tissue culture technique on structure and growth behaviour of date palm *Phoenix dactylifera,* L) explants to find out the most suitable treatment for its micropropagation (*Phoenix dactylifera,* L). Zaghloul cv was sued. Some biochemical constituents were also examined.

MATERIAL AND METHODS

The explants were obtained from faculty of Agric.Cairo Univ. carried out at the laboratory of the Agric. BotanyDep., Faculty of Agric. Mansoura Univ. during the seasons of 1999-2002.

Callus initiation (starting)

Sterilized explants (Shoot tip segments, axillary buds and leaf primordia) were initially cultured on MS basal nutrient media Murashige &Skoog. (1970)in absence and presence of the following growth substances (mg/l):

1- MS alone (control) 2- MS + 100 2, 4-D
3- MS + 50 2, 4-D + 3 2iP 4- MS + 50 2, 4-D + 3 BA
5- MS + 100 NAA 6- MS + 50 NAA+ 3 2iP
7- MS + 50 NAA+ 3 BA 8- MS + 5 NAA + 10 2,4-D + 3BA
9- MS + 5 NAA + 10 2,4-D + 5BA

Each treatment was replicated 6 times (6 jars), each of them was used for sterilized specific explant used.

The swelled explants were subcultured three times, eight weeks intervals, tell the production of soft and smooth callus (friable callus).

All media were supplemented with 100 mg/l meyoinositol + 0.4 mg/l thiamine-HCl + 170 mg/l + NaH2PO4 + 40 mg/l adenine sulphate + 30 g/l sucrose + 3 g/l activated charcoal as recommended by Abo El-Nil (1986). The pH of the prepared medium was adjusted to 5.7-5.8 prior to addition of 1.0 g gelrite as a gelling agent. All cultures jars were maintained in complete darkness to minimize browning, to a least degree, at 27-28c^{o} for 32 weeks with three sub-culturing to corresponding fresh specific media every eight weeks. Surviving and swelling values in the different explants cultures were recorded after eight weeks from incubation. In addition, at each culturing date, average values of initiated callus/explant was scored (Pottino, 1981)

Negative result (-) = 1 Below average result (+) = 2
Average results (++) = 3 Good results (+++) = 4

Callus growth, development and differentiation:

The primary calli were separated from the initiated explants. They were subcultured two times every 8 weeks. The MS basal nutrient media was also used. The effect of different concentrations of the following growth substances (mg/l) on callus growth, development and differentiation were examined.

1 - MS alone (control)
2- MS +10 2, 4-D
3- MS +5 2, 4-D + 2.5 2iP
4- MS + 5 2,4-D + 3 BA
5- MS + 10 NAA
6- MS + 5 NAA+ 2.5 2iP
7- MS + 5 NAA+ 5 BA
8- MS+2.5 NAA + 5 2,4-D+5 BA
9- MS + 2.5 NAA + 5 2,4-D + 3 2iP

Each treatment was replicated six times and each of them contained one piece of friable callus from the specific explant used and incubated in complete darkness at 27-28c^{o}. At each culturing date, friable callus growth/explant was scored visually (Pottino, 1981). In addition, the friable callus developed embryogenic callus (somatic embryo) as described by Tisserat (1982) was determined and scored visually (Pattino, 1981). The determination was carried out as average increase in embryogenic callus formation per explant. Tisserat (1982) described the embryogenic callus as white nodular callus with globular structures.

3-3-4- Mature embryoes production

The resultant friable callus which composed emryogenic tissues (developed calli; Mater, 1986a and b) were divided into pieces at approximately 1x1 cm and cultured on the specific medium. Each treatment was replicated six times and each replicate contained one piece of embryogenic callus.

All cultured jars were incubated for 8 weeks at 27-28c^{o}on white fluorescent intensity of about 1500 Lux for 16 hrs/day. The friable yellowish callus were subcultured on fresh specific media used in the callus growth treatments which previously mentioned for another eight weeks. At each culturing date, (8 and 16 weeks) average number of mature embryo/explant (longer than 5 mm) was recorded. According to Veramendi and Navarro (1997) the minimum size that allowed embryo germination was that longer than 5 mm.

3-3-5- Shoot multiplication (shooting)

The developed embryo explants which resultant from germinated mature embryos having small cluster 1.5-2 cm in height and containing 3-4 developed shoots were used as the explants material for shooting under the following treatments:

1- MS alone (control)
2-MS + 10 2iP
3- MS + 1 2, 4-D + 10 2 iP
4- MS + 1 NAA + 10 2 iP
5- MS + 10 BA
6- MS + 1 2,4-D + 10 BA
7- MS + 1 NAA + 10 BA
8- MS+1 NAA+ 1 2,4-D + 20 2iP
9- MS + 1 NAA + 1 2,4-D + 20 BA

Each treatment was replicated six times, each of them contained one developed explant. All cultured jars were incubated under the same conditions mentioned before. The explants were subcultured three times, six weeks intervals, on fresh specific media. Shoot multiplication was took place

till forming shoots having 3-4 foliage leaves. At each culturing date, average shoot numbers and average shoot length/explant were recorded. In addition, average number of the secondary mature somatic embryos which developed and produced on the basal part of developed shoot explants were scored visually according to Pottino (1981).

3-3-6- Rooting of developmental shoots

Developed shoots of date palm Zaghloul cv which were obtained and formed from different explant sources at shooting stage and having 2-3 foliage leaves; 5-7 cm in length, were transferred and cultured in tubes (25x150 mm) containing 15 ml of MS basal nutrient medium supplemented with or without auxins (mg/l) as follows:

1 - MS alone (control) 2- MS + 1 NAA
3- MS + 1 IBA 4- MS + 2 NAA
5- MS + 2 IBA

All these treatments were examined in the presence and absence of the activated charcoal (3 g/l). Each treatment was replicated three times and each replicate contained one developed shoot.

The specific explants tubes were incubated at the same conditions which described in shooting stage for 16 weeks with one culture at eight weeks on fresh specific media. At the end of incubation, average of shoot length, number of leaves, number of the adventitious roots/plantlet and root length were recorded.

RESULT AND DISCUSSION

I -Starting (initiation)stage

I -Surviving and swelling values

Table1 shows that all treatments increased survival percentage compared with the control (MS alone). Shoot tips explants were superior in this respect followed by leaf primordia and axillary buds. The treatments which recorded 100% were MS + 100 2,4-D, MS + 100 NAA, MS + 50 NAA + 3 2iP, in shoot tips and leaf primordia culture as well as MS + 50 2,4-D + 3 2iP in shoot tips cultures. Those treatments produced about 100% compared with 50% in the control. The increase survival percentage of Zaghloul cv at any treatment used compared with the control (MS alone) was true in leaf primordia, shoot tips and axillary buds in descending order.

Regarding the swelling percentage, data in the same table show that it dependent on the explants used and the treatment Swelling percentage values were less than that of surviving in all explants used. However, shoot tips recorded 100% swelling with MS + 50 2,4-D + 3 2 iP treatment, whereas leaf primordia showed 100% swelling with the treatments of MS + 100 2,4-D and MS + 100 NAA. Data in Table1 showed also that 2,4-D gave positive effects on swelling compared with the effects of NAA. However, 2 iP was the more effective on increasing surviving and swelling than BA in the different explants used

I-2 Callus initiation

1

Gabr and Tisserat (1985), Khan et al. (1982), Tisserat (1979) and Woingkorw *et al .* (1991) found that 2,4-D, NAA + 2 iP gave best growth of palm callus. The importance of auxins and cytokinins was also stated by Dass *et al .* (1989).

Tisserat (1982) showed that friable yellow-white callus composed of minute nodules was initiated from leafy tissues of shoot tips and lateral buds of date palm within 2-4 months after explant introduction to nutrient media containing 100 mg/l, 2,4-D + 3 mg/l 2iP and 0.3% activated charcoal. Veramendi and Navarro (1997) detected that friable callus was produced from all types of date palm explants used except non-leaf buds when cultured on MS medium containing 453 µM 2,4-D + 14 µM 2iP + 3 g/l activated charcoal. They added that explants from shoot tips and leaf buds that possessed meristimatic areas gave the best results for callus formation and further proliferation without necrosis. Furthermore, Sharma et al. (1984) recorded that callus establishment was obtained when axillary buds and shoot tip cultured on modified MS media containing activated charcoal (0.3%), NaH2PO4 (170 mg/l), KH2PO4 (200 mg/l), 2,4-D (100 mg/1), BA (5 mg/l) and thiamin (1 mg/l). Shakib (1994) found that increasing the auxin concentration in culture media enhanced the callus production from shoot tip explants of date palm variety Estamaram. Producing being highest (55%) on MS medium with 100 mg/l 2,4-D + 3 mg/l BA + 3g/l activated charcoal.

Moreover, Omar (1988a) reported that, leaf segments of date palm explant grown on media containing 3 mg isopentenyladenin and 100 mg NAA/litter induced callus initiation. Sahavacharin and Suwanaro (1987) mentioned that date palm calli multiplied by culturing in an agar media composed of MS salts + 100 mg/l mesoinositol, 0.4 mg/l thiamine-HCl + 40 mg/l NAA + 0.4 mg/l kinetin + 30 g/l sucrose and subculturing every 8 weeks.

As for the effect of auxins, Gabr and Tisserat (1985) recorded that increasing auxins level (1-100 mg/l) caused an increase in callus production from shoot tip explant of date palm. IAA and NAA were notably less effective than 2,4-D in callus production. However, NAA was found to be more effective than IAA. Nodular callus was found to be produced after 4-6 weeks in media, continued reculture caused the original explant to become obliterated by subsequent callus development

П-Callus Growth , Development and Differentiation

Data in table Ш show that all treatments increased friable callus growth /explant compared with the control in different explants used .Shoot tips explants produced more callus growth than that of axillary buds and leaf brimordia, respectively. Using MS + 2.5 NAA + 5 2,4-D + 3 2iP gave the best results in all explants used (shoot tips, axillary buds and leaf primordia) throughout the experimental period.

The results in the same table show also that callus formation/explant was a positive correlated with the auxin types and their levels (2,4-D or NAA) as well as the present of 2 iP. In addition, it was found that friable callus growth/explant was increased with an increase in culturing period in all explants used. Similar results were reported by Gabr and Tisserat (1985) using 2,4-D and 2 iP; as well as Dass et al. (1989) with NAA and BA and Mater (1986a) using NAA.Khan *et al* . (1982)

Table 3: Effects of MS basal nutrient media supplemented with or without different growth substances (mg/l) on friable callus growth/explant cultures formed from shoot tip (S.T.) axillary buds (A.B.) and leaf primordia (L.P.) of date palm, (*Phoenix dactylifera,* L) Zaghloul Cv during different culturing period (weeks).

	Callus growth explants						Mean			
	S.T.		A.B.		L.P.					
	Weeks		Weeks		Weeks					
	8	16	8	16	8	16	S.T.	A.B	L.P.	
Ms alone (control)	1.16	1.33	1.16	1.16	1.00	1.16	1.25	1.16	1.08	1.16I
MS + 10 2,4-D	2.00	2.33	1.66	1.83	1.50	1.66	2.16	1.99	1.58	1.83g
MS + 5 2,4-D+ 2.5 2iP	2.66	3.00	2.16	2.50	1.83	2.00	2.83	2.33	1.91	2.35c
MS + 5 2,4-D+ 3 BA	2.16	2.66	1.66	1.83	1.66	1.83	2.41	1.84	1.74	1.96f
MS + 10 NAA	1.66	2.00	1.50	1.66	1.33	1.16	1.83	1.58	1.24	1.55h
MS + 5 NAA +2.5 2 iP	2.50	2.83	2.00	2.33	1.66	1.83	2.66	2.16	1.74	2.19d
MS + 5 NAA + 5 BA	2.33	2.83	1.66	2.16	1.83	2.00	2.58	1.91	1.91	2.13e
MS+2.5 NAA+52,4-D+5 BA	2.50	3.16	2.33	2.66	2.16	2.50	2.83	2.49	2.33	2.55b
MS+2.5 NAA+52,4-D+32 iP	2.83	3.33	2.50	2.83	2.33	2.50	3.08	2.66	2.41	2.72a
Mean	2.10	2.60	1.84	2.10	1.70	1.84	2.50A	2.01B	1.77C	
							Week	8	16	
								1.88B	2.18A	

Means in the same column or row having different superscripts are significantly differ at P≤0.05

Friable callus is type of callus was originated from the aggregated callus in the form of small white colonies (Mater, 1986a). Globular embryos were recognized by their smooth spherical profile and translucence (Verma and Dougall, 1977). It is an embryonic development phase before the production of mature embryos (Dodds and Roberts, 1982).

П-2 Embryogenic callus formation

Data in table IV show that repeated subculture of friable callus on MS basal nutrient media supplemented with different auxins and cytokinins on embryonic callus formation showed an increase on embryonic callus formation compared with the control throughout the experimental period.

Generally, data indicate that the embryonic callus was initiated from friable callus during the most of subculturing period. All explants used showed similar trend with the different treatment throughout the experimental period.

Table 4: Effects of MS basal nutrient media supplemented with or without different growth substances (mg/l) on embryogenic callus (somatic embryo)/explant cultures formed from shoot tips; S.T., axillary buds; A.B. and leaf primordia; L.P. of date palm (*Phoenix dactylifera,* L) Zaghloul cv during different culturing period (weeks).

Treatment mg/l	Callus growth explants						Mean			
	S.T.		A.B.		L.P.					
	Week		Week		Week					
	8	16	8	16	8	16	S.T.	A.B.	L.P.	
Ms alone (control)	1.50	1.66	1.33	1.50	1.16	1.16	1.58	1.41	1.16	1.38i
MS + 10 2,4-D	1.83	2.16	1.50	1.83	1.33	1.50	1.99	1.66	1.41	1.69g
MS + 5 2,4-D+ 2.5 2iP	2.50	3.00	2.16	2.66	1.50	1.83	2.75	2.41	1.66	2.27c
MS + 5 2,4-D+ 3 BA	2.00	2.16	1.66	1.83	1.33	1.50	2.08	1.74	1.41	1.74f
MS + 10 NAA	1.50	2.00	1.33	1.66	1.16	1.33	1.75	1.49	1.24	1.49h
MS + 5 NAA +2.5 2 iP	2.33	2.83	1.83	2.50	1.50	1.83	2.58	2.16	1.66	2.13d
MS + 5 NAA + 5 BA	2.16	2.66	2.00	2.33	1.66	2.00	2.41	2.16	1.83	2.13e
MS + 2.5 NAA + 5 2,4-D+5BA	2.83	3.33	2.16	2.66	1.83	2.00	3.08	2.41	1.91	2.46b
MS + 2.5 NAA + 5 2,4-D+3 2iP	3.16	3.66	2.50	2.83	2.00	2.33	3.41	2.66	2.16	2.74a
Mean	2.20	2.60	1.83	2.20	1.50	1.72	2.40A	2.01B	1.60C	
							Week	8	16	
								1.84B	2.17A	

Means in the same column or row having different superscripts are significantly differ at P≤0.05

The treatment of 2.5 NAA + 5 2,4-D + 3 2iP obtained the best results for all explants used (shoot tips, axillary buds and leaf primordia) throughout the differentiation of the embryonic callus (somatic embryo). Shoot tips explants, obtained the highest value for somatic embryo in all treatments compared with 1.66/explant in the control. It followed by axillary buds and leaf primordia in a descending order. MS + 2.5 NAA + 5 2,4-D + 5 BA treatment gave good results in this respect.

Data in the same table show that 2 iP and BA gave an additive effect to the auxins used on increasing somatic embryos/explant. These results are true for all explants used throughout the experimental period. Therefore, all explants grown in 2iP and BA media showed higher values than that grown in auxins media (2,4-D and NAA alone) throughout the experimental period. 2 iP was the most effective in this respect especially in the presence of auxins. Moreover, Prolonging culturing period increased somatic embryos/explant regardless the explants used.

From the obtained results, it could be concluded that the presence of auxins and cytokinins balanced in combinations gave best results for date palm in the stages of callus formation and callus differentiation. The balances between these substances are very important in these stages. MS alone gave the lowest values in this respect. The exogenous supply of the growth substances was recommended.

Sharma et al. (1980) indicated that somatic embryos may be produced on medium without growth substances while, Mater (1986b) observed somatic embryos on medium containing 0.1 NAA plus charcoal. Falcon and Marchoschi (1988) used NAA (10 mg/l) or 2,4-D (1mg/l) for producing embryogenic callus. Hormone free medium was also used by Omar and Novak (1990) who noticed that root and shoot development was stimulated in the presence of 0.45 µM NAA and 0-0.5 µM BA. Similar results were recorded by Tisserat (1979, 1981 and 1984b), Khan *et al.* (1982a), Sharma *et a*l. (1984), Gabr and Tisserat (1985), Mater (1986a), Brackpool (1986), Dass *et al .* (1989), Hervan *et al* . (1991), Shakib et al . (1994), Veramendi and Navarro (1997), Al-Khargi and Al-Maarri (1997) and Ibrahim (1999).

Callus is basically a more or less non organized tumour tissue which usually arises on wounds of differentiated tissue and organs. The initiation of callus formation is referred to as callus induction. It is possible with many different plant species to produce a callus and then to grow further on a new medium.

In exceptional conditions and sometimes spontaneously, the regeneration of adventitious organs and/or embryos can occur from a callus (Pierik, 1987). If there are only differentiated cells present in an isolated explant than de-differentiation must take place before cell division can occur paranchima cells usually undergoing this de-differentiation. If the explant already continuous meristimatic cells when isolated, this can be divided immediately without de-differentiation. De- differentiation plays a very important role enabling mature cells in an explant isolated from adult plant to be determined. In this process adult cells (temporarily) able to revert from the adult to the juvenile state (rejuvenation). This rejuvenation can have very important consequences, cells are induced to divided intensively, rejuvenated have a greater growth and division potential than adult cells, rejuvenated cells are able, under special circumstances to regenerate into organs and/or embryos. After de-differentiation the cells may begin to divide intensively under the influence of such factors as regulators present in nutrient medium, a tumor tissue is then formed when is known as a callus. Callus is principle , a non-organized and little differentiated tissue, however, differentiated tissues can be present especially in larger clump of callus tissue. Examined callus culture showed homogenous of tissues, differentiated and non-differentiated (Pierik, 1987).

Monocotyledons react differently when considering callus introduction generally being less likely to form callus tissue than dicotyledons, it was often only necessary to addd auxin as the hormonal stimulation for callus induction (Tisserat et al., 1979a and Ibrahim 1999). An exogenous supply of regulators is often recommended to initiate callus formation on an explant. The exogenous regulator requirement (type of regulator, concentration, auxin/cytokinin ratio) depends strongly on the genotype and endogenous hormone content. These requirements can in principle be split three types. Auxin only needed (especially monocotyledons).

Cytokinin alone required and both auxin and cytokinin required (Pierik, 1987) for callus formation. Callus is understood as a rapidly growing tissue which is

relatively easy to grow if plants are to be propagated vegetatively by the means of callus.

The regenerative potential should not be lost after repeated subculture. It appears that the potential of a callus to form organs and embryos can be lost (Tulecke and Mc Gramahan, 1985).

Gabr and Tisserat (1985) reported that, a dosage growth-response relationship was found between auxin concentrations and callus production from cultured embryos when subcultured on a medium devoid of auxin, the aggregate have rise to leaves or not able to development and died.

П-3 Mature somatic embryos production

Data in TableV show that all treatments increased average number of mature somatic embryo/explant of date palm Zaghloul cv during different culturing period compared with the control (MS alone). Average number of mature somatic embryo/explant was found to be high with shoot tips followed by axillary buds and leaf primordia in a descending order. Moreover, this parameter was increase with increasing culturing period from 8 to 16 weeks of embryognic callus in all treatments over all explants used. The different between number of mature somatic embryo/explant of the first culturing period (8 weeks) was found to be less than that at 16 weeks

Table 5: Effects of MS basal nutrient media supplemented with or without different growth substances (mg/l) on average number of mature somatic embryos/explant cultures formed from shoot tips (S.T.), axillary buds (A.B.) and leaf primordia (L.P.) of date palm (*Phoenix dactylifera,* L) Zaghloul cv during different culturing period (6 and 12 weeks).

Treatment mg/l	S.T.			A.B.			L.P.			Mean
	Number of mature embryo		mean	Number of mature embryo		mean	Number of mature embryo		mean	
	8	16		8	16		8	16		
Ms alone (control)	1.33	1.50	2.41	1.33	1.50	1.41	1.16	1.33	1.24	1.68i
MS + 10 2,4-D	2.00	2.33	2.17	1.66	2.00	1.83	1.50	1.66	1.58	1.86g
MS + 5 2,4-D+ 2.5 2iP	1.83	2.00	2.08	1.66	1.83	1.44	1.50	1.66	1.88	1.80h
MS + 5 2,4-D+ 3 BA	2.00	2.33	2.16	1.66	2.16	1.91	1.66	2.00	1.83	1.96f
MS + 10 NAA	2.16	3.00	2.58	1.83	2.66	2.24	1.50	2.16	1.83	2.36b
MS + 5 NAA +2.5 2iP	2.16	2.66	2.41	1.83	2.33	2.08	1.33	2.00	1.66	2.05e
MS + 5 NAA + 5 BA	2.33	2.83	2.58	2.16	2.66	2.41	2.00	2.50	2.25	2.41a
MS + 2.5 NAA + 5 2,4-D+5BA	2.00	2.5	2.25	1.83	2.33	2.08	1.66	2.16	1.91	2.08c
MS + 2.5 NAA + 5 2,4-D+3 2iP	2.33	2.83	2.58	1.66	2.16	1.91	1.50	1.83	1.66	2.05d
Means	2.02	2.44	2.23 A	1.73	2.18	1.92 B	1.55	1.90	1.76 C	2.03

Means in the same column or row having different superscripts are significantly differ at P≤0.05

The best treatment producing average number of mature somatic embryos/explant was dependent on the explants used as well as the exogenous growth substances (type, level and auxin/cytokinin ratio). In shoot tips explants, the best treatment producing mature embryos/explant was MS + 2.5 NAA + 5 2,4-D + 3 2 iP followed by MS + 5 NAA + 5 BA. However, the later treatment produced higher number of mature embryos/explant of axillary

buds and leaf primordia than the other treatments throughout the experimental period.

П-4 Shoot multiplication (Shooting).

Data in TableVI indicate that all treatments increased average of shoot numbers, shoot length and secondary mature somatic embryos overall explants used throughout the experimental period. The values were found to dependent on the growth substances used in the media and the explants. The best treatment inducing shooting number from shoot tips explant was found with MS basal nutrient media supplemented with 2 iP, whereas that with BA were preferable when using axillary buds and leaf primorida. Similar results were recorded for the average number of the secondary mature somatic embryos which developed on the basal part of the developed shoot explants; throughout the culturing period. The presence of auxins; NAA and 2,4-D, with the superiority of the former, may play a helper factor for obtaining good results and showed an additive effects in this respect. The role of hormones on induction shooting from the somatic embryogenesis appears to be directly interacting with factors including ionic currents which control cell polarity.

These results are true for all different sources of the explants used formed mature embryos. However, the highest number of shoots were produced from shoot tips explants followed by axillary buds explants and the lowest average number of shoots was recorded from leaf primordia explants.

Data also indicate that shoot number formed from different sources of the explants used as well as average number of the secondary mature somatic embryos which developed and produced on the basal part of the developed shoot explants in all treatments were increased with an increase in culturing period up to the 3rd one. Therefore, repeated subculture explants on a fresh specific medium stimulated shoot formation/explant.

The highest multiplication rate was recorded at the 2nd subcultures in MS alone (control). Less increase rate of multiplication are showed at the 3rd culturing period (TableVI). Subculturing in the presence of growth substances used encouraged the growth of axillary buds and increased shoot multiplication rate till the 3rd subculturing period comparing with those rate in the MS alone. This effect was to be dependent on the explants used. While, 2 iP encouraged rate of shoot multiplication from shoot tips explants, it was found that BA induced this parameter from axillary buds and leaf primordia.

Data in the same table show that average values of the developed shoots length is depending on the constituents of the culture media and the explants used.The best results were recorded with media MS + 1 NAA + 1 2,4-D + 20 BA in all. explants used throughout the culturing period followed by treatment MS + 1 NAA + 1 2,4-D + 20 2 iP. The highest average length value was formed from shoot tip explant recorded from shoot tips followed by axillary buds and leaf prmoridia in a descending order. However, the lowest average length was produced when used leaf primordia as an explant and grown in MS media alone ·

Table 6: Effects of MS basal nutrient media supplemented with or without different growth substances (mg/l) on average shoot numbers/explants and shoot length (cm) of the germinated embryos as well as average number of the secondary mature somatic embryos/explant cultures which developed and formed on the basal part of developed shoot explants formed from shoot tips (S.T), axillary buds (A.B.) and leaf primordia(L.P) of date palm (*Phoenix dactylifera,* L.) Zaghloul cv during different culturing period (6, 12 and 18 weeks).

Treatment mg/l	Average shoot number/explant												Mean			Average shoot number/ explant
	Explants												Week			
	S.T.				A.B.				L.P.							
	Weeks				Weeks				Weeks							
	6	12	18	Mean	6	12	18	Mean	6	12	18	Mean	6	12	18	
MS alone (control)	5	11	24	13.33	3	7	18	9.3	1	4	13	6	3	7.33	18.33	9.54h
MS + 10 2IP	10	20	35	21.66	6	15	29	16.6	3	9	20	10.66	6.33	14.66	28	16.30g
MS+ 1 2,4-D+10 2IP	11	24	37	24.00	9	20	34	21.3	7	16	27	16.66	9.00	17	32.66	19.55d
MS+ 1 NAA+10 2IP	13	27	42	27.3	10	22	38	23.3	8	17	29	18	10.33	22	36.33	22.88b
MS + 10 BA	8	21	35	21.3	7	17	30	18	4	11	21	12	6.33	16.33	28.66	17.10f
MS+1 2,4-D+ 10 BA	10	23	36	23.0	8	20	31	19	6	15	26	15.4	8	19.33	31	19.44e
MS + 1 NAA + 10 BA	11	25	39	25	9	21	33	21	8	16	28	17.33	9..33	20.66	33.33	21.11c
MS + 1 NAA + 1 2,4-D + 20 2iP	14	33	49	32	10	26	39	25	8	21	35	21.33	10.66	20.66	41	26.11a
MS + 1 NAA + 1 2,4-D + 20 BA	12	37	39	26	11	27	42	4225.6	9	22	36	22.33	10.66	28.66	39	26.1a
Mean	10.44	23.44	37.3	33.72 A	8.11	19.44	32.66	20.07B	6	14.55	26.1	15.55 C	8.18C	19.18 B	32.03 A	19.79

Treatment mg/l	Average shoot length (cm)												Mean			Average shoot number/ explant
	Explants												Week			
	S.T.				A.B.											
	Weeks				Weeks											
	6	12	18	Mean	6	12	18	Mean	6	12	18	Mean	6	12	18	
MS alone (control)	2.18	4.59	8.16	4.97	2.11	4.42	7.98	8.40	2.04	4.34	7.72	4.76	2.11	4.45	7.95	4.83i
MS + 10 2IP	2.54	4.88	8.44	5.28	2.44	4.74	8.25	5.13	2.31	4.62	8.02	4.95	2.43	4.74	8.23	5.12h
MS+ 1 2,4-D+10 2IP	2.58	5.04	8.76	5.45	2.52	4.86	8.52	5.3	2.48	4.74	8.24	5.10	2.52	4.76	8.47	5.28g
MS+ 1 NAA+10 2IP	2.69	5.14	8.92	5.80	2.62	4.72	8.72	5.38	2.60	4.78	8.64	5.30	2.63	4.88	8.76	5.42e
MS + 10 BA	2.61	5.94	8.68	5.40	2.52	4.78	8.43	5.20	2.43	4.68	8.68	5.20	2.52	4.8	8.59	5.27f
MS+1 2,4-D+ 10 BA	2.64	5.03	8.98	5.50	2.58	4.82	8.78	5.30	2.46	4.73	9.98	5.39	2.56	4.86	8.91	5.40d
MS + 1 NAA + 10 BA	2.72	5.24	9.14	5.70	2.66	5.16	8.94	5.58	2.52	4.88	9.14	5.40	2.54	5.09	9.07	5.47c
MS + 1 NAA + 1 2,4-D + 20 2iP	2.84	5.45	9.38	5.80	2.76	5.34	9.16	5.75	2.60	5.04	9.38	5.70	2.76	5.27	9.30	5.73b
MS + 1 NAA + 1 2,4-D + 20 BA	2.88	5.53	9.68	6.03	2.82	5.12	9.14	5.60	2.79	4.97	9.68	5.81	2.83	5.20	9.48	5.81a
Mean	2.63	5.1	8.90	5.52A	2.56	4.88	8.56	5.33B	2.51	4.75	8.66	5.25C	2.54C	4.86B	8.75A	5.37

Treatment mg/l	Average number of secondary mature somatic embryos/explant												Mean			Average number of secondary mature somatic embryos /explants
	Explants												Week			
	S.T.				A.B.				L.P.							
	Weeks				Weeks				Weeks							
	6	12	18	Mean	6	12	18	Mean	6	12	18	Mean	6	12	18	
MS alone (control)	1.00	1.16	1.50	1.20	1.00	1.16	1.33	1.16	1.00	1.00	1.16	1.05	1.00	1.11	1.33	1.14i
MS + 10 2IP	1.00	1.33	1.83	1.38	1.00	1.16	1.66	1.27	1.00	1.16	1.50	1.22	1.00	1.21	1.66	1.29h
MS+ 1 2,4-D+10 2IP	1.00	1.50	1.50	1.5	1.00	1.33	1.83	1.38	1.00	1.16	1.66	1.27	1.00	1.33	1.83	1.38f
MS+ 1 NAA+10 2IP	1.00	1.66	1.66	1.66	1.00	1.50	2.00	1.500	1.00	1.33	1.83	1.38	1.00	1.50	2.05	1.51d
MS + 10 BA	1.00	1.33	1.44	1.44	1.00	1.33	1.83	1.38	1.00	1.33	1.66	1.33	1.00	1.33	1.83	1.38g
MS+1 2,4-D+ 10 BA	1.00	1.50	1.66	1.66	1.00	1.33	2.16	1.49	1.00	1.33	2.00	1.44	1.00	1.38	2.22	1.53e
MS + 1 NAA + 10 BA	1.00	1.66	1.77	1.777	1.00	1.50	2.33	1.61	1.00	1.50	2.16	1.55	1.00	1.55	2.38	1.64c
MS + 1 NAA + 1 2,4-D + 20 2iP	1.00	2.00	2.05	2.05	1.00	1.66	2.66	1.77	1.00	1.50	2.33	1.61	1.00	1.72	2.71	1.81b
MS + 1 NAA + 1 2,4-D + 20 BA	1.00	1.83	1.94	1.94	1.00	1.83	2.83	1.88	1.00	1.66	2.66	1.77	1.00	1.77	2.83	1.86a
Mean	1.00	1.55	2.33	1.62B	1.00	1.42	2.07	1.50A	1.00	1.33	1.88	1.40C	1.00C	1.43B	2.09A	1.50

Means in the same column or row having different superscripts are significantly differ at P□0.05

EL-Hennawy and Wally (1980), Zaid and Tisserat (1983b), gabr and Tisserat (1985), Nasir *et al .* (1994), Al-Kharyl and Al-Maarri (1997) and El-Hammadi et al. (1999) mentioned that the capability of regeneration are necessary for plant propagation using tissue culture method. The ability to regenerate and formation of adventitious organs or embryos in vitro are determined by genotype, the environmental conditions (nutrient supply, regulators and physical conditions) and the developmental stage of the plant.

It is well known that the same families and genera have high regeneration ability. Juvenile plants have a greater regeneration capacity than adult plants. Since, adult plants are generally used for vegetative propagation, especially in the woody species, this means that an attempt should be made to rejuvenate them before use. Tisserat (1984a) recorded that production of plantlets from shoot– tips and buds by organogenesis process is less well developed technique compared to sexual embryogenesis.

Rejuvenation, by means of meristem culture, despite the difficulties associated with the method, is still the most favoured technique since it maintains genetic stability, eliminates fungi and bacteria and can sometimes result in the additional advantage of obtaining virus-free material (Pierik, 1987). Tisserat (1984 a) reported that producing plantlets of date palm through organogenesis process should be clonal and less risk of genetic variation than callus derived plantlets. Somatic embryogenesis has been carried with a high degree of success with a number of plants. For example, the date palm (Tisserat, 1979, Mater, 1986a, Dass *et al .,* 1989, Shakib *et al.,* 1994 and Ibrahim, 1999) and the oil palm (Jones, 1983, Litz *et al* ., 1984 and Black, 1983) are cloned at the moment on a large scale by callus, embryo and shoot formation.

Belal *et al*. (1993) found that shoot multiplication, starting from shoot tip explants isolated from the mother plant of two Egyptian date palm cultivars (Zaghloul and Samani) were induced by using MS medium containing high cytokinin level (30 mg/l 2iP) and low auxin level (0.1 – 1 mg/l NAA).

Belal and El-Deed (1997) recorded that using medium containing 0.5 mg/l IAA, 0.5 mg/l NAA and 10 mg/l BAP and 5 mg/l 2 iP enhanced axillary shoot formation while, using hormone free medium stimulated shoot growth.

П-5 Rooting of developmental shoots

Data in Table VП indicate that highest shoot length and number of leaves were recorded in plantlets developed from shoot tips explants, followed by axillary buds and leaf primordia. Both of IBA and NAA treatments increased these parameters in all explants and the increase was a concentration dependent, IBA was preferable in this respect. Data in the same table reveal that activated charcoal showed an additive effects to that of either NAA or IBA on increasing all parameters recorded from different explants used.

As for the effects of auxins on number of adventitious roots and root length of the plantlets formed from the different developed shoots, data in the same table show that all treatments increased all these parameters over all explants used. Plantlets formed from shoot tips explants gave high values in this respect followed by that from axillary buds and leaf

primordia in a descending order. NAA gave higher values than IBA and the increase was a concentration dependent. The highest root number and lowest root length was found in plantlets formed from shoot tips explants followed by axillary buds and leaf primordia, respectively. The presence of activated charcoal increased all these parameters over all explants used in all treatments compared with the control.

Table 7 : Effects of MS basal nutrient media supplemented with or without different auxins (mg/l) in the presence and absence of the activated charcoal (A.C.) on average of shoot length (cm), number of leaves/plantlet, number of adventitious roots/plantlet and root length (cm) of the date palm (*Phoenix dactylifera,* L.) Zaghloul cv plantlets formed from shoot tips (S.T.), axillary buds (A.B.) and leaf primordia(L.P) at the end of shooting incubation period (16 weeks).

Treatments mg/l	Explants												Means			
	S.T.				A.B.				L.P.							
	Shoot length	No. of leaves	No. of roots	Root length	Shoot length	No. of leaves	No. of roots	Root length	Shoot length	No. of leaves	No. of roots	Root length	Shoot length	No. of leaves	No. of roots	Root length
Without A.C.																
MS alone (control)	8.50	2.00	1.00	3.40	8.20	2.00	1.00	4.10	7.80	2.00	1.00	3.90	8.16	2.00	1.00	3.80
Ms + 1 NAA	10.60	2.00	2.00	6.10	10.80	2.00	2.00	5.80	8.60	2.00	2.00	5.50	10.0	2.00	2.00	5.80
MS + 2 NAA	11.20	2.00	3.00	6.30	9.10	2.00	2.00	6.10	9.00	2.00	2.00	6.40	9.76	2.00	2.33	6.26
MS + 1 IBA	10.90	2.00	1.00	9.30	9.90	2.00	1.00	8.10	8.10	2.00	1.00	8.80	9.63	2.00	1.00	8.73
MS + 2 IBA	12.30	2.00	2.00	9.20	8.30	2.00	1.00	9.60	7.60	2.00	1.00	9.80	9.4	2.00	1.33	9.53
Mean	10.16	2.00	1.80	7.04	9.26	2.00	1.40	6.74	8.22	2.00	1.40	6.88	8.82B	2.00	1.53 B	6.82B
With A.C.																
MS alone (control)	8.80	2.00	1.00	4.90	8.50	2.00	1.00	4.60	8.20	2.00	1.00	4.50	8.50	2.00	1.00	4.66
Ms + 1 NAA	11.30	2.00	3.00	6.80	11.50	2.00	2.00	9.20	9.90	2.00	2.00	6.50	10.90	2.00	2.33	7.50
MS + 2 NAA	14.30	2.00	4.00	6.10	9.60	2.00	3.00	6.10	7.20	2.00	2.00	7.80	10.36	2.00	3.00	6.66
MS + 1 IBA	11.80	2.00	2.00	9.40	9.10	2.00	2.00	10.80	7.40	2.00	2.00	11.40	9.4	2.00	2.00	10.50
MS + 2 IBA	15.20	2.00	2.00	10.20	10.20	2.00	2.00	11.30	8.50	2.00	1.00	12.60	11.3	2.00	1.66	11.36
Mean	12.28	2.00	2.40	7.48	9.78	2.00	2.00	8.40	8.24	2.00	1.60	8.56	10.09 A	2.00	1.99 A	8.18A
Total mean																
MS alone (control)	8.65	2.00	1.00	4.15	8.35	2.00	1.00	4.35	8.00	2.00	1.00	4.20	8.33 e	2.00	1.00 d	4.23 e
Ms + 1 NAA	10.95	2.00	2.50	6.45	11.15	2.00	2.00	8.85	9.25	2.00	2.00	6.00	10.45a	2.00	2.16 b	6.65c
MS + 2 NAA	12.75	2.00	3.50	6.20	9.35	2.00	2.50	6.10	8.10	2.00	2.00	7.10	10.06c	2.00	2.66 a	6.46 d
MS + 1 IBA	11.35	2.00	1.50	9.35	9.50	2.00	1.50	9.45	7.75	2.00	1.50	10.10	9.51 d	2.00	1.50 c	9.61 b
MS + 2 IBA	13.75	2.00	2.00	9.70	9.25	2.00	1.50	10.45	8.05	2.00	1.00	11.20	10.35b	2.00	1.50 c	10.44a
Mean	11.22A	2.00	2.10 A	7.16C	9.52B	2.00	1.70 B	7.84B	8.23C	2.00	1.50 C	7.72A	9.74	2.00	1.76	7.50

Means in the same column or row having different superscripts are significantly differ at P≤0.05

The positive effects of the activated charcoal was more pronounced in the presence of NAA than IBA represented by an increase in root initiation, number of strong roots as they were shorter and thicker than grown in presence of IBA (TableVΠ).

Tisserat (1982), Zaid and Tisserat (1983a), Tisserat (1984b) and Madhuri (1988) found that the best rooting results were noticed on medium containing 0.1 mg/l auxin for 8-16 weeks prior to transplanting to the soil. However, Kocher *et al .* (1989) suggested that plantlets could transfer to a modified MS medium containing 2 mg/l NAA and 2 mg/l IBA for sharpening.

On the other hand, Omar (1988a) found that incubation of clusters under 16 hrs daily exposure to 1000 LUX for 6 weeks improved their subsequent survival. He added that, when the plants reach the desired size, they are hardened of activated charcoal as compared with media without activated charcoal. In the latter, the roots were increased in number and were thicker. Nasir *et al* . (1994) used MS medium supplemented with 0.05, 1.0, 3.0 or 5.0 mg/l NAA for rooting and found that the longest roots were formed on media containing 3.5 mg/l NAA.

Tisserat (1981), Sharma *et al* . (1986) and El-Hamady *et al* . (1999) reported that, in order to improve in vitro adventitious rooting, the isolated plantlets were cultured on media containing 0.1, 1.0 and 10.0 mg/l IAA or NAA in various physical conditions. Optimum adventitious rooting and subsequent plant survival was obtained by culturing plantlets in medium containing 0.1 mg/l NAA for 8-16 weeks prior to soil transplanting (Tisserat, 1982). Similar results were obtained by (Omar, 1988a) on date palm. The normally phased for rooting cultures for date palm was 5.7 or 5.8 cm in length (Reuveni, 1972, Tisserat, 1979&1984, Sharma *et al.*, 1984, Mater, 1983&1986, Nasir *et al* . 1994 and Veramer and Navorro, 1997)

REFERENCE

Abo El-Nil, M.M. (1986). The effect of amino acids on growth of date palm callus. 2nd Syp. of Date Palm, King Faisal University, Al-Hassa, Saudia Arabia: 59-65

Al-Kharagi, J.M. and Al-Maarri, K.W. (1997). Effect of seasonal variation on the regeneration capacity of date palm. *In vitro*, 33(3): 22-26.

Ammar, S. and Benbadis, A. and Tripathi, B.K. (1987). Floral induction in date palm seedlings (*Phoenix dactylifera,* L.) var. Deglet Nour cultured *in vitro.* Canadian J. of Botany, 65(1): 137-142.

Anjarne, M. and Zaide, A. (1993). The effects of various growth regulators levels on early rooting in date palm (*Phoenix dactylifera,* L).

Belal, A.H. and El-Deeb, M.D. (1997). Direct organogenesis of the date palm (*Phoenix dactylifera,* L). Assuit J. of Agric. Sci. 28(2): 1997

Belal, A.H; A.M.Sabour; Ibrahim, I.A. and N.H. El-Barakouky (1993). Shoot tip differentiation *in vitro* for (*Phoenix dactylifera,* L). The 3rd Symp. on Date Palm, King Faisal Univ, Al-Hassa, Jan, 17-20.

Black, J. (1983). Tissue culture propagation of coconut, date palm and oil palm. In : Tissue culture of trees. (J.H. Dodds, ed). Pp, 29-50 Groom Ehlm 1td, Beckenham, Kent, UK.

Brackpool, A.; Branton, R.L. and Blacke, J. (1986). Regeneration in plants. Cell culture and somatic cell genetic of plants, 3: 1986

Calero, N; Blanc, A. and Benbais, A. (1990). The combined effects of ABP and red light on the somatic embryogenesis on the cotyledonary sheath of date palm (*Phoenix dactylifera,* L) cultured *in vitro.* Bulletin-de-la-Botanique-de-france-letters-botaniques, 137(1): 13-19.

Dass, H.C.; Kaul, R.K.; Joshi, S.P. and Bhansali, R.R. (1989). *In vitro* regeneration of date palm plantlets. Current Sci., 58(1): 22- 24.

Drira, N. (1983). Vegetative Propagation of date palm (*Phoenix dactylifera,* L) by *in vitro* culture of axillary buds and of leaves originating for them (Biblographic Citation). Ompts Rendus-des-seancs-de-l'Academie-des-Sci, III-Sci-de-la-vie., 296(22): 1077-1082.

Dodds, J.H. and Robert, L.W. (1982). Experiments in plant tissue culture. Published by the Press Syndicate of the University of Cambridge, pp 88-97.

El-Hammady, A.M. ; Wanas, F.H.; ABo-Rawash, M. and Awad A.A. (1999). *In viro* propagation of date palm. 2- Factors affecting rooting and acclimatization of *in vitro* proliferated shoots of date palm "Sewy: cv. (c.f. El-Sharabasy, 2000).

El-Hennaway, H.M. and Wally, Y.A. (1980). Vegetative propagation of date palm by expalnt culture *in vitro*. Egypt J. Hort, 7(2): 211-220.

Falcone, A.M. and Marcheschi, G.L. (1988). *In vitro* somatic embryogenesis from tissue of date palm (*Phoenix dactylifera,* L). Preliminary results. Rivista di Agricultura subtrobicalle, 82(1-2): 379-389 (c.f. Hort. Abst, 60(10), 85:62).

Gabr, M. and Tisserat, B. (1985). Propagation of plants *in vitro* with special emphasis on the date palm (*Phoenix dactylifera,* L). Sci. Hort., 25(3): 255-262. Herman, B.E. (1987). Contamination of plant tissue culture. Toward control of micropropagation. Special Issue, Agicell Rep., 9(5): 33-34.

Hervan, E.M.; Shakib, A.;Afshari, M.; Khoshkam, S. and Nazeri, S. (1991). Study of callus induction from. *In vitro* culture of different explants of date palm. Plant Breeding Abst., 63(6): 6712.

Ibrahim, A.I. (1999). Somaclonal variation during micropropagation of date palm via embryogenesis. The first international Conf.; on plant tissue culture and it's application, Cairo, Egypt, 189.

Jones, L.H. (1983). The oil palm and its clonal propagation by tissue culture.
Biologist, 30(4): 181-184.

Joseph, H.C.L. and John-Stabu, E. (1979). Effects of precursors on serially propagated *Digitalis anata* and root cultures. Phytochemistry, 18: 1913-1916.

Khan, M.A.; Khalil, M.S. and Al-Kahtani, M.S. (1982a). *In vitro* culture of different tissue of date palm (*Phoenix dactylifera,* L) offshoot. 1st Symp. on date palm, King Faisal University, Al-Hassa, Saudi Arabia 152-157.

Kocker, N.L.; Solanki, K.R. and Joshi, S.P. (1989). Microprpagation of the date palm (*Phoenix dactylifera,* L) cv Khadraw using tissue culture technique. Annals of Arid Zone, 28(1-2):137-141.

Litz, R.E. (1984). *In vitro* somatic embryogenesis from nuclear callus of monoe monoembryonic Mango. Hort. Sci., 19(5): 715-717.

Madouhuri, and Shankar, P.C. (1998). Somatic embryogenesis and plant regeneration from leaf primordia of (*Phoenix dactylifera,* L) cv Tokubi. Indian J. of Experimental Biology, 36(5): 526-529 (c.f. Hort. Abst., 68:10).

Mater, A. (1983). Plant regeneration from callus culture of (*Phoenix dactylifera,* L). Date Palm., J 2(1): 57-77.

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

Mater, A.A. (1986b). A histological study of *in vitro* propagated date Palm. Dirasat, 13 (8): 96-120.

Moore, S. and Stein, W.H. (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem., 211: 907-913.

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

Nasir, I.A.; Khan, M.A. and Butt, S.J. (1994). *In vitro* culture of date palm varieties; Estamaran and Kabkab. Seed and Plant, 8(3-4): 16-20.

Omar, M.S. (1988a). Callus initiation, a sexual embryogenesis and plant regeneration in (*Phoenix dactylifera,* L). Date Palm J., 6: 265-275.

Omar, M.S. and Novak, F.J. (1990). *In vitro* plant regeneration and ethylmethonesulphonate (EMS) uptake in somatic embryos of date palm (*Phoenix dactylifera,* L). Plant Cell Tissue and Organ Culture, 20:(3): 185-190.

Pierik, R.L.M. (1987). *In vitro* culture of higher plants, Martinus, Nijhoff Pub. Dordrecht, Boston, Lancaster, p 66-79.

Pottino, B.G. (1981). Methods in Plant Tissue Culture. Dept. of Hort. Agric. Collage, Maryland Univ., Collage, Park, Maryland, USA 8-29.

Pregl, F. (1945). Quantitative Organic Micro-Analysis. 4th Ed. J. and A. Churchill Ltd., London.

Reuveni, O. and Lilien-Kipnis (1974). Date palm. Volcani Inst. Agric. Res. Div. Sci., Publ. Pam (1960-1969), pp: 143-180 BetDagen, Israel (c.f. Al-Dawayati, Maiada, M., 1999).

Sahavacharin, O. and Suwanaro, T. (1987). Clonal propagation of date palm (*Phoenix dactylifera,* L). Umaly, R.C. Umboh-I; Halos-S; Noor, N.M.E.DS.). The application of tissue culture technique in economically important tropical trees Bogor (Indonesia). SEAMEO. Bio Trop., 88: 157-163 (c.f. El-Dawayati, Maiada, 1999).

Schroeder, C.A. (1970). Tissue culture of date shoots and seedling. Report of the 47th . Annual Date Geowers Inst., 47: 25-27.

SEAMEO.BioTrop.,88:157-163(c.f.El-Dawayati, Maiada, 1999).

Sharma, D.R.; Dawrara, S and Chowdury, J.B. (1984). Somatic embryogeneisis and plant regeneration in date palm (*Phoenix dactylifera,* L). cv Khadravi through tissue culture. Indian J. of Exp. Biol., 22: 596-598.

Sharma, D.R.; Deepak, S. and Chowduary, J.B. (1986). Regeneration of plantlets from somatic tissue of the date palm. (*Phoenix dactylifera,* L). Indian J. of Experimental Biology, 24: 763-766.

Sharma, D.R.; Kamari, R and Chowdury, J.B. (1980). *In vitro* culture of female date palm (*Phoenix dactylifera,* L). tissue. Euphytica, 29: 169-174.

Shakib, A.M.; Khoshhkam, S. and Hervan, M. (1994). Plant regeneration of date palm variety Estamaran by tissue culture. Seed and Plant, 9(3-4): 8-11.

Smith, R.H. and Murashige, T. (1970). *In vitro* development of isolated shoot apical meristem of Angiosperms. A.J. Bot., 57: 562-568.

Tisserat, B. (1979a). Tissue culture of the date palm. J. of Heredity, 70(3): 221-222.

Tisserat, B. (1979b). Propagation of date palm (*Phoenix dactylifera,* L). *in vitro* J. of Exp. Bot., 30: 1275-1283.

Tisserat, B. (1981a). Date palm tissue culture. USDA/ARS Advances in Agriculture Technology, Western Series, 17. Agric. Res. Ser. Oakland, California.

Tisserat, B. (1981b). Production of free-living palms through tissue culture. Date palm J., 1: 43-54.

Tisserat, B. (1982). Development of new tissue culture technology to aid in the cultivation crop improvement of new date palm first. 1st Symp. on Date Palm. King Faisal Univ., Saudia Arabia: 126-139.

Tisserat, B. (1984a). Propagation of date palms by shoot tip cultures. Hort. Sci., 19(2): 230-231.

Tisserat, B. (1984b). Date Palm in Sharp, W.R.; Evans, D.A.; Ammirato, P.V. and Yamado, Y. (eds). Hand Book of Plant Cell Culture, 2, Mc Millian, New York, p 505-545.

Tulecke, W. and McGranahan, G. (1985). Somatic embryogenesis and plant regeneration from cotyledons of walnut, *Juglans regia* L.. Plant Sci., 40: 57-63.

Veramendi, J. and Navarro, L. (1997). Influence of explant sucrose of adult date palm (*Phoenix dactylifera,* L) on embryogenic callus formation. J. of Hort. Sci., 75(5): 665-671.

Verma, D.C. and Dougall, D.K. (1977). Influence of carbohydrates on quantative aspect of growth and embryo formation in wild carrot suspension cultures. Plant Physiol., 59: 81-85.

Wangkaew, P.; Pienngarm, B. and Polthampitak, T. (1991). Tissue culture of date palm. Kaenkaset Khon Kaen Agric. J. 19(4): 191-200.

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

Zaid, A. (1984). *In vitro* browning of tissues and media with special emphasis to date palm cultures. A review Date palm J., 3(1): 269-275.

تـأثير بعض عوامـل النمـو علـى التركيـب وسـلوك النمـو فـى مـزارع القمـم الطرفيـة لساق نخيل البلح

محمد نصر الدين هلالى و حنان رشاد الحسينى
قسم النبات – كلية الزراعة- جامعة المنصورة

هدفت تجارب البحث إلى دراسة أثر العديد من عوامل النمو لمراحل الاكثار الدقيق بالطريقةالغير مباشرة لنبات نخيل البلح (صنف زغلول) لمزارع القمم الطرفية مقارنة بالبراعم الجانبية والبادءات الورقية وتضمنت هذه العوامل دراسة تركيب البيئة المحفزةلتكوين النبتات ونوع الجزء النباتي المأخوذ من فسائل بعمر 2-4 سنوات لنباتات ام منزرعه بمزرعه كليه الزراعه جامعه القاهرة ولقد درست مراحل تكوين الأجنة الجسدية المنتجة من الكالس الناتج عند تحفيز القمم الطرفية والبراعم الجانبية و البادءات الورقية.كما تم انبات الأجنة الجسدية المتحصل عليها حتى تكوين البادرات الصغيرة. واوضحت النتائج ان المعاملات المستخدمة ادت الي زيادة نسبة استجابة المنفصلات النباتية لتكوين الكالس مقارنة بالكنترول .وقد تفوقت القمم الطرفية في هذا الخصوص عن البادءات الورقية و تلاها فى ذلك البراعم الجانبية .

كما أدى إضافة الأكسينات والستيوكينينات إلى بيئة النمو الطبيعية المنزرعة بها الكالس الهش ، إلى تكوين الكالس الجنينى ، بدرجات متباينة ،، معتمداً على التركيز المستخدم ، والمنفصلات النباتية، وفترة التجربة0 وقداعطت القمم الطرفيه أعلي قيم لأعداد الأجنه الجسديه مقارنة بالمنفصلات النباتيه الأخري ،تحت تأثير نفس المعاملة .كما تفوقت البيئة المضاف اليها الأيزوبنتيل ادنين عن غيرها من البيئات المضاف اليها الأكسينات في جميع فترات التجربه .و يبدو أن الاتزان بينهما لعب دورا هاما في هذا الشأن ،كما وجد أن تطويل فترة التحضين زاد من معدل تكوين الأجنة الجسدية..

Table I: Effects of MS basal nutrient media supplemented with or without different growth substance (mg/l) on percentages of surviving and swelling values/explant cultures of shoot tips (S.T.) axillary buds (A.B.) and leaf primordia (L.P.) of date palm (*Phoenix dactylifera,* L) Zaghloul cv after 8 weeks of incubation period.

Treatments mg/l	Explants												mean			
	S.T.				A.B.				L.P.							
	Surviving		Swelling		Surviving		Swelling		Surviving		Swelling		Surviving		Swelling	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
MS alone (control)	3.00	50	1.00	16.66	2.00	33.33	1.00	16.66	2.00	33.33	1.00	16.66	2.33f	38.88f	1g	16.66h
MS+100 2,4-D	6.00	100	5.00	83.33	5.00	83.33	3.00	50.00	6.00	100	6.00	100.00	5.66a	94.44a	4.66a	77.77a
MS+50 2,4-D + 3 2iP	6.00	100	6.00	100	4.00	66.66	3.00	50.00	5.00	83.33	4.00	66.66	5.00b	83.33b	4.33b	72.22b
MS+50 2,4-D + 3 BA	5.00	83.33	4.00	66.66	3.00	50.0	2.00	33.33	4.00	66.66	2.00	33.33	4.00d	66.66d	2.66e	44.44f
MS+100 NAA	6.00	100	5.00	83.33	5.00	83.33	3.00	50.00	6.00	100	6.00	100.00	5.66a	94.44a	4.66a	77.77a
MS+50 NAA + 3 2iP	6.00	100	5.00	83.33	5.00	83.33	4.00	66.66	6.00	100	4.00	66.66	5.66a	94.44a	4.33b	72.22c
MS+50 NAA + 3 BA	5.00	83.33	3.00	50	4.00	66.66	3.00	50.00	4.00	66.66	3.00	50.00	4.33c	72.22c	3.00d	50.00e
MS+5 NAA + 10 2,4-D+3 BA	5.00	83.33	3.00	50	3.00	50.0	2.00	33.33	2.00	33.33	2.00	33.33	3.33e	55.55e	2.33f	38.88g
MS+5 NAA + 10 2,4-D+5 BA	5.00	83.33	5.00	83.33	4.00	66.66	3.00	50.00	3.00	50	3.00	50.00	4.00d	66.66d	3.66c	61.16d
Mean	5.22A	87.03 A	4.11A	68.5	3.89C	64.8C	2.66C	44.44	4.00B	70.37B	3.44B	57.4	4.37	74.06	3.40	62.78

Means in the same column or row having different superscripts are significantly differ at P≤0.0

Table 2: Effects of MS basal nutrient media supplemented with or without different growth substances (mg/l) on average values of initiated callus/explant cultures formed from shoot tips (S.T) axillary buds (A.B) and leaf primordial (L.P) of date palm (*Phoenix dactylifera,* L); Zaghloul cv during different culturing period (weeks).

Treatment mg/l	Explants															Mean			
	S.T . culture period (week)					A.B. Culture period (week)					L.P. culture period (week)					S.T.	A.B.	L.P.	AV.
	8	16	24	32	Mean	8	16	24	32	Mean	8	16	24	32	Mean				
MS alone (control)	1.00	1.00	1.16	1.50	1.16	1.00	1.00	1.16	1.33	1.12	1.00	1.00	1.00	1.16	1.04	1.16	1.20	1.04	1.01i
MS+100 2,4-D	1.16	1.50	2.16	2.83	1.91	1.00	1.16	1.50	2.00	1.41	1.00	1.16	1.33	1.66	1.29	1.90	1.40	1.29	1.53g
MS+50 2,4-D + 3 2iP	1.66	2.33	3.00	3.66	2.66	1.33	1.66	2.16	2.50	1.91	1.16	1.33	1.83	2.00	1.58	2.66	1.90	1.58	2.05c
MS+50 2,4-D + 3 BA	1.33	1.83	2.66	3.00	2.2	1.16	1.16	2.00	2.33	1.66	1.00	1.16	1.50	1.83	1.37	2.20	1.66	1.37	1.74d
MS+100 NAA	1.16	1.33	2.00	2.50	1.75	1.00	1.16	1.16	1.83	1.29	1.16	1.16	1.33	1.50	1.29	1.74	1.29	1.29	1.44h
MS+50 NAA + 3 2iP	1.83	2.16	3.16	3.5	2.66	1.33	1.83	2.33	2.50	2.00	1.33	1.50	1.66	2.16	1.66	2.66	2.00	1.66	2.10b
MS+50 NAA + 3 BA	1.16	1.50	2.33	2.66	1.91	1.16	1.50	1.83	2.16	1.66	1.16	1.16	1.33	1.83	1.37	1.90	1.66	1.37	1.64f
MS+5 NAA + 10 2,4-D+3 BA	1.33	1.50	2.33	2.66	1.95	1.00	1.33	1.50	2.50	1.58	1.16	1.33	1.66	1.83	1.49	1.95	1.58	1.49	1.67e
MS+5 NAA + 10 2,4-D+5 BA	1.5	2.50	3.16	3.83	2.75	1.33	1.83	2.33	2.83	2.08	1.33	1.50	1.83	2.16	1.70	2.75	2.08	1.70	1.17a
Mean	1.35	1.74	2.44	2.90	2.10	1.15	1.40	1.77	2.22	1.63	1.14	1.25	1.5	1.79	1.42	2.1A	1.63B	1.42C	
Week																8	16	24	32
																1.27	1.46	1.90	2.30
																D	C	B	A

Means in the same column or row having different superscripts are significantly differ at P≤0.05