J. Agric. Sci. Mansoura Univ., 33 (4): 2803 - 2815, 2008

MICROPROPAGATION OF EGYPTIAN DATE PALM C.V SELMY THROUGH FLORAL BUDS CULTURE.

Hegazy, A. E.
Genetic Engineering and Biotechnology Research Institute (GEBRI), Plant Biotech. Sec., Minufiya University, Sadat City, Egypt

ABSTRACT

The totally closed date palm (*Phoenix dactylifera* L) female spathe was surface sterilized by sprayed with ethyl alcohol 70 % prior to aseptic conditions transfer, then flamed. To minimize fast oxidative browning, inflorescence stalks were shortened down to 3 cm then immersed in sterilized antioxidant solution containing citric and ascorbic acids 150 mg/L each for 2 hours, prior to culture.

Modified MS medium supplemented with NOA (5 mg/L) + NAA (5 mg/L) + 2iP (1 mg/L) + BA (1 mg/L) was superior in explant survival %, callus formation %, creamy callus colour % and subsequently embryogenic callus formation % after 4 months of incubation. The modified medium (3/4 salts strength) supplemented with IBA (0.3 mg/L) and 2iP (0.5 mg/L) was recorded higher percentage values of embryos formation and the highest significant percentage values of number of embryos as well as embryos fresh weight (g) after 6 weeks of incubation. Embryos cultured on the previous medium in addition to putrescine (100 mg/L) obtained significant values of multiplication rate and growth value as well as total soluble protein and PAL activity after 3 weeks of incubation.

Individual shootlets cultured on basal MS medium (3/4 salts strength) supplemented with putrescine (100 mg/L) and IBA (0.5 mg/L) achieved the highest significant values of root formation %, number of roots and root length (cm) as well as PAL activity after 2 months of incubation.

On the other hand, using of soil culture containing compost and perlite (1:1, v/v) significantly recorded the highest survival percentage, number of leaves/plantlet and leaf length (cm) for plantlets after three months of acclimatization.

Keywords: *Phoenix dactylifera* L., embryogenesis, *in vitro*, tissue culture, inflorescence,
PAL enzyme. TDZ (thidizuron), CPA (p-chlorophenoxyacetic acid), acclimatization.

INTRODUCTION

The primary advantage of micropropagation is the rapid production of high quality, disease-free and uniform planting material. Date palm micropropagation was extensively studied via different explants material. Several workers attempted to culture shoot tip (Al-Khayri and Al-Bahrany, 2001), leaf primordial (Hegazy *et al.* 2006) and floral bud (Drira 1981 and Drira and Benbadis 1985). Inflorescences of several species have been cultured *in vitro* (Nitsh, 1963). Date palm ovules, carpel tissue, parthenogenetic endosperm, and the fruit stalk blackened within 24 hours after culturing on nutrient media, and subsequently died (Reuveni and Kipnis, 1974). Also cultures of date palm floral bud reproductive tissues and especially male anthers, usually turned brown and died after a few weeks in culture (Tisserat *et al.,* 1979). A high auxin level was speculated to be necessary to disrupt normal date palm development (Eeuwens and Blake, 1977). Tisserat and De Mason

(1980) confirmed that *in vitro* applications of auxins to media increase the frequency of visible expanded carpel's developing from supposedly date palm male flowers. Vestigial female date carpels on surviving male flowers enlarged and became quite prominent (Tisserat, 1979). White friable callus usually initiated from the floral bud strand (Tisserat *et al.,* 1979). In some cases, roots and embryoids were initiated from explants of *Cocos* inflorescences rachillae (Eeuwens, 1978) and from date palm (Tisserat, 1979). Roots have not been initiated on inflorescence rachis explants, which lack leaf or meristem tissue. Morphogenetic responses of date palm inflorescence culture were found dependent on the origin and physiological stage of the explant (Drira 1981). In addition, transfers of plantlets to greenhouse are depending primarily upon the quality and type of materials produced in the previous stages. Plantlets performance during acclimatization was determined to a large extent by the degree of autotrophy (Kozai, 1993).

The aim of this work was to study the availability of micropropagate the high quality date palm c v. Selmy through floral bud culture (fresh cultivars < 30% moisture content in their fruits), which enable to produce sufficient amount of offshoots for commercial plantations is a rather new approach.

MATERIAL AND METHODS

This work was carried out in the Plant Tissue Culture Department of the Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minufiya University during the period 2004- 2006.

In this work, explant materials were obtained from around 15 years- old tree of female date palm c. v Selmy have high quality fruits grown at, Abo Sultan, Esmalia governorate, during the winter season in February, invisible closed date palm spathe around 15 cm long, which early growing in leaf axil, were separated carefully from the mother trees without scratch and used as stock plant materials. After excision, it was immediately transferred to the laboratory. The MS basal medium (Murashige and Skooge, 1962) modified with [casein hydrolyzed (1.0 g/L), glutamine (200 mg/L), bioten (0.5 mg/L), thiamine-HCl (10 mg/L), glycine (3.0 mg/L), Ca-pantothenate (5.0 mg/L), a ascorbic acid (75 mg/L), citric acid (75 mg/L), Polyvinylpyrrolidone (1.5 g/L), adenine sulfate (80 mg/L), NaH_2PO_4. $2H_2O$ (170 mg/L) and raised KH_2PO_4 up to (220 mg/L) and sucrose up to (40 g/L)] was used.

Plant material sterilization: In a trial to avoid contamination, the totally closed spathe were surface sterilized by sprayed with ethyl alcohol 70 % prior to transfer to the hood cabinet. Once again under aseptic conditions, were sprayed with alcohol then, flamed. Spathe sheath removal was carried out using sterilized sharp knife to open and subsequently inflorescence stalks appear. To minimize fast oxidative browning, inflorescence stalks were removed from the opened spathe and shortened down to 3 cm then immersed in filtered sterilized antioxidant solution containing citric acid and ascorbic acid each at the concentration of 150 mg/L for 2 hours, prior to culture.

Exp. I: Callus formation:

Explants were cultured on modified MS medium supplemented with activated charcoal (1.5 g/L) in addition to the combination of auxin [NAA

(naphthaleneacetic acid) & NOA (naphthoxy acetic acid)] and cytokinin [BA(6-benzylaminopurine) & 2iP (isopentenyladenine) were used (mg/L) ;

Control (free hormone), NAA (10), NOA (10), {NAA: 2iP: BA, 10:1:1}, {NOA: 2iP: BA, 10:1:1} and {NOA: NAA: 2iP: BA, 5:5:1:1}. And solidified with phyto- agar (6 g/L)

Cultures were incubated in total darkness in a growth room at 25±1°C., and recultured monthly. After 2 months, cultures were exposed to a 16-h photoperiod using fluorescent tubes with a light intensity of 1500 lux for another 2 months. After 4 months, data of callus formation %, callus colour % (white or creamy) and embryogenic callus formation % were recorded.

Exp. II: Somatic embryos formation:

Embryogenic callus were cultured on modified MS medium (3/4 salt strength) supplemented with activated charcoal (1.5 g/L) in addition to auxin [IBA (indole-3-butyric acid)] in combination with different types of cytokinins [2iP, BA, kin (6- forfurilaminopurine), TDZ (thidiazuron)] were used (mg/L); control (free hormone), {IBA: 2iP, 0.3:0.5}, {IBA: Kin, 0.3:0.5}, {IBA: BA, 0.3:0.5} and {IBA: TDZ, 0.3:0.5}. Incubation in a growth room was carried out at 25±1°C with 16 h photoperiod (1500 lux). After 6 weeks, data of embryos formation %, number of embryos as well as embryos fresh weight (g) were recorded.

Exp. III: Embryos growth and development:

Healthy embryos resulted from the previous treatments were cultured on modified MS medium (3/4 salts strength) supplemented with the best concentrations {IBA: 2iP, 0.3:0.5 mg/L} in addition to putrescine at the concentration of 0, 50, 100, 150 (mg/L) were used. Cultures were incubated under the same embryos formation conditions. After 3 weeks, data of growth analysis i.e. embryos multiplication rate and embryos growth value as well as chemical analysis i.e. total soluble protein and phenylalanine ammonialyase (PAL) enzyme activity were recorded.

Exp. IV: Root formation:

Individual shootlets were cultured on basal MS medium (3/4 salts strength) supplemented with putrescine (100 mg/L) in addition to different types of auxins at the same concentration (0.5 mg/L) were used; control (free hormone), IAA, IBA, NAA and CPA (p-chlorophenoxyacetic acid) Incubation in a growth room was carried out at 25±1°C with 16 h photoperiod (3000 lux). After 2 months, data of root formation %, number of roots, root length (cm) were recorded as well as PAL enzyme activity were analyzed.

Exp. V: Acclimatization:

Plantlets produced from rooting medium were removed from the gelling media and rinsed under tap water and then the entire plantlet was completely immersed in distilled water for 2 h. Then, the plantlet roots were only immersed for 5 min in Benlate solution (0.5 %, w/v) containing 2 drops of Tween 20 as a fungicide treatment. Plantlets were individually planted with care in plastic pots (5 cm in diameter and 18 cm in length) filled with a soil mixture as follows: peat moss, peat moss and sand (1:1, v/v), peat moss and perlite (1:1, v/v) and finally compost and perlite (1:1, v/v). The plantlets were covered with transparent polyethylene sheets to raise the relative humidity around the plantlets. The potted plantlets were incubated for 25 days in

acclimatization room at 25 ± 1ºC, relative humidity of (80-90 %) and 16 h photoperiod with a light intensity of 1500 lux. Acclimatization of plantlets was achieved through removing the plastic sheets progressively longer period each day till it totally removed after 25 days from transplanting. Plantlets were fertigated with nutrient solution containing commercial fertilizer of NPK (Nitrolive, 1.0 g/L) at a ratio of 20: 20: 20. Plantlets were transferred to plastic greenhouse and were left to grow for another two months. After 3 months, all pots for each treatment were taken and the survival percentage, number of leaves/plantlet and leaf length (cm) were recorded.

Growth value: Embryos growth value of cultured explant was estimated according to the equation of **Ziv (1992).**

Chemical analysis: The colorimetric method of Folin as described by Lowry *et al.* (1951) was employed for determination of total soluble proteins. The corresponding amount of total soluble proteins was calculated from standard curve prepared from bovine serum albumin (BSA). Extraction and assay of phenylalanine ammonialyase (PAL) were done according to Lamb *et al.* (1979).

Statistical analysis: Data were statistically analyzed by one factorial randomized complete design using the SAS (1988) package. The Least Significant Differences among levels of each treatment were compared using L.S.D. test at 5%, according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Exp. I: Callus formation: The totally closed spathe were efficiently surface sterilized by sprayed with ethyl alcohol 70 % prior to transfer under aseptic conditions then, flamed (Fig. 1-A). To minimize fast oxidative browning, inflorescence stalks were shortened down to 3 cm then immersed in filtered sterilized antioxidant solution containing citric acid and ascorbic acid each at the concentration of 150 mg/L for 2 hours (Fig. 1- B) prior to culture.

Concerning the effect of auxins / cytokins addition to the, modified MS medium on explant growth and morphogenesis, regardless of the type and the concentration. The results in Table (1) and Figures (1- C) showed that addition of auxin / cytokinin was found to have significantly enormous effect on explant survival % as compared to the control medium. In this concern, Ziv (1991) stated that *in vitro* explant culturing necessitates a continuous supply of growth regulators to the culture medium. The most commonly used growth regulators to the culture medium are auxins and cytokinins supplied either singly or in combination at diverse ratios, depending on the species and the type of explant. The possibility of different hormonal receptors controlling growth and development is another new question, as well as whether different hormone types may compete for common receptor or at least operate in separate signaling pathways (Timpte *et al.*, 1995).

Table (1): Effects of some types of auxins and cytokinins on embryogenic callus formation of date palm inflorescence c.v Selmy cultured *in vitro* for 4 months.

Treatment (mg/L)		Growth characters %				
				Callus colours		
		Survival	Callus formation	White	Creamy	Embryogenic callus formation
Control (Free hormone)		11.11[b]	00.00 [c]	00.00 [b]	00.00 [b]	00.00 [b]
NAA (10)	----	100.00[a]	66.67 [ab]	100.00 [a]	00.00 [b]	00.00 [b]
	+2iP (1) + BA (1)	100.00[a]	77.78 [ab]	85.71 [a]	14.29 [b]	14.29 [b]
NOA (10)		100.00[a]	44.44 [b]	100.00 [ab]	00.00 [b]	00.00 [b]
	+2iP (1) + BA (1)	100.00[a]	88.89 [a]	75.00 [a]	25.00 [b]	25.00 [b]
NAA (5) + NOA (5) + 2iP (1) + BA (1)		100.00[a]	100.00 [a]	44.44 [b]	55.56 [a]	55.56 [a]
L. S. D. $_{0.05}$		0.12	35.32	44.21	29.55	29.55

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

Generally the obtained data showed that addition of NOA (5 mg/L), NAA (5 mg/L), 2iP (1 mg/L) and BA (1 mg/L) to the cultured medium recorded the highest percentage value of survival, callus formation as well as the highest significant value of creamy callus colour % and embryogenic callus formation % as compared with the other studied treatments (Fig 1-D). Also, from the obtained results, it could be simply noticed that addition of cytokinins to the culture medium were recorded the highest percentage values of creamy callus colour % and subsequently embryogenic callus formation %, regardless of auxin type/ concentration. Moreover, white callus colour was failed to regenerate embryogenic callus. In this regard, Jiaqiang *et al*. (2003) reported that cytokinin plays a critical role in plant growth and development by stimulating cell division and cell differentiation. Despite many years' research efforts, our current understanding of this hormone is still limited regarding both its biosynthesis and signaling. On the other hand, in carrot, Tokuji and Kuriyama (2003) reported that purine riboside, an anticytokinin, inhibited direct somatic embryogenesis, and this effect was nullified by the application of cytokinin. They added that cytokinin regulates the early stage of auxin-induced somatic embryogenesis in carrots.

Exp. II: Somatic embryos formation: The formation of somatic embryo from the embryogenic callus, could mainly affected by the type and the concentration of both auxin and cytokinin. The results of Table (2) and Figure (1- D) indicated that after 6 weeks of culture duration, modified MS medium (3/4 salt strength) supplemented with IBA (0.3 mg/L) and 2iP (0.5 mg/L) was recorded higher percentage values of embryos formation as well as the highest significant percentage values of number of embryos as well as embryos fresh

weight (g) as compared with the other studied treatments. On the other hand, using of TDZ (0.5 mg/L) as a sole source of cytokinin in combination with IBA (0.3 mg/L) significantly reduced somatic embryo formation %, number of embryos as well as embryos fresh weight (g) as compared with the other studied treatments**.**

Table (2): Effects of IBA with different types of cytokinins (0.5 mg/L) on embryos regeneration from embryogenic callus c.v selmy cultured *in vitro* for 6 weeks.

Treatment (mg/L)		Growth characters (Embryos)		
		Formation %	No.	fresh weight (g)
Control (Free hormone)		100.00 [a]	4.00 [b]	0.61 [b]
IBA (0.3) +	2iP	100.00 [a]	5.89 [a]	0.88 [a]
	kin	55.56 [b]	2.00 [c]	0.28 [c]
	BA	55.56 [b]	1.89 [c]	0.30 [c]
	TDZ	11.11 [c]	0.44 [d]	0.05 [d]
L. S. D. 0.05		34.79	1.29	0.18

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

Meanwhile, somatic embryogenesis precedes directly or indirectly after exposure of responsive explants to critical concentrations of exogenously supplied plant growth regulators during the initial culture phase (Gupta and Grob, 1995). Ammirato (1986) reported that embryo could be developed indirectly from a cell or tissue with previous callus formation. The cells from which the embryo develops are called induced-embryonic determined cells (IEDCs). On the other hand, Abo El-Nil (1980) stated that processes involved in controlling embryogenic competence are not fully understood. The expression of embryogenesis seemed to be controlled by manipulating auxin levels. High exogenous levels of auxins induced cell division and unorganized growth while low levels stimulated organization of embryoids. Nomura and Komamine (1985) suggested that auxin might have two different roles in embryogenesis. During the first phase, auxin required for the transition from single cells to embryogenic cell clusters. During the second phase, auxin is inhibitory for the development of embryogenic cell clusters to embryos. Recently, Nissen *et al.* (2003) indicated that the appearance of Glycine max L. Merr. cv Jack somatic embryos is preceded by dedifferentiation of the cotyledon during the first 2 weeks on auxin. The arrangement of the new cells into organized structures might depend on a genetically controlled balance between cell proliferation and cell death.

Exp. III: Embryos growth and development: Regarding the effects of polyamine data presented in Table (3) and Figure (1- E) indicated that, embryos cultured on modified MS medium (3/4 salt strength) supplemented with IBA (0.3 mg/L) and 2iP (0.5 mg/L) in addition to putrescine (100 mg/L) were recorded the highest significant values of growth analysis; embryos multiplication rate and embryos growth value as well as chemical analysis i.e. total soluble protein and phenylalanine ammonialyase (PAL) activity after 3

weeks of incubation as compared with the other studied treatments. Srivastava (2002) published that, polyamines (PAs) are generally recognizeed as active regulators of plant growth. They are present in all cells, and their millimolar titer is responsive to physiological effects caused by many agents, such as hormones, light, and stress, but their precise mode of action in plant growth and development is still unclear.

Table (3): Effects of polyamine on growth and development as well as (Chemical analysis) total soluble proteins and PAL activity of Selmy embryos cultured *in vitro* for 3 weeks.

Treatment (mg/L)		Growth characters		Chemical analysis	
		Embryos		Total soluble proteins (µg/g f.wt.)	PAL Activity (nkat/ g protein)
		multiplication rate	growth value		
Control		2.50 d d	3.24 d	93.21 d	36. 55 c
Putrescine	50	2.74 c c	3.77 c	94.08 c	41.90 b
	100	3.19 a a	4.88 a	96.51 a	51.85 a
	150	2.95 b b	4.19 b	95.01 b	43.62 b
L. S. D. 0.05		0.13	0.37	0.75	4.74

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

On the other hand, elevated putrescine concentration up to 150 mg/L recorded significant reversible effect in all growth characters as compared with the best treatment 100 mg/L. In this concern, Srivastava (2002) summarized that, PAs metabolism is affected by auxins, cytokinins, and gibberellins in several plant systems and that PAs are essential for many of the growth responses attributed to these hormones. The specific roles of PAs in these responses are unknown. On the other hand, Vasil and Vasil (1980) reported that it seems that auxins are required to induce embryogenesis, but this substance is not necessary and may even have an inhibitory effect on embryo development.

Exp. IV: Root formation: Regarding the effects of auxin types data presented in Table (4) and Figure (1- F and G) indicated that, individual shootlets cultured on basal MS medium (3/4 salts strength) supplemented with putrescine (100 mg/L) in addition to IBA (0.5 mg/L) were recorded the highest significant values of growth characters i.e. root formation %, number of roots, root length (cm) as well as chemical analysis i.e. PAL activity comparing with the control and the other auxin types treatments. Results are in accordance with those obtained by Srivastava (2002) who reported that, because auxin application caused a large increase in PAs content, it was suggested that auxins act through PAs to promote growth in this tissue. Similarly, IBA-induced root formation on mung bean hypocotyl cuttings was accompanied by 2 fold increase in putrescine content. Inhibition of this increase by PAs synthesis inhibitors decreased root formation.

Table (4): Effects of different types of auxins on rooting stage and (Chemical analysis) of date palm shootlets c.v Selmy cultured *in vitro* for 2 months.

Treatment (0.5 mg/L)	Growth characters			Chemical analysis
	Roots			PAL activity (nkat/ g protein)
	Formation %	No.	Length (cm)	
Control(Free hormone)	11.11 ^{c}	0.11 ^{c}	0.44 ^{c}	131.28 ^{d}
IAA	22.22 ^{be}	0.22 ^{c}	0.72 ^{c}	137.11 ^{c}
IBA	100.00 ^{a}	2.67 ^{a}	5.42 ^{a}	145.25 ^{a}
NAA	55.56 ^{b}	1.22 ^{b}	2.56 ^{b}	141.60 ^{b}
CPA	11.11 ^{c}	0.22 c	0.22 ^{c}	129.00 ^{d}

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

The decrease was reversed by the exogenous application of arginine or ornithine, (PAs synthesizer) suggesting that PAs are necessary for IBA-induced root formation. On the other hand, CPA (0.5 mg/L) was recorded significantly the lowest activity in growth characters as well as chemical analysis among all auxin types tested.

Exp. V: Acclimatization:

Data presented in Table (5) and Figure (1- H) showed that, soil mixture containing compost and perlite significantly increased plantlet survival %, number of leaves/plantlet and leaf length (cm) as compared with those produced under the other soil culture types.

The superiority of compost and perlite treatment on induction of higher plantlets survival % could be ascribed to their effects on sparring more suitable conditions for the growing roots. Perlite could hold three to four times its weight of water as well as it was most useful in increasing aeration in mixture (Hartmann *et al*., 1990 and Hegazy *et al.*, 2006).

Table (5): Effect of soil types on survival percentage, number of leaves and leaf length of Selmy plantlets cultivated *ex vitro* for 3 months.

Soil type		Growth characters		
		Survival %	Leaves	
			No.	Length (cm)
Peat moss	----	16.67 ^{b}	0.47 ^{b}	2.76 ^{b}
	+ Sand (1:1, v/v)	30.00 ^{b}	0.83 ^{b}	4.63 ^{b}
	+ Perlite (1:1, v/v)	36.67 ^{b}	1.10 b	5.93 ^{b}
Compost + Perlite (1:1, v/v)		63.33 ^{a}	2.03 ^{a}	10.43 ^{a}

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

Compost might increase the organic matter content, which in turn improved the soil physical condition in such way, increase the water holding capacity, prevented nutrients leaching and added mineral nutrients, might be a consequence of an increase in root surface area which in turn could increase water and mineral uptake from the soil. In this concern, Al-Jibouri *et al*. (1988) studied the optimal conditions for successful transfer of *in vitro* regenerated date palm plants of 7 cultivars, using 3 different potting substrates. They found that plant survival reached about 29, 76 and 88 % by using either of vermiculite

or sphagnum peat or (1:1) mixture of both, respectively. Picoli *et al*. (2001) mentioned that failure of hyperhydric plants to grow when transferred to soil may often be due to malfunctioning of the leaf rather than the poor rootability. Reasons for this leaf malfunctioning are absence of epicuticular wax, stomatal abnormalities and reduced development of palisade tissue. However, Al-Salih *et al.* (1986) suggested that, success or fail of transferred plantlets to greenhouse are dependent primarily upon the quality and type of materials produced in the previous stages of *in vitro* propagation. Moreover, Hegazy (2003) reported that soil culture represents the main source of water and nutrients for growing roots. Therefore, it must retain enough moisture, has sufficient porous so that excess water drains away, permitting adequate aeration to the roots and finally retains the nutrients in available form for plantlets to uptake. Thus, it appears that soil type consisted of compost and perlite could spare the aforementioned requirements, since perlite holds three to four times its weight of water, whereas compost may represent permanent source of available nutrients for the growing plantlets, which was reflected on the higher plantlets survival values obtained from this treatment.

Conclusion

The totally closed spathe were surface sterilized by sprayed with ethyl alcohol 70 % prior to transfer under aseptic conditions then, fired. To minimize fast oxidative browning, inflorescence stalks were shortened up to 3 cm then immersed in filtered sterilized antioxidant solution containing citric acid and ascorbic acid each at the concentration of 150 mg/L for 2 hours, prior to culture.

- Modified MS medium supplemented with NOA (5 mg/L) + NAA (5 mg/L) + 2iP (1 mg/L) + BA (1 mg/L) significantly recorded the highest percentage values in survival, callus formation %, creamy callus colour % and subsequently embryogenic callus formation % after 4 months of incubation.
- Modified MS medium (3/4 salt strength) supplemented with IBA (0.3 mg/L) and 2iP (0.5 mg/L) was recorded higher percentage values of embryos formation % as well as the highest significant percentage values of number of embryos as well as embryos fresh weight (g) as compared with the other studied treatments after 6 weeks of incubation.
- Embryos cultured on the previous medium in addition to putrescine (100 mg/L) were recorded the highest significant values of multiplication rate and growth value as well as total soluble protein and phenylalanine ammonialyase (PAL) activity after 3 weeks of incubation.
- Individual shootlets cultured on 3/4 MS basal medium supplemented with putrescine (100 mg/L) in addition to IBA (0.5 mg/L) were recorded the highest significant values of root formation %, number of roots, root length (cm) as well as PAL activity after 2 months of incubation.
- Soil mixture containing compost and perlite significantly increased plantlet survival %, number of leaves/plantlet and leaf length (cm) as compared with the other soil culture types.

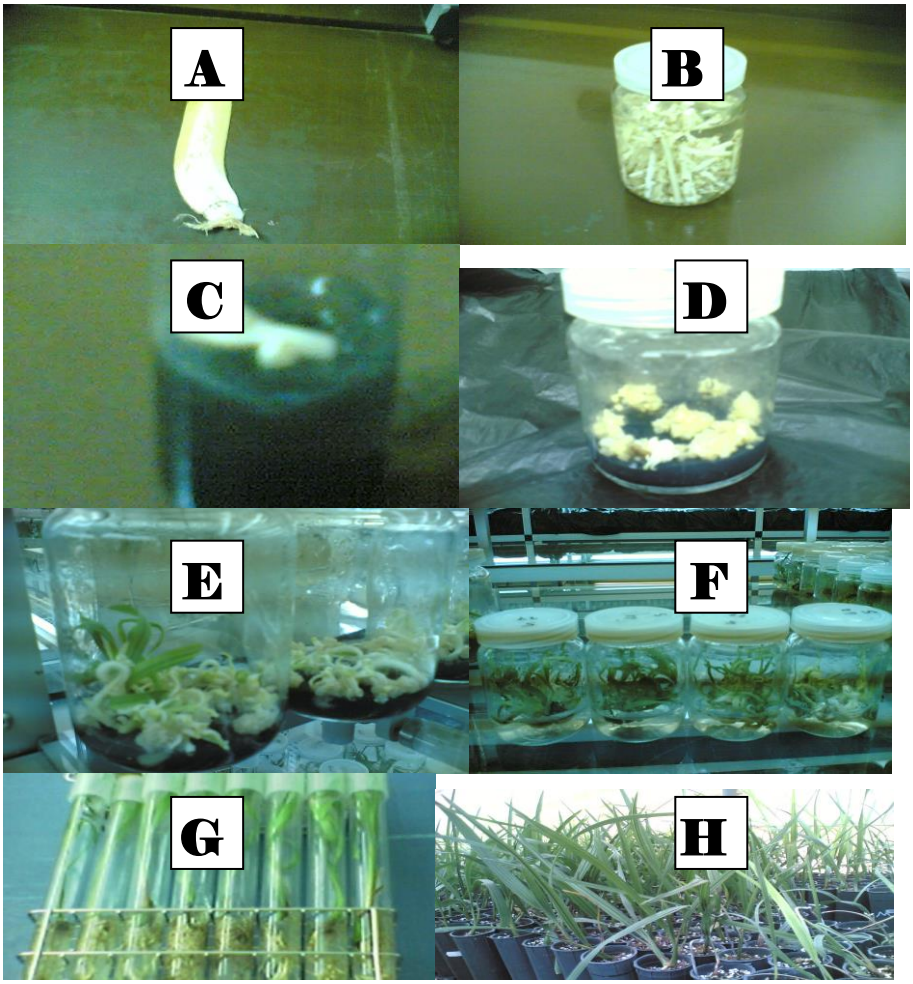

Fig (1): Micropropagation of date palm c.v Selmy through floral bud culture:-

A – Floral bud after isolation and sterilization.
B – Inflorescence stalk in antioxidant solution after spathe covers removal.
C – Explant (flowers) cultured in starting stage
D – Yellow embryogenic callus regenerated somatic embryos.
E – Repitative embryos during multiplication stage.
F – Embryos growth and development.
G –.Plantlets during rootig stage.
H – Healthy plantlets after 3 months in acclimatization.

REFERENCES

Abo El-Nil, M. M. (1980). Embryogenesis of gymnosperm forest trees, U. S. Patent 4, 217:730.

Al-Khayri, J. M. and Al-Bahrany, A. M. (2001). Silver nitrate and 2-isopentyladenine promote somatic embryogenesis in date palm (*Phoenix dactylifera* L.). Sci. Hort., 89: 4, 291-298.

Al-Jibouri, A. J. M.; Salman, R. M. and Omar, M. S. (1988). Transfer of *in vitro* regenerated date palms to the soil. Date Palm J., 6 (2):390-400.

Al-Salih, A. A.; Bader, S. M.; Jarrah, A. Z. and Al-Qadi, M. T. (1986). A comparative morphological and anatomical study of seed and embryo culture-derived seedling of *Phoenix dactylifera* L. Date Palm J., 4: 153-162.

Ammirato, P. V. (1986). In: Withers and Alderson. MacMillan, New York. 23-45.

Drira , N. (1981). Multiplication Vegetative et micropropagation du palmier dattier (*Phoenix dactylifera L*.) a partir d, organs preleves sur la phase adulte cultives *in vitro* These pour le titre de Docteur de Specialite. Faculte des Sciences de Tunis. 138.

Drira , N. and Benbadis , A. (1985). Vegetative multiplication of date palm (*Phoenix dactylifera* L.) by reversion of *in vitro* cultured female flower budy. J. Plant Physiol., 119: 227-253.

Eeuwens, C. J. (1978). Effect of organic nutrient and hormones on growth and development of tissue explants from coconut (*Cocos nucifera* L.) and date palms (*Phoenix dactylifera* L.) cultured *in virto*. Physiol. Plant., 42: 173-17

Eeuwens, C. J. and Blake, J. (1977). Culture of coconut and date palm tissues with a view to vegetative propagation. Acta horticulturae 78:277- 286.

Gupta, P, K. and Grob, J. A. (1995). Somatic Embryogenesis in Conifers. In: Somatic Embryogenesis in Woody Plants. I. (Jain, S.M.; P,K. Gupta and R. J. Newton, ed.). Kluwer Academic, The Netherlands. 81-98.

Hartmann, T. T.; Kester, D. E.; Fred, T. and Davies, J. (1990). Plant Propagation Principles And Practices. Principles of Tissue Culture for Micropropagation. Englewood Cliffs, New Jersey, 475-478.

Hegazy, A. E. (2003): Some Physiological Studies on Date Palm Micropropagation Through Direct Somatic Embryogenesis. Ph. D. Thesis. Plant Physiol. Dep. Fac. of Agri. Cairo Univ. Egypt.

Hegazy, A. E; Nesiem, M. R. A.; Ibrahim, I. A. and EL-Ghamrawy, N. K. (2006). Direct Somatic Embryos of Date Palm II- Acclimatization and Genetic Stability. The Third International Date Palm, Abu Dhabi, United Arab Emirates; 19-21. February, 39.

Jiaqiang Sun, Qi-Wen Niu, Petr Tarkowski, Binglian Zheng, Danuse Tarkowska, Göran Sandberg, Nam-Hai Chua, and Jianru Zuo (2003). The Arabidopsis AtIPT8/PGA22 Gene Encodes an Isopentenyl Transferase That Is Involved in De Novo Cytokinin Biosynthesis. Plant Physiol., 131: 167-176.

Kozai, T. (1993). Micropropagation under photoautotrophic conditions. In micropropagation. Technology and Application (Debergh P.C. and Zimmerman, R.H. eds). Kluwer Academic, Netherland, pp. 477-469.

Lamb, C. J.; Merritt, T. K. and Butt, V. S. (1979). Synthesis and removal of phenylalanine ammonia-lyase activity in illuminated discs of potato tuber parenchyme. Biochim. Biophys. Acta., 2: 196-212.

Lowry, O. H.; Rosebrough, N. J.; Forr, A. L. and Randell, R. J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem.,193, 265 - 275.

Murashige, T. and Skoog, F. A. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol., 15 : 433-479.

Nissen, T. F.; Shealy R. T.; Khanna, A. and Vodkin, O. L. (2003). Clustering of Microarray Data Reveals Transcript Patterns Associated with Somatic Embryogenesis in Soybean. Plant Physiol, 132, 118-136.

Nitsh, J. (1963). Tha *in vitro* culture of flowers and organ culture symposium:198- 214.

Nomura, K. and Komamine, A. (1985). Identification and isolation of single cells that produce somatic embryos at a high frequency in a carrot suspension culture. Plant Physiol., 79: 988.

Picoli, E. A. T.; Otoni, W. C.; Figueira, M. L.; Carolino, S. M. B.; Almeida, R. S.; Silva, E. A. M.; Carvalho, C. R. and Fontes, E. P. B. (2001). Hyperhydricity in *in vitro* eggplant regenerated plants: structural characteristics and involvement of BiP (Binding Protein). Plant Sci., 160: 5, 857-868.

Reuveni, O. and H. L. Kipnis (1974). Studies of the *in vitro* culture of date palm (*Phoenix dactylifera* L.) tissues and organs. Volcani Inst. Agric. Res. Pamphalet, 145: 3-39.

SAS (1988): Statistical analysis system SAS User's Guide: Statistics SAS Institute Inc., Cary, N. S., ed.

Sharma, D. R.; Dawra, S. and Chowdhury, J. B. (1984). Somatic embryogenesis and plant regeneration in date palm cv. Khadrawy through tissue culture. Ind. J. of Exp. Biol., 22: 596-598.

Srivastava, L. M. (2002). Plant Growth and Development: Hormones and Environment. Academic Press. An imprint of Elsevier, San Diego, California, USA, 245-248.

Steel, R. G. and Torrie, J. H. (1980). Principles and Procedures of Statistics, a Biomerical Approach. Mc Grow- Hill Book Company, New York, 469-517.

Timpte, C.; Lincoln, C.; Pickett, F. B.; Turner, J. and Estelle, M. (1995). The AXRI and AUXI genes of Arabidopsis function in separate auxin-response pathways. Plant. J. 8: 561-568.

Tisserat, B. (1979). Propagation of date palm (*Phoenix dactylifera* L.) *in vitro*. J. Exp. Bot., 30 (119): 1275-1283.

Tisserat, B. and D. De Mason (1980), « A histological study of development of asexual embryos in organ cultures of *Phoenix dactylifera* L. ». Annals of Bot, 46 : 465 - 472.

Tisserat, B.; G. Foster and D. De Mason (1979). Plantlet production *in vitro* from *P.dactylifera* L. Date Grow. Inst. 45 : 19- 23.

Tokuji, Y. and Kuriyama, K. (2003). Involvement of gibberellin and cytokinin in the formation of embryogenic cell clumps in carrot (Daucus carota). J. Plant Physiol., 160 (2): 133- 141.

Vasil, I. K, and Vasil, V. (1980). Clonal Propagation. Intl. Rev. of Cytol. Suppl., 11 A, 145-173.

Ziv , M (1991). Virtification morphological and disardors of *in vitro* plants . In : Micropropagation technology and application (eds. P.C. Deberegn and R.H . Zimmerman),

Kluwer Academic publishers Dordrecht . Boston , London. 45-69 Ziv, M. (1992): The use of growth retardants for the regulation and acclimatization of *in vitro*
plants. In: Karssen, C.M.; Van loon, L.C. and vreugdenhil, D. (eds). Progress in Plant Growth Regulation, 809-817. Inflorescence

الإكثار الدقيق لنخيل البلح المصرى صنف سلمى من خلال زراعة البراعم الزهرية

عادل السيد أحمد حجازى
معهد الهندسة الوراثية والتكنولوجيا الحيوية ـ جامعة المنوفية

تهدف الدراسة إلى إكثار نخيل البلح من خلال البراعم الزهرية وذلك عن طريق زراعة الازهار معمليا. وقد تناولت الدراسة عدة عوامل طبيعية وكيميائية خلال المراحل المختلفة لتكون الاجنة وتكاثرها ونموها معمليا.

أدى رش البرعم الزهرى المغلق تماما بكحول الايثيل ٧٠% ثم ا شعاله الى التخلص تماما من التلوث. كما أدى تقصير الشماريخ الزهرية (٣ سم) ثم تقعها لمدة ساعتين فى مضاد الاكسدة يحتوى على حمض الستريك (١٥٠ ملليجرام/لتر) وحمض الأسكوربيك (١٥٠ ملليجرام/لتر) الى التحكم الكامل فى ظاهرة التلون البنى للنسيج خلال الزراعة والتحضين.

الازهار المزروعة على بيئة موراشيجى وسكوج ١٩٦٢ المعدلة بالاضافة الى نفثوكسى حمض الخليك (٥ملليجرام/لتر)، نفثالين حمض الخليك، ايزوبنتينيل ادنين (١ ملليجرام/لتر) و بنزايل ادنين (١ ملليجرام/لتر) حققت اعلى نسبة معنوية فى البقاء حية، تكون الكالس، تكون الكالس الكريمى وتكون الكالس الجنينى بعد ٤ شهور من الزراعة.

اظهرت النتائج أن ٤/٣ املاح بيئة موراشيجى وسكوج المعدلة بالاضافة الى اندول حمض البيوتريك (٠,٣ملليجرام/لتر) وايزوبنتينيل ادنين (٠,٥ ملليجرام/لتر) قد سجلت اعلى نسية لتكون الاجنة ، واعلى نسبة معنوية فى عدد ووزن الاجنة بعد ٦ اسابيع من التحضين. وباضافة بيتريسين (١٠٠ ملليجرام/لتر) الى البيئة السابقة سجلت زيادة معنوية فى قوة النمو والتضاعف العددى للاجنة كما صاحب ذلك زيادة فى نشاط انزيم الفنيل الانين بعد ٣ اسابيع من التحضين.

أشارت النتائج أن استخدام ٤/٣ بيئة الاساس لموراشيجى وسكوج مع اندول حمض البيوتريك (٠,٥ ملليجرام/لتر) فى بيئة تحتوى على اجار (٦ جم/لتر) كان لها أكبر الأثر فى تحقيق أعلى نسبة معنوية للتجذير وعدد وطول الجذور كما صاحب ذلك زيادة فى نشاط انزيم الفنيل الانين بعد شهرين من التحضين.

كما أظهرت النتائج ان التربة المكونة من خليط من الكمبوست مع البيرليت (١:١ حجم/حجم) أعطت أعلى نسبة نجاح للنبيتات في البقاء حية وكذلك اكبر عدد وطول لاوراق النبيتات يعد ثلاثة أشهر من الأقلمة بالمقارنة بباقي التراكيب المختلفة لأنواع التربة تحت الدراسة٠