MICROPROPAGATION OF SELECTIVE OLD SEEDLING DATE PALM TREES BY USING INFLORESCENCES

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Abstract

This invistigation has been conducted through successive period from 2000 to 2004 at the Tissue Culture Laboratory, Central Laboratory for Date palm Research and Development, Agricultural Research Center, Ministry of Agriculture. Female inflorescence was used as an explant material via tissue culture technique to propagate the elite Upper Egyptian seedling date palm that did not produce new offshoots. It was recommended that, spikes of 2 - 4 cm in length gave the best results for browning %, swelling % and embryogenic callus formation %. Whereas, spikes of 12– 15 cm in length recorded the worest results. In addition, 50 g/L sucrose recorded the highest values, while, 30 g/L sucrose and 40 g/L recorded low values. Palm No. 5 showed the highest values of swelling % and embryogeric callus formation %, while palm No. 4 recorded the lowest value of browning %. Also, media composition No. 12 (Greshoff and Doy inorganic salts medium, 1972) supplemented with in mg/L, 10.0 2,4-D, 0.5 NOA and 0.3 2ip) recorded the highest value of swelling and embryogenic callus formation. MS medium with 0.1 mg/L or free hormone inhanced the number of somatic embryos. MS medium containing 0.2 mg/L BA recorded the highest leaves number and plantlet number, while 0.6 mg/L BA recorded the highest leaf length. MS medium containing 3.0 mg/L NAA or IBA in the presence of 3.0 g/L activated charcoal showed the highest rooting percentage and root number, while 1.0 mg/L NAA or IBA recorded the highest root length. The highest value of survival percentage, leaves number and plant length was obtained after 3 months of acclimatization.

INTRODUTION

Micropropagation of date palm (*Phoenix dactylifera* L.) has been subjected by numerous studies. Also, many researches had employed, through this technique, different types of explants, i.e., shoot tip, leaf primordia, axillary bud... etc. from small shoots (Tisserat, 1982 & 84, Al Salih *et al.*, 1986, Mater, 1986, Aljibouri *et al.*, 1988, Quraishi *et al.*, 1997, Zaid, (1999, 2003). In addition, inflorescences as starting material has been rarely described (Tisserat, 1981 and Bhaskaran and Smith, 1992 & 1995).

Immature floral buds showed different responses depending on the physiological stage of inflorescences, that lengthed 3.5 cm to mature (Tisserat, 1981). In early stage of inflorescence development, some floral initials produced callus from the carpels which produced

subsequently embryogenic callus likely embryogenic callus produced from shoot tip (Bhaskaran and Smith, 1992). Then, white fariable callus having granular appearance was able to develop into somatic embryos which in turn developed into complete and separated plantlets (Tisserat, 1984, Drira and Benbadis, 1985, Bhaskaran and Smith, 1995, Loutfi and Chlyah, 1998, Abhaman, 1999 and Abo El-Soaud, 2003).

Abhaman (1999) and Abo El-Soaud (2003) found that best method of sterilization of inflorescences was 0.1 % mercuric chloride for 5 minutes contniud by 20.0 % sodium hypochlorite for 10 minutes.

MS salt medium (1962) and Greshoff and Doy salt medium (1972) were the most uses for induce inflorescence callus (Bhaskaran and Smith, 1995, Loutfi and Chlyah, 1998, Abhaman, 1999, and Abo El-Soaud, 2003). Also, sucrose was used in nutrient medium with concenterations from 30 to 50 g/L (Al-Dawayati, 2000 and Abo El-Soaud, 2003).

Incubation under dark conditions for 3-6 months at 25±2 °C induced callus formation, and dialy exposure to light intensity of 2000-9000 lux illumination induced the development of embryos to plantlets with good root system under. (El-Kosary, 1998, 2004, Al-Dawayati, 2000 and Abo El-Soaud, 2003).

Quaraish *et al.* (1997), El Sharabasy (2000), Abo El-Soaud *et al.* (2002) and Gadalla (2003) found that plantlets that having 2-3 leaves with 10 cm in length which transplanted in acclimitization stage onto soil mixture of peat and vermiculite (1:1 v/v) were suitable for the best survival percentage and obtaining free living date palm seedling plants.

The aim of this work was trying to find a new way to propagate the elite Upper Egyptian date palm that did not produce new offshoots through using inflorescence as an explant material *via* tissue culture technique.

MATERIALS AND METHODS

This investigation was carried out through successive period from 2000 to 2004 at Pomology department, Faculty of Agriculture, Cairo Unviersity Giza, Egypt, and The experimental work was performed at the Tissue Culture Laboratory, Central Laboratory for Date palm Research and Development.

1-Establishment stage:

1-1-Plant material:

The clonal material of date palm (*Phoenix dactylifira* L.) was obtained from old female seedling date palm trees grown in Assuit Governorate. The used date palm trees are commonly known as a superior ones that characterized by good fruit quality and yield but did not produce new offshoots (Metwaly, 1999).

1-2- Preparation of explants:

The mature leaves (fronds) were removed acropetally with tapestry knife. When the first inflorescence was appeared, it was carefully removed with its protective sheath (spathe) intact and placed immediately in an antioxidant solution (150 mg/L citric acid and 100 mg/L ascorbic acid) over night to avoid tissues browning (Loutfi and Chlyah, 1998). Inflorescences thereafter were washed by soap under running tap water for 30 minutes and sterilized with mercuric chloride ($HgCl_2$) at 0.1% for 10 min, under asepict conditions and thoroughly washed with sterilized distilled water for one time then sterilized with 20% Clorox (5.25% sodium hypochlorite NaOCl) with two drops of Tween-20, for 10 min then washed three times with sterilized distilled water. Secondly, inflorescence was removed from the sheath and the spikelets were cut into 1.5-2.5 cm long pieces or completely intact cultured if the spikelets are less than 2.5 cm in length. All explants were cultured into different treatments through starting experiments. Inflorescences were divided into three physiological growth stages on base of inflorescence length in cm as follows:

1- Inflorescence length 9-10 cm with spike length 2-4 cm.

2- Inflorescence length 16-17 cm with spike length 6-8 cm.

3- Inflorescence length 28-30 cm. with spike length 12-15 cm.

Explants which employed in this investigation and cultured upon the various media types were spike segments of 1.5 - 2.5 cm.

1-3-Inflorescence age:

A morphological and physiological studies were performed to define the optimal inflorescence length and its spathe which employed in this investigation (photo. No. 1).

1- 4-Media preparation:

The basal nutrient medium throughout this stage contained Greshoff and Doy inorganic salts medium (1972) supplemented with (in mg/L): 200 glutamine, 0.5 pyridoxine-HCl, 0.5 thiamine-HCl, 0.5 nicotinc acid, 100 myo-inositol, 100 L-cysteine, 6000 agar, 1500 activated charcoal and 3 concentrations of sucrose (30000, 40000 and 50000).

1- 5- Plant growth regulators:

2,4-dichlorophenoxyacetic acid (2,4-D) and naphthoxy acetic acid (NOA) as auxins and 2-iso pentenyladenine (2ip) and 6-benzyladenine (BA) as cytokinins were used as shown in Table (1).

Table 1. The combinations between auxins and cytokinins used in establishment.

Media number	Auxin		Cytokinin	
	2,4-D	NOA	2ip	BA
1	0.0	0.0	0.0	0.0
2	2.5	0.5	0.1	0.2
3	2.5	0.5	0.2	0.1
4	2.5	0.5	0.3	0.0
5	2.5	0.5	0.0	0.3
6	5.0	0.5	0.1	0.2
7	5.0	0.5	0.2	0.1
8	5.0	0.5	0.3	0.0
9	5.0	0.5	0.0	0.3
10	10.0	0.5	0.1	0.2
11	10.0	0.5	0.2	0.1
12	10.0	0.5	0.3	0.0
13	10.0	0.5	0.0	0.3

1- 6- Sucrose concentrations:

Effect of sucrose concentrations on growth and development of date palm inflorescence explant were investigated. Various sucrose concentrations (30, 40 and 50 g/L) were employed.

1- 7- culture conditions:

Each treatment cotained 15 replicates, and each replicate (one jar) contains 3 explants. In vitro cultures was incubated under darkness in a temperature controlled room at 27±2°C. Data collection and reculturing were performed every 6 weeks. during these stages, different observations were recorded through 3 subcultures (18 weeks): browning percentage, swelling percentage and embryogenic callus formation percentage (photo. No. 2).

Data were scored visually according to Pottino (1981) as follows:

Negative results (-) = 0

Bellow average results (+) = 1

Average results (++) = 2

Good results (+++) =3

Very good results (++++) =4

2- Proliferation stage:

Indirect somatic embryoids which initiated from initial flowers at early stage were instructed into proliferation, differentiation and enlargement stages during steps as follow, small masses of white fariable embryoginc callus tissues were transferred from the establishment media to proliferation and differentiation media which contained Murashige and Skoog (MS) inorganic salts (1962) supplemented with (in mg/L): 40 adenine sulphate-$2H_2O$, 170 NaH_2PO_4, 200 KH_2PO_4-$2H_2O$, 100 myo-inositol, 0.5 nicotinic acid, 0.5 pyridoxine-HCl, 1.0 thiamine-HCl, 2.0 Glycine, 6000 agar, 1500 activated charcoal (A.C.) and 30000 sucrose. For embryos formation (photo. No. 3), in mg/L, M1: free hormone, M2: 0.1 NAA, M3: 0.1 BA + 0.1 NAA, M4: 0.1 2ip + 0.1 NAA, M5: 0.05 BA + 0.1 NAA, M6: 0.05 2ip + 0.1 NAA, M7: 0.2 2ip. For embryos

development, 2ip, BA and Kin were the cytokinins used in concentraions in mg/L, 0.0, 0.2, 0.4, 0.6 and 0.8, pH was adjusted at 5.7 ± 0.1. Media were dispensed onto small culture vessels (small jar 150 ml) in aliquots of 35 ml per jar and were capped with polypropylene closures. Media were then autoclaved for 20 minutes at 1.5 kg/cm^2 pressure and 121°C. All cultures were incubated in growth room at 25 ± 1 °C under 16 hours daily exposured to low light intensity of 1000 lux illumination. Then, individually germinating embryos were picked up when they reached approximately 1.0 cm. in length. This growth stage was performed when germination of embryos was exhausted, where, several morphological forms of embryos were initiated because of their different potentials for regeneration of embryos. The next step, individual embryos were transferred to the same media composition to germinate and develop shoots and, may be, also, secondary embryos produced. Cultures were incubated in growth room at 27 ± 2 °C under 16 hours daily exposure to light intensity of 2000 lux illumination.

In this stage: multiplication of embryo cultures, regenerative types (secondary embryos and shoot formation) and complete individual plantlets (5-10 cm in length) were obtained.

Thereafter, regenerative embryos were employed for proliferation stage, and the individual plantlets were employed for enlargement stage. Clusters composed of 3-5 shoots (5-10 cm in length) were selected and separated from proliferation cultures and then cultured in large Jars (350 ml) as shown in photo. No. 4. One cluster was cultured onto medium and all cultures were incubated in growth room under 16 hours daily exposure to light intensity of 2000 lux illumination at 27 ± 2 °C (Abo-El-Soaud, 1999 and Zaid 2003).

At the end of proliferation stage, plantlets were transferred to MS basel medium (Free hormone) with activated charcoal 1.5 g/L as enlargement medium for two subcultures.

3- Rooting of developed plantlets:

Date palm plantlets at length 7-10 cm derived from proliferation stage with initial primary roots about 1.0 cm in length were cultured on ¾ MS (salt strength) supplemented with (in mg/L): 3000.0 A.C., 1.0 GA3, 2.0 Ca-panthothianic acid, 2.0 glycine, 0.4 thiamine –HCl, 0.5 nicotinic acid, 0.5 pyridoxine-HCl, 100 myo-inositol, 200 glutamine, 40000 sucrose, 6000 agar and auxins NAA or IBA, in concentraions, 1.0, 2.0 and 3.0.

The plantlets were cultured in test tubes (25 x 150 mm), each tube contained 15 ml of nutrient medium and one plantlet was cultured in each tube. Tubes were plugged with polypropylene lid. All culture tubes were incubated in the growth room for 6 weeks under 27 ± 2 °C and 16 hrs. illumination of 6000 lux. Plantlets were recultured on the same medium for another 6 weeks under the same incubated conditions. Then, plantlets were transferred to large tubes (25x250 mm) containing 25 ml of the second rooting medium consists of ¾ MS medium supplemented with (in mg/L): 3.0 NAA or 3.0 IBA, 2 Ca-panthothianic acid, 0.4 thiamine-HCl, 2.0 glycine, 0.5 nicotinic acid, 0.5 pyridoxine-HCl, 100 myo-inositol, 200 glutamine, 3000 A.C., 40000 sucrose and 6000 agar. Plantlets of each treatment were transferred and repeatedly

recultured for 2 recultures every 6 weeks into fresh medium of the same composition (photo. No. 5).

The date palm plantlets were rinsed with sterile distilled water to remove excess adherent media, under aseptic conditions and then transferred to the third rooting medium, consists of ¾ MS basal liquid medium without growth regulators and sugars supplemented with (in mg/L): 0.4 hiamine-HCL, 0.5 nicotinic acid, 0.5 pyridoxine-HCl, 2.0 glycine and 100 myo-inositol. Each tube (25 x250 mm) contained 25 ml and tubes were covered with aluminum foil caps. The cultures were incubated in the growth room under the same conditions for 4 weeks.

4- Preacclimatization of date palm plantlets:

The plantlets lengthed 10-12 cm with distinct tap root and 2-3 leaves produced from the previous stage were treated with preacclimatization process as follows: plantlets were transferred to medium consists of vermiculite and ¾ MS basal liquid medium without growth regulators and sugars under aseptic conditions. Each plantlet was cultured in one tube (25 x 250 mm) contained 30 ml of media and tubes were covered with aluminum foil caps. The cultures were incubated in growth room under 16 hrs. illumination of 9000 lux and 27 ± 2 °C for 2 weeks.

Then plantlets were transferred in the same tubes and media to the greenhouse under control conditions, where, the ventilation process was done in stages, first after one week by punching holes in the aluminum cap (one hole each one day for a week, followed by complete removal for a week).

5- Acclimatization stage:

The date palm plantlets produced from preacclimatization process were transferred exvivo to a mixture of peat moss: vermiculite: sand as 1 :1 :1 (v/v) before planting. Plants were rinsed thoroughly with tap water, and then imersed in 0.5% Topsin-M 70% WP fungicide solution for 5 min., plants were transplanted individually in plastic pots (5x18 cm) and watered a quarter strength MS solution with high humidity (90-95%) just for two weeks. the pots were embedded in sand bath and covered with a plastic bell (transparent tent for two weeks with exposure the plants to normal conditions in greenhouse). The plants were watered every other day with distilled water and once a week with quarter strength MS solution during the first two months of development. After two weeks, the transparent cover was removed and after two months the plants were treated as date palm seedlings (photo.No. 6).

RESULTS AND DISCUSSION

Browning percentage:

Data presented in Table (2) and Fig (1) indicate that, browning percentage was affected significantly by different date palm trees, spikes length, sucrose concentrations and different

media composition. Palm No. 4 showed the lowest value of browning (44.25%), while palm No.1 had the highest value of browning (45.55%). In addition, spikes 2-4 cm in length recorded the lowest value of browning (12.46%), while, spikes 12-15 cm in length showed the highest value of browning (83.77%).

Regarding to sucrose concentration, 30 g/L reduced the value of browning (40.65%) incidence in all cultures. In respect to media composition, medium No. 4 gave the lowest value of browning (34.60%) followed by medium No. 3 (36.17%), medium No. 2 (40.20%), medium No. 7 (42.90%), medium No. 8 (43.86%), medium No. 5 (43.99%), medium No. 12 (45.13%), medium No. 9 (46.56%), medium No. 1 (46.72%), medium No. 11 (48.88%), medium No. 6 (49.99%), medium No. 10 (50.36%) and medium No. 13 (55.16%).

Regarding the interaction between different date palm trees, spikes length, sucrose concentrations and different media composition, spikes of 2-4 cm in length drived from all palm trees with 30 g/L sucrose on medium composition No. 1, 2, 3 and 4 recorded the best cultures browning free, while spikes 12-15 cm in length drived from all palm trees with 40 and 50 g/L sucrose on medium No. 6 and 13 recorded the highest value of browning (100.0%).

These results are in harmony with many workers who showed that browning was noticed in late of growth stage of date palm inflorescence explants where browning considered the most important obstacle in the *in vitro* – palm establishment that interferes with the absorption of nutrients from ambient medium (Reuveni et al., 1972, Drira and Benbadis, 1985, Bhaskaran and Smith, 1992,95, Drira and Al-Shaary, 1993, Loutfi and Chlyah, 1998, Abhaman, 1999 and Abo El-Soaud, 2003).

Swelling percentage:

Data presented in Table (3) and Fig (2) indicate that, swelling percentage was affected significantly by different date palm trees, spikes length, sucrose concentrations and different media composition. Palm No. 5 recorded the highest value of swelling (67.15%), while palm No.4 showed the lowest value of swelling (47.18%). In addition, spikes of 2-4 cm in length appeared the highest value of swelling (70.77%), while spikes of 12-15 cm in length gave the lowest value of swelling (41.30%).

Regarding to sucrose concentration, 50 g/L induced explant swelling (59.21%), comparing with 40 g/L (53.47%), while 30 g/L sucrose gave 54.57% of explant swelling. In respect to media composition, medium No. 12 promoted the highest explant swelling (73.52%), followed by medium No. 11 (67.92%), medium No. 10 (63.36%), medium No. 13 (62.26%), medium No. 7 (59.19%), medium No. 8 (57.73%), medium No. 9 (57.72%), medium No. 4 (57.21%), medium No. 6 (54.80%), medium No. 3 (49.86%), medium No. 2 (49.25%), medium No. 5 (48.44%), whereas, medium No. 1 gave the lowest value of swelling (23.64%).

Regarding the interaction between different date palm trees, spikes length, sucrose concentrations and different media composition, spikes of 2-4 cm in length from palm No. 5 and

6 with 50 g/L sucrose on medium No. 12 recorded the highest value of swelling (99.47%). Also, spikes of 6-8 cm in length from palm No. 5 and 6 with 50 g/L sucrose on medium No. 12 recorded the swelling percentage 90.07%, whereas, spikes of 12-15 cm in length from palm No. 5 with 30 g/L sucrose on medium No. 12 recorded swelling percentage 72.80%.

These results are in accordance with those found by Drira and Benbadis (1985), Bhaskaran and Smith (1992,95), Loutfi and Chlyah (1998), Abhaman (1999) and Abo El-Soaud (2003) who reported that swelling percentage is paramount factor towards early indication in growth and development of the culture explants on the culture media which contained the optimal hormonal combination from auxin (0.1-10.0 mg/L) and cytokinin (0.1-1.0 mg/L).

Embryogenic callus formation percentage:

Data presented in Table (4) and Fig (3) indicate that embryogenic callus formation percentage was affected significantly by different date palm trees, spikes length, sucrose concentrations and different media composition. Palm No. 5 developed the highest value of embryogenic callus formation (37.48%), while palm No. 2 showed the lowest value of embryogenic callus formation (25.23%). In addition, spikes 2-4 cm in length recorded the highest value of embryogenic callus formation (59.09%), whereas, spikes 12-15 cm in length showed the lowest value (9.49%).

Regarding to sucrose concentrations, 50 g/L increased the value of embryogenic callus formation (45.74%), while 30 g/L reduced its value (19.71%). In respect to media composition, medium No. 12 recorded the highest value of embryogenic callus formation (49.91%), followed by medium No.11 (42.58%), medium No. 13 (38.01%), medium No. 10 (37.68%), medium No. 4 (35.40%), medium No. 8 (34.35%), medium No. 3 (33.05%), medium No. 7 (28.86%), medium No. 2 (28.22%), medium No. 9 (23.27%), medium No. 5 (23.15%), medium No. 6 (19.94%), while medium No. 1 recorded the lowest value of embryogenic callus formation (3.65%).

Regarding the interaction between palm trees, spikes length, sucrose concentrations and media composition, spikes of 2-4 cm in length derived from all palm trees at 50 g/L sucrose on media composition Nos. 11,12 and 13 showed the highest value of embryogenic callus formation (100.0%), whereas, spikes of 12-15 cm in length derived from palm Nos. 1,2,3,5 and 6 at 30 g/L sucrose on media Nos. 1, 2, 3, 4, 5, 6, 9, 10, 12 and 13 recorded the lowest value of embryogenic callus formation (0.0%) .

These results are in agreement with Tisserat (1984), Drira and Benbadis (1985), Drira and El-Shaary (1993), Bhaskaran and Smith (1995), Loutfi and Chlyah (1998), Abhaman (1999) and Abo El-Soaud (2003) who reported that physiological stage of inflorescences affected callus formation, in early stage, some floral buds produced callus from the carpels which produced embryogenic callus characterized with white fariable callus having a granular appearance.

Embryos formation stage:

It was clearly obtained that MS medium supplemented with 0.1 mg/L NAA enhanced the number of embryos (7.33), followed by MS free hormone which recorded 6.67 embryos, MS + 0.05 mg/L BA + 0.1 mg/L NAA (4.67), MS + 0.1 mg/L 2ip + 0.1 mg/L NAA (4.00) and MS + 0.05 mg/L 2ip + 0.1 mg/L NAA (4.00), while MS medium supplemented with 0.1 mg/L BA and 0.1 mg/L NAA recorded the lowest number of embryos (3.67). Data not tabulated and the results were significant at 5% level L.S.D.

The obvious results are in parallel with many workers who reported that, white fariable callus which had a granular appearance can develop into somatic embryos when cultured upon MS medium free hormone or MS medium supplemented with 0.1 mg/L NAA. (Tisserat, 1981, 84, Drira and El-Shaary, 1993, Loutfi and Chlyah, 1998, El-Kosary, 1998, 2004, Abo El-Soaud, 2003, Gadalla, 2003 and Abd El-Sattar, 2005).

Proliferation stage:

Leaves number and leaf length were affected significantly by different types and concentrations of cytokinins (Table 5). In addition, BA recorded the highest leaves number (2.01), while Kin recorded the lowest leaves number (1.79) and 2ip recorded intermediate value (1.82). In respect to concentration of different cytokinins used, 0.4 mg/L recorded the highest number of leaves (2.10), followed by 0.2 mg/L (2.09), 0.6 mg/L (2.00), 0.8 mg/L (1.94) and MS free cytokinin (1.23), respectively.

In respect to interaction between cytokinin type and concentrations used, BA at 0.2 and 0.4 mg/L recorded the highest value of leaves number (2.54 and 2.24, respectively). Also, 0.4 mg/L Kin and 0.8 mg/L 2ip gave intermediate values in this regard (2.13 and 2.12, respectively), while control (free cytokinin) recorded the lowest value of leaves number (1.23).

Regarding to leaf length as shown in Table (5), BA recorded the highest leaf length (5.36 cm), while 2ip gave the lowest value (3.74 cm). In respect to different concentrations of cytokinin used, 0.4 mg/L gave the highest leaf length (5.42 cm) comparing with control (2.56 cm). Regarding to the interaction between cytokinin type and concentrations, BA at 0.6 mg/L recorded the highest value of leaf length (6.69 cm) followed by 0.8 mg/L Kin (5.39 cm) and 0.2 mg/L 2ip (4.80 cm).

Regarding to plantlets number, Table (5) shows that, BA recorded the highest number of plantlets (2.73), followed by 2ip and Kin (2.54 and 2.53, respectively). In respect to, cytokinin concentration, 0.2 mg/L recorded the highest value of plantlets number (2.84) followed by, 0.6 mg/L (2.79), 0.4 mg/L (2.77), 0.8 mg/L (2.72), while control (free cytokinin) recorded the lowest value of plantlets number (1.87). In respect to the interaction between cytokinin type and different concentrations used, BA at 0.2 mg/L recorded the highest value of plantlets number(3.35), whereas, 0.8 mg/L 2ip and 0.4 mg/L Kin recorded 2.92 and 2.88 plantlets, respectively.

These results are in harmony with Saker *et al.* (1998), Wong *et al.* (1999), El Sharabasy (2000), Gadalla (2003), Zaid (2003), El Kosary (2004), Abd El-Sattar (2005) and Zein El-Den (2005) who reported that shoots increased with increasing subculture number, also, using high levels of growth regulators produced malformed shoots and the growth was greatly inhibted. They also recorded that individual young plantlets when transferred to MS media containing 0.01 mg/L BA to improved the shoot system.

Rooting stage:

Data presented in Table (6) show that, rooting percentage, root number and root length were affected significantly by auxin type, auxin concentration and the interaction between auxin and concentration. In addition, using NAA improved root percentage (80.83%), root number (3.83) and root length (2.93 cm), whereas, using IBA recorded (79.58%, 2.70 and 2.75 cm, respectively) but without significant differences in root percentage and root length. Concerning different concentrations used from two auxins, 3.0 mg/L recorded the highest root percentage (100%) and root number (5.17), while 1.0 mg/L recorded the highest root length (3.93 cm) than all concentrations used.

The interaction between auxin type and its concentrations, 1.0 mg/L NAA and 2.0 mg/L IBA was the best treatments to improve the root percentage (100%) and root length (4.48 cm with NAA and 4.12 cm with IBA). Also, 2.0 mg/L NAA and 3.0 mg/L IBA was the best treatment to induce root number (5.00 and 5.33, respectively).

These results are in agreement with Nasir *et al.* (1994), El Sharabasy (2000), Gadalla (2003) and El-Kosary (2004) who found that, rooting percentage, root number and root length seemed to increase when plantlets cultured upon MS media containing NAA or IBA in concentrations between 1.0 and 3.0 mg/L in presence the of 1.0 - 3.0 g/L A.C.

Acclimatization stage:

Data presented in Table (7) indicate that survival percentage, leaves number, leaf length and plant length were affected significantly by acclimatization period, preacclimatization treatment and media composition.

Regarding to acclimatization period, survival percentage recorded the highest percentage (80.0%), while leaves number, leaf length and plant length recorded the lowest values (2.35, 15.32 cm and 16.66 cm, respectively), whereas, survival percentage reduced and recorded the lowest value after 3 months of acclimatization (35.0%), while leaves number, leaf length and plant length showed the highest values (2.85, 19.87 cm and 22.27 cm, respectively).

Concerning preacclimatization, treatment with preacclimatization showed the highest values of survival percentage, leaves number, leaf length and plant length (60.0%, 2.57, 18.33 cm and 20.35, respectively), comparing with treatment without preacclimatization (46.67%, 2.44, 16.40 cm, 17.92 cm, respectively), without significant differences for survival percentage and leaves number.

Regarding to media (soil mixture), using medium no.1 [soil mixture of peat : sand : vermiculite (1 : 1 : 1, v/v)] gave the highest value of survival percentage, leaves number, leaf length and plant length (80.0%, 2.77, 18.21 cm and 19.96 cm, respectively), while using medium no.2 [soil mixture of peat : sand (1 : 1, v/v)] showed the lowest values (26.66%, 2.23, 16.51 cm and 18.30 cm, respectively).

Concerning the interaction between acclimatization period, preacclimatization treatment and media composition, it was clearly noticed that the highest value of leaves number, leaf length and plant length and high value of survival percentage (3.40, 23.86 cm, 26.36 cm and 80.0%, respectively) was obtained after 3 months of acclimatization within plantlets treated with preacclimatization on medium No.1.

These results are in agreement with many investigators who showed evidence that, plantlets characterized with several expended photosynthetic leaves that were at least 10 cm with a well developed root system composed of adventitious roots gave the best survival ratio. Also, mixture of peat and vermiculite (1 : 1, v/v) was suitable for obtaining free living date palm plants (Sharma *et al.,* 1990, Shakib *et al.,* 1994, Quaraish *et al.,* 1997, Madhuri *et al.,* 1998, Sharon and Shankar, 1999, El-Sharabasy, 2000, Abo El-Soaud, 2003 and Gadalla, 2003).

CONCLUSION

From the present investigation, it could be concluded that, inflorescences in early stage of growth and development can be used as a starting material *via* tissue culture techniqe to propagate the ellite upper Egyptian seedling date palms which did not produce new offshoots, whereas, it is not preferable to use an inflorescence as a disirable source for explant in date palm micropropagation at later age of growth and development. Inflorescence explants lengthed 2-4 cm from palm No. 5 with medium composition consistis of Greshoff and Doy basal medium (1972) supplemented with in mg/L 10.0 2,4-D, 0.5 NAA and 0.3 2ip and 50 g/L sucrose recorded the best responses for swelling percentage and embryogenic callus formation percentage, also, it recorded the lowest value of browning percentage. The embryogenic callus which obtained differentiated to somatic embryos developed to complete plantlets which acclamitized and developed to free life plants.

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الإكثار الدقيق للسلالات البذرية المنتخبة لنخيل البلح المسن باستخدام النورات الزهرية

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٢- *المعمل المركزي للأبحاث و تطوير نخيل البلح – مركز البحوث الزراعية*

اجري هذا البحث خلال الفترة الزمنية من ٢٠٠٠ حتى ٢٠٠٤ في معمل زراعة الأنسجة النباتية المعمل المركزي للأبحاث و تطوير نخيل البلح – مركز البحوث الزراعية حيث هدف البحث إلي دراسة إمكانية استخدام النورات الزهرية المؤنثة لنخيل البلح كمنفصلات نباتيه من خلال تقنية الزراعة النسيجية لإكثار أشجار نخيل البلح البذرية الفاخرة و التي فقدت قدرتها على إنتاج فسائل جديده. و قد تم الحصول على مزارع معقمه خاليه من التلوث لمنفصلات النورات الزهرية. أعطت المنفصلات النباتية للنورات الزهرية بطول ٢-٤ سم أقل تلون بني و أفضل انتفاخ و تكوين للكالس الجنيني. بينما المنفصلات النباتية للنورات الزهرية بطول ٦-٨ سم سجلت استجابات متوسطة. كما سجلت المنفصلات النباتية للنورات الزهرية بطول ١٢-١٥ سم أقل الاستجابات. كذلك سجل تركيز ٥٠ جرام/ لتر سكروز أعلى الاستجابات. بينما سجل تركيز ٣٠ جرام / لتر سكروز وأيضا تركيز ٤٠ جرام / لتر سكروز أقل. أظهرت نخلة رقم ٥ أعلى استجابات لكل من الانتفاخ و تكوين الكالس الجنيني. بينما سجلت نخلة رقم ٤ أقل تلون بنى. البيئة رقم ١٢ المكونة من بيئة أملاح جرشوف و دوي ١٩٧٢ مضافا إليها بالميللجرام / لتر: ١٠,٠ (2,4-D) ، ٠,٥ (NOA) ، ٠,٣ (2ip) سجلت أعلى الاستجابات لكل من الانتفاخ و تكوين الكالس الجنيني. لاتمام عملية تكشف الأجنة، شجعت البيئة MS المحتوية على ٠,١ ملليجرام / لتر (NAA) أو الخالية تماما من الهرمونات على تكوين و تكشف الأجنة الجسمية و زيادة عددها. أما البيئة MS المحتوية على ٠,٢ ملليجرام / لتر (BA) فقد سجلت زيادة في عدد الأوراق و عدد النباتات. بينما سجلت بيئة MS المحتوية على ٠,٦ ملليجرام / لتر (BA) أعلى طول للأوراق. كما أظهرت البيئة MS المحتوية على ٣ ملليجرام / لتر (NAA) أو(IBA) بالإضافة إلى ٣ جم / لتر فحم نباتي نشط أعلى نسبه مئوية للتجذير للنباتات و أطول جذور. بينما أعطى تركيز ٠,١ ملليجرام / لتر (NAA) أو(IBA) أعلى طول للجذور. في عملية الأقلمة سجلت النباتات التي تم معاملتها بمعاملات ما قبل الأقلمة و زرعت على خليط التربة (بيت : رمل : فيرموكوليت بنسبة ١ : ١ : ١ حجما) أفضل نسبه مئوية للحيوية و أكبر عدد للأوراق و أفضل طول للأوراق و أفضل طول للنبات. هذه النباتات الناتجة من عملية الأقلمة تكون قادرة على تحمل الظروف الطبيعية.