IN VITRO MICROPROPAGATION PROTOCOL FOR ROOT EXPLANTS OF DATE PALM CV. SEWI

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Abstract

Developing high frequency of somatic embryos and normal plantlets from root segment explants of date palm cv. Sewi has been established in this study. Root segment explants were excised from roots of 3-5 years old offshoots. Different surface sterilization treatments were investigated. In this study, the sterilized roots segment explants were excised as two different explants, the first one was 1-5 cm in length including the root tip (RT) and the second one was 1-5 cm in length without root tip (RS) . Root explants (RT and RS) were cultured on modified MS basal nutrient medium supplemented with 100mg/l 2,4-D+3mg/l 2iP (M_1) or 10mg/l 2,4-D+3mg/l 2iP (M_2). The best surface sterilization treatments after 8 weeks in culture medium was by dipping root explants in 70% ethyl alcohol then transferred and dipping in 20% (w/v) calcium hypochlorite (CaOCl) for 5 minutes as the survival percentage was 25.83%.Culture medium (M_2) and RT explants showed the higher value of browning and swelling.Callus formation percentages increased with increasing subculture number to 7. Culture medium (M_2) and RT explants gave the higher percentage of callus induction. Embryogenic callus developed from each culture medium (M_1 and M_2), during subculture 5,6,7 and 8 were transferred and cultured onto differentiation nutrient medium which consists of MS basal nutrient medium supplemented with 0.1mg/l NAA. RT explants showed the higher percentage of embryogenic callus formation. In addition, percentage of normal somatic embryos (individual somatic embryos and multi-somatic embryos), number of individual somatic embryos, number of multi somatic embryos increased with increasing subculture number to 7. Culture medium (M_1) and RT explants showed the higher normal somatic embryos percentage .Culture medium (M_2) and RT explants resulted in the highest number of individual somatic embryos and multi somatic embryos. Number of leaves and roots per plantlet developed from individual somatic embryo increased with increasing subculture to 7. Culture medium (M_2) gave higher number of leaves per plantlet while culture medium (M_1) gave the higher number of roots per plantlet. RT explant gave the higher number of leaves and roots per plantlet.

Key Words: Date Palm *(Phoenix dactylifera L.)*, *In vitro*, Micropropagation, Root explant, Sewi, Somatic embryogenesis

INTRODUCTION

Date palm is a dioecious monocotyledonous fruit tree with a high degree of heterozygosity. Conventional vegetative propagation, made through offshoots, is very

slow and laborious. The number of offshoots produced by individual date palm trees is highly variable (0-30), it is affected by cultivar and cannot be induced *in vivo* (Munier, 1973). In addition, the number of offshoots established in soil is slow (30-80%) and cultivar dependent (Saaidi *et al.*,1979).

Therefore, the development of micropropagation protocol is very important for this species. Several attempts have been made based on organogenesis and somatic embryogenesis. Organogenesis in date palm has a low efficiency due to the low number of explants that respond *in vitro*, the long time required for the initiation phase, the low multiplication rate and the strong influence of variety (Poulain *et al.*, 1979, Beauchesne, 1982). Embryogenic callus has been obtained from ovules and zygotic embryo (Reuveni, 1979) and from shoot tip and bud excised from offshoots (Daguin and Letouze, 1988, Mater 1986, Sharma *et al.*, 1980, 1984, 1986, Tisserat, 1979, 1984, Zaid and Tisserat, 1983).

Callus formed at the root tip region of young date palm seedlings produced leaves and shoots (Smith 1975). On the other hand, Schroeder (1970) observed that root pieces of date palm cultured *in vitro* developed secondary roots but did not produce shoots. Eeuwens and Blake (1977) found that roots excised from *in vitro* cultured explant of date palms continued to grow and produce lateral roots when subcultured on liquid media. Sharma *et al.*, (1980) observed no growth in cultured date palm roots, and the explants experienced severe browning and death within the first few weeks of culture. Zaid and Tisserat (1983) obtained some callus from roots of date palm, such callus failed to exhibit any morphogenic response.

In other plants like in oil palm *Elaeis guineensis* used apical root tip cultured *in vitro* for root initiation (Starisky, 1970). However, Ong, 1977 and Martin *et.al.*, (1972 a,b) observed root elongation and callus from root explant cultured *in vitro*. Rohani and Paranjothy (1995) used axenic root cultures *in vitro* for long term. Root growth was best in liquid medium. Root explant with root tips (RT) multiplied by root tip elongation and axiallry root production, whereas root explants without tips (RS) multiplied by axiallry root production only. Explants were maintained in liquid medium for 18 months by subculturing 4 times. RT explants sustained better growth than RS plants during subculturing but growth vigorous declined with successive subcultures.

Chen *et al.*, (1987) showed that somatic embryogenesis in *Carica papaya L.* tissue culture derived only on callus from root explant (after 3 months on culture). Plants were regenerated from somatic embryo. Callus cultured for 2 years maintained its high regenerative capacity. Mandal *et al.*, (1994) used root explant of plantlets cultured *in vitro* in *Carica papaya* L. Callus formation was highest when root explant were cultured in half MS medium. Shoot regeneration was highest for root derived

callus grown in full strength modified MS medium and rooted shoots were successfully transferred to the field.

The present study aimed to developing high frequency of somatic embryogenesis and plantlet formation from root explants of date palm cv. Sewi.

MATERIALS AND METHODS

This study was carried out during of 2002-2005 in the Laboratory of the Central Laboratory for Date Palm Researches and Development (CLDRD), Agriculture Research Center (ARC), Egypt.

Preparation of plant materials:

Root segment with about 1-10 cm in length including root tip were excised from 3-5 years old offshoots of date palmt cv. Sewi cultured in El-Wahaat El-Baharia in Giza. Root segments were immediately washed by running tap water for one hour and then submerged in anti-oxidant solution containing 100 mg/l ascorbic acid and and 150 mg/l citric acid. The root segment explants were removed from the anti-oxidant solution and then proceeding the following surface sterilization treatments:

1 -Root segment explants were dipped in 70% ethyl alcohol for 1-5 seconds and then rinsed with sterilized distilled water three times one min. for each.

2 -Root segment explants were dipped in 70% ethyl alcohol for 1-5 seconds and then transferred and dipped in 0.1 % mercuric chloride (HgCl2) and two drops of tween 20 for 5 min. Explants were then rinsed with sterilized distilled water three times one min. for each.

3 -Root segment explants were dipped in 70% ethyl alcohol for 1-5 seconds then transferred and dipped in 10% Clorox (5.25 % sodium hypochlorite NaOCl) and two drops of tween 20 for 5 min. and then the explants were rinsed with sterilized distilled water three times one min .for each one.

4 -Root segment explants were dipped in 70% ethyl alcohol for 1-5 seconds and then transferred and dipped in 20% (w/v) calcium hypochlorite CaOCl, and two drops of tween 20 for 5 min. and then the explants were rinsed with sterilized distilled water three times one min. for each one.

5 -Root segment explants were dipped in 70% ethyl alcohol for 1-5 second and then transferred and dipped in 0.1 % mercuric chloride (HgCl2)and two drops of tween 20 for 2.5 min. and then the explants were rinsed with sterilized distilled water one time, and then transferred to 10% Clorox (5.25 % sodium hypochlorite NaOCl) and two drops of tween 20 for 2.5 min and then the explants were rinsed with sterilized distilled water three times one min. for each one.

6 -Root segment explants were dipped in 70% ethyl alcohol for 1-5 second and then transferred and dipped in 0.1 % mercuric chloride (HgCl2) and two drops of tween 20 for 2.5 min. and then the explants were rinsed with sterilized distilled water one time, and then transferred to 20% (w/v) calcium hypochlorite CaOCl and two drops of tween 20 for 2.5 min and then the distilled water three times one min. for each one.

After the previous surface sterilization treatments the sterilized root segment explants were excised to two different explant types. The first one was about 1-5 cm in length including the root tip (RT) and the second one was about 1-5 cm in length without root tip (RS).

Media components:

The previous sterilized root explants were cultured on Murashige and Skoog salts MS (1962) basal nutrient medium with the following modification in mg/l: 170 NaH_2PO_4, $2H_2O$, 200 glutamine, 40 Adenine sulfate, 0.4 thiamine-HCl. The basal nutrient medium was supplemented with mg/l: 3000 Activated charcoal, 30000 Sucrose, and 6000 Agar.

All explants were cultured on two basal nutrient medium as follow:

1 - Culture medium 1 (M_1) all nutrient medium supplemented with 100 mg/l 2, 4-D + 3 mg/l 2iP as described by Matar (1986).

2 - Culture medium 2 (M_2) all nutrient medium supplemented with 10 mg/l 2, 4-D + 3mg/l 2iP as described by Tisserat (1984).

The pH of all culture media was adjusted to 5.8 ± 0.1 prior to the addition of agar, and then 35 ml of medium was dispensed into small jars (150ml). The culture jars were sealed with caps of polyvenylpropelin. The jars were autoclaved at 121 °C, and 1.1 kg/cm^2 for 20min.

One sterilized explant was cultured individually in each culture jar. The explant was cultured horizontally with a good contact with the surface of the culture medium.

Each treatment consists of 3 replicates, each replicate consists of 6 culture jars, and each jars containing one explant, culture jars were incubated in a temperature controlled room at 24 °C± 3 under complete darkness condition.

Data of each surface sterilization treatment were collected after 8 weeks in culture, as the survival percentage.

Callus induction:

Uncontaminated and survived explants were subcultured to fresh medium every 6-8 weeks interval for at least 8 subcultures.

Data were collected at the end of each subculture. Three parameters:

browning degree, swelling degree and callus induction were evaluated. Browning degree and swelling degree were calculated visually as score (according to Pottino, 1981).

Negative results (-) = 1 below average results (+) = 2

Average results (++) = 3 Good results (+++) = 4

Embryogenic callus and somatic embryo development:

Embryogenic callus developed from each culture medium (M_1 and M_2), during subculture 5,6,7 and 8 were transferred and cultured onto differentiation nutrient medium which consists of MS basal nutrient medium supplemented with 0.1mg/l NAA. The embryoganic callus of each culture medium and each subculture was subcultured to a fresh differentiation medium every 6-8 weeks intervals for 2 subcultures.

Data collected at the end of each two subcultures, four parameters embryogenic callus percentages, normal somatic embryo percentages (individual and multi somatic embryos), number of normal individual somatic embryo, number of multi-somatic embryo were evaluated. Normal somatic embryo :(Individual embryo): Small seedling with primary root and shoot as described by (George, 1993). Multi-somatic embryo: (Secondary or accessory embryos) which are 3-4 embryos can occur on base of the original embryo as described by (George, 1993 and Zaid, 2003). Two parameters: number of leaves/plantlet and number of roots/plantlet were evaluated for different individual somatic embryos.

Experimental design

The experiments were performed utilizing complete randomized block design with factorial arrangement. The results were subject to analyzed for variance and the means were compared using L.S.D at 5% level according to Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

Table (1) showed the effect of surface sterilization methods, culture medium and explant type on survival percentage of date palm cv. Sewi. Data clearly showed that the methods of using Ethyl alcohol 70% + CaOCl 20% for surface sterilization showed the highest significant survival percentage (25.83%) compared with the other surface sterilization methods. Concerning to the effect of culture medium, data clearly showed that, no significant difference between the survival percentage of the two culture medium under investigation. With regard to the effect of expalnt type, it could be noticed that RT explant showed the higher survival percentage (12.56%) compared

with the survival percentage of RS explant (8.78%).

Browning degree value:-

Data in Table (2) showed the effect of subculture number, culture medium and explant type on browning degree value of date palm cv. Sewi. The results indicated that subculture no. 6,4 and 5 showed the highest value of browning degree (1.88, 1.85 and 1.83 respectively) compared with other subculture number. Concerning the effect of culture medium, the data clearly showed that the medium M_2 resulted in the higher degree value of browning (1.68) compared with the browning degree value (1.61) resulted from medium M_1. With regard to the effect of explant type, the data revealed that RT explant showed the higher value of browning degree (1.99) compared with the browning degree value produced from RS expellant (1.30).

Swelling degree value:-

Data in Table (3) and Fig. (1) showed the effect of subculture number, culture medium and explant type on swelling degree of date palm cv. Sewi. Concerning the effect of subculture number, data clearly showed that subculture no. 1 and 2 showed the lowest significant degree of swelling (1.00, for each) compared with the swelling degree. Subculture no.6, 7 and 5 which showed the highest significant swelling value of (2.19, 2.17 and 2.14, respectively). With regard to the effect of culture medium data revealed that culture medium (M_2) showed the higher value of swelling (1.74) compared with culture medium (M_1) (1.59). Concerning the effect of explant type, it could be noticed that, the RT explant resulted in the higher degree of swelling (1.78) compared with the RS explant which showed the lower value of swelling (1.59).

Callus induction percentage:-

Friable callus began to appear Fig. (1) at the end of the third subculture from different explant type on different culture medium. Table (4) showed the effect of culture medium and explant type on callus induction percentage of date palm cv. Sewi through different subcultures. Callus induction percentage increased significantly by increasing the subculture number from, third subculture to the seventh subculture. (9.33%, 14.08%, 20.92%, 32.17% and 41.50%, respectively). Increasing the subculture number from seven to the eighth decreased significantly the callus percentage from 41.50 to 37.67%. Regarding the influence of culture medium on callus induction percentage, culture medium (M_2) supplemented with 10 m/l 2,4-D + 2ip m/l gave the best results (27.58%) compared to culture medium (M_1) supplemented with 100 m/l 2,4-D + 3 m/l 2ip (24.31%). Data also showed a significant difference for the effect of explant type on callus induction percentage. However the best results obtained from using RT explant (29.94%) compared with the callus induction percentage were obtained by RS explant (24.94%).

These findings are in agreement with those of Smith (1975) who reported that callus was formed at the root region of young date palm seedlings, Zaid and Tisserat (1983) who obtained some callus from roots of date palm Ong (1977) and Martin *et al.*, (1972 a ,b) who observed root elongation and callus from root explant cultured *in vitro* in oil palm Mandal *et al.*, (1994) who mentioned that callus formation was highest, when root explant of *Carica paparya* cultured in half MS medium and Handro *et al.*, (1988) who reported that callus was induced from root explant of Brazilian medicinal plant *in vitro*.

Embryogenic callus percentage:

The embryogenic callus Fig. (2) appeared on different explant types cultured on medium M_1 or M_2 at the end of the 5 th subcultures. The embryogenic callus developed during subculture nos. 5,6,7and 8 from different explants type on different culture media were transferred and cultured on differentiation medium (MS basal medium supplemented with 0.1 mg/l NNA). Data tabulated in Table (5) indicated that no significant differences were observed among embryogenic callus percentage during subcultures 7, 8 and 6 as the embryogenic callus reached to the highest significant percentage (22.83%, 21.25% and 21.00%, respectively). Whereas during subculture no. 5 the embryogenic callus percentage reduced significantly to 12.75%. The obtained results showed also that no significant difference was observed among embryogenic callus percentage of explant cultured on medium M_1 supplemented with 100 mg/l 2,4-D + 3 mg/l 2ip with (19.13%) or culture medium M_2 supplemented with 10 mg/l 2,4-D + 3 mgl/ 2ip (19.79%). However, the embryogenic callus percentage was increased significantly by using RT explant compared to using RS explant.

These results are in parallel to those obtained by Komai and Masuda (2002) who found that root segments of spanich seedling efficiently produced embryogenic calluses and produced somatic embryos, Faryua and Hosoki (2004) who found that embryogenic callus induced from root segments of *Oenanthe javanica*. Contrary to results of Zaid and Tisserat (1983)who showed that callus obtained from root explants of date palm failed to exhibit any marphogenic response.

Percentages of normal somatic embryo:-

Results in Table (6) showed the formation of normal somatic embryo from embryogenic callus cultured on differentiation medium (MS basal medium supplemented with 0.1 mg/l NNA) .The percentages of normal somatic embryos (individual and multi-somatic embryo) were significantly highest at the end of subculture no. 7 (83.58%). Hence, the reduced of subcutlure number from 7 to 6 and 5 reduced significantly the production of normal somatic embryo as the percentage reduced from 83.58% to 65.67 and 68.50%, respectively. Also increasing the

subculture number from 7 to 8 reduced significantly the production of normal somatic embryo from 83.58 to 59.67% respectively. Data also indicated that, no significant difference could be observed between the formation of normal somatic embryo on culture medium M_1 supplemented with 100 mg/l 2,4-D+3 mg/l 2ip or culture medium M_2 supplemented with 10 mg/l 2,4-D+3 mg/l 2ip as the percentage of normal somatic embryo were 71.13 and 67.58, respectively. Results also showed that, RT explant formed higher significant normal somatic embryo (80%) than those formed from RS explant (58.71%).

Similar results for production of embryogenic callus from root explants were obtained with Chaudhuri *et al.*, (2004) who mentioned that the somatic embryos production for *Tylophora indica* by root explant, 89% of fariable embryogenesis callus produced globular somatic embryos. Also, Furuya and Hosoki (2004) found that many somatic embryos of *Oenanthe javanica* were developed into normal plants.

Number of individual somatic embryo:-

Data tabulated in Table (7) revealed significant differences in number of individual somatic embryo for different subcultures number. Where the number of individual somatic embryo was significant highest (11.42 embryo/ explant) at the end of subculture no. 7. This number reduced significantly with reducing the subculture number to 6 or 5 (6.00 and 2.33 embryo/explant ,respectively) or with increasing the subculture number to 8 (4.42 embryo/ explant). On the other hand, the number of individual somatic embryo was not affected significantly by culturing explants on medium M_1 or M_2. Regarding, the influence of explant type, the obtained data indicated that RT explants formed the highest significant number of individual somatic embryo culture (9.5 embryo / explant) than those formed by RS explant (2.54 embryo / explant).

Number of multi- somatic embryo:-

Multi- somatic embryo : (Secondary or accessory embryos) which are 3-4 embryos can occur on base of the original embryo as described by George, (1993) and Zaid, (2003). Data tabulated in Table (8) revealed significant differences in number of multi-somatic embryo for different subculture number. The number of multi- somatic embryo was significantly highest (3.17 somatic embryo/ explant) at the end of subculture no.7. This number reduced significant with reducing the subculture number to 6 or 5 (1.17 and 0.33 embryo/ explant, respectively) or with increasing the subculture number to 8 (1.00 embryo/ explant). On the other hand, the number of multi- somatic embryo was not affected significantly by culturing explants formation on medium M_1 or M_2. Regarding, the influence of explant type, the obtained data indicated that RT explants formed the more significant number of multi- somatic

embryo (1.88 embryo/ explant) than those formed by RS explant (0.96 embryo / explant).

Leaves number of plantlet developed from normal individual somatic embryo:-

The results in Table (9) and Fig. (3) clearly showed increments in leaves number/plantlet (2.83, 2.54 and 2.46 leaves/plantlet) developed from individual somatic embryo during subculture nos. 7,6 and 8 respectively as compared to the leaves number developed from individual somatic embryo during the 5 subculture (2.03 leaves/plantlet). In regard to the effect of embryo production from different culture medium, data indicated that no significant differences were observed among the leaves number/plantlet developed on medium M_1 and M_2 (2.40 and 2.53 leaves/plantlet respectively). On the other hand, number of leaves/plantlet was affected significantly by explant type since the number of leaves/plantlet developed from RT explant (2.77) was significantly higher as compared to number of leaves/plantlet developed by RS explant (2.17).

Root number of plantlet developed from normal individual somatic embryo:-

The results in Table (10) and Fig (3) revealed that no significant differences were observed among the root numbers developed from indiviual somatic embryo during the subcultures number 7,6 and 5 as the root number/plantlet were 2.75, 2.50 and 2.33,respectively. Data showed that no significant difference was noticed between number of root/plantlet developed from individual somatic embryo during subculture number 5 and 8 as the root number/plantlet were 2.33 and 1.83 respectively. Data also indicated no significant difference among root number/plantlet developed from individual somatic embryo cultured on M_1 and M_2 medium (2.54 and 2.17, respectively). On the other hand, individual somatic embryo developed from RT explant showed higher number of root/plantlet compared with those developed from RS explant (2.83 and 1.88 root/plantlet, respectively).

It could be concluded from all the previous results that root segments explant excised with or without root tip of date palm cv. Sewi efficiently produced embryogenic callus, somatic embryos and these somatic embryos able to develop normal plantlets.

These results are in agreement with those found by Smith (1975) who described that callus formed at the root tip region of date palm seedlings produced leaves and shoots. These results are in contrary with the previous results reported by Schroeder (1970) who observed that root pieces of date palm cultured *in vitro* developed secondary roots, but did not produce shoots. Eeuwens and Blake (1977) who found

that roots excised from *in vitro* cultured explant of date palm continued to grow and produced laterals root when subcultured on liquid media. Sharma *et al.*, (1980) who observed no growth in cultured date palm roots, and the explants experienced severe browning and death within the first few weeks of culture. Zaid and Tisserat (1983) who obtained some callus from root of date palm such callus failed to exhibit any morphogenic response.

Table 1. Effect of different surface sterilization treatments(using70% ethyl alcohol, $HgCl_2$, Clorox (NaOCl) and $Ca(OCl)_2$, culture medium and explant type on survival percentages of date palm cv. Sewi, (after 8 weeks in culture).

Treatment (A) Concentration %		Medium (B) M_1 Explant type (C) RT	M_1 RS	M_1 Mean	M_2 Explant type (C) RT	M_2 RS	M_2 Mean	Mean (A)
ethyl	70.00	13.33	06.67	10.00	10.00	5.33	07.67	08.33
ethyl+ MC	70.00 00.10	15.33	08.00	11.50	17.33	07.00	12.17	11.83
ethyl+ Clorox	70.00 10.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
ethyl+ $Ca(OCl)_2$	70.00 20.00	28.33	21.67	25.00	30.00	23.33	26.67	25.83
ethyl+ $HgCl_2$+ Clorox	70.00 00.10 10.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
ethyl + $HgCl_2$+ $Ca(OCl)_2$	70.00 00.10 20.00	16.67	16.67	16.67	20.00	16.67	18.33	17.50

Mean (B)	M_1	M_2
	10.53	10.81
Mean (C)	RT	RS
	12.56	8.78
Mean (B xC)	M_1 x RT	M_1 x RS	M_2 x RT	M_2 x RS
	12.22	8.83	12.89	8.72

Mean separation by L.S.D. at 0.05

A=1.47	B= N.S	C=0.85
AxB=2.08	AxC=2.08	BxC=1.20
AxBxC=2.94		

Table 2. Effect of subculture number, culture medium and explant type on browning degree value of date palm cv. Sewi.

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
1	1.60	1.00	1.30	1.53	1.00	1.27	1.28
2	1.63	1.00	1.32	1.50	1.00	1.25	1.28
3	1.77	1.40	1.58	2.00	1.27	1.63	1.61
4	1.90	1.67	1.78	2.20	1.67	1.93	1.86
5	2.07	1.50	1.78	2.30	1.47	1.88	1.83
6	2.23	1.47	1.85	2.50	1.33	1.92	1.88
7	2.13	1.23	1.68	2.20	1.40	1.80	1.74
8	2.03	1.17	1.60	2.20	1.27	1.73	1.67
Mean (B)	M_1			M_2			
	1.61			1.68			
Mean (C)	RT			RS			
	1.99			1.30			
Mean (B xC)	M_1 x RT		M_1 x RS		M_2 x RT		M_2 x RS
	1.92		1.30		2.05		1.30

Mean separation by L.S.D. at 0.05

A=0.09	B=0.04	C =0.04
AxB=0.12	AxC=0.12	BxC=0.06
AxBxC=0.17		

*Values determined as described by Pottino 1981

Table 3. Effect of subculture number, culture medium and explant type on swelling degree value of date palm cv. Sewi.

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3	1.30	1.20	1.25	1.47	1.50	1.48	1.37
4	1.60	1.33	1.47	2.00	1.53	1.77	1.62
5	2.03	2.03	2.03	2.30	2.20	2.25	2.14
6	2.23	1.87	2.05	2.60	2.07	2.33	2.19
7	2.47	1.77	2.12	2.53	1.90	2.22	2.17
8	2.00	1.63	1.82	2.00	1.67	1.83	1.83
Mean (B)	M_1			M_2			
	1.59			1.74			
Mean (C)	RT			RS			
	1.78			1.59			
Mean (B xC)	M_1 x RT		M_1 x RS		M_2 x RT		M_2 x RS
	1.70		1.48		1.86		1.61

Mean separation by L.S.D. at 0.05

A=0.08	B=0.04	C=0.04
AxB=0.12	AxC=0.12	BxC=0.06
AxBxC=0.17		

*Values determined as described by Pottino 1981

Table 4. Effect of subculture number, culture medium and explant type on callus induction percentage of date palm cv. Sewi

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
3	09.00	08.00	08.50	11.33	09.00	10.17	09.33
4	14.67	12.00	13.33	14.67	15.00	14.83	14.08
5	22.33	20.00	21.17	21.00	20.33	20.67	20.92
6	35.00	26.33	30.67	41.67	25.67	33.67	32.17
7	45.00	30.67	37.83	57.00	33.33	45.17	41.50
8	36.00	32.67	34.33	51.67	30.33	41.00	37.67
Mean (B)	M_1			M_2			
	24.31			27.58			
Mean (C)	RT			RS			
	29.94			21.94			
Mean (B xC)	M_1 x RT		M_1 x RS		M_2 x RT		M_2 x RS
	27.00		21.61		32.89		22.28

Mean separation by L.S.D. at 0.05

A=1.56 B=0.90 C=0.90

AxB=2.20 AxC=2.20 BxC=1.27

AxBxC=3.11

Table 5. Effect of subculture number, culture medium and explant type of friable embryogenic nodular callus cultured on differentiation medium (after 2 subcultures 6-8 weeks for each)on embryogenic callus percentage of date palm cv. Sewi.

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
5	11.33	10.00	11.67	11.78	16.00	13.83	12.75
6	21.67	21.33	21.50	21.67	19.33	20.50	21.00
7	30.00	16.67	23.33	31.67	13.00	22.33	22.83
8	25.00	15.00	20.00	28.33	16.67	22.50	21.25
Mean (B)	M_1			M_2			
	19.13			19.77			
Mean (C)	RT			RS			
	22.92			16.00			
Mean (B xC)	M_1 x RT		M_1 x RS		M_2 x RT		M_2 x RS
	22.50		15.75		23.33		16.25

Mean separation by L.S.D. at 0.05

A=2.36 B= N.S C=1.67

AxB=3.33 AxC=1.90 BxC=3.80

AxBxC=5.38

Table 6. Effect of subculture number, culture medium and explant type of friable embryogenic nodular callus cultured on differentiation medium (after 2 subcultures 6-8 weeks for each) on normal somatic embryo percentage of date palm cv. Sewi .

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
5	74.00	72.33	73.17	88.67	39.00	63.83	68.50
6	77.00	43.33	60.17	88.67	53.67	71.17	65.67
7	86.00	78.33	82.17	90.33	79.67	85.00	83.58
8	74.67	63.33	69.00	60.67	40.00	50.33	59.67
Mean (B)	M_1			M_2			
	71.13			67.58			
Mean (C)	RT			RS			
	80.00			58.71			
Mean (B xC)	M_1 x RT		M_1 x RS		M_2 x RT		M_2 x RS
	77.92		64.33		82.08		53.08

Mean separation by L.S.D. at 0.05

A=13.35	B=N.S	C=9.44
AxB=18.88	AxC=12.90	BxC=25.80
AxBxC=36.48		

Table 7. Effect of subculture number, culture medium, and explant type of friable embryogenic nodular callus cultured on differentiation medium (after 2 subcultures 6-8 weeks for each)on number of normal individual somatic embryo of date palm cv. Sewi

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
5	2.23	1.00	1.67	5.00	1.00	3.00	2.33
6	8.67	1.33	5.00	12.67	1.33	7.00	6.00
7	15.00	5.67	10.33	19.67	6.33	12.50	11.42
8	8.00	2.67	5.33	6.33	1.00	3.50	4.42
Mean (B)	M_1			M_2			
	5.58			6.50			
Mean (C)	RT			RS			
	9.54			2.54			
Mean (B xC)	M_1 x RT		M_1 x RS		M_2 x RT		M_2 x RS
	8.50		2.67		10.58		2.42

Mean separation by L.S.D. at 0.05

A=1.41	B= N.S	C=1.00
AxB=2.00	AxC=3.68	BxC=1.84
AxBxC=5.21		

Table 8. Effect of subculture number, culture medium and explant type of friable embryogenic nodular callus cultured on differentiation medium (after 2 subcultures 6-8 weeks for each)on number of normal multi-somatic embryo of date palm cv. Sewi .

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
5	0.67	0.33	0.50	0.33	0.00	0.17	0.33
6	1.67	0.33	1.00	2.00	0.67	1.33	1.17
7	3.67	2.00	2.83	4.33	2.67	3.50	3.17
8	1.33	0.67	1.00	1.00	1.00	1.00	1.00

Mean (B)	M_1	M_2
	1.33	1.50
Mean (C)	RT	RS
	1.88	0.96

Mean (B xC)	M_1 x RT	M_1 x RS	M_2 x RT	M_2 x RS
	1.83	0.83	1.92	1.08

Mean separation by L.S.D. at 0.05

A=0.48	B= N.S	C=0.34
AxB=0.68	AxC=0.42	BxC=0.85
AxBxC=1.20		

Table 9. Effect of subculture number, culture medium and explant type of white friable embryonic nodular callus on leaves number of normal individual somatic embryo cultured onto germination and development medium (after 2 subculturs 4 weeks for each)of date palm cv. Sewi .

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
5	2.27	1.93	2.10	2.33	1.60	1.97	2.03
6	2.93	1.57	2.25	3.17	2.50	2.83	2.54
7	3.00	2.60	2.80	3.20	2.53	2.87	2.83
8	2.60	2.30	2.45	2.63	2.30	2.47	2.46

Mean (B)	M_1	M_2
	2.40	2.53
Mean (C)	RT	RS
	2.77	2.17

Mean (B xC)	M_1 x RT	M_1 x RS	M_2 x RT	M_2 x RS
	2.70	2.10	2.83	2.23

Mean separation by L.S.D. at 0.05

A=0.45	B= N.S	C=0.32
AxB=0.64	AxC=0.31	BxC=0.64
AxBxC=0.87		

Table 10. Effect of subculture number, culture medium and explant type of white friable embryonic nodular callus on root number of normal individual somatic embryo cultured onto germination and development medium (after 2 subculturs 4 weeks for each) of date palm cv. Sewi .

Subculture no. (A)	Medium (B)						Mean (A)
	M_1			M_2			
	Explant type (C)			Explant type (C)			
	RT	RS	Mean	RT	RS	Mean	
5	3.00	2.00	2.50	3.00	1.33	2.17	2.33
6	4.67	1.00	2.83	2.33	2.00	2.17	2.50
7	3.00	2.67	2.83	2.33	3.00	2.67	2.75
8	2.33	1.67	2.00	2.00	1.33	1.67	1.83
Mean (B)		M_1			M_2		
		2.54			2.17		
Mean (C)		RT			RS		
		2.83			1.88		
Mean (B xC)		M_1 x RT		M_1 x RS		M_2 x RT	M_2 x RS
		3.25		1.83		2.42	1.92

Mean separation by L.S.D. at 0.05

A=0.67	B= N.S	C=0.48
AxB=0.95	AxC=0.21	BxC=0.41
AxBxC=0.58		

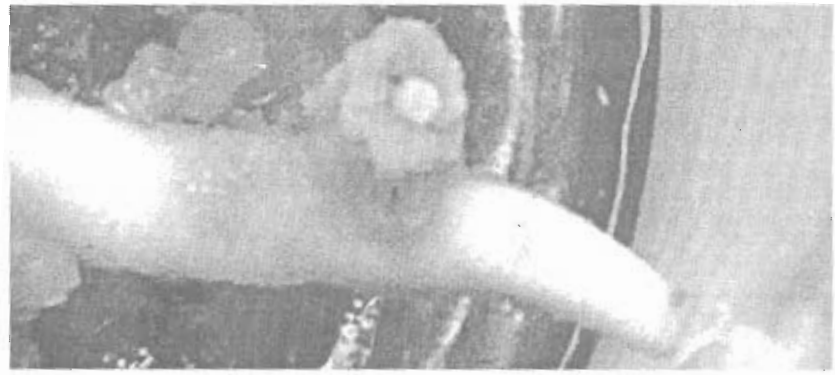

Fig. 1. Callus induction and swelling from root tip explants (RT) of date palm cv. Sewi cultured on MS modified medium supplemented with 10mg/L 2,4-D+ 3mg/L 2iP after 2 subcultures (6-8 weeks for each).

Fig. 2. Embryogenic callus and normal somatic embryos developed from root tip explants palm cv. Sewi cultured on differentiation medium after 2 subcultures (6-8 weeks for each).

Fig. 3. Number of leaves and roots/plantlet developed form root tip explants of date palm cv. Sewi after 2 subcultures (6-8 weeks for each).

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برتوكول الإكثار الدقيق بزراعة الأنسجة باستخدام المنفصلات النباتية للجذور لنخيل البلح صنف سيوي

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بدأت هذه الدراسة لتحديد اعلي معدل لنمو الأجنة الجسمية والنبيتات الطبيعية الناتجة من استخدام منفصلات نباتية من جذور نخيل البلح صنف سيوي .فصلت المنفصلات النباتية للجذور من جذور فسائل نخيل البلح عمر ٣-٥ سنوات وتم استخدام معاملات مختلفة من التعقيم السطحي لها . المنفصلات النباتية للجذور المعقمة قسمت إلي جزأين وهما الجزء الأول بطول ١-٥ سم يحتوي علي القمة الجذرية (RT)أما الجزء الثاني بطول١-٥ سم لا يحتوي علي القمة الجذرية (RS). زرعت المنفصلات النباتية للجذور علي أوساط غذائية معدلة من بيئة موراشيج وسكوج (MS)مضافاً اليها اما 100ملليجرام/ لتر 2و 4كلورو فينوكس حامض الخليك + 3 ملليجرام/لتر ايزوبنتيل ادنين (M_1) أو10ملليجرام/ لتر 2و4 كلوروفينوكس حامض الخليك + 3 ملليجرام/لتر ايزوبنتيل ادنين (M_2) .أفضل معاملة للتعقيم السطحي بعد 8أسابيع علي وسط الزراعة هو غمس المنفصلات النباتية الجذرية في70 % كحول ايثانول ثم بعد ذلك نقلها وغمسها في20 % (وزن/حجم) هيبوكلوريت كالسيوم لمدة 5 دقائق والتي أعطت نسبة مئوية للمنفصلات النباتية الحية 25.83 % . تم ملاحظة اعلي قيمة للتلوين البنى والإنتفاخات علي وسط الزراعة M_2 والمنفصلات النباتية المحتوية علي القمة الجذرية RT. تم نقل الكالس الجنيني النامي على كلاً من وسط الزراعة الغذائي M_1 و M_2 فى نقلات الزراعة رقم ٥ ، ٦ ، ٧ ، ٨ إلى وسط زراعة التكشف المعدلة من بيئة موراشيج وسكوج MS مضافاً إليها ٠,١ مليجرام/لتر نفثا لين حامض الخليك وكانت النسبة المئوية لتكوين الكالس الجنيني تزيد بزيادة عدد نقلات الزراعة حتى النقلة السابعة . وسط الزراعة M_2 والمنفصلات النباتية المحتوية علي القمة الجذرية RT أعطت اعلي نسبة مئوية من استحداث الكالس واعلي نسبة مئوية لتكوين الكالس الجنيني .لوحظ زيادة النسبة المئوية للأجنة الجسمية (الأجنة الجسمية الفردية والأجنة الجسمية المتعددة) وعدد الأجنة الجسمية الفردية وعدد الأجنة الجسمية المتعددة بزيادة عدد نقلات الزراعة حتى النقلة السابعة .لوحظ أن وسط الزراعة M_1 والمنفصلات النباتية المحتوية علي القمة الجذرية RT أعطت اعلي نسبة مئوية من الأجنة الجسمية الطبيعية . أما وسط الزراعة M_2 والمنفصلات النباتية المحتوية علي القمة الجذرية RT سجلت اعلي نتيجة من عدد الأجنة الجسمية الفردية والأجنة الجسمية المتعددة . زيادة عدد الأوراق والجذور للنبيتات المتكونة للأجنة الجسمية الفردية بزيادة عدد نقلات الزراعة حتى النقلة السابعة .أعطي وسط الزراعة M_2 اعلي عدد من الأوراق للنبيتات بينما وسط الزراعة M_1 أعطي اعلي عدد من الجذور للنبيتات . أعطت المنفصلات النباتية المحتوية علي القمة الجذرية RT اعلي عدد من الأوراق والجذور للنبيتات المتكونة.