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SOME ASPECTS OF *IN VITRO* MICROPROPAGATION OF DATE PALM (*Phoenix dactylifera* L.)

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ABSTRACT

The effect of callus source (shoot tip, axillary bud and leaf primordium), medium (growth regulators) and number of subcultures on both somatic embryogenesis and shoot formation were studied. Moreover, the anatomical features of roots and leaves of regenerated plantlets were also studied in comparison with those of seedlings.

Results revealed that shoot tip callus on a medium containing 2mg/L IBA gave the best somatic embryogenesis. Hormone free medium and that containing 5 mg/L 2, ip did not cause somaclonal variation, while on other media it ranged between 14.42 and 32.15%. On transferring onto free hormone medium, 20% of the abnormal embryos developed normal plantlets. The highest rate of shoot multiplication was obtained with shoot tip callus on medium containing 3mg/L 2,ip.

Comparative anatomy of *in vitro* and *ex vitro* leaves and roots, indicated that the leaves of the *in vitro* regenerated plantlets had well developed tissues. On the contrary, their roots were characterized by poorly developed cortex and vascular bundles, which may be the main causes of the observed problems with plantlet acclimatization.

Abbreviations.

2,4-D: 2,4 dichlorophenoxyacetic acid; NAA: naphthaleneacetic acid; IBA: indole-3- butyric acid; 2, ip: (2-dimethylallayl – amino)- purin; GA_3: gibberellic acid.

INTRODUCTION

Date plam (*Phoenix dactylifera* L.) is considered one of the most important fruit trees cultivated in Egypt and other Middle East and Arab Countries (Mohammad, 1983; Moursy and Saker, 1996). The cultivated grown cultivars are used for food, fiber, fuel and shelter (Al- Bakr, 1972; Zohary and Spiegel – Roy, 1975). Recently in Egypt, more attention is directed to date palm as being a promising crop for the cultivation in new reclamised soils at the regions of high temperature, water deficit and/or salinity (Bouchreib and Clarck, 1997). Moreover, some cultivars were imported to Egypt from the Gulf area for their super fruit qualities. Therefore, the need for a propagation protocol which insures the mass production of true to type plants became very necessary . The conventional Method of vegetative propagation using offshoots is slow and expensive (Pareek, 1984). The use of tissue culture techniques in the clonal propagation of date palm has been investigated by many workers since the pioneer work of Tisserat (1979). Two micropropagation protocols were adopted : a) by somatic embryogenesis via callus as reported by Tisserat, 1982; Sharma *et al*. 1986;

Daguin and Letouzé, 1988; Zaid and Hughes, 1995; Diwaker *et al.* 1998 and b) by axillary bud multiplication as reported by Poulain *et al*. 1979; Beauchesne, 1982; Gabr and Tisserat, 1985; Nasir *et al.* 1994; Al-Karyi and Al-Maarri, 1997.

It has been reported that organogenesis and bud multiplication are less efficient when compared with somatic embryogenesis 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 the cultivar. (Poulain *et al*. (1979); Beauchesne, (1982)).

Although the use of tissue culture techniques for the mass propagation of date plam is already adopted in many countries, yet the adoption level of these techniques is still low. The main reasons are; the absence of felt need among farmers for the incompatibility of this technology, uncertainity about its results and impacts, and the wide spread of the seedless fruits phenomenon in certain varieties when propagated by tissue culture (Al-Sakran, 2001).

The present study aimed to elucidate some aspects of both somatic embryogenesis and organogenesis in tissue cultures of the local variety of date palm (Sewi) including an evaluation of the phenotypic and anatomical variations of the *in vitro* produced plants.

MATERIALS AND METHODS

This work was performed at the Laboratory of Tissue Culture, Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza.

Plant materials: Offshoots of the cv. Sewi were secured from farms at Giza region, each weighed 5-10 kg, 60-100 cm in height, 25-30 cm in diameter and with 7-15 leaves. Leaves were completely removed with their bases to expose the axillary buds and the shoot tip of each offshoot.

Shoot tips (5-7 X 3-4 cm) and axillary buds (0.5-1 X 0.3-0.5 cm) were carefully removed, washed with tap water for 30 minutes and were surface sterilized in 95% ethanol for 5 min. Thereafter they were dipped in 20% (v/v) commercial bleach (2.25% sodium hypochlorite) for 30 min., and finally in 0.1% mercuric chloride (MC) for 5 min. The explants thereafter were rinsed three times with sterile distilled water. Under aseptic conditions, the axillary buds (AB) were intactly cultured after cutting the destructed broad base. The outer 3-4 leaf primordia (LP), lengthed about 3cm, were dissected from the apical shoot tips, then the shoot tips (ST) were longitudinally cut into 3-4 sectors. The explants were cultured on MS medium (Murashige and Skoog 1962) supplemented with (in mg/L): 0.5 nicotinic acid; 0.5 pyridoxine- HCl; 1.0 thiamin HCl; 100 myoinositol; 2.0 glycine, 3000 agar (Win-Lap); 3000 activated charcoal and 30000 sucrose. The medium pH was adjusted to 5.7 before autoclaving at 121°C. For callus initiation and proliferation, the medium was supplemented with 30 mg/L 2, 4-D and 20 mg/L 2,ip such medium proved the most suitable for callus growth when compared with other media. The cultures were incubated at 25-27°C in darkness, at 6 weeks intervals. To study somatic embryogenesis, proliferated callus at the age of

32 weeks was transferred to the previously mentioned MS medium, supplemented with five different additions of growth regulators (in mg/L) (A) 5mg 2, ip; (B) 1 mg 2,4-D + 1mg GA_3; (C) 2 mg IBA; (D) 1 mg NAA and (E) no regulators were added . The cultures were also incubated in darkness and recultured on fresh medium at 6 weeks intervals. The numbers of normal and abnormal embryos per culture jar were recorded at the end of each subculture. The formed embryos on all media were systematically isolated and cultured on free hormone medium and incubated at 16 hrs light photoperiod regime with 3000 Lux light intensity to stimulate embryos germination into normal plantlets.

To study organogenesis and shoot multiplication, the callus was transferred to a medium contained 10 mg/L 2,4-D and 3 mg/L 2,ip. The formed shoots were cultured in multiplication medium supplemented with 3 mg/L 2,ip without activated charcoal. The growing shoots were counted throughout 3 successive subcultures each of 3 weeks, and the multiplication rate was estimated. The off types observed through the regenerated shoots were grouped into different morphological groups according to leaf shape, and were counted. Cultures of shoot multiplication were kept under the previously mentioned light regime. Rooting of the regenerated shoots was achieved on a medium containing 0.1 mg/L NAA and 3 mg/L activated charcoal.

The experiments were performed in a complete randomized design, and treatments were replicated five times. The results were analysed using the analysis of variance method, according to Steel and Torrie (1980).

For anatomical study, specimens 1 cm long of root, and 1 cm^2 of leaves (from the middle zone for both), of *in vitro* regenerated plantlets and those of normal seedlings were taken at the stage of two simple foliage leaves (24 weeks old). Specimens were killed and fixed in F.A.A., washed in 50% alcohol, dehydrated in normal butyl and embbeded in paraffin wax (55 °C mp), (Sass 1958). Cross sections, 20µ thick were cut, and stained by crystal violet/ erythrosin combination and mounted in Canada balsam (Jackson, 1926). Slides were microscopically examined. Measurements of different tissues were taken, and averages of 10 readings from 5 slides were calculated.

RESULTS AND DISCUSSION

1- Somatic embryogenesis:

Data concerning the response of somatic embryogenesis to the source of callus, medium and number of subcultures are presented in Tables (1) and (2). As shown in Table (1) there were significant differences between average numbers of normal embryos/Jar, developed from calli of different sources. The shoot tip (ST) and axillary bud (AB) calli were significantly more regenerable than leaf primordium (LP) callus giving averages of 5.4, 4.5 and 1.9 embryos/ Jar, respectively. Concerning the effect of medium, it is evident that there were significant differences between the used media which could be averaged, depending on their stimulative effect on somatic embryogenesis, as media C, A, (D & B) and E; with the averages 5.67, 4.48,

3.86, 3.73 and 2.70 embryos/ Jar, respectively. This indicates that the induction of somatic embryogenesis started on the medium of callus proliferation which contained 2,4-D at relatively high level. Further development of somatic embryos did not need any hormones as it occurred on hormone free medium (E), however the addition of growth regulators, either auxin or cytokinin, at relatively low level encouraged the process and increased the number of developed embryos. The embryogenic capacity of the callus significantly increased with increasing number of subcultures. The recorded averages were 1.79, 2.46, 4.31, 5.48 and 6.42 embryos/Jar for subcultures S_1, S_2, S_3, S_4 and S_5 , respectively. All interactions between the three studied factors were significant, which means the necessity of taking all these factors in consideration in somatic embryogenesis protocols.

Data in Table (2) show the effect of the studied factors on the percentage of abnormal embryos. With respect to the medium effect, it is evident that the free hormone medium (E) and medium containing only 2,ip (A) did not cause any abnormality in somatic embryogenesis. This held true with all kinds of callus and throughout five successive subcultures which suggests that the type of growth regulator in the medium is the main factor affecting abnormality in somatic embryogenesis. On the other hand, the presence of auxin type regulators caused different degrees of variation. The highest percentage of abnormal embryos (32.15%) resulted on medium (D) containing 1 mg/L NAA, which suggested the need of using lower concentration of this regulator. Percentages of 23.04% and 14.42 of abnormal embryos were found in media B and C, respectively. Results showed that there were significant differences between percentages of abnormal embryos recorded at different subcultures. However, it is difficult to elucidate constant trend of abnormality with increasing subcultures. The callus source significantly affected somatic embryo abnormality. Percentages of 10.2, 12.8 and 18.7 were recorded with shoot tip, axillary bud and leaf primordium calli, respectively.

The abnormal embryos were morphologically observed and calculated at the end of each subculture (Fig. 1). The abnormal embryos were collected and transferred on hormone free medium. Results indicated that about 20% of those abnormal embryos turned normal and grew into normal plantlets, while the rest (about 80%) produced abnormal plantlets or browned callus (Figs. 2 and 3). The obtained results indicate the sensitivity of somatic embryogenesis in date palm to the *in vitro* culture factors, which could cause high percentage of embryo abnormalities. In conclusion, the best protocol for obtaining maximum number of normal somatic embryos includes the use of shoot tips for initiating callus on a medium containing 30 mg/L 2,4-D and 20 mg/L 2,ip, then transferring such callus to embryo induction medium containing 2 mg/L IBA, and finally, for embryo further growth, the embryos must be transferred to a hormone free medium.

1,2

Fig. (1): Normal embryo (First on left side), and abnormal embryos observed on somatic embryogenesis media at the 1st subculture (6 weeks old).

In accordance with our results many authors used juvenile parts for initiating embryogenic callus. Mater (1987), Shyamala *et. al.* (1992) used callus initiated from shoot tips and immature inflorescence explants for developing somatic embryos. Daikh and Demarly (1987) used leaf explants. Falcone and Marscheschi (1988), Nazeri *et al.* (1993) used shoot tips, lateral buds and young leaves.

With respect to growth regulators in embryogenesis medium, wide ranges of regulators were used. Sharma *et. al.* (1986) induced somatic
embryos on medium without growth regulators, while Mater (1987) observed somatic embryos on a medium containing 0.1 NAA plus charcoal. Falcone and Marscheschi (1988) used NAA (10 mg/L) or 2,4-D (1 mg/L) for producing embryogenic callus. Hormone free medium was also used by Omar and Novak (1990) who noticed that root and shoot development was stimulated with the presence of 0.45 μm NAA at 0.05μm BA.

2- Organogenesis

Shoot formation:

Studying the phenomenon of organogenesis in date palm callus in the present work, started, by observation of shoot formation in callus grown on medium containing 2,4-D and 2,ip used for callus proliferation. The number of formed shoots ranged between 2 to 3 shoots/Jar. These shoots were transferred to a medium contained 3 mg 2,ip as growth regulator. On such medium, the shoots grew and new axillary shoots appeared. Data in Table (3) represent the mean number of shoots and means of multiplication rates recorded at 3 successive subcultures as affected by callus source and subculturing. It is evident that there were significant differences between average number of shoots formed from calli originated from different sources. The highest average number of shoots recorded was with shoot tip callus, followed by calli from axillary buds and leaf primordia giving averages of 18.25, 13.75 and 13.25 shoots/Jar., respectively. Considering the multiplication rate, shoot tip callus recorded the highest rate (1.8 shoots)

which significantly differed from the rates 1.6 and 1.5 shoots recorded for AB and LP calli, respectively. As for the effect of subculturing on shoot multiplication, results indicate that, the number of shoots significantly increased with subculturing, giving averages of 5.7, 18.3 and 33.3 shoots/ for the 1st , 2nd and 3rd subcultures, respectively. With respect to the rate of multiplication at the successive subcultures, it was significantly increased from 1.8 shoots at 1st subculture to 3.2 shoots at the 2nd subculture, however it was significantly decreased to 1.7 shoots at the 3rd subculture. This indicates the quick decline in the shoot regeneration capacity of the callus.

Table (3) : Effect of callus source and subculturing on *in vitro* shoot formation of date palm cv. Sewi.

No. of subcultures	0		1		2		3		Average (callus source)	
Callus source	No. of shoots	MR	No. of shoots	MR	No. of shoots	MR	No. of shoots	MR	No. of shoots	MR
Shoot tip (ST)	3.0	-	7.0	2.3	23.0	3.3	40.0	1.7	18.25	1.8
Axillary bud (AB)	3.0	-	5.0	1.6	17.0	3.4	30.0	1.7	13.75	1.6
Leaf primordium (LP)	3.0	-	5.0	1.6	15.0	3.0	30.0	1.7	13.25	1.5
Average (Subcultures)	3.0	-	5.7	1.8	18.3	3.2	33.0	1.7	-	-

MR: multiplication rate

L.S.D. (0.05) **Callus source: (A) 0.40 n.s**
Subcultures : (B) 0.34 0.27
Interaction: (C) 0.20 n.s

Fig. (2) and (3): Types of abnormal plantlets, compared with the normal plantlet (first on left side) at the 2nd subculture (12 weeks old)

From the previously mentioned results related to shoot formation and multiplication, it could be suggested that the presence of auxin type regulators in the callus medium inhibits shoot formation, while cytokinins, like 2, ip, encouraged growth of axillary buds, i.e. shoot multiplication. Fig. (4).

Fig. (4) : Multiplication stages (2nd subculture).

Results presented in Table (4) show the frequencies of ten different morphological off types which were observed at early stage of plantlet life (12 weeks). Fig. (5). As shown in the Table, it is evident that the total percentages of somaclonal variants ranged from 4.3% in ST to 6.03% in AB and 6.10% in LP plantlets. The highest frequencies 6.4% and 3.3% were recorded for types 3 and 9, respectively. Mean values 1.66-1.66-2.3% were recorded for types No. 1,2 and 10 , respectively. The rest types recorded less values ranging from 0.3% (minimum value) for type 6 to 0.6% for type 5 and 7 and 1.0% for types 4 and 8. It could be concluded that the percentage of somaclonal variation by using shoot multiplication protocol, was less than that observed with using somatic embryogenesis protocol.

It this respect Tisserat (1984a) recorded that production of plantlets from shoot tips and buds by organogenesis process is less well developed technique compared to embryogenesis. But producing plantlets of date plam through organogenesis process should be clonal and less risky of genetic variation than callus derived plantlets. Belal *et.al .* (1993) applied shoot multiplication starting from shoot tip explants, isolated from the mother plants of two Egyptian date plam cultivars, (Zaghloul and Semani) using MS medium containing high cytokinin level (30 mg/L 2,ip) and low auxin

level (0.1 mg/L- 1 NAA). Belal and El-Deeb (1997) recorded that using medium containing 0.5 mg/L IAA, 0.5 mg/L NAA, 10 mg/L BAP and 5 mg/L 2, ip enhanced axillary shoot formation, while using hormone free medium stimulated shoot growth.

Table (4): Frequencies of different off types observed at 300 plants at the 3rd subculture of shoot multiplication in date palm.

Off types / Source	1	2	3	4	5	6	7	8	9	10	total	%
Shoot tip (ST)	3	-	3	1	1	-	-	1	2	2	13	4.30
Axillary bud (AB)	1	5	4	2	-	-	-	1	4	2	19	6.03
Leaf primordium (LP)	1	-	7	-	1	1	2	1	5	3	21	6.10
Total	5	5	14	3	2	1	2	3	11	7		
Averages	1.66	1.66	4.60	1.00	0.60	0.30	0.60	1.00	3.30	2.30		
L.S.D. (0.05)	0.87	0.56	1.20	0.56	0.50	0.35	0.43	0.61	0.79	0.75		

Fig. (5): Types of abnormal leaves of date palm (T_1 – T_9) developed at multiplication stages compared with the control normal leaf (C)

Anatomical structure of the leaf:

Cross sections in the first foliage leaves of the seedling (a); and the *in vitro* plantlet developed from somatic embryo (EL) and the *in vitro* developed shoot (SL) were shown in Fig. (6) a,b and c. Measurements of the main anatomical features of those leaves were presented in Table (5) . It is obvious that the seedling leaf was wider, thinner and had thicker cuticle than

both EL and SL leaves. Comparing with the seedling leaf, there was an appreciable increase in thickness of the mesophyll layer, its thickness averaged 112.5μ and 156.5 μ in El and SL leaves; respectively compared with 78.3 μ in the seedling leaf. The mesophyll of the seedling leaf contained more number of tannin containing cells than in both types of *in vitro* leaves. The band of expansion cells in the midrib region of the seedling leaf consisted of a well arranged band of cells with adaxial group of small sclereid cells, whereas in the *in vitro* developed leaves, this band appeared wider and consisted mostly of 3-4 layers of large cells. Concerning the vascular and mechanical tissues, the seedling leaf had more vascular bundles, comparing with *in vitro* developed leaves. However, the diameter of the vascular bundles, including the midrib bundle was larger in the *in vitro* developed leaves than in the seedling leaf. Such difference was mainly attributed to the development of wider xylem vessels under the *in vitro* conditions. There was no appreciable differences between *in vivo* and *in vitro* leaves in relation to number and orientation of the fibrous strands. The previously mentioned observations revealed that the *in vitro* grown leaves were characterized by well developed mesophyll, vascular and mechanical tissues comparing with the *in vivo* seedling leaf. The present findings are contradicting with the results of Al-Salih *et. al.* (1986) who indicated the weak development of the vascular tissues in the *in vitro* developed date plantlets.

Fig. (6): Transections in leaves of the date palm plantlets developed from: a) seedling leaf. (X80).

Fig. (6): (Cont.) (X80)
b) Somatic embryo.
c)*In vitro* shoot.

Table (5): Measurements of certain microscopical features in transverse sections of the leaflet of *in vitro* plantlets and seedling of date plam cv. Sewi. (Averages of 10 readings)

Characters	Seedling leaf	Somatic embryo leaf (LE)	Shoot leaf (SL)
Upper epidermis thickness (μ)	11.6	13	15
Lower epidermis thickness (μ)	11.3	13	15
Cuticle thickness of upper epidermis (μ)	3.13	2.43	1.7
Number of rows of motor cells	2	2	2
Thickness of mesophyll (μ)	78.3	112.5	156.5
Thickness of midrib zone (μ)	103.3	150.7	165.8
Thickness of midrib bundle (μ)	62.9	110.6	114.5
Thickness of xylem area in midrib bundle (μ)	21.9	32.5	37.5
Range No. of xylem vessels in midrib bundle	10-12	9-11	10-12
Thickness of phloem in midrib (μ)	12.5	18.7	12.5
No. of lateral vascular bundles	24	17	14
Thickness of lateral bundles (μ)	33.5	62.5	69.6
Leaf width (mm)	14.0	6.0	8.0

Anatomical structure of the root:

Cross sections in the roots of seedling and *in vitro* developed plantlets are shown in Fig. 7 (a,b,c and d). Measurements of the main anatomical features of these roots presented in Table (6). It is evident, that the primary root of the somatic embryos was characterized by the weak development of parenchyma in the cortex layer which was composed of a lesser number of long lacunae, comparing with layers of well developed lacunae in the seedling root. Moreover the embryo root contained a lesser number of vascular bundles; 7-9, comparing with 14-15 in the seedling root, and finally, the embryo root had no pith and its center was occupied by a metaxylem vessels.

The adventitious roots developed on the regenerated shoots in the rooting medium, were greatly different from those in the seedling root. At the beginning it was shown that some of adventitious roots were abnormal double roots. Such roots appeared in their transections as one root with two vascular steles as being two lateraly united roots. In general, the adventitions roots were characterized by the absence of the cortical parenchyma, lesser number of cortical fibral strands, the weak development of endodermis, pericycle and the vascular tissues, especially the xylem vessels.

The present observation on root structure revealed that the *in vitro* developed roots are characterized with a conspicuous reduction in the development of endodermis and vascular tissues especially the xylem tissue.

These results are in agreement with those observed by Al-Salih *et.al.* (1986) in date palm. In general, it could be suggested that the acclimatization problem of the *in vitro* generated plantlets of date palm may be mainly due to the weak development of the root tissues and not to the modifications in the leaf structure. Ibrahim *et. al.* (1999) mentioned that, survival of regenerated plantlets of Zaghloul cultivar of date palm depends mainly on the root system of the plantlets. They recorded that plantlets

produces from rooting on a medium containing ¾ MS salts, 50 g/L sucrose without activated charcoal, had achieved the best survival percentage.

Fig. (7) : Transections in the root of the date palm plantlets developed from : (X 80)

a) Somatic embryo (primary root)

b) *In vitro* shoot (adventitious root)

Fig. (7): (Cont.) (X80)
c)*In vitro* shoot (adventitious double root)
d) Seedling (primary root)

Table (6): Measurements of certain microscopical features in transections of the root of *in vitro* plantlets and seedling of date palm cv. Sewi. (Averages of 10 readings).

Characters	Seedling root	Embryo root	Adventitious roots of regenerated shoot		
			Single root	Double root	
Root diameter (μ)	787.5	750	184.6	700	
Gortex thickness (μ)	352.4	300.8	74.5	306.6	312.4
No. of cortex layers	20-24	16.19	15-17	20-23	14-16
Exodermis thickness (μ)	47.5	31.3	25.3	48.6	47.8
No. of exodermis layers	6-8	5-6	5-6	7-8	6.-7
Mesodermis thickness (μ)	295.5	263.2	42.8	257.5	263.7
No. of mesodermis layers	15-17	10-13	10-12	15-17	12-14
Endodermis thickness (μ)	9.4	6.26	6.26	-	-
Vascular cylinder diameter (μ)	162.5	150.5	39.3	87.4	75.5
No. of vascular bundles	14-15	7-9	9-10	10-12	7-9
Metaxylem diameter (μ)	49	34.4	6.46	9.4	6.2
No. of lacunae layers	2	1	-	-	-
No of lacunae	50	19	-	-	-
Lacunae area (μ)	36.4	46.9	-	-	-
Pith diameter (μ)	57.5	-	26.6	30.1	27.3

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بعض خصائص الأكثار الدقيق لنخيل البلح

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تمت دراسة تأثير مصدر الكلس (قمة الساق – البراعم الجانبية- بدايات الأوراق) والبيئة (هرمونات النمو) وعدد النقلات على كل من تكون الأجنة الجسدية وتكوين الأفرع الخضرية وبالإضافة إلى ذلك تمت دراسة الخصائص التشريحية للجذور والأوراق والنباتات المتخلقة معملياً ومقارنتها بمثيلاتها الناتجة من البادرات البذرية.

- أشارت النتائج بأن الكلس الناتج من قمة الأفرع الخضرية على بيئة تحتوى على 2مجم/لتر من حمض الأندول بيوتاريك أعطى أفضل تكوين للأجنة الجسدية.
- البيئة الخالية من الهرمونات والبيئة المحتوية على 5 مجم/لتر من (2, ip) لم تحدثا اختلافات فى التباينات الجسمية بينما أعطت على بيئات أخرى اختلافات تتراوح بين 14.42-32.15%.
- عند نقل الأجنة على بيئات خالية من الهرمون فان ذلك أدى إلى تطور 20% من الأجنة الغير طبيعية إلى نباتات طبيعية.
- تم الحصول على أعلى معدل لتضاعف الأفرع الخضرية من الكلس الناتج من قمة الأفرع الخضرية على بيئة تحتوى على 3 مجم/لتر من (2,ip).
- اظهرت المقارنة التشريحية لأوراق البادرات البذرية أنها كانت أكثر أتساعاً وأقل سمكاً ومغطاة بطبقة كيوتيكل أكثر سمكاً عن مثيلاتها الناتجة معملياً سواء من الأجنة الجسدية أو الأفرع الخضرية واحتوى نسيج الميزوفيل لأوراق البادرات البذرية على أعداد أكبر من الخلايا التانينية والحزم الوعائية بينما كان نسيج الميزوفيل فى الأوراق الناتجة معملياً أكثر سمكاً.

أوضحت المقارنة التشريحية لجذور النباتات الناتجة معمليا مع جذور البادرات البذرية أنها كانت ذات باراشيمة أقل تطوراً فى طبقات القشرة التى تكونت من عدد قليل من الفجوات المستطيلة كما كان عدد الحزم الوعائية بها أقل عن جذور البادرات البذرية وكانت أوعية الخشب التالى تحتل مركز الجذور حيث لا يوجد نخاع. وقد تكون هذه التطورات الملحوظة فى جذور النباتات الناتجة معمليا هى السبب الرئيسى فى المشكلات الخاصة بأقلمة تلك النباتات.

Table (1) : Effect of callus source, medium and subculturing on somatic embryogenesis; (normal embryos/Jar); of date palm cv. Sewi.

Media	A (5mg 2,ip)					B (1mg 2,4-D +1mg GA_3)					C (2 mg IBA)					D (1 mg NAA)					E(hormone free)					Averages
Callus source	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4	S_5	
Shoot tip (ST)	4.2	5.9	9.2	8.3	7.1	1.5	1.3	2.7	7.1	9.3	3.2	4.4	8.7	9.2	12.1	2.4	4.2	5.0	5.5	7.8	1.2	2.7	3.8	4.5	5.1	5.4
Axillary bud (AB)	2.4	3.7	8.1	7.9	7.8	1.5	1.4	2.6	5.8	8.2	2.6	4.0	7.6	9.1	10.0	2.4	2.5	3.7	6.0	7.2	1.0	2.7	3.7	4.4	5.0	4.5
Leaf primordium (LP)	1.6	1.1	0.0	0.0	0.0	0.5	0.1	4.2	4.4	5.4	1.6	1.2	2.3	4.1	5.0	0.4	1.3	1.4	3.8	4.4	0.3	0.4	1.7	2.1	2.0	1.9

S = subculture

L.S.D. (0.05)

Callus source	(A)	0.17
Media	(B)	0.23
Subcultures	(C)	0.23
	(AB)	0.40
	(AC)	0.40
	(BC)	0.50
	(ABC)	0.89

	Averages				
Subcultures	1.79	2.46	4.31	5.48	6.42
Media	4.47	3.73	5.67	3.86	2.70

Table (2) : Effect of callus source, medium and subculturing on somatic embryogenesis; (percentage of abnormal embryos/Jar); of date palm cv. Sewi.

Media	A (5mg 2,ip)					B (1mg 2,4-D +1mg GA_3)					C (2 mg IBA)					D (1 mg NAA)					E(hormone free)					Averages
Callus source	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4	S_5	
Shoot tip (ST)	0.0	0.0	0.0	0.0	0.0	33.3	0.0	37.0	16.9	15.0	25.0	4.5	0.0	0.0	0.0	29.0	26.0	26.0	23.6	17.9	0.0	0.0	0.0	0.0	0.0	10.2
Axillary bud (AB)	0.0	0.0	0.0	0.0	0.0	26.6	0.0	26.9	17.2	14.6	23.1	45.0	15.7	5.4	0.0	29.2	24.0	37.8	26.6	29.2	0.0	0.0	0.0	0.0	0.0	12.8
Leaf primordium (LP)	0.0	0.0	0.0	0.0	0.0	20.0	0.0	40.4	47.7	50.0	12.5	50.0	17.4	9.7	8.0	50.0	30.7	50.0	42.8	38.6	0.0	0.0	0.0	0.0	0.0	18.7

S = subculture

L.S.D. (0.05)

Callus source	**(A)**	**0.50**
Media	**(B)**	**0.64**
Subcultures	**(C)**	**0.64**
	(AB)	**1.12**
	(AC)	**1.12**
	(BC)	**1.45**
	(ABC)	**2.50**

	Averages				
Subcultures	16.60	12.00	16.74	12.66	11.55
Media	0.0	23.04	14.42	32.15	0.0

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