

## Evaluation of Fruit Flesh Explants of Zaghoul and Samany Date Palm Cultivars for Micropropagation

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THIS INVESTIGATION began as an attempt to micropropagate the desirable cultivars of date palm (Zaghoul and Samany) beginning from segments of fruits flesh to obtain initial callus, compact callus and meristematic center cells. For this purpose, a study was conducted to define micropropagation protocol based on the cell totipotency to obtain formation of callus. Sodium hypochlorite was very effective for surface sterilization of explants. The best method of surface sterilization was achieved by using treatment containing NaOCl 70% that produced the lowest percentage of contamination 1.33% for Zaghoul and 1.58% for Samany explants by time expanding during sterilization period from 10 to 30 min. It was observed that the highest percentage of callus was produced at Khalal stage than other stages of fruit development of Zaghoul and Samany cv., when culturing fruit flesh in MS medium containing 100mg/l of 2,4-D or 30mg/l of Picloram (Pic). The effect of physiological stage of fruit and growth regulators on callus browning was investigated. The results indicate that the treatment supplemented with Pic (30mg/l) produced the highest amount of total soluble phenols in callus 3.73 mg/100g F.W for Zaghoul at Rutab stage and 3.42 mg/100g F.W for Samany at kimri stage. On the other hand, there were significant differences between all stages on total soluble phenols of callus in Zaghoul. While there was no any significant difference among Khalal and Rutab stage on callus formation from Samany explants. The stages of initial callus formation and callus percentages of date palm (*Phoenix dactylifera L.*) were studied from the 2-10 months-old cultures in MS medium containing 2,4-D or Pic at different concentrations. The study revealed that the compact callus was formed from the friable callus and meristematic center cells. The number of these center cells were unequally distributed inside the compact callus.

**Keywords:** 2,4-D, Picloram, Anatomy, Callus, Date palm, Phenols, Tissue culture

Date palm (*Phoenix dactylifera L.*) is affected by a disease syndrome known as Bayoud, primarily caused by *Fusarium oxysporum* f.sp. *albedinis*, which has already destroyed 3 millions of trees (Djerbi, 1988).

The efficiency of tissue culture for competitive cell line selection is hampered by the loss of cell totipotency in a relatively short time. The cell

proliferation in prolonged time and the maintenance of cell totipotency is an essential step for cell manipulation.

Numerous papers have been published that described somatic embryogenesis regeneration in date palm using various explant sources (Ammar & Benbadiss, 1977, Drira, 1985, Tisserat, 1979 and Scarnec, 1991).

The use of somatic cell genetic techniques such as protoplast regeneration, cell hybridization and cell selection exhibit resistance to applied stress has been widely recognized as a tool to improve species (Karp *et al.*, 1990). To explore the ability to grow plant cells by isolation, an efficient regeneration system is necessary to genetically transform species. Although monocotyledonous species have been shown to be recalcitrant in *in vitro* culture, plants can be regenerated from embryogenic cell suspensions and protoplasts from number of species (Morris *et al.*, 1987).

The first histological study of embryogenic callus in date palm was mentioned by Tisserat & DeMeason (1980). They reported that the embryogenic callus composed of compact aggregates were dispersed among friable tissue. Also, the meristematic clusters probably derived from single cells were embedded in the callus and divided to form loci that acquired polarity when transferred to low auxin medium. Concerning the fruit anatomy, Al-Awdat and El-Deajy (1992) mentioned that the date palm fruits are simple succulent berry. Endocarp is consisting of single layer of small cells which could be seen in an early stage of fruit development. Mesocarp which comprise most of the part of fruit, is consisted of enlarged parenchymatous cells. The mesocarp is divided into outer-mesocarp and inner-mesocarp, between them there are 3-10 layers of tannin ferous cells. The exocarp consists of epidermis covered with cuticle, 3-5 layers of parenchymatous cells and stone cells in radial orientation.

The objective of present investigation is to study the cell totipotency of fruit flesh explants of date palm cultivars Zaghoul and Samany in callus formation at different developmental stages influenced by age of fruit and other determining factors such as different forms and concentrations of auxins.

### Materials and Methods

The explants were collected from two varieties of date palm (*Phoenix dactylifera* L.) namely Zaghoul and Samany grown in Horticulture Reserch Station at El Kanater El Khayreia, Kalubia Gvernorate, Egypt to study the potentiality of fruit flesh in callus formation at different developmental stages viz. Hababok, Kimri, Khalal and Rutab.

#### *Sterilization experiment*

The explants were washed several times with liquid soap and water then placed under running tap water for 1-2 hours. Fruit flesh explants were prepared

3-5 cm in length and were transferred to the laminar flow cabinet for further sterilization process. Sterilization was done by dipping explants in 70 % ethanol for 5 minutes then transferred to different concentrations (15%, 30% & 70%) of sodium hypochlorite (NaOCl) solution for 10, 20 and 30 minutes. The explants were rinsed three times with sterilized distilled water ten minute for each and the contamination percentage was recorded. All tissue culture experiments were carried out during 2011-2013 in the tissue culture Laboratory, Horticulture Research Institute, Giza, Egypt.

The fruit flesh explants were cultured in small jars contained basal nutrient medium Murashige and Skoog (MS, 1962) for different *in vitro* experiments. The MS medium was supplemented with plant growth regulators 2ip (3 mg/L), 2,4-D (10, 50 and 100 mg/L and Pic (10, 20, 30 mg/L). The PH was adjusted at  $5.7 \pm 0.1$  by adding few drops of either 0.1 KOH or 0.1 HCl before sterilization. The cultured explants were incubated at  $27 \pm 2^\circ\text{C}$ . Each treatment contained six replications each replicate has three explants .

#### *Callus formation*

The cultures were incubated in dark at 27 to 29 °C until callus was formed. After 18 to 24 weeks of culture, the fruit explants producing friable and compact callus were subcultured at 4-weeks intervals on the same medium. The data was collected from 24<sup>th</sup> to 36<sup>th</sup> week for callus induction percentage at different fruit growth stages.

#### *Chemical analysis*

Plant samples were obtained for chemical analysis at different stages (Hababok, Kimri, Khalal and Rutab) of Zaghoul and Samany date palm cultivars.

#### *Determination of total soluble phenols*

The colorimetric method of folin-denis as described by Dani and George (1972) was employed for determination of total soluble phenols of fruit flesh tissue samples (0.1g) extracted by boiling in 80% aqueous ethanol and stored in cold conditions for 24h. The extracts were filtered and final solution was made to 50 ml in measuring flask with ethanol 80%. For estimation of total soluble phenols, 0.5 ml of folin-Denis reagent was added to 1 ml of ethanolic tissue extract and the tube was thoroughly shaken for 3 min. Then, 1 ml saturated  $\text{Na}_2\text{CO}_3$  (25% w/v) and 17.5 ml distilled water was added. The mixtures were kept at 30-40°C for one hour. The rate of total soluble phenol absorbance was determined at 730 nm by using Spectrophotometer. The concentrations of total phenols in different samples were calculated as mg/100g FW.

#### *Anatomical study*

The samples of mature fruits were dissected and fixed in 70% FAA solution, then dehydrated with ethyl alcohol series and infiltrate to study fruit anatomy. The samples were subsequently embedded in paraffin wax (M.P. 58°C). According to Willey (1971) by using a rotary microtome, the sections (20  $\mu$ )

were obtained and stained with Safranin and Fast-Green before being mounted in Canada balsam. Finally, the sections were examined microscopically and the different tissues were measured by eyepiece micrometer.

#### *Statistical analysis*

In all experiments the complete randomized design was used. Each treatment was replicated three times and every replicate was represented by six vessels (jars) each vessels had three explants. The data obtained from experiments devoted for studying effect of sterilization, callus production and callus content of phenol subjected to statistical analysis of variance. The separation of means among treatments was determined using LSD test at 5%, according to Steel and Torrie (1980).

### **Results and Discussion**

#### *Sterilization experiment*

This experiment was carried out in order to evaluate the effect of different NaOCl concentrations and times to obtain contamination free cultures of fruit flesh explants of Zaghoul and Samany date palm cultivars.

The data presented in Table 1 shows that the rate of contamination decreased in both cultivars (Zaghoul and Samany) by increasing sterilization time. The lowest contamination percentage was observed 2.39 % in Zaghoul and 2.22% in Samany cultivars at 30 min sterilization time.

The effect of NaOCl concentrations to make explants contamination free showed significant differences among all of them, which resulted in decreasing contamination percentage in explants from 3.83% to 2.06% for Zaghoul and from 3.78% to 2.56% for Samany .

Whereas the interaction effects between NaOCl concentration and the sterilization time, clearly showed that the treatment containing 70% NaOCl produced the lowest contamination percentage for Zaghoul (1.33%) and for Samany (1.50%) by time expanding during steriliation period from 10 to 30 min.

It could be concluded from the results mentioned above that the best survival rate and less contamination percentage was achieved by using 70 % NaOCl (commercial bleach) with the sterilization period of 30 min. These results are similar to those of Shatanawi *et al.* (1997) who used the NaOCl at concentration (1.32 and 2.65%) and mercuric chloride (0.1%) as initial sterilizer for explant sterilization of *Phoenix dactylifera* L. obtained from axillary buds. They reported that the mercuric chloride gave the lowest contaminated buds and the highest survival (91%) followed by NaOCl at 2.65%. Awad (1999) reported that the highest survival percentage (90%) achieved when shoot tip and leaf primordia explants of date palm Sewy cultivar were disinfected with 2.6% NaOCl for 25 min followed by 1.3% NaOCl for 15 min. While, the survival percentage

decreased to 75% when disinfected with 2.6% NaOCl alone for 25 min. Abo-Hatem (2002) found that the best treatment for sterilization, which reduce contamination percentage explants to 12.5%, was achieved by exposing shoot tip explants to double sterilization by NaOCl firstly at 60% for 20 min and secondly at 40% for 15 min, after immersion in 70% ethanol for 5 seconds and 0.1% mercuric chloride (HgCl<sub>2</sub>) for 5 min.. Islam (2007) reported that the sterilization period significantly increased the number of survived explant shoot tips and consequently the rate of contamination decreased for Zaghoul and Samany cultivars. It revealed the significant increase in explants survival rate at sterilization periods of 20 and 25 min as compared to 5 and 10 min by using 25% NaOCl concentration. However, there was no significant difference between 25 and 30 min sterilization period.

**TABLE 1. Effect of different concentrations of NaOCL solution, sterilization time and their interaction on contamination percentage of fruit flesh explants of Zaghoul and Samany date pam cultivars.**

Time Treat	Zaghoul			Avg.	Samany			Avg.
	10	20	30		10	20	30	
15 %	4.50 a	3.67 b	3.33 cd	3.83 a	4.33 a	4.00 ab	3.50 bc	3.78 a
30 %	3.50 bc	2.83 de	2.50 e	2.94 b	3.83 bc	3.00 cd	2.67 de	3.00 b
70 %	2.67 de	2.17 e	1.33 f	2.06 c	3.17 cd	2.00 ef	1.50 f	2.56 b
Avg.	3.56 a	2.89 b	2.39 c		3.94 a	3.17 b	2.22 c	

L.S.D at 0.05

L.S.D at 0.05

Treat. A 0.4663

Treat. A 0.4867

Time. B 0.4663

Time. B 0.4867

Treat x time AB 0.8076

Treat. x time. AB 0.8431

\*Mean in each column, row or interaction has the same letters are not significantly different at 5% level for each cultivar .

*Effect of growth regulators and different fruit stages on callus formation percentage in Zaghoul and Samany date palm cultivars*

The results presented in Table 2 show that callus formed in Zaghoul and Samany cultivar explants was affected significantly by different treatments of growth regulators during different fruit flesh developmental stages. The highest percentage of callus produced (4.42% in Zaghoul and 4.50% in Samany) when fruit flesh explants were cultured on MS medium supplemented with 2,4-D (100mg/l). There were significant differences among all 10mg/l, 50mg/l and 100mg/l concentrations of 2,4-D for both cultivars .

The effect of fruit flesh developmental stages revealed that the highest percentage of callus was produced at Khalal stage as compared to other stages in both cultivars . Regarding the interaction between fruit flesh developmental stages and different concentration of 2,4-D, it was clearly noticed that the treatment containing 2,4-D (100mg/l) produced the highest percentage of callus (5.50%) for Zaghoul followed by 5.33% for Samany cultivar at Khalal stage.

The results presented in Table 3 show that callus formed in Zaghoul and Samany cultivar explants was affected significantly by different treatments of

picloram during different fruit flesh developmental stages. The highest percentage of callus was produced (4.63% in Zaghoul and 3.95% in Samany) when fruit flesh explants were cultured on MS medium supplemented with Pic (30mg/l).

The effect of fruit flesh developmental stages revealed that the highest percentage of callus was produced at Khalal stage as compared to other stages in both cultivars.

Regarding the interaction between fruit flesh developmental stages and different concentrations of Pic, it was observed that the treatment containing Pic (30mg/l) produced the highest percentage of callus 5.00% in Zaghoul and 4.83% in Samany cultivar at Khalal stage .

These results are in agreement with those of Beyl and Sharma (1983) showed that the Picloram was more effective than 2,4-D for callogenesis, embryo induction and final yield of embryos in *Gasteria* and *Haworthia*. Li-Chun and Dauh-Lian (1988) who found that the maximum callus formation from shoot tip of banana (*Musa sapientum*) was achieved with 2,4-D (optimum concentration  $5 \times 10^{-4}$  mM), which was only half of that obtained with Pic at  $5 \times 10^{-3}$  mM. Omar and Novak (1990) noticed that another auxin with properties similar to 2,4-D is Picloram. the Picloram has been successfully applied for callogenesis in date palm.

In this concern, Le-Thi-Lan-Hong *et al.* (1999) indicated that 12 weeks-old cultures on MS basal medium supplemented with 100 $\mu$ M Pic and 9.5 $\mu$ M kinetin produced embryogenic callus with very compact, pale yellow and nodular structures on zygotic embryo and shoot tip explants of *Phoenix canariensis*. Masmoudi *et al.* (2003) found that the callus formation from juvenile leaves and inflorescences of Deglet Nour date palm cultivar required between 4 to 8 months after cultured on MS liquid medium supplemented with 2,4-D (100 mg/l) and activated charcoal (300 mg/l). In addition, the medium was positively affected the differentiation of somatic embryos. Kaur and Kothari (2004) reported that Pic (10mg/l) in combination with kinetin (5mg/l) was found most effective for callus formation on immature inflorescences of Kodo millet (*Paspalum Scrobiculatum* L. cv. GPUK-3) than 2,4-D. Moreover, in addition to pale yellowish callus, the compact embryogenic callus was developed from the cut ends of explant after 2 weeks of culture.

Islam (2007) reported that the treatment containing 30mg/l of Pic and 3mg/l of 2ip produced the highest amount of callus (5.67%) in Zaghoul followed by Samany (5.33%) after 12 week of culture from shoot tip explants. However, there was no significant difference between the callus percentages obtained from Zaghoul and Samany shoot tip explants cultured on the same medium containing 30mg/l of 2,4-D and 3mg/l of 2ip.

**TABLE 2. Effect of different concentrations of 2,4-D, fruit growth stages and their interaction of explants on callus formation percentage of Zaghoul and Samany date palm cultivars.**

Fruit growth Stages	Zaghoul				Samany			
	2,4-D mg/L			Avg.	2,4-D mg/L			Avg.
	10	50	100		10	50	100	
Hababok	3.66 de	2.83 e	3.83 de	3.44 b	2.66 g	2.50 g	3.66 ef	2.94 c
Kimri	3.66 de	3.16 cd	4.00 cd	3.61 b	4.17 cd	3.33 fg	4.33 bc	3.94 b
Khalal	4.66 ab	4.16 bc	5.50 a	4.78 a	5.16 ab	3.83 ef	5.33 a	4.78 a
Rutab	4.50 ab	3.00 de	4.33 b	3.94 b	3.66 ef	3.00 fg	4.66 ab	3.78 b
Avg.	4.13 a	3.29 b	4.42 a		3.92 b	3.17 c	4.50 a	

L.S.D at 0.05                      L.S.D at 0.05  
 Stages. A    0.6713                      Stages. A    0.5711  
 Auxin. B    0.5814                      Auxin. B    0.4946  
 Stag x Aux AB 1.163                      Stag. x Aux. AB 0.9892

\*Mean in each column, row or interaction has the same letters are not significantly different at 5% level for each cultivar .

**TABLE 3. Effect of different concentrations of Pic ,fruit growth stages and there interaction of explants on callus formation percentage of Zaghoul and Samany date palm cultivars.**

Fruit growth Stages	Zaghoul				Samany			
	Pic mg/L			Avg.	Pic mg/L			Avg.
	10	20	30		10	20	30	
Hababok	2.83 f	3.33 ef	4.33 cd	3.50 c	3.00 de	2.66 e	3.16 cd	2.94 c
Kimri	3.16 ef	3.50 ef	4.66 ab	3.78 bc	3.66 cd	3.00 de	4.16 bc	3.61 b
Khalal	5.00 ab	4.66 ab	5.33 a	5.00 a	4.50 ab	3.83 bc	4.83 a	4.39 a
Rutab	4.50 bc	4.33 cd	4.16 de	4.33 b	3.00 de	3.00 de	3.66 cd	3.22 bc
Avg.	3.88 b	3.96 b	4.63 a		3.54 ab	3.12 b	3.95 a	

L.S.D at 0.05                      L.S.D at 0.05  
 Stages. A    0.6034                      Stages. A    0.6034  
 Auxin. B    0.5226                      Auxin. B    0.5226  
 Stag x Aux AB 1.045                      Stag. x Aux. AB 1.045

\*Mean in each column, row or interaction has the same letters are not significantly different at 5% level for each cultivar.

The data in Table 4 shows that the total soluble phenols in callus formed in Zaghoul and Samany date palm explants was affected significantly by different auxin treatments during different stages of explant used in this study.

The effect of fruit flesh developmental stage revealed that the amount of total phenols of callus was increased significantly by different fruit flesh stages were maximum amounts are found in Zaghoul (2.88 mg/100g F.W) and Samany cultivar (3.07 mg/100g F.W) at Rutab stage.

The data presented in Table 4 also show the effect of different concentrations of 2,4-D on total soluble phenols production from callus. It was noticed that the treatment containing 100mg/l of 2,4-D produced the highest amount of total

soluble phenol in callus of Zaghoul (3.49 mg/100g F.W) and Samany (3.17 mg/100g F.W) as compared to other treatments used.

Regarding the interaction between fruit developmental stages and different auxin treatments, it was observed that the treatment supplemented with 100mg/l of 2,4-D produced the highest amount of total soluble phenol in callus of Zaghoul (3.96 mg/100g F.W) at Khalal stage and Samany (3.70 mg/100g F.W) at Rutab stage.

The data presented in Table 5 shows that the total soluble phenols in callus formed from Zaghoul and Samany date palm explants was affected significantly by different Pic-treatments during different stages of fruit flesh explants. The effect of different fruit developmental stages revealed that the amount of total phenols in callus was increased significantly by different fruit flesh stages in Zaghoul (3.16 mg/100g F.W) and Samany cultivar (2.93 mg/100g F.W) at Rutab stage. Moreover, there were significant differences between all the stages of total phenol of callus in Zaghoul, while there was no significant difference between Khalal and Rutab stage for callus formation in Samany explants.

The data presented in Table 5 shows the effect of different concentration of Pic on total soluble phenols production. It was observed that the treatment containing 30mg/l of Pic produced the highest amount of total soluble phenols of callus in Zaghoul (2.96 mg/100g F.W) and Samany (2.92 mg/100g F.W) as compared to other treatments used.

Regarding the interaction between developmental stages and different Pic treatments, it was observed that the treatment supplemented with Pic (30mg/l) produced the highest amount of total soluble phenols of callus in Zaghoul (3.73 mg/100g F.W) at Rutab stage and in Samany (3.42 mg/100g F.W) at Kimri stage.

**TABLE 4. Effect of different concentrations of 2,4-D, fruit growth stages and there interaction of explants on callus content of phenols (mg/100g fresh weight) of date palm Zaghoul and Samany cv.**

Fruit growth Stages	Zaghoul				Samany			
	2,4-D mg/L			Avg.	2,4-D mg/L			Avg.
	10	50	100		10	50	100	
Hababok	2.36 e	2.17 hi	2.96 d	250 d	1.84 i	2.60 ef	2.73 de	2.39 d
Kimri	2.29 gh	2.24 gh	3.32 c	2.61 c	2.01 hi	2.58 ef	3.12 bc	2.57 c
Khalal	2.21 gh	2.03 i	3.96 a	2.74 b	2.21 gh	2.86 cd	3.12 bc	2.73 b
Rutab	2.57 e	2.35 eg	3.70 b	2.88 a	3.13 b	2.39 fg	3.70 a	3.07 a
Avg.	2.36 b	2.20 c	3.49 a		2.30 c	2.61 b	3.17 a	

L.S.D at 0.05

L.S.D at 0.05

Stages. A 0.0818

Stages. A 0.1515

Auxin. B 0.0708

Auxin. B 0.1312

Stag x Aux AB 0.1417

Stag. x Aux. AB 0.2623

\*Mean in each column, row or interaction has the same letters are not significantly different at 0.05% level for each cultivar

**TABLE 5. Effect of different concentrations of Pic ,fruit growth stages and there interaction of explants on callus content of phenols (mg/100g fresh weight) of date palm Zaghloul and Samany cv.**

Fruit growth Stages	Zaghloul				Samany			
	Pic mg/L			Avg.	Pic mg/L			Avg.
	10	20	30		10	20	30	
Hababok	2.27 f	2.61e	2.16 fg	2.35 d	2.14 ef	2.43 de	2.08 fg	2.22 c
Kimri	2.62 e	2.19 fg	2.90 cd	2.57 c	1.82 g	2.12 fg	3.42 a	2.45 b
Khalal	2.07 g	3.06 b	3.03 bc	2.71 b	2.58 d	2.73 cd	3.03 bc	2.78 a
Rutab	2.82 d	2.92 cd	3.73 a	3.16 a	2.53 d	3.12 ab	3.16 ab	2.93 a
Avg.	2.44 c	2.70 b	2.96 a		2.27 c	2.60 b	2.92 a	

L.S.D at 0.05

L.S.D at 0.05

Stages. A 0.08179

Stages. A 0.1855

Auxin. B 0.07084

Auxin. B 0.1606

Stag x Aux AB 0.1410

Stag. x Aux. AB 0.3213

\*Mean in each column, row or interaction has the same letters are not significantly different at 5% level for each cultivar .

These results are in agreement with Forrest (1969) showed that phenolics, especially the most common polyphenol, cause oxidative browning in explants, which lead to discoloration of the culture medium. Blake and Euwens (1982) found that the 2,4-D was the most effective for callus formation in date palm as compared to other auxins. However, Fitch *et al.* (1983) found that the Picloram produced more phenolics from cut surfaces and was slower for callogenesis than 2,4-D in sugarcane.

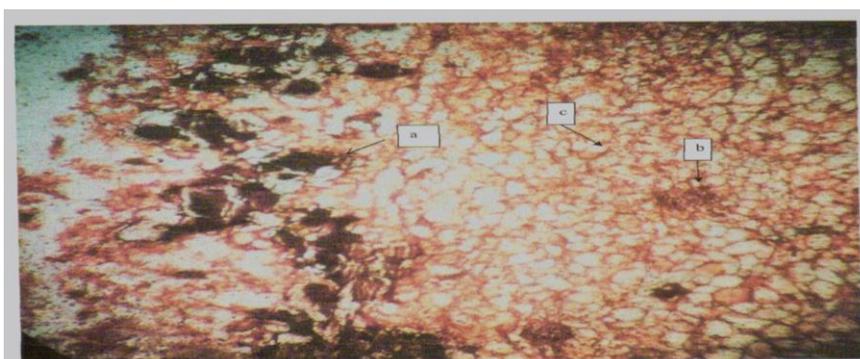
Sugimura and Salvana (1989) obtained similar results in coconut inflorescence explants of 1 mm in size, which showed 32% browning compared to 11% browning in 0.5 mm thick coconut inflorescence explants. Preece and Compton (1991) reported the synthesis of phenolics, tannins or oxidized polyphenols and flavonoid through shikimic acid pathways. Moreover, they stated that these substances are abundantly present in some plants and act as inhibitory agents. Furthermore, Bhat & Chandel (1991) and Trautmann & Visser (1991) reported that the oxidized phenolic compounds which frequently exuded into the medium by injured woody tissues cause lethal browning or blackening of explants. Jesty and Francis (1992) who reported that auxin at high doses (5mg/l or 3mg/l) was necessary for callus induction in palm, especially on medium supplemented with 1-3g/l AC.

Abed El-Hamid (2002) revealed that there is negative correlation between dry weight and flavonoids or total phenols content of callus derived from shoot tip tissues of Sewy date palm cultivar. He reported that the highest value obtained was 0.412mg/100g total phenols and 0.1797mg/100g total Flavonoids under light condition. The lowest value obtained was 0.0921 mg/100g total phenols and 0.421 mg/100g total flavonoids under dark condition. Sukanto (2011) in his investigation for the factors that effect browning content of antipodal and micropylar of coconut endosperm tissues showed that the

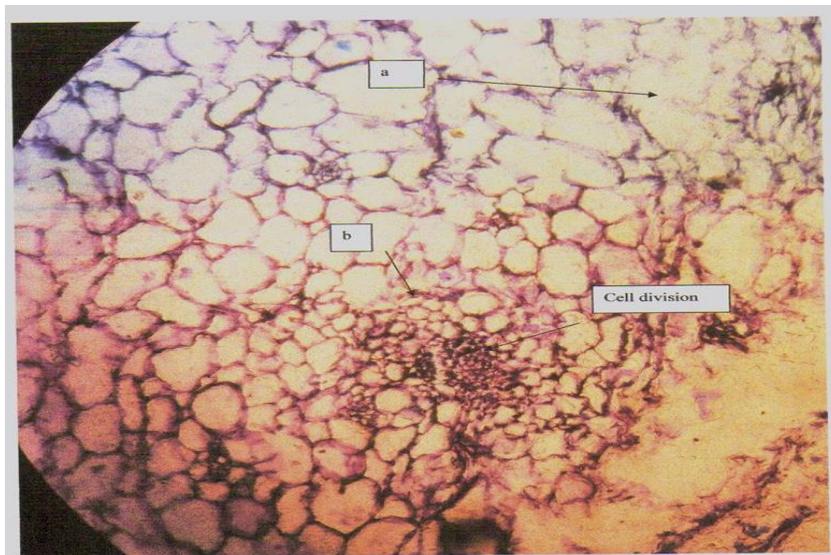
browning in antipodal tissues was more significant than micropylar tissues after 9, 26, and 31 weeks of culturing on MS medium supplemented with different concentrations of 2,4-D and Picloram. He reported that it could be due to antipodal tissues which were earlier formed (older) and thicker than micropylar tissues.

#### *Histological investigations*

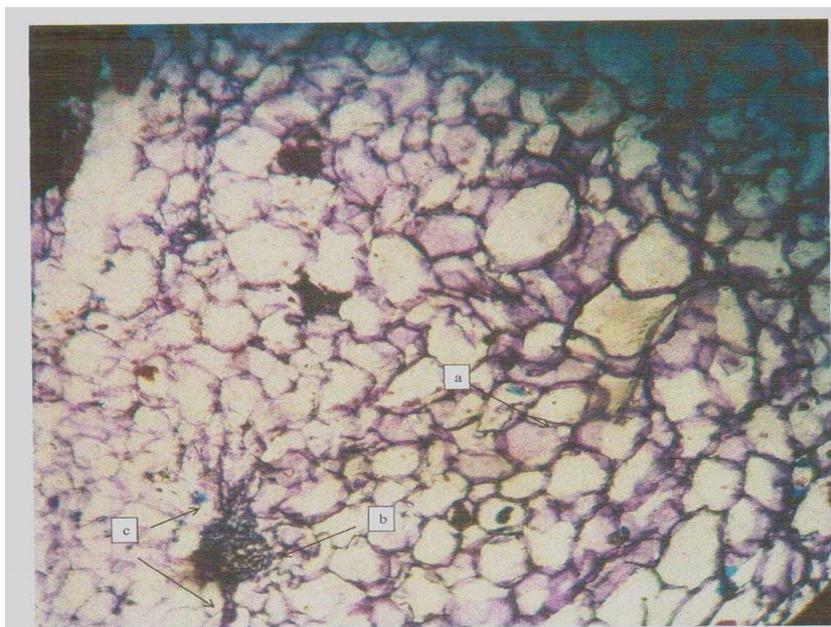
One to three months-old cultures on MS medium supplemented with 2,4-D (100mg/l) or Picloram (30mg/l) showed that the initial callus started its growth to friable callus formation in the mesocarp. Whereas, after four to six months of culturing in the same medium the compact callus was formed from the friable callus and meristematic center cell emerge from the outer mesocarp in both the cultivars. The number of meristematic centers were increased in Zaghoul than Samany cultivar. However, most of the meristematic centers were formed inside the compact callus masses as shown in Fig 1- 5. These results are coincided with Williams and Maheswarian (1980) who suggested that the indirect embryogenesis requires redetermination of differentiated cells & callus proliferation. The development of embryogenically determined cells require growth regulators not only for re-entry into mitosis but also for determination of the embryogenic state. In addition, Abdumunain (2003) found that the number of meristematic centers were increased in compact callus masses. Sakr *et al.* (2010) reported that the highest value for maximum thickness of stone cell layers was 132 $\mu$  in Kuboshy, while the lowest value was 94 $\mu$  in Hayany cultivar. the stone cells were found in continuous layers in all cultivars (Samany, Amhat, Bint-Aisha, Hayany, Amry and Kuboshy) under investigation except in the Zaghoul cultivar, where they were found in groups (discontinuous), this character is unique to Zaghoul cultivar. The highest average thickness of outer-mesocarp was recorded 1.68, 1.14 and 1.05 mm in Kuboshy, Amhat and Zaghoul respectively. Whereas, the lowest values were noticed in Bint-Aisha (0.37 mm) and Hayany (0.49 mm) respectively. The average thickness of tannins region between outer and inner-mesocarp ranged 0.31 to 1.05 mm in Zaghoul and Samany cultivars respectively .



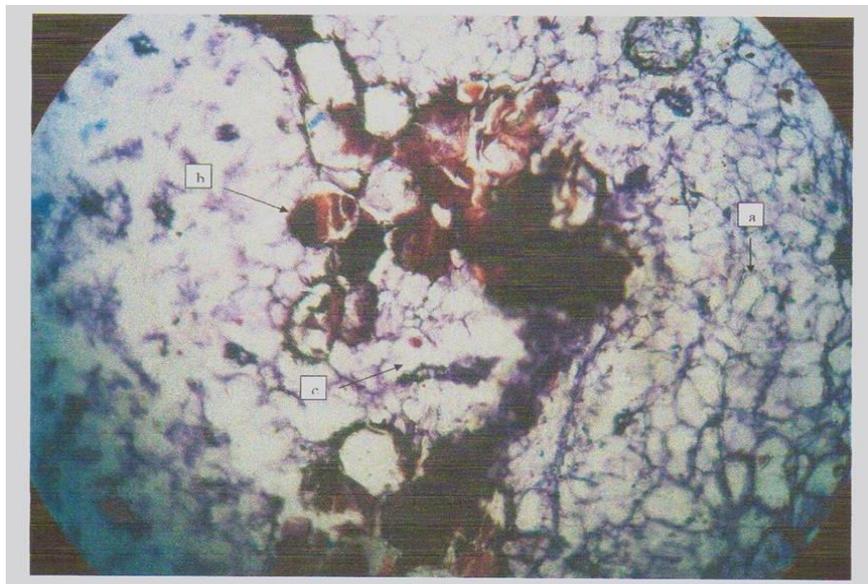
**Fig. 1. Formation of callus from wounded surface of flesh fruit Zaghoul cv. Snowea tannins centers (a) and some meristematic centers (b) inside the compact callus (c) magnified X= 200**



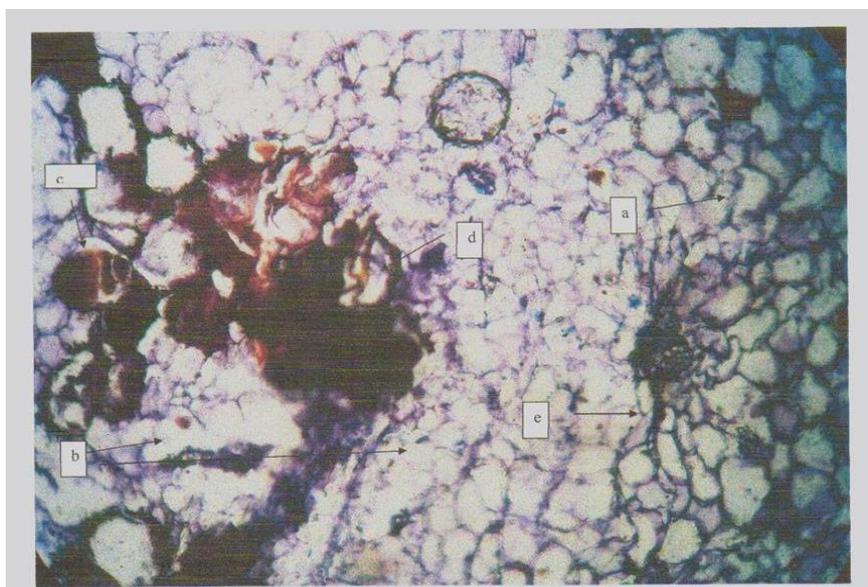
**Fig. 2.** A Cross section of callus growth showed of flesh fruit (mesocarpe) of Zaghoul c.v. magnified x= 400



**Fig. 3.** The observers section at the compact callus (a) of flesh fruit (mesocarpe) of Zaghoul c.v. illustrated some meristematic centers (b) inside the compact callus, that developed into organs (c) magnified x= 400



**Fig. 4.** A cross at the compact callus (a) of Samany flesh showed the stone cells (b) and the friable callus (c) that separated a part of the compact callus by a necrotic area magnified  $\times= 400$



**Fig. 5.** A cross section at the callus of flesh fruit Samany cv showed the two types of callus (a: compact callus and b: friable callus ), stone cells (c) the separated zone (d) , some meristematic centers that developed into organs (e) magnified  $\times= 400$

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### تقييم المنفصلات النباتية لحم الثمرة لصنفى نخيل البلح الزغول والسمانى للأكثر الدقيق

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أجريت هذه الدراسة لمحاولة الأكثر النقيق لنخيل البلح صنفى الزغول والسمانى باستخدام أجزاء لحم الثمرة للحصول على بداية الكالس والكالس المتناسك الذى يحتوى بداخله المراكز المرستيمية. لهذا الغرض تم وضع برتوكول يعتمد على قدرة الخلية الجسدية على تكوين الكالس من المنفصلات النباتية لحم الثمرة.

أوضحت النتائج أن صوديوم هيبوكلوريت أكثر تأثيراً للتعقيم السطحي للأجزاء النباتية. كانت أفضل طريقة للتعقيم السطحي هى المعاملة بـ ٧٠٪ صوديوم هيبوكلوريت حيث أعطت أقل نسبة تلوث ١,٣٣٪ للزغول و ١,٥٨٪ للسمانى عند زيادة وقت التعقيم من ١٠ إلى ٣٠ دقيقة. أظهرت تأثير مرحلة تطور الثمار أعلى نسبة تكون كالس كانت عند مرحلة الخلال أكثر من المراحل الأخرى لزغول والسمانى عند زراعة لحم الثمرة على بيئة تحتوى على ١٠٠مجم/لتر ٤,٢ داى كلوروفينوكسى أسيتك أسيد أو ٣٠مجم/لتر بيكلورام على التوالى. تم دراسة إمكانية المرحلة الفسيولوجية لنمو الثمرة وتأثير منظمات النمو على التلون البنى للكالس. أشارت النتائج إلى أن المعاملة بـ ٣٠مجم/لتر بيكلورام أدت للحصول على أعلى كمية من الفينولات الذاتية فى الكالس ٣,٧٣ مجم / ١٠٠ جرام مادة رطبة بالنسبة للزغول عند مرحلة الرطب و ٣,٤٢ مجم / ١٠٠ جرام مادة رطبة بالنسبة للسمانى عند مرحلة الكمرى. من ناحية أخرى يوجد اختلافات معنوية بين كل المراحل فى الزغول فى محتوى الفينولات الكلية الذاتية، بينما لا يوجد اختلافات معنوية بين مرحلتى الخلال والرطب فى الكالس المتكون من أجزاء السمانى. مراحل بداية تكون الكالس ونسبة الكالس تم دراستها تشريحياً بداية من شهرين وحتى ١٠ شهور من زراعة لحم الثمرة فى بيئة مورايشجى وسكوج المحتوية عند التركيزات المختلفة. أشارت الدراسة لأن الكالس المتناسك والمراكز المرستيمية تتكون من الكالس الرخو. عدد هذه المراكز المرستيمية غير متساوى فى التوزيع داخل الكالس المتناسك.

**الكلمات المفتاحية:** بيكلورام - داى كلوروفينوكسى أسيتك أسيد - تشريح- كالس - نخيل البلح-فينول- زراعة الأنسجة.