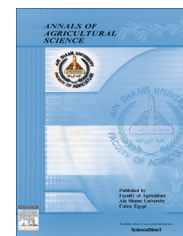




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Morphogenesis of immature female inflorescences of date palm *in vitro*



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Histology

Abstract Plant regeneration from immature female inflorescences of date palm (*Phoenix dactylifera* L.) cultivar Siwi (semi-dry cv.), via somatic embryogenesis was achieved. Immature inflorescences explants were cultured on Murashige and Skoog (MS) medium supplemented with TDZ at 1.0 mg/l. Endogenous hormones (GA₃, IAA, Zeatin and ABA), total soluble sugars (reducing and non-reducing sugars), free amino acids, indoles and phenols were determined. The levels of GA₃ and IAA in explant reached the highest values and then decreased in callus stage. Zeatin, IAA, and ABA levels were higher in embryogenic callus. GA₃ and zeatin in mature somatic embryo maintained a relatively high level while IAA and ABA dropped. The highest significant values of total soluble sugars and their fractions were noted in explant stage. Embryogenic callus stage induced a significant increase in total soluble sugars, free amino acids, phenols and indoles concentrations as compared to callus and mature somatic embryo. Callus induction was achieved by TDZ after 4–5 weeks of culture. The callus differentiated into embryogenic calli on free cytokinin medium. Light microscope observations revealed that the callus consisted of two different types of cells, soft vacuolated cells which did not implicate in embryo formation and compact aggregate cell masses which have the ability to generate the somatic embryos.

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Introduction

The date palm (*Phoenix dactylifera*) is a perennial dioecious ‘tree’ of the Arecaceae family, and has a great value particularly in North Africa and the arid regions of the Middle East by its economic importance and environmental impact (Kriaa et al., 2012). Traditionally, palm groves were planted

from off-shoots produced by the date palms in the early part of their life. *In vitro* micropropagation thus soon became an essential and effective means to ensure the renewal and the extension of palm plantations (Smith and Aynsley, 1995). For some years the vegetative propagation of date palm by *in vitro* culture has been the subject of numerous studies using techniques such as somatic embryogenesis and organogenesis (Sharma et al., 1986; Bouguedoura et al., 1990; El Hadrami et al., 1995). In most cases the cultured explants were taken from young tissues of basal offshoots. Use of inflorescences as starting material has been rarely described by Drira and

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Benbadis (1985), Bhaskaran and Smith (1992), Loutfi and Chlyah (1998); and Ebigman (1999) indicated that, micropropagation technique of date palm using floral tissues depending on culturing floral spicks in early stage in growth onto culture media aids to convert these tissues from floral state to vegetative one. These tissues are considering alternative source of tissues derived from offshoots without the risk of a definitive loss, particularly of the head clone genotypes limited only to one plant. Zayed (2011) tested many female inflorescences in different stages of development as explants and cultured on different concentrations of plant growth regulators. The results indicated that the most responsive starting material for initiation embryogenic cultures was the immature female inflorescence with TDZ. Plant growth regulators have a major influence on tissue culture success as they are involved in the regulation of cell division, tissue and organ differentiation (Jennifer et al., 2010). However there is no consistent result published for discussing the relationship between the levels of endogenous plant hormones and reversion of the floral state of date palm cv. Siwi into vegetative state. So that, this study aimed to make a correlation between hormonal, biochemical and morphogenesis changes *via* somatic embryogenesis and to better understand physiological and histological states of the explant (female inflorescences and its development to somatic embryogenesis).

Materials and methods

The present study was performed during the years of 2013–2014 at the Central Laboratory of Date Palm Researches and Development, Agricultural Research Center (ARC), Giza, Egypt, and Agricultural Botany Department, Faculty of Agriculture, Ain Shams University. This investigation was conducted to study the physiological and histological characters which concerning with each stage via somatic embryogenesis of immature female inflorescences of *P. dactylifera* cv. Siwi.

Plant material and tissue culture protocol

Immature female inflorescences of date palm (*P. dactylifera* L.) cv. Siwi were taken from adult female trees which grow in the field of agriculture ministry at El-Badarashein, Egypt. The explants were collected at 15–30th January the average spathe length 6–7 cm. Explants sterilization is performed by soaking in 40% of commercial clorox (5.25% sodium hypochlorite) for 20 min and then rinsed with sterilized distilled water three times, and then protective sheath and part of base were removed. Sterilized inflorescence explant was divided longitudinally into 2–3 equal segments (spikes with part of inflorescence base) for use as explants as described by Zayed (2011).

Explants divided longitudinally into 3–4 segments each explant was cultured individually in each jar. The explants were cultured horizontally with a good contact with the surface of the best basal inductive culture medium used in our present study which was chosen from previous studies made by Zayed (2011). The culture medium contains of basic salts and vitamins of Murashige and Skoog (1962) supplemented with sucrose (3%) used as carbon source, 200 mg/l glutamine, 40 mg/l adenine sulfate and TDZ 1.0 mg/l. The pH was adjusted to 5.7 ± 0.1 before autoclaving. Media were

dispensed into culture small jars (150 ml) at the 35 ml/jar and were capped with polypropylene closures. Medium was then autoclaved for 20 min at 121 °C 15 lb/in²; the jars were autoclaved for 20 min at 121 °C 15 lb/in². Each treatment contained 3 replicates and each replicate contained one explant. Cultures were incubated in temperature controlled room at 27 ± 2 °C under darkness and transferred to fresh media every 6 weeks.

Biochemical components

Four samples were taken at different morphogenesis stages (immature female inflorescences, callus, embryogenic callus and mature somatic embryos) of date palm cv. Siwi for the endogenous plant hormones (GA₃, IAA, Zeatin and ABA) and chemical (total soluble sugars, free amino acids, total phenols and indoles) analyses.

Determination of phytohormones

Phytohormones (GA₃, IAA, Zeatin and ABA) analysis was carried out according to the following procedures: the extraction procedure was followed as described by Shindy and Smith (1975). GA₃, IAA, Zeatin and ABA were estimated by HPLC.

Total soluble sugars estimation

Determination of total soluble sugars, reducing and nonreducing sugars was carried out according to the method of Shales and Schales (1945).

Determination of free amino acids

Total amino nitrogen or free amino acids were determined according to the method of Jayraman (1984).

Determination of total indoles

The total indoles were determined according to Larsen et al. (1962).

Determination of total phenols

Phenols determination was carried out according to Malik and Singh (1980).

Histological study

Plant samples were harvested for histological study at different morphogenesis stages (immature female inflorescences, callus, embryogenic callus and mature somatic embryos) of date palm cv. Siwi. All previous samples were killed and fixed in FAA solution (Formalin, acetic acid and 50% ethyl alcohol, 5:5:90 by volume) for 24 h. The schedule of the paraffin method as described by Johansen (1940) was followed. Serial transverse and longitudinal sections (6–8 μm) in thickness were made by LEICA rotary microtome model RM 2125 RTS and fixed on slides by means of Haupt's adhesive. The sections were stained with a Safranin–Fastgreen combination, and then mounted in Canada balsam (Sass, 1951).

Anatomical examination and photomicrograph were achieved using OPTICA binocular model SZM-2 and a LEICA light microscope research (microscope model DM 2500 supplied with a digital camera).

A complete randomized design with three replicates was used, using L.S.D. test at 5% according to [Snedecor and Cochran \(1989\)](#).

Results

Phytohormones

Concerning different morphogenesis stages ([Table 1](#)), the highest value of endogenous gibberellic acid (GA_3) was recorded by female inflorescences explant (18.88 mg/100 g FW) followed by callus, embryogenic callus and mature somatic embryo respectively (3.96, 1.28 and 0.113 mg/100 g FW).

Regarding endogenous auxin (IAA), data clearly revealed that the highest value of endogenous auxin (896.9 μ g/100 g FW) was observed with female inflorescences explant while the mature somatic embryo produced the lowest value (8.2 μ g/100 g FW). There was a gradual increase in endogenous auxin from callus stage toward the embryogenic callus stage (32.63–204.1 μ g/100 g FW).

In developmental stages, a gradual increase in endogenous zeatin was noticed from immature female inflorescence to the

embryogenic callus stages (14.26, 209.04, 212.66 μ g/100 g FW). Then, mature somatic embryo decreased in endogenous zeatin as compared to callus and embryogenic callus stages.

As for endogenous ABA (Abscisic Acid), endogenous ABA in immature female inflorescence and embryogenic callus was found with large amounts (30.79 and 50.01 μ g/100 g FW) when compared to other developmental stages (callus and mature somatic embryos; 8.93 and 17.34 μ g/100 g FW). The embryogenic callus produced the highest value of endogenous ABA when compared with the immature female inflorescence.

Biochemical components

[Table 2](#) indicates levels of total sugars, reducing and non-reducing sugars in different morphogenesis stages. Immature inflorescence female stage led to significant increase in total soluble sugars and fractions (reducing and non-reducing sugars) as compared to the other developmental stages. The lowest significant value of total soluble sugars and fractions was showed with callus stage as compared with the explant stages. The level of total soluble sugars and fractions is decreasing from callus stages (3.8, 0.9 and 2.9 mg/g DW) and then increasing in embryogenic callus and mature somatic embryo stages (6.9, 2.4, 4.5 and 6.3, 2.6, 3.7 mg/g DW respectively).

Data in [Table 3](#) showed levels of free amino acids, indoles and phenols in different morphogenesis stages *in vitro*. It could be

Table 1 The levels of endogenous hormones in the immature female inflorescence and its subsequent developmental stages of date palm cv. Siwi *in vitro*.

Morphogenesis stages	GA_3 (mg/100 g FW)	IAA (μ g/100 g FW)	Zeatin (μ g/100 g FW)	ABA (μ g/100 g FW)
Imm. Fem. Inflore.	18.880	896.909	14.261	30.793
Callus	3.961	32.63	209.04	8.93
Embryogenic callus	1.281	204.11	212.66	50.01
Mature somatic embryo	0.113	8.20	68.39	17.34

Imm. Fem. Inflore. = Immature female inflorescences.

Table 2 The levels of total sugars, reducing sugars and non-reducing sugars in the immature female inflorescence and its subsequent developmental stages of date palm cv. Siwi *in vitro*.

Morphogenesis stages	Total soluble sugars (mg/g DW)	Reducing sugars (mg/g DW)	Non-reducing sugars (mg/g DW)
Imm. Fem. Inflore.	17.2	7.0	10.2
Callus	3.8	0.9	2.9
Embryogenic callus	6.9	2.4	4.5
Mature somatic embryo	6.3	2.6	3.7
L.S.D.	4.9	1.3	0.02

Imm. Fem. Inflore. = Immature female inflorescences.

Table 3 The levels of free amino acids, indoles and phenols in the immature female inflorescence and its subsequent developmental stages of date palm cv. Siwi *in vitro*.

Morphogenesis stages	Free amino acids (mg/g DW)	Indoles (mg/g FW)	Phenols (mg/g FW)
Imm. Fem. Inflore.	3.9	1.0	0.4
Callus	0.6	1.3	0.5
Embryogenic callus	4.6	1.6	0.5
Mature somatic embryo	0.9	0.9	0.3
L.S.D.	2.5	0.03	0.1

Imm. Fem. Inflore. = Immature female inflorescences.

noticed from Table 3 that the embryogenic callus and immature female inflorescence stages showed a significant increase in free amino acids concentration comparing with the other developmental stages (Callus and mature somatic embryos stages).

Regarding the indole and phenol concentrations, data revealed that embryogenic callus and callus stages were the most effective in increasing indole and phenols. On the other hand, the other developmental stages (immature female inflorescence and mature somatic embryos) showed reduction in this concern.

Histological study

Structure of the immature female inflorescence

Morphology of the immature female inflorescence shows that the flower primordia and their subtending bracts firstly appear at the base of rachillae and are initiated solitary in an acropetal sequence as the rachillae elongate (Fig. 1a). Light microscope observations revealed that the immature pistillate flowers appear as small mass of meristematic cells subtending with small bracts (Fig. 1b and c). These masses have only a single distinct tunica layer covering the corpus. All lateral primordial appears to be initiated by periclinal divisions in the peripheral zone under the tunica.

Callus induction

Since the explants were cultivated on induction media supplemented with TDZ, remarkable morphogenetic transformations were observed. After three weeks of culture, the immature pistillate flowers went a slight increase in size. Subsequently, callus induction was achieved after 4–5 weeks of culture. This callus has a translucent appearance with irregular borders. It grows all over the tissues of immature pistillate flowers (Fig. 1d). It was initiated from the epidermal and subepidermal layers (Fig. 1e). Occasionally the callus consist separated soft fragments. At the end of the fifth week most of the pistillate flowers with their growing callus masses have been separated from the inflorescence axis and fall on the media (Fig. 1f and g).

Ontogeny and development of somatic embryos

These bulky masses of callus resulting from the flowers after its splitting from the rachillae were transformed onto free cytokinin medium for 6 weeks. During this time, small parts of this translucent calli evolved and acquired new abilities to differentiate into embryogenic calli. The latter consisted of granules of different sizes, usually white in color and attached or separated by undefined borders. They are characterized by regular surfaces and hard consistencies (Fig. 2a). Light microscope observations revealed that the callus consisted of two different types of cells, soft vacuolated cells and compact aggregate ones. The friable portion of the callus was composed of disorganized masses of highly vacuolated cells ranging in diameter from 30 to 60 μm . This tissue did not implicate in embryo formation and was generally found surrounding by the aggregate masses that serve as the source of embryooids. These masses were composed of meristematic and rich cytoplasmic cells from 6 to 25 μm in diameter. All of the embryonic phases could be observed in these aggregations from a single cell, 2 celled, 4 celled, globular to bipolar structures (Fig. 2c–f). Then the last

stage “the mature somatic embryo” could be achieved at the end of 4th to 6th week of the second subculture (Fig. 2g).

Discussion

Differences between immature female inflorescence and subsequent developmental stages in endogenous hormone concentrations and exogenous TDZ could explain these different morphogenetic responses. The findings presented in the current study revealed that the immature female inflorescence contained the highest level of GA₃. This result matched well with Liu et al. (2008), who observed that the high GA levels coincided with the presence of floral primordia. This increase in gibberellins appeared to play an important role in inflorescence development. Also, they reported that ABA increased in the same stage to maintain a certain balance between the inhibitor level and GA-like substances for controlling the growth rate of inflorescence and floral parts. Al-Khassawneh and Karam (2006) reported that exogenous GA₃ promoted flowering in the black iris (*Iris nigricans* Dinsm.), which may be attributed to the growth-promotion effect of GA₃ in stimulating and accelerating cell division, increasing cell elongation and enlargement, or both. Gibberellins can be affected by TDZ. The latter could mediate endogenous GA and stimulated somatic embryogenesis in many species by GA-synthesis inhibitors (Hutchinson et al., 1996; Murch and Saxena, 1997).

Data presented here showed that, endogenous IAA reaches to its highest level in immature female inflorescences followed by embryogenic callus and then decreased reaching to its lowest level in mature somatic embryos. These data are in agreement with George et al. (2008) who reported that auxin is abundant in young leaves, developing floral organs since the meristematic cells occurred. High levels of IAA were necessary for adequate histo-differentiation in early stages of development. Also, immature somatic embryos contain the highest concentration of IAA, during the early development phase and decrease in mature embryos (Zein El Din, 2010).

Requirement of auxin or other plant growth regulators for the initiation of somatic embryogenesis is largely determined by the developmental stage of the explant tissue (Kutschera, 1994). In geranium hypocotyl and peanut seedlings, TDZ modulated endogenous auxins, modifying their biosynthesis and consequently inducing the differentiation of somatic embryos (Visser et al., 1992).

The obtained data revealed that callus which cultured on TDZ medium contains the lowest levels of endogenous ABA. The endogenous ABA level has been increased again in the embryogenic callus. These results are in harmony with Zein El Din (2010) who found that embryogenic callus of date palm contained much higher levels of endogenous ABA and declined in the individual somatic embryo. Similar results have been reported in carrot where the endogenous levels of ABA increased during induction of embryogenesis (Kamada and Harada, 1981). ABA promotes normal development of somatic embryos *in vitro* by stimulating reserve substance accumulation and inhibiting precocious germination. Manipulation of endogenous and/or exogenous ABA levels increases the frequency of embryos reaching maturity and can assist the handling of the large populations of somatic embryos which can be required for mass propagation (Ammirato, 1988).

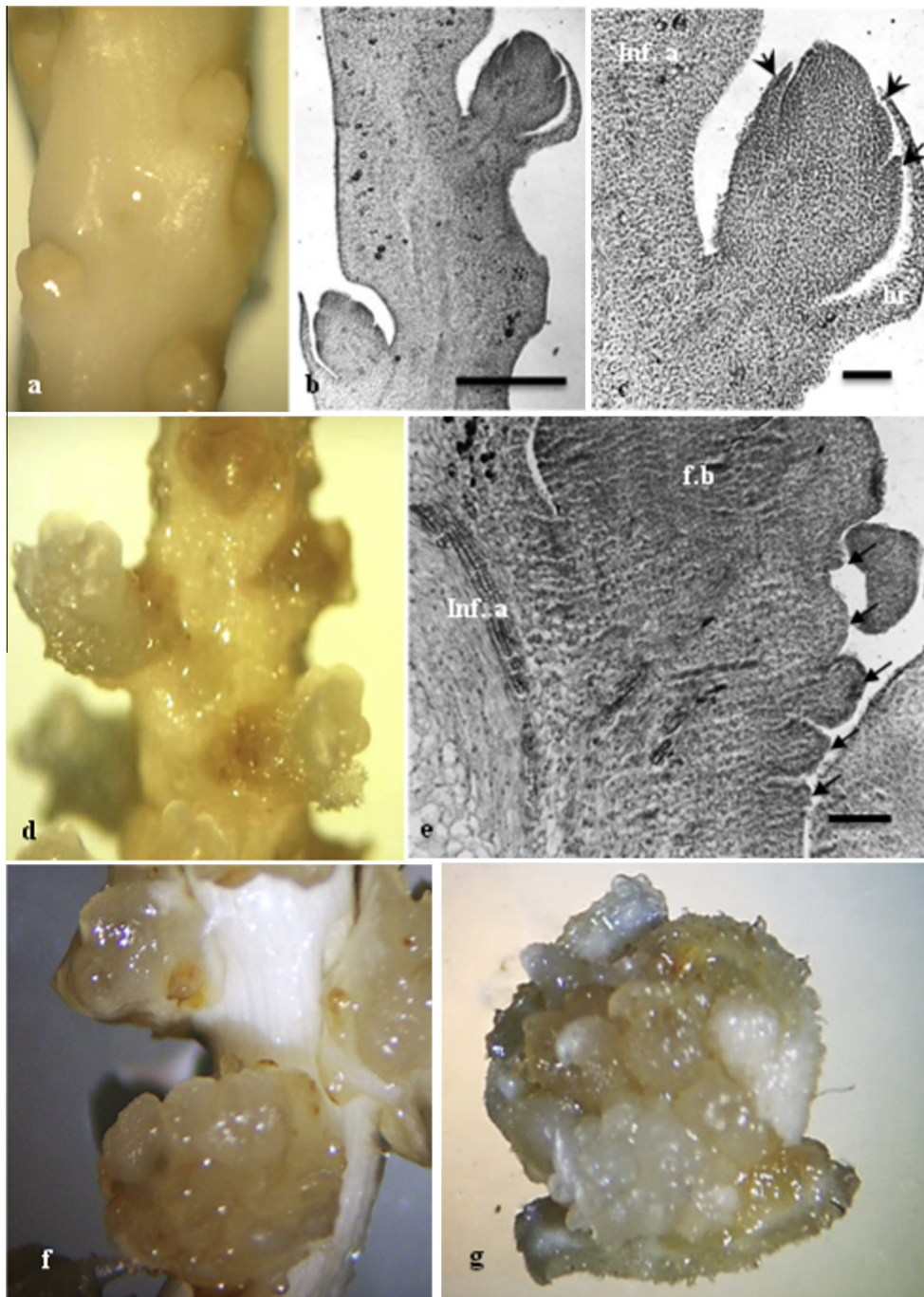


Fig. 1 Morphology and anatomy of immature female inflorescence of date palm cv. Siwi (a–c) and callus induction (d–g). (a) Morphology of floral buds on rachilla of immature female inflorescence. (b) Longitudinal section of rachilla showing the meristematic state of the floral bud subtending with small bract. (c) Magnified part of (b), and arrowheads indicate tepals primordia. (d) Two to three weeks after culturing on media supplemented with TDZ showing development of callus all over the floral buds with increasing in flower size. (e) Longitudinal section in the same stage illustrating the initiation of callus from the epidermal and subepidermal layers of the floral buds, and the arrows indicate small callus masses arise from the floral bud. (f) Bulky masses of callus resulting from the flowers after 4–5 weeks culturing. (g) One floral bud separated from the inflorescence axis with numerous callus masses at the end of 4–5 weeks of culturing. Bar = 300 μ m in (b) and 100 μ m in (c and d). Magnification in a, d, f and g: X15. Abbreviations: br, bract; inf. a, inflorescence axis; f. b, floral bud.

The present data showed that endogenous zeatin concentration was at its lowest level in the explant “immature inflorescence” then, increased at the subsequent developmental

stages and reached to its highest concentration at embryogenic callus. Exogenous supply with TDZ is responsible for the remarkable increases in zeatin which indicates the active extend

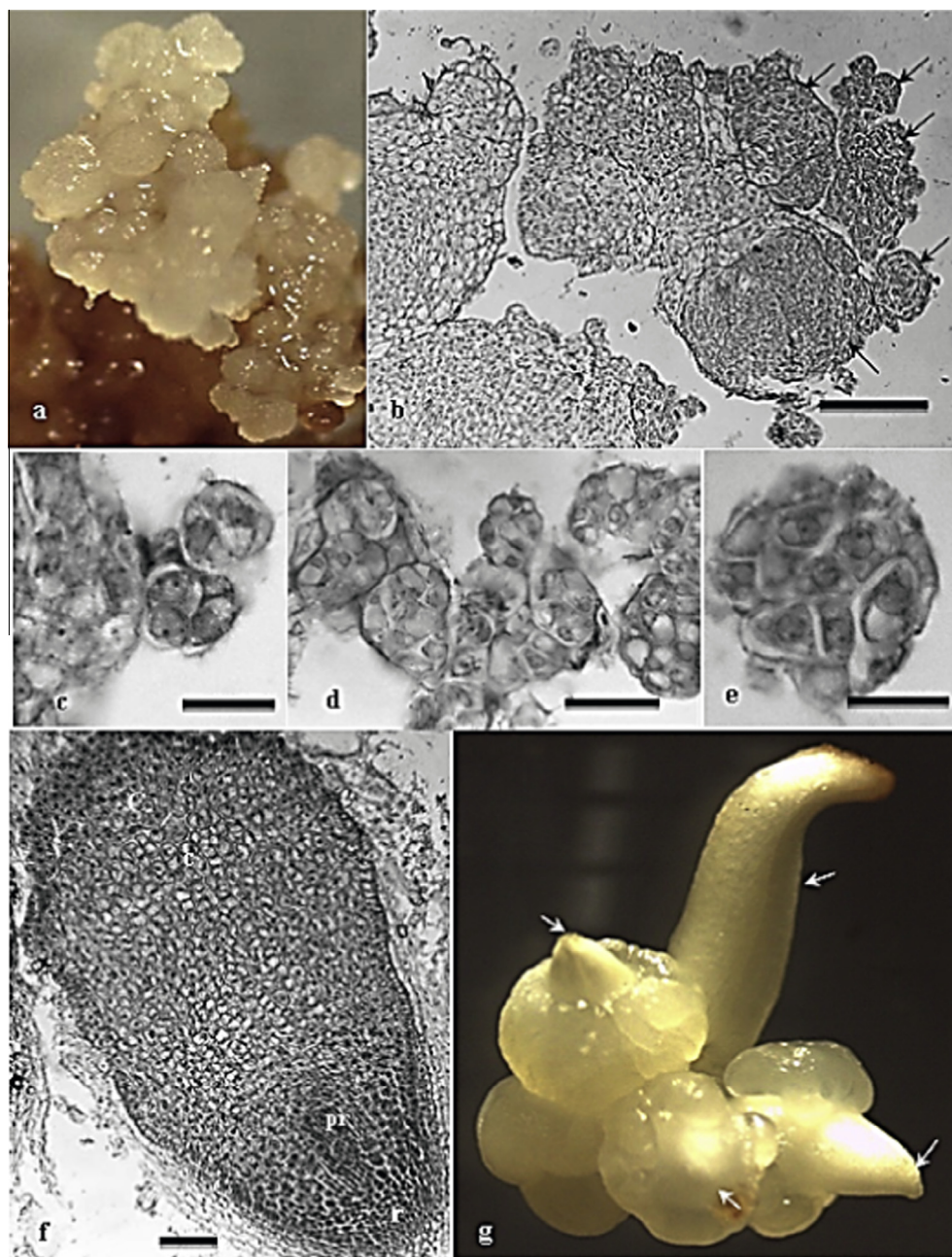


Fig. 2 Ontogeny of somatic embryos. (a) Differentiation of date palm embryogenic callus from female floral bud 6 weeks after culture on medium free of cytokinin. Note the nodular appearance of the callus. (b) Transverse section through the same callus shows several disorganized callus masses. Note the occurrence of two types of cells in these masses, the first is composed of more vacuolated cells and the second is meristematic aggregates with thick walled fragmentation lines (black arrows). (c) Transverse division of single embryogenetic cell producing two-celled embryo, a further division yields a four celled embryo. (d and e) Multicellular globular embryos. (f) Longitudinal section in the bipolar embryo, note the polarity structure which consists of a meristematic end “the root tip (r)” with a procambium (pr) strand and a more vacuolated tip “the cotyledon (c)”. (g) Three mature embryos with germinated one (arrows) note the root pole region is embedded within the callus. Bar = 50 μ m in (b) and 10 μ m in (c–f). Magnification in a & g: X15. Abbreviations: c; cotyledon, pr; procambium strand, r: root tip.

of cell division and metabolism of plant (Casanova et al., 2004; Zhang et al., 2005). This greater effectiveness of TDZ might be due to its slow metabolism in tissue culture systems (Mok and Mok, 1985). Also, TDZ is a urea based cytokinin and therefore, is non-degradable by cytokinin oxidase enzymes in plant

tissues. This quality causes TDZ to be persistent in tissues, hence, transforming them from cytokinin dependence to cytokinin autonomy. The outstanding increase in zeatin riboside of petals induced by TDZ may result from its ribotide, since TDZ promotes the conversion of cytokinin ribotides to

ribosides in callus tissues. This increase in zeatin riboside has been observed in petals without, or with few meristematic cells that might undergo proliferation (Dobrev et al., 2002). TDZ induced calli of *Scutellaria baicalensis* responded in high fraction of IAA and it is possible that TDZ altered indirectly endogenous hormonal values, particularly IAA/cytokinin ratio which is responsible for calli growth and bud formation (Zhang et al., 2005). Therefore, thidiazuron has been reported to modulate endogenous levels of plant growth hormones (Hutchinson and Saxena, 1996; Hutchinson et al., 1996; Murthy et al., 1998).

The present results showed that immature female inflorescences contained much high levels of total sugars, reducing and non-reducing sugars, while callus contains the lowest level among the other developmental stages then, the level of total sugars, reducing and non-reducing sugars increase in the embryogenic callus. These data are in agreement with Catarina et al. (2003) who found high level of total sugars in the globular embryoides. This increase of total sugars could also have occurred as a consequence of the uptake and metabolism of the carbohydrates supplied exogenously. The highest concentration of reducing sugars was obtained when the translucent callus transformed into embryogenic callus. Cell division demands a high amount of carbon in order to supply the ATP necessary for cellular metabolism (Martin et al., 2000; Yoshida, 2003).

Results of the present study reported that level of free amino acids, indoles and phenols in embryogenic callus is increased. This increase may be attributed to high mitotic activity during this stage. The obtained data insure the previous data obtained by Zein El Din (2010) who observed that embryogenic callus contained the much high level of free amino acids and phenols, while, callus contained the lowest level of free amino acids content. The accumulation of amino acids during somatic embryogenesis was also reported for alfalfa, anise, and cowpea (Ramakrishnan et al., 2005). Rawal and Mehta (1982) found that shoot formation from haploid tobacco callus occurred as the content of natural phenolic substances declined. Phenols in plant cells may include a participation in auxin catabolism, an ability to modulate the free polyamine levels by their conjugation and the inhibitory effects of some phenolic acids on cell division (Cviková et al., 1998). Fry (1986) observed that important role of phenolic acids is their involvement in the modulation of the cell wall composition. Also, phenolic acids incorporated into cell wall help restrict cell expansion, which is essential for cell division.

Histology

The key of the technique presented in the current study, which is based on completely undifferentiated female inflorescence, is the attainment of the callus. In fact, reversion of the floral meristematic cells into vegetative cells is a complicated process that requires a high control of various physicochemical and hormonal factors. In this context, the exogenous TDZ which has the ability to stimulate the meristematic cells to reach the embryogenic callus is also an important factor. This result is in line with that previously reported (Capelle et al., 1983; Hutchinson et al., 1996). *In vitro* alteration of auxin to cytokinin ratio induces somatic embryogenesis from somatic cells, whereas TDZ alone has the potential to induce somatic embryogenesis in several species (Murthy et al., 1998).

Indeed, the histological observations of date palm tissues showed the continuous effect of TDZ as a cytokinin not only in reaching the embryogenic callus but also in promoting successive divisions which led to the polarity of the proembryos. This was recorded by well-developed shoot and root tip and a cotyledon (the dipoles). This result is agreed with those of Sané et al. (2006) and Abd El Bar and El Dawayati (2014).

With respect to the explant (immature female inflorescence), the flower primordia are constituted of meristematic cells; in this case the meristems make the reversion process easier. The histological observations of cultured explant revealed that callus originated from the surface meristematic cells, and it started at the fourth week of culture. Several studies have also reported that the differentiation degree of the date palm flowers is an important factor defining their ability to develop (Drira and Benbadis, 1985; Kriaa et al., 2012). Moreover, they found that the mature female flowers required 8 weeks to start callus formation process. This confirms that the mature flowers needed long time to acquire the meristematic characteristics than the immature flowers. The later endowed with the best capacities of proliferation and plant regeneration.

The possible use of immature female inflorescences is therefore an attractive alternative since a single tree can produce many inflorescences per year that can be used as explants and offer several highly valued advantages, namely the possibility of reversion from the reproductive to the vegetative state. This deviation in morphogenetic behavior may be attributed to the age of inflorescences and/or the exogenous plant growth regulators. TDZ may modify endogenous plant growth regulators either directly or indirectly and produce reactions in cells and tissue necessary for its division and regeneration (Guo et al., 2011). As compared to the vegetative tissue conferred to the floral tissues which characterizing with morphogenetic plasticity stands in contrast with the morphogenetic rigidity characterizing vegetative tissues in regeneration processes of date palm (Drira and Benbadis, 1985). Moreover, this readily available material is accessible in large quantities every year, easy to collect without harming or sacrificing the mother plant, and carry no undesirable contaminative effects due to their protective spathe (Loutfi and Chlyah, 1998; Kriaa et al., 2012).

References

- Abd El Bar, O.H., El Dawayati, M., 2014. Histological changes on regeneration *in vitro* culture of date palm (*Phoenix dactylifera*) leaf explants. *Aust. J. Crop Sci.* 8 (6), 848–855.
- Al-Khassawneh, N.M., Karam, N.S., 2006. Growth and flowering of black iris (*Iris nigricans* Dinsm.) following treatment with plant growth regulators. *Sci. Hortic.* 107, 187–193.
- Ammirato, P.V., 1988. Role of ABA in regulation of somatic embryogenesis. *Hort Sci.* 23 (3), 520.
- Bhaskaran, S., Smith, R., 1992. Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* L. cv. Barhee. *Plant Cell Rep.* 12, 22–25.
- Bouguedoura, N., Michaux-Ferriere, N., Bompar, J.L., 1990. Comportement *in de* bourgeons axillaires de type indéterminé du palmier-dattier (*Phoenix dactylifera*). *Can. J. Bot.* 68, 2004–2009.
- Capelle, S.C., Mok, D.W.S., Kirchner, S.C., Mok, M.C., 1983. Effects of thidiazuron on cytokinin autonomy and the metabolism of N6-(Δ^2 -isopentenyl) [8- 14 C] adenosine in callus tissues of *Phaseolus lunatus* L. *Plant Physiol.* 73, 796–802.

- Casanova, E., Valdés, A., Fernández, B., Moysset, L., Isabel, M., 2004. Levels and immunolocalization of endogenous cytokinins in thidiazuron induced shoot organogenesis in carnation. *J. Plant Physiol.* 161, 95–104.
- Catarina, C.S., Randi, A.M., Viana, A.M., 2003. Growth and accumulation of storage reserves by somatic embryos of *Ocotea catharinensis* Mez. (Lauraceae). *Plant Cell Tiss. Organ Cult.* 74, 67–71.
- Cvikrová, M., Malá, J., Eder, J., Hrubcová, M., Vágner, M., 1998. Abscisic acid, polyamines and phenolic acid in sessile oak somatic embryos in relation to their conversion potential. *Plant Physiol. Biochem.* 36 (3), 247–255.
- Dobrev, P., Motyka, V., Gaudinová, A., Malbeck, J., Trávníčková, A., Kamínek, M., Vanková, R., 2002. Transient accumulation of *cis*- and *trans*-zeatin type cytokinins and its relation to cytokinin oxidase activity during cell cycle of synchronized tobacco BY-2 cells. *Plant Physiol. Biochem.* 40, 333–337.
- Drira, N., Benbadis, A., 1985. Vegetative multiplication of date palm (*Phoenix dactylifera* L.) by reversion of *in vitro* cultured female flower buds. *J. Plant Physiol.* 119 (3), 227–235.
- Ebigman, E., 1999. Rapid micropropagation of date palm using floral tissues. In: *The International Conference on Date Palm*, Assiut. vol. 9, no. 11, pp. 385–388.
- El Hadrami, I., Cheikh, R., Baaziz, M., 1995. Somatic embryogenesis and plant regeneration from shoot-tip explants in *Phoenix dactylifera* L. *Biol. Plant.* 37, 205–211.
- Fry, S.C., 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Ann. Rev. Plant Physiol.* 37, 165–186.
- George, E., Hall, M., De Klerk, G.J., 2008. *Plant Propagation by Tissue Culture*, third ed. Springer, US, ISBN 978-1-4020-5005-3.
- Guo, B., Abbasi, B.H., Zeb, A., Xu, L.L., Wei, Y.H., 2011. Thidiazuron: a multi-dimensional plant growth regulator. *Afr. J. Biotechnol.* 10 (45), 8984–9000.
- Hutchinson, J.M., Saxena, P.K., 1996. Acetylsalicylic acid enhances and synchronizes thidiazuron-induced somatic embryogenesis in geranium tissue cultures. *Plant Cell Rep.* 15, 512–515.
- Hutchinson, M.J., KrishnaRaj, S., Saxena, P.K., 1996. Morphological and physiological changes during thidiazuron-induced somatic embryogenesis in geranium (*Pelargonium hortorum* Bailey) hypocotyl cultures. *Int. J. Plant Sci.* 157, 440–446.
- Jayraman, J., 1984. *Laboratory Manual in Biochemistry*. Willey Eastern Limited, New Delhi.
- Jennifer, K.C., Reflini, Harry, E.I., Brian, P.F., Stephen, P.C., Peter, D.S., 2010. Effects of Picloram in Inflorescence Culture of Oil Palm. *Sumatra Bioscience*, Singapore, pp. 71–78.
- Johansen, D.A., 1940. *Plant Microtechnique*. McGraw-Hill Book Co., New York, pp. 126–156.
- Kamada, H., Harada, H., 1981. Changes in the endogenous level and effects of abscisic acid during somatic embryogenesis of *Daucus carota* L. *Plant Cell Physiol.* 22, 1423–1429.
- Kriaa, W., Sghaier, B., Masmoudi, F., Benjemaa, R., Drira, N., 2012. The date palm (*Phoenix dactylifera* L.) micropropagation using completely mature female flowers. *C.R. Biol.* 335, 194–204.
- Kutscheram, V., 1994. The current status of the acid-growth hypothesis. *New Phytol.* 126, 549–569.
- Larsen, P., Harbo, A., Klungsoer, S., Asheim, T.C., 1962. On the biogenesis of some indole compounds in *Acetobacter xylinum*. *Physiol. Plant.* 15, 552–655.
- Liu, T., Yunqian, H., Xiaoxian, L., 2008. Comparison of dynamic changes in endogenous hormones and sugars between abnormal and normal *Castanea mollissima*. *Nat. Sci.* 18, 685–690.
- Loutfi, K., Chlyah, H., 1998. Vegetative multiplication of date palms from *in vitro* cultured inflorescences: effect of some growth regulator combinations and organogenetic potential of various cultivars. *Agronomic* 18, 573–580.
- Malik, E.P., Singh, M.B., 1980. *Plant Enzymology and Histochemistry*, first ed. Kalyani Publishers, New Delhi, pp. 286.
- Martin, A.B., Cuadrado, Y., Guerra, H., Gallego, P., Hita, O., Martin, L., Dorado, A., Villalobos, 2000. Differences in the contents of total sugars, reducing sugars, starch and sucrose in embryogenic and non-embryogenic calli from *Medicago orborea* L. *Plant Sci.* 154, 143–151.
- Mok, M.C., Mok, D.W., 1985. The metabolism of [14C]-thidiazuron in callus tissues of *Phaseolus lunatus*. *Physiol. Plant.* 65, 427–432.
- Murashige, T., Skoog, F.A., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15, 473–479.
- Murch, S.J., Saxena, P.K., 1997. Modulation of mineral and fatty acid profiles during thidiazuron mediated somatic embryogenesis in peanuts (*Arachis hypogaea* L.). *J. Plant Physiol.* 151, 358–361.
- Murthy, B.N., Murch, S.J., Saxena, P.K., 1998. Thidiazuron: a potent regulator of *in vitro* plant regeneration. *In Vitro Cell. Dev. Biol. Plant* 34, 267–275.
- Ramakrishnan, K., Gnanam, R., Sivakumar, P., Manickam, A., 2005. *In vitro* somatic embryogenesis from cell suspension cultures of cowpea (*Vigna unguiculata* L. Walp). *Plant Cell Rep.* 24 (8), 449–461.
- Rawal, S.K., Mehta, A.R., 1982. Changes in enzyme activity and isoperoxidases in haploid tobacco callus during organogenesis. *Plant Sci. Lett.* 24, 67–77.
- Sané, D., Aberlence- Bertossi, F., Gassama-Dia, Y.K., Sagna, M., Trouslot, M.F., Duval, Y., Borgel, A., 2006. Histocytological analysis of callogenesis and somatic embryogenesis from cell suspensions of Date Palm (*Phoenix dactylifera*). *Ann. Bot.* 98, 301–308.
- Sass, J.E., 1951. *Botanical Microtechnique*, second ed. The Iowa State College Press, pp. 5–77.
- Shales, O., Schales, S.S., 1945. A simple method for the determination of glucose in the blood. *Arch. Biochem. Biophys.* 8, 285–292.
- Sharma, D.R., Deepak, S., Chowdhury, J.B., 1986. Regeneration of plantlets from somatic tissues of the date palm (*Phoenix dactylifera* L.). *Ind. J. Exp. Bot.* 24, 763–766.
- Shindy, W.W., Smith, O.E., 1975. Identification of plant hormones from cotton ovules. *Plant Physiol.* 55, 550–554.
- Smith, D.L., Aynsley, J.S., 1995. Field performance of tissue cultured date palm (*Phoenix dactylifera*) clonally produced by somatic embryogenesis. *Principes* 39, 47–52.
- Snedecor, G.W., Cochran, W.C., 1989. *Statistical Methods*, eighth ed. Iowa State University, Press, Iowa, USA, pp. 297.
- Visser, C., Qureshi, J.A., Gill, R., Saxena, P.K., 1992. Morphoregulatory role of thidiazuron. *Plant Physiol.* 99, 1704–1707.
- Yoshida, S., 2003. Molecular regulation of leaf senescence. *Curr. Opin. Plant Biol.* 6, 79–84.
- Zayed, E.M.M., 2011. Propagation of *Phoenix dactylifera* L. *Chamaerops humilis* L. and *Hyophorbe verschaftelii* L. palms by using tissue culture technique. Ph.D. Thesis, Department of Horticulture, Faculty of Agriculture, Cairo University, Egypt, pp. 130–136.
- Zein El Din, A.F.M., 2010. Physiological and Biotechnological Studies on Date Palm Micropropagation. *Plant Physiology Department, Faculty of Agriculture, Cairo University, Egypt*, pp. 84–87.
- Zhang, C.G., Li, W., Mao, Y.F., Zhao, D.L., Dong, W., Guo, G.Q., 2005. Endogenous Hormonal levels in *Scutellaria baicalensis* calli induced by thidiazuron. *Russ. J. Plant Physiol.* 52 (3), 345–351.