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

Eman M.M. Zayed [a], **Ola H. Abd Elbar** [b,*]

[a] *The Central Laboratory of Date Palm Researches and Development, Agriculture Research Center, Cairo, Egypt*
[b] *Agricultural Botany Department, Faculty of Agriculture, Ain Shams University, Egypt*

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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 (GA_3, IAA, Zeatin and ABA), total soluble sugars (reducing and non-reducing sugars), free amino acids, indoles and phenols were determined. The levels of GA_3 and IAA in explant reached the highest values and then decreased in callus stage. Zeatin, IAA, and ABA levels were higher in embryogenic callus. GA_3 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

* Corresponding author.
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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 Ib/in^2; the jars were autoclaved for 20 min at 121 °C 15 Ib/in^2. 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 (GA_3, IAA, Zeatin and ABA) and chemical (total soluble sugars, free amino acids, total phenols and indoles) analyses.

Determination of phytohormones

Phytohormones (GA_3, 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). GA_3, 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).

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