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INDUCTION OF SOMATIC EMBRYOGENESIS BY USING IMMATURE INFLORESCENCE OF DATE PALM CV. ZAGHLOUL

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ABSTRACT

The effect of combinations of auxins and cytokinins on the induction of somatic embryos of date palm (*Phoenix dactylifera* L.) cv. Zaghloul (soft cultivar) from inflorescence explant. Explants were cultured on Murashige and Skoog (MS) medium supplemented with different plant growth regulators. The best combination for induction of somatic embryo from inflorescence explant which contained 0.25 mg/l naphthoxyacetic acid (NOA), 0.25 mg/l α -naphthalene acetic acid (NAA), and 2.5 mg/l 6-(γ,γ -dimethylallylamino) purine (2iP), 2.5 mg/l 6-benzyl aminopurine (BAP).

Key words: 2iP, BAP, date palm, immature inflorescence, NAA, NOA, *Phoenix dactylifera* L., somatic embryogenesis.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is probably the most ancient cultivated tree for its edible sweet fruit. Date palm is a diploid ($2n = 2x = 36$), perennial, and monocotyledonous plant. Date palm is dioecious, meaning it has separate female and male trees. *Phoenix dactylifera* L., belongs to, family: Palmae (*Arecaceae*) which is one of the oldest fruit trees in the world. *Arecaceae* family is contains over 200 genera and over 2500 species. (El Hadrami and Al-Khayri, 2012)

Somatic embryogenesis is currently the most efficient technique regarding rates of multiplication and production and, therefore,

commercially attractive to completely replace traditional vegetative propagation practices. Direct somatic embryogenesis avoids passage through callus and thus avoids the genetic instability often associated with somatic embryos obtained indirectly, through callus (**Rai and McComb, 2002**).

Sidky and Eldawyati (2012) stated that induce direct somatic embryogenesis from female inflorescences explants of date palm was on MS medium containing 5 mg/l 2, 4-D, 0.5 mg/l 2iP and abscisic acid 1.5 mg/l. The aim of this experiment was to study the effect of combinations of auxins and cytokinins on the induction of somatic embryos of date palm (*Phoenix dactylifera* L.,) cv. Zaghloul from inflorescence explant.

MATERIALS AND METHODS

This work was carried out during the period 2012-2018 in the Central Laboratory of Date Palm Researches and Development (CLDRD), Agriculture Research Center (ARC), Giza, Egypt, and Plant Physiology Section, Faculty of Agriculture, Cairo University, Egypt.

a- Plant material

The propagation process was started with female inflorescence isolated from mother palm trees soft cultivar, Zaghloul grown in Agriculture Research Center at South Giza, Giza Governorate. All female inflorescence were collected in mid of January. Some leaves surrounded the head of tree were cut to get spathe in length 12-19 cm (**Fig. A**).

b- Inflorescence sterilization

Explant preparation was done inside the laminar flow hood. The spathes sprayed with 95% ethanol and burned before opened. The spathes were gently opened from outer side with sterilized scalpel and soaked in 0.1% mercuric chloride at for 60 min., rinsed three times with sterilized distilled water then soaked in sterile anti-oxidant solutions of 100 mg/l ascorbic acid and 150mg/l citric acid before culturing under aseptic conditions (**Fig. B**).

The lengths of all sterilized explants were ranged from 3-4 cm. The explants were transformed to MS basal culture medium. After 6 weeks, the explants succeeded free-contamination cultured for further studied experiments.

c- Culture medium

The MS basal medium (**Murashige and Skooge, 1962**) was used through this study. However, MS medium was modified by the addition

of biotin 0.2 mg/l, and adenine sulfate 40 mg/l, 30 g/l sucrose was added and the acidity of the final medium was adjusted to pH 5.7 ± 0.1 prior to adding the agar (6 g/l) to solidify culture medium. Each culture jar (250 ml) was supplied with 45 ml of MS culture medium and the jars were capped with polypropylene closures and autoclaved at 121 °C at 1.05 kg/cm² for 20 min. Cultured jars were incubated at $27 \pm 1^\circ\text{C}$ in darkness for 18 weeks. Cultures were transferred to fresh medium every 6 weeks.

In this study the visual characteristics at the end of each studied stage were recorded from all studied treatments during both parts are visually scored according to scale proposed by **Bottino (1981)** as Table (1) :

Number	Results
1	Low results (+)
2	Average results (++)
3	Good results (+++)
4	Very good results (++++)

The sterilized inflorescence explants were cultured on MS basal medium supplemented with 40 mg/l adenine sulphate, 170 mg/l NaH₂PO₄.2H₂O, 200 mg/l glutamine, 2 g/l PVP, 30 sucrose, 6 g/l agar and plant growth regulators as following (0.01 mg/l 2, 4-D, 0.01 mg/l NAA, 0.01 mg/l 2ip, 0.01mg/l BAP) as a control for 6 weeks. After that explants were culture on medium contain MS basal medium supplemented with 40 mg/l adenine sulphate, 170 mg/l NaH₂PO₄.2H₂O, 200 mg/l glutamine, 2 g/l PVP, 30 sucrose and 6 g/l agar. Twelve treatments (T1-T12) of hormonal combinations as well as control were studied. Each culture medium treatment contained two auxins, i.e. 2, 4-D and NAA and two cytokinins, i.e. 2ip and BAP. Four concentrations of either 2, 4-D or NAA, i.e. 0.25, 0.5, 1.0 and 2 mg/l were applied. Meanwhile, three concentration of either 2ip or BAP, i.e. 0.1, 0.5 and 2.5 mg/l were added to the culture medium as presented in **Table (2)**

The developed explants induced from previous stage were cultured on MS basal medium supplemented with 40 mg/l adenine sulphate, 100 mg/l KH₂PO₄.2H₂O, 2 g/l PVP, 30 g/l sucrose and 6 g/l agar. Twelve treatments (D1-D12) of hormonal combinations as well as control were studied. Each culture medium treatment contained two auxins, i.e. NOA and NAA and two cytokinins, i.e. 2iP and BAP. Four concentrations of either NOA or NAA, i.e. 0.25, 0.5, 1.0 and 2 mg/l were applied.

Meanwhile, three concentrations of either 2iP or BAP, i.e. 0.1, 0.5 and 2.5 mg/l were added to the culture medium as tabulated in **Table (3)**.

Statistical analysis

The data were tabulated and statistically factorial analyzed according to the randomized complete block design with three replicates according to **Snedecor and Cochran (1980)**. The mean values were compared using LSD test at the 5% level of probability.

RESULTS AND DISCUSSION

Date palm explant (female inflorescence) was used to induce indirect somatic embryos. After 18 weeks from the starting, there was observation of callus was formed on explants, greatly increased 3.33 when auxins (2, 4-D& NAA) were added for each at 0.25 mg/l and cytokinins (2iP& BAP) were added for each at 0.1 mg/l. In this **Table (2)** the lowest significant degree of swelling and callus formation (1.00) when auxins were added at 2 mg/l and cytokinins either 0.1 mg/l or 0.5 mg/l (**Fig. D, E, F**). Finally, degree of browning increased to (2.67) when auxins (2, 4-D& NAA) were added at 1.0 mg/l for each of them and cytokinins (2iP& BAP) were added for each at 2.5 mg/l.



Fig. (A) Inflorescence explant

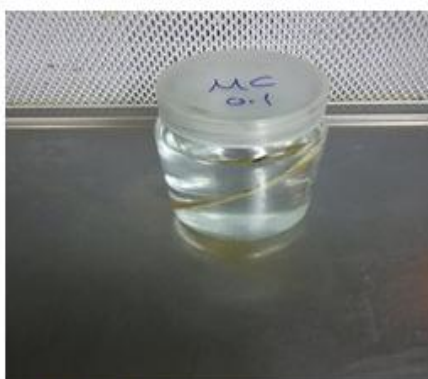


Fig. (B) Sterilization stage

Table (2). Effect of diversity concentration of combination between auxins and cytokinins on degree of swelling ,browning as well as callus induction of date palm explant (Zaghloul cv.), after three subculture (18 weeks).

Treatment	mg/l				Swelling	Browning	vitrification	Callus
	2,4-D	NAA	2ip	BAP				
control	0.01	0.01	0.01	0.01	2.67 a-c	1.33 bc	1.67 bc	1.33 bc
T1	0.25	0.25	0.1	0.1	2.00 b-e	1.33 bc	2.00 bc	3.33 a
T2	0.25	0.25	0.5	0.5	2.33 a-d	1.00 c	1.00 c	1.00 c
T3	0.25	0.25	2.5	2.5	3.00 ab	1.00 c	2.67 ab	2.00 b
T4	0.5	0.5	0.1	0.1	3.00 ab	1.33 bc	2.67 ab	2.00 b
T5	0.5	0.5	0.5	0.5	1.67 c-e	1.33 bc	1.67 bc	1.67 bc
T6	0.5	0.5	2.5	2.5	3.33 a	1.33 bc	3.33 a	2.00 b
T7	1	1	0.1	0.1	3.00 ab	1.67 bc	1.67 bc	1.33 bc
T8	1	1	0.5	0.5	2.67 a-c	1.00 c	2.67 ab	1.67 bc
T9	1	1	2.5	2.5	1.33 de	2.67 a	2.00 bc	1.67 bc
T10	2	2	0.1	0.1	1.00 e	2.00 ab	1.33 c	1.00 c
T11	2	2	0.5	0.5	1.00 e	1.00 c	1.33 c	1.00 c
T12	2	2	2.5	2.5	2.67 a-c	1.33 bc	2.00 bc	1.00 c
					1.071	0.9276	1.079	0.6741

L.S.D. (0.05)

Several studies have shown that somatic embryogenesis in date palm has shown that floral explants excised from immature inflorescences needed low concentrations of auxins for callus induction. **Drira and Benbadis (1985)** stated that callus formation was obtained from very young inflorescence tissues culture medium containing 0.5 mg/l IBA, 0.3-0.5 mg/l 2, 4-D and 0.2 mg/l BA. However, floral tissues excised from mature inflorescence required high auxin concentrations for callus initiation.

On the other hand, **Loutfi (1998)** reported that 100 mg/l 2, 4-D was required for callus induction from emerged inflorescence tissues of 'Jihel' and 'Iklane' Moroccan cultivars. In addition, **Karun et al. (2004)** found that use of 68 μ M 2, 4-D did not succeed to induce callus formation from immature inflorescence of the palm *Areca catechu* L. In contrast, they obtained embryogenic callus with 200 μ M Picloram.



Fig. (C) spikelet explants



Fig. (D) Swelling stage



Fig. (E) Callus induction



Fig. (F) Embryogenic callus

Data in **Table (3)** illustrated that the best combinations for callus multiplication was medium contain 0.5 mg/l for each plant growth regulators (NOA, NAA, 2iP, BAP). While the best combinations for maturation and germination of embryos were medium contained 0.25 mg/l NOA, 0.25 mg/l NAA, 2.5 mg/l 2iP and 2.5 mg/l BAP. (**Fig. G, H**).

Table (3). Effect of diversity concentration of combination between auxins and cytokinins on degree of browning, vitrification as well as multiplication of callus and maturation of embryos of date palm explant (Zaghloul cv.), after three subculture (30weeks).

Treatment	mg/l				Browning	vitrification	callus	Embryo
	NOA	NAA	2iP	BAP				
control	0.01	0.01	0.01	0.01	1.33 b	1.33 b	2.00 b-d	1.00 c
D1	0.25	0.25	0.1	0.1	1.33 b	1.33 b	1.33 cd	1.67 b
D2	0.25	0.25	0.5	0.5	2.00 ab	1.00 b	1.00 d	1.00 c
D3	0.25	0.25	2.5	2.5	2.00 ab	1.00 b	1.33 cd	3.33 a
D4	0.5	0.5	0.1	0.1	1.667 b	1.33 b	2.00 b-d	1.00 c
D5	0.5	0.5	0.5	0.5	1.67 b	1.33 b	3.67 a	1.00 c
D6	0.5	0.5	2.5	2.5	2.00 ab	1.33 b	2.33 bc	1.00 c
D7	1	1	0.1	0.1	1.33 b	1.33 b	2.33 bc	1.00 c
D8	1	1	0.5	0.5	2.00 ab	1.00 b	1.33 cd	1.33 bc
D9	1	1	2.5	2.5	1.33 b	1.67 b	1.00 d	1.00 c
D10	2	2	0.1	0.1	1.33 b	2.67 a	3.00 ab	1.00 c
D11	2	2	0.5	0.5	1.67 b	1.33 b	1.33 cd	1.00 c
D12	2	2	2.5	2.5	3.33 a	1.00 b	2.33 bc	1.00 c
					1.515	0.8392	1.242	0.657

L.S.D. (0.05)



Fig. (G) Embryo formation



Fig. (H) Shoot formation

Biochemical analysis

Total, reducing and non- reducing sugars:

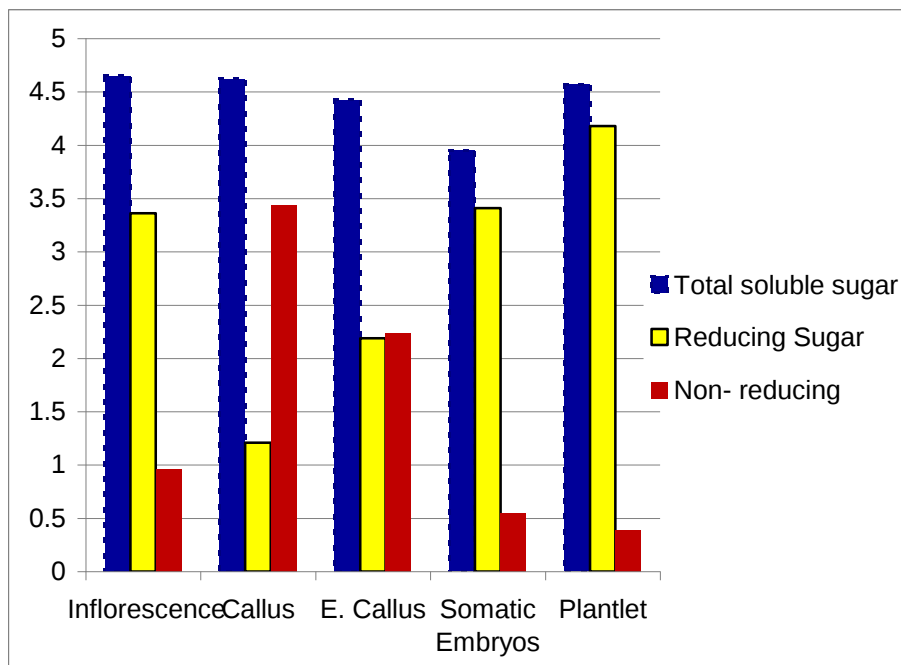
Indicates levels of total sugars, reducing and non-reducing sugars in different morphogenesis stages cleared that the highest level of total sugars in immature inflorescence and decreased in other developmental stage (callus, embryogenic callus and embryos), then increased again in plantlet.

Immature inflorescence explants at zero time and dividing cells of callus tissue contain nearly the same and highest level of total sugars (4.65 and 4.62 mg/g FW). However, fully developed SE contains the lowest level of total sugars (3.95 mg/g FW). Reducing sugars form around the most percentage of total sugars within the plantlets (4.18 mg/g FW). The highly dividing cells of callus tissue contain the lowest of reducing sugars (1.21 mg/g FW).

Non-reducing sugars reduced (0.39 mg/g FW) in plantlets derived from immature inflorescence explants *via* somatic embryogenesis protocol. The obtained data insure the previous data obtained by **Zayed, (2015)** who observed that immature inflorescence in zero stage contained the much high level of total sugars.

It is well known that carbohydrates play an important role in *in vitro* cultures as an energy and carbon source, as well as gene expression (**Koch, 1996 and George et al 2008**). Sucrose is almost universally used for micropropagation purposes as it is so generally utilisable by tissue cultures (**George et al 2008**).

Fig. (I). The contents of total soluble, reducing and non-reducing sugars (mg/g FW) within different developmental stages of date palm somatic embryos derived from immature inflorescence explants.



Baud *et al* (2002) stated that the mobilization of sugars in the early stages of embryogenic development is of major importance, because they act mainly as a source of metabolic energy, of carbon skeletons and/or for signalling.

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استحداث الاجنة الجسدية باستخدام البراعم الزهرية الغير ناضجة لنخيل البلح صنف زغلول

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تهدف هذه التجربة لمعرفة تأثير التداخل بين الأكسينات والسيتوكينينات علي استحداث الاجنة الجسدية الغير المباشرة من البراعم الزهرية المؤنثة الغير ناضجة لنخيل البلح صنف زغلول (من الاصناف الرطبة). تم زراعة المنفصلات النباتية علي وسط غذائي لمورشيحي وسكوج مضاف اليه بعض منظمات النمو المختلفة وتشير النتائج الي ان افضل التداخلات لاستحداث الاجنة الجسدية كانت مع الوسط الغذائي الذي احتوي علي 0.25 ملليجرام /لتر نفتوكسي اسيتك اسيد، 0.25 ملليجرام / لتر نفتالين اسيتك اسيد وتركيزات 2.5 ملليجرام /لتر 6 جاما داي ميثيل امينوبيورين و 2.5 ملليجرام/لتر بنزيل امينو بيورين.

الكلمات الدالة : نخيل البلح ، نفتوكسي اسيتك اسيد، نفتالين اسيتك اسيد، داي ميثيل امينوبيورين، بنزيل امينو بيورين، البراعم الزهرية، تكوين الاجنة الجسدية.