

Effect of Naphthaleneacetic acid and Indole-3-acetic acid on somatic embryogenesis of female inflorescence explants of date palm (*Phoenix dactylifera* L.) cv. Aseel

*Adel Ahmed Abul-Soad, **Ghulam Sarwar Markhand and **Saeed Ahmed Shah

adelaboelsoaud@gmail.com

*Horticulture Research Institute, Agriculture Research Center, Cairo, Egypt.

**Date Palm Research Institute, Shah Abdul Latif University, Sindh, Pakistan.

Key words: Date palm, inflorescence, plant growth regulators, somatic embryogenesis, tissue culture.

Abstract

Effect of basal modified nutrient medium supplemented with combination of NAA (0.0 – 1.0 – 5.0 mg l⁻¹) and IAA (0.0 – 0.1 – 1.0 mg l⁻¹) on somatic embryos regeneration from inflorescence explants of date palm (*Phoenix dactylifera* L.) was investigated. Spikelet explants of cv. Aseel around 3.0 cm in length were surface sterilized with 1.0 % sodium hypochlorite solution for 3 minutes. All explants were incubated at 23 ± 2 °C° under full darkness in controlled chamber. The explants have been transferred every 3 weeks onto fresh nutrient medium for 3 subcultures. The obtained results revealed that the fungal and bacterial contamination percentage was zero. As well as, no browning generally was observed in most treatments. Therefore, swelled spikelet explants produced white compact callus onto the nutrient medium containing 1.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IAA. Mostly the callus formation was linked to swelled explants. On the other hand, some spikelet explants which were cultured onto the basal medium included 5.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IAA produced friable yellowish callus combined with direct somatic embryos. Interestingly, presence of NAA in the nutrient medium of spikelet explants mostly accompanied with some browning. Simultaneously, production of white compact callus stimulated by IAA whether endogenous (control treatment) or exogenous addition (other treatments).

Introduction

In all forms of plant embryogenesis certain criteria have to be fulfilled before initiation. The species or genotype has to have the genetic potential to form embryos from somatic cells and one or a few cells of the plant/explant have to be competent to receive a signal (endogenous or exogenous) that triggers the pathway of embryogenic development (commitment) leading to embryo formation even in the absence of further signals. For the *in vitro* forms of somatic embryogenesis, these conditions (potential, competence, induction, commitment) have to be experimentally optimized (Feher, 2005).

In several culture systems, such as in *Arachis hypogaea* seedlings (Victor *et al.*, 1999), *Juglans regia* embryonic axes (Fernandez *et al.*, 2000) and sunflower zygotic embryos (Thomas *et al.*, 2004), the morphogenic pathway can be oriented through either shoot organogenesis or somatic embryogenesis, only by modifying the plant growth regulator (PGR) composition of the culture medium (Jimenez and Thomas, 2005).

In spite of using the explants derived from offshoots causes many difficult problems like bacterial contamination with endogenous bacteria, browning, long-term of such technique and somaclonal variations. Therefore, it is stated that using the offshoots in micropropagation of date palm takes around three years, if the protocol well-known (**Abul-Soad *et al.*, 2004a**). Moreover, most of the commercial laboratories in all over the world are using somatic embryogenesis (**Tisserat, 1979; Al-Khayri, 2001; Abul-Soad *et al.*, 2002**). For all these reasons, the micropropagation of date palm is becoming surrounded by many risks until now.

After then, specific studies used the high possibilities of inflorescence explants to produce direct (**Abul-Soad *et al.*, 2004b**) and in-direct shoot of date palm (**Drira and Al-Sha'ary, 1993; Abul-Soad *et al.*, 2005**).

The individual role of some auxins and cytokinins was studied by (**Abul-Soad, 2007**). The study explored the effect of NAA, IAA, 2,4-D, BA and 2iP on the early age of spikelets of female date palm inflorescence. BA and 2iP had effect to produce organized structures. However, these structures couldn't be able to proceed their development and produce embryos or shoots. Interestingly, incorporating 2,4-D into the nutrient medium gave these structures to produce globular embryos on the basal parts of elongated sepals of small florets. However, the role of combinations of these plant growth regulators and accurate timing still needs investigation. The aim of this study is to investigate the role of initial pulse by auxins, particularly IAA, NAA to trigger the pathway of somatic embryogenesis in the explants of date palm inflorescence.

Materials and Methods

This work was carried out in the biotechnology lab. of Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Sindh, Pakistan in 2007.

Plant materials:

One immature inflorescence was excised from female mother palm of dominant variety Aseel which is grown at Khairpur district. The inflorescence excised from mother plant on 27 of January at 12 cm in length. The excised inflorescence was kept in clean plastic cover and manipulated carefully from an open field to the lab.

Surface sterilization:

The whole inflorescence was washed under the running tap water for a few minutes. The intact inflorescence was subjected to surface sterilization treatment under aseptic conditions. The inflorescence immersed in 1 % sodium hypochlorite solution for 1 minute with few drops of Tween-20 solution. After then, the inflorescence washed gently with the distilled sterilized water 3 times. In the next step, the outer cover of the inflorescence was perfectly removed and the spikelet explants subjected individually into the different treatments.

Media preparation:

The modified-basal nutrient medium employed through this study contained macro-salts of **Gamborg's B-5 medium (Gamborg *et al.*, 1968)** and micro-salts of **Murashige & Skoog, 1962 (MS)** supplemented with (in mg l⁻¹):

100.0 myo-inositol; 1.0 nicotinic acid; 1.0 pyridoxine-HCl; 1.0 thiamine-HCl; 2.0 glycine; 200.0 glutamine; 40.0 adenine sulfate; 2100.0 agar (Agar Technical, Oxoid, Inc.); 1300.0 Gel (Gellan Gum, Caisson Laboratories, Inc.) and 30000.0 sucrose. The

basal modified nutrient medium supplemented with combination of NAA (0.0 – 1.0 – 5.0 mg l⁻¹) and IAA (0.0 – 0.1 – 1.0 mg l⁻¹).

After preparation of the medium the pH was adjusted to 5.7 ± 0.1 before the autoclaving. Media were dispensed into small culture tubes (25 × 150 mm) in aliquots of 15 ml per tube and were capped with aluminum foil. Media were then autoclaved for 20 minutes at 1.11 Kg/Cm² and 121°C.

Culture conditions:

In vitro explants were incubated under darkness in a temperature-controlled chamber at 23 ± 2 °C. Data collection and sub-culturing were performed at 3 weeks intervals. During various subcultures, some observations have been taken. As well as, the following data were recorded:

1. Browning, swelling and callus formation [it is expressed as scores and presented as +, ++, +++, - represent poor, moderate, high and no response, respectively, according to the method described by **Abul-Soad et al., (2002)** and **Mujib et al., (2005)**].
2. Fresh weight (grams/explant).

Statistical analysis:

In each treatment, 9 tubes containing 1 explant (spikelet). Factorial Randomized Complete Block Design was used and data were subjected to analysis of variance. Separation of means among treatments was determined using L.S.D test at 5% according to **Steel and Torrie (1980)**.

Results and Discussion

Auxins have multiple roles in tissue culture, according to their chemical structure, their concentration, and the plant tissue being affected. Auxins cause the production of callus stimulates cell elongation, cell division in cambium tissue (**Victor, 2005**). Role of plant growth regulators (PGRs) NAA and IAA was investigated in this study throughout the obtained effect. Some properties of cultured cultures which expose the effect of PGRs on the explants were determined. One of the best varieties in Pakistan has been selected cv. Aseel. This variety has high quality properties according to **Markhand and Abul-Soad (2007)**.

Table 1. Effect of modified basal nutrient medium supplemented with different concentrations of NAA and IAA on browning, swelling and callus formation of spikelet explants of female date palm cv. Aseel, after 9 weeks in culture.

PGR		Browning	Swelling	Callus formation
NAA	IAA			
0.0	0.0	-	+++	+++
1.0	0.1	-	+++	+++
1.0	1.0	++	++	++
5.0	0.1	+++	-	+
5.0	1.0	+	+	+

Each treatment had 9 replicates (tubes). +, ++, +++, - represent poor, moderate, high and no response, respectively.

Browning:

In general, minimal browning was observed of the inflorescence explants, particularly in the well-responded explants (Fig. 1). This result is in agreement with **Abul-Soad *et al.* (2005)** who reported that Negligible browning was observed on the juvenile spikes that cultured onto nutrient medium amended with 2,4-D and IBA together. However, no browning was noticed on the spikes that cultured onto control medium (a basal medium without auxins). The reason could be because the low temperature during the spring season when the explants have been excised. Similarly, **Loomis and Battaile (1966)** had demonstrated that the low temperature results in decreasing the activity of several enzymes, which are widely distributed in plant and oxidize phenols to quinines (often toxic to the plant tissues) e.g., mono phenol oxidase (tyrosinase) and polyphenol oxidase (catechol oxidase).

Data in Table 1 showed that there is a difference among different treatments. Since, increasing the NAA and decreasing IAA in the nutrient medium increased the browning. Where, the spikelet explants which cultured onto nutrient medium containing 5.0 mg l^{-1} NAA produced the highest percentage of browning. However, increasing the concentration of IAA lighten the effect of NAA and reduce the browning. Browning of the original palm tissue is common. Under this topic available publications of many researchers in the field of tissue culture, regarding their attempts to prevent or minimize releasing and oxidation of endogenous phenolic compounds, which included either through antioxidant or adsorbent substances. **Reuveni and Kipins (1974)** clarified that, date palm tissue cultures like those of many other plant have been commonly observed to release discoloring substances into the medium which inhibit their own growth.



Fig. 1. Minimal browning was noticed of the inflorescence explants of cv. Aseel.

Swelling:

Data displayed in Table 1 indicated that increasing the Plant Growth Regulators (PGRs) in the nutrient medium reduced the swelling of cultured explants (Fig. 2). The highest swelling has been occurred onto free growth regulators medium (control treatment). Simultaneously, the explants which cultured onto 1.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IAA exhibited same swelling. On the other side, no swelling was observed for the spikelet explants which cultured onto the nutrient medium included 5.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IAA. However, increasing the IAA in the nutrient medium has increased the swelling of explants. It seems that addition of exogenous IAA stimulate the swelling more than NAA. These results are in agreement with **Abul-Soad *et al.* (2002)** who reported that swelling of date palm explants of shoot tip represents a good sign for date palm explants capability to produce viable callus. Moreover, **Abul-Soad (2007)** postulated that the addition of auxins particularly 2,4-D into the inflorescence nutrient medium greatly increased swelling of spikes explants.



Fig. 2. Comparison between the cv. Aseel explants cultured onto two NAA treatments, 1.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IAA (left tube), and 5.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IAA (right tube), after 3 weeks from initial culture.

Callus formation:

It was noticed that the callus formation was increased by increasing the level of IAA in the nutrient medium. However, no obvious difference was observed in between the treatment containing 1.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IAA in comparison with control treatment. It seemed that the endogenous phytohormones within the explant induced the explant onto control treatment to produce callus. This result is in harmony with **Abul-Soad *et al.* (2005)** who mentioned that the presence of auxin particularly in the early age of inflorescence explants stimulated callus formation.

This callus was white and compact and didn't give somatic embryos during the period of this study (12 weeks). On the other hand, the spikelet explants which cultured onto

treatments have high concentration from NAA produce small amount of callus. However, this callus was small in amount yellowish in color. Nevertheless, this callus produce friable callus after 12 weeks in culture. Moreover, the explants which cultured onto 5.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IAA produced friable callus mixed with direct somatic embryos, after 12 weeks from the initial culture (Fig.3).

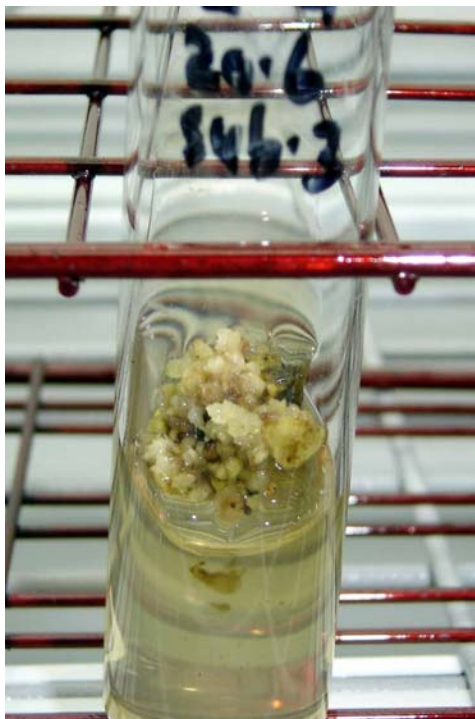


Fig. 3. Somatic embryos formation from female inflorescence explants of date palm.

There is a relation between swelling and callus formation so that an auxin causing swelling and division of explant cells. Auxin regulates cell division in the vascular cambium, which of course is involved in secondary growth, and auxin can have either a positive or a negative effect on any of these phenomena, depending on its concentration.

Table 2. Fresh weight (grams/explant) of spikelet explants of female date palm cv. Aseel during different subcultures of somatic embryogenesis process.

PGR		Fresh weight			Mean
NAA	IAA	Sub ₁	Sub ₂	Sub ₃	
0.0	0.0	0.078	0.129	0.158	0.121c
1.0	0.1	0.167	0.224	0.310	0.233a
1.0	1.0	0.099	0.161	0.211	0.157b
5.0	0.1	0.084	0.067	0.195	0.115c
5.0	1.0	0.074	0.048	0.050	0.057d
Mean		0.100c	0.126b	0.275a	0.167

Values followed by same letters aren't significantly different.

Fresh weight

Data in Table 2 showed the effect of the different treatments and subculture number on the fresh weight of spikelet explants. The obtained results indicated that the

swelled and callusing explants significantly increased in the fresh weight. Since, the explants which were cultured onto the treatment containing 1.0 mg l^{-1} NAA + 0.1 mg l^{-1} IAA resulted in the highest fresh weight (0.233) in comparison with other treatments. It was observed that the treatments which had high concentrations of NAA exhibited less fresh weights.

On the other hand, the average number of the fresh weight is increased by increasing the subculture number. Where, the average of fresh weight from sub-culture 1 (sub1) to sub-culture 3 (sub3) ranged from 0.1 g/explant to 0.275 g/explant.

It can be mentioned that, the fresh weight is increasing by presence of IAA and by increasing the subculture number as well.

It can be concluded that the presence of NAA in the nutrient medium of spikelet explants mostly accompanied with some browning (Fig. 3). But generally, no browning was observed in the tissue culture of the inflorescence explants. Simultaneously, production of white compact callus stimulated by IAA whether endogenous (control treatment) or exogenous addition (other treatments). As well as, mostly the callus formation was linked to swelled explants.

References

- Abul-Soad, A. A. (2007) Inflorescence tissue culture utilization for date palm (*Phoenix dactylifera* L.) micropropagation. The 4th Symposium on Date Palm in Saudi Arabia. 5 - 8 May 2007, King Faisal University Date Palm Research Center Hofuf, Kingdom of Saudi Arabia.
- Abul-Soad, A. A., I. A. Ibrahim, N. R. El-Sherbeny, and S. I. Baker (2004a) Improvement and characterization of somatic embryogenesis in date palm (*Phoenix dactylifera* L.). Proceedings of The International Conference of Genetic Engineering & its Applications, The Egyptian Society of Genetics and Suez Canal University, Sharm El Sheikh City, South Sinai, Egypt, 8-11 April 2004. 359 - 373.
- Abul-Soad, A. A., N. R. El-Sherbeny, and S. I. Baker (2004b) Organogenesis in female inflorescence of date palm (*Phoenix dactylifera* L. cv. Zaghoul). 2nd International Conference on Date Palm, Suez Canal University Faculty of Environmental Agricultural Sciences, El-Arish, Egypt, 6-8 October 2004. 139 - 163.
- Abul-Soad, A. A., N. R. El-Sherbeny, and S. I. Baker (2005) Date palm (*Phoenix dactylifera* L. cv. Zaghoul) propagation using somatic embryogenesis of female inflorescence. 3rd Conference on Recent Technologies in Agriculture, Cairo University, Egypt, 14-16 November 2005. 3: 423 - 441.
- Abul-Soad, A. A., Z. Zaid, A. Salah, and R. Sidky (2002) Tissue culture of date palm (*Phoenix dactylifera* L.). The 3rd Scientific Conference of Agricultural Science, Assiut, Egypt, October 2002. 327 - 341.
- Al-Khayri, J. M. (2001) Optimization of biotin and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera* L.). *In Vitro Cell. Dev. Biol., Plant.* 37453 - 37456.
- Drira, N. and A. Al-Sha'ary (1993) Analysis of date palm female floral initials potentials by tissue culture. Third Symposium on Date Palm, King Faisal University, Al-Hassa, Saudi Arabia. 161 - 170.
- Feher, A. (2005) Why somatic plant cells start to form embryos? In: A. Mujib and J. Samaj (eds.) *Somatic Embryogenesis*. Springer-Verlag Berlin Heidelberg. 85 - 101.
- Fernandez H, Perez C, Sanchez-Tames R. (2000) Modulation of the morphogenic potential of the embryonic axis of *Juglans regia* by cultural conditions. *Plant Growth Regulators*. 30: 125 - 131.
- Gamborg, O. L., R. A. Miller, and K. Ojima (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* 50: 151 - 158.
- Jimenez, V. M. and Thomas, C. (2005) Participation of plant hormones in determination and progression of somatic embryogenesis. In: A. Mujib and J. Samaj (eds.) *Somatic Embryogenesis*. Springer-Verlag Berlin Heidelberg. 103 - 118.
- Loomis, W. D. and J. Battaile (1966) Plant phenolic compounds and the isolation of plant enzymes. *Phytochem.* 5: 423 - 438.
- Markhand, G. S. and A. A. Abul-Soad (2007) Fruit characterization of Pakistani dates. The 4th Symposium on Date Palm in Saudi Arabia. 5 - 8 May 2007, King Faisal University Date Palm Research Center Hofuf, Kingdom of Saudi Arabia.
- Mujib, A., S. Banjee and P. D. Ghosh (2005) Origin, development and structure of somatic embryos in selected Bulbous ornamentals: BAP as inducer. In: A.

- Mujib and J. Samaj (eds.) Somatic Embryogenesis. Springer-Verlag Berlin Heidelberg. 15 – 24.
- Murashige, T. and F. A. Skoog (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473 - 479.
- Reuveni, O. and H. L. Kipins (1974) Studies of the *in vitro* culture of date palm (*Phoenix dactylifera* L.) tissue and organs. Pamphlet. 145: 3 - 39.
- Steel, R. G. and Torrie J. H. (1980) Principles and Procedures of Statistics, a Biomedical Approach. Mc Grow- Hill Book Company, New York: 469 - 517.
- Tisserat, B. (1979) Propagation of date palm (*Phoenix dactylifera* L.) *in vitro*. *J. of Exp. Bot.* 30: 1275 - 1283.
- Thomas, C., Meyer, D., Himber, C. and Steinmetz, A. (2004) Spatial expression of a sunflower SERK gene during induction of somatic embryogenesis and shoot organogenesis. *Plant Physiol Biochem.* 42: 35 – 42.
- Victor, J. M. R., Murch, S. J., KrishnaRaj S. and Saxena, P. K. (1999) Somatic embryogenesis and organogenesis in peanut: The role of thidiazuron and N⁶ – benzylaminopurine in the induction of plant morphogenesis. *Plant Growth Regulators.* 28: 9 – 15.
- Victor, P. G. (2005) Plant growth regulators in plant tissue culture and development. In: Trigiano, R. N. and D. J. Gray (eds.) *Plant Development and Biotechnology*. CRC Press LLC, 2000 N. W. Corporate Blvd., Boca Raton, Florida 33431. 8: 87 – 100.

تأثير نفضالين حامض الخليك و أندول حامض الخليك علي التخلق الجسمي للأجزاء النباتية للنورة المؤنثة لنخيل التمر (*Phoenix dactylifera L.*) صنف أصيل

عادل أحمد ابوالسعود* ، غلام سرور مركند** ، سعيد أحمد شاه**

* معهد بحوث البساتين ، مركز البحوث الزراعية ، مصر.
** معهد بحوث النخيل ، جامعة شاه عبد اللطيف ، السند ، باكستان.

الملخص:

بُحِثَ تأثير الوسط الغذائي الأساسي المعدل و المزود بتوليفات من نفضالين حامض الخليك بتركيزات (٠,٠, ١,٠, ٥,٠, ١٠,٠, ١٠٠,٠) ملليجرام/لتر) و أندول حامض الخليك (٠,٠, ١,٠, ٥,٠, ١٠,٠) ملليجرام/لتر) علي تكوين الأجنة الجسمية من الأجزاء النباتية للنورة الزهرية لنخيل التمر (*Phoenix dactylifera L.*). تم عمل التطهير السطحي للأجزاء النباتية للشمرخ الزهري لصنف الأصيل بطول ٣ سم باستخدام ١,٠% محلول هيبوكلوريت الصوديوم لمدة ٣ دقائق. حُضِنَت جميع الأجزاء النباتية داخل حضانة علي 23 ± 2 درجة مئوية تحت ظروف الظلام الدامس. نُقِلَت الأجزاء النباتية كل ٣ أسابيع علي وسط غذائي طازج لمدة ٣ نقلات. دلت النتائج المتحصل عليها علي أن النسبة المئوية للتلوث البكتيري و الفطري للأجزاء النباتية كان صفر. أيضاً لم يلاحظ أي تلون بني عموماً في معظم المعاملات. علاوة علي ذلك اعطت الأجزاء النباتية للشماريخ الزهرية كتلة من الكالس أبيض و ذلك علي الوسط الغذائي المحتوي علي ١,٠ ملليجرام/لتر نفضالين حامض الخليك + ٠,١ ملليجرام/لتر اندول حامض الخليك. و غالباً ما ارتبط تكوين الكالس الأبيض اللون بالأجزاء النباتية المنتفخة. علي الجانب الأخر، اعطت بعض الأجزاء النباتية للشمرخ الزهري والتي تمت زراعتها الوسط الغذائي الأساسي المحتوي علي ٥,٠ ملليجرام/لتر نفضالين حامض الخليك + ٠,١ ملليجرام/لتر اندول حامض الخليك كالس متفكك مصفر اللون مختلطاً بأجنة جسمية مباشرة.

من الجدير بالذكر، غالباً ما يرتبط وجود النفضالين حامض الخليك في الوسط الغذائي للأجزاء النباتية للشمرخ الزهري ببعض التلون البني. في الوقت ذاته ينشط تكوين كتل الكالس الأبيض باستخدام اندول حامض الخليك سواء كان للمحتوي الداخلي من الهرمون (المعاملة بدون إضافات) أو بالإضافة الخارجية (باقي المعاملات).

الكلمات الدلييلة: نخلة التمر ، النورة الزهرية ، منظمات النمو النباتية ، تخلق الأجنة الجسمية ، زراعة الأنسجة النباتية.