

## Micropropagation of Date Palm (*Phoenix dactylifera* L.) Khadhrawy cv.

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Received: 06/04/2019

Accepted: 03/08/2019

### Abstract

This research was conducted at the Tissue Culture Laboratory, Department of Genetic Engineering, Directorate of Agriculture Research, Ministry of Science and Technology, Iraq, in 2017. The main objective was to establish the best method for *In Vitro* micropropagation of Khadhrawy economical Iraqi date palm cultivar. Shoot tips, lateral buds and leaf primordial were detached from the offshoots and surface was sterilized with 1% sodium hypochlorite with Tween 20. Explants were cultured on modified MS medium supplemented with 10 mg.l<sup>-1</sup> of NAA, 10 mg.l<sup>-1</sup> 2,4-D, 10 mg.l<sup>-1</sup> NAA+ 2 mg.l<sup>-1</sup> 2,4-D or 10 mg.l<sup>-1</sup> 2,4-D + 2 mg.l<sup>-1</sup> NAA for callus initiation. Induced calli were transferred to regeneration medium. Regenerated shoots were rooted on medium supplemented with 1 mg.L<sup>-1</sup> IBA. The results showed that the highest quality of calli was induced on medium supplemented with 10 mg.l<sup>-1</sup> 2,4-D + 2 mg.l<sup>-1</sup> NAA which was significantly different from the other treatments. Moreover, the shoot tips were the best explants to induce calli from Khadhrawy cultivar compared with leaf primordial and lateral buds. The interaction analysis showed that the highest quantity of calli was produced from shoot tips cultured on medium supplemented with 10 mg.l<sup>-1</sup> 2,4-D + 2 mg.l<sup>-1</sup> NAA which was highly significant from all other combinations, while the lowest quantity of calli was produced from lateral buds cultured on medium supplemented with 10 mg.l<sup>-1</sup> NAA. The average number of plants regenerated from 0.5 g calli was 30 after 2 months. The rooted shoots were successfully acclimatized and transferred to the nursery.

**Keywords:** Date palm, Khadhrawy c.v., Micropropagation, 2,4-D, NAA

### Introduction:

Date palm (*Phoenix dactylifera* L.) is the most important tree in Iraq for its economical, nutritional and historical value. Unfortunately, the number of date palm trees in Iraq has been reduced drastically due to several wars and bad agriculture managements. Date palm is a dioecious, cross pollinated tree, therefore plants resulting from seeds are not true to type besides about 50% of the plants are male (Abahmane, 2011). Furthermore, up to date there is no method for sexing date palm trees at early growing stage. Therefore, elimination of male trees will be at the fruiting stage only. Traditionally it is propagated by offshoots which are produced from axillary buds located at the base of the trunk during juvenile life of the palm. However, offshoots number is limited during the lifetime of the tree depending on the cultivar.

Moreover, offshoots price is high especially for the desirable cultivars and transplanting success is low and highly affected by environmental conditions (Ibrahim, 2011). With all these facts, *In Vitro* propagation is the most promising method for fast propagation of date palm to overcome the reduction in the number of the desirable cultivars (Mujib *et al.*, 2004; AL-Khayri and Naik, 2017).

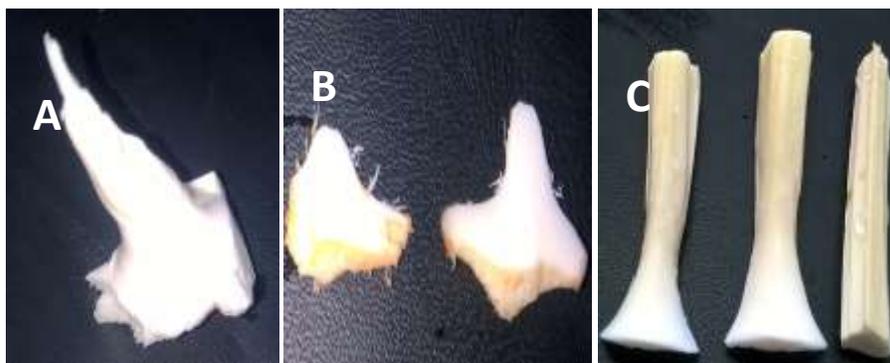
There is a vast literature on the successful micropropagation of date palm in different parts of the world. Generally, two pathways were used either organogenesis (Sharon and Shankar, 1999; Khierallah and Bader, 2007) or somatic embryogenesis (Al-Khayri, 2003; Aslam *et al.*, 2011). Several explants have been used to initiate date palm culture such as shoot tips (Zaid and Tisserat, 1983; Eke, *et al.*, 2005), inflorescences (Zaid *et al.*, 2007; Abahmane, 2010), leaf segments (Sane *et al.*, 2012), lateral buds, mature and immature embryos. Some successes have been achieved using all types of explants of different cultivars. Apparently, shoot tips and lateral buds are the most responsive to culture and the most frequently used in date palm propagation. Researchers investigated the factors affecting culture initiation and subsequent steps of propagation and reported detailed protocols (Tisserat, 1982; Fki, *et al.*, 2003; Badawy, *et al.*, 2005). However, those protocols are specific for the cultivars under investigation. Since there are more than 3000 date palm cultivars in the world and at least 624 of them are in Iraq (Mohan Jain, 2007), there is a strong demand to optimize the protocol for each cultivar or at least the desirable cultivars. Khadhrawy cultivar is of high quality, precocious flowering, early maturity, and moisture tolerance, thus, mass propagation of this important cultivar is of great interest. The main objective was to establish the best method for *in vitro* micropropagation of Khadhrawy economical Iraqi date palm cultivar

#### Materials and Methods:

Offshoots of Khadhrawy Mandily date palm cultivar were purchased from Ministry of Agriculture, Horticulture station/ Iraq in 2017. The outer leaves were removed and the shoot apex was taken skillfully and without any injury (Figure 1-A). Each shoot apex was resized and divided longitudinally into four equal pieces. Lateral buds and leaf primordial were also excised and used as explants in this study (Figure 1- B, C). All explants were washed with running tap water for 20 min to remove the entire dirt, then samples were kept in antioxidant solution consisted of 150 mg. L<sup>-1</sup> of both ascorbic acid and citric acid to prevent browning. Explants were surface-sterilized for 12 min. in 1% sodium hypochlorite solution with one drop of Tween- 20 per 100 ml of the solution. Samples were washed three times with sterilized distilled water 5 min each, before they were cultured on modified MS (Murashige and Skoog, 1962) by the addition of 100 mg.l<sup>-1</sup> Myo-inositol, 200 mg.l<sup>-1</sup> L-Tyrosine, 3 mg.l<sup>-1</sup> Thiamine- HCl, 0.5 mg.l<sup>-1</sup> Nicotinic acid, 0.5 mg.l<sup>-1</sup> Pyridoxine- HCl, 2 mg.l<sup>-1</sup> Glycine, 3 mg.l<sup>-1</sup> N6-(2-Isopentenyl) adenine (2iP), 30 g.l<sup>-1</sup> sucrose and 3 g.l<sup>-1</sup> active charcoal. Two growth regulators were used of different concentrations and combinations naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) for callus induction. Before adding the agar, the pH was adjusted at 5.8, the media were poured in special jars (300 ml capacity) at 50 ml per jar and were autoclaved at 121 °C for twenty minutes. Explants were cultured and kept in the culture room at 26± 2 °C in the dark and subculture every month for 6 months before data collection. Calli were removed from the explants and the fresh weight was measured. Equal amount of calli (500 mg.l<sup>-1</sup>) was transferred to regeneration and proliferation medium in jars which is consisted of free hormone MS salts, 20 g.l<sup>-1</sup> sucrose without charcoal and kept in the light for 16 h. and 8 h of darkness. Shoots were rooted in the light on half strength MS medium supplemented with 1 mg.l<sup>-1</sup> IBA, 3 g.l<sup>-1</sup> activated charcoal and 20 g.l<sup>-1</sup> sucrose in jars. For acclimatization, plantlets were then washed with

tab water, treated with fungicide solution and planted in small pots filled with sterilized peat moos and covered. Adapted plants were transferred to polythene bags containing peat moos and potting soil mixture (1:1) for their further growth and development in the plant nursery.

The experiment was conducted in Completely Randomized Design (C.R.D). Collected data were analyzed using Gene stat software. The means were compared using L.S.D test at  $p \geq 0.05$  level of probability.



**Figure 1. Explants excised from offshoots of Khadrawy Mandily date palm cultivar. A: Shoot tip, B: Lateral buds, C: Leaf primordia.**

### Results and Discussion:

Callus was induced from all explants after four transferred on callus induction media (Figure 2- A, B, C). Leaf primordia and the shoot tip expanded and increased in size first before callus formation at the bases of the leaves (Figure 2- B, C). Calli were detached from the explants after six months of culture and the fresh weight was measured. The results in Table (1) shows that the shoot tips gave the highest quantity of callus per explants (1441 mg) which was significantly different than the quantity induced from leaf primordia and lateral buds (892 mg and 444 mg) respectively. Also leaves primordia were significantly different from the lateral buds in the quantity of induced calli. These results are in agreement with the previously reported results which indicated that shoot tips are the best explants for callus induction in other date palm cultivars (Zaid and Tisserat, 1983; Badawy *et al.*, 2005). On the other hand, callogenesis of date palm leaves is highly dependent on genotype (Sane *et al.*, 2012). In the current study, leaf primordia followed by the shoot tips in the callogenesis ability. Since there is only one shoot tip per offshoot, several offshoots have to be sacrificed to initiate good quantity of callus as a starting step for micropropagation. Therefore, it is recommended to use leaf primordia of Khadrawy Mandily cultivar along with the shoot tips to increase the quantity of callus and reserve the offshoots.

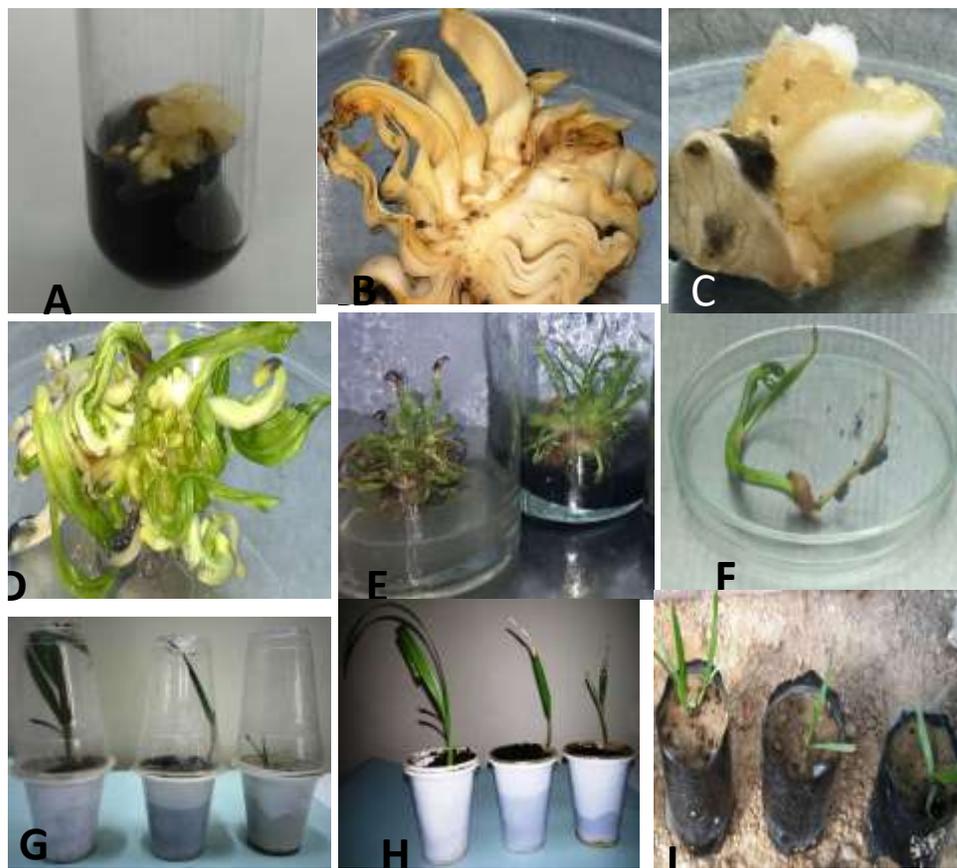
**Table 1. Effect of explants and auxins on callus fresh weight (mg) of date palm Khadrawy Mandily cultivar.**

Explants	Auxin ( $\text{mg.l}^{-1}$ )				mean
	10 (NAA)	10 (NAA) + 2 (2,4-D)	10 (2,4-D)	10 (2,4-D) + 2 (NAA)	
Shoot tips	408	590	1597	3170	1441
Leaf primordia	180	408	985	1993	892
Lateral buds	153	155	430	1037	444
mean	247	384	1004	2067	2777
L.S.D 0.05	Explant= 205.2		Auxin= 237	Explant X Auxin = 410.5	

The results in Table (1) also shows that there is great effect for the growth regulators on the quantity of calli induced from the explants under investigation. The highest quantity of calli (2067 mg) were induced on medium supplemented with  $10 \text{ mg.l}^{-1}$  2,4-D +  $2 \text{ mg.l}^{-1}$  NAA which was significantly different from the other treatments. Using  $10 \text{ mg.l}^{-1}$  2,4-D without NAA was significantly different from  $10 \text{ mg.l}^{-1}$  NAA alone or with low concentration of 2,4-D. The lowest quantity of calli (247 mg) was induced by the treatment of  $10 \text{ mg.l}^{-1}$  NAA. The type and the concentration of the growth regulators are important factors for callus induction and subsequently plant regeneration. Researchers used different growth regulators to induce callus and somatic embryos from date palm explants such as 2,4-D and 2,4,5-T or chlorophenoxy-acetic acid (CPA) (Aslam and Khan, 2009); NAA and IBA (Al-Khayri, 2003). Moreover, it has been found that NAA increases the percentage of somatic embryos with roots only, while IBA enhanced the percentage of embryos that formed plantlets (Al-Khayri, 2003). Although 2,4-D has been used widely for callus induction from different explants of date palm, high concentration of this growth regulator induce genotoxicity in callus cells which was detected by protein and RAPD analyses (Abass *et al.*, 2017). Genetic stability is very important in date palm micropropagation to regenerate true to type plants. Therefore, in the current study high concentration of 2,4-D was avoided to reduce callus heterogeneity and insure genetic stability.

The interaction analysis (Table 1) showed that the highest amount of calli were produced from shoot tips cultured on medium supplemented with  $10 \text{ mg.l}^{-1}$  2,4-D +  $2 \text{ mg.l}^{-1}$  NAA which was highly significant than all other combinations. On the other hand, there were no significant differences in the callus amount induced from shoot tips cultured on medium supplemented with  $10 \text{ mg.l}^{-1}$  2,4-D and the amount induced from leaf primordial cultured on medium supplemented with  $10 \text{ mg.l}^{-1}$  2,4-D +  $2 \text{ mg.l}^{-1}$  NAA and they were significantly different from the other combinations. Moreover, the lowest amount of calli was produced from lateral buds cultured on medium supplemented with  $10 \text{ mg.l}^{-1}$  NAA. Other researchers reported that 2, 4-D at  $100 \text{ mg.l}^{-1}$  with  $3 \text{ mg.l}^{-1}$  2iP produced the highest amount of calli from shoot tip explants (Badawy *et al.*, 2005). The response of different explants is varied with the presence of different growth regulators. Some explants contain endogenous hormones which might interact with the exogenous hormones added to the culture medium and stimulate callus induction.

Equal amount (500 mg) of calli produced from the explants under investigation was transferred to regeneration medium. There were no significant differences in the number of plants regenerated from the callus of the varied explants. The number of shoots ranged from 24 – 37 per 500 mg of callus from all sources in two months which was multiplied by shoots proliferation in the next subculture (Figure 2-D). In the current study, shoots were obtained on hormone free medium while other researchers found that  $10 \text{ mg.l}^{-1}$  of NAA is the best treatment for shoot formation (Zaid and Tisserat, 1983). Although during the shoot development and elongation stage some of the shoots formed roots on the hormone free medium, most of the shoots were rooted successfully after elongation in the presence of  $1 \text{ mg.l}^{-1}$  IBA and reduced amount of sucrose (Figure 2-E, F). However, in other date palm cultivars somatic embryos were germinated to complete plant in one step by using half strength MS medium with low concentration of growth regulator 0.2 to  $0.4 \text{ mg.l}^{-1}$  IBA (Al-Khayri, 2003). These variations in the published protocols depend on the cultivar under investigation. Finally, plantlets were acclimatized in the growth room before they were transplanted in small pots and moved to the nursery (Figure 1-B). The plants were strong and survived in the nursery with minimum attention.



**Figure 2. Stages of Khadrawy Mandily cv. micropropagation, Callus initiation from A: Lateral bud, B: Expanded leaf primordial, and C: Shoot tip; D: Embryo germination and shoot formation; E: Shoot elongation and rooting; F: Rooted plant; G: Acclimatization; H: Acclimatized plants; I: Regenerated date palm plants in the nursery .**

### Conclusion:

Micropropagation method had been established for Khadrawy Mandily date palm cultivar. Shoot tips were the best explants for callus induction followed by leaf primordial on MS supplemented with  $10 \text{ mg.l}^{-1}$  2,4-D +  $2 \text{ mg.l}^{-1}$  NAA. Somatic embryos were germinated on hormone free medium and the shoots were rooted on MS supplemented with  $1 \text{ mg.l}^{-1}$  IBA. Regenerated plants are grown in the nursery after successful acclimatization.

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الإكثار الدقيق لنخيل التمر *Phoenix dactylifera* L. صنف خضراوي

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تاريخ القبول: 2019/08/03

تاريخ الاستلام: 2019/04/06

## الملخص

تم إجراء هذا البحث في مختبر زراعة الأنسجة النباتية التابع لقسم الهندسة الوراثية في دائرة البحوث الزراعية، في وزارة العلوم والتكنولوجيا، في العراق في العام 2017. كان الهدف الرئيسي تحديد أفضل طريقة للإكثار الدقيق في المختبر لصنف خضراوي من نخيل التمر العراقي الاقتصادي. فصلت القمة النامية، والبراعم الجانبية، وبادئات الأوراق عن الفسائل، وعقمت سطحياً باستخدام 1% من هايبيكلوريدات الصوديوم مع المادة الناشرة Tween 20. زرعت الأجزاء النباتية على الوسط الغذائي MS المعدل والمجهز بالمعاملات التالية: 10 ملغ.لتر<sup>-1</sup> NAA و 10 ملغ.لتر<sup>-1</sup> 2,4-D و 10 ملغ.لتر<sup>-1</sup> NAA + 2,4-D أو 10 ملغ.لتر<sup>-1</sup> 2,4-D + 2 ملغ.لتر<sup>-1</sup> NAA لنشوء الكالس. نقل بعدها الكالس المستحدث إلى وسط الإخلاف. جذرت الأفرع المتولدة من الكالس في وسط التجذير المجهز بـ 1 mg L<sup>-1</sup> IBA. بينت النتائج أن أعلى كمية من الكالس المستحدث كانت في الوسط الذي أضيف له 10 ملغ.لتر<sup>-1</sup> من 2,4-D + 2 ملغ.لتر<sup>-1</sup> من NAA والذي كان مختلفاً بشكل معنوي عن باقي المعاملات. علاوةً على ذلك فإن القمة النامية هي أفضل جزء نباتي لحث الكالس للصنف خضراوي مقارنةً بباقي الأجزاء. وأظهرت نتائج التداخل أن أعلى كمية من الكالس نتجت من القمة النامية المزروعة على الوسط المجهز بـ 10 ملغ.لتر<sup>-1</sup> 2,4-D + 2 ملغ.لتر<sup>-1</sup> NAA والتي تفوقت بصورة معنوية عالية بالمقارنة مع بقية التوليفات، في حين تم الحصول على كمية من الكالس أنتجت من البراعم الجانبية والمزروعة على وسط مجهز 10 ملغ.لتر<sup>-1</sup> من NAA. بلغ متوسط أعداد النباتات الناتجة من 0.5 غ كالس هو 30 بعد شهرين. كانت الأقلية ناجحة للفروع المجذرة وتم نقلها الى الحاضنة.

الكلمات المفتاحية: نخيل التمر، الصنف خضراوي، إكثار دقيق، 2,4-D، NAA.