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Anatomical Study Of Adventitious Bud Regeneration From Shoot Tip Of Date Palm (*Phoenix dactylifera* L.) C.V Barhee In Vitro

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Abstract

Anatomy is highly important as a linking medium between several key branches of modern plant science. Detailed anatomical studies allow a more detailed understanding to achieve the ambitious goal of acquiring a holistic knowledge of plant development and aspects of cell and tissue differentiation. This study was carried out in the laboratories of Date Palm Research Centre at Basra University during the period from 2022-2023. The histological process of adventitious bud regeneration from the shoot tip explants of *Phoenix dactylifera* L 'Barhee' was reported in this study. Shoot regeneration was obtained from Murashige and Skoog (MS) supplemented with 1 mg l^{-1} naphthalene-acetic acid (NAA), 0.5 mg l^{-1} 6-benzyladenine (BA), and 0.5 mg l^{-1} kinetin (Kn) at a 16 hours photoperiod. The anatomical study of the responded explants revealed the presence of a mechanism for the differentiation of adventitious buds from shoot-tip. Further, a histological study showed that there were multiple vascular bundles around the adaxial side of explants, and the adventitious buds directly originated from the parenchymatous cells around the vascular bundles without the intervening callus phase. The parenchymatous cells started dividing and meristemoids formed thereafter. The meristematic cells continued division and subsequently gave rise to bud primordia. Well-developed shoot buds through direct organogenesis were achieved after 28 weeks of culture.

Keywords: Anatomical development; Direct organogenesis; Meristematic tissue cells; Parenchyma cells; Plant growth regulator

Introduction

Date palm *(Phoenix dactylifera* L.) is one of the oldest cultivated fruit crops in the world and belongs to the family Arecaceae. It is a multipurpose tree that has food, medicinal, and ornamental importance. It is believed to have originated in Mesopotamia at least 6000 years ago. Then, cultivation has expanded and has become a main crop in the Arabian Peninsula, North Africa, and Middle Eastern countries (Chao and Krueger 2007). Date palm is propagated through seeds or offshoots, but both methods suffer from some inadequacies and limitations (Al-Khateeb, 2006; Asemota *et al.,* 2007). Therefore, it becomes necessary to propagate date palm using alternative biotechnology methods. Date palm micropropagation enables the rapid and large-scale propagation of uniform plant pathogen-free plants, during plant material exchange, and true-to-type (Al-Mayahi, 2014; Awad *et al.,* 2020). The success of plant tissue culture and development are affected by several factors, including the composition of the culture medium used (Al-Asadi *et al.,* 2024). Plant growth regulators are synthetic compounds that act as natural plant hormones that are often manipulated in tissue culture experiments (Jiménez 2001). Auxin, cytokinins, and auxin-cytokinin interactions are usually the most important and generally required to regulate growth and organize development in plant tissue and organ cultures. In previous studies, some factors affecting the efficiency of shoot regeneration in *P. dactylifera* L. were discussed (Al-Mayahi, 2022 a, b; Al-Asadi *et al.,* 2024). The environment promotes morphological, physiological, and anatomical modifications in plants, which adapt them to changes in their environment. Nowadays along with other branches, anatomy is essential to validate and understand many aspects of plant biology (Sokoloff *et al.,* 2021). Anatomical features have also played a very important role in determining phylogenetic relationships. Hence, it is necessary to clarify the stages during shoot regeneration and understand how and where adventitious bud originated and formed in date palm. Clarification of this method will facilitate the enhancement of the efficiency of the regeneration of date palm.

Materials and Methods

The experiments for this study were carried out in the Laboratories for Date Palm Research Centre at Basra University during the period from 2022-2023.

Initiation stage

prepare of explant

Three-four-year-old offshoots of date palm cv Barhee were detached from healthy mother plants grown from Abu al-Khasib area south of Basrah (Fig. 1). The shoot tips were excised from the offshoot heart and trimmed to the size of about 0.8– 1.0 cm in width and 1–2 cm in length and used as starting material for current work. Sterilization of explants was performed using 70% ethanol for 1 min and 5% sodium hypochlorite for 20 min. Explants were then rinsed three times with sterile distilled water. To stimulate callus induction, explants were cultured on the MS basal medium (Murashige and Skoog 1962). It was combined with 100 mg l^{-1} glutamine, 5 mg l^{-1} thiamine HCl, 1 mg l^{-1} biotin, 30 g l^{-1} sucrose, and solidified with agar at 6.0 g l^{-1} and 2 g l^{-1} activated charcoal, with the addition of growth regulators Naphthalene Acetic Acid (NAA) at 1.0 mg l^{-1}, 6-benzyladenine (BA) at 0.5 mg l^{-1}, and 0.5 mg l^{-1} kinetin (Kn). Cultures were incubated under complete darkness at 27 ± 2 °C. The cultures were transferred to fresh media, with the same composition every 6 weeks until buds initiation.

Fig 1. Preparation of date palm offshoots and bud regeneration

Histological studies

Histological examinations during bud formation were carried out as Microtome slide preparation and observation were made using paraffin wax following the methods described by Drury et al. (1967), and included: fixation, dehydration, clearing, infiltration, embedding and sectioning: The paraffin-embedded models were cut with a 10 µm thickness using Microtomn Rotatory device {REICHERT-JUNG (820-II) 820 Histocut Rotary Microtome}. Then slide Preparation and photography took place as the slides were photographed using a compound optical microscope

type Olympus with a camera, then an Ocular Micrometer was used for the purpose of completing the measurements after being calibrated with the Stage Micrometer.

Result and Discussion

The histological process of adventitious bud regeneration from the shoot tip explants of *Phoenix dactylifera* L. 'Barhee' was reported in this study. At first histological change, several types of cells, including parenchyma cells adjacent to vascular bundles, were characterized by a dense cytoplasm and a large, prominent nucleus in the middle of cells, and showed an increase in size. Also, some of those cells adjacent to vascular bundles started initial division while the cells further apart with vascular bundles remained unchanged (Fig. 2).

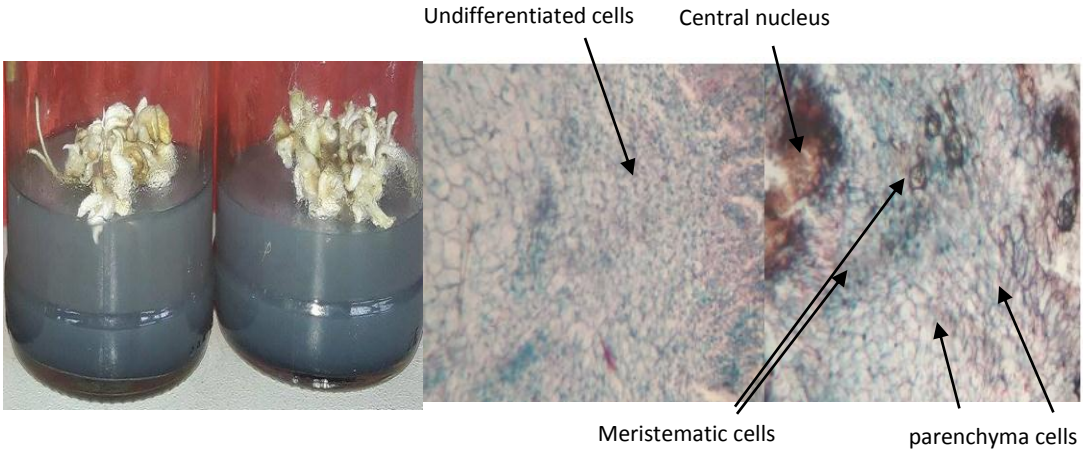
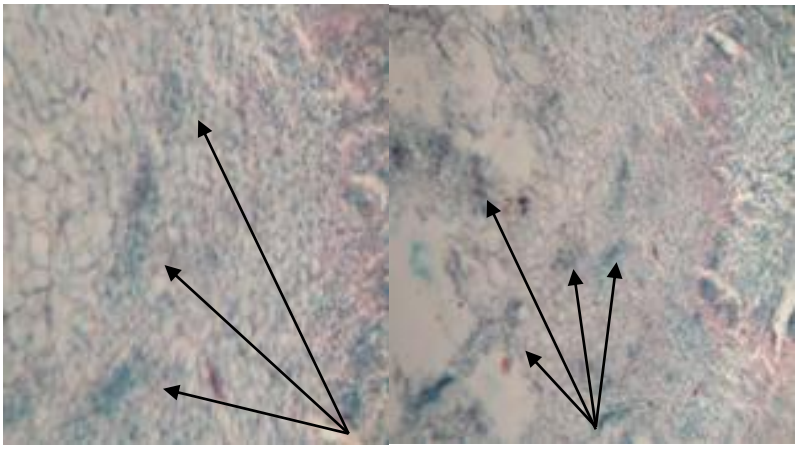


Fig 2. The continued development of plant tissue and the formation of meristems composed of small cells with clear nuclei.

The second phase was characterized by the emergence of meristems. Due to successive dedifferentiation and division of parenchymatic cells in all directions, meristemoids composed of small tightly packed cells appeared in these regions of the explants (Fig. 3). Those meristemoid cells were noted to be much smaller than the surrounding cells in terms of densely stained cytoplasms with little vacuoles, a large and prominent centrally positioned nucleus, and a high nucleus- to-cell area ratio (Fig 4).



Cell division and multiplication

Fig 3. Cell division and multiplication.

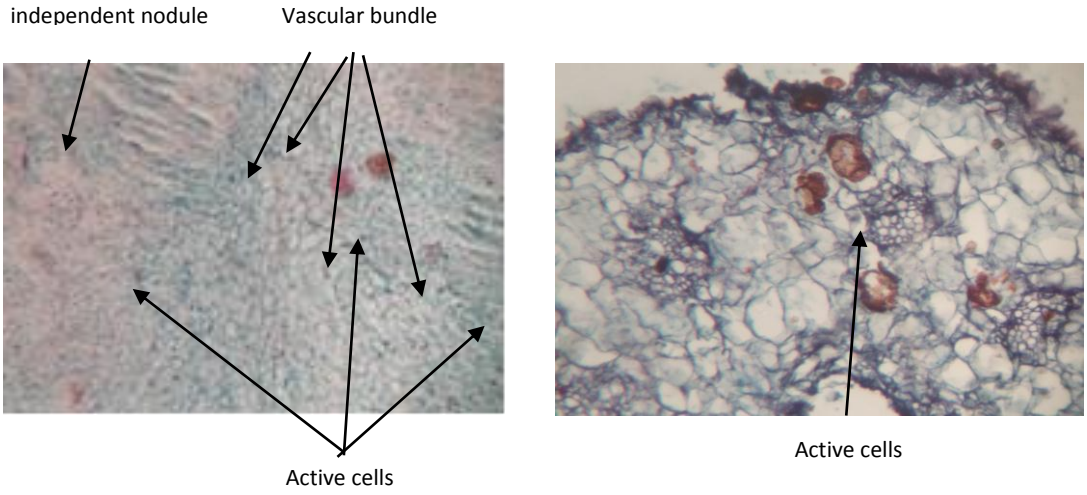


Fig 4. The initial stages of differentiation of adventitious buds and the formation of fine and effective cells

Regeneration of primordia and adventitious shoots were the traits of this period which appeared after 28 weeks of culture. At this phase, the meristematic zones divided constantly and gave rise to single or multiple (Fig. 5) bud primordia with obvious leaf primordia and apical meristems without an intervening callus phase. After incubation in a differentiation medium, buds with apical meristems, leaves, and evident vascular connected to the explants were observed. The adventitious shoot with leaf primordial (L) and shoot meristems (MR) formed (Fig 6) .

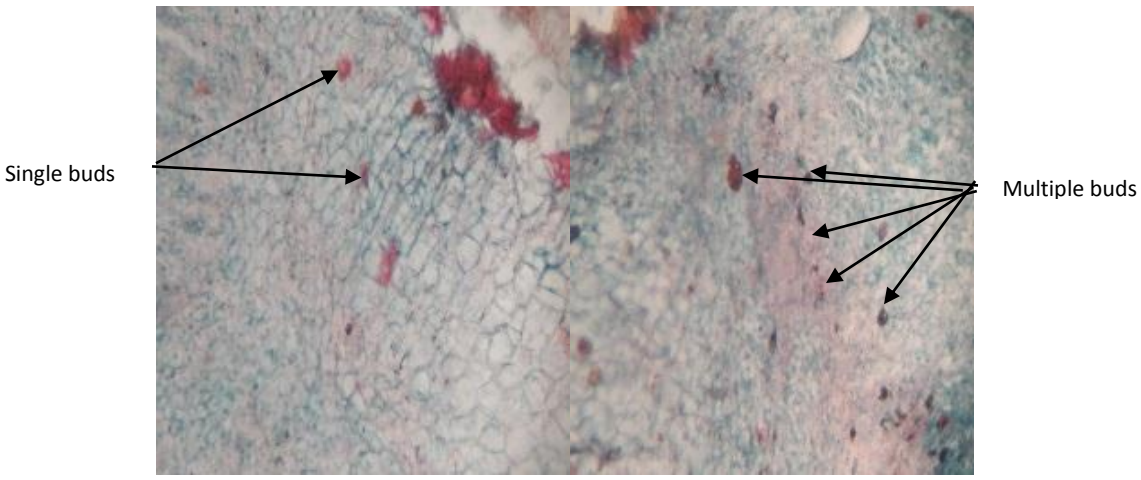


Fig 5. Meristematic zones continually divide and single or multiple buds appear

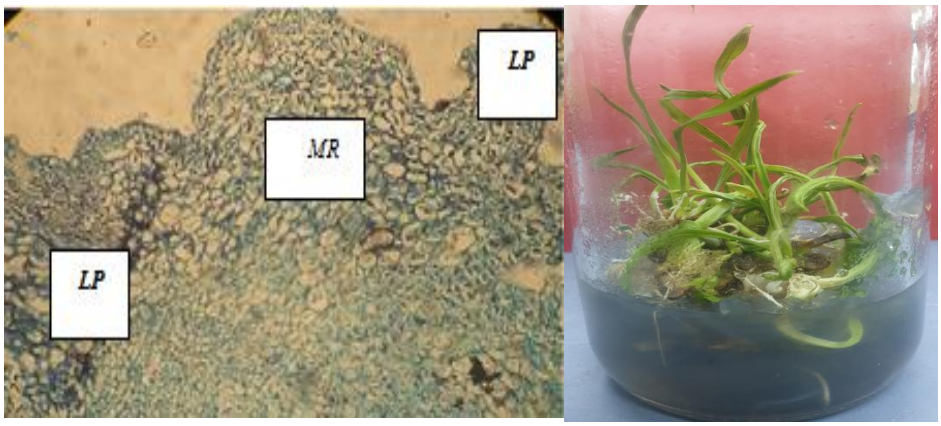


Fig 6. Development of shoot primordia (LP) and shoot meristems (MR) .

In addition, the regeneration of adventitious buds was asynchronous and continuous throughout the whole culture period. For example, some adventitious buds were already well developed, while the parenchymatous cells adjacent to vascular tissues differentiated continuously and gave rise to new meristemoids, and a few of them failed to develop into normal shoots. Plant regeneration could occur through organogenesis directly or indirectly (Fernando *et al.*, 2016; Al-Asadi *et al.*, 2019). The adventitious shoots of date palms were differentiated from the meristematic zones established from parenchymatous cells around the vascular bundles. Hence, the adventitious shoot proliferation from *in vitro* apical buds explants of *P. dactylifera* 'Barhee' by organogenesis could be confirmed. Buding is considered the most efficient proliferation process for date palm micropropagation (Al-Mayahi, 2021; Al-Mayahi and Ali, 2021). It is

reported to be a quick and efficient technique for large-scale propagation of date palm and could also be highly beneficial for propagation programs (Al-Mayahi, 2022 b;c).

It is well confirmed that cytokinins and auxins play a role in buds' regeneration, perhaps mediated by signals cascade triggered by these exogenous cytokinins and auxins (Jasim *et al.,* 2009; Resan *et al.,* 2023). The hormonal balance made the pro meristemic tissue continue its active division which led increase in the volume of this pro meristemic tissue. The appearance of the new buds from the meristemic tissue and its conversion into bud-generating tissue is caused by the increase of the cytokenines to the auxins. Anatomical sections showed that, during the early stages of the pro-culture period (i.e., before the appearance of meristemoid structure), cell division of bud culture was mostly located in hypodermal layers, whereas for the bud cultures, cell division occurred randomly in all tissues. Where active cell division occurs, certain cells of the meristems undergo divisions in such a way that one product of a division becomes a new body cell, called a derivative and the other remains in the meristem, called initials (Attaha *et al.,* 2012).

The meristemoid formation could be revealed on the top of the meristematic area on some peripheral callus sectors. Consequently, this tissue developed into primitive meristemic tissue, which is qualified by its quick division and increase in mass. The divisions led to the genesis of meristematic zones. These consisted of little cells with a dense cytoplasm and prominent nucleus. Thus, the adventitious buds were developed from meristemoid zones, as the result of the mitotic activity of cells. Meristemoids are differentiated and are later transformed into cyclic nodules from which shoots or roots develop (Al-Khalifah and Shanavaskhan, 2012).

Conclusion

The histological studies detected that the adventitious buds directly originated from the parenchymatous cells around the vascular bundles without the intervening callus stage. The parenchymatous cells began dividing and meristemoids formed thereafter. The meristematic cells continued division and subsequently gave rise to bud primordia. well-developed shoot during direct organogenesis was attained after 28 weeks of culture.

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دراسة تشريحية لتوالد البراعم العرضية من البراعم القمية لنخيل التمر (*Phoenix dactylifera* L.) صنف البرحي

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الخلاصة

يعد علم التشريح حلقة وصل بين العديد من الفروع الرئيسية لعلم النبات الحديث. كما تسهم الدراسات التشريحية بفهم أكثر تفصيلاً للمعلومات المتعلقة بتطور النبات وجوانب تمايز الخلايا والأنسجة. أجريت هذه الدراسة في مختبرات مركز أبحاث النخيل في جامعة البصرة خلال الفترة من 2022–2023. تم الإبلاغ في هذه الدراسة عن العملية النسيجية لتجديد البراعم العرضية من اطراف الافرع shoot tip في نخيل التمر *Phoenix dactylifera* L صنف البرحي 'Barhee' . تم الحصول على توالد البراعم المباشر على وسط موراشيكي وسكوك Murashige و Skoog (MS) مع إضافة 1 ملغم .لتر $^{-1}$ نفثالين حامض الخليك (NAA) و 0.5 ملغم .لتر $^{-1}$ بنزيل أدينين (BA) و 0.5 ملغم .لتر $^{-1}$ كينيتين (Kn) . كشفت الدراسة التشريحية للأجزاء النباتية المستأصلة عن وجود آلية لتمايز البراعم العرضية من أطراف البراعم. علاوة على ذلك، أظهرت الدراسة النسيجية أن هناك حزمًا وعائية متعددة حول الجانب المحوري للأجزاء النباتية المستأصلة، وأن البراعم العرضية نشأت مباشرة من الخلايا البرنكيمية حول الحزم الوعائية دون المرور بمرحلة الكالس. بدأت الخلايا البرنكيمية بالانقسام وتكوّنت بعد ذلك الخلايا الميرستيميّة. واصلت الخلايا المرستيمية الانقسام وأدت بعد ذلك إلى ظهوربادئات البراعم. تم الحصول على البراعم الخضرية المتطورة من خلال توالد الأعضاء المباشر بعد 28 أسبوعًا من الزراعة.

الكلمات المفتاحية: التطور التشريحي، توالد الأعضاء المباشر ، الخلايا البرنكيمية، خلايا الأنسجة المرستمية، منظمات النمو النباتية .