

Case reports

Open Access



A rare case of spontaneous inflorescence formation during micropropagation of date palm *Phoenix dactylifera* L cv. Khinazi.

Khaled Elmeer¹, Trechelle Joy Soliven²

¹ Libyan Authority for Scientific Research, Tripoli, Libya.

² Almarani for Agricultural Services, Doha, Qatar.

*Corresponding author: E-mail addresses: elmeer@gmail.com
Department Name, Libyan Authority for Scientific Research, Tripoli, Libya.

Received: 2026-07-23

Accepted: 2026-08-10

Publish online: 2026-09-10

الباحث الاول¹: خالد المير، الهيئة الليبية للبحث العلمي، طرابلس ليبيا
الباحث الثاني: تريشيل جوي سوليفان، المراني للخدمات الزراعية، الدوحة قطر .

Abstract

Commercial micropropagation of date palm (*Phoenix dactylifera* L.) aims to maintain vegetative development to ensure clonal fidelity and efficient multiplication. The induction of reproductive structures under *in vitro* conditions is therefore considered undesirable and extremely rare. In this study, we report a well-documented case of premature inflorescence formation observed during hormone-free subculture in date palm cv. Khinazi. Among 3658 culture jars maintained for a cumulative period of 471 days, only a single culture (0.027%) developed a floral structure. The culture exhibiting this unusual response originated from an offshoot collected while the mother palm was fruiting, and during initiation it had been given fairly high levels of 2,4-D. Moreover, this particular culture skipped the intermediate hormone-reduction stage altogether, it was moved straight from callus proliferation to the hormone-free medium, instead of going through the gradual step-down normally built into the protocol. We believe this early flowering was likely driven by several factors together leftover hormone effects, the donor plant's condition, and the sudden stop in growth regulators. This case shows how flexible date palm tissue can be after long periods in culture, and highlights why careful hormone management and donor selection matter in commercial micropropagation.

Keywords: Auxin; developmental instability; *in vitro* flowering; micropropagation; *Phoenix dactylifera*; somaclonal variation

حالة نادرة لتكوين نورة زهرية تلقائية تحت الظروف المخبرية أثناء الإكثار الدقيق لنخيل التمر *Phoenix dactylifera* L.

المستخلص: يهدف الإكثار الدقيق التجاري لنخيل التمر (*Phoenix dactylifera* L.) إلى الحفاظ على نمط نمو خضري بحت يضمن الثبات الوراثي والاستساخي وكفاءة تكاثر مرتفعة. وعليه، يُعد تحفيز التراكيب التكاثرية تحت الظروف المخبرية (*in vitro*) أمراً غير مرغوب فيه ونادر الحدوث للغاية. تعرض هذه الدراسة حالة موثقة بدقة لتكوين نورة زهرية مبكرة، رُصدت أثناء الإكثار الفرعي في وسط خالي من الهرمونات لنخيل التمر صنف "خينزي". فمن أصل 3658 وعاء زراعة جرى الحفاظ عليها لفترة تراكمية بلغت 471 يوماً، لم يتطور تركيب زهري إلا في مزرعة واحدة فقط، بنسبة قدرها 0.027%. وقد نشأت هذه المزرعة الشاذة من فسيلة جُمعت خلال طور الإثمار، وكانت قد تعرّضت لتراكيز مرتفعة نسبياً من حمض 2,4-ثنائي كلوروفينوكسي حمض الخليك (D-2,4) خلال مرحلة البدء الزراعي. علاوة على ذلك، نُقلت المزرعة مباشرة من طور تكاثر الكالوس إلى وسط خالي من الهرمونات، متجاوزة بذلك مرحلة التخميف الهرموني التدريجي الوسيطة المُتبعة عادة ضمن البروتوكول. ونفترض أن التأثير المشترك لتراكم بقايا الهرمونات، والحالة الفسيولوجية للنبات الأم، والسحب المفاجئ لمنظمات النمو الخارجية المنشأ، قد يكون وراء التنشيط المبكر لمسارات النمو التكاثري. وتُبرز هذه الملاحظة المرونة النمائية (**plasticity**) لأنسجة نخيل التمر في ظل ظروف الزراعة المخبرية المطوّلة، وتؤكد أهمية ضبط التحولات الهرمونية بعناية ودقة انتقاء النبات الأم في أنظمة الإكثار الدقيق التجارية.

الكلمات المفتاحية: الإزهار في الانابيب، اضطرابات النمو، الإكثار الدقيق، الأكسينات، التباين الجسدي، نخيل التمر.



INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most important perennial fruit crops in arid and semi-arid regions, particularly in the Middle East and North Africa, where it plays a significant economic and cultural role (Al-Habsi, 2025). Traditional propagation by offshoots is slow and limited in scale, restricting the rapid dissemination of elite cultivars (Manikandan et al., 2025; Shukla and Shukla, 2024). Consequently, micropropagation has become an important tool for large-scale clonal propagation of date palm, allowing year-round production of uniform planting material (Nimavat and Parikh, 2024).

Despite its advantages, prolonged *in vitro* culture may induce developmental abnormalities due to hormonal imbalance, physiological stress, or genetic and epigenetic instability. These abnormalities may include vitrification, somaclonal variation, abnormal shoot formation, and other morphological deviations. Among these phenomena, premature flowering under *in vitro* conditions is extremely rare in palms and is generally considered undesirable because it disrupts vegetative multiplication and may indicate instability in developmental regulation (Poethig and Fouracre, 2024; Sadka, 2025).

In vitro flowering has been reported in several plant species under specific physiological conditions or hormonal treatments (Das et al., 2025); however, reports in date palm are scarce. Such flowering events are often associated with hormonal stress, prolonged culture duration, or physiological signals originating from donor tissues. In particular, this phenomenon is often thought to arise from an interaction between synthetic auxins such as 2,4-D and the physiological memory retained by the excised donor tissue, a possibility further explored in light of the present findings.

The present study documents a rare case of premature inflorescence formation during micropropagation of date palm cv. Khinazi and examines the possible physiological and hormonal factors that may have contributed to this developmental deviation.

To our knowledge, documented instances of spontaneous *in vitro* inflorescence formation in date palm are extremely rare, and reports describing such events under commercial micropropagation conditions remain limited.

MATERIALS AND METHODS

A basal offshoot of date palm *Phoenix dactylifera* L. cv. Khinazi was collected from a healthy mother plant located in northern Qatar during the fruiting stage. After removal of external tissues, the apical meristem was excised and subjected to standard surface sterilization procedures before *in vitro* culture. Explants were cultured on MS medium (Murashige and Skoog, 1962). The culture protocol consisted of five sequential stages with varying hormonal compositions and durations (Table 1):

Table. 1: Stages, media composition, and exposure times during *in vitro* micropropagation of date palm.

Stage		Medium	Duration	Notes
1	Initiation (Figure 1)	MS + 2,4-D 100 mg L ⁻¹ + 2-iP 3 mg L ⁻¹	40 days	Dark conditions
2	Callus induction	MS + 2,4-D 30 mg L ⁻¹ + 2-iP 1.5 mg L ⁻¹	60 days	Two subcultures
3	Soft Callus proliferation (Figure 2)	MS + NAA 5 mg L ⁻¹ + 2-iP 0.5 mg L ⁻¹	220 days	Monthly subculture
4	Multiplication	MS + NAA 2 mg L ⁻¹ + 2-iP 0.23 mg L ⁻¹	18 months	Standard protocol

5	Free growth regulators	MS without plant growth regulators	16 cycles (30 days each)	Critical transition stage
---	------------------------	------------------------------------	--------------------------	---------------------------

Cultures in multiplication stage were maintained at $26 \pm 2^\circ\text{C}$ under a 16 h photoperiod with light intensity ranging between 2000–3000 lux.

Monitoring of cultures

A total of 3,658 culture jars were monitored during the hormone-free stage over a cumulative period of 471 days. Cultures were visually inspected during each subculture cycle for morphological abnormalities, including any signs of reproductive structure formation.

RESULTS AND DISCUSSION

Among all cultures examined, only one culture jar (1 out of 3,658; 0.027%) displayed premature inflorescence formation during the hormone-free stage. No additional reproductive structures were observed in any other cultures throughout the entire experimental period.

The structure observed exhibited characteristics consistent with early floral differentiation, including the presence of compact meristematic tissues and bract-like structures (Figure 3), suggesting that developmental instability arose from a combination of hormonal and physiological factors. Notably, the affected culture had been transferred directly from Stage 3 to Stage 5, omitting the gradual hormonal reduction provided by Stage 4. This abrupt transition may have accelerated the shift from vegetative to reproductive development.



Figure 1: Early responsive stage of date palm (*Phoenix dactylifera* L.) explants on initiation medium, prior to embryonic callus formation.



Figure 2: Onset of embryonic callus formation in date palm (*Phoenix dactylifera* L.) explants.



Figure 3. Rare premature inflorescence formation observed during hormone-free subculture (Stage 5), indicating morphogenic deviation from the expected vegetative pathway.

The extremely low frequency of this event suggests that *in vitro* flowering in date palm is not a spontaneous or common occurrence, but rather the outcome of a specific combination of physiological and hormonal conditions.

Contributing factors: Several factors may have contributed to this developmental deviation.

First, the relatively high concentration of 2,4-D applied during the initiation stage may have altered the developmental competence of the cultured tissue. Synthetic auxins are known to influence cellular reprogramming, and prolonged exposure to them may induce physiological or epigenetic changes (Abd Elaziem et al., 2022)..

Second, the donor offshoot was collected during the fruiting stage of the mother palm. It is possible that endogenous reproductive signals or developmental cues present in the donor tissue were partially retained during the early stages of culture. Such physiological carryover has been suggested in other plant systems where explant developmental stage influences *in vitro* morphogenesis (Mirani et al., 2024).

Third, the omission of the intermediate hormonal-reduction stage may have created an abrupt withdrawal of exogenous plant growth regulators. This sudden hormonal transition could act as a stress signal, potentially triggering developmental reprogramming and promoting the activation of reproductive pathways.

Finally, prolonged *in vitro* culture may increase the likelihood of developmental instability and somaclonal variation. Extended exposure to *in vitro* conditions can modify cellular regulation and may occasionally lead to unexpected morphogenic responses (Alemayehu, 2023).

Taken together, these factors may have interacted to disrupt the mechanisms that normally maintain vegetative identity in date palm tissue culture, leading to premature activation of floral meristem pathways.

4. Implications for commercial micropropagation

From a commercial perspective, premature flowering during micropropagation represents an undesirable developmental deviation because it reduces multiplication efficiency and may indicate underlying physiological instability. The present observation suggests that careful control of hormonal transitions, particularly during the transfer to hormone-free media, is essential to maintain vegetative growth.

In addition, the physiological status of donor plants should be considered during explant collection. Selecting donor material from non-reproductive phases may help minimize the potential carryover of endogenous signals that could influence *in vitro* developmental pathways. This interpretation is consistent with previous reports indicating that excessive auxin exposure and physiological memory can induce developmental reversion or phase transitions in palms (Al-Mazroui et al., 2006; Nimavat and Parikh, 2024).

CONCLUSION

This study documents a rare case of premature inflorescence formation during the micropropagation of date palm cv. Khinazi, occurs at a frequency of only **0.027%** among more than three thousand cultures. Although rare, premature flowering during micropropagation carries significant commercial implications, as it reduces clonal multiplication efficiency and may indicate underlying epigenetic instability that could affect other traits. Our findings emphasize the importance of optimizing auxin concentrations, selecting donor material from non-reproductive phases, and adhering strictly to step-wise hormonal reduction protocols to maintain developmental stability in date palm micropropagation systems. Future investigations employing molecular markers for floral identity genes and epigenetic profiling may further elucidate the mechanisms underlying such rare developmental shifts.

ACKNOWLEDGEMENT: The authors thank Al-Marani Company for providing laboratory facilities and logistical support.

ETHICS

This research complies with relevant ethical and institutional guidelines. All experimental work followed Al-Marani Company's biosafety protocols. Offshoot material was collected with the farm owner's permission, causing no harm to the parent palm. As a widely cultivated species, date palm (*Phoenix dactylifera*) is not endangered or protected. All *in vitro* cultures were maintained and disposed of per standard biosafety procedures.

DUALITY OF INTEREST: The authors declare no conflicts of interest.

FUNDING: This research received no funding from public, commercial, or not-for-profit agencies.

AUTHOR CONTRIBUTIONS: Khaled Elmeer and Trechelle Joy Soliven conducted the tissue culture experiments, T. J. Soliven documented the anomalous inflorescence formation. K. Elmeer and T. J. Soliven analyzed the data and interpreted the results. K. Elmeer wrote the original draft of the manuscript. Both authors reviewed and contributed to the final version of the manuscript.

REFERENCES

Abd Elaziem, T.M., Ahmed, M.E.S.A. and Abou El-Dis, G.R., 2022. *In vitro* propagation for conservation of the rare date palm (*Phoenix dactylifera* L.) “Amri” using immature inflorescence. *In Vitro Cellular & Developmental Biology-Plant*, 58(6), pp.1048-1056.

Alemayehu, M., 2023. Growth requirements and propagation of date palm. In: *Date Palm*, pp.141-178.

Al-Habsi, N., 2025. Date palm (*Phoenix dactylifera* L.) fruit: strategic crop for food security, nutritional benefits, postharvest quality, and valorization into emerging functional products. *Sustainability*, 17(16), p.7491.

Al-Mazroui, H.S., Zaid, A. and Bouhouche, N., 2006. Morphological abnormalities in tissue culture-derived date palm (*Phoenix dactylifera* L.). In: *III International Date Palm Conference* 736, pp.329-335.

Das, J.B.B., Bharti, S., Singh, R.P., Singh, D., Singh, L., Ranganna, G., Sikarwar, P.S. and Kumar, S., 2025. Role of plant growth regulators in flower induction and quality enhancement: a review. *Plant Archives*, 25(2), pp.515-523.

Manikandan, S.K., Gowda, N.K., Nair, V., Al-Ruzouq, R., Gibril, M.B.A., Lamghari, F., Klironomos, J., Al Hmoudi, M., Sheteiwy, M. and El-Keblawy, A., 2025. Advancing date palm cultivation in the Arabian Peninsula and beyond: addressing stress tolerance, genetic diversity, and sustainable practices. *Agricultural Water Management*, 307, p.109242.

Mirani, A.A., Harikrishna, J.A., Teo, C.H. and Solangi, N., 2024. Factors influencing somaclonal variation in date palm, detection and selection for application in the plantation. In: *Somaclonal Variation: Basic and Practical Aspects*, pp.169-197. Cham: Springer International Publishing.

Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), pp.473-497.

Nimavat, N. and Parikh, P., 2024. Innovations in date palm (*Phoenix dactylifera* L.) micropropagation: detailed review of in vitro culture methods and plant growth regulator applications. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 159(1), p.6.

Poethig, R.S. and Fouracre, J., 2024. Temporal regulation of vegetative phase change in plants. *Developmental Cell*, 59(1), pp.4-19.

Sadka, A., 2025. Flowering and flower development in plants. *Annual Plant Reviews*.

Shukla, S. and Shukla, S.K., 2024. Micropropagation for crop improvement and its commercialization potential. In: *The Potential of Microbes for a Circular Economy*, pp.271-287. Academic Press.

Author's Contribution Form

Please Tick ✓ in the appropriate box for the author:

S.N	Author's Name*	Conceived & design of the study	Col- lected the data	Contributed data or anal- ysis tools	Performed the analy- sis/ inter- pretation of data	Drafting the manuscript/re- vising for im- portant intellec- tual context	Approval of the final version of the manuscript**
1	Khaled Elmeer	✓		✓	✓	✓	✓
2	Khaled Elmeer		✓		✓		✓

*list the authors' First name followed by surname, ** This is required for all authors

S.N	Author's name	Email	Author's signature
1	Khaled Elmeer	elmeer@gmail.com	
2	Khaled Elmeer	trechellesoliven@gmail.com	