Menara Perkebunan 2024, 92(1), 131-140
p-ISSN: 0125-9318/ e-ISSN: 1858-3768

http://dx.doi.org/10.22302/iribb.jur.mp.v92i2.588
Accreditation Number: 164/E/KPT/2021

Callus induction and regeneration of date palm (*Phoenix dactylifera* L.) cv. Zambli through somatic embryogenesis from four layers of young leaves explant

Masna Maya SINTA[1*)], Rizka Tamania SAPTARI[1)], Imron RIYADI[1)] & SUMARYONO[1)]

[1)]Indonesian Oil Palm Research Institute, Bogor Unit, Jalan Taman Kencana 1, Bogor 16128, Indonesia

Received 5 Aug 2024 / Revised 8 Oct 2024 / Accepted 29 Oct 2024

Abstract

The Zambli variety of date palm shows potential for cultivation in tropical regions, as its fruits are edible during the Rutab stage. However, large-scale production of Zambli seedlings presents a significant challenge. In vitro propagation offers a solution for producing large quantities of clonal planting material. This study focuses on inducing callus formation from the four-layered shoot tips of young leaves and regenerating these calli into plantlets through somatic embryogenesis. Explants were cultured on a modified MS medium with 10, 50, or 100 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), combined with 1 or 3 mg L^{-1} N6-(2-isopentenyl)adenine (2-iP). Embryo maturation was performed on the same medium without 2,4-D, while a hormone-free medium was used for plantlet regeneration. The results indicated that the highest callus induction occurred from the younger leaf layer (layer 1) in the medium containing 100 mg L^{-1} 2,4-D and 1 mg L^{-1} 2-iP, achieving a callus formation rate of 82.3%. Successful callus induction was achieved from the first, second, and third layers of young leaves. Somatic embryo maturation and plantlet regeneration were also completed, producing vigorous, well-rooted plantlets. Additionally, the development of date palm cv. Zambli in vitro culture through somatic embryogenesis was confirmed through histological analysis.

[Keywords: 2,4-D, 2-iP, embryogenic callus, in vitro propagation]

Introduction

Date palm (*Phoenix dactylifera* L.) is an essential plant in the Middle East and Africa. This plant grows in dry areas with very low humidity but requires sufficient water (Chao & Krueger, 2007). This plant is mainly cultivated for its very sweet fruit (dates), which has a high nutritional content. Dates contain various types of carbohydrates, such as maltose, glucose, fructose, and sucrose, whose composition depends on the date variety and the fruit's maturity level. There are five maturity levels of dates: Hababouk, Kimri, Khalaal, Rutab, and Tamar (Al-Alawi et al., 2017; Idowu et al., 2020).

Dates are essential or sacred fruits to Muslims, and consumption increases during the holy month of Ramadhan, especially in countries with significant Muslim populations. Indonesia contributes 13% of Southeast Asia and become the world's third-largest Muslim population. However, Southeast Asian countries rarely planted date palms, so the importation fulfilled the demand for dates. Indonesia is the world's fifth-largest date palm importer with 50,133 tons of dates were imported in 2021 (BPS, 2022).

The rare date palm plantations in Southeast Asia mainly were due to unsuitable climates for date palm cultivation. The date palm is well adapted to the desert environment, where a dry and warm climate is essential for fruit maturity and ripening (Chao & Krueger, 2007). Therefore, date palm cultivations in most Southeast Asia countries with tropical climates rarely produce fruits or do not support maturation until the final stage (Tamar stage), but only in the Khalaal or early Rutab stage (Sudarsono, 2020). Therefore, date palm planting materials that are adaptive to tropical climates or varieties in which the fruits taste good in the Rutab stage are necessary to establish date palm cultivation in tropical countries, particularly Southeast Asia. Zambli is one of the commercial date palms from Saudi Arabia which has high productivity (60-80 kg per plant per year) and can be consumed in both Tamar and Rutab stages (Gurra_Down, 2022), which is suitable for cultivation in the tropical climate (Sudarsono, 2020). Thus, the availability of Zambli date palm seedlings is essential for opening up date palm plantations in Indonesia.

*)Corresponding author: mayasinta77@gmail.com

Menara Perkebunan is DOAJ indexed Journal and accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3125)
How to Cite: Sinta, M. M., Saptari, R. T., Riyadi, I., & Sumaryono. (2024). Callus induction and regeneration of date palm (*Phoenix dactylifera* L.) cv. Zambli through somatic embryogenesis from four layers of young leaves explant. *Menara Perkebunan*, 92(2), 131-140.
http://dx.doi.org/10.22302/iribb.jur.mp.v92i2.588

Date palms are usually propagated from either seeds or offshoots (Mazri & Meziani, 2015). However, seed propagation is not ideal because of the variety of seeds produced. Since date palms are dioecious, they have distinct male and female plants, but their gender can only be identified once they start flowering, which takes about 3 to 5 years after planting (Chao & Krueger, 2007). This makes the palm gender identification before flowering is challenging. While gender identification at the early phase through molecular analysis has been widely studied (Al-Mahmoud et al., 2012; Elmeer & Mattat, 2012; Zhao et al., 2012; Cherif et al., 2013), the methods are expensive. On the other hand, offshoot propagation is limited by the small number of offshoots produced. Date palm trees generally grow upwards and offshoots only appear during the early years of plant growth (Eke et al., 2005).

Clonal propagation of date palms through in vitro tissue culture offers an effective solution. This method allows the production of large numbers of uniform, disease-free, and true-to-type plants, with the gender already determined during the selection of explants. Date palm tissue culture can be done through organogenesis and somatic embryogenesis (Al-Khayri & Naik, 2017; Sidky, 2017). Somatic embryogenesis (SE) can be defined as plant regeneration through direct or indirect embryo formation from somatic cells. It is useful for plant breeding, especially through genetic transformation and genome editing (Bortesi & Fischer, 2015; Donmez et al., 2013; Yin et al., 2017), and germplasm storage through cryopreservation (Bhatia & Bera, 2015; le Roux et al., 2016; Martínez et al., 2022). In monocots, including date palms, SE development can be observed through histological analysis (Santos et al., 2018; Wehbi et al., 2022).

Somatic embryogenesis in date palms can be initiated from the inflorescence or apical meristem as explants (Boufis et al., 2014; Mazri et al., 2018). However, using inflorescences as explants depends on the flowering season, which competes with the harvesting period for dates. The apical meristem also provides a limited number of explants. To overcome this limitations, young leaves can be used as an alternative source of explants, as they offer more tissue for *in vitro* culture. These young leaves are located in the base of offshoot, surrounding the apical meristem (Sidky, 2017).

The in vitro propagation of some commercial date palm varieties, such as Degla Beida, Mejhoul, and Barhee, has been widely studied (Al-Khayri, 2012; Boufis et al., 2014; Mazri et al., 2018). However, the success of propagation varied depending on the variety. Thus, this research focuses on inducing somatic embryogenesis in Zambli date palms and regenerating the somatic embryos into plantlets.

Materials and Methods

Plant material

The planting material was an offshoot taken from a 4-year-old female Zambli date palm, identified by its flowers. The planting material was collected in October 2017 from the Jonggol Date Farm, Bogor, West Java, Indonesia (Figure 1A and 1B). After removal from the mother plant, the offshoot was sterilized at the plant tissue culture laboratory of the Indonesian Oil Palm Research Institute in Bogor, West Java. The pre-sterilization process involved cleaning the offshoot from soil, washing it with detergent, and spraying it with 70% alcohol. The outer sheath was then peeled off, followed by the surface sterilization process in the Laminar Air Flow (LAF) cabinet. The sterilization consisted of soaking the offshoot in 0.5% Benlox (50% benomyl) for 30 min, followed by immersion in 15% Bayclean® (5.2% NaOCl) for 30 min, and in antibiotic solution (15 mg L^{-1}) for 20 min. After each step, the offshoot was rinsed with sterile distilled water three times.

Callus induction

The sterilized offshoot was peeled into four layers of young leaves (Figure 1C). The leaves were sliced into 1 x 1 cm pieces (Figure 2A) and used as explants. The innermost layer was labeled as layer 1, while layer 4 was the outermost. The explants were cultured in test tubes containing 12.5 mL callus induction medium. The experiment used six different formulations of callus induction medium, designed as KR1 to KR6. These media were based on modified MS basal minerals (as described by Al Khayri, 2007) and enriched with 40 g L^{-1} sucrose, 1.5 g L^{-1} activated charcoal, 3.5 g L^{-1} Gelzan.

Table 1. Treatments for date palm callus induction

Medium	2,4-D concentration (mg L^{-1})	2-iP concentration (mg L^{-1})
KR1	10	1
KR2	50	1
KR3	100	1
KR4	10	2
KR5	50	2
KR6	100	2

The media also contained varying concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) at 10; 50; or 100 mg L^{-1}, and N6-(2-isopentenyl)adenine (2-iP) at 1 or 3 mg L^{-1}, combined as treatments (see Table 1 for details). The pH of the media was adjusted to 5.8 and the media were sterilized by autoclaving at 121°C for 20 min. The cultures were stored in a dark room at 26 ± 2°C until callus formation was observed.

Callus maturation, embryo germination, and plantlet regeneration

Callus multiplication and maturation were done by subculturing the primary calli into the same fresh basal media (supplemented with 6 mg L^{-1} 2-iP and 10 mg L^{-1} naphtalena acetic acid (NAA)). The embryogenic calli (EC) were then maturated to become somatic embryos (SE) by transferring them in media with the same composition, but with lower 2-iP (1.5 mg L^{-1}) (Saptari & Sumaryono, 2018). Embryo germination and plantlet regeneration were achieved by transferring the SE to free-hormone basal media. All media used throughout the culture stages were enriched with 40 g L^{-1} sucrose, and the pH was adjusted to 5.7. Gelzan (3.5 g L^{-1}) was used as gelling agent for all stages, except for final plantlet regeneration, where liquid media were used for root induction. Subcultures were performed every six weeks. The cultures were maintained under 36-watt TL lamps with a 12-hour photoperiod.

Histological analysis

Histological analysis was performed on EC and SE, following the method described by Kladnik (2013). Samples of EC and SE were placed into vials, fixed in a buffered formaldehyde solution (formaldehyde, alcohol, acetic acid) for 48 hours, stored in 50% ethanol for 24 hours, and dehydrated in a series of tert-butanol (TBA). For embedding, the samples were placed in molten paraffin (Paraplast Plus). TBA was partially removed by pouring molten Paraplast Plus into vials containing TBA, and the samples were kept in an oven at 56–60°C until fully embedded. The TBA-paraffin mixture was carefully pipetted out and replaced with fresh molten paraffin. The vials were left uncovered overnight to allow the remaining TBA to evaporate. Next, the samples were embedded into paraffin blocks and sliced into thin sections (~15 µm) using a microtome. These sections were mounted onto glass slides and stained with 1% Safranin and 1% Fast Green (Merck, Germany), both dissolved in 95% alcohol. Cover slips were then added to the slides. The prepared histological samples were observed under a Nikon SMZ75T digital stereo microscope, and images were captured using a Nikon Digital Sight 1000 camera installed on the microscope.

Data analysis

The experiments in this study were conducted with at least 12 replicates, each in a separate tube. Data were analyzed using a T-test and Duncan's Multiple Range Test (DMRT) at a 5% significance level ($\alpha = 0.05$).

Results and Discussion

Callus induction

The 4-year-old date palm cv. Zambli mother plant had eight leaves (Figure 1A). Young leaf offshoots were isolated from the plant (Figure 1B), sterilized, and sliced according to their layers as described in the methods. The sliced explants were then cultured on callus induction medium. In this study, 18 explant slices were obtained from layers 1 and 2, while 24 slices were taken from layers 3 and 4 (Figure 1C). Layer 4, being the outermost and largest, provided more explant slices than the inner layers.

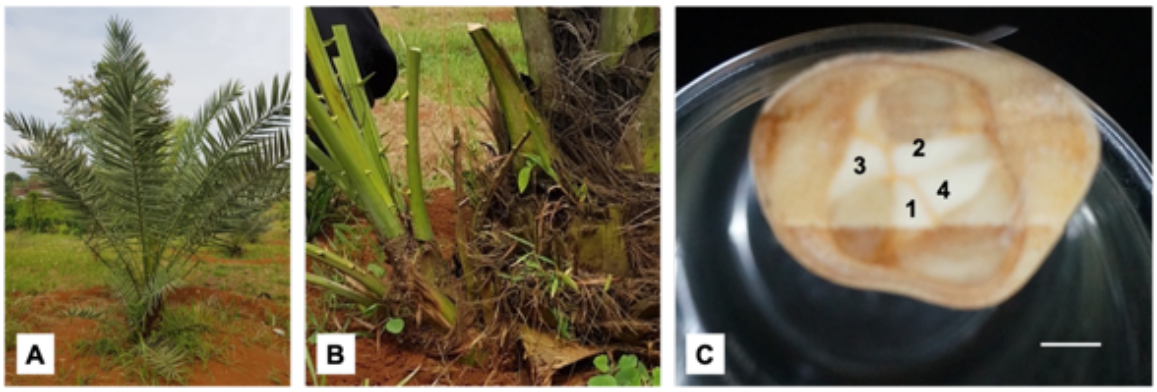

Figure 1. Source of explants: A) The Zambli date palm mother plant, B) offshoot as explants material, C) explant layer 1-4. Bar length 1 cm.

The callus induction medium supplemented with 100 mg L^{-1} 2,4-D and 1 mg L^{-1} 2-iP (KR3) successfully produced callus (50-82.3%) after 12 to 24 weeks of culture (Figure 2). Explants that failed to form callus turned brown (browning). The highest callus induction occurred on the KR3 medium (Figure 2 and 3). KR1 medium induced callus formation (16.7%) only in explants from layer 1 but failed to induce callus in outer layers. Lower concentrations of 2,4-D resulted in reduced or no callus formation, with KR4 medium failing to induce any callus. The highest callus induction was observed on KR3, followed by KR6 (50–82.3%). KR1 and KR4 contained the lowest 2,4-D concentration (10 mg L^{-1}), while KR3 and KR6 had the highest (100 mg L^{-1}). The success of callus induction was also influenced by the explant layer used (Figure 3B).

The highest percentage of callus induction was observed on the KR3 medium, followed by KR6 and KR2 (Figure 3A). Both KR3 and KR6 contained 100 mg L^{-1} 2,4-D, with different 2-iP concentrations. Overall, 1 mg L^{-1} 2-iP combined with 2,4-D was more effective for callus induction than 3 mg L^{-1} at the same 2,4-D concentration.

The most responsive explants for callus formation came from leaves isolated from the first layer (Figure 2 and 3B). A similar trend occurs in oil palm callus induction, where leaf phyllotaxis number -4 to -9 (very young leaves) are preferred as explants (Hashim et al., 2018). Inner leaf layers contain younger, more meristematic tissues compared to outer layers, which enhances their responsiveness.

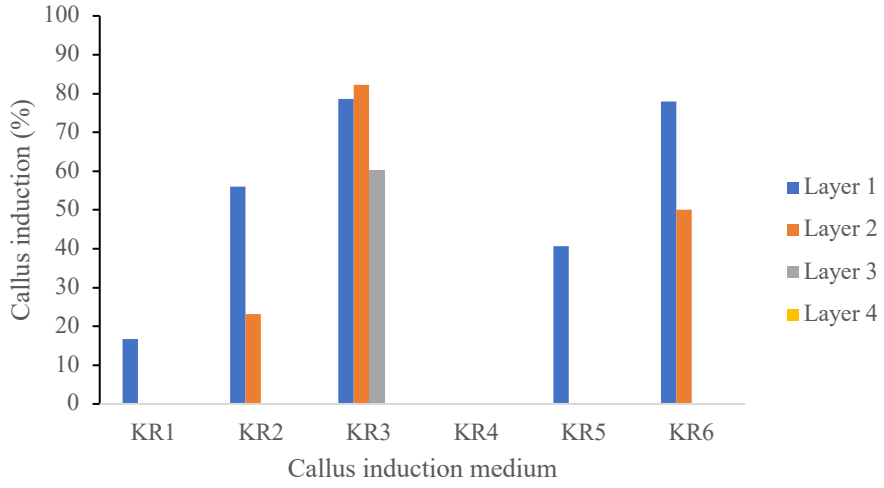

Figure 2. Zambli's callus induction rates on six different media and young leaf layers after 24 weeks of culture

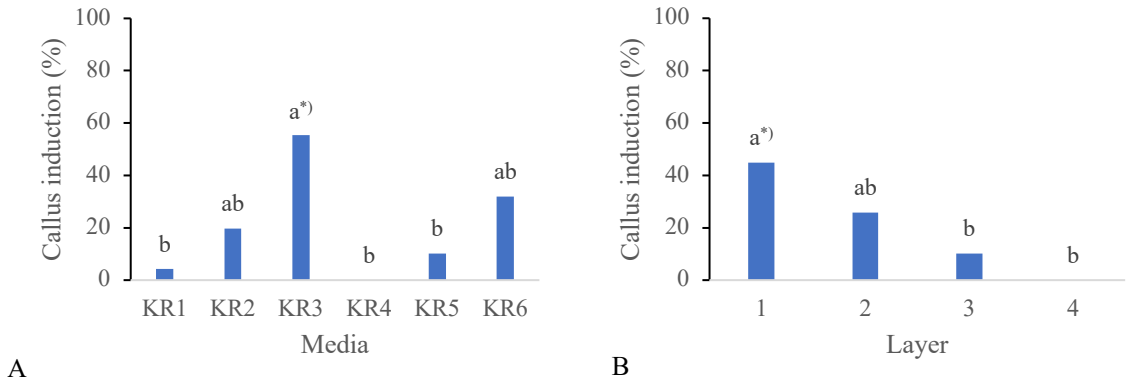

Figure 3. Callus induction percentage based on A) media, and B) explant layers after 24 weeks of culture. KR1: 10 mg L^{-1} 2,4-D and 1 mg L^{-1} 2-iP; KR2: 50 mg L^{-1} 2,4-D and 1 mg L^{-1} 2-iP; KR3: 100 mg L^{-1} 2,4-D and 1 mg L^{-1} 2-iP; KR4: 10 mg L^{-1} 2,4-D and 3 mg L^{-1} 2-iP; KR5: 50 mg L^{-1} 2,4-D and 3 mg L^{-1} 2-iP; KR6: 100 mg L^{-1} 2,4-D and 3 mg L^{-1} 2-iP. Layer 1: innermost layer, layer 4: outermost layer.
*) Different letter labels indicate statistical significance based on Duncan's Multiple Range Test ($\alpha = 0.05$)

Hormone 2,4-D is commonly used for callus induction of palm trees, with 100 mg L^{-1} frequently employed for date palm (Eke et al., 2005; Al-Khayri, 2012). However, some studies have reported that lower concentrations were more effective (Othmani et al., 2009; Saptari & Sumaryono, 2018). In addition to 2,4-D, Picloram has also been used as an alternative hormone for callus induction (Mazri et al., 2018). In this study, media with 1 mg L^{-1} 2-iP were more effective at inducing callus than those with 3 mg L^{-1}. This result aligns with previous research on Ajwa dates, where 1 mg L^{-1} 2-iP yielded better outcomes (Saptari & Sumaryono, 2018). However, optimal hormone concentrations appear to vary by variety or cultivar. For example, Zebia, Loko (Eke et al., 2005), and Barhee (Al-Khayri, 2012) dates responded better to 3 mg L^{-1} 2-iP for callus induction.

In this study, media containing 100 mg L^{-1} 2,4-D combined with 1 mg L^{-1} 2-iP successfully induced callus formation in Zambli date palm explants up to the third layer. This result expands the potential explant sources. Typically, only three explants are obtained from a single offshoot (Mazri et al., 2018), but this research yielded 84 pieces of explants. Prior studies (Sinta et al., 2023) have shown that the responsiveness of different explant layers varies by variety. For example, callus induction in Ajwa dates occurs up to the second layer, while for Barhee, Jarvis, and Khalas dates, it is restricted to the first layer. A novel finding in this study is that Zambli date palm explants can respond to callus induction up to the third layer.

Regeneration of date palm calli into plantlets

Callus formation in the explants (Figure 4A) began with swelling, which occurred six weeks after culture initiation. The primary or initial calli (PC) emerged after 12 weeks, characterized by a translucent cell mass that appeared on the basal part of the explant in contact with the medium (Figure 4B). The PC were then subcultured to a callus proliferation medium, where they developed into embryogenic calli (EC). Zambli date palm EC formed after 24 weeks and was characterized by its friable and loose structure (Figure 4C). In the callus proliferation medium, EC showed a relatively high proliferation rate, with a three- to four-fold increase in fresh biomass every six weeks.

On the maturation medium, EC developed to somatic embryos (SE) after 30 weeks. These SEs were characterized by the various developmental stages, including globular (G), elongated (E), scutellar (S), and coleoptilar (C) embryos (Figure 4E). The embryos germinated over 42 weeks on a hormone-free solid medium, with subsequent shoot regeneration taking place on a hormone-free liquid medium without a gelling agent (Figure 4F and 4G). The growth of Zambli shoots was relatively slow, requiring at least 4-5 subcultures for complete plantlet regeneration.

In general, in vitro regeneration of date palms is a slow and time-consuming process. For example, the Boufeggous variety required up to seven months for callus formation (Othmani et al., 2009), while the Degla Beida variety took ten months for embryogenic callus induction and an additional four months for somatic embryo formation (Boufis et al., 2014). The influence of different varieties on the success of in vitro propagation is also observed in other palm species, such as sago (Sinta et al., 2018) and oil palm (Santos et al., 2018), with the response of explants varying based on hormone treatments.

Despite the slow process, in vitro propagation of date palms can produce more seedlings compared to conventional offshoot propagation, which yields fewer planting materials. However, this method generally involves high concentrations of hormones, particularly 2,4-D (100 mg L^{-1}) during the early stages of callus induction (Al-Khayri, 2007; Boufis et al., 2014). However, plant abnormalities are still an issue with the use of high concentrations of hormones (Abass et al., 2017). In this study, moderate concentration of 2,4-D (50 mg L^{-1}) was tested as an alternative, in combination with explants from the deepest layer (layer 1) using medium KR2 or KR5. However, the callus induction percentage achieved with this treatment was relatively low. Further improvements in callus proliferation could involve the use of a temporary immersion bioreactor system (Saptari et al., 2020), which reduces the risk of abnormalities by limiting the explants' contact with hormone-enriched medium. In other plants, such as oil palm, abnormalities have been linked to prolonged exposure of explants to hormone-containing media. To address this, reducing the number of subcultures or implementing a temporary immersion system has been shown to prevent abnormalities (Marbun et al., 2015).

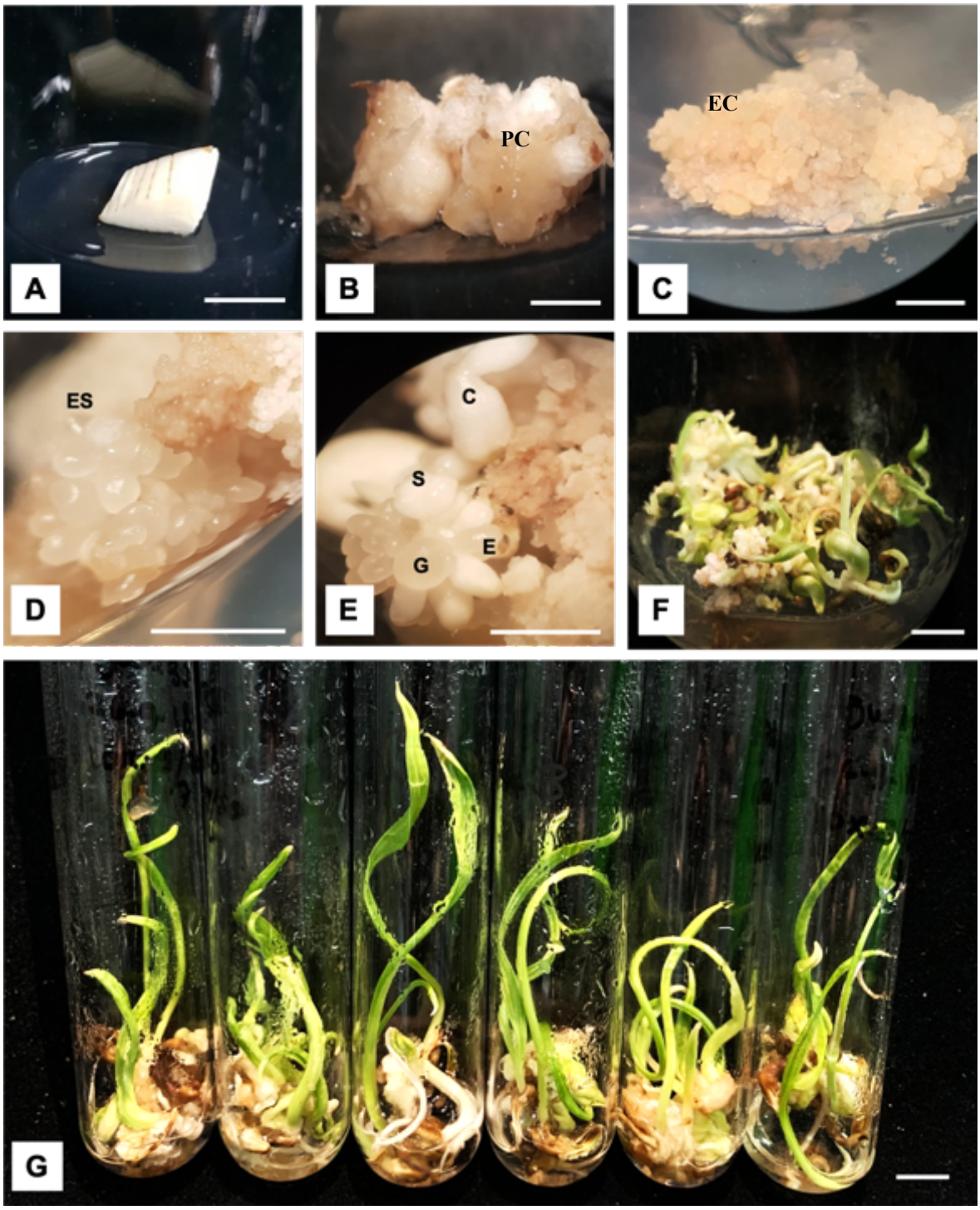

Figure 4. Somatic embryogenesis of Zambli date palm: A) explant, B) primary callus (PC) appears on KR3 medium after 12 weeks and becomes C) embryogenic callus (EC) after 12 weeks on proliferation medium, and become D-E) somatic embryo (SE) six weeks on maturation medium, F) germination on hormone-free medium, G) plantlets regeneration in liquid medium, SE: somatic embryo, G: globular embryo, E: elongated embryo, S: scutellar embryo, C: coleoptelar embryo. Bar length 1 cm.

Histological analysis on the embryogenic callus and somatic embryos

Histological analysis of Zambli dates calli and somatic embryos revealed structural differences at each developmental phase (Figure 5), confirming that in vitro regeneration in this study proceeded through somatic embryogenesis. The embryogenic calli (EC) were characterized by cells with small vacuoles and dense cytoplasm, visualized by the thick red staining (Figure 5A). The EC then progressed into globular somatic embryos, with some embryos separating into single globular forms (Figure 5B), while others remained fused with adjacent cells (Figure 5C). This fusion indicated early vascular tissue formation (V) and increasingly condensed cytoplasm (Figures 5B and 5C).

The globular embryos elongated into ovoid forms, marking the elongation phase (Figure 4E and 5D). The next stage was the scutellar phase (Figure 5E), characterized by a polarized structure, signifying further embryo development with an enhanced vascular system. The final phase of somatic embryo development was the coleoptelar phase (Figures 5F and 5G), during which germination began, as indicated by the formation of leaf primordia (LP). These primordia eventually developed into shoots (Figure 4F) and matured into plantlets (Figure 4G).

In vitro propagation of date palms can occur through two main pathways: organogenesis or somatic embryogenesis. In the organogenesis pathway, such as in the Zaghlool variety (Bekheet, 2013), vegetative buds are cultured to initiate organogenesis, leading to shoot proliferation and plantlet regeneration. In addition to buds, inflorescences can also serve as explants, as demonstrated in the organogenesis of Medjool, Barhee, and Selmi varieties (Taha et al., 2021). In this research, histological analysis confirmed that the micropropagation of Zambli date palm followed the SE pathway, where mature somatic embryos exhibited bipolar structures. Somatic embryogenesis involves the formation of embryo-like structures from somatic tissues, which develop into complete plants (Bhatia & Bera, 2015). The SE pathway typically takes longer than organogenesis and comprises six phases: callus induction, callus proliferation, embryo induction, embryo maturation, germination, and plantlet regeneration. In this research, the entire process, from callus induction to plantlet regeneration, took nearly two years. This duration is faster than in other palm species, such as oil palm, which can take three to five years to regenerate plantlets fully.

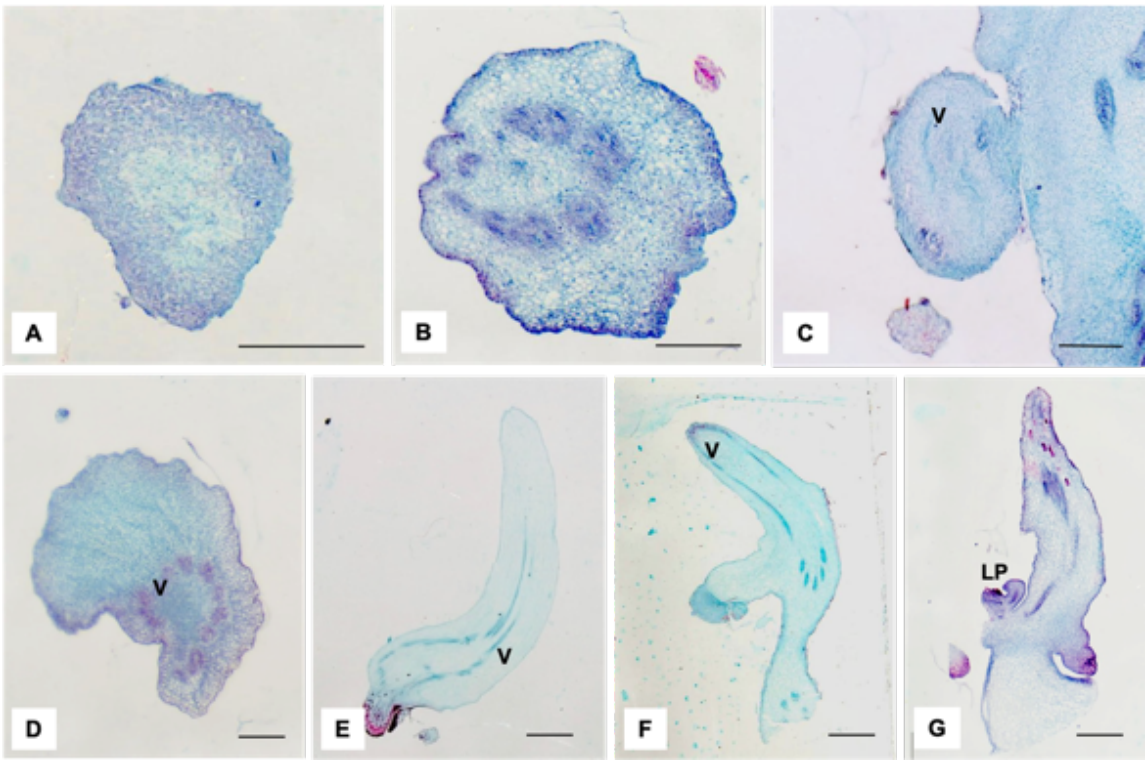

Figure 5. Histological sections presenting successive developments of somatic embryogenesis in Zambli date palm: A) embryogenic callus, B-C) globular somatic embryo, D). elongated somatic embryo, E) scutellar somatic embryo, F-G) coleoptelar somatic embryos. V: vascular system, LP: leaf primordia. Bar length 0.2 mm.

According to Bhatia & Bera (2015), SE offers several advantages, including the ability to produce plants in large quantities and simultaneously generate both roots and shoots, eliminating the need for a separate root induction phase. It is particularly advantageous for large-scale vegetative propagation of monocots. The utilization of SE is easily expandable and amenable to subculturing. Moreover, the culture can be manipulated to synchronize embryo formation and germination, maximizing plant production while minimizing labor costs. Additionally, SE allows embryos to enter a dormant state, making the process suitable for long-term storage.

Conclusions

The highest callus induction was achieved on media containing 100 mg L^{-1} 2,4-D and 1 mg L^{-1} 2-iP, using young leaf explants from the first layer. Callus formation in Zambli date palms was successfully induced from leaf layers 1 to 3. Lower concentrations of 2,4-D (10 mg L^{-1}) also promoted callus induction, but only in deeper-layer explants (layers 1 and 2). The development of Zambli date palm in vitro culture via somatic embryogenesis was confirmed through histological analysis. Propagation was successfully accomplished using indirect somatic embryogenesis, characterized by the formation of friable embryogenic calli and distinct developmental stages, including globular, elongated, scutellar, and coleoptelar embryos.

Acknowledgments

We thank the support of Jonggol Date Farm for supplying the plant material.

References

Abass, M. H., Al-Utbi, S. D., & Al-Samir, E. A. R. H. (2017). Genotoxicity assessment of high concentrations of 2,4-D, NAA and Dicamba on date palm callus (*Phoenix dactylifera* L.) using protein profile and RAPD markers. *Journal of Genetic Engineering and Biotechnology*, *15*(1), 287–295. https://doi.org/10.1016/j.jgeb.2016.12.003

Al-Alawi, R. A., Al-mashiqri, J. H., Al-nadabi, J. S. M., & Al-shihi, B. I. (2017). Date palm tree (*Phoenix dactylifera L.*)*: Natural Products and Therapeutic Options*. *8*, 1–12. https://doi.org/10.3389/fpls.2017.00845

Al-Khayri, J. (2007). Date palm *Phoenix dactylifera* L. micropropagation. In S. Jain & H. Haggman (Eds.), *Protocols for Micropropagation of Woody Trees and Fruits* (pp. 509–526). Springer International Publishing. https://link.springer.com/chapter/10.1007/978-1-4020-6352-7_46

Al-Khayri, J. M. (2012). Determination of the date palm cell suspension growth curve, optimum plating efficiency, and influence of liquid medium on somatic embryogenesis. *Emirates Journal of Food and Agriculture*, *24*(5), 444–455.

Al-Khayri, J. M. & Naik, P. M. (2017). Micropropagação de tamareira: Avanços e aplicações. *Ciencia e Agrotecnologia*, *41*(4), 347–358. https://doi.org/10.1590/1413-70542017414000217

Al-Mahmoud, M. E., Al-Dous, E. K., Al-Azwani, E. K., & Malek, J. A. (2012). DNA-based assays to distinguish date palm (arecaceae) gender. *American Journal of Botany*, *99*(1), 7–10. https://doi.org/10.3732/ajb.1100425

Amine Mazri, M., & Meziani, R. (2015). Micropropagation of date palm: A Review. *Cell & Developmental Biology*, *04*(03), 3–7. https://doi.org/10.4172/2168-9296.1000160

Bekheet, S. (2013). Direct organogenesis of date palm (*Phoenix dactylifera* L.) for propagation of true-to-type plants. *Scientia Agriculturae*, *4*(3), 85–92.

Bhatia, S., & Bera, T. (2015). Somatic embryogenesis and organogenesis. In *Modern Applications of Plant Biotechnology in Pharmaceutical Sciences*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802221-4.00006-6

Bortesi, L. & Fischer, R. (2015). The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology Advances*, *33*(1), 41–52. https://doi.org/10.1016/j.biotechadv.2014.12.006

Boufis, N., Khelifi-Slaoui, M., Djillali, Z., Zaoui, D., Morsli, A., Bernards, M. A., Makhzum, A., & Khelifi, L. (2014). Effects of growth regulators and types of culture media on somatic embryogenesis in date palm (*Phoenix dactylifera* L. cv. Degla Beida). *Scientia Horticulturae*, *172*, 135–142. https://doi.org/10.1016/j.scienta.2014.04.001

BPS. (2022). *Impor: Statistik Perdagangan Luar Negeri*. Badan Pusat Statistik, Indonesia.

Chao, C. C. T. & Krueger, R. R. (2007). The date palm (*Phoenix dactylifera* L.): Overview of biology, uses, and cultivation. *HortScience*, *42*(5), 1077–1082. https://doi.org/10.21273/hortsci.42.5.1077

Cherif, E., Zehdi, S., Castillo, K., Chabrillange, N., Abdoulkader, S., Pintaud, J. C., Santoni, S., Salhi-Hannachi, A., Glémin, S., & Aberlenc-

Bertossi, F. (2013). Male-specific DNA markers provide genetic evidence of an XY chromosome system, a recombination arrest and allow the tracing of paternal lineages in date palm. *New Phytologist*, *197*(2), 409–415. https://doi.org/ 10.1111/nph.12069

Donmez, D., Simsek, O., Izgu, T., Aka Kacar, Y., & Yalcin Mendi, Y. (2013). Genetic transformation in citrus. *The Scientific World Journal*, *2013*. https://doi.org/10.1155/ 2013/491207

Eke, C. R., Akomeah, P., & Asemota, O. (2005). Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) from apical meristem tissues from "zebia" and "loko" landraces. *African Journal of Biotechnology*, *4*(3), 244–246.

Elmeer, K. & Mattat, I. (2012). Marker-assisted sex differentiation in date palm using simple sequence repeats. *3 Biotech*, *2*(3), 241–247. https://doi.org/10.1007/s13205-012-0052-x

Gurra_Down. (2022). *Field trial date palm*. https://www.gurradowns.com.au/field-trial-date-palms

Hashim A. T., Ishak, Z., Rosli, S. K., Ong-Abdullah, M., Ooi, S. E., Husri, M. N., & Bakar, D. A. (2018). *Step wise protocols for somatic embryogenesis of important woody plants. Chapter 18 Oil Palm (Elaeis guineensis Jacq.) Somatic Embryogenesis* (Vol. 85). Springer International Publishing. https://doi.org/10. 1007/978-3-319-79087-9

Idowu, A. T., Igiehon, O. O., Adekoya, A. E., & Idowu, S. (2020). Dates palm fruits: A review of their nutritional components, bioactivities and functional food applications. *AIMS Agriculture and Food*, *5*(4), 734–755. https://doi.org/ 10.3934/agrfood.2020.4.734

le Roux, M. L., Botha, A. M., & van der Vyver, C. (2016). Somatic embryogenesis and cryopreservation of South African bread wheat (*Triticum aestivum* L.) genotypes. *South African Journal of Botany*, *106*, 78–88. https://doi.org/ 10.1016/j.sajb.2016.05.018

Marbun, C. L. M., Toruan-Mathius, N., Reflini, Utomo, C., & Liwang, T. (2015). Micropropagation of embryogenic callus of oil palm (*Elaeis guineensis* Jacq.) using temporary immersion system. *Procedia Chemistry*, *14*, 122–129. https://doi.org/10.1016/ j.proche. 2015.03.018

Martínez, M. T., Suárez, S., Moncaleán, P., & Corredoira, E. (2022). Cryopreservation of holm oak embryogenic cultures for long-term conservation and assessment of polyploid stability. *Plants*, *11*(9), 1–16. https://doi.org/ 10.3390/plants11091266

Mazri, M. A., Meziani, R., Belkoura, I., Mokhless, B., & Nour, S. (2018). A combined pathway of organogenesis and somatic embryogenesis for an efficient large-scale propagation in date palm (*Phoenix dactylifera* L.) cv. Mejhoul. *3 Biotech*, *8*(4), 1–8. https://doi.org/ 10.1007/s13205-018-1235-x

Othmani, A., Bayoudh, C., Drira, N., Marrakchi, M., & Trifi, M. (2009). Somatic embryogenesis and plant regeneration in date palm *Phœnix dactylifera* L., cv. Boufeggous is significantly improved by fine chopping and partial desiccation of embryogenic callus. *Plant Cell, Tissue and Organ Culture*, *97*(1), 71–79. https://doi.org/10.1007/s11240-009-9500-7

Santos, I. R., Maximiano, M. R., Almeida, R. F., da Cunha, R. N. V., Lopes, R., Scherwinski-Pereira, J. E., & Mehta, A. (2018). Genotype-dependent changes of gene expression during somatic embryogenesis in oil palm hybrids (*Elaeis oleifera* x *E. guineensis*). *PLoS ONE*, *13*(12), 1–15. https://doi.org/10.1371/journal.pone.02094 45

Saptari, R. T., Sinta, M. M., Riyadi, I., Priyono, P., & Sumaryono, S. (2020). Propagasi in vitro tanaman kurma (*Phoenix dactylifera* L.) pada bioreaktor dengan perendaman sesaat. *Menara Perkebunan*, *88*(2), 90–99. https://doi.org/10. 22302/iribb.jur.mp.v88i2.394

Saptari, R. T., & Sumaryono, . (2018). Embriogenesis somatik dari pucuk tunas tanaman kurma (*Phoenix dactylifera* L.). *Menara Perkebunan*, *86*(2), 81–90. https:// doi.org/10.22302/ iribb.jur.mp.v86i2.313

Sidky, R. (2017). Optimized direct organogenesis from shoot tip explants of date palm. *Methods in Molecular Biology*, *1637*, 37–45. https://doi.org/ 10.1007/978-1-4939-7156-5_4

Sinta, M. M., & Amanah, D. M. (2019). Acclimatization and early growth of tissue culture-derived *Stevia rebaudiana* at low altitude area in Bogor, Indonesia. *Menara Perkebunan*, *87*(1), 68–76. https://doi.org/10.22302/iribb.jur. mp.v87i1.326

Sinta, M. M., Riyadi, I., & Sumaryono. (2018). Somatic embryogenesis of sago palm (*Metroxylon sagu* Rottb.) from different origins in Indonesia. *IOP Conference Series: Earth and Environmental Science*, *183*(1). https://doi.org/10.1088/1755-1315/183/1/01200 8

Sinta, M., Saptari, R., Riyadi, I., & Sumaryono, S. (2023). Tissue culture of four varieties of date

palm grown in Indonesia. *IOP Conference Series: Earth and Environmental Science*, *1255*. https://doi.org/10.1088/1755-1315/1255/1/012019

Sudarsono (2020). Kurma ruthob Indonesia bisa jadi peluang bisnis baru. *IPB Today*, 354. https://biofarmaka.ipb.ac.id/news/2623-tips-ipb-kurma-ruthob-indonesia-bisa-jadi-peluang-bisnis-baru-2020

Taha, R. A., Allam, M. A., Hassan, S. A. M., Bakr, B. M. M., & Hassan, M. M. (2021). Thidiazuron-induced direct organogenesis from immature inflorescence of three date palm cultivars. *Journal of Genetic Engineering and Biotechnology*, *19*(1). https://doi.org/ 10.1186/s43141-021-00115-4

Wehbi, H., Soulhat, C., Morin, H., Bendahmane, A., Hilson, P., & Bouchabké-Coussa, O. (2022). One-Week scutellar somatic embryogenesis in the monocot B*rachypodium distachyon. Plants*, *11*(8). https://doi.org/10.3390/plants11081068

Yin, K., Gao, C., & Qiu, J. L. (2017). Progress and prospects in plant genome editing. *Nature Plants*, *3*(July), 1–6. https://doi.org/10.1038/nplants.2017.107

Zhao, Y., Williams, R., Prakash, C. S., & He, G. (2012). Identification and characterization of gene-based SSR markers in date palm (*Phoenix dactylifera* L.). *BMC Plant Biology*, *12*(December). https://doi.org/10.1186/1471-2229-12-237