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Effectiveness of Culture Medium Type on the Propagation of Date Palm (*Phoenix dactylifera* L.)

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I. Abstract

Date palm (*Phoenix dactylifera* L.) is considered one of the most important strategic crops in arid and semi-arid regions due to its high economic, nutritional, and environmental value, particularly in the Arab world. Given the limitations associated with conventional propagation through offshoots, plant tissue culture has emerged as an effective alternative for producing uniform and disease-free plants.

The culture medium plays a critical role in the success of micropropagation, as it directly influences callus induction, somatic embryogenesis, and the growth and multiplication of vegetative shoots. Previous studies have demonstrated that variations in mineral salt composition and plant growth regulator concentrations lead to significant differences in tissue responses. Among the commonly used media, Murashige and Skoog (MS) and Woody Plant Medium (WPM) have shown superior performance in promoting embryogenic callus formation and shoot proliferation.

Furthermore, liquid media have been reported to enhance initial callus growth, whereas solid media provide more suitable conditions for embryo differentiation. Continuous advancements in the formulation of culture media and the regulation of plant growth regulators represent a crucial step toward developing more efficient and standardized protocols for date palm micropropagation, ultimately improving productivity in response to increasing global demand.

This study provides a comprehensive overview of the culture media used in plant tissue culture, highlighting their characteristics as key determinants of successful in vitro propagation and their essential role in regulating growth, differentiation, and cell division in plant explants.

Keywords: Date palm, Plant tissue culture, Culture medium, Somatic embryogenesis, Callus induction, Plant growth regulators

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II. Introduction

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, dioecious plant and one of the most important fruit crops due to its high nutritional and economic value (Vardareli *et al.*, 2019). It is widely cultivated in tropical and subtropical regions across West Asia, North Africa, and the Middle East (Krueger *et al*., 2021).

In vitro propagation of date palm can be achieved either through organogenesis using shoot tips and axillary buds or through somatic embryogenesis, which involves callus formation followed by the development of embryogenic callus and subsequent somatic embryos. These embryos ultimately give rise to plantlets when cultured on artificial nutrient media under aseptic conditions (Kujur *et al*., 2024).

Plant tissue culture techniques are widely used for the large-scale propagation of various plant species under controlled laboratory conditions. In addition, they play a significant role in producing stress-tolerant genotypes, such as salt-tolerant varieties, and in conserving rare plant species (Bhatia, 2015). This technology has proven highly efficient in generating large numbers of plants from a single explant while maintaining genetic fidelity to the mother plant (Al-Khayri and Naik, 2017).

The composition of the culture medium, particularly the type and concentration of plant growth regulators, is a key factor influencing the success of tissue culture (Ibrahim *et al*., 2013). Culture media are among the most critical determinants of successful in vitro propagation, as they provide essential nutrients required for growth, differentiation, and cell division, with careful consideration needed to maintain a proper balance among their components (Al-Mayahi, 2022).

Accordingly, numerous media formulations have been developed for different plant species, varying in their macro- and micronutrient composition, types of vitamins, and concentrations of plant growth regulators to optimize tissue culture performance (Al-Mayahi, 2021; Al-Miyahi, 2023). Plant growth regulators are vital tools for improving crop productivity and quality, as they directly influence physiological processes such as cell division and elongation. Therefore, selecting the appropriate type, concentration, and timing of application is essential to achieve an optimal balance between auxins and cytokinins. This balance significantly affects the efficiency of somatic embryogenesis and, consequently, enhances propagation efficiency, plant quality, and productivity (Baldissera *et al*., 2025; Al-Mir, 2020).

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Economic Importance of Date Palm

Date palm (*Phoenix dactylifera* L.) is widely cultivated in tropical and subtropical regions around the world, particularly in North Africa, the Middle East, and South Asia. However, intensive cultivation is mainly concentrated within latitudes 10°–35° north of the equator, extending from the Indus River basin in Pakistan to the Canary Islands in the Atlantic Ocean. Climatic factors play a crucial role in palm growth and fruit production, both quantitatively and qualitatively. Optimal yields are achieved in regions characterized by high temperatures, low humidity, and absence of rainfall during the fruiting period. These conditions are typically found within the ecological belt extending between latitudes 16°–27° north of the equator, commonly referred to as the date palm belt (Fernandez-Lopez *et al*., 2022). In Iraq, date palm cultivation extends from Mandali and Tikrit at latitude 35°N to Al-Faw at latitude 30°N, with widespread distribution across most provinces (Ibrahim, 2019). Date palms are highly tolerant to harsh environmental conditions, including high temperatures, water scarcity, and soil salinity They also represent a rich nutritional source compared to other fruits due to their high caloric value and content of sugars, proteins, fibers, calcium, potassium, phosphorus, iron, and vitamins Additionally, dates possess significant health-promoting properties, including antioxidant compounds such as carotenoids, polyphenols, and tannins (Al-Khayri *et al*., 2015; Al-Adeeb, 2021; Tuaema, 2025).

Iraq is among the leading date-producing countries worldwide, and the date production sector has experienced continuous growth. Recent statistics indicate that global date production has reached approximately 8.46 million tons annually, with a steady increase (Albarrak *et al*., 2022). Furthermore, the number of date-producing countries increased to 37 in 2020, and production is projected to rise significantly, reaching 22.57 million tons by 2050, with an average global yield of 40 kg per palm (El-Mausly, 2022).

Date Palm Propagation

Date palm can be propagated through several methods, including seed propagation, offshoot propagation, and tissue culture propagation. Offshoot propagation is the most commonly used method for commercial production; however, it faces several limitations. These include the limited number of offshoots produced by the mother plant within a specific period during its juvenile stage (Mayahi, 2019; Gupta, 2020). Offshoot production is restricted to the vegetative phase and generally does not exceed the first 15 years of the palm's life. Consequently, some rare cultivars may surpass this stage without producing offshoots, making their propagation and conservation difficult (Hegazi *et al*., 2021).

Moreover, vegetative propagation through offshoots is a slow process that limits the number of plants produced per tree, is associated with low survival rates of new offshoots, and carries a risk of transmitting diseases and pests between orchards. Additionally, it requires a long time to reach fruiting (Al-Khalifa, 2011; Askari and Gantait *et al*.,

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2018). To achieve large-scale commercial expansion of date palm cultivation, a rapid and efficient propagation method is required. Tissue culture has proven to be a highly effective technique due to its ability to produce a large number of uniform plants within a short period. Moreover, the regenerated plants are genetically identical to the mother plant, free from pathogens and pests, and can be produced year-round. For these reasons, considerable attention has been directed toward tissue culture propagation (Alwael *et al*., 2017; Ribeiro *et al*., 2020).

Plant Tissue Culture

Plant tissue culture is defined as the in vitro growth of plant cells, tissues, or organs in glass or plastic containers containing artificial nutrient media under sterile and controlled environmental conditions (Qabil, 2015). Micropropagation refers to the technique of culturing a small piece of living plant tissue on an artificial medium enriched with chemical substances that stimulate cell division and proliferation, ultimately leading to the formation of complete plant organs (Al-Mayahi, 2019).

It is also defined as the science of culturing plant cells, tissues, or organs isolated from the mother plant under aseptic conditions, using artificial nutrient media and maintaining controlled environmental parameters such as temperature, light, and humidity, allowing the cultured explant to develop toward a desired objective (Smith, 2013; George *et al*., 2008). Plants produced through tissue culture are initially heterotrophic, meaning they are unable to synthesize their own and depend entirely on the nutrients provided in the culture medium (Mazri *et al*., 2016; Ikenganyia *et al*., 2017). This technique is carried out in specialized laboratories equipped for sterilization and precise environmental control.

Among the major advantages of tissue culture are the production of a large number of plantlets from a limited number of mother plants, the generation of disease-free propagules, and the ability to obtain fruiting plants within a relatively short period (approximately four years). Additionally, plantlets can be directly transplanted into the field without requiring a nursery stage, and they are easy to handle and transport while maintaining uniformity and health status. Tissue culture also enables the propagation of elite or rare genotypes that have lost their ability to produce offshoots (Abdalla *et al*., 2022; Fki *et al*., 2011).

The importance of plant tissue culture has increased significantly as one of the key biotechnological tools for plant propagation and improvement, both quantitatively and qualitatively. It also allows continuous production of secondary metabolites independent of seasonal constraints (Al-Amery et al., 2023).

This technique has contributed to a substantial increase in global date palm cultivation, with an estimated annual production of approximately 2.5 million tissue-cultured palms (Zaid, 2010). Currently, the global population of date palms is estimated at around 150 million trees (ICAR, 2023).

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Plant tissue culture represents the foundation of modern plant biotechnology, as nearly all biotechnological applications ultimately rely on the successful in vitro culture of plant cells, tissues, or organs. It has become an increasingly important tool for both scientific research and commercial applications in recent years (Kathayat, 2021; Oseni *et al*., 2018; Sharma; Kumar and Reddy, 2011).

Stages of Micropropagation

Micropropagation can be divided into the following stages:

1-Initiation Stage

This stage involves the selection of the appropriate explant, its surface sterilization, and its establishment on a suitable nutrient medium under aseptic conditions.

2-Multiplication Stage

This stage focuses on rapid proliferation and increasing the number of plantlets. Tissue cultures are repeatedly subcultured onto fresh media under sterile conditions until the desired number of plants is achieved.

3-Rooting Stage

Rooting may occur on the same medium used for shoot multiplication; however, in many cases, it is necessary to modify the medium composition, including nutrient balance and plant growth regulator concentrations, to induce strong root formation.

4-Acclimatization Stage

This stage involves transferring in vitro plantlets to soil or natural conditions. During this phase, plants transition from heterotrophic to autotrophic growth as they adapt to external environmental conditions.

Role of Plant Growth Regulators in the Efficiency of Date Palm Micropropagation

Plant growth regulators (PGRs) are a group of non-nutritional organic compounds that include both naturally occurring substances produced within plants and synthetic compounds applied exogenously. These compounds act at very low concentrations to regulate physiological and morphological processes responsible for plant growth and development (Al-Khafaji, 2014). They are classified into five major groups: auxins, cytokinins, gibberellins, ethylene, and abscisic acid.

PGRs play a pivotal role in stimulating cell division, differentiation, and organ formation in plant tissue culture systems (Al-Sumidaie, 2017). The type and concentration of growth regulators, in addition to the composition of the culture medium, are critical determinants of the efficiency of date palm propagation and its in vitro response (Hassan *et al*., 2021).

Auxins, particularly 2,4-D, have been shown to play a key role in enhancing embryogenic responses, improving embryo maturation, increasing their ability to develop into complete plantlets, and reducing dependence on genetic variability (Gang *et al*., 2025). Secondary somatic embryogenesis (SSE) represents a fundamental mechanism in plant biotechnology, enabling the repeated production of somatic embryos from primary embryos, thereby enhancing propagation efficiency and providing a continuous source of regenerable tissues. SSE develops through two pathways: a direct pathway of unicellular origin, which minimizes somaclonal variation and supports genetic transformation applications, and an indirect pathway involving embryogenic callus. Despite its importance, SSE is influenced by genetic factors and may exhibit abnormalities in embryo development and a decline in efficiency with repeated subculturing (Bogdanović & Čuković, 2025).

Significant advances in plant tissue culture have been achieved through successful callus induction from various plant explants and the development of single cells or cell aggregates into callus masses, facilitating large-scale plant propagation and improvement (Zaid and de Wet, 2005). Numerous studies have investigated callus induction from shoot tips and axillary buds of date palm offshoots.

For instance, Al-Maari and Al-Ghamdi (1998) demonstrated the role of NAA in stimulating cell division and growth when using apical and axillary buds of the Hilali cultivar. Similarly, El-Hammady et al. (1999) successfully induced primary callus from apical buds of the Siwi cultivar using a culture medium supplemented with 20 and 50 mg L^{-1} NAA and 2 mg L^{-1} 2iP. Jasim (1999) obtained both primary and embryogenic callus in the Sayer cultivar using MS medium supplemented with 25 mg L^{-1} NAA and 2 mg L^{-1} 2iP.

Yadav *et al*. (2001) reported callus induction from apical buds of Khadrawy, Medjool, and Halawi cultivars using a medium containing 100 mg L^{-1} 2,4-D and 1 mg L^{-1} 2iP. Apical buds showed faster callus formation compared to other explants, likely due to wounding effects that stimulate cellular responses. Bhargava *et al*. (2003) highlighted genotypic variation in callus induction response, while Eshraghi *et al*. (2005) confirmed the importance of auxin type and concentration in callus induction of the Kheneizi cultivar.

Al-Mayahi (2008) successfully induced primary callus from different explants (apical buds, axillary buds, and leaf primordia) of several date palm cultivars using a medium supplemented with 50 mg L^{-1} NAA and 3 mg L^{-1} 2iP, with the Khasab cultivar showing superior response. Zayed (2017) demonstrated that mature buds are an excellent source for callus formation and somatic embryogenesis using MS medium supplemented with 10 mg L^{-1} 2,4-D and 3 mg L^{-1} 2iP.

Muhsen *et al*. (2023) reported successful callus induction from immature inflorescences of the Barhee cultivar using MS medium supplemented with 1 mg L^{-1} picloram and 1 mg L^{-1} TDZ. The combination of 0.1 mg L^{-1} NAA and 0.5 mg L^{-1} TDZ resulted in the highest somatic embryo induction, germination, and proliferation. Following transfer to hormone-free medium for 45 days, acclimatized plantlets achieved a survival rate of 65%.

Recent studies indicate that embryogenic callus development and somatic embryo production depend on complex interactions among morphological, physiological, and environmental factors. Liquid media enhance initial callus growth, whereas solid media provide optimal conditions for embryo differentiation (Al-Karkhi, 2023).

Tuaimah (2025) demonstrated that low concentrations of cytokinins significantly promote embryogenic callus induction and somatic embryo formation. Specifically, 0.5 mg L^{-1} of both zeatin and 2iP resulted in the shortest induction periods (42.2 and 33.63 days, respectively) and an average of 7.3 embryos. Cytokinins also enhanced shoot regeneration, with 2 mg L^{-1} zeatin or 2iP reducing shoot formation time to 27.4 days and increasing shoot number to 5.73 per explant. Additionally, tissues derived from secondary somatic embryogenesis exhibited higher responsiveness to hormonal treatments. Biochemical changes, particularly the activity of superoxide dismutase (SOD), play a crucial role in successful induction. These findings provide a solid scientific basis for developing more efficient micropropagation protocols for economically important plants (Husain *et al*., 2025).

Role of Culture Medium Type in the Efficiency of Date Palm Micropropagation

The culture medium is one of the most critical factors directly influencing the success of plant tissue culture. For successful in vitro growth of any explant, it is essential to supply all necessary nutrients, as cultured cells and tissues are unable to perform photosynthesis and rely entirely on the external nutrient supply. Nutritional requirements vary not only among plant species but also within the same species depending on developmental stage. Therefore, the composition and physical nature of the culture medium are key determinants of successful tissue culture (Al-Mayahi, 2023). Growth and differentiation of plant tissues can be regulated by modifying the composition of the culture medium, which depends on the plant species, type of explant, developmental stage, and the objective of the culture (Al-Mayahi, 2022; Abera and Sulaiman, 2019). Accordingly, careful optimization of medium components and their physical state is essential to meet the nutritional demands of explants at each stage, thereby influencing subsequent developmental stages (Al-Karkhi, 2023).

Since the development of the Murashige and Skoog (MS) medium in 1962, numerous culture media formulations have been developed for various plant species, differing in macro- and micronutrient composition, vitamin content, and plant growth regulator concentrations (Shi, 2014; Al-Mayahi, 2014, 2019a, 2019b, 2021; Al-Mayahi *et al*., 2020). Among the most commonly used media are MS, Woody Plant Medium (WPM), and Gamborg's B5 medium.

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The MS medium was originally designed for herbaceous plants such as tobacco (Nicotiana), gerbera (Gerbera), ferns, begonias, and lilies, but it has since been successfully applied to a wide range of plant species. WPM, developed by Lloyd and McCown, is particularly suitable for woody plants and differs from MS in having lower concentrations of sodium and chloride ions, as well as reduced levels of ammonium nitrate (75%) and potassium (60%) compared to MS (Al-Hadidi, 2000).

Numerous studies have demonstrated the differential effects of culture media. For example, Baker et al. (2006) found that GD medium was superior to MS in terms of explant response and embryogenic callus formation, whereas MS produced a higher number of embryos and plantlets. Daradkah (2006) reported that MS medium supplemented with NAA and BAP significantly increased somatic embryo formation. Khairallah (2007) showed that MS medium provided the highest explant response and shoot proliferation rates in date palm inflorescences. Hamad and Jasim (2011) found that MS medium supplemented with auxins and cytokinins resulted in higher callus biomass compared to B5 medium. Kinawy (2015) demonstrated the superiority of WPM in embryogenic callus proliferation and embryo formation.

Al-Najm *et al*. (2018) reported that WPM supplemented with cytokinins achieved the highest embryogenic callus formation (93%) and improved shoot regeneration and rooting efficiency. Meziani *et al*. (2019) found that MS medium without growth regulators enhanced shoot elongation and rooting, with acclimatization success reaching 90%.

Recent studies have confirmed that WPM is often superior for shoot multiplication and growth, whereas MS remains highly effective for embryo production. Additionally, liquid media enhance biomass accumulation, while alternating between liquid and solid media can optimize embryogenesis (Malik *et al*., 2025).

Furthermore, Gang *et al*. (2025) demonstrated that MS medium supplemented with 1.0 mg L^{-1} BAP and 3.472 mg L^{-1} AMP achieved 100% somatic embryogenesis with an average of 9.5 embryos per explant.

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III. Conclusions

This review clearly demonstrates that the success of date palm micropropagation programs is closely associated with the type, composition, and hormonal balance of the culture medium. Both Murashige and Skoog (MS) and Woody Plant Medium (WPM) formulations have been widely proven to be effective; however, their superiority varies depending on the tissue culture stage and plant genotype.

Auxins and cytokinins play a central role in regulating cell division and tissue differentiation, making the optimization of their concentrations a fundamental step in establishing an efficient micropropagation protocol.

Recent studies highlight that somatic embryogenesis, particularly secondary somatic embryogenesis (SSE), represents a promising approach for the future of commercial date palm propagation. This technique enables rapid, repetitive, and genetically uniform plant production, thereby meeting the increasing demands of agricultural, industrial, and pharmaceutical sectors.

Furthermore, there is a growing need to develop optimized culture media that balance embryogenic induction and plant development while minimizing somaclonal variation. The integration of dual culture systems combining liquid and solid media is also recommended to enhance growth efficiency and overall propagation success.

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