

## The Role of Culture Medium Type and Auxin Concentration in Improving In Vitro Rooting of Shoots of Date Palm (*Phoenix dactylifera* L. cv. Barhi)

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### Abstract

This study was conducted at the Date Palm Research Center, University of Basrah, Iraq, during 2023–2024 to improve in vitro rooting of organogenesis-derived shoots of date palm (*Phoenix dactylifera* L. cv. Barhi). A factorial experiment in a completely randomized design compared two basal media (MS and WPM) and two auxins (NAA and IBA) at three concentrations (0, 0.1, and 0.5 mg L<sup>-1</sup>) with 10 replicates per treatment (replicate unit = one jar containing three shoots). Rooting percentage, number of roots per shoot, and mean root length were recorded after the second subculture (n = 5 replicates per treatment evaluated). Basal medium and auxin concentration significantly influenced rooting responses, with WPM and 0.5 mg L<sup>-1</sup> generally producing superior rooting performance, while auxin type effects depended on the measured trait. These findings provide a practical protocol option for enhancing rooting efficiency of micropropagated cv. Barhi plantlets prior to acclimatization.

**Keywords:** Culture medium; In vitro ; rooting; shoots; Naphthaleneacetic acid (NAA); Indole-3-butyric acid (IBA)

### Introduction

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous dioecious plant representing one of the most important fruit crops due to its high nutritional and economic value [1]. Tissue culture propagation of date palm can be achieved through two methods: organogenesis, via the induction of adventitious organs from cultured apical meristems and axillary buds, or somatic embryogenesis, via the formation of somatic embryos through a callus and embryogenic callus phase, by culturing plant tissues in sterile artificial nutrient media [2].

The culture medium composition and its constituents are considered critical factors

determining the success of plant tissue culture. Therefore, careful attention must be given to the selection and formulation of culture media because of their direct impact on the success of tissue culture programs [3]. This is one of the most important factors directly affecting the success of plant tissue culture. Plant tissue growth and differentiation can be controlled through the selection of appropriate medium type and composition. The composition of the culture medium varies depending on the plant species to be propagated, the plant organ used as explant, its developmental stage, and the objective of the culture procedure [4,5]. Multiple medium formulations have been developed by researchers for various plant

species, differing in the proportions of mineral nutrients (including macronutrients and micronutrients), the concentration of plant growth regulators, and the vitamin types used for successful tissue culture [6,7]. Among the most commonly used media are Murashige and Skoog (MS) medium [8], which is the most widely used in laboratory experiments for plant tissue culture, and Woody Plant Medium (WPM) [9], which is used for the propagation of woody plants and other species [10].

Plant growth regulators and culture medium type play vital roles in plant growth and differentiation. The type and concentration of growth regulators are important factors in date palm propagation [11]. The rooting phase of regenerated shoots is critical to the success of plant tissue culture. Among the most commonly used growth regulators for this phase are auxins, particularly naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA). The response of shoots to adventitious root formation differs depending on the plant species [12]. [13] in a study investigating the effects of cytokinins and auxins on the growth and rooting of

vegetative shoots of two date palm cultivars (Hallawi and Ashgar) in vitro using three types of auxins at different concentrations, found that NAA at  $0.75 \text{ mg L}^{-1}$  significantly increased the rooting percentage of Hallawi shoots to 80% and that of Ashgar shoots to 90%, outperforming other auxin types and concentrations. [14] achieved rooting of Medjool date palm shoots using NAA and IBA at various concentrations (0, 0.5, 1, 2, and  $3 \text{ mg L}^{-1}$ ) added to full-strength MS medium. Results showed that NAA significantly exceeded IBA in producing the highest mean root number (3.44 roots), and the  $1.5 \text{ mg L}^{-1}$  NAA treatment gave the highest mean root number (6.5 roots), while the  $1 \text{ mg L}^{-1}$  NAA treatment produced the longest roots (9.6 cm). Based on these findings, Objective: Accordingly, this study aimed to evaluate the factorial effects of basal medium (MS and WPM), auxin type (NAA and IBA), and auxin concentration (0, 0.1, and  $0.5 \text{ mg L}^{-1}$ ) on the in vitro rooting performance of organogenesis-derived shoots of date palm (*Phoenix dactylifera* L. cv. Barhi).

## Materials and Methods

### 1- Experimental Location and Period

The experiment was conducted in the Plant Tissue Culture Laboratory at the Date Palm Research Center, University of Basrah, Iraq, during 2023–2024.

### 2- Plant Material and Preparation of Culture Media

Organogenesis-derived shoots of date palm (*Phoenix dactylifera* L. cv. Barhi) were obtained from the multiplication stage maintained on MS medium supplemented

with cytokinins. Two basal media were evaluated: Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) and Woody Plant Medium (WPM) (Lloyd & McCown, 1981), both supplied by Caisson Labs (USA) with vitamins.

Media were prepared by dissolving salts of each basal medium, macro- and micronutrients, plant growth regulators, sucrose ( $30 \text{ g L}^{-1}$ ), and agar ( $6 \text{ g L}^{-1}$ ) as shown in Tables 1 and 2. Media composition was adjusted to pH 5.7 using 0.1 N NaOH or 0.1 N HCl. Additional media components

included adenine sulfate ( $40 \text{ mg L}^{-1}$ ), which enhances cytokinin-like activity and promotes shoot survival and axillary bud elongation during rooting transition; activated charcoal ( $1 \text{ g L}^{-1}$ ), which absorbs phenolic compounds and reduces browning during rooting while potentially enhancing root differentiation [7]; and glutamine ( $100 \text{ mg L}^{-1}$ ), which serves as a rapid nitrogen source supporting organogenesis and alleviating ammonium toxicity in higher-salt media [7].

Media were heated to  $90^{\circ}\text{C}$  with magnetic stirring, distributed into 500 mL glass jars at 50 mL per jar, sealed with plastic lids, and autoclaved at  $121^{\circ}\text{C}$  and  $1.05 \text{ kg cm}^{-2}$  for 20 minutes. After autoclaving, media were allowed to cool and solidify at room temperature before use.

#### Rooting Experiment: Factorial Design

Shoots obtained from the multiplication stage were excised and transferred to rooting media containing factorial combinations of:

- Factor A (Basal Medium): MS and WPM
- Factor B (Auxin Type): NAA and IBA
- Factor C (Auxin Concentration): 0, 0.1, and  $0.5 \text{ mg L}^{-1}$

This generated  $2 \times 2 \times 3 = 12$  treatment combinations, each replicated 10 times ( $n = 120$  shoots total). Each replicate unit

consisted of one 500 mL jar containing three in vitro-derived shoots of uniform size (approximately 3–4 cm in length with 2–3 leaves).

### 3- Culture Conditions

The rooting phase is a critical stage of micropropagation, during which an adventitious root system is formed on vegetative shoots generated from previous culture stages. To encourage root formation on regenerated shoots, an experiment was conducted to evaluate the effect of two culture media (as shown in Table 1) supplemented with two auxins, NAA and IBA, at three concentrations (0, 0.1, and  $0.5 \text{ mg L}^{-1}$ ) each, on the in vitro rooting of vegetative shoots derived from callus tissue induced from immature inflorescences of the Barhi cultivar. Ten replicates per treatment were cultured and incubated at  $27 \pm 1^{\circ}\text{C}$  under a 16-hour photoperiod with light intensity of 1000 lux, followed by 8 hours of darkness daily. Subculturing was performed every 4–6 weeks, and after the second subculture, five replicates per treatment were selected for evaluation of the following characteristics:

1. Rooting percentage (%) =  $(\text{Number of rooted vegetative shoots} / \text{Total number of shoots}) \times 100$
2. Mean number of roots per plantlet
3. Mean root length per plantlet (cm), measured using a ruler

Table (1) Composition of nutrient media based on (mg L<sup>-1</sup>)

Substance name	MS	WPM
<b>NH<sub>4</sub>No<sub>3</sub> Ammonium nitrate</b>	1650	400
<b>Calcium nitrate CaNo<sup>3</sup></b>	-	556
<b>KNo<sub>3</sub> Potassium nitrate</b>	1900	-
<b>So<sub>4</sub>No<sub>3</sub> Ammonium sulfate</b>	-	-
<b>KSo<sub>4</sub> Potassium sulfate</b>		990
<b>MgSo<sub>4</sub>.H<sub>2</sub>O Magnesium sulfate</b>	370	180
<b>CaCl<sub>2</sub> Calcium chloride</b>	440	72.47
<b>KHPo<sub>4</sub> Potassium phosphate</b>	170	170
<b>NaHPo<sub>4</sub> Sodium phosphate</b>	-	-
<b>H<sub>3</sub>BO<sub>3</sub> Boric acid</b>	6.20	6.20
<b>CoCl<sub>2</sub>.6H<sub>2</sub>O Cobalt chloride</b>	0.025	0.160
<b>MnSo<sub>4</sub> Manganese sulfate</b>	22.3	22.30
<b>CuSo<sub>4</sub>.5H<sub>2</sub>O Copper sulfate</b>	0.025	0.160
<b>NaMoO<sub>4</sub>.2H<sub>2</sub>O Sodium molybdate</b>	0.25	0.25
<b>KI Potassium iodide</b>	0.83	-
<b>ZnSo<sub>4</sub>.7H<sub>2</sub>O Zinc sulphate</b>	8.60	8.60
<b>FeSo<sub>4</sub>.7H<sub>2</sub>O Aqueous ferrous sulfate</b>	27.84	27.85
<b>Na<sub>2</sub>EDTA chelating substance</b>	36.7	37.30
<b>Thiamine HCl</b>	0.5	1
<b>Nicotinic acid</b>	0.5	0.5
<b>Pyridoxine</b>	0.5	0.5
<b>Claesin</b>	2	2
<b>My inositol</b>	100	100

Table (2) Concentpercentns of Additives to Nutrient Media

Substance	Quantitative mg l <sup>-1</sup>
Sucrose	30000
Sodium hydrogen orthophosphates	170
Adenine sulphates	40

Amino acid glutamine	100
Activated charcoal	1000
Agar	6000

### Statistical Analysis

Data were analyzed as a factorial experiment in a completely randomized design (CRD) with three factors: basal medium (MS and WPM), auxin type (NAA and IBA), and auxin concentration (0, 0.1, and 0.5 mg L<sup>-1</sup>). Analysis of variance (ANOVA) was performed using Genstat (Version 12.1). When F-tests were significant ( $P \leq 0.05$ ), treatment means were separated using Fisher's least significant difference (LSD) test at the 0.05 probability level. The main effects of each factor and their two-way and three-way interactions were examined.

### Results and Discussion

#### 1- Effect of Basal Medium and Auxin Concentration on Rooting Percentage

Basal medium type and auxin concentration significantly affected rooting percentage (Table 3), while auxin type and all interaction effects showed no significant differences. WPM promoted rooting more effectively than MS, achieving 56.7% rooting compared to 38.9% with MS. This

46% improvement reflects the lower salt concentrations in WPM, particularly the reduced ammonium nitrogen content, which favors root organogenesis over shoot proliferation [3][4]. At the control treatment (0 mg L<sup>-1</sup> auxin), rooting was minimal (20%), indicating that exogenous auxin is essential for efficient root initiation in date palm shoots derived from organogenesis. The 0.5 mg L<sup>-1</sup> auxin concentration yielded the highest rooting percentage (65%), a significant improvement over the 0.1 mg L<sup>-1</sup> concentration (58.3%) and the control (20%). This concentration-dependent response is consistent with the auxin sensitivity range reported for date palm in earlier studies [5][6].

The lack of significant difference between NAA and IBA in promoting rooting percentage suggests that at the concentrations tested (0–0.5 mg L<sup>-1</sup>), both auxins are similarly effective for initiating root primordia in cv. Barhi. However, this does not preclude differential effects on root quality or growth parameters, as evidenced by the subsequent measurements.

Table 3. Effect of culture medium type, auxin type and concentration, and their interactions on rooting percentage (%)

A type of media	B type auxin	C concentration auxin mg l <sup>-1</sup>			A average	A*B
		0	0.1	0.5		
MS	NAA	13.3	46.7	53.3	38.9	37.8
	IBA	13.3	46.7	60		40
WPM	NAA	26.7	60	73.3	56.7	53.3
	IBA	26.7	80	73.3		60
MS*C		13.3	46.7	56.7		
WPM*C		26.7	70	73		
B*C	NAA	20	53.3	63.3		
	IBA	20	63.3	66.7		
Average NAA		45.6				
Average IBA		50				
Average C		20	58.3	65		
L.S.D.=0.05	A=9.99			A*B=NS		
	B=NS			A*C=NS		
	C=12.24			B*C=NS		
	A*B*C=NS					

## 2- Effect of Basal Medium and Auxin Type on Average Number of Roots

WPM again demonstrated superiority over MS, producing 1.66 roots per shoot compared to 1.4 roots with MS (Table 4). IBA promoted a higher average root number (1.67 roots) compared to NAA (1.4 roots), a significant difference that highlights auxin-type specificity for root primordium proliferation. The 0.5 mg L<sup>-1</sup> concentration produced the highest average root count (1.75 roots), significantly exceeding the 0 mg L<sup>-1</sup> control (1.2 roots) and marginally surpassing the 0.1 mg L<sup>-1</sup> level (1.65 roots). These findings indicate that the increased root number at higher auxin

concentrations reflects both enhanced initiation (as seen in rooting percentage) and post-initiation proliferation of root primordia.

No significant interaction effects were observed between basal medium and auxin type, medium and concentration, or three-way interactions, suggesting additive rather than synergistic effects of these factors on root number within the tested parameter ranges. This additivity is physiologically reasonable because basal medium primarily influences the osmotic and mineral environment, while auxin concentration affects hormone-mediated developmental signaling [9].

Table 4. Effect of culture medium type, auxin type and concentration, and their interactions on mean root number per plantlet

A type of nutrient media	B type of auxin	concentration auxin mg l <sup>-1</sup> C			A average	A*B
		0	0.1	0.5		
MS	NAA	1	1.4	1.4	1.4	1.267
	IBA	1.2	1.6	1.8		1.533
WPM	NAA	1.2	1.6	1.8	1.667	1.533
	IBA	1.4	2	2		1.8
MS*C		1.1	1.5	1.6		
WPM*C		1.3	1.8	1.9		
B*C	NAA	1.1	1.5	1.6		
	IBA	1.3	1.8	1.9		
Average NAA		1.4				
Average IBA		1.67				
C average		1.2	1.65	1.75		
L.S.D.= 0.05	A=0.188			A*B=NS		
	B=0.188			A*C=0NS		
	C=0.230			B*C=NS		
	A*B*C=NS					

3- Effect of Basal Medium and Auxin Concentration on Average Root Length

Basal medium and the interaction between medium and auxin concentration both significantly influenced root length (Table 5). WPM produced longer roots (9.5 cm average) than MS (9.15 cm). although this difference was modest. Importantly, the two-way interaction between medium and concentration revealed that WPM supplemented with  $0.5 \text{ mg L}^{-1}$  auxin produced the longest roots (11.65 cm), suggesting optimal synergy between the nutrient-rich environment of rooting media at intermediate salt concentrations and the auxin threshold for sustained root elongation.

Regarding auxin type, NAA at  $0.5 \text{ mg L}^{-1}$  and IBA at  $0.1 \text{ mg L}^{-1}$  both achieved exceptional root lengths (11.74 cm and 11.62 cm, respectively), indicating concentration-dependent differential responsiveness. This trade-off between NAA (requiring higher concentration) and IBA (effective at lower concentration) may

reflect different auxin receptor affinities or differential polar transport and perception in cv. Barhi tissue. The interaction between medium and auxin concentration (medium  $\times$  concentration) showed that WPM with NAA at  $0.5 \text{ mg L}^{-1}$  produced the longest roots (12.44 cm), suggesting that the combination of low basal salt (from WPM) and optimal auxin concentration may maximize the developmental window for root cell elongation.

The physiological basis for WPM's superiority in root quality likely stems from its lower macronutrient concentrations, particularly the reduced nitrogen content, which shifts the carbohydrate-to-nitrogen ratio in favor of carbohydrate accumulation and reduced vegetative growth [10]. In rooting media, elevated carbohydrate availability (from the  $30 \text{ g L}^{-1}$  sucrose) combined with limited nitrogen may promote differentiation of root cells and enhance their elongation potential relative to callus or undifferentiated tissue formation that occurs in higher-salt media such as MS.

Table 5. Effect of culture medium type, auxin type and concentration, and their interactions on mean root length (cm)

type of nutrient media A	type of auxin B	C Concentration auxin mg l <sup>-1</sup>			averag A e	A*B
		0	0.1	0.5		
MS	NAA	5.44	10.94	11.04	9.157	9.14
	IBA	5.44	11.44	10.64		9.173
WPM	NAA	5.5	10.92	12.44	9.503	9.62
	IBA	5.5	11.8	10.86		8.387
MS*C		5.44	11.19	10.84		
WPM*C		5.5	11.36	11.65		
B*C	NAA	5.47	10.93	11.74		
	IBA	5.47	11.62	10.75		
Average NAA		9.38				
Average IBA		9.28				
C average		5.47	11.275	11.245		
L.S.D.= 0.05	A=0.136			A*B=NS		
	B=NS			A*C=0.235		
	C=0.166			B*C=0.235		
	A*B*C=0.334					

**Comparison with Previous Studies and Practical Implications**

Previous work on date palm micropropagation [5][6] and other woody plants [11] consistently shows that basal media with lower salt concentrations promote rooting efficiency. Our findings for cv. Barhi align with this paradigm and extend the evidence base to include direct comparison of NAA and IBA within a factorial design. The superior performance of IBA over NAA in promoting root number, combined with the comparable root length outcomes, suggests that future optimizations might emphasize IBA for number-based metrics and explore concentration reductions below 0.5 mg L<sup>-1</sup> to identify IBA optima.

The practical significance of this study is that rooting of organogenesis-derived shoots of cv. Barhi can be substantially improved by selecting WPM as the basal medium and 0.5 mg L<sup>-1</sup> auxin (either NAA or IBA, with

IBA preferred for maximizing root number). This protocol offers a robust basis for scaling up micropropagation and reducing the time and resources required for plantlet establishment prior to acclimatization, thereby enhancing the economic viability of commercial tissue culture propagation of this important cultivar.

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