International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

First Use of the Biocoupler Temporary Immersion System for Micropropagation of *Argania spinosa* (L.) Skeels : A Step Toward Sustainable Plant Biotechnology

Rajaa TESSE[1], Bouchaib BAHLAOUAN[2], Oumaima Mrabet[1], Said EL ANTRI[1], Nadia BOUTALEB[1]*

1 University of Casablanca, Laboratory of Biochemistry, Environment and Agri-Food, LBEA URAC36, 20650 Morocco
2 Higher Institutes of the Nursing Professions and Techniques of Health ISPITS Casablanca 22500, Morocco

*Corresponding author's email: nadia.boutaleb@fstm.ac.ma

ARTICLE INFO

Article history:

Received: 28 February 2025,
Received in revised form: 12 May 2025,
Accepted: 21 May 2025,

Article type:

Research paper

Keywords:

Argania spinosa,
Biocoupler,
Hyperhydricity,
Meta-topolin

ABSTRACT

This study presents the Biocoupler temporary immersion system as an innovative approach for the micropropagation of Argania spinosa, a species of considerable horticultural and ecological importance. The primary objective was to optimize in vitro propagation conditions to enhance plant growth while ensuring sustainability. A 1-min immersion cycle was identified as optimal for stimulating shoot development. By adjusting immersion intervals across three critical growth stages, problems associated with waterlogging and vitrification were effectively mitigated. In addition, supplementation of the culture medium with 0.8 mg L^{-1} meta-topolin, combined with a low concentration of IBA, significantly increased shoot biomass, indicating a synergistic effect on growth regulation. Compared with conventional micropropagation techniques, the Biocoupler system allowed more precise control of moisture and nutrient delivery, thereby substantially reducing the incidence of hyperhydricity. Collectively, these results represent a significant advancement in sustainable plant biotechnology, providing a scalable and efficient strategy for both the conservation and commercial propagation of argan trees.

Abbreviations: Agence Nationale pour le Développement des Zones Oasiennes et de l'Arganier (ANDZOA), analysis of variance (ANOVA), American Public Health Association (APHA), Adenosine Triphosphate (ATP), 6-Benzylaminopurine (BAP), Hydrogen Dioxide (H_2O_2), Indole-3-Butyric Acid (IBA), Light Emitting Diodes (LED), Murashige and Skoog medium (MS), Meta-Topolin (MT), α-naphtalene Acetic Acid (NAA), Sodium hypochlorite (NaClO), Temporary Immersion Systems (TIS), United Nations Educational, Scientific and Cultural Organization (UNESCO)

Introduction

The argan tree (*Argania spinosa* L. Skeels), a member of the Sapotaceae family, is an endangered species native to Morocco, where it holds substantial ecological and socioeconomic significance (Zahidi et al., 2013). It is the sole representative of its genus and naturally occurs in the arid and semi-arid landscapes of southwestern Morocco (Faouzi et al., 2015). In recognition of its importance, UNESCO designated the argan tree as a protected species in 1998, and in 2021 the United Nations General Assembly declared May 10th as International Argan Day. Beyond its native range, efforts have been made to introduce the species into several regions across Europe, America, and Asia (Koufan et al., 2020). Renowned for its remarkable longevity, the argan tree can survive for up to 200 years and tolerate extreme temperatures exceeding 50 °C. Its exceptional drought resistance is attributed to a deep

taproot system that enables access to groundwater in highly arid environments, allowing survival with as little as 120 mm of annual rainfall (Badreddine, 2016). The kernels of the tree yield argan oil, a globally valued product rich in bioactive compounds such as phytosterols, with wide nutritional and medicinal applications (El-Monfalouti et al., 2013; Charrouf and Guillaume, 2018). The growing international demand for argan oil has significantly boosted Morocco's economy (ANDZOA, 2011). However, this demand, coupled with deforestation, intensive agriculture, and climate change, has contributed to a severe decline in argan populations (Ait Aabd, 2022). Plant biotechnology has emerged as a promising conservation strategy to counter this decline, particularly through in vitro propagation, which enables large-scale production of genetically uniform plants. This approach not only supports the preservation of genetic diversity but also facilitates sustainable utilization (Mazri et al., 2022). Nonetheless, conventional micropropagation techniques face persistent challenges, the most notable being hyperhydricity—a physiological disorder that results in abnormal shoot development and poor survival during ex vitro acclimatization (Aguilar, 2019). To overcome these limitations, Temporary Immersion Systems (TIS) have been developed as innovative alternatives to traditional liquid culture methods. TIS operate by periodically immersing plant explants in nutrient solutions, followed by aeration phases that enhance gas exchange, nutrient absorption, and overall plant development while significantly reducing hyperhydricity (Georgiev et al., 2014; García-Ramírez, 2023). Over the past decades, various TIS models have been designed and widely adopted for the commercial micropropagation of economically valuable plant species. Prominent examples include RALM, SETIS, PLAN TIMA, PLANTFORM, Box-in-Bag, FA-Bio bioreactor, WAVE, Twin-Flask, and the Automatic Temporary Immersion bioreactor (RITA®) (Watt, 2012; Georgiev et al., 2014; Mohammadpour Barough et al., 2024). These systems have demonstrated their efficiency by reducing production costs by up to 70%, largely through eliminating the need for agar, optimizing explant–nutrient interactions, improving propagation rates, and facilitating the management of physiochemical parameters (Welander et al., 2017). Despite their advantages, the high cost and technical complexity of automated TIS can limit their accessibility, particularly in resource-constrained settings. This highlights the need for more affordable alternatives, such as manually operated TIS, which offer many of the same benefits at reduced cost (García-Ramírez, 2023). Manually operated systems periodically immerse plant tissues in nutrient solutions, thereby improving nutrient uptake and gas exchange while minimizing hyperhydricity common in continuous immersion cultures. Their simplicity and cost-effectiveness make them particularly suitable for laboratories and nurseries with limited resources (Welander et al., 2017). A variety of manual TIS designs have been developed, each adapted to specific plant species and propagation objectives.

The Biocoupler is an innovative temporary immersion bioreactor designed to simplify the micropropagation process and provide an effective solution for plant tissue culture. It combines the advantages of temporary immersion systems with improved accessibility for facilities operating under budgetary constraints. Successful implementation of manual TIS requires careful optimization of immersion cycles, nutrient composition, and environmental conditions to achieve consistent results. Although these systems demand precise management, their scalability, cost-effectiveness, and capacity to improve plant quality position them as valuable tools in sustainable plant biotechnology (García-Ramírez, 2023). This study introduces the Biocoupler Manual Temporary Immersion System as a pioneering approach for the micropropagation of *Argania spinosa* in vitro plants. By refining in vitro growth conditions, the system seeks to promote shoot development, increase biomass production, and reduce the incidence of hyperhydricity, while maintaining both economic feasibility and sustainability. The findings present a scalable and practical strategy to advance conservation initiatives and support large-scale reforestation programs for this endangered species.

Materials and Methods

Plant materials

In this study, *in vitro* shoots (nodal sections) were derived from *Argania spinosa* seeds collected in September 2024 from the Oued Essafa area in the Chtouka Ait Baha region. Following the procedure described by Alouani and Bani-Aameur (2014), the seeds were dried, dehulled, and stored at 4 °C. For surface sterilization, seeds were immersed in a 12% sodium hypochlorite (NaClO) solution for 15 min and subsequently rinsed three times with sterile distilled water. This was followed by a one-hour treatment with 100% hydrogen peroxide (H_2O_2), as outlined by Tesse et al. (2024), after which seeds were again rinsed thoroughly with sterile water.

The disinfected seeds were cultured on a modified half-strength Murashige and Skoog (½ MS) medium, in which all macro- and micronutrient concentrations were reduced by half. This medium was devoid of plant growth regulators. Cultures were incubated under cold white LED light at an intensity of 228.13 µmol m^{-2} s^{-1}, at 25 ± 2 °C, with a photoperiod of 16 h light and 8 h dark. Germination began on d 6, peaked around d 14, and then stabilized, reaching a

final germination rate of 73%. The resulting shoots were subcultured every three weeks onto fresh ½ MS medium without growth regulators and were used as the plant material for subsequent experimental procedures.

Description of the temporary immersion system (Biocoupler)

The Biocoupler is a novel yet simplified temporary immersion bioreactor specifically designed to enhance the efficiency of plant micropropagation. It comprises two interconnected glass jars fitted with an integrated filter that enables smooth and controlled flow of culture medium between compartments. Standing 20 cm tall, the device accommodates up to 150 mL of culture medium. A defining feature of the Biocoupler is its microporous aeration filter, which equilibrates internal pressure with atmospheric conditions. This mechanism minimizes contamination risk while maintaining sterility within the system.

The Biocoupler is engineered to support key micropropagation processes such as mixing, wetting, agitation, coating, and the controlled movement of plant propagules, all of which contribute to improved growth conditions. Its design sustains consistently high humidity, facilitates air exchange, and ensures the delivery of filtered gas—factors essential for the successful development of in vitro plant cultures.

A significant advantage of the Biocoupler lies in its ability to operate independently of additional infrastructure, such as external piping, electronic controls, timers, or valves. This feature makes it a highly cost-effective and user-friendly solution, particularly well-suited to laboratories and nurseries with limited resources. While its manual operation is ideal for small-scale applications, the system is also adaptable to industrial-scale production. When combined with the BioTilt—a specialized support system—multiple Biocoupler units can be operated simultaneously, enabling automated movement and enhanced efficiency. This scalability renders the Biocoupler a compelling option for large-scale plant propagation, offering a balance of affordability, simplicity, and performance (Fig. 1).

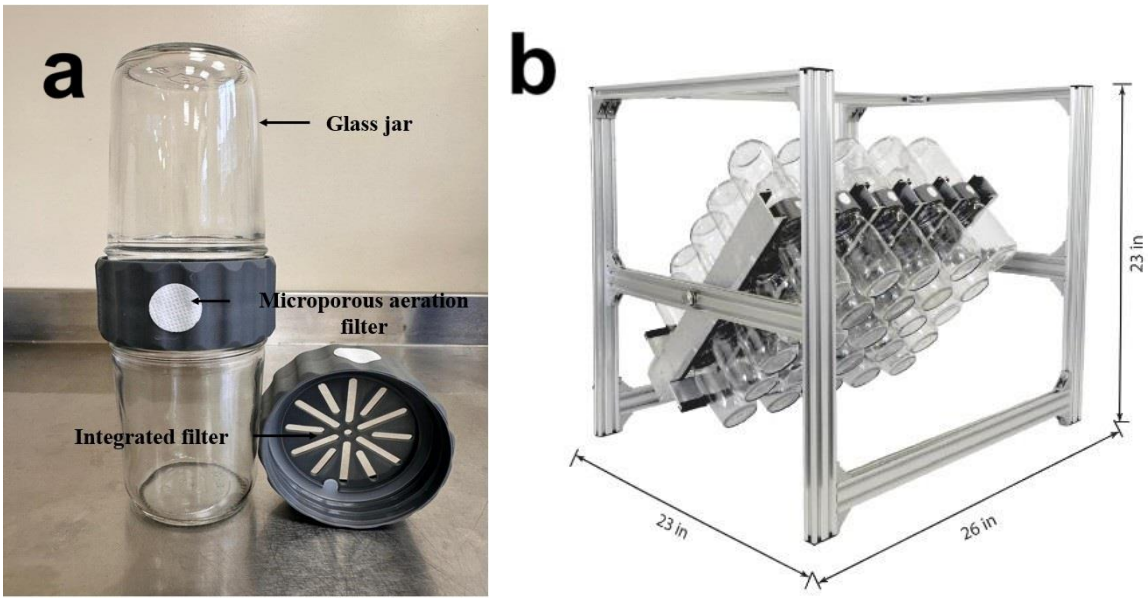

Fig. 1. (a) Manual Temporary Immersion Bioreactor: Biocoupler; (b) The BioTilt automate the rotation of Biocouplers.

In vitro multiplication trials

Analysis of the influence of contact time on the development of Argan in vitro shoots in a temporary immersion system

A modified Murashige and Skoog (MS) medium was prepared by supplementing it with 1 mg L^{-1} meta-topolin and 0.01 mg L^{-1} indole-3-butyric acid (IBA), and omitting agar to maintain a liquid consistency. The pH of the medium was carefully adjusted to 5.7 prior to dispensing 100 mL into each temporary immersion system (TIS) container. To ensure sterility, the medium was autoclaved at 121 °C for 20 min.

For the experimental setup, five in vitro shoots—each approximately 1.5 cm in length—were gently placed in the upper chamber of the Biocoupler (Fig. 2a). The immersion protocol tested a range of immersion durations—1, 2, 3, 4, 5, 6, and 7 min—applied once daily. Cultures were maintained under strictly controlled environmental conditions, including a 16-h light/8-h dark photoperiod, an ambient temperature of 25 ± 2 °C, and illumination

provided by cold white LED tubes with a light intensity of 228.13 µmol m^{-2} s^{-1}. After a cultivation period of three weeks, key growth parameters were recorded to evaluate the multiplication rate, providing insights into the system's effectiveness in promoting shoot development and optimizing *in vitro* propagation conditions using the following formula:

$$TM = \frac{Nf}{Ni}$$

where TM represents the multiplication rate, Nf represents the total number of newly formed shoots after three weeks of culture, and Ni denotes the initial number of *in vitro* shoots used at the beginning of the experiment. Shoot length (cm) was measured using a ruler in conjunction with millimeter graph paper to ensure precision. Fresh weight (g) was recorded immediately after harvesting by weighing the shoots, while dry weight (g) was determined through a sequential drying process. The harvested shoots were first dried at 105 °C for 2 h, followed by continuous drying at 80 °C until a constant dry weight was achieved, following the method described by Huang et al. (2019). To ensure accuracy and reproducibility, the entire experimental procedure was conducted in triplicate.

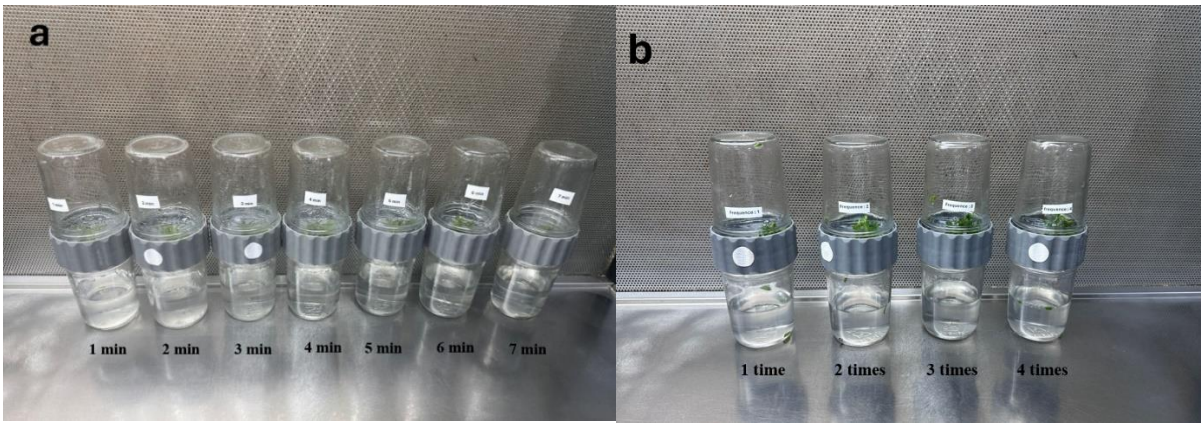

Fig. 2. (a) Impact of contact time on Argan *in vitro* plants; (b) Impact of immersion frequency on Argan *in vitro* plants in Biocoupler.

Analysis of the influence of immersion frequency on the development of Argan in vitro shoots in a temporary immersion system

The culture medium and environmental conditions were maintained as previously described. In this experiment, however, the immersion frequency was varied to examine its effect on shoot development. A fixed immersion duration of 1 min was applied at different frequencies: once, twice, three times, and four times per day, with 4-h intervals between each immersion cycle (Fig. 2b). After three weeks of cultivation, growth parameters were recorded to evaluate the influence of immersion frequency. These included the total multiplication rate (TM), shoot length, fresh weight, and dry weight of the in vitro shoots. To ensure statistical reliability, the experiment was conducted in triplicate.

Synergistic effect of meta-topolin and auxins on the proliferation of Argania spinosa in vitro

Previous studies have demonstrated that meta-topolin outperforms other commonly used cytokinins—such as benzylaminopurine (BAP) and zeatin—in promoting the proliferation of *Argania spinosa* shoots (Tesse et al., 2024). Building on this evidence, the current study aimed to determine the optimal concentration of meta-topolin in combination with auxins to maximize the in vitro proliferation of argan shoots. For this purpose, a modified MS medium was supplemented with meta-topolin and two auxins: naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA), as detailed in Table 1.

The pH of the medium was precisely adjusted to 5.7 prior to sterilization, ensuring both nutrient stability and physiological compatibility with the explants. In vitro shoots, each approximately 1.5 cm in length, were cultured under controlled environmental conditions at 25 ± 2 °C. The temporary immersion system was programmed for 1-min immersion cycles every 8 h to facilitate adequate nutrient absorption and gas exchange. A 16-h light/8-h dark photoperiod was applied to support optimal photosynthetic activity.

After a three-week cultivation period, key growth parameters were measured, including multiplication rate, shoot length, and fresh weight of the regenerated shoots. These data provide valuable insights into the synergistic effects of meta-topolin and auxins, and help identify the most effective hormonal combination for enhancing the micropropagation of *Argania spinosa*.

Table 1. Combinations of IBA and NAA with meta-topolin at different concentrations.

	Cytokinins	Auxins	
Base medium modified MS	**(MT mg L^{-1})**	**IBA mg L^{-1}**	**NAA mg L^{-1}**
M0	0	0	0
M1	0.2	0.01	0
M2	0.3	0.01	0
M3	0.5	0.01	0
M4	0.7	0.01	0
M5	0.8	0.01	0
M6	1	0.01	0
M7	1.1	0.01	0
M8	1.3	0.01	0
M9	0.2	0	0.01
M10	0.3	0	0.01
M11	0.5	0	0.01
M12	0.7	0	0.01
M13	0.8	0	0.01
M14	1	0	0.01
M15	1.1	0	0.01
M16	1.3	0	0.01

MS: Murashige and Skoog, NAA: Naphthaleneacetic acid, IBA: Indole-3-butyric acid; mT: méta-topolin.

Analysis of nutrient absorption by Argania spinosa in vitro shoots in a temporary immersion system

The morphological growth responses of *Argania spinosa* in vitro plants cultivated in a temporary immersion system are closely influenced by the uptake and depletion of mineral nutrients from the culture medium. To elucidate these interactions, a comprehensive assessment was conducted to evaluate nutrient consumption and its relationship to plant development. Key physicochemical parameters—including pH, electrical conductivity, and mineral nutrient concentrations—were measured in the culture medium both prior to the introduction of plant material and following the 21-d growth period.

The analysis encompassed both macronutrients (nitrogen [N], phosphorus [P], potassium [K], calcium [Ca], and magnesium [Mg]) and micronutrients (iron [Fe], copper [Cu], manganese [Mn], cobalt [Co], molybdenum [Mo], and zinc [Zn]). By comparing nutrient concentrations before and after cultivation, the study aimed to identify specific nutrient uptake patterns exhibited by *Argania spinosa* in vitro shoots. These findings offer valuable insights for optimizing culture medium formulations, enhancing growth efficiency, and refining nutrient management strategies to improve the success of micropropagation efforts (Table 2).

Table 2. Methods for analyzing chemical and nutritional parameters.

Parameters	**Methods Used**	**References**
pH	pH/ORP meter HI 2211 (Hanna Instruments)	APHA
Conductivity	Conductimeter HI98312 (Hanna Instruments)	APHA
Nitrogen (N)	Nessler calorimetric method	Bradstreet (1965)
Phosphorus (P)	Metavanadate calorimetric method	APHA
Potassium (K)	Atomic Absorption Spectrophotometry	Smith et al. (1972)
Magnesium (Mg)		
Calcium (Ca)		
Manganese (Mn)		
Zinc (Zn)		
Cobalt (Co)		
Copper (Cu)		
Molybdenum (Mo)		
Iron (Fe)		

Comparison of micropropagation methods

Three micropropagation methods were compared: culture on solid medium, liquid medium, and manual temporary immersion system (TIS). A modified MS medium was used, enriched with 0.8 mg L^{-1} meta-topoline and 0.01 mg L^{-1} IBA, with a pH adjusted to 5.7. In the TIS method, 100 mL of medium was poured into a dedicated container. In the liquid medium, cotton was used as a support to avoid complete immersion of the explants. For the solid medium, 6.4 g L^{-1} of agar was added before autoclaving at 121 °C for 20 min. Five explants measuring 1.5 cm were cultured in each system. The *in vitro* shoots in TIS were immersed for 1 min every 8 h. The cultures were incubated at 25 °C under cold white LED tubes (228.13 µmol m^{-2} s^{-1}) with a photoperiod of 16 h.

After 21 d of culture, the hyperhydration index (HI) was determined using the fresh-to-dry weight ratio:

$$HI = \frac{FW}{DW}$$

where FW is the fresh weight (g) and DW is the dry weight (g).

The hyperhydration percentage (HH%) was calculated as:

$$HH(\%) = (\frac{Nh}{Nt}) \times 100$$

where Nh is the number of hyperhydrated shoots and Nt is the total number of evaluated shoots (Vinoth et al.,2019).

The dry matter content (DM%) was determined using:

$$DM(\%) = (\frac{DW}{FW}) \times 100$$

where DW is the dry weight which was obtained after drying the *in vitro* shoots at 105 °C for 2 h and then continuously at 80 °C until achieving a constant dry weight (Huang et al., 2019) and FW is the fresh weight.

The multiplication rate was calculated using the following formula:

$$TM = \frac{Nf}{Ni}$$

where TM represents the multiplication rate, N_f is the total number of new shoots obtained after multiplication after three weeks of culture, and N_i is the initial number of cultured *vitro* shoots, shoot size, fresh weight, and dry weight.

Data analysis

Statistica® 10 software was used for generating response surface plots to perform statistical data analysis, including principal component analysis (PCA) (StatSoft, Inc., https://www.statsoft.de/en/data-science-applications/tibco-statistica/Multiple). Comparisons for one-way ANOVA with repeated measures (3 repetitions) were conducted, followed by Tukey's HSD test. The significance level was set at 0.05.

Results

Influence of contact time and immersion frequency on the development of Argan in vitro shoots

Our findings demonstrated that both the duration of contact with the liquid medium and the frequency of immersion significantly influenced the growth dynamics of *Argania spinosa* in vitro plants cultivated in a temporary immersion system. From the first min of exposure, the shoots began efficiently absorbing water and nutrients, resulting in a fresh weight of 0.68 g and a dry weight of 0.07 g. However, by the second min, a decline in fresh weight to 0.53 g was observed, likely reflecting an initial physiological adjustment. Stabilization occurred between the third and fifth min, during which fresh weight fluctuated between 0.57 and 0.65 g. A notable peak in fresh weight (0.79 g) was recorded at 6 min, followed by a slight reduction to 0.72 g at 7 min, suggesting the onset of tissue saturation due to excessive water uptake.

Dry weight followed a steady upward trend, reaching a maximum of 0.11 g at the 6-min mark. Shoot elongation displayed a distinct pattern: rapid initial growth to 2.10 cm in the first min was followed by a temporary slowdown between the second and third min. Growth then resumed, culminating in a final shoot length of 2.20 cm at 7 min, indicating a gradual adaptation of the plant tissues to immersion conditions over time.

The multiplication rate showed notable fluctuations throughout the treatment. It began relatively high at 1.40, decreased to 1.01 at 3 min, and then rose again to 1.47 by the seventh min. This pattern suggests that while excessive immersion may temporarily hinder proliferation, prolonged immersion—when carefully regulated—can confer physiological advantages, underscoring the importance of optimizing immersion parameters to enhance micropropagation efficiency (Fig. 3 and 4).

When evaluating the effect of immersion frequency, our results revealed that limited immersion events (1–2 times per day) supported moderate water and nutrient uptake, maintaining a relatively stable fresh weight between 0.60 g and 0.62 g, and a dry weight ranging from 0.11 to 0.12 g. Increasing the immersion frequency to three times per day

significantly enhanced biomass accumulation, resulting in the highest recorded fresh weight of 0.80 g and a dry weight of 0.15 g. However, further increasing the frequency to four immersions per day appeared to promote excessive water absorption, which may have limited dry matter accumulation due to potential physiological stress.

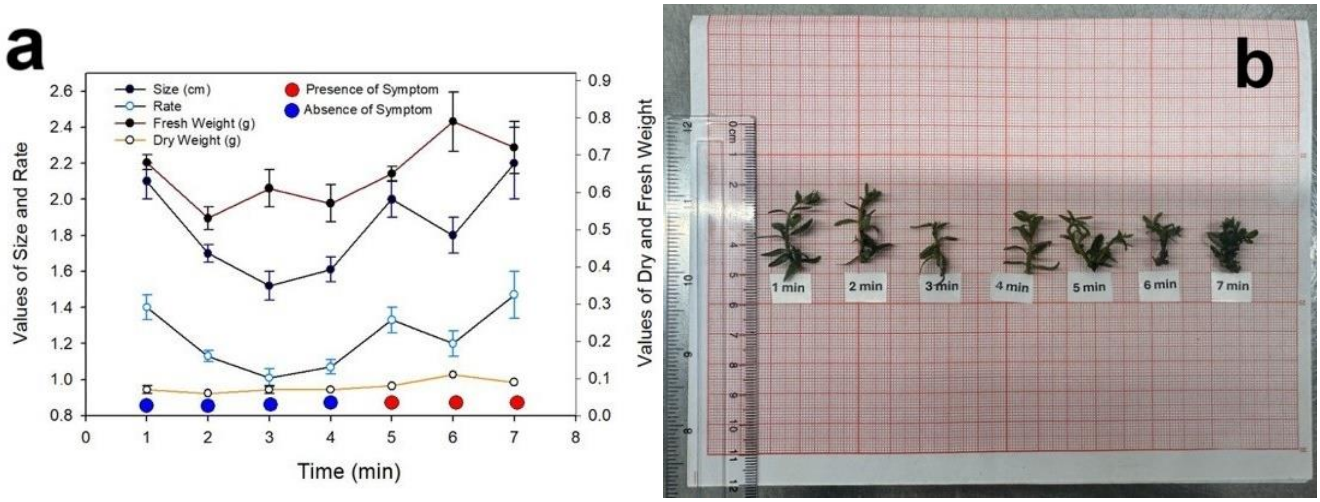

Fig. 3. (a) Impact of immersion time on growth measurements; (b) visual analysis of the morphology of Argan *in vitro* shoots according to immersion time.

Shoot elongation followed a comparable trend, reaching a maximum length of 2.63 cm under three immersions per day, while both lower and higher frequencies were less favorable for shoot growth. Similarly, the multiplication rate peaked at three immersions per day (1.76), indicating an optimal balance between nutrient availability and aeration. In contrast, increasing the frequency to four immersions resulted in a slight decline in the multiplication rate to 1.67, possibly due to waterlogging and reduced oxygen availability.

These findings underscore the importance of precisely regulating both immersion duration and frequency in temporary immersion systems. Achieving an optimal balance is essential to maximizing the in vitro growth and propagation efficiency of *Argania spinosa*, while minimizing the adverse effects of excessive moisture, such as reduced biomass accumulation and stress-related growth inhibition.

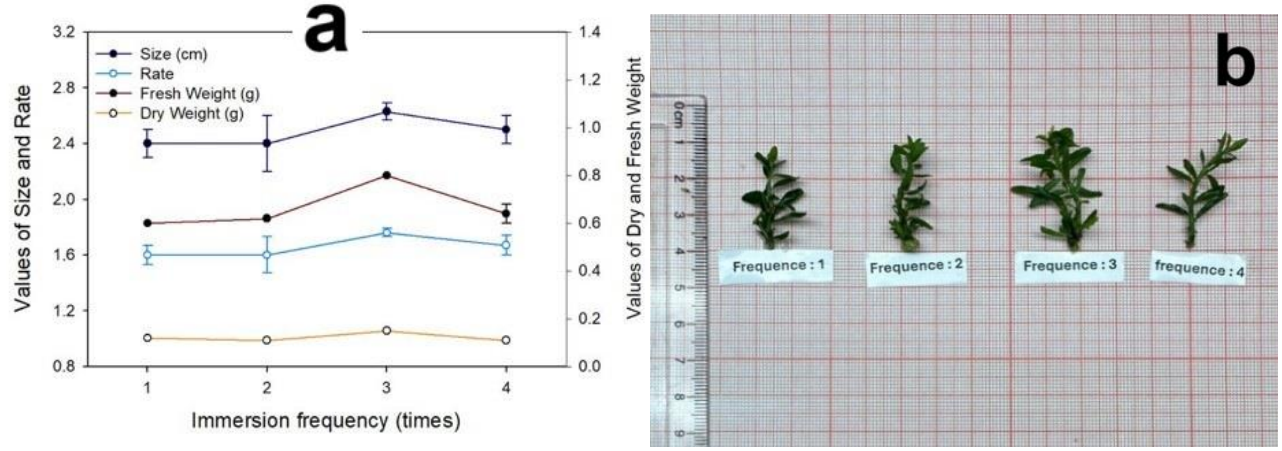

Fig. 4. (a) Impact of immersion frequency on growth measurements; (b) visual analysis of the morphology of Argan *in vitro* shoots according to immersion frequency.

Synergistic effect of meta-topolin and auxins on the in vitro proliferation of Argania spinosa

Our findings demonstrate that the application of meta-topolin at a concentration of 0.8 mg L^{-1} (M5) significantly enhanced the growth performance of *Argania spinosa in vitro* plants. Under these conditions, an average shoot length of 2.34 cm, a fresh weight of 1.36 g, and a multiplication factor of

1.88 were achieved, surpassing the results obtained with other tested media. These outcomes underscored the superior efficacy of this specific concentration in promoting *in vitro* proliferation. However, when the meta-topolin concentration exceeded 1 mg L^{-1} (M7 and M8), a slight decline in growth parameters was observed, likely due to metabolic saturation or an inhibitory effect, a phenomenon that has been documented in previous studies. Additionally, supplementing the culture medium with a low dose of IBA (0.01 mg L^{-1}) generally led to a modest improvement in plant growth. While this auxin contributed positively, its impact remained secondary to that of meta-topolin. In contrast, the inclusion of NAA (0.01 mg L^{-1}) in certain media formulations (M9 to M16) did not produce a significant influence on growth parameters. This suggests that, among the auxins tested, IBA is more effective in stimulating the *in vitro* development of argan plant tissues. These findings provided valuable insights into the optimization of growth regulator formulations for enhancing the micropropagation efficiency of this economically and ecologically significant species (Table 3).

Table 3. Effect of meta-topolin and auxins on the growth and multiplication of argan *in vitro* shoots.

Medium N°	Growth parameters		
MS Modifie	Size (Length of the stems) cm^{-1}	Fresh weight of the plants g^{-1}	Rate
M 0	1.64 ± 0.15^{a}	0.84 ± 0.09^{a}	1.17 ± 0.13^{a}
M1	1.85 ± 0.36^{ab}	1.02 ± 0.27^{ab}	1.41 ± 0.37^{b}
M 2	1.91 ± 0.32^{ab}	1.05 ± 0.16^{ab}	1.45 ± 0.22^{b}
M3	1.89 ± 0.24^{ab}	1.13 ± 0.16^{b}	1.55 ± 0.22^{c}
M4	1.90 ± 0.24^{ab}	1.09 ± 0.15^{b}	1.50 ± 0.21^{bc}
M5	2.34 ± 0.47^{c}	1.36 ± 0.21^{c}	1.88 ± 0.29^{d}
M6	2.05 ± 0.35^{bc}	1.20 ± 0.19^{bc}	1.65 ± 0.25^{d}
M7	2.27 ± 0.16^{c}	1.23 ± 0.38^{bc}	1.62 ± 0.62^{d}
M8	2.17 ± 0.20^{c}	1.26 ± 0.42^{bc}	1.74 ± 0.58^{d}
M9	1.89 ± 0.26^{ab}	0.88 ± 0.03^{a}	1.20 ± 0.04^{a}
M10	1.79 ± 0.16^{ab}	1.12 ± 0.16^{b}	1.48 ± 0.22^{bc}
M11	1.68 ± 0.19^{a}	0.89 ± 0.09^{a}	1.24 ± 0.13^{a}
M12	1.67 ± 0.08^{a}	0.93 ± 0.07^{a}	1.36 ± 0.15^{b}
M13	1.64 ± 0.06^{a}	0.97 ± 0.15^{ab}	1.34 ± 0.20^{b}
M14	1.74 ± 0.07^{ab}	0.99 ± 0.09^{ab}	1.31 ± 0.15^{b}
M15	1.75 ± 0.14^{ab}	1.08 ± 0.21^{b}	1.55 ± 0.30^{c}
M16	1.66 ± 0.04^{a}	0.95 ± 0.07^{ab}	1.31 ± 0.10^{b}

Average ± standard deviation; values with same letters (^{a}, ^{b}, ^{c}, ^{d}, ^{e}, or ^{f}) represent homogeneous groups; in each column, different letters indicate a significant difference at $P < 0.05$ using ANOVA and Tukey's Post Hoc.

Nutrient absorption analysis

Nutrient absorption analysis is as follows (Table 4).

The present study revealed significant variations in nutrient concentrations, indicating efficient nutrient uptake by *Argania spinosa* in vitro plants cultivated in a temporary immersion system (TIS). After three weeks of culture, substantial nutrient assimilation was observed, with no visible signs of nutrient deficiency or depletion. A 22.62% reduction in electrical conductivity (EC) reflected active ion absorption, while a gradual decline in pH from 5.5 to 4.69 suggested increased mineral bioavailability due to medium acidification.

Among the macronutrients, nitrogen showed the highest uptake at 75%, followed by potassium (65.02%), calcium (26%), and phosphorus (24.66%), underscoring their essential roles in cellular processes, photosynthesis, and protein synthesis. Micronutrient uptake varied: iron (82.19%), zinc (55.63%), and manganese (32.44%) were absorbed at higher levels, whereas cobalt (1.15%), copper (0.00%), and molybdenum (0.00%) exhibited minimal or no uptake. The limited absorption of certain trace elements may reflect either a lower physiological demand or sufficient baseline concentrations in the medium to meet metabolic needs. These results highlight the importance of optimizing nutrient formulations in TIS-based micropropagation to support robust growth and development of *Argania spinosa* in vitro plants.

Table 4. Average decline in mineral nutrients in the culture medium at the beginning and end of the growth period (three weeks) of Argan *in vitro* plants in a temporary immersion system.

Parameters	Initial Concentration (mg L^{-1})	Final Concentration (mg L^{-1})	F-Statistic	P-Value
pH	5.5 ± 0.1	4.69 ± 0.03	2539.65*	1.38e-49*
Conductivity	5.94 ± 0.03	4.6 ± 0.09	5969.41*	3.44e-60*
Nitrogen (N)	555 ± 2	138.75 ± 3.35	347819.53*	2.86e-111*
Phosphorus (P)	38 ± 0.95	28.63 ± 0.04	3903.94*	6.63e-55*
Potassium (K)	731 ± 1.15	255.85 ± 0.09	6451868.59*	4.75e-148*
Magnesium (Mg)	73 ± 1.53	32.919 ± 0.32	20728.73*	8.76e-76*
Calcium (Ca)	122 ± 2	90.230 ± 0.17	9800.30*	2.18e-66*
Manganese (Mn)	12.66 ± 0.07	8.551 ± 0.17	36528.82*	6.63e-83*
Zinc (Zn)	7.06 ± 0.03	3.134 ± 0.05	155535.29*	3.89e-101*
Cobalt (Co)	0.0200 ± 0.000	0.01977 ± 0.000	-	-
Copper (Cu)	0.02 ± 0.01	0.02 ± 0.00	2.62	0.11
Molybdenum (Mo)	0.198 ± 0.00	0.198 ± 0.00	-	-
Iron (Fe)	18.4 ± 0.03	3.278 ± 0.03	5662660.71*	2.09e-146*

Note: The results indicated a significant difference between initial and final concentrations for most parameters ($P < 0.05$). For cobalt and molybdenum, the concentration values are constant, rendering the ANOVA test not app licable. Copper does not show a significant difference between initial and final concentrations ($P = 0.11$). *Signif icant difference between concentrations.

Comparison of micropropagation methods

A comparative evaluation of three in vitro culture methods for *Argania spinosa* is presented in Figure 5, covering parameters such as fresh weight (FW), dry weight (DW), fresh weight gain (ΔFW), growth rate, shoot length, FW/DW ratio, hyperhydricity percentage, and hyperhydricity index.

In a liquid medium, in vitro plants achieved the highest fresh weight (1.19 g) but exhibited a comparatively low dry weight (0.173 g). The resulting FW/DW ratio was 6.88, accompanied by a high hyperhydricity rate of 85.41%, indicating excessive water retention—a frequent challenge in hydroponic systems. In contrast, plants grown on solid medium displayed lower fresh (0.70 g) and dry weights (0.144 g), but the hyperhydricity rate declined to 79.42%. The reduced FW/DW ratio (4.86) reflected improved water balance, although overall growth was less vigorous.

Among the tested systems, the Temporary Immersion System (TIS) yielded the most favorable outcomes. Plants cultivated in TIS achieved a fresh weight of 0.81 g and a dry weight of 0.154 g. Shoot length reached 2.5 cm, and the growth rate was 1.67. TIS also maintained a moderate hyperhydricity rate of 80.91% and a hyperhydricity index of 2.36. These results suggest that TIS provides an optimal balance between moisture regulation, biomass accumulation, and controlled hyperhydricity, making it the most efficient method for promoting the in vitro development of *Argania spinosa*.

Discussion

This study investigated the influence of immersion parameters and growth regulators on the in vitro propagation of *Argania spinosa* using a temporary immersion system (TIS). Given the species' considerable agronomic and environmental importance (Aziz et al., 2013), optimizing culture conditions is critical for successful large-scale propagation. A comprehensive assessment was undertaken to examine the interactions between medium composition, culture parameters, and plant productivity across different developmental stages. Specifically, the effects of immersion duration and frequency on plant growth within a TIS were analyzed, alongside the role of growth regulators in influencing nutrient uptake throughout the cultivation period. Additionally, a comparative evaluation of three culture systems—liquid, solid, and TIS—was conducted to determine their relative efficiencies in promoting cell division and biomass accumulation, both of which are vital for robust plant development.

The results demonstrated that the initial minutes of immersion played a pivotal role in promoting optimal shoot growth. Within the first minute, rapid absorption of water and nutrients supported substantial tissue development without inducing vitrification. This finding corroborates earlier observations by Pérez-Tornero et al. (2001), who reported that short immersion periods enhance nutrient uptake while minimizing the risk of water stress. However, by the second minute, a decline in fresh weight and shoot size indicated the onset of overhydration, which began to interfere with cellular expansion. These results are consistent with those of Franck et al. (2004), who noted that prolonged immersion can lead to tissue oversaturation, thereby limiting elongation potential.

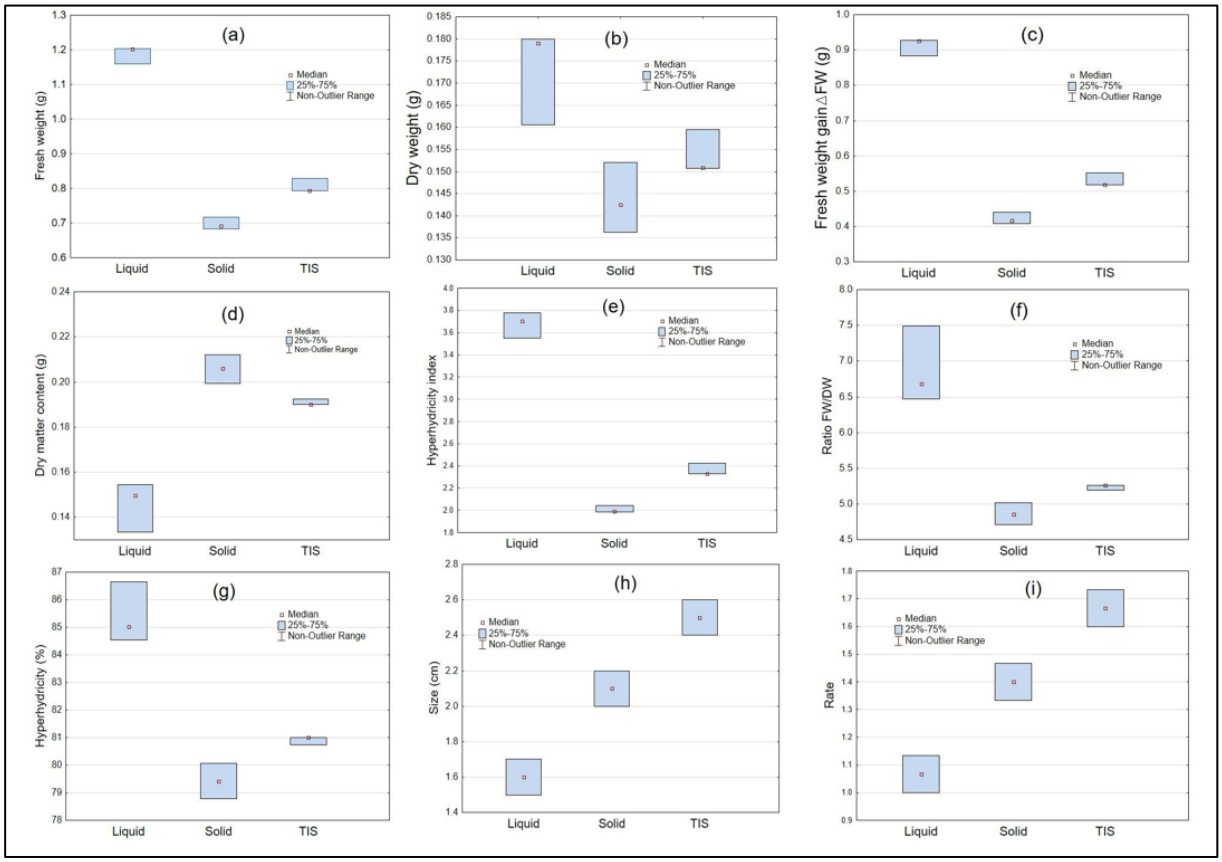

Fig. 5. Comparative evaluation of *in vitro* multiplication techniques: Growth and development analysis of argan plants in liquid, solid, and temporary Immersion Systems. (a) fresh weight (g); (b) dry weight (g); (c) fresh weight gain (ΔFW); (d) dry matter content (g); (e) hyperhydricity index; (f) ratio FW/DW; (g) hypehydricity (%); (h) size (cm); (i) rate.

Beyond five minutes of immersion, growth continued, but signs of vitrification—excessive tissue hydration resulting in metabolic disruption—became increasingly apparent. This phenomenon intensified at six and seven minutes, where, despite greater shoot size, excessive internal water content potentially compromised plant vigor. Such effects align with the findings of Polivanova and Bedarev (2022), who identified vitrification as a recurrent challenge in immersion-based in vitro systems. Therefore, a one-minute immersion duration emerged as the optimal condition for supporting healthy shoot development in *Argania spinosa* under TIS conditions.

Immersion frequency also had a significant impact on plant performance. An immersion frequency of three cycles per day proved most effective in promoting growth. Lower frequencies (one to two cycles per day) resulted in insufficient nutrient uptake, while higher frequencies (four cycles per day) caused waterlogging and reduced oxygen availability, leading to physiological stress. These outcomes support the conclusions of Etienne and Berthouly (2002), who emphasized the importance of balancing immersion frequency to ensure both adequate nutrient delivery and aeration. The adverse effects associated with four daily immersions reaffirm the findings of Ziv (2005), who demonstrated that excessive immersion events can oversaturate tissues, reduce oxygen diffusion, and suppress metabolic activity.

In contrast, plants subjected to three daily immersions exhibited the highest dry biomass accumulation and optimal shoot elongation, suggesting that this frequency provides an ideal balance between hydration and aeration. These results are in agreement with studies by Albarrán et al. (2005) and Pérez-Alonso et al. (2009), who found that moderate immersion frequencies are most effective in avoiding both water stress and hypoxic conditions. Overall, the findings underscore the importance of finely tuned immersion parameters in TIS-based in vitro culture. Achieving the right balance of immersion duration and frequency is essential for maximizing the healthy growth and propagation efficiency of *Argania spinosa*.

Meta-topolin, a cytokinin known for its regulatory role in plant growth and development, has been extensively documented for its effectiveness in promoting in vitro plant proliferation. The results of

the present study confirmed that concentrations below 1 mg L^{-1} were particularly effective in stimulating shoot development in *Argania spinosa*, consistent with the findings of Jayaprakash et al. (2021). However, higher concentrations of meta-topolin led to growth inhibition, a pattern similarly reported in other woody species such as *Musa* (banana) by Escalona et al. (2003).

In terms of auxin application, a low concentration of indole-3-butyric acid (IBA) at 0.01 mg L^{-1} significantly enhanced biomass accumulation and shoot elongation, supporting the observations of Hesami et al. (2018) in *Ficus religiosa*. In contrast, α-naphthalene acetic acid (NAA) at the same concentration showed no notable improvements in growth performance, aligning with the findings of Benbya et al. (2019) on *Argania spinosa* tissue cultures. Among the treatments tested, the combination of 0.8 mg L^{-1} meta-topolin with 0.01 mg L^{-1} IBA proved to be the most effective for promoting in vitro proliferation. These results further corroborate the work of Faisal et al. (2007) and Moradi et al. (2016), who emphasized the importance of maintaining an optimal cytokinin-to-auxin ratio to enhance micropropagation in valuable and endangered woody species such as *Tylophora indica* and *Epipactis veratrifolia*.

Beyond the use of growth regulators, optimizing culture conditions and TIS parameters also played a pivotal role in improving propagation outcomes, as highlighted by Mahdavi et al. (2023). Nutrient uptake was found to be fundamental in supporting key physiological and metabolic processes required for *Argania spinosa* development. Phosphorus was particularly critical for energy metabolism and biosynthesis, contributing to ATP production, glucose-6-phosphate formation, membrane stability, and enzyme activation necessary for starch synthesis (Georges et al., 2008). Potassium played a central role in enzyme regulation and carbohydrate metabolism, especially by influencing carbon-processing enzyme activity (Chakrabarty et al., 2008). Magnesium, as a core component of chlorophyll in photosystems I and II, was essential for photosynthetic activity.

Nitrogen was vital for the synthesis of amino acids, proteins, and porphyrins, including chlorophyll and cytochrome enzymes—key components of both photosynthesis and respiration (George et al., 2008). Calcium contributed not only to cell membrane stability but also to cell wall integrity and intracellular signaling processes. The study's findings revealed a well-balanced nutrient absorption pattern, aligning with the physiological requirements of *Argania spinosa* throughout its development in a TIS.

Although trace elements such as cobalt, copper, and molybdenum exhibited minimal uptake, their presence remained essential for enzymatic activation and functioning as metabolic cofactors, as suggested by Chajer et al. (2008). Collectively, these results underscore the critical importance of optimizing both the composition of the nutrient medium and the immersion parameters in TIS-based systems to support vigorous and sustainable in vitro growth of *Argania spinosa*.

The analysis of the results underscored the distinct characteristics of each culture method with respect to growth performance and moisture regulation. The liquid medium, while enabling substantial fresh weight accumulation, was notably affected by hyperhydration, as reflected in the high FW/DW ratio and a hyperhydration rate of 85.41%. This excessive water retention likely compromised the structural integrity and overall development of plant tissues. Similar issues have been documented in hydroponic systems by Bayraktar et al. (2020) and Polivanova et al. (2022), reinforcing the observation that liquid-based systems frequently struggle with excessive moisture retention.

In contrast, the solid medium demonstrated superior moisture control, as evidenced by its lower FW/DW values and reduced hyperhydration percentage. However, it appeared to restrict overall plant growth, yielding a fresh weight of only 0.70 g. Although this method effectively minimized water uptake, it was less efficient in supporting biomass accumulation compared to the liquid medium. These findings are consistent with those of Manokari et al. (2021), who reported that solid culture systems improve tissue quality at the expense of growth potential.

The temporary immersion system (TIS) emerged as the most balanced approach, effectively regulating moisture while promoting superior plant growth. This method achieved a growth rate of 1.67 and a maximum shoot length of 2.5 cm, highlighting its capacity to support robust plant development. Furthermore, the hyperhydration rate was significantly reduced to 80.91%, and the hyperhydration index remained relatively low at 2.36, indicating a more controlled environment for water and nutrient uptake. These outcomes align with findings by Ramírez-Mosqueda and Bello-Bello (2021), who emphasized the advantages of TIS in enhancing nutrient absorption while mitigating the adverse effects of hyperhydration, ultimately improving plant vigor.

Beyond its application in in vitro argan propagation, TIS has demonstrated promising results in the micropropagation of various plant species, enhancing proliferation efficiency and lowering production costs. For instance, a recent study on *Phalaenopsis* orchid micropropagation (Mohammadpour Barough et al., 2024) reported that incorporating banana powder and activated charcoal (SM2) into a TIS-FA-Bio system significantly improved seedling development while reducing

costs by 72.5% compared to more expensive systems such as TIS-RITA®.

While both liquid and solid media offer specific advantages in moisture regulation and hyperhydration control, the TIS method proved the most effective for the in vitro culture of *Argania spinosa*, providing an optimal balance between hydration and growth while minimizing hyperhydration-related complications. The implementation of TIS for large-scale propagation of argan tree seedlings presents a promising opportunity for sustainable agriculture, both locally and globally. Nevertheless, further research is needed to ensure the successful acclimatization of propagated plants in natural environments and to refine protocols for large-scale production. Advancing research in this area may play a pivotal role in combating desertification and promoting sustainable agricultural practices.

Conclusion

This study highlighted the significant influence of immersion parameters and growth regulators on the in vitro development of *Argania spinosa*. The results demonstrated that an initial immersion duration of 1 minute, applied three times per day, enhanced nutrient absorption and promoted optimal plant growth. In contrast, higher immersion frequencies induced hypoxia and water stress, underscoring the importance of optimizing both immersion time and frequency to avoid physiological imbalances.

The application of growth regulators—particularly the combination of meta-topoline and indole-3-butyric acid (IBA)—proved effective in stimulating shoot elongation and biomass accumulation, with the optimal concentration identified as 0.8 mg L^{-1}. Efficient nutrient absorption was essential in supporting key metabolic functions such as energy metabolism and photosynthesis. A balanced uptake of essential macronutrients, including phosphorus, potassium, magnesium, nitrogen, and calcium, was critical for healthy development, while trace elements functioned as enzymatic cofactors, further emphasizing the need for an optimized nutrient medium.

Moreover, the comparative evaluation of different culture systems revealed that the temporary immersion system (TIS) offered the most effective balance between hydration control and growth promotion. TIS successfully minimized the incidence of hyperhydration while maintaining sufficient nutrient availability, making it the most efficient approach for in vitro argan propagation.

The findings underscore the importance of refining culture protocols to enhance plant viability and support the large-scale propagation of *A. spinosa*, thereby contributing to sustainable agroecological practices, food security, and the resilience of rural communities. Beyond its immediate relevance to argan propagation, this study reinforces the broader significance of plant biotechnology in advancing sustainable agricultural systems and fostering a circular bioeconomy. The integration of biocoupling techniques into forestry and agroforestry could further improve ecosystem sustainability and strengthen food systems. Future research should focus on diversifying methodological approaches and incorporating a broader literature base to further develop in vitro propagation techniques and expand their applications in plant biotechnology.

Acknowledgments

The authors would like to thank the Research Centre for Materials and Energy at Hassan II University of Casablanca and CNRST for their financial and technical support.

Author contributions

Conceptualization, BN, BB, MO, and ES; methodology, TR; formal analysis, TR; data curation, TR. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Aguilar M, Garita K, Kim Y, Kim J, Moon H. 2019. A Simple Protocol for the Micropropagation of Teak (*Tectona grandis* Linn.) in Semi-Solid and Liquid Media Using RITA® Bioreactors and *Ex Vitro* Rooting. American Journal of Plant Sciences 10, 1121-1141. https://doi.org/10.4236/ajps.2019.107081

Ait Aabd NA, Tahiri A, Qessaoui R, Mimouni A, Bouharroud R. 2022. Self- and Cross-Pollination in the Argan Tree and their Implications on Breeding Programs. Cells 11, 828. https://doi.org/10.3390/cells11050828

Albarrán J, Bertrand B, Lartaud M, Etienne H. 2005. Cycle Characteristics in a Temporary Immersion Bioreactor Affect Somatic Embryo Regeneration, Morphology, Hydric and Mineral Status in Coffee (*Coffea arabica*). Plant Cell, Tissue and Organ Culture 81, 27–36. https://doi.org/10.1007/s11240-004-2618-8

Alouani M, Bani-Aameur F. 2014. Viabilité et vieillissement des semences d'arganier (*Argania spinosa* (L.) Skeels). African Science 10(1), 245-255.

American Public Health Association (APHA). 2012. American Water Works Association and Water Environment Federation. Standard Methods for the Examination of Water and Wastewater, 22nd edition. https://www.standardmethods.org/

ANDZOA. 2011. Argan Tree Sector. Available at: https://andzoa.ma/filiere-de-larganier/

Aziz A, Mormont M, & Allali K. 2013. Effects of Argan Tree Commercialization on the Socioeconomic Life of the Populations in the Moroccan Argan Tree Biosphere. Electronic Review in Environmental Sciences 13(1). https://doi.org/10.4000/vertigo.13445

Badreddine A. 2016. Preparation and Characterization of Extracts from *Argania spinosa* and Argan Oil and Evaluation of Their Neuroprotective Effects In Vivo and *In Vitro*. Doctoral Thesis, Faculty of Sciences and Techniques, Hassan I University – Settat – Morocco; Burgundy Franche-Comté University – Dijon – France. https://theses.hal.science/tel-01499664

Bayraktar H, Korkmaz SE, Gül S, & Yücel M. 2020. Study on the Micropropagation of Olive under TIS, Showing a Reduction in Hyperhydricity. South African Journal of Botany 134, 87-94. https://doi.org/10.1016/j.sajb.2019.11.022

Benbya A, Mdarhri Alaoui M, Gaboun F, Delporte F, Chlyah O, Cherkaoui S. 2019. Vegetative Propagation of *Argania spinosa* (L.) Skeels Cuttings: Effects of Auxins and Genotype. Adv. Hort. Sci 33(4), 519-527. https://doi.org/10.13128/ahsc-8131

Bradstreet RB. 1965. The Kjeldahl Method for Organic Nitrogen. New York, NY: Academic Press Incorporated, 39-88.

Chajer AK, Sharma M, Kachhwaha S, & Kothari L. 2008. Micronutrient optimization resulting in highly improved *in vitro* plant regeneration in kodo (*Paspalum scrobiculatum* L.) and finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Cell Tissue Organ Culture 94, 105-112. https://doi.org/10.1007/s11240-008-9392-y

Chakrabarty D, Dewir YH, Hahn EJ, Datta SK, Paek KY. 2008. Nutrient Utilization Dynamics and Root Growth of 'M9 EMLA' Apple in Temporary or Continuous Immersion Bioreactors. Plant Growth Regulation 51, 11-19. https://doi.org/10.1007/s10725-006-9115-5

Charrouf Z, Guillaume D. 2018. The argan oil project: going from utopia to reality in 20 years. Oilseeds & fats Crops and Lipids 25(2), D209. https://doi.org/10.1051/ocl/2018006

El Monfalouti H, Guillaume D, Denhez C, Charrouf Z. 2013. Therapeutic Potential of Argan Oil - A Review. Journal of Pharmacy and Pharmacology 62, 1669-1675. https://doi.org/10.1111/j.2042-7158.2010. 01190.x

Escalona M, Cejas I, Gonzáles-Olmedo J, Capote I, Roels S, Cañal M.J, Rodriguez R, Sandoval J.A, Debergh P. 2003. Effect of Meta-Topoline on the Propagation of Plantain Banana in an Immersion Bioreactor. Infomusa 12(2), 28-30.

Etienne H, & Berthouly M. 2002. Temporary Immersion Systems in Plant Micropropagation. Plant Cell, Tissue and Organ Culture 69(3), 215-231. https://doi.org/10.1023/A:1015668610465

Faisal M, Ahmad N, & Anis M. 2007. An Effective Micropropagation System for *Tylophora indica*: A Medicinally Important Endangered Plant. Reports on Plant Biotechnology 1(3), 155-161. https://doi.org/10.1007/s11816-007-0025-4

Faouzi K, Rharrabti Y, Boukroute A, Mahyou H, Berrichi A. 2015. Mapping the Distribution Area of the Argan Tree (*Argania spinosa* L. Skeels) in the Eastern Region of Morocco Using GPS Combined with GIS. Revue Nature & Technologie, C- Sciences de l'Environnement 12, 16-24.

Franck T, Kevers C, Gaspar T, Dommes J, Deby C, Greimers R, Serteyn D, & Deby-Dupont G. 2004. Hyperhydricity of *Prunus avium* shoots cultivated on gelrite: a controlled response to stress. *Plant* Physiology and Biochemistry 42(6), 519-527. https://doi.org/10.1016/j.plaphy.2004.05.003

García-Ramírez Y. 2023. Temporary immersion system for *in vitro* propagation via organogenesis of forest plant species. *Trees* 37, 611-626. https://doi.org/10.1007/s00468-022-02379-w

George EF, & Klerk GJ. 2008. The Components of Plant Tissue Culture Media I: Macro- and Micro-Nutrients. In: George E.F. et al., eds. *Plant* Propagation by Tissue Culture. 3rd ed. Dordrecht, The Netherlands: Springer, 65-113. https://doi.org/10.1007/978-1-4020-5005-3_3

Georgiev V, Schumann A, Pavlov A, Bley T. 2014. Temporary immersion systems in plant biotechnology. Life Sciences Engineering 14(6), 607-621. https://doi.org/10.1002/elsc.201300166

Huang W, Ratkowsky D, Hui C, Wang P, Su J, Shi P. 2019. Leaf Fresh Weight Versus Dry Weight: Which is Better for Describing the Scaling Relationship between Leaf Biomass and Leaf Area for Broad-Leaved Plants?. Forests. https://doi.org/10. 256. 10.3390/f10030256

Hesami M, Daneshvar MH, Yoosefzadeh-Najafabadi M, Alizadeh M. 2018. Effect of Plant Growth Regulators on Indirect Organogenesis in *Ficus religiosa* Shoots through Petiole Segments

Derived from Seedlings. Journal of Genetic Engineering and Biotechnology 16, 175–180. https://doi.org/10.1016/j.jgeb.2017.11.001

Jayaprakash K, Manokari M, Badhepuri MK, Cokul Raj M, Abhijit Dey & Mahipal S. 2021. Influence of Meta-Topoline on *In Vitro* Propagation and Micro-Morpho-Anatomical Development of Leaf Structures in *Oxystelma esculentum* (Lf) Sm. Plant Cell, Tissue and Organ Culture 147, 325-337. https://doi.org/10.1007/s11240-021-02126-y

Koufan M, Mazri MA, Essatte A, Moussafir S, Belkoura I, El Rhaffari L, Toufik I. 2020. A novel regeneration system through micro grafting for *Argania spinosa* (L.) Skeels, and confirmation of successful rootstock-scion union by histological analysis. Plant Cell Tissue Organ Culture 142, 369-378. https://doi.org/10.1007/s11240-020-01868-5

Mahdavi Z, Daylami SD, Fadavi A, Vahdati K. 2023. Artificial seed production of *Phalaenopsis* orchid: effect of encapsulation materials, temperature, light spectra, and storage period. Plant Cell Tissue Organ Culture 155, 797–808. https://doi.org/10.1007/s11240-023-02600-9

Manokari M, Kurniawan M, Purnamasari D, & Santoso S. 2021. Utilization of Liquid Systems to Improve the Micropropagation of *Vanilla planifolia*. Plant Cell, Tissue and Organ Culture 145(2), 405-417. https://doi.org/10.1007/s11240-021-02044-z

Mazri MA, Koufan M, Moussafir S. 2022. Recent advances in argan propagation: a review. Trees - Structure and Function 36, 1455–1476. https://doi.org/10.1007/s00468-021-02262-0

Mohammadpour Barough A, Dianati Daylami S, Fadavi A, Aliniaeifard S, Vahdati K. 2024. Enhancing photosynthetic efficiency in *Phalaenopsis amabilis* through bioreactor innovations. BMC Plant Biology 24, 1166. https://doi.org/10.1186/s12870-024-05767-1

Mohammadpour Barough A, Dianati Daylami S, Fadavi A, Vahdati K. 2024. Enhancing *Phalaenopsis* orchid production: a comparative study of permanent and temporary immersion bioreactors. In Vitro Cellular Development and Biology Plant 60, 523–537. https://doi.org/10.1007/s11627-024-10439-8

Moradi S, Dianati Daylami S, Arab M, Vahdati k. Direct somatic embryogenesis in *Epipactis Veratrifolia*, a temperate terrestrial orchid. The Journal of Horticultural Science and Biotechnology 92(1), 88-97 https://doi.org/10.1080/14620316.2016.1228434

Murashige T, & Skoog F. 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Plant Physiology* 15, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Pérez-Alonso N, Wilken D, Gerth A, Jähn A, Nitzsche HM, Kerns G, Capote-Perez A, & Jiménez E. 2009. Cardiotonic Glycosides from Biomass of *Digitalis purpurea* L. Cultivated in Temporary Immersion Systems. Plant Cell, Tissue and Organ Culture 99, 151–156. https://doi.org/10.1007/s11240-009-9587-x

Pérez-Tornero O, Egea J, Olmos E, & Burgos L. 2001. Control of Hyperhydricity in Micropropagated Apricot Cultivars. *In Vitro Cellular &* Developmental Biology - Plant 37(2), 250-254. https://doi.org/10.1007/s11627-001-0044-8

Plant Cell Technology. Biocoupler™ glass set. 2023. https://plantcelltechnology.com/products/biocoupler-glass-set

Plant Cell Technology. BioTilt™.2024. https://plantcelltechnology.com/pages/biotilt?_pos=1&_psq=bioti&_ss=e&_v=1.0

Polivanova O, Arkhipova T, & Zhelezova E. 2022. Analysis of Hyperhydricity in *In Vitro* Cultures. Plants 11(23), Article 3313. https://doi.org/10.3390/plants11233313

Ramírez-Mosqueda R, & Bello-Bello J. 2021. Comparison Between Conventional Systems and TIS for the Propagation of *Vanilla planifolia*. *Acta Physiologiae Plantarum* 43(7), Article 227. https://doi.org/10.1007/s11738-021-03227-z

Smith DL, & Schrenk WG. 1972. Application of Atomic Absorption Spectroscopy to Plant Analysis. I. Comparison of Zinc and Manganese Analysis with Official AOAC Colorimetric Methods. Journal of Association of Official Analytical Chemists 55(4), 669–675. https://doi.org/10.1093/jaoac/55.4.669

Tesse R, Bahlaouan B, El Antri S, & Boutaleb N. 2024. Plant Biotechnology for the Conservation of *Argania spinosa* (Skeels L): Optimization of Propagation and Sustainable Valorization. International Review of Environmental Studies, 1–17. https://doi.org/10.1080/00207233.2024.2405461

Vinoth, A., Ravindhran, R. Reduced hyperhydricity in watermelon shoot cultures using silver ions. In Vitro Cell.Dev.Biol.-Plant 51, 258–264 (2015). https://doi.org/10.1007/s11627-015-9698

Watt Paula M. 2012. The status of temporary immersion system (TIS) technology for plant micropropagation.Afr.J.Biotechnol, 11, 14025–14035. https://doi.org/10.5897/AJB12.1693

Welander M, Sayegh A, Hagwall F, Kuznetsova T, Holefors A. 2017. Technical improvement of a new bioreactor for large scale micropropagation of

several Vaccinium cultivars. Acta Hortic 1180, 387–391. https:// doi. org/ 10. 17660/ ActaH ortic. 2017. 1180. 53

Zahidi A, Bani-Aameur F, El Mousadik A. 2013. Morphological variability of the fruiting branches in *Argania spinosa*: Effects of seasonal variations, locality and genotype. Journal of Horticulture and Forest 5, 168–18. https://doi.org/10.5897/JHF2013.0311

Ziv M. 2005. Simple Bioreactors for Massive Plant Propagation. In: Hvoslef-Eide AK, & Preil W, eds. Liquid Culture Systems for *In Vitro* Plant Propagation, Springer-Verlag, Dordrecht, 79-94. https://doi.org/10.1007/1-4020-3200-5_5