

Cite this article: Maiada M. Eldawayati, Zeinab E. Zayed, Walid B. Abdelaala, Eman M. Zayed, AgNPs: A superior alternative to AgNO₃ for the optimal plantlets production by the indirect somatic embryogenesis protocol for date palm 'Barhee', *RP Cur. Tr. Agri. Env. Sci.* **4** (2025) 25–31.

Original Research Article

AgNPs: A superior alternative to AgNO₃ for the optimal plantlets production by the indirect somatic embryogenesis protocol for date palm 'Barhee'

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ABSTRACT

ARTICLE HISTORY

Received: 5 April 2025 Revised: 25 June 2025 Accepted: 25 June 2025 Published online: 27 June 2025

KEYWORDS

Date palm micropropagation; AgNP toxicity; Root Morphogenesis; Protein quantification; Oxidative stress.

1. Introduction

Date palm (Phoenix dactylifera L.) is a crucial agricultural resource in the deserts and arid regions of the Arab world, where its economic significance extends beyond its cultivation, as its fruits constitute a substantial dietary source with a high profile of human nutritional needs. Moreover, the valorization of date palm by-products, encompassing the utilization of seed and frond, presents avenues for sustainable resource management and the development of diverse industrial applications [1, 2]. While micropropagation offers a highthroughput method for date palm propagation, in vitro cultures frequently encounter challenges such as tissue browning, hyperhydricity, premature and abnormal embryo germination, poor shoot development, and inadequate rooting [3, 4]. This research explores the potential of nanotechnology to improve date palm micropropagation. Nanomaterials, characterized by dimensions within the 1-100 nm range, possess unique physical and chemical properties that may enhance plant tissue culture outcomes.

The application of nanomaterials in agriculture has shown promise in various areas, including enhanced seed germination, increased plant growth and yield-facilitated genetic transformation, and improved photosynthetic efficiency [5, 6]. In plant tissue culture, nanoparticles have demonstrated benefits in reducing microbial contamination, and promoting callus formation, shoot regeneration, stimulating bioactive compound production [7] and overall plant growth 8 - 10]. In different date palm cultivars the application of carbon

The economic and cultural importance of date palms necessitates efficient propagation methods. While Phoenix dactylifera micropropagation offers a pathway to high-volume production, its implementation is hindered by persistent in vitro developmental obstacles. This research evaluated the potential of silver nanoparticles (AgNPs) as an alternative to silver nitrate (AgNO₃) for improving complete P. dactylifera tissue culture protocol for somatic embryogenesis production shoots, proliferation and rooting stages. 'Barhi' explants were exposed to varying concentrations (0.01-2.0 mg/L) of AgNPs and AgNO₃. Findings indicated that 0.1 mg/L AgNPs effectively promoted somatic embryo formation, whereas 0.5 ml L⁻¹reduced the incidence of hyperhydricity. Furthermore, 0.5 ml L⁻¹ AgNPs significantly enhanced shoot proliferation where root development parameters (root no., adventitious root no. , leaf no. and leaf width) were significantly improved at 0.5 ml L⁻¹. Inductively Coupled Plasma (ICP) analysis demonstrated that residual silver levels remained within safe limits at lower AgNP concentrations (0.1 and 0.5 mg L⁻¹), correlating with positive biological outcomes. Consequently, AgNPs present a viable strategy for optimizing P. dactylifera tissue culture and enhancing micropropagation efficiency.

nanotubes [11], iron nanoparticles [12], and zinc oxide nanoparticles [13] for improving somatic embryogenesis. Specifically, studies have reported the use of silver nanoparticles (AgNPs) for microbial control [14] and enhancing somatic embryogenesis [15]. Silver ions, supplied as silver nitrate (AgNO₃), and AgNPs are recognized for their roles in regulating plant growth [16]. These substances can influence ethylene activity, a key hormone in plant development. Research indicates that AgNPs may positively impact micropropagation by modulating ethylene production [8, 9]. Furthermore, studies have explored the use of silver nanoparticles to stimulate plant regeneration and multiplication in various species [17]. Optimal AgNP concentrations have been shown to enhance in vitro growth and development in several plant systems [9, 18, 19]. This study investigates the comparative potential of AgNPs and AgNO3 as oxidative stress inhibitors during the indirect somatic embryogenesis protocol of date palm cv. Barhi. The research objective is to evaluate their impact on decreasing in vitro issues related to browning, hyperhydricity, abnormal somatic embryo germination, poor shoot proliferation, and insufficient root system formation for a successful micropropagation process. Biochemical analysis of protein content during shoot cluster proliferation and the chlorophyll a, b, and carotenoid contents of produced plantlets were determined for the different studied treatments as physiological change indicators. Inductively coupled plasma (ICP) was applied to demonstrate the residual silver levels within the produced plantlet tissues received from the different



Copyright: © 2025 by the authors. Licensee Research Plateau Publishers, India This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/). AgNP treatments to determine the use of nanoparticles in safe limits.

2. Materials and methods

The micropropagation protocol, encompassing sterilization and establishment, adhered to the guidelines outlined by El-Dawayati and Zayed [3]. The study was structured into three distinct stages of date palm micropropagation via indirect somatic embryogenesis: (1) callus differentiation and somatic embryo induction, (2) multiplication of differentiated shoot clusters, and (3) shoot elongation and root development.

Nanomaterial composition

A 10 ml vial contained a solution composed of 9.0% nitrogen, 6.0% potassium, and 1.0% nano silver (Sigma-Aldrich). The nano-silver component was characterized as silver oxide (Ag₂O) with an average particle size of 50 nm (Figure 1).



Figure 1: 50 nm average particulate size silver nano.

1. Callus differentiation and somatic embryo induction

Embryonic calluses, derived from shoot tip cultures on Murashige and Skoog (MS) medium (1962) supplemented with 200 mg L⁻¹ glutamine, 4 mg L⁻¹ thiamine HCl, 1 mg L⁻¹ biotin, 40 g L⁻¹ sucrose, 7.0 g L⁻¹ agar, 0.1 g L⁻¹ activated charcoal, and 1 mg L-1 naphthalene acetic acid (NAA), were used. To evaluate the effects of AgNPs and AgNO3 on callus growth and development, varying concentrations of each (0.01, 0.1, 0.5, 1.0, and 2.0 ml L⁻¹) were incorporated into the callus growth and development medium across three subculture intervals of four weeks. The medium's pH was adjusted to 5.7-5.8 before agar addition. Media were dispensed into culture vessels and autoclaved at 121 °C and 1.04 kg/cm² for 20 minutes. Cultures were incubated at 27 ± 2 °C under a 16-hour light/8-hour dark photoperiod, provided by 100 µmol m⁻²/s white fluorescent lamps. Each treatment consisted of three replicates, with each replicate comprising three culture vessels, each containing 1.0 g of embryonic callus. Data were collected following the three subcultures, focusing on callus growth value, browning intensity, hyperhydricity level, differentiation percentage, and the number of normal and abnormal embryos. The growth value was calculated as (Final fresh weight-Initial fresh weight) / Initial fresh weight, as per Zaved et al. [20].

Browning and hyperhydricity were assessed visually using a scoring system: (-). A negative result, (+) Below average result, (++) Average result, (+++) Good result, (++++) Excellent result, based on the methods of Pottino [21], Mujib et al. [22], and Eldawayati et al [2].

2. Multiplication of the differentiated shoot clusters

Primary smallshoot clusters (4-5 shoots, 0.5-0.7 cm length) obtained from the indirect somatic embryogenesis stage

were cultured on the culture nutrient (MS)medium, with growth regulators adjusted to 0.05 mg L⁻¹ BA and 0.1 mg L⁻¹ NAA. The same AgNPs and AgNO₃ concentrations were used to investigate their impact on shoot cluster multiplication and selected phytochemical properties. Each treatment comprised three replicates, each with three culture vessels, each containing one shoot cluster explant. Cultures were incubated at 27 \pm 2 °C under a 16-hour light/8-hour dark photoperiod, provided by 100 µmol m⁻²/s white fluorescent lamps. Data were recorded after three subcultures for the morphological appearance of shoot number, shoot length (cm), and biochemical analysis of total protein content and chlorophyll content.

Total protein content was determined using the Bradford method [23].

3. Shoot elongation and root development.

Elongated shoots (shootlets) measuring 5 cm in length with two expanded leaves were cultured on the previously described medium, with sucrose increased to 50 g L⁻¹ and growth regulators adjusted to 0.4 mg L⁻¹ paclobutrazol (PBZ), 1.0 mg L⁻¹ NAA, and 1.0 mg L⁻¹ IBA. The same AgNPs and AgNO₃ concentrations were employed. Each treatment included three replicates, each with three culture tubes, each containing one shootlet explant. All culture tubes were incubated under 200 µmol m⁻²/s fluorescent light for a 16-hour light/8-hour dark photoperiod at 27 ± 2 °C. Data were recorded after three subcultures, including shoot length, root number, root length (cm), leaf number, leaf width (cm), adventitious roots number, and plantlet growth vigor, assessed using the browning and hyperhydricity scoring system.

Chlorophyll A, B, and carotenoid content (mg/g) were measured spectrophotometrically at 660, 640, and 440 nm, respectively, using a Thermo Scientific Orion Aqua Mate 8000 UV-Visible Spectrophotometer, as described by Lichtenthaler and Buschmann [24].

Silver Content Determination via Inductively Coupled Plasma (ICP)

Silver content was measured at the Micro Analytical Center, Faculty of Science, Cairo University. Samples were dried at 65 °C for 72 hours, and 1 g was digested in 20 mL of concentrated nitric acid at 125 °C until complete consumption of organic matter. The resulting solution was cooled, filtered, diluted to 50 mL with deionized water, and analyzed using ICP against a silver standard calibration curve.

Statistical Analysis

Experiments were conducted using a Complete Randomized Block Design (CRD). All data were subjected to analysis of variance (ANOVA) using SPSS version 18.0, and significant differences between treatments were determined according to Steel et al. [25].

3. Results and discussion

1. The comparative potential of AgNPs and AgNO $_3$ during the callus differentiation and somatic embryo induction

The effect of silver nanoparticles silver nitrate (AgNO₃) on the growth and development of the embryonic callus of date Palm CV Barhe was evaluated in Figures 2(a,b,c,d,e,f).



Figure 2: Illustrates the comparative influence of AgNPs and AgNO₃ concentrations on Callus differentiation and somatic embryo induction through evaluating Figure 2(a) browning, Figure 2(b) the callus growth value, Figure 2(c) hyperhydristy, Figure 2(d) the number of normal somatic embryos differentiation, and Figure 2(f) the differentiation percentage of the somatic embryo.

The results showed that using silver, either as silver nanoparticles or silver nitrate, had a significant effect in all parameters compared to the control treatment. In contrast, the browning degree in Figure 2(a) decreased with increasing silver concentration; 2.0 mL⁻¹ AgNPs achieved the lowest value of the browning degree. The browning of callus often correlates with excessive accumulation of phenolic compounds. Thus, the accumulation of phenolic compounds in the culture medium adversely affects the growth and survival of explants under in vitro conditions. The ethylene produced during in vitro culture impairs plant growth and development and could limit in vitro propagation of several plants. Accordingly, the findings of this study may ethylene inhibitors suggest that AgNO₃ particularly alleviated the negative effects

of ethylene on the growth of banana culture in vitro. Silver nitrate allowed a significant reduction of the browning of callus derived from petal and staminode whatever the cocoa genotype. The effect of AgNPs and AgNO₃ on the growth value of callus data in Figure 2(b) decided that there are highly significant differences between AgNPs, AgNO₃, and control treatments. AgNPs at 0.5 ml/l gave the best result of the growth value of callus, followed by 1.0- and 2.0-ml L⁻¹ AgNPs, and there are no significant differences between them, while AgNPs at 0.1 ml/L and AgNO₃ at 2.0 ml L⁻¹ recorded the same result, Figure 2(b). The growth value of the callus of date palm increased in response to the increase of silver nitrate, reaching a maximum of 0.7 g on 75 mg silver nitrate. Further, an increase in silver nitrate concentration reduced callus growth.

50 mL⁻¹ ZnO-NPs gave the highest callus induction (the highest percentage of callus-producing buds and the average bud formation per callus of date palm. Hyperhydricity (or vitrification) is a common physiological disorder encountered in date palm in vitro regeneration systems based on either somatic embryogenesis or organogenesis. This waterlogged appearance is the result of the accumulation of water in the cultured explants El Dawayati and Zayed [3]. In these studies, the hyperhydricity of embryonic callus was evaluated in Figure 2(c) the results showed that the Hyperhydiricity degree significantly decreased by increasing AgNPs or AgNO₃ concentration in the culture medium whereas. the hyperhydricity degree decreased from 2.00 to 0.25 by treating embryonic callus with different concentrations of AgNPs also hyperhydiricity degree decreased from 2.33 to 0.88 by treating embryonic callus with AgNO3 concentrations. To reduce hyperhydricity and resume normal growth. Dianthus chinensis, the addition of silver nitrate is routinely recommended in the culture medium. However, AgNP treatment of hyperhydric explant (4 weeks) resulted in high retroversion paralleled with reduced relative water content. Supplementation of 100 µg L⁻¹ AgNPs in MS medium significantly reduced the percentage of hyperhidricity to 13.3%, in contrast to the control (100%) Sreelekshmi et al. 2022. The addition of 0.2g/l AgNO₃ to the culture medium of date palm cv. Gundela decreased the hyperhydricity percentage to 22.22% compared with the control medium (55.55%) [20]. The differentiation percentage of somatic embryos was the same and the highest at concentrations of 0.1 and 0.5 mg L⁻¹ AgNPs without significant differences between (100%) followed by AgNO₃ at 2.0 mg L^{-1} concentration (90.22%) Figure 2(d). Data in Figure 2 (e and f) showed the effect of AgNPs and AgNO₃ with the different concentrations on the number of normal and abnormal somatic embryos differentiation, the highest number of normal somatic embryos was obtained when either AgNPs or AgNO₃ was applied whereas, control treatment recorded the maximum value of abnormal somatic embryos (8.66). There was no significant difference between 2.0 m L⁻¹ of AgNO₃ and 0.1 mL⁻¹ concentration of AgNPs on the differentiation percentage or number of somatic embryos, these results agree with Seif et al [26] who found that There was no significant difference between 100 ppm of silver nitrate and 60 ppm concentration of silver nano on the shoot silver concentration. Therefore, the permeability of nano-silver is far greater than silver nitrate. The reason for this matter is the small size of nanoparticles, which causes more adhesion of nanoparticles to plant tissues. Considering the lesser use of silver in nano silver, this treatment can be used instead of other combinations of silver. El-Kosary et al [15] noticed that MS medium supplemented with 125µg L⁻¹ AgNPs recorded the highest percentage of direct somatic embryo formation in date palm cultivars (Sewi and Medjool) compared with the other concentrations also somatic embryo multiplication and germination were significantly affected by different AgNPs the concentrations produced the highest embryo lower multiplication and germination value in both cultivars.

Table 1: The comparative influence of AgNPs and AgNO₃ on shoot cluster proliferation of date palm 'Barhee' Means in each column followed by different letters are significantly different atp _ 0.05).

Treatments ml L ⁻¹		No. of shoot	Shoot length (cm)	Growth vigor degree	
Control	0.00	18.25g	5.00e	3.00d	
AgNPs	0.01	25.00ef	5.75cd	3.75b	
	0.1	38.00c	7.50a	4.00a	
	0.5	49.50a	6.50b	4.00a	
	1.00	44.75b	6.25bc	3.50c	
	2.00	33.25d	5.00e	3.00d	
AgNI ₃	0.01	22.75ef	4.75f	3.00d	
	0.1	27.75e	4.55f	3.75b	
	0.5	35.25cd	4.75ef	3.75b	
	1.00	45.25b	6.25bc	3.75b	
	2.00	40. 22c	5.60cd	3.00d	

2. The comparative potential of AgNPs and AgNO₃ of (AgNPs) and silver nitrate (AgNO₃) during shoots cluster proliferated

Using silver as nano silver or silver nitrate significantly affected the number of shoots, shoot length (cm), number of leaves, and growth vigor of date palm shoot proliferated (Table 1).

The results showed that increasing silver concentration as nano silver or silver nitrate reduced the number of shoots where the highest number of shoots recorded (49.50) at 0.5 m L^{-1} AgNPs concentration followed by 1.0 m L^{-1} AgNO₃ (45.25) and 1.0 ml L^{-1} AgNPs (44.75) without significant differences between Figure 3.

The control treatment recorded the lowest value of shoot proliferation (18.25). These results harmonize with Seif et al [26] declared that silver concentration in the shoot of Borago officinalis L. was increased by all treatments (either nano silver or silver nitrate); however, the higher concentration of silver in the shoot was obtained by silver nitrate treatment in comparison to others.



Figure 3: The highest number of shoots recorded at 0.5 m L^{-1} AgNPs concentration(a), followed by 1.0 m L^{-1} AgNO₃ (b) and 1.0 ml L^{-1} AgNPs (c), without significant differences between.

There was no significant difference between 100 ppm of silver nitrate and 60 ppm concentration of nanosilver on the shoot silver concentration. Therefore, the permeability of nanosilver is far greater than silver nitrate. The reason is explained by the small size of nanoparticles, which causes more adhesion of nanoparticles to plant tissues. Considering the lesser use of silver in nano silver, this treatment can be used instead of other combinations of silver. In addition, there was no significant difference between 0.1 mL⁻¹ concentration of AgNPs (38.00) and 2.0 mL⁻¹ of AgNO₃ (40.22). Regarding shoot length (cm), data showed that AgNPs at 0.1 obtained the highest shoot length (7.5 cm), followed by 0.5 mL⁻¹ AgNPs and 1.0 mL⁻¹ of AgNO₃ without significant difference between them (6.50 and 6.25 cm). The lowest value of shoot length was recorded at 0.01, 0.1, and 0.5 mL⁻¹AgNO₃ without significant differences among them (4.75, 4.55, and 4.75 cm), respectively. On the other hand, the number of shoot clusters proliferated was the greatest at 0.5 and 1.0 mL⁻¹ AgNPs concentration without significance between (105.25 and 99.75) followed by 86.0 at 1.0 m $^{L-1}$ AgNO₃. While control treatment and 0.01 mL⁻¹ AgNO₃ gave the lowest number of leaves (36.33 and 42,25). The same maximum value of growth vigor of the shoot cluster proliferated was achieved at 0.1 and 0.5 mL⁻¹ AgNPs (4.0). In the control treatment, 2.0 mL⁻¹ AgNPs and 2.0 mL⁻¹ AgNO₃ recorded the same minimum value of growth vigor of the shoot cluster proliferated (3.0).



Figure 4: The influence of silver nanoparticles (AgNPs) and silver nitrate (AgNO₃) on the protein contents of developed shoot clusters of date palm Barhee cultivar.

It is evident from the results in Figure 3 that the amount of total content protein gradually increases by increasing AgNPs but decreases at high concentrations of AgNPs (2.0 m L^{-1}). The highest amounts of protein obtained were 1.60 and 1.63 mg/g FW with concentrations of 0.5 and 1.0 m L^{-1} AgNPs without significant differences, followed by AgNO₃ at 2.0 L^{-1} (1.41 mg\g FW). Control treatment and AgNO₃ at 0.01 L^{-1} recorded 0.99 and 1.11 mg/g FW as the lowest amount of protein without significant differences between.

Table 2: The comparative influence of AgNPs and AgNO3 on the rooting development of shootlets of date palm 'Barhee'. Means in
each column followed by different letters are significantly different at $p_0.05$)

Treatmen ml L ⁻¹	ıts	Shoot Length (cm)	No. of Root	Root Length (cm)	No. of adventitious roots	No. of Leaf	Leaf Width (cm)	Growth Vigor
Control	0	11.22e	2.25d	7.50f	5.50g	2.22e	0.51e	2.25
AgNPs	0.01	14.33cd	3.33bc	11.92b	8.33c	2.66d	0.78cd	3.00
	0.1	15.90b	3.75ab	11.92b	6.33e	3.00c	0.98abc	3.33
	0.5	16.50b	4.33a	11.75bc	10.00a	3.66a	1.11a	3.5
	1	18.33a	4.33a	12.33a	10.33a	3.83a	0.78cd	4.00
	2	13.33d	3.33bc	11.75bc	6.50de	3.33b	0.77cd	3.33
AgNO ₃	0.01	14.50c	3.00c	10.88e	6.75d	2.83d	0.77cd	2.75
	0.1	14.33cd	3.33bc	11.33d	6.75d	3.16c	0.79cd	3.33
	0.5	14.75c	3.30c	11.33d	7.50d	3.33b	0.89bc	3.00
	1	14.33cd	4.33a	11.50cd	6.00ef	3.66a	0.89bc	3.66
	2	16.00b	4.00ab	11.90b	9.33b	3.33b	0.98abc	3.66

Effect of silver nanoparticles (AgNPs) and silver nitrate (AgNO₃) on the rooting of shootlets

The addition of silver nanoparticles or silver nitrate in the rooting culture medium induced significant growth parameters compared to the control treatment (table 2) Figure (5) and Figure (6).

The highest shoot length (cm) was obtained by adding 1.0 mL^{-1} AgNPs to the rooting medium (18.33 cm), and no significant differences in addition 0.1. 0.5 mL^{-1} AgNPs and 2.0 mL^{-1} AgNO₃ to the rooting medium were recorded as 15.90, 16.50, and 16.00 cm, respectively. The number of roots 0.5, 1.0 0 mL^{-1} AgNPs, and 1.0 mL^{-1} AgNO₃ were recorded as the maximum and the same value of the root number (4.33) and followed by 0.1 mL^{-1} AgNPs and 2.0 mL^{-1} AgNO₃ (3.75 and 4.00). Furthermore, the root length was the longest with adding 1.0 0 mL^{-1} AgNPs to the rooting medium, at AgNPs 0.5 and 1.0 0 mL^{-1} gave the best results in the number of hairy roots without significant differences.



Figure 5: A comparison between the control and the best treatments of AgNPs and AgNO₃ during the rooting stage of date palm cv. Barhee.



Figure 6: The different effects of AgNPs during the rooting stage of date palm 'Barhee'.

On the other hand, data showed that there are no significant differences in the number of leaves by adding 0.5, 1.0 mL⁻¹ AgNPs, and 1.0 mL⁻¹ AgNO₃ to the rooting medium (3.66, 3.83, and 3.66 respectively), which achieved the highest results. Moreover, the low concentrations of AgNPs or AgNO₃ appeared to decrease the number of leaves. While the control treatment recorded the lowest value in leaves number (2.22). Regarding the leaf width of date palm plantlets, data observed that increasing AgNP concentration from 0.001 to 0.5 increased the leaf width, and AgNPs at 0.1 0 mL⁻¹ and AgNO₃ at 2.0 0 mL⁻¹ recorded the same result (0.98). There are no significant differences between 0.5 mL⁻¹ AgNPs and 2.0 mL⁻¹ AgNO₃ in the growth and development of date palm shootlets; therefore, the permeability of nano-silver is far greater than silver nitrate due to the small size of nanoparticles, which causes more adhesion of nanoparticles to plant tissues.





The chlorophyll content a, b and Carotenoids as shown in Figure 7(a) decreased with the lowest AgNP concentration (0.01 mL^{-1}) compared to the control then it gradually increased with increasing the AgNP concentration from 0.1 to 0.5 to 1.0 ml/l then decreased again with increasing its concentration to 2.0 ml L⁻¹ but still higher than the control. The maximum chlorophyll a, b, and carotenoid content were recorded at 1.0 mL⁻¹ AgNPs with 1.814, 0.695, and 2.217, respectively. Figure 7(b) shows the increase of chlorophyll content with increasing AgNO₃ at 2.0 mlL⁻¹ recorded the maximum value of chlorophyll content a, b, and Carotenoids (1.153, 0.747, and 2.145 respectively) while the control treatment gave the lowest value of chlorophyll content a, b, and Carotenoids (0.428, 0.260, and 0.637 respectively).

The results regarding silver content in tissues of the date palm plants were recorded in Figure 8. As expected, data showed that the maximum content of silver element accumulation was 1.9 mg/g DW with the use of the highest concentration AgNPs (2.0 mL^{-1}) in comparison to the control treatment which has the least value (0.015 mg/g DW). Nanoparticles, characterized by their unique physicochemical properties arising from their nanoscale dimensions. However, worries about nanomaterials' possible effects on the environment and human health have also grown in tandem with their increasing prevalence. To guarantee their safe and responsible use, a comprehensive grasp of their toxicological profiles is required [27].



Figure 8: The demonstration of residual silver levels within plantlet tissues of the different AgNP concentrations.

4. Conclusions

In this study, AgNPs optimized the date palm 'Barhee' micropropagation protocol. Nanobiotechnology is emerging as a prominent and promising field for date micropropagation with excellent potential towards plant improvement. The effects of AgNPs can vary depending on factors such as

nanoparticle size, concentration, and the specific date palm cultivar. There is a requirement for more targeted researches in date palm Nanotechnology to clarify and streamline the process to harness only the beneficial aspects without exposure to the adverse effects.

Authors' contributions

The author read and approved the final manuscript.

Conflicts of interest

The author declares no conflict of interest.

Funding

This research received no external funding.

Data availability

No new data were created.

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