



Effect of microcontainer enriched with zinc nanoparticle (ZnO-NPs) on the acclimatization and development of micropropagated seedlings of *Paliavana sericiflora* Benth

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Received: 30 October 2025 / Accepted: 30 May 2026 / Published online: 10 June 2026
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Abstract

This study investigated the effect of incorporating zinc oxide nanoparticles (ZnO-NPs) on the acclimatization and growth of micropropagated seedlings of *Paliavana sericiflora* Benth. The seedlings were grown in 4.5 cm³ microcontainer in polypropylene cups using a mini-incubator system. The experimental factor was the concentration of ZnO-NPs added to the polymer in the tubes (PLA), with the following levels: 0, 25, 50, 75, 100 and 1000 mg kg⁻¹. After the acclimatization period, the plants were transplanted into 250 cm³ tubes. The results indicated that the optimal concentration of ZnO-NPs was 100 mg kg⁻¹, which promoted greater plant vigour, higher dry matter accumulation, and superior development in terms of height, stem diameter, and leaf area, while also altering the nutritional composition by increasing the levels of N, P, Ca, Mg, and S. This concentration resulted in 100% seedling survival. In contrast, the concentration of 1000 mg kg⁻¹ of ZnO-NPs had a phytotoxic effect, highlighting the need for more in-depth studies into the relationship between the concentration of ZnO-NPs and the specific characteristics of each plant species. The high correlation between vegetative variables and the ideal concentration of ZnO-NPs suggests that incorporating ZnO-NPs into microcontainer could be a promising strategy for optimizing the acclimatization and growth of micropropagated seedlings of *Paliavana sericiflora* and other plant species.

Keywords Zinc oxide · *Campo Rupestre* · Vulnerable to extinction · In vitro · Polylactic acid (PLA)

Communicated by Mohammad Faisal

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Introduction

In vitro cultivation the plants offer a wide range of benefits for various sectors. This makes it possible to obtain plants with high genetic and health quality on a large scale, boosting crop productivity and profitability. In addition, micropropagation is an important tool for the preservation of endangered species, enabling their recovery and reintroduction into the natural environment. Currently, India stands out as the main producer and exporter of micropropagated plants in general, with an estimated annual production of 5 million units (Shukla and Shukla 2024).

Micropropagation consists of the multiplication of plants from explants of organs, tissues or cells, cultivated in vitro under aseptic conditions in suitable culture media, with strict control of environmental and nutritional variables (George et al. 2008; Thorpe 2007). This tissue culture technique stands out due to its many applications in the forestry sector (Alfenas et al. 2004), above all by minimizing genetic variability in relation to the original material (Nehra et al. 2005), inducing rejuvenation of plant tissue and increased physiological vigour (Dutra et al. 2009), and the possibility of creating germplasm banks (Leite et al. 2024).

Paliavana sericiflora Benth. belongs to the family Gesneriaceae and is an endemic species associated with the campo rupestre ecosystem, particularly ferruginous environments characterized by nutrient poor substrates and adverse environmental conditions. The species has ornamental potential due to its attractive floral characteristics and ecological importance because of its contribution to biodiversity maintenance in highly specialized habitats. Furthermore, anthropogenic disturbances and habitat fragmentation reinforce the need for conservation strategies for this species (Rossini et al. 2020; SiBBR. 2020).

Previous studies involving *P. sericiflora* have established protocols for in vitro propagation, including multiplication, rooting, and acclimatization procedures, demonstrating the feasibility of large scale seedling production (Leite et al. 2024, 2025). In addition, previous studies showed the effectiveness of mini-incubator systems during the transfer of plantlets to greenhouse conditions. Despite these advances, limited information is available regarding nutritional approaches capable of improving acclimatization performance and subsequent plant development under ex vitro conditions.

One of the main challenges of growing plants in vitro is the high mortality rate during the acclimatization stage. This phenomenon stems from the inadequate control of transpiration, since, in general, plants grown in vitro do not have functional stomata, which are responsible for regulating water loss to the external environment (Manokari et al. 2021; Miranda et al. 2024). To minimize transpiration and

ensure effective acclimatization, a commonly used technique is the mini-incubator system (Brondani et al. 2018). This approach optimizes the survival of plants in the ex vitro environment. The way the nutrients are made available, and the period of application can significantly influence future results. In this context, nano fertilizers enable the efficient delivery of nutrients and optimize their use by crops (Jakhar et al. 2022; Haydar et al. 2024). The application of fertilizers not only helps with ex vitro rooting and the consequent survival of the plants but also contributes to enhancing their physiological vigour. Several studies have already explored the use of nano fertilizers in different plant species, and many of them are obtained through green synthesis (Haydar et al. 2024), featuring slow and controlled release mechanisms, which favour the gradual absorption of nutrients by plants.

Among the essential nutrients for plants, zinc (Zn) stands out as a micronutrient of crucial importance, although its availability in tropical soils is often limited. Its high mobility within the plant organism enables action in various redox reactions (Araujo et al. 2016). Zinc plays a fundamental role in electron transfer and energy transformation and may even be involved in chlorophyll synthesis in some plant species (Taiz et al. 2017). In addition, this micronutrient is essential for the synthesis of tryptophan, a precursor of auxins, plant hormones that regulate plant growth and development (Brondani et al. 2012).

Adequate zinc supply can therefore encourage new shoots and stimulate root growth, optimizing seedling development. On the other hand, a deficiency of this element can cause disturbances in auxin production, leading to inhibition of stem elongation and a reduction in leaf size (Natarelli et al. 2021). Given the importance of zinc for plant growth, the use of nanoparticles containing this micronutrient into acclimatization is a promising alternative for boosting the production of high-quality clonal seedlings.

Zinc (Zn) can be made available in the form of zinc oxide nanoparticles (ZnO-NPs), with diameters between 1 and 100 nanometers (Rai 2013). A strategy for delivering ZnO-NPs to plants consists of directly incorporating these nanoparticles into the polymer of the growing container, such as polylactic acid (PLA). PLA stands out as a biodegradable bioplastic, with wide availability and the ability to decompose without causing damage to the environment (Taib et al. 2023).

The objective of this study was to optimize the acclimatization process and maximize the survival of micropropagated plantlets of *Paliavana sericiflora* Benth. by investigating the effect of incorporating zinc oxide nanoparticles (ZnO-NPs) into the microcontainers used during the acclimatization stage.

Materials and methods

Study site and plant material

The studies were carried out at the Laboratory for the in vitro Cultivation of Forest Species and the Forest Nursery, located in the Department of Forestry Sciences at the Lavras School of Agricultural Sciences (ESAL/UFLA), Federal University of Lavras (UFLA), in Lavras, Minas Gerais, Brazil. The plant material used in this study originated from in vitro germinated seedlings established in previous studies involving *Paliavana sericiflora* (Leite et al. 2024, 2025; code ACF88E2 in SisGen). The material was maintained under in vitro conditions through periodic subcultures, totalling approximately 12 subculture cycles before the establishment of the present experiment.

Obtaining and evaluating nanoparticles

The production of ZnO-NPs followed the methodology described by Alves et al. (2012). Two aqueous solutions were prepared, one containing zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] and the other containing sodium hydroxide (NaOH), both at a concentration of 1 mol L^{-1} . The solutions were mixed and homogenized under moderate stirring

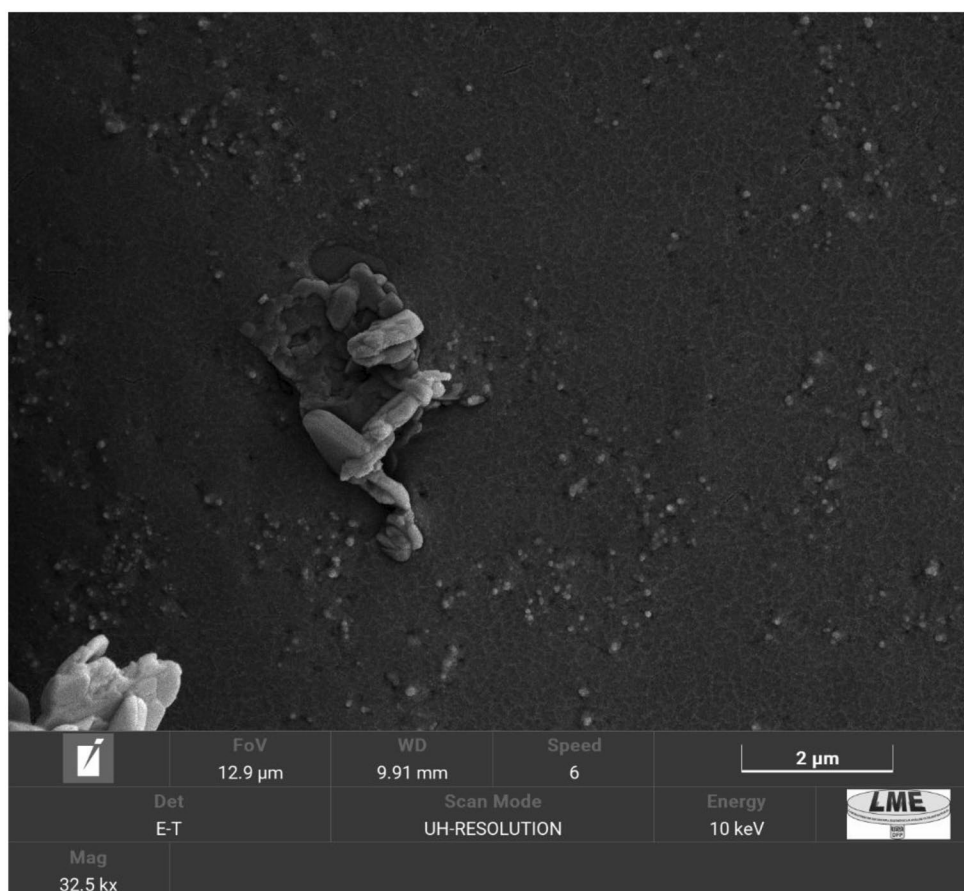
and heating at 80°C for two hours. After this period, the white precipitate obtained was collected by filtering through filter paper and washed successively with ethanol and plenty of deionized water. The precipitate was then dried at 60°C for 48 hours and stored under vacuum. The dried material was macerated and analysed by scanning electron microscopy (SEM) (Fig. 1).

Filament and microcontainer production

The PLA filament was enriched with ZnO-NPs in concentration of 25, 50, 75, 100 and 1000 mg kg^{-1} of PLA. For each treatment, 1 kg of PLA granules was used for filament production, and ZnO-NPs were incorporated according to the respective concentrations. To do this, the ZnO-NPs was dispersed in chloroform and added to the PLA granules under constant stirring. The mixture was then dried in an oven at 60°C for 48 hours. After drying, the granules were added to the FILMAQ3D CV extruder to make the filament at a temperature of 100°C . On leaving the extruder, the filament was pulled by the FTR1, which was responsible for cooling the filament and measuring its diameter.

The 4.5 cm^3 microcontainers (protected by patent application BR 10 2023 007189 9), 4 cm high and 1.2 cm in

Fig. 1 Zinc oxide (ZNO-NPS) nanoparticles after the maceration process. Bar: $2 \mu\text{m}$



diameter, were produced by a 3D printer (Orion Delta) using zinc-enriched filament according to each treatment.

Acclimatization and hardening-off

Plantlets used for acclimatization originated from in vitro germinated seedlings maintained under active growth conditions. Before transfer to ex vitro conditions, the plantlets exhibited fully developed shoots and roots suitable for acclimatization. Complete in vitro grown plantlets (aerial part and root) were used in acclimatization treatments. The seedlings were transplanted into 4.5 cm³ and about 1 g, microcontainer in transparent polypropylene cups using a mini-incubator system (Fig. 2). The substrate used was decomposed of pine bark. The plants were grown under a 16-hour photoperiod and an irradiance of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 30 days. After this period, plant survival rates were assessed, and the formation of adventitious roots and plant tissues was visualized.

After the acclimatization period in microcontainer, the plants were transplanted into 280 cm³ tubes. The substrate used consisted of decomposed pine bark and medium vermiculite in a ratio of 1:1 (v/v). No supplemental fertilization or nutrient solution was applied during the acclimatization and hardening-off stages. The tubes were kept in a greenhouse with irrigation every other day for 60 days. The experiment was set up in a completely randomized design with 20 replications per treatment. The experimental factor was the concentration of ZnO-NPs added to the polymer in the tubes (PLA), with the following levels: 0, 25, 50, 75, 100 and 1000 mg kg⁻¹. The following were assessed: vigour (grading scale), number of leaves, number of shoots, height (cm), neck diameter (cm), shoot dry mass (g), root dry mass

(g), total dry mass (g), Dickson Quality Index (IQD), leaf width (cm), leaf length (cm), leaf area (cm²), photosynthetic pigment content [chlorophyll *a*, *b*, total (*a*+*b*), carotenoids ($\mu\text{g mg}^{-1}$)], macro and micronutrients.

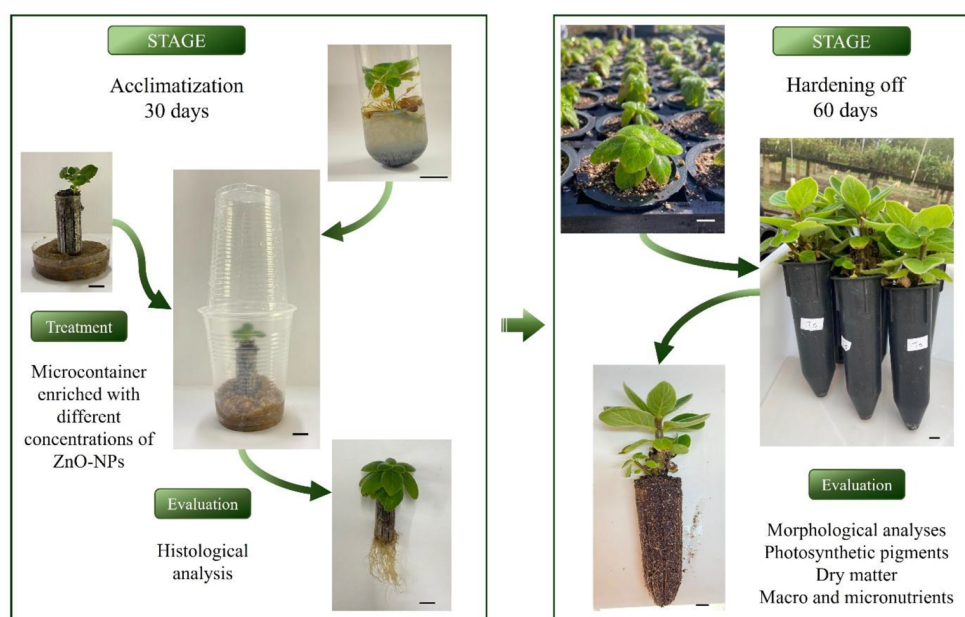
To evaluate vigour in a comprehensive and objective way, a predefined grading scale from 1 to 5 was developed: 1 – excellent (a): vigorous shoot induction, active growth and no signs of nutritional deficiency, 2 – good (b): shoot induction (or not), reduced growth compared to the ideal, no visible symptoms of nutritional deficiency, 3 – fair (c): reduced growth, leaves with necrotic or yellowed spots, 4 – bad (d): low growth, appearance of visual symptoms of nutritional deficiency, and necrosis of plant tissues, 5 - very bad (e): death of more than 75% of leaf and shoot tissues (Fig. 3).

Leaf analysis, photosynthetic pigments and histological analysis

The leaf area, length and width of the leaves were measured using image J software and the images were obtained using an HP Deskjet Ink Advantage 1516 scanner. Twenty leaves per treatment were evaluated, taken from the 3rd and 4th pair of young leaves, the aerial part towards the coleus.

To quantify the photosynthetic pigments (chlorophylls *a* and *b*, carotenoids) in the plant leaves, a method based on Lichtenthaler (1987) and Wellburn (1994) was used. Leaf discs of 25 mg were removed and submerged in 5 mL of dimethyl sulfoxide (DMSO) solution for 48 hours in a dark environment, ensuring the preservation of the pigments. The absorbance of the solutions was measured in triplicate on a Genesys 10UV spectrophotometer (ThermoScientific, USA) in quartz cuvettes with an optical path of 10 mm,

Fig. 2 Schematic representation of the experimental procedure for acclimatization and hardening off of *Paliavana sericiflora*, illustrating the transfer of in vitro grown plantlets into microcontainers using a mini-incubator system and their subsequent development under ex vitro conditions. Bar: 1 cm



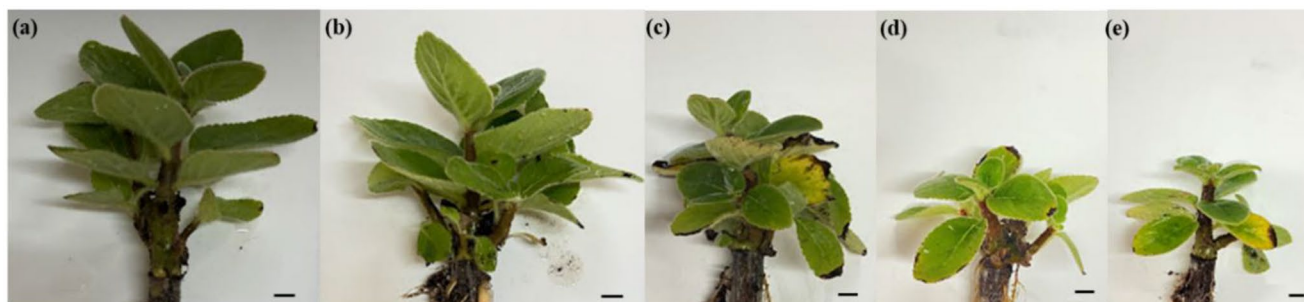


Fig. 3 Scale of vigour at 60 days of hardening off *Paliavana sericiflora*, (a) excellent, (b) good, (c) fair, (d) bad end (e) very bad. Bar: 1 cm

using specific wavelengths: 665 nm (chlorophyll *a*), 649 nm (chlorophyll *b*) and 480 nm (carotenoids). The concentrations of chlorophylls *a*, *b*, total and carotenoids were calculated using the equations of Wellburn (1994).

To make the slides, fragments of approximately 1 cm² were taken from the median regions of the leaves and the base of the stem of the treatment with the best morphological responses. The material was subjected to ethanolic dehydration (70, 80, 90, 100%) at room temperature (Johansen 1940). It was then immersed in a pre-infiltration solution consisting of a solution of 100% ethanol and base resin, in a 1:1 ratio, according to the manufacturer's instructions (Leica Historesin Kit) for 24 hours. After this process, the base resin was infiltrated for 15 days at 4 °C.

The Historesin Kit (hydroxyethyl methacrylate, Leica, Heidelberg) was used for polymerization. The sections were taken on a semi-automatic rotating microtome and then stained in 1% Toluidine Blue, pH 6.7 (Feder and O'Brien 1968). The images were obtained using a Nikon Eclipse E100 light microscope coupled with an Infinity capture camera.

Determining the macro and micronutrients

After the evaluations, the material was oven-dried at 50 °C to constant weight, ground, and sent to the Soil Analysis Laboratory of the Department of Soil Science at the Federal University of Lavras for chemical determination (Teixeira et al. 2017).

Statistical analysis

Nanoparticle size was analyzed descriptively, while the other variables were checked for homoscedasticity and normal distribution of residues using the Hartley and Shapiro-Wilk tests, respectively, with a 5% probability of error. The results obtained were analyzed by analysis of variance (ANOVA), followed by Tukey's test to compare the means. The program R version 4.0.3 (2020) and the functions contained in the packages easyanova (Arnhold 2013)

and AgroR (Shimizu et al. 2024) were used, and principal component analysis was carried out using the packages Factorextra, and FactoMineR (Kassambara and Mundt 2020).

Results

During 30 days of acclimatization, the seedlings produced in vitro showed 100% survival, in all treatments tested, demonstrating a successful acclimatization process. The vigour of the seedlings, assessed on a scale of scores from 1 to 5 (where 5 represented underdeveloped plants), was significantly lower at the lower concentration of ZnO-NPs, resulting in plants with underdevelopment, chlorosis and leaf fall. In contrast, the concentration of 100 mg kg⁻¹ of ZnO-NPs provided the most vigorous seedlings (Fig. 4a) with the best growth. In association with vigour, the accumulation of aerial and total dry mass (Fig. 4b and c) showed a similar trend, with the treatment with the most vigorous plants (100 mg kg⁻¹ of ZnO-NPs) showing the greatest accumulation of dry matter.

Initially, the microcontainer (Fig. 4d) weighed approximately 1.5 g. After 90 days of cultivation, differences in microcontainer mass were observed among treatments. These variations may be associated with factors such as differential biodegradation rates of the polymer matrix, water absorption, or interactions with environmental conditions and microorganisms. However, these mechanisms were not investigated in the present study, and further studies are required to clarify the factors responsible for the observed differences among treatments.

The biodegradation of the tubes may have influenced the release of the ZnO-NPs nanoparticles, promoting the absorption of the nutrient by the seedlings. The association with fungi and bacteria is one of the possibilities of interaction with the environment that can accelerate the decomposition of the material and the release of nanoparticles. The increase in mass in the container with better seedling growth suggests that the microorganisms helped absorb water and

Fig. 4 Characteristics assessed at 60 days of hardening off *Paliavana sericiflora* as a function of the concentrations of ZnO-NPs used in the microcontainer (0, 25, 50, 75, 100 and 1000 mg kg⁻¹). (a) Vigour, (b) aerial dry matter (g), (c) total dry matter (g), (d) microcontainer weight after drying (g). Means followed by the same letter do not differ significantly according to Tukey's test. Bars represent the standard error of the sample

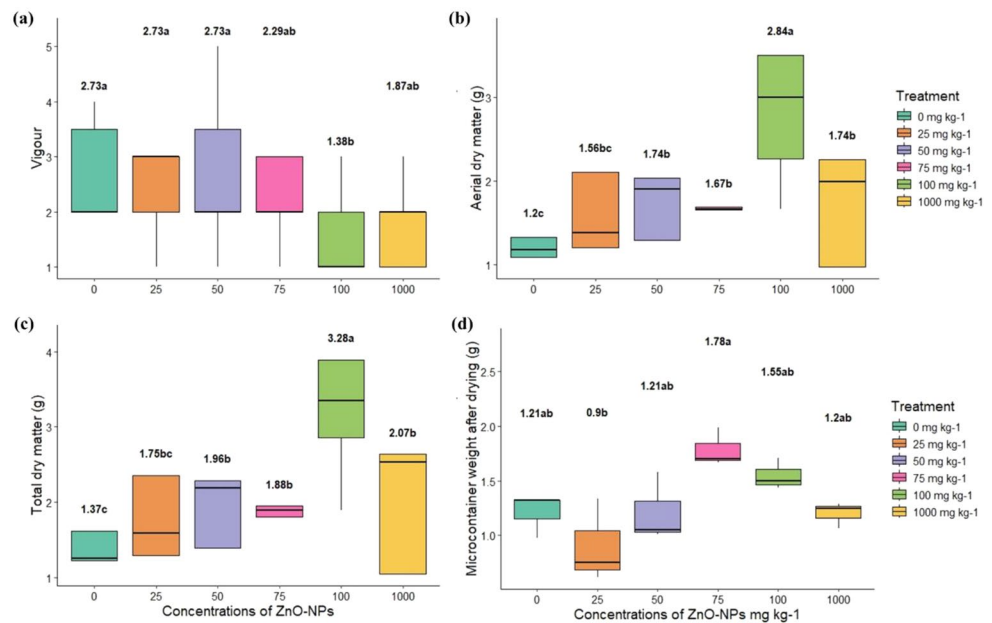
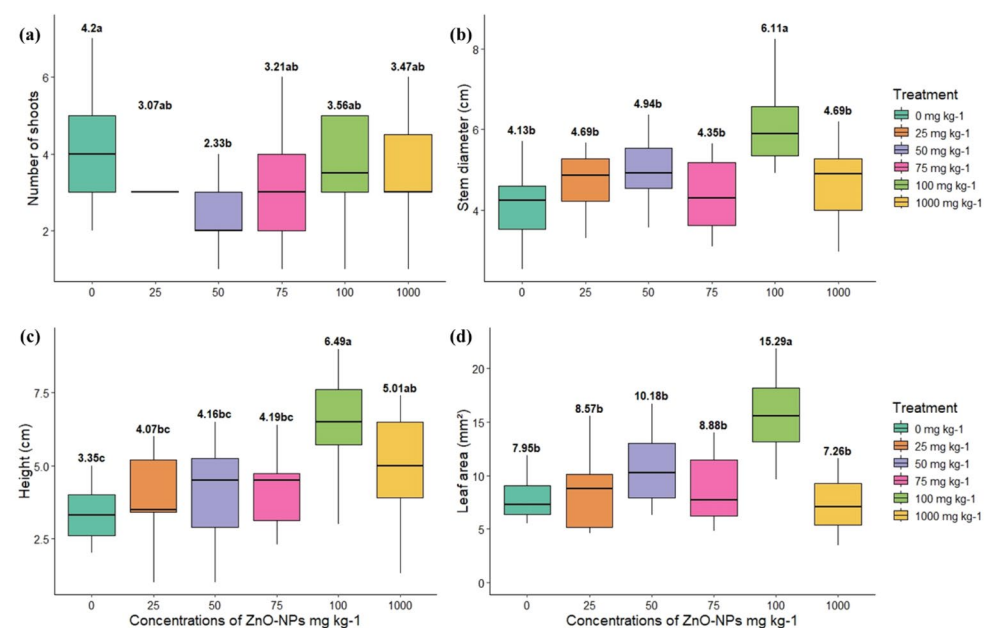


Fig. 5 Characteristics assessed at 60 days of hardening off *Paliavana sericiflora* as a function of the concentrations of ZnO-NPs used in the microcontainer (0, 25, 50, 75, 100 and 1000 mg kg⁻¹). (a) Number of shoots, (b) stem diameter (cm), (c) height (cm), (d) leaf area (mm²). Averages followed by the same letter do not differ significantly according to Tukey's test. Bars represent the standard error of the sample



nutrients, as well as releasing the ZnO-NPs, favouring plant development.

Although the control treatment showed a numerically higher number of shoots (Fig. 5a), this response was not associated with superior overall plant development. Furthermore, a greater number of shoots does not necessarily indicate greater plant vigour, since these plants exhibited reduced growth in height (Fig. 5c). Stem diameter and plant height (Fig. 5b and c) showed similar response patterns, with the treatment containing 100 mg kg⁻¹ ZnO-NPs promoting the greatest stem diameter at ground level and the highest plant height among the evaluated treatments. These results suggest that the application of ZnO-NPs at

appropriate concentrations favored biomass allocation and overall plant development.

Leaf size is crucial for the survival of *Paliavana sericiflora* of hardening off. Larger leaf areas, after hardening off, maximize the production of photo assimilates, favouring the plant's growth and development. In line with the results for height and diameter, the area the leaves (Fig. 5b–d) showed higher values in the treatment with 100 mg kg⁻¹, showing the positive effect of this concentration of ZnO-NPs on the leaf development of the species.

The photosynthetic pigments responsible for converting light into chemical energy showed different responses to the concentrations of ZnO-NPs tested. The treatment with

1000 mg kg⁻¹ resulted in the highest content of chlorophyll *a*, carotenoids and total chlorophyll (Fig. 6a, c and d), not differing from 50 mg kg⁻¹ for total chlorophyll and carotenoids, suggesting that these treatments present the best establishment responses. For chlorophyll *b*, the concentration of 50 mg kg⁻¹ provided the highest content, with the other pigments also standing out. These results indicate that the application of ZnO-NPs in appropriate concentrations can optimize the content of photosynthetic pigments in *Paliavana sericiflora*, favouring photosynthesis and plant development.

Another relevant point is that the increase in leaf area was not associated with a corresponding rise in photosynthetic pigments. The treatment with 100 mg kg⁻¹, which exhibited the largest leaf area, nevertheless showed low levels of all evaluated pigments. Even so, this treatment also presented the highest dry mass accumulation, suggesting a greater efficiency in the use of available pigments for biomass production.

Principal component analysis (PCA) (Fig. 7) explained more than 50% of the variability in the data. In Dimension 1, the variables total dry matter, shoot dry matter and height captured the greatest variability in the data. In Dimension 2, photosynthetic pigments covered the variability of the residual data. The treatment containing 100 mg kg⁻¹ ZnO-NPs showed greater association with morphological and biomass related variables. These responses may reflect not only the influence of ZnO-NPs but also possible interactions involving nutrient uptake, nutrient balance, and physiological processes associated with plant development. Therefore, the PCA results suggest relationships between treatments and plant responses, indicating that the observed effects are likely associated with a combination of factors. The

treatments with 25, 50 and 100 mg kg⁻¹, on the other hand, showed a higher correlation with photosynthetic pigments. Vigour showed a high correlation with the treatment without added zinc, indicating that higher values for this variable represent worse conditions for the plants.

The use of 75 mg kg⁻¹ showed a high correlation for the number of leaves and number of shoots and differed from the other ZnO-NPs concentrations. These results suggest that the application of ZnO-NPs can influence the morphological development, physiology, and vigour of *Paliavana sericiflora*, optimizing the growth and productivity of the species.

The use of zinc oxide nanoparticles (ZnO-NPs) in the microtubes significantly influenced the absorption pattern of macro- and micronutrients in *Paliavana sericiflora* plants (Table 1). It was observed that the concentration of 100 mg kg⁻¹ promoted the highest accumulation of N, P, Ca, Mg, and S, indicating a beneficial effect of ZnO-NPs on the assimilation and translocation of essential nutrients. On the other hand, potassium (K) showed its highest content at 1000 mg kg⁻¹, suggesting that the absorption of this element follows a distinct dynamic, possibly associated with increased ion flux and the regulation of membrane channels.

Among the micronutrients, the concentrations of Fe and Mn reached their maximum values at 100 mg kg⁻¹, while Cu exhibited a similar trend, highlighting the role of ZnO-NPs in modulating the absorption and transport of essential metals. Nevertheless, foliar zinc contents did not increase proportionally with the applied concentrations and did not directly correspond to improved plant growth. Although control plants showed relatively higher Zn concentrations, superior development was observed in plants treated with 100 mg kg⁻¹ ZnO-NPs, which also exhibited increased

Fig. 6 Characteristics evaluated at 60 days of hardening off *Paliavana sericiflora* as a function of the concentrations of ZnO-NPs used in the microcontainer (0, 25, 50, 75, 100 and 1000 mg kg⁻¹). (a) Chlorophyll *a* (µm mg⁻¹), (b) chlorophyll *b* (µm mg⁻¹), (c) total chlorophyll (µm mg⁻¹) and (d) carotenoids (µm mg⁻¹). Averages followed by the same letter do not differ significantly according to Tukey's test. Bars represent the standard error of the sample

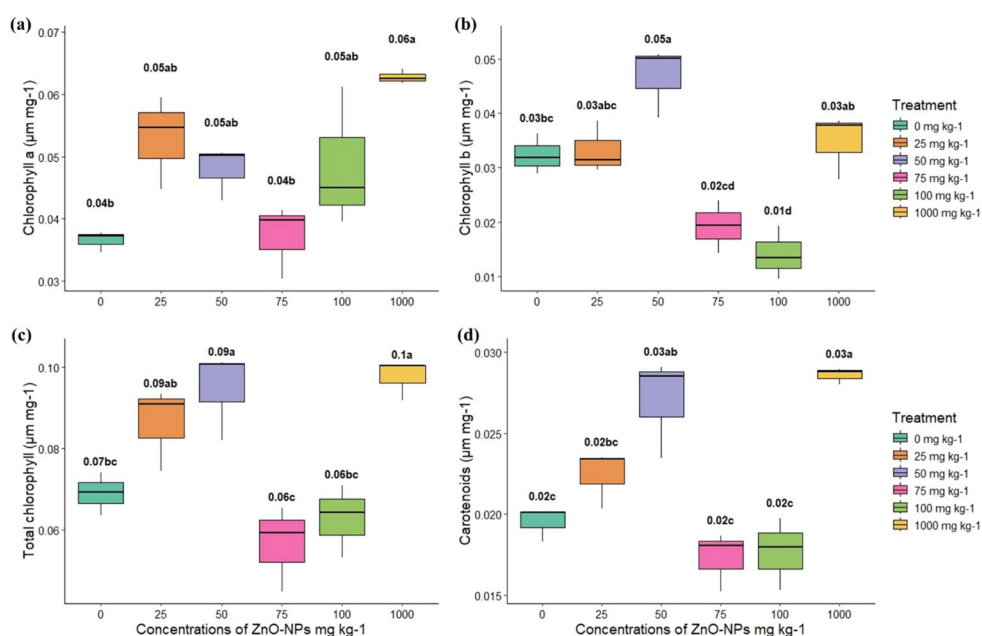
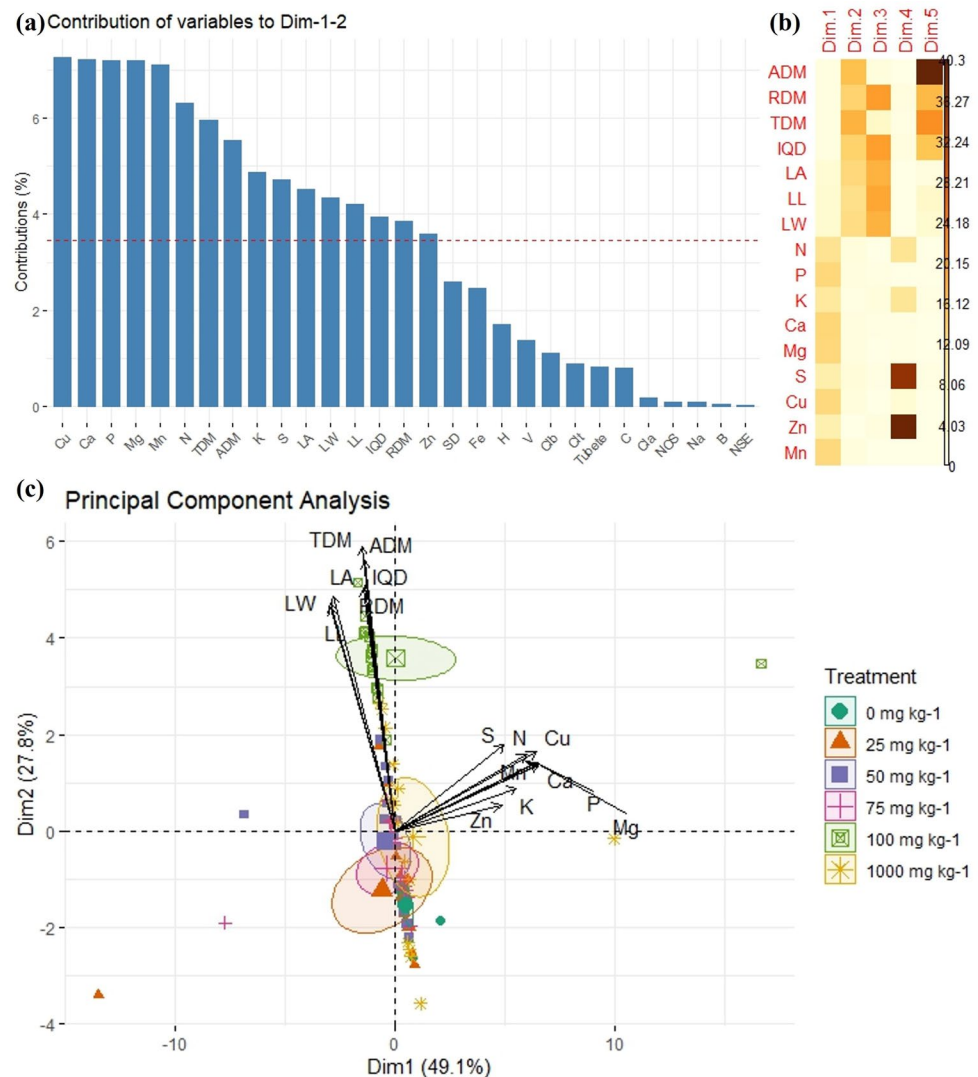


Fig. 7 Characteristics of *Paliavana sericiflora* seedlings after 60 days of hardening off in micro-container with different concentrations of ZNO-NPs, (T1: 0; T2: 25; T3: 50; T4: 75; T5: 100; and T6: 1000 mg kg⁻¹). (a) Contribution of variables to Dim1-2; (b) Correlation of variables in Dim1-5; (c) Principal component analysis. Dim1=principal component 1 (PC1), Dim2=principal component 2 (PC2), Dim3=principal component 3 (PC3), Dim4=principal component 4 (PC4), Dim5=principal component 5 (PC5), ADM=aerial dry matter, C = carotenoids, Cla = chlorophyll *a*, Clb = chlorophyll *b*, Clt - total chlorophyll, H = height, IQD=Dickson quality index, LA=leaf area, LL=leaf width, LW=leaf length, NOS = number of shoots, NSE = number of leaves, RDM=root dry matter, SD = stem diameter, TDM=total dry matter, Tubete = microcontainer, V = vigour, B = boron, Ca=calcium, Cu=copper, K = potassium, Mg=magnesium, Mn=manganese, N = nitrogen, Na = sodium, P = phosphorus, S=sulfur, Zn=zinc



levels of several essential nutrients. These findings suggest that plant performance was more strongly associated with nutritional balance and nutrient-use efficiency than with foliar zinc concentration alone.

In addition, a marked increase in Na was observed in plants exposed to 100 mg kg⁻¹, which may indicate an osmotic adjustment associated with the presence of nanoparticles. Conversely, higher concentration (1000 mg kg⁻¹) did not result in significant increases in mineral contents, possibly due to saturation effects or physiological limitations. Thus, moderate concentrations of ZnO-NPs appear to favour mineral nutrition and the early growth of *Paliavana sericiflora*, whereas excessive concentration tend to reduce the efficiency of nutrient absorption and utilization.

Qualitative analysis of the root anatomy revealed good formation and connection of the adventitious roots with the vascular cambium in all treatments (Fig. 8a arrows), indicating efficient root development. In addition, continuous

cell formation was observed to have a good connection with the vascular exchange.

The leaf mesophyll showed differences between the treatments, with greater structural complexity in the treatments with ZnO-NPs nanoparticles compared to the treatment without ZnO-NPs (Fig. 8b and c).

Discussion

Determining the ideal dose of zinc nanoparticles (ZnO-NPs) varies according to the plant species under study, since plant responses to ZnO-NPs may differ due to variations in physiological characteristics and nutritional requirements. Therefore, the responses observed in *Paliavana sericiflora* should be interpreted considering species-specific traits. Studies show that the appropriate dose can promote photosynthesis, enzyme activity and the absorption of other nutrients

Table 1 Concentration of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Na, Cu, Zn, Fe, Mn, and B) in *Paliavana sericiflora* leaves during acclimatization stage under different concentrations of zinc oxide nanoparticles used in the microcontainers

Treatments (mg kg ⁻¹)	(g kg ⁻¹)					(mg kg ⁻¹)						
	N	P	K	Ca	Mg	S	Na	Cu	Zn	Fe	Mn	B
0	16.2e	3.30c	15.90c	20.20c	12.20c	1.30d	727.5d	8.7c	18.2a	275.0f	70.1c	42.3a
25	14.2f	2.63f	15.30d	15.97f	9.21f	1.21f	844.8b	7.2f	12.2f	287.7e	46.8f	37.6b
50	17.0c	2.71e	14.77e	17.21e	10.00e	1.55b	770.6c	8.2d	12.9e	669.2b	56.3e	31.9e
75	16.5d	2.77d	14.19f	17.35d	10.90d	1.29e	675.3e	7.9e	13.5d	371.4d	61.1d	29.7f
100	19.9a	4.27a	16.64b	25.10a	15.25a	1.73a	871.4a	10.6a	15.9c	796.4a	86.6a	34.1d
1000	19.4b	3.69b	16.86a	23.67b	13.96b	1.48c	664.2f	9.3b	16.3b	411.2c	74.2b	34.3c

In the column, means followed by the same letter do not differ from each other at the 5% probability level according to Tukey's test

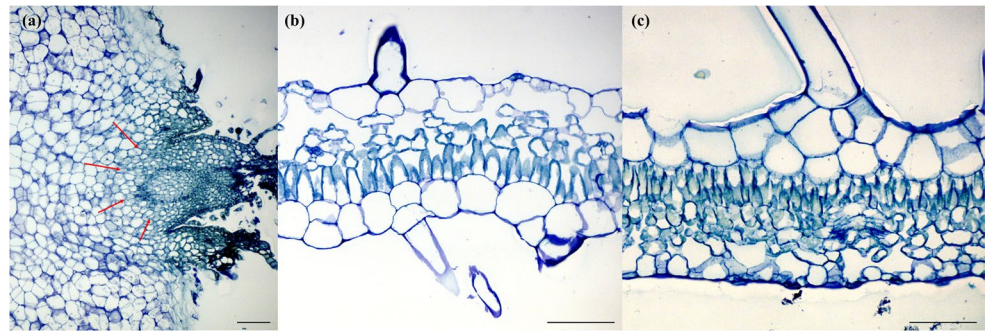
essential for plant growth (Lv et al. 2022). This optimization of nutrition can be reflected in the vigour of the seedlings, as evidenced in a study where the highest concentration within a certain limit of ZnO-NPs resulted in greater plant vigour (Burman et al. 2013). However, it is crucial to consider that excessive use of ZnO-NPs can lead to toxicity. A study with chickpea seedlings showed that the application of 1.5 mg L⁻¹ of ZnO-NPs resulted in a significant increase in the root system, while the application of 10 mg L⁻¹ significantly reduced root growth (Burman et al. 2013). The same study showed toxic effects at even higher concentrations.

Similar responses have been reported in other micro-propagated species exposed to ZnO-NPs. Studies involving *Eriobotrya japonica* and *Coleus forskohlii* showed that nanoparticle application promoted improvements in plant development, morphophysiological characteristics, and acclimatization-related responses. These findings reinforce the potential of ZnO-NPs as a strategy for optimizing growth responses in micropropagated plants, although the magnitude and nature of these effects may vary according to species-specific physiological and nutritional characteristics (Ghareb et al. 2025; Manokari et al. 2026).

The increase in plant dry matter varies according to the dose of ZnO-NPs applied. Studies show that lower concentration generally result in a greater increase in the dry matter content of the aerial part and total plant (Saleem et al. 2023). In a study with nine different crops (wheat, maize, radish, beans, lettuce, tomatoes, peas, cucumbers and beet), the application of ZnO-NPs in two types of soil, at concentrations ranging from 20 to 200 mg kg⁻¹, resulted in the greatest increase in crop biomass and the yield of some grains (García-Gómez et al. 2018; Patil et al. 2024). On the other hand, higher concentrations, between 225 and 1000 mg kg⁻¹, reduced the increase and yield of some crops.

Specific studies with the species in question have shown that the application of 100 mg kg⁻¹ of ZnO-NPs gave the best results in terms of growth and productivity, while treatment with higher concentration resulted in a reduction in yield. This reduction may be related to phytotoxicity, a harmful effect caused by excess ZnO-NPs in the plant. The positive effect of zinc nanoparticles (ZnO-NPs) on plant growth has been corroborated in several studies. A notable example is the case of guinea grass (*Panicum maximum*), where the application of 100 mg kg⁻¹ of ZnO-NPs resulted in a significant increase in the length of the aerial part, leaf area and number of leaves, compared to the dose of 500 mg kg⁻¹ which already showed a reduction compared to the control, suggesting possible phytotoxicity (Gomes et al. 2023). In addition, the 100 mg kg⁻¹ dose stood out in relation to the 500 mg kg⁻¹ dose for variables such as stalk thickness, plant length, productivity, total levels of carbohydrates and proteins, and photosynthesizing pigments.

Fig. 8 Cross-sections of the stem (a) and mesophyll (b without ZnO-NPs and c with ZnO-NPs), observed during the acclimatization of *Paliavana sericiflora*. Arrows show the connection between the root and the stem. Bars: 1 μm



This complex structure of the mesophyll, seen in, may contribute to better responses in the hardening off of the seedlings, as it increases the photosynthetic leaf area and the efficiency of light absorption. These results suggest that the application of ZnO-NPs in adequate concentrations can influence the growth and development of *Paliavana sericiflora*, optimizing the plant's characteristics for propagation and field management purposes. Application of zinc nanoparticles (ZnO-NPs) in low concentrations proves to be more efficient than the use of zinc sulphate (ZnSO_4 in conventional use). Studies show that it is possible to achieve maximum productivity or similar effects with the application of ZnO-NPs in 70% lower quantities compared to conventional inputs (Du et al. 2019; Sun et al. 2020b).

Several studies have shown that the application of zinc nanoparticles (ZnO-NPs NPs) induces an increase in the content of chlorophylls and carotenoids in plants, resulting in improvements in their growth species (Faizan et al. 2018; Kolenčik et al. 2020; Saleem et al. 2023). In a study with soybean plants, the application of 10, 100 and 1000 mg L^{-1} of ZnO-NPs resulted in a significant increase in the production of chlorophylls a and b, with an increase in the ratio between them of 21, 29 and 23%, respectively (Leopold et al. 2022). It is important to note that this study differs from the one presented, as the concentrations (75 and 100 mg L^{-1}) of ZnO-NPs showed the lowest chlorophyll levels compared to the other treatments.

Carotenoids act in photoprotection of plant cells, fighting oxidative damage and acting as adjuncts in light capture. In stressful situations, as demonstrated in soybean plants that showed signs of stress when ZnO-NPs were applied (Leopold et al. 2022), carotenoids act as antioxidant. This intense antioxidant function may explain the paradox observed in *Paliavana* seedlings, where the treatments with the highest carotenoid content showed lower increases in mass and lower vigour. It is believed that the excessive allocation of resources to carotenoid production to the detriment of vegetative growth may be linked to some kind of stress.

The application of nanoparticles (NPs) to supply nutrients to plants is gaining prominence. In addition to zinc, ZnO-NPs (zinc oxide) NPs can stimulate the absorption of

other essential nutrients, such as iron and manganese (Sun et al. 2020a; Lv et al. 2022). When doped with copper, ZnO-NPs can also have a nematicide, antifungal (Tryfon et al. 2022) and even antibacterial action (Lv et al. 2022; Mirza et al. 2023). In seedling production, it is crucial to pay attention to the correct concentration of NPs to avoid phytotoxicity effects, which can result in low plant vigour. The incorporation of zinc oxide nanoparticles (ZnO-NPs) into the microtubes significantly altered the nutritional composition of *Paliavana sericiflora*. The concentration of 100 mg kg^{-1} promoted the highest levels of N, P, Ca, Mg, and S, elements directly related to protein synthesis, energy metabolism, and the structural integrity of cell membranes. This increase may be associated with the activation of redox enzymes and the stimulation of root growth, which enhance the absorptive surface area and the efficiency of nutrient assimilation (Lv et al. 2022; Ntarelli et al. 2021).

The increase in N and P contents reflects the role of ZnO-NPs in nitrogen fixation and phosphate metabolism, processes essential for ATP production and structural protein formation, thereby explaining the greater biomass accumulation. Similarly, the elevation of Ca and Mg indicates improved cell wall integrity and higher photosynthetic efficiency, as observed in woody species micropropagated and treated with metallic nanoparticles (Faizan et al. 2022).

Regarding the micronutrients, the 100 mg kg^{-1} dose increased the levels of Fe and Mn, elements essential for water photolysis and electron transport in photosynthesis. These results corroborate the findings of Sun et al. (2020a), who highlighted the synergistic role of ZnO-NPs in modulating the metabolism of essential metals. On the other hand, foliar zinc content did not show a proportional increase, indicating the presence of homeostatic and exclusion mechanisms such as vacuolates compartmentalization and cell wall deposition that prevent Zn toxicity (Kolenčik et al. 2020). At the highest concentration (1000 mg kg^{-1}), reductions in P, Ca, and Mg were observed, suggesting saturation or ionic competition and possible induction of oxidative stress (Leopold et al. 2022). Overall, moderate concentrations of ZnO-NPs improved the nutritional status of *Paliavana sericiflora*, promoting greater vigour and

field establishment potential. The controlled application of these nanoparticles in biodegradable containers emerges as a sustainable and technically feasible strategy to optimize growth and nutrient-use efficiency in ecological restoration programs.

In conclusion, this research demonstrated the successful synthesis and incorporation of zinc oxide nanoparticles (ZnO-NPs) into filaments to produce microcontainers. The use of this approach supported the acclimatization process, resulting in 100% plant survival under the conditions evaluated. The concentration of 100 mg kg⁻¹ ZnO-NPs was associated with greater plant vigour, biomass accumulation, and improved growth characteristics, including plant height, stem diameter, and leaf area, together with increased levels of N, P, Ca, Mg, and S. In contrast, the concentration of 1000 mg kg⁻¹ may have negatively affected plant development, suggesting possible phytotoxic effects at higher concentrations. The results suggest that ZnO-NPs-enriched microcontainers positively influenced plant growth and acclimatization responses, likely through effects on nutrient dynamics and plant physiological processes. Therefore, ZnO-NPs-enriched microcontainers represent a promising strategy for improving acclimatization and early plant development in *Paliavana sericiflora*. Further studies are required to better understand the physiological mechanisms involved and the performance of this technology under field conditions.

Acknowledgements We thank the National Council for Scientific and Technological Development, Brazil (“Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq”), Coordination for Improvement of Higher Education Personnel, Brazil (“Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES – Código de Financiamento 001”), and Foundation for Research of the State of Minas Gerais, Brazil (“Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG, Rede Mineira de Biotecnologia em Multiplicação e Clonagem de Plantas”). We also thank the Research and Innovation Unit in Ferruginous Rupestrine Grassland (‘Unidade de Pesquisa e Inovação em Campos Rupestres Ferruginosos’), and GERDAU Açominas S.A. (‘Convênio DICON/UFLA: 008/2020’).

Authors contributions DML conducting experiments, statistical analysis, writing, and discussion. TAS conducting experiments, writing, and discussion. MVFD carrying out anatomical analyses. LFP carrying out anatomical analyses. JCU production of zinc oxide nanoparticles. ARSN assistance in the experiment. GEB supervisor, was responsible for the obtention of funds and the administration of project, review, and discussion. All authors revised the manuscript and approved the final version.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). Convênio DICON/UFLA 008/2020; CNPq 302505/2022-9; FAPEMIG RED-00225-23; Acordo UFLA 006/2025.

Data availability The original data presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare that they have no conflict of interest.

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