

The Interactive Effects of Culture Vessels and Paclobutrazol on in Vitro Rooting and Acclimatization of ‘Grand Nain’ Banana: A Potential Study for Commercial Propagation

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Abstract. *Musa* spp. is an essential global food crop, yet its large-scale production is often hindered by the physiological stress that occurs during the transition from in vitro to ex vitro conditions. We sought to overcome this critical bottleneck by developing an integrated, cost-effective micropropagation protocol. The effects of paclobutrazol (PBZ) concentrations [0.0 (control), 0.5, and 1.0 mg·L⁻¹] and various culture vessel types were evaluated regarding in vitro root development and subsequent greenhouse acclimatization of ‘Grand Nain’ bananas. Our results demonstrate that the use of round Magenta vessels without PBZ (0.0 mg·L⁻¹) provided the optimal environment. This treatment enhanced transplant quality significantly, including height (18.6 cm), stem diameter (5.2 mm), and leaf number ($n = 6.4$), while achieving a superior photosynthetic efficiency (variable fluorescence/maximum fluorescence = 0.782) and a 100% survival rate. The novelty of this research lies in the combined analysis of vessel-induced micro-environmental factors and PBZ-mediated growth regulation, an interaction previously undocumented for this cultivar. This protocol offers a reliable and standardized method for the mass production of high-quality banana transplants suitable for both local cultivation and international export.

Introduction

Banana (*Musa* spp.) is a member of the Musaceae family and originated in Southeast Asia. It is widely cultivated in tropical and subtropical countries throughout the world and is the fourth most important global stable food crop after rice, wheat, and maize (Talla et al. 2022; Uma et al. 2023a). It is one of the world’s four major produced and consumed fruit, along with oranges, grapes, and apples. Bananas supply more than 25% of the world’s daily carbohydrate needs (Omari et al. 2024a). They provide food, livelihood, employment, and income security in its main production

areas for a significant population. Bananas are very popular globally for their flavor; impressive, high nutritional value; therapeutic benefits; and economic potential (Saraswathi et al. 2025). In addition to its delicious fruit, the agricultural wastes of banana plants such as rhizomes, leaves, peels, and pseudostem are highly used in various sustainable applications as food, medicine, bioethanol stock, biofertilizers, and fiber, and in textile industries (Akatwijuka et al. 2024). However, the expansion of banana-growing areas and commercial production is hampered by the lack of quality clonal planting materials for the desired varieties (Saraswathi et al. 2024). The ever-increasing demand for planting materials

and the need for greater productivity to feed the growing population have led to the development of advanced, cost-efficient and sustainable technologies for the production of high-quality transplants for intensive agriculture. One of these technologies is banana tissue culture (Ngullie and Deb 2024; Omari et al. 2024b; Uma et al. 2023b).

As a vegetative propagated fruit, ‘Grand Nain’ banana (*Musa paradisiaca* AAA) has traditionally used suckers for cultivation. However, this method has its limitations, including transmission of diseases to the new fields by soil-borne pathogens, low productivity, a very slow rate of sucker multiplication, and poor preservation of the parent plant’s genetic material (Justine et al. 2022). Moreover, this traditional method of propagation is season dependent, time-consuming, and produces nonuniform “off-type” plants (Subrahmanyeswari et al. 2022). Hence, the production of tissue-cultured planting material for banana cultivars is a crucial issue. In vitro tissue culture techniques have been established commercially for successful large-scale propagation for a variety of high-demand banana cultivars. It is one of the fruits that has been multiplied extensively through micropropagation to produce millions of true-to-type, genetically uniform, disease-free, superior planting materials year-round regardless of seasonal variations (Saraswathi et al. 2025; Shehu et al. 2026). This has improved crop performance in the field and profitability (Mohanta et al. 2026). However, there is still a dire need to improve the micropropagation protocols for specific high-value cultivars and economically important plant species for disease free and large-scale availability of quality planting materials (Talla et al. 2022). In addition, micropropagated banana plantlets are grown in conditions characterized by high relative humidity and limited gaseous exchange between the internal atmosphere and the surrounding environment. These conditions usually induce physiological imbalances and result in the accumulation of ethylene and reactive oxygen species (ROS). This leads to oxidative stress, which makes the banana plants ultimately unable to adapt when transferred to ex vitro conditions such as greenhouse or field environments and leads to high rates of mortality during acclimatization (Hazarika 2006a). Therefore, an efficient photosynthetic system, improved antioxidant capacity, vigorous vegetative growth, and well-developed roots of in vitro raised plants are crucial for successful acclimatization because they influence plant performance and survival directly under field conditions (Talla et al. 2022).

Acclimatization is the most crucial stage in the micropropagation protocol for banana plants, which results in banana transplants, and its success determines the survival rate, vigor, and field performance of the transplants (Saraswathi et al. 2025). However, during acclimatization, in vitro plantlets still exhibit reduced functional leaves, low nutritional reserves, limited energy, very fragile roots, and a poorly developed cuticle, all of which may cause a decreased survival rate under ex vitro conditions (Sanó et al. 2022). Chemical fertilizers (Bai et al. 2025), bio-organic fertilizers (Liu et al. 2024), plant growth-promoting microorganisms (Sanó et al. 2022;

Sun et al. 2025), and plant growth retardants (Shalaby et al. 2022) have been used to improve the photosynthetic apparatus and enhance root growth. The consequent development of plants has produced high-quality seedlings with a high survival rate. Paclobutrazol (PBZ), a growth retardant, is a synthetic inhibitor that restricts gibberellin biosynthesis by inhibiting kaurene oxidase and blocking the oxidation of ent-kaurene to ent-kaurenoic acid (Abdalla et al. 2021; Shahzad et al. 2023). Thus, it can decrease endogenous gibberellic acid contents, reduce cell elongation, increase stem diameter, decrease plant length, increase chlorophyll content, increase the number of leaves, enhance photosynthesis (mainly if it added to the shoot multiplication medium) (Zhang et al. 2025), and stimulate root development, resulting in thick roots and increased root number, quality, and survival rate of in vitro rooted plantlets (Wen et al. 2013).

The physiological modifications induced by PBZ result in improving acclimatization success and micropropagation efficiency directly through morphological alterations such as compact shoots, thicker and shorter leaves, and an increased thickness of the leaf wax layer, reducing the overall shoot surface area available for transpirational water loss, which decreases water loss during transplanting (Li et al. 2009; Plaza-Wüthrich et al. 2016; Wen et al. 2013). It also causes anatomic shifts (enhancing the root architecture and creating a favorable shoot-to-root ratio necessary for ex

vitro survival) and alters biochemical processes (hormonal modulation through the increase of endogenous abscisic acid and cytokinins, which actively induce rapid stomatal closure when atmospheric humidity drops suddenly). Osmo-protection is provided by the accumulation of cellular protectants such as soluble carbohydrates and proline (Nagar et al. 2021), preventing excessive electrolytes or ion leakage during acute transplant stress. Moreover, PBZ upregulates protective antioxidant enzyme activities, suppressing lipid peroxidation and keeping the plant's structural proteins functional while it adapts to its new environment under ex vitro conditions (Nagar et al. 2021).

The concern regarding how PBZ-induced antioxidant enzyme activity upregulation translates into improved micropropagation efficiency and acclimatization success highlights a critical issue in plant tissue culture. This mechanism lies in mitigating transplant-induced oxidative shock through the activation of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO) enzymes that maintain cell turgor and protect chloroplasts, develop photosynthetic apparatuses, and stabilize cellular membranes, thereby enhancing survival and growth quality (Chakrabarty et al. 2007; Hazarika 2006).

Although PBZ can be absorbed via stems or leaves, its primary and most efficient physiological route of uptake is through the root system (Percival and Salim AlBalushi 2007). The bioavailability of PBZ is highly dependent on the medium matrix. PBZ adsorbs strongly to organic matter and clay minerals in soil or solid media (Vaz et al. 2015). It is a lipophilic, moderately hydrophobic triazole compound. It enters the root through passive apoplastic or symplastic pathways, across the lipid bilayers of roots and root hairs (Soumya et al., 2017). The rate of PBZ accumulation at its active site (the subapical meristem) is directly proportional to the volume and velocity of the transpirational stream (Maheshwari et al. 2022). Environmental conditions that promote open stomata and a high vapor pressure deficit accelerate the transpirational stream, effectively facilitating faster movement of PBZ from the rooting medium up to the shoot tip. Vessel gas exchange characteristics—specifically the movement of air, water vapor, and dissolved gases—alter PBZ translocation and distribution directly. High gas exchange and low stomatal resistance allow rapid xylem translocation of PBZ upward into the stem (Percival and Salim AlBalushi 2007).

The physical and chemical environments of culture vessels strongly influence the growth rate as well as other morphological and physiological features of in vitro cultured plantlets. Optimizing the gas exchange rate of culture vessels could prevent or reverse physiological and morphological disorders, hence increasing the survival rate and quality of transplants during ex vitro acclimatization (Mohamed et al. 2023).

We aimed to enhance photosynthetic efficiency and antioxidant enzymatic status while improving vegetative growth and rooting capacity of in vitro propagated 'Grand Nain' banana plantlets by modifying the composition

of the rooting medium [Murashige and Skoog (1962) (MS) medium fortified with 1.0 mg·L⁻¹ indole-3-butyric acid (IBA)] by adding different concentrations of PBZ.

We hypothesized that the interaction between culture vessel-induced microenvironmental characteristics and PBZ concentrations would regulate the morphological and physiological adaptation significantly of 'Grand Nain' banana. Specifically, we expected that optimized vessel gas exchange conditions, combined with tailored PBZ-mediated growth regulation, would improve in vitro root development and ex vitro survival. Furthermore, we hypothesized that the specific combination of these factors would stabilize the photosynthetic apparatus [the variable fluorescence (Fv)-to maximum fluorescence (Fm) ratio] and mitigate transplant-induced stress, thereby defining an optimal micropropagation protocol for high-quality mass banana production. Furthermore, our aims included examining the effect of different types of culture vessels on the subsequent ex vitro acclimatization rate and quality characteristics of banana transplants. By optimizing these factors, our goal was to produce large-scale, high-quality planting material that would enable intensive agricultural production of this highly important banana cultivar.

Materials and Methods

This study was carried out at the Physiology and Breeding of Horticultural Crops Laboratory, Horticulture Department, Faculty of Agriculture, Kafrelsheikh University, Egypt.

Plant material. Preestablished in vitro shoot cultures of banana (*Musa paradisiaca* L. cv. Grand Nain) were cultured on MS medium containing 3% sucrose and supplemented with 3.0 mg·L⁻¹ 6-benzylamino purine and 1.0 mg·L⁻¹ kinetin. The culture medium was solidified with 0.2% Gelrite (Duchefa Biochemie Co, Haarlem, Netherlands). The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C and 118 kPa for 15 min. The cultures were incubated for 4 weeks at 25.0 ± 1.0 °C under a 16-/8-h photoperiod provided by cool-white fluorescent tubes at a 25-μmol·m⁻²·s⁻¹ photosynthetic photon flux density (PPFD) for shoot multiplication. The microshoots were used as plant material in the following experiments to examine the in vitro rooting capacity of banana shoots and ex vitro acclimatization of plantlets in response to different treatments of PBZ and different types of culture vessels.

In vitro rooting of banana microshoots. In vitro shoots of 'Grand Nain' banana were cultured on MS medium solidified with 0.7% agar containing 3% sucrose and supplemented with 1.0 mg·L⁻¹ IBA. The effect of three concentrations of PBZ (Titan Biotech LTD, Rajasthan, India) were investigated at 0.0, 0.5, and 1.0 mg·L⁻¹, where 0.0 mg·L⁻¹ PBZ served as the control. These concentrations of PBZ were selected based on our previous study (Mohamed et al. 2023) on *Gypsophila paniculata* L. Different culture vessels, such as glass jars with a 350-mL capacity; round Magenta boxes (350 mL; Magenta LLC, Chicago, IL, USA), and rectangular Magenta boxes (575 mL) with

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During the preparation of this work, we did not use any AI program, even to generate initial outlines and draft sections. We reviewed and edited the content without help from AI programs to ensure accuracy and clarity, and take full responsibility for the content of this publication.

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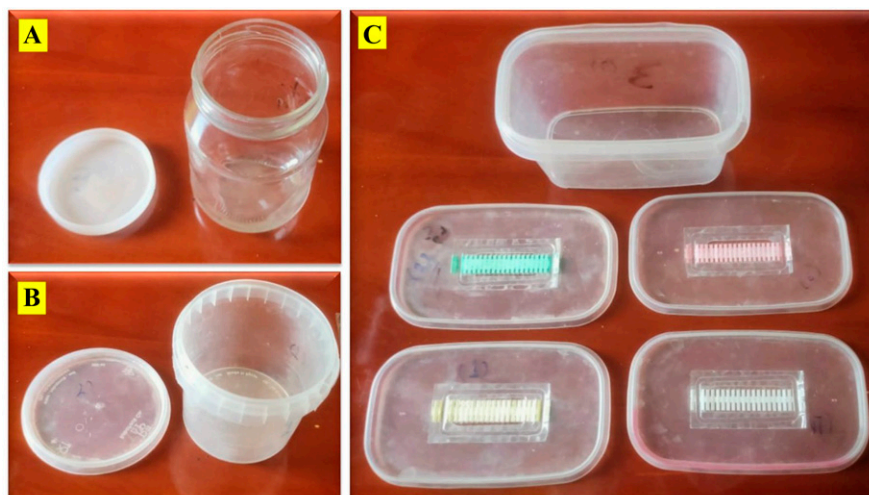


Fig. 1. The culture vessels used in our study included glass jars (A), round Magenta vessels (B) (Magenta LLC, Chicago, IL, USA), and rectangular Magenta vessels (C) with green (top left), rose (top right), yellow (bottom left), and white (bottom right) filters.

four different filters (Sac O₂, Deinze, Belgium) were examined for root induction as well as for improving the quality of in vitro-raised plantlets and, consequently, transplants. The filters had four different gas exchange rates [white, 7.44; yellow, 9.14; rose, 10.83; and green, 62.87 gram equivalents (GE)/d] (Figs. 1 and 2). The culture vessels contained 75, 75, and 150 mL medium for glass jars, round Magenta boxes, and rectangular Magenta boxes, respectively. The cultures were kept at 25.0 ± 1.0 °C in a culture room at a 16-/8-h photoperiod at a 60 μmol·m⁻²·s⁻¹ PPFD. After 4 weeks of culture, plantlet length (measured in centimeters), stem diameter (measured in millimeters), number of leaves per plantlet, root length (measured in centimeters), number of roots per plantlet,

plantlet fresh weight (measured in grams), and chlorophyll content of shoots [using a soil plant analysis development (SPAD) chlorophyll meter (SPAD 502 Plus; Japan)] were recorded.

Analysis of antioxidant enzyme activity. To assess the activity of the antioxidant enzymes, 0.5 g of fully expanded leaves from in vitro rooted plantlets were homogenized using a precooled mortar and pestle under liquid nitrogen with 1.5 mL of extraction buffer. The mixture was filtered and centrifuged at 22,000 rpm for 20 min at 4 °C. The supernatant, which was recentrifuged at 22,000 rpm for 20 min at 4 °C, was used for detection of antioxidants. CAT (EC 1.11.1.6) activity was measured by the consumption of H₂O₂ at 240 nm (Aebi 1984). One unit of CAT activity

was defined as a 0.01 decrease in absorbance at 240 nm·mg⁻¹ protein·min⁻¹. POX (EC 1.11.1.7) activity was determined according to the procedure proposed by Hammerschmidt et al. (1982). Enzyme activity of POX was expressed as an increase in absorbance per minute per gram fresh weight. PPO (EC 1.10.3.1) activity was determined according to the method described by Malik and Singh (1980). PPO enzyme activity was expressed as an increase in absorbance per minute per gram fresh weight.

Acclimatization of 'Grand Nain' banana in vitro plantlets. 'Grand Nain' banana in vitro plantlets (9–10 cm) at the five- to six-leaf stage were collected from culture vessels, washed directly with running tap water, then dipped for 2 min in a fungicide solution [1.0 g·L⁻¹ double 56% wettable powder that contains 16% w/w 5-methylisoxazol-3-ol and 40% w/w dimethyl 4,4-(o-phenylene) bis (3-thioallophanate); (Starchem Co, El-Giza, Egypt)]. The in vitro plantlets were transplanted in a greenhouse into 5-cm black plastic pots filled with growing medium consisting of peatmoss and vermiculite (2:1). The in vitro plantlets were covered with a clear plastic lid (thickness, 50 μm) in a small tunnel. The clear plastic lid was partially removed after 20 d, and then removed completely after 25 d. The in vitro plantlets were grown under a 50% shade net and were fertilized with a compound fertilizer (19N–19P₂O₅–19K₂O; Rosasol; Rosier, Moustier, Belgium) at 0.5 g·L⁻¹ once after 30 d as 20 mL fertilizer solution per plant. The initial environmental conditions in the plastic greenhouse were 30 ± 2 °C and 70% to 80% relative humidity. This was adjusted in the small tunnel with a mist system to 28 ± 2 °C and 90% to 100% relative humidity in the first 20 d of the experiment, then was decreased gradually to 70% to 80% relative humidity thereafter. The survival percentage, transplant height (measured in centimeters), number of leaves per transplant, stem diameter (measured in millimeters), leaf area (measured in square centimeters), chlorophyll content, and photosynthesis efficiency (Fv/Fm) were measured after 40 d of acclimatization. The equation used to measure photosynthetic efficiency was

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m},$$

where F_m is the maximum chlorophyll fluorescence at >3500 mol·m⁻²·s⁻¹ and F₀ is the minimum chlorophyll fluorescence at 0 photosynthetic photon flux (PDF) observed in a dark-adapted state.

Experimental design and statistical analyses.

The in vitro rooting and ex vitro acclimatization experiments were organized in a completely randomized design. They were factorial experiments with two factors. The first factor was PBZ added to the culture medium in three concentrations; the second factor was the type of culture vessel (six types: glass jar, round Magenta vessel, rectangular Magenta vessels with white, yellow, rose, and green filters). For the in vitro rooting experiment, there were five replicates per treatment. Each replicate was a single culture of 10 individual shoots, giving a total of 50 shoots per treatment. For the

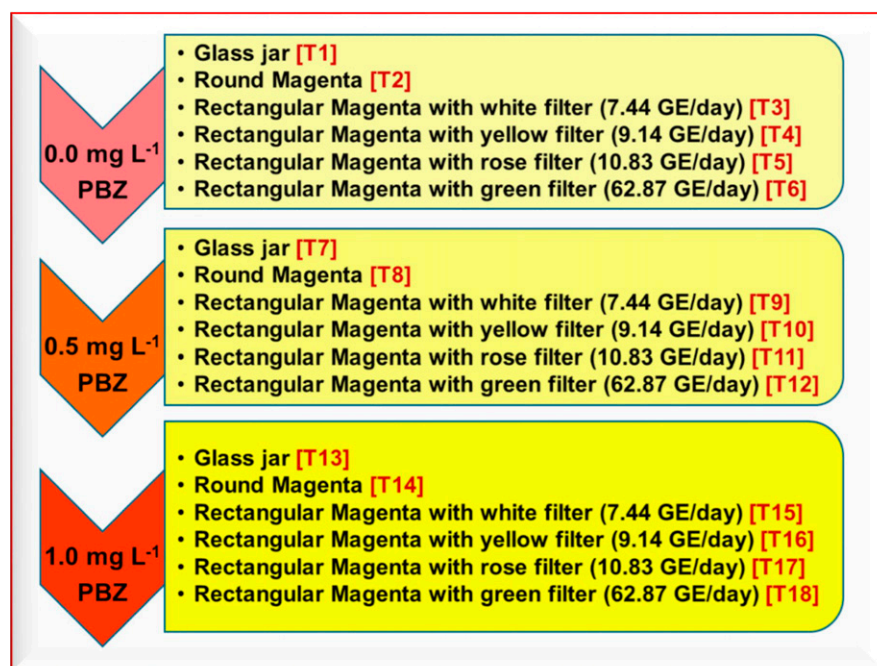


Fig. 2. A flowchart of the experimental design of the study, including the number for each treatment (T). GE = gram equivalents; PBZ = paclobutrazol.

Table 1. Effect of paclobutrazol (PBZ) and vessel type on in vitro rooting, morphology, and chlorophyll content of 'Grand Nain' banana plantlets after 4 weeks of culture.ⁱ

Treatment								
PBZ (mg·L ⁻¹) and significance	Vessel type and interaction	Plantlet length (cm)	Plantlet fresh wt (g)	Stem diam (mm)	No. of roots/plantlet	Root length (cm)	No. of leaves/plantlet	SPAD
0.0 (control)	Jar	13.0 ± 1.03 ab ⁱⁱ	3.9 ± 0.64 g	2.4 ± 0.48 h	13.3 ± 0.75 a	9.8 ± 0.54 a	4.0 ± 0.47 a	6.5 ± 0.64 i
0.0 (control)	Round Magenta	14.3 ± 1.33 a	8.2 ± 0.17 a	4.4 ± 0.10 g	12.7 ± 1.9 ab	9.4 ± 0.25 a	5.0 ± 0.0 a	7.4 ± 0.17 hi
0.0 (control)	White	10.7 ± 0.288 cd	5.5 ± 0.32 c-e	4.7 ± 0.38 fg	10.0 ± 1.0 a-c	10.3 ± 1.0 a	4.3 ± 0.40 a	8.0 ± 0.32 fg
0.0 (control)	Yellow	11.5 ± 0.37 bc	5.5 ± 0.413 c-e	4.3 ± 0.23 g	10.7 ± 0.9 b-d	10.7 ± 0.28 a	5.0 ± 0.40 a	8.2 ± 0.41 gh
0.0 (control)	Rose	11.5 ± 1.08 bc	7.6 ± 0.27 ab	5.9 ± 0.28 de	11.3 ± 1.6 a-c	10.8 ± 0.1 a	4.7 ± 0.62 a	8.0 ± 0.27 gh
0.0 (control)	Green	10.5 ± 1.14 cd	6.6 ± 0.82 bc	4.2 ± 0.13 g	10.0 ± 0.47 c-e	8.7 ± 0.40 a	5.3 ± 0.25 a	11.3 ± 0.82 bc
0.5	Jar	9.0 ± 0.0 de	3.8 ± 0.36 g	4.7 ± 0.38 fg	8.7 ± 0.62 d-g	7.7 ± 0.82 a	5.0 ± 0.40 a	9.8 ± 0.36 de
0.5	Round Magenta	9.3 ± 0.62 de	4.6 ± 0.39 e-g	5.2 ± 0.17 ef	8.0 ± 0.47 e-g	7.7 ± 0.6 2 a	4.7 ± 0.40 a	8.9 ± 0.39 e-g
0.5	White	9.5 ± 0.43 de	6.0 ± 0.39 cd	7.1 ± 0.27 b	11.3 ± 0.4 a-c	7.2 ± 0.042 a	5.7 ± 0.28 a	8.6 ± 0.39 e-h
0.5	Yellow	8.7 ± 0.25 e	4.5 ± 0.48 e-g	6.8 ± 0.65 bc	8.7 ± 0.64 d-g	7.7 ± 0.15 a	5.0 ± 0.47 a	8.5 ± 0.48 f-h
0.5	Rose	9.3 ± 0.288 de	4.3 ± 0.204 fg	6.2 ± 0.39 cd	9.7 ± 0.23 c-f	8.2 ± 0.51 a	7.0 ± 0.47 a	9.7 ± 0.20 d-f
0.5	Green	9.3 ± 0.239 de	3.7 ± 0.40 g	5.4 ± 0.18 d-f	7.0 ± 0.81 g	6.5 ± 0.25 a	7.0 ± 0.47 a	11.2 ± 0.40 c
1.0	Jar	6.3 ± 0.5 f	4.0 ± 0.39 g	4.6 ± 0.18 fg	7.7 ± 0.40 e-g	7.0 ± 0.08 a	3.7 ± 0.25 a	12.6 ± 0.39 b
1.0	Round Magenta	8.2 ± 0.28 e	4.0 ± 0.12 g	5.3 ± 0.14 ef	9.0 ± 0.0 c-g	6.8 ± 0.31 a	4.7 ± 0.28 a	10.8 ± 0.12 cd
1.0	White	10.7 ± 0.25 cd	4.2 ± 0.06 g	9.9 ± 0.53 a	7.3 ± 0.62 fg	6.4 ± 0.20 a	5.0 ± 0.47 a	12.5 ± 0.06 b
1.0	Yellow	9.0 ± 0.64 de	4.8 ± 0.18 e-g	7.2 ± 0.08 b	10.7 ± 0.28 b-d	5.3 ± 0.64 a	5.3 ± 0.28 a	10.3 ± 0.18 cd
1.0	Rose	9.2 ± 0.55 de	5.3 ± 0.24 d-f	6.2 ± 0.09 cd	9.0 ± 0.0 c-g	7.3 ± 0.4 a	5.7 ± 0.28 a	12.5 ± 0.24 b
1.0	Green	8.5 ± 0.23 e	4.7 ± 0.62 e-g	5.7 ± 0.08 de	9.0 ± 0.25 c-g	5.8 ± 0.31 a	4.7 ± 0.40 a	14.3 ± 0.62 a
Significance	PBZ	***	***	***	***	***	***	***
Significance	Vessel	NS	***	***	NS	NS	**	***
Significance	PBZ × vessel	***	***	***	*	NS	NS	**

ⁱ The values represent the mean ± standard error of four replicates.

ⁱⁱ Values followed by the same letters in the same column are not significantly different.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively, according to analysis of variance followed by Duncan's multiple range tests. SPAD = soil plant analysis development.

acclimatization experiment, each replicate was five plants, with a total of 25 plants per treatment. Data were subjected to analysis of variance (ANOVA) using two-way ANOVA and CoStat v. 6.303 (CoHort Software, Monterey, CA, USA). The mean separations were calculated using Duncan's multiple range test at $P \leq 0.05$.

Results

In vitro rooting, morphology, and chlorophyll content of 'Grand Nain' banana plantlets

The data presented in Table 1 indicate that PBZ had a highly significant effect on all recorded parameters. The effect of vessel type was significant for all measurements except plantlet length, number of roots per plantlets, and root length. The interaction between PBZ and vessel type was significant for all parameters except root length and chlorophyll content. The longest plantlet (14.3 cm) was recorded for round Magenta vessels in the control treatment; the shortest plantlet (6.3 cm) was recorded for glass jars at 1.0 mg·L⁻¹ PBZ (Figs. 3–5). Regardless of the vessel type, increasing PBZ concentration decreased the length of plantlets (by mean values of 11.91, 9.18, and 8.65 cm for the control, 0.5-, and 1.0-mg·L⁻¹ PBZ treatments, respectively). The maximum fresh weight of plantlets (8.2 g) was seen in round Magenta vessels in the control treatment. The minimum fresh weight (3.7 g) was observed for Magenta vessels with green filters at 0.5 mg·L⁻¹ PBZ, without significant differences seen in glass jars in the control and 0.5-mg·L⁻¹ PBZ treatments, and glass jars, round Magenta vessels, and Magenta vessels with white filters in the 1.0-mg·L⁻¹ PBZ treatment. The stem diameter of plantlets was greatest and least (9.9 and 2.4 mm,

respectively) in the 1.0-mg·L⁻¹ PBZ treatment for white-filter Magenta vessels and for the control treatment in glass jars, respectively. Contrary to plantlet length, increased concentrations of PBZ from control to 1.0 mg·L⁻¹ increased the stem diameter of the plantlets (average, 4.32, 6.20, and 6.48 mm for the control, 0.5-, and 1.0-mg·L⁻¹ PBZ treatments, respectively). The number of roots per plantlet was greatest ($n = 13.3$) with the control treatment for glass jars and least ($n = 7.0$) in the 0.5-mg·L⁻¹ PBZ treatment for Magenta vessels with green filters. The effect of PBZ and vessel type was not significant on root length or number of leaves/plantlets. The highest

chlorophyll content (SPAD index, 14.3) was seen in a Magenta vessel with a green filter in the 1.0-mg·L⁻¹ PBZ treatment. The lowest value (SPAD index, 6.5) was seen in glass jars in the control treatment. Magenta vessels used in the control treatment enhanced plantlet height, plantlet fresh weight, and number of roots per plantlets significantly.

Antioxidant enzyme activity of in vitro rooted plantlets

Antioxidant enzyme activity of CAT, POX, and PPO were measured in in vitro rooted 'Grand Nain' banana plantlets directly after

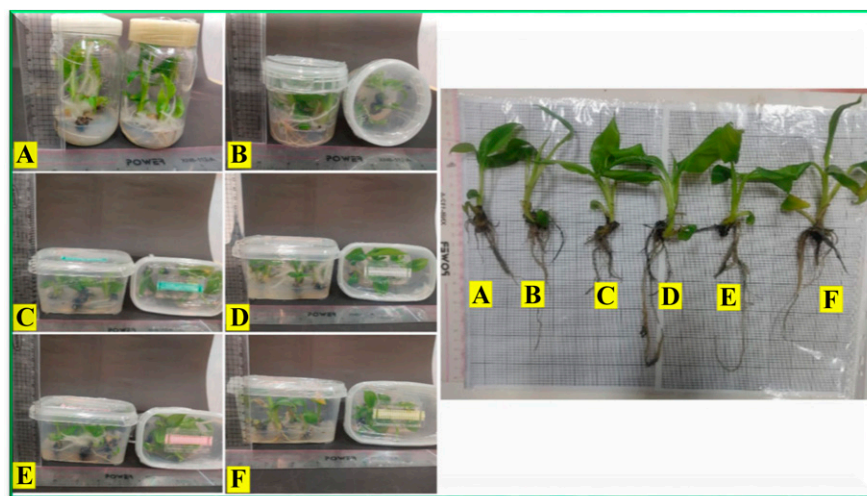


Fig. 3. In vitro rooting of 'Grand Nain' banana microshoots with the control treatment in glass jars (A, left), round Magenta vessels (Magenta LLC, Chicago, IL, USA) (B, left), and rectangular Magenta vessels (C–F, left) with green, white, rose, and yellow filters, respectively. (A–F, right) View of microshoots removed from vessels, respectively.

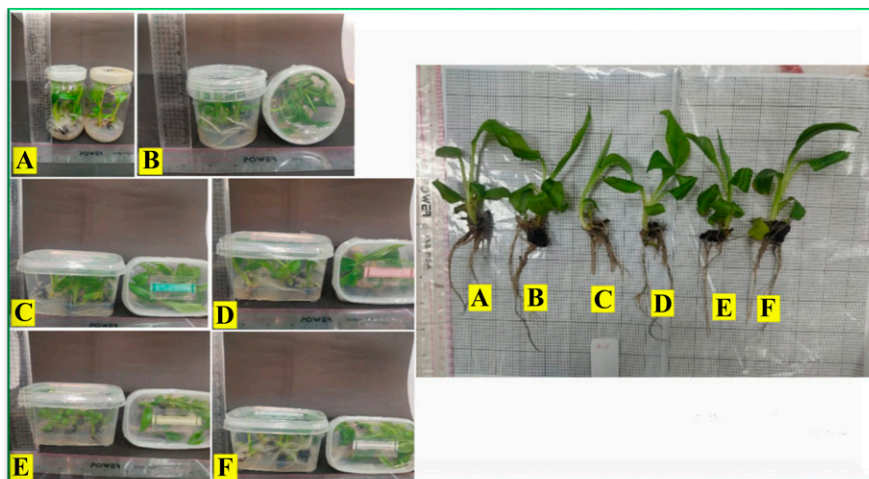


Fig. 4. In vitro rooting of 'Grand Nain' banana microshoots with 0.5 mg·L⁻¹ paclobutrazol in glass jars (A, left), round Magenta vessels (Magenta LLC, Chicago, IL, USA) (B, left), and rectangular Magenta vessels (C–F, left) with green, rose, yellow, and white filters; respectively. (A–F, right) View of microshoots removed from vessels, respectively.

removing the plantlets from the culture vessels and before acclimatization (Figs. 6–8). The antioxidant activity of CAT was enhanced and reached its maximum value during treatment (T) 8, followed by T16, T7, and T12. The lowest CAT activity was recorded for T5. In general, 0.5 mg·L⁻¹ PBZ was the best concentration of PBZ to increase CAT antioxidant activity; it reflected growth, development, and the physiological status of the in vitro plantlets. POX activity was less using PBZ and vessel treatments than that of CAT. The maximum value of POX activity was for T13, followed by T7. PPO activity was relatively high in the 0.5- and 1.0-mg·L⁻¹ PBZ treatments for all culture vessels compared with the control. The highest PPO activity was recorded for T11 and T13, which had the same value of antioxidant activity, followed by T7. The lowest activity was observed for T2.

Acclimatization of in vitro 'Grand Nain' banana plantlets

Effect of PBZ and culture vessels on survival percentage. Acclimatization performance is a critical process in the micropropagation protocol and relies on activities of the newly developed hairy roots to regulate some fundamental processes such as water uptake, nutrient absorption, photosynthesis, and gas exchange to achieve successful ex vitro acclimatization with a high survival rate. The 0.5 mg·L⁻¹ PBZ treatment demonstrated a 100% survival rate for all culture vessels used in our study (Fig. 9). The same result was obtained at 0.0 mg·L⁻¹ PBZ for all culture vessels except the glass jars. PBZ at 1.0 mg·L⁻¹ showed 100% survival for Magenta vessels with white, rose, and green filters, followed by round Magenta vessels (98%), then by glass jars and Magenta vessels with white filters (95% survival rates).

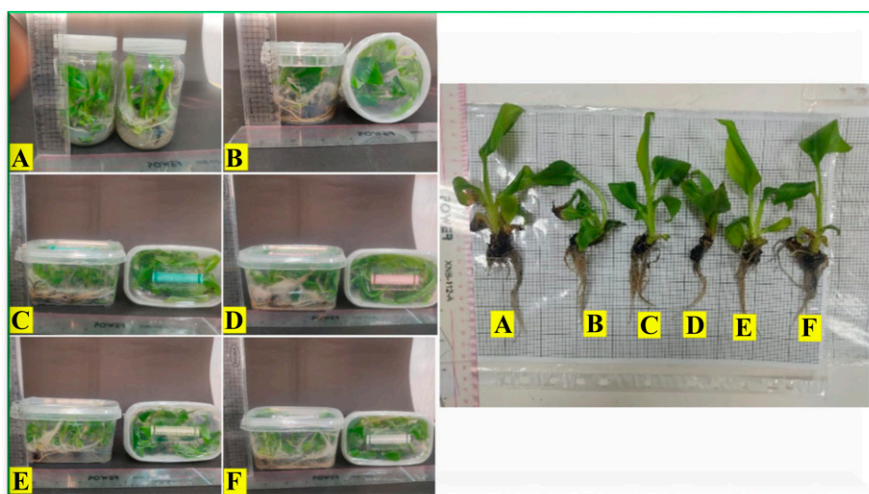


Fig. 5. In vitro rooting of 'Grand Nain' banana microshoots with 1.0 mg·L⁻¹ paclobutrazol in glass jars (A, left), round Magenta vessels (Magenta LLC, Chicago, IL, USA) (B, left), and rectangular Magenta vessels (C–F, left) with green, rose, yellow, and white filters; respectively. (A–F, right) View of microshoots removed from vessels, respectively.

Effect of PBZ and culture vessels on quality of 'Grand Nain' banana transplants. The effects of culture vessels and PBZ treatments (0.0, 0.5, and 1.0 mg·L⁻¹) added to in vitro rooting media on quality characteristics of 'Grand Nain' banana transplants after acclimatization are presented in Table 2 and Fig. 10. The double interaction of culture vessels and PBZ concentrations showed significant differences for all parameters except the stem diameter of the acclimatized transplants. The maximum significant values for transplant height and number of roots per transplant (20.9 cm and n = 10.0, respectively) were seen in glass jars in the control treatment. Transplant height was only half the maximum (10.8 cm) in Magenta vessels with yellow filters with 1.0 mg·L⁻¹ PBZ. Differences in stem diameter were not significant, but the largest (5.7 mm) was for Magenta vessels with white filters at 0.5 mg·L⁻¹. Root length was significantly longest (13.0 cm) in Magenta vessels with rose filters in the control treatment. The minimum values for number of roots per transplant and root length (n = 3.7 and 3.3 cm, respectively) were seen in Magenta vessels with green filters at PBZ concentrations of 0.5 and 1.0 mg·L⁻¹, respectively. Our results indicate that the acclimatized plants showed no significant variations in transplant stem diameter, the most important characteristic for high-quality transplants.

Effect of PBZ and culture vessels on photosynthetic capacity of 'Grand Nain' banana transplants. Significant differences were found by treatment on photosynthesis-related parameters (Table 3). The control PBZ and round Magenta vessels recorded the maximum values for number of leaves per transplant and photosynthetic efficiency (n = 6.4 and 0.782, respectively). Leaf area was the maximum (127.8 cm²) for the control treatment in glass jars. Chlorophyll content was greatest in Magenta vessels with white filters in the control treatment. Results indicate that control PBZ broadly resulted in improved photosynthetic capacity of the acclimatized banana transplants. The lowest value for number of leaves per transplant (n = 4.3) and chlorophyll content (SPAD index, 22.7) was for seen in the 0.5-mg·L⁻¹ PBZ treatment and glass jars. Leaf area and photosynthetic efficiency were least (71.8 cm² and 0.754, respectively) in the 1.0 mg·L⁻¹ PBZ treatment in glass jars and in Magenta vessels with rose filters, respectively.

Discussion

Bananas are among the most important food and trade fruit cultivated globally (Leonel et al. 2024; Tegen and Mohammed 2016). To fulfill the gap between high demand and limited supply of improved, vigorous, and high-quality planting materials, micropropagation techniques have been applied commercially to banana plants (Abacı et al. 2026). Acclimatization is recognized as the most frequently critical and limiting stage in micropropagation. Successful, optimized rooting is essential because it determines directly the subsequent acclimatization success (Asayesh et al. 2017; Leite et al. 2021; Patial et al. 2024; Selvakumar

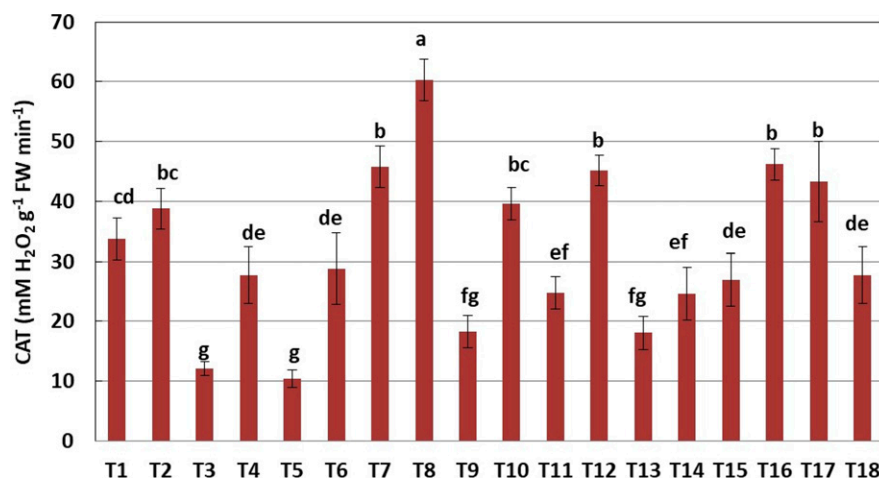


Fig. 6. Antioxidant activity of catalase (CAT) enzyme in in vitro rooted 'Grand Nain' banana plantlets. Treatment 1 (T1) through T6, 0.0 mg·L⁻¹ paclobutrazol (PBZ; control); T7 through T11, 0.5 mg·L⁻¹ PBZ; and T12 through T18 at 1.0 mg·L⁻¹ PBZ in glass jars, round Magenta vessels (Magenta LLC, Chicago, IL, USA), and rectangular Magenta vessels with white, yellow, rose, and green filters for each PBZ concentration. FW = fresh weight.

and Parasurama 2020). Poor acclimatization survival hinders the commercialization of the micropropagation protocol and reduces the economic feasibility of tissue culture industries (Selvaskanthan et al. 2025; Soni et al. 2021; Thakur et al. 2006).

Environmental conditions such as light, temperature, relative humidity, and nutrient levels required for ex vitro growth under acclimatization are significantly different from those in in vitro culture under controlled conditions, so they can affect the ex vitro acclimatization of plants negatively (Asayesh et al. 2017; Hazarika 2006b). Plant growth retardants (i.e., PBZ, uniconazole, triapenthenol, triadimefon, and hexaconazole) reduce plant height effectively while promoting strong growth (retaining dark-green leaves and thick roots) and enhancing antiwilting characteristics. This results in healthy plantlets and leads to better growth and survival under ex vitro conditions (Cha-um et al. 2009; Thakur et al. 2006).

Plant growth retardants can be used for many applications (i.e., enhancing shoot multiplication and/or rooting efficiency) (Roussos et al. 2016; Žiauka and Kuusienė, 2010), embryo germination and seedling development (Xu et al. 2022), increased survival percentages under in vitro (Cha-um et al. 2009) and ex vitro (Aziz and Al-Taweel 2019) acclimatization, enhanced drought stress tolerance (Duan et al. 2008; Iqbal et al. 2020), and reduced damage by increasing proline, glycine betaine, α-tocopherol, and protein contents (Hajhashemi and Ehsanpour 2013). They can also promote waterlogging tolerance (Qiu et al. 2005), salt tolerance (Kandil and Eleiwa 2008), and extreme temperature tolerance (Kaneko and Suzuki 2006); retard vegetative growth; and induce dwarfing for the potted flowering plant industry (Teto et al. 2016). Prior investigations have demonstrated that gibberellins play a key role in regulating plant height in several species, including bananas

(Tong et al. 2024). PBZ acts as a gibberellin antagonist (Huang et al. 2011). It has been reported to inhibit plant cell division and elongation by blocking gibberellin biosynthesis. PBZ is often used to delay plant growth, inhibit stem elongation, shorten internodes, and decrease plant height (Luo et al. 2018). Moreover, PBZ treatments have produced high-quality tomato seedlings, which were greener, more compact, and had a better developed root system (Shalaby et al. 2022).

PBZ has been observed to induce adventitious root formation in tissue culture systems (Vaičiukynė et al. 2025), increase the scavenging of ROS to alleviate biotic and abiotic stresses in calli culture (Hajhashemi and Ehsanpour 2014), and promote direct somatic embryogenesis in plants (Moradi et al. 2017). Paclobutrazol is a widely used and highly active triazole-type plant growth inhibitor that effectively suppresses gibberellin biosynthesis and reduces vegetative growth (Desta and Amare, 2021), which causes slow growth and decreases the number of subcultures in plant tissue cultures, as well as increases the period between them. Thus, PBZ could be applied in slow-growth in vitro storage to decrease the frequency of contamination and reduce the loss of plant material (Benelli et al. 2022). Moreover, PBZ has been widely applied to reduce growth and induce early flowering in vitro (Abdalla et al. 2021). In vitro germplasm conservation of citrus species would be easier with PBZ, which acts as a growth reducer, thereby reducing contamination and increasing its maintenance period as well as reducing the costs associated with labor and materials (Mendes et al. 2021). PBZ has been applied to induce microbulb formation (Topaloglu and Öztürk 2021).

PBZ acts primarily by altering the balance of endogenous hormones and redirecting plant nutrient distribution through adjusting the source-sink balance (Huang et al. 1995). PBZ reduces significantly the accumulation of carbohydrates in leaves, and enhances the rapid and effective transfer of the products of photosynthesis to the roots, making them thicker and stronger. Many studies have confirmed that PBZ promotes the storage of nutrients in roots and the growth of roots (e.g., Jie et al. 2017). PBZ has recently been discovered to act as a multistress protectant against challenges such as heat, chilling, drought, waterlogging, and heavy metals (Maheshwari et al. 2022). Paclobutrazol may mitigate environmental stresses by influencing physiological and biochemical pathways, such as modulating electrolyte leakage, phytohormone levels, glutathione status, and lipid peroxidation, thereby helping plants grown in field or in vitro conditions to better manage these stresses (Sasi et al. 2021). PBZ improves plant survival, especially under drought stress, through decreased transpiration rates resulting from reduced leaf area, thicker leaves, smaller stomatal pores, less water use, and increased antioxidant activity (Kumar et al. 2023).

PBZ is widely applied to culture media to suppress shoot elongation and improve the growth, development, and quality of in vitro cultured plants. Increasing chlorophyll content

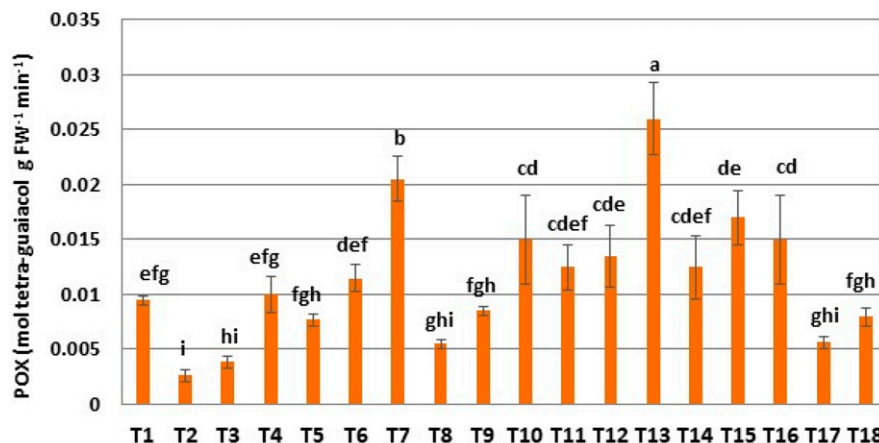


Fig. 7. Antioxidant activity of peroxidase (POX) enzyme in in vitro rooted 'Grand Nain' banana plantlets. Treatment 1 (T1) through T6, 0.0 mg·L⁻¹ paclobutrazol (PBZ; control); T7 through T11, 0.5 mg·L⁻¹ PBZ; and T12 through T18 at 1.0 mg·L⁻¹ PBZ in glass jars, round Magenta vessels (Magenta LLC, Chicago, IL, USA), and rectangular Magenta vessels with white, yellow, rose, and green filters for each PBZ concentration. FW = fresh weight.

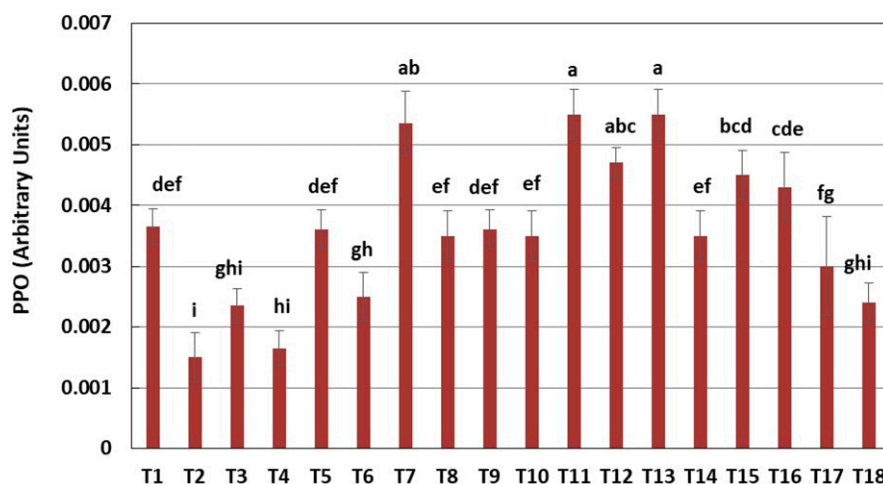


Fig. 8. Antioxidant activity of polyphenol oxidase (PPO) enzyme in in vitro rooted 'Grand Nain' banana plantlets. Treatment 1 (T1) through T6, 0.0 mg·L⁻¹ paclobutrazol (PBZ; control); T7 through T11, 0.5 mg·L⁻¹ PBZ; and T12 through T18) at 1.0 mg·L⁻¹ PBZ in glass jars, round Magenta vessels (Magenta LLC, Chicago, IL, USA), and rectangular Magenta vessels with white, yellow, rose, and green filters for each PBZ concentration.

and enhancing protein accumulation, increasing absorption of mineral nutrients, reducing water consumption, and increasing photosynthetic capacity are the key effects of PBZ (Mohamed et al. 2023). Earlier studies have reported that incorporation of PBZ in multiplication media reduced the excessive growth of stems and leaves of bananas significantly, induced shoot clusters, and improved the multiplication coefficient in bananas, yams, and potatoes. In addition, PBZ increased the survival rate during the acclimatization stage in chrysanthemums and philodendrons (Albany et al. 2005). PBZ caused a significant shortening in seedling height and stem length in cucumbers. Moreover, as the concentration of PBZ increased, the decrease in seedling height and stem length continued (Aktaş et al. 2024). These findings are consistent with the results obtained in our study. PBZ improved physiological (chlorophyll content and chlorophyll fluorescence ratio) and morphological (shoot and root fresh and dry weights) parameters significantly of cucumber seedlings (Ramin 2009). It also ameliorated

damage caused by heat stress and increased the heat tolerance of cucumber seedlings. However, the application method and concentration of PBZ could affect plant physiology and performance, so these factors should be taken into consideration (Baninasab and Ghobadi 2011). The application of PBZ not only reduces plant height, it also increases stem diameter and chlorophyll content, and affects plant growth and flowering quality by increasing the flower diameter and fresh weight in herbaceous peonies (Wu et al. 2022). PBZ at 1.5 mg·L⁻¹ increased seedling stem thickness, reduced its height, and led to the highest chlorophyll (SPAD) value in *Gypsophila bicolor* (Freyn & Sint.) Grossh (Parlakova Karagoz et al. 2023).

PBZ reduced plant height and increased stem diameter as observed in the results of our study. On the other hand, a reduction in root number and root length at greater PBZ concentrations was also noticed. It has been reported that the effects of PBZ on root systems are highly dose dependent (Amien et al. 2022).

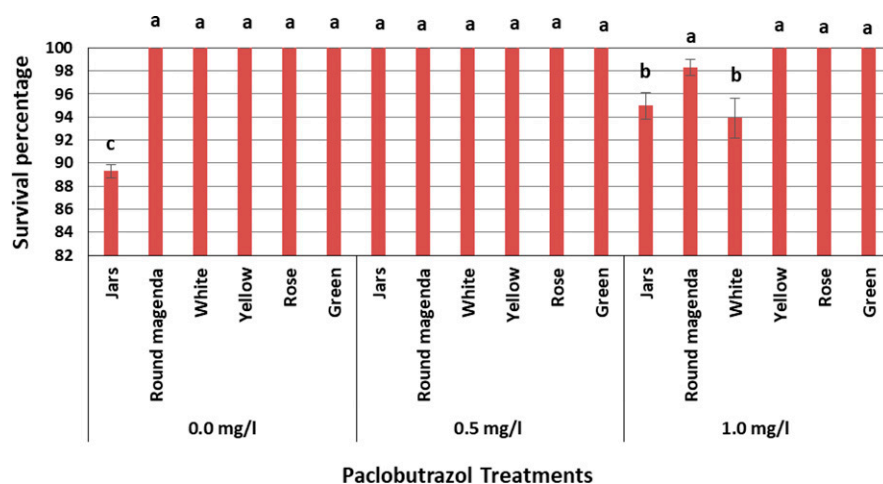


Fig. 9. Survival percentage of 'Grand Nain' banana in vitro plantlets during ex vitro acclimatization. The same letters indicate the values are not statistically different.

High concentrations often trigger significant morphological abnormalities in the roots, causing a substantial obstacle for subsequent ex vitro survival (Wen et al. 2013). When high concentrations of PBZ severely exhaust gibberellin levels, suppression in root elongation occurs, leading to drastically shorter roots (Amien et al. 2022; Wen et al. 2013). Reduction in root number could be observed as a result of the alterations in the auxin-to-cytokinin ratio at high PBZ doses (Li et al. 2023), which can also cause radial thickening, leading roots to become stubby, thick, and anatomically restricted (Amien et al. 2022; Wen et al. 2013). A reduction in root number and length at greater PBZ concentrations limits the plant's overall root surface area, thereby affecting water uptake negatively and limiting nutrient absorption, worsening transplant shock and leaf desiccation (Amien et al. 2022; Hassan et al. 2014). Because root development is a critical parameter for successful acclimatization and efficient micropropagation systems, balancing a compact shoot morphology with root quality is a crucial consideration when selecting PBZ concentrations.

The production of healthy transplants that can acclimatize efficiently to ex vitro conditions is a highly needed requirement for a successful micropropagation protocol. Therefore, altering environmental conditions inside the culture vessels to improve transplants without any physiological and morphological disorders to achieve high acclimatization rates is a high-impact strategy in micropropagation, and it is tremendously important for the horticulture industry (Asayesh et al. 2017). Micropropagated plantlets are grown in a culture vessel. The type of culture vessel can affect growth and development of in vitro plantlets by regulating light penetration, ventilation rate, humidity, and gas exchange during the production of in vitro plantlets (Malik et al. 2022). The most critical physical properties of plant tissue culture vessels are the air exchange rate (the hourly volumetric ventilation rate of the vessel divided by the air volume of the vessel), light transmittance, and spectral irradiance. The most important criterion for choosing tissue culture vessels is to ensure the containers provide homogeneous and sufficient light quality, isolate the cultures from water loss and prevent contamination, allow gas ventilation, and provide an adequate growing area (Huang and Chen 2005; Naik et al. 2020).

Different in vitro methodologies have been developed to enhance the photosynthetic capacity, growth, and quality of micropropagated plants (Bortolotti da Silva et al. 2014). The gas exchange rate is particularly related to the flow of carbon dioxide, oxygen, ethylene, and water vapor in and/or out of the vessel (Vollmer et al. 2024). Gas exchange vessels (Magenta vessels; specialized culture vessels equipped with filters with different gas exchange rates) reduce the relative humidity within the culture vessels (Pérez-Tomero et al. 2001), decrease water availability, increase the gas exchange rate with the outer atmosphere, and provide optimal environmental conditions to support photoautotrophic micropropagated plants (Bortolotti da Silva et al. 2014; Kiferle et al. 2014; Mamedes-Rodrigues et al. 2019).

Table 2. Effect of paclobutrazol (PBZ) and vessel type on the quality characteristics of ‘Grand Nain’ banana transplants after 40 d of acclimatization.ⁱ

Treatment		Transplant	Stem diam	No. of roots/	Root length
PBZ (mg·L ⁻¹) and significance	Vessel type and interaction	ht (cm)	(mm)	transplant	(cm)
0.0 (control)	Jar	20.9 ± 1.15 a ⁱⁱ	5.1 ± 0.44 a	10.0 ± 1.15 a	8.2 ± 0.16 c
0.0 (control)	Round Magenta	18.6 ± 1.7 a–d	5.2 ± 0.59 a	7.0 ± 0.0 b	7.8 ± 0.39 cd
0.0 (control)	White	16.4 ± 1.18 c–e	5.3 ± 0.13 a	6.0 ± 0.57 b–d	10.7 ± 1.09 b
0.0 (control)	Yellow	18.0 ± 0.72 a–d	5.0 ± 0.40 a	6.0 ± 0.57 b–d	11.8 ± 0.16 ab
0.0 (control)	Rose	17.2 ± 1.60 b–e	5.1 ± 0.50 a	6.5 ± 0.28 bc	13.0 ± 0.54 a
0.0 (control)	Green	19.9 ± 1.12 ab	5.2 ± 0.41 a	6.0 ± 0.57 b–d	7.8 ± 0.44 d
0.5	Jar	13.1 ± 0.56 f–h	4.2 ± 0.38 a	5.3 ± 0.33 d–f	5.0 ± 0.0 e
0.5	Round Magenta	15.6 ± 1.01 d–f	5.0 ± 0.45 a	4.7 ± 0.33 d–g	5.0 ± 0.0 e
0.5	White	18.7 ± 0.97 a–c	5.7 ± 0.45 a	6.0 ± 0.0 b–d	4.3 ± 0.33 e–g
0.5	Yellow	18.6 ± 0.84 a–d	3.7 ± 0.35 a	4.7 ± 0.33 d–g	4.9 ± 0.57 e
0.5	Rose	17.9 ± 0.82 a–d	4.9 ± 0.30 a	5.7 ± 0.33 b–e	6.7 ± 0.33 d
0.5	Green	17.2 ± 0.49 b–e	5.0 ± 0.35 a	3.7 ± 0.33 g	4.0 ± 0.0 e–g
1.0	Jar	11.9 ± 0.50 gh	4.2 ± 0.31 a	4.0 ± 0.57 fg	5.0 ± 0.57 e
1.0	Round Magenta	12.0 ± 0.37 gh	4.2 ± 0.17 a	5.0 ± 0.57 d–g	4.7 ± 0.33 ef
1.0	White	13.1 ± 0.56 f–h	5.5 ± 0.12 a	4.3 ± 0.33 b–e	4.7 ± 0.33 ef
1.0	Yellow	10.8 ± 0.61 h	4.4 ± 0.34 a	6.0 ± 0.0 b–d	3.7 ± 0.33 fg
1.0	Rose	14.6 ± 0.48 e–g	5.6 ± 0.27 a	4.7 ± 0.33 d–g	5.0 ± 0.0 e
1.0	Green	13.2 ± 0.78 f–h	4.9 ± 0.29 a	4.7 ± 0.33 d–g	3.3 ± 0.33 g
Significance	PBZ	***	NS	***	***
Significance	Vessel	NS	**	**	***
Significance	PBZ × vessel	***	NS	***	***

ⁱ The values represent the mean ± standard error of four replicates.ⁱⁱ Values followed by the same letters in the same column are not significantly different.NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively, according to analysis of variance followed by Duncan’s multiple range tests.

The type of vessel used can have significant effects on culture growth and development. However, vessel types vary widely, depending on species-specific requirements (Kavanagh et al. 1991). PBZ is widely applied to enhance acclimatization and quality of seedlings/transplants for in vitro-grown plants (Díaz-Pérez et al. 1995; Gimenes et al. 2018; Kim et al. 2005; Kozak 2006; Kucharska and Orlikowska 2008; Wen et al. 2013). The quality characteristics of banana transplants include morphological parameters (i.e., transplant height, transplant diameter, number of roots, number of leaves), and physiological parameters (i.e., chlorophyll content, photosynthetic efficiency, antioxidant enzyme activity). All support the transplants and allow them to transfer successfully to ex

vitro conditions with a high survival rate. In our study, the round Magenta containers were the best at improving the ‘Grand Nain’ banana survival rate after acclimatization, followed by glass jars, both with the PBZ control treatment. The glass vessels resulted in a higher multiplication rate for ‘Grand Nain’ banana shoots, whereas polycarbonate plastic vessels had an undesirable effect on banana shoot proliferation (Aktaş et al. 2024).

Unlike the banana plant, filtered Magenta vessels with a gas exchange rate of 62.87 GE·d⁻¹ are highly preferred for improved growth of shoots and roots, reduced electrolyte leakage, and enhanced antioxidant enzyme activity to obtain high quality in vitro-micropropagated *Gypsophila paniculata* L. (Wen et al. 2013).

In vitro tobacco plants (*Nicotiana tabacum* L.) cultured in Magenta GA-7 vessels had significantly greater chlorophyll a and b contents and β-carotene levels than those seen in tightly closed glass vessels (Vollmer et al. 2024). Other plants required filtered Magenta vessels, such as *Ficus carica* L. cv. Sarilop (Aktaş et al. 2024). Plastic vessels supplied with a high-efficiency particulate air filter that allows a high gas exchange rate increased plant height, stem diameter, leaf area, and chlorophyll content, and reduced water content in the tissues of in vitro potato plants (Vollmer et al. 2024). Culture vessels sealed with three-holed caps filled with a sponge proved to be efficient for improving morphological and physiological parameters, especially leaf size, by eliminating excess water and reducing ethylene accumulation, resulting in well-developed, healthy, and high-quality in vitro potato seedlings (Szarvas and Dobránszki 2025).

The physical properties of culture vessels such as material, closure type, and vessel volume have a significant influence on growth and quality of in vitro plantlets. Vessel volume alters the headspace microenvironment fundamentally above the medium and affected the growth, morphology, and physiological quality substantially of in vitro plants. Smaller vessel volumes allow accumulation of both ethylene and carbon dioxide gases. They also increase the relative humidity inside the vessels, causing senescence, leaf yellowing, and hyperhydricity, limiting photosynthetic potential. Vessels of larger volume could decrease these toxic affects. Also, larger vessels allow a greater volume of nutrient media to be used and more space for biomass expansion, supporting better growth, survival, and quality (Vollmer et al. 2024).

It is worth noting that the culture vessels evaluated in our study differed inherently not only in gas exchange capacities but also in physical volume and medium capacity. In plant tissue culture, these factors collectively constitute the vessel-induced microenvironment. Although this structural linkage makes it challenging to isolate the independent effects of ventilation entirely from the headspace and nutrient availability, the pronounced variations observed in photosynthetic efficiency (Fv/Fm) and stomatal regulation strongly indicate that gas exchange dynamics and humidity control were primary drivers of the physiological adaptations. Nonetheless, future studies using uniform volumes with modified ventilation septa could further decouple these microenvironmental components.

Crucially, the vessel-dependent gas exchange characteristics regulated the physiological transport and systemic distribution of PBZ directly within the plantlets. In tissue culture vessels with high ventilation and gas exchange rates, the removal of excess water vapor lowers the relative humidity in the headspace, creating a greater vapor pressure deficit. This microenvironmental change accelerates the transpirational stream of the plantlets, effectively generating a stronger acropetal “pull” that facilitates the faster translocation of dissolved PBZ from the rooting medium up to the subapical meristems. Conversely, in poorly ventilated vessels, the stagnant, saturated atmosphere suppresses transpiration,

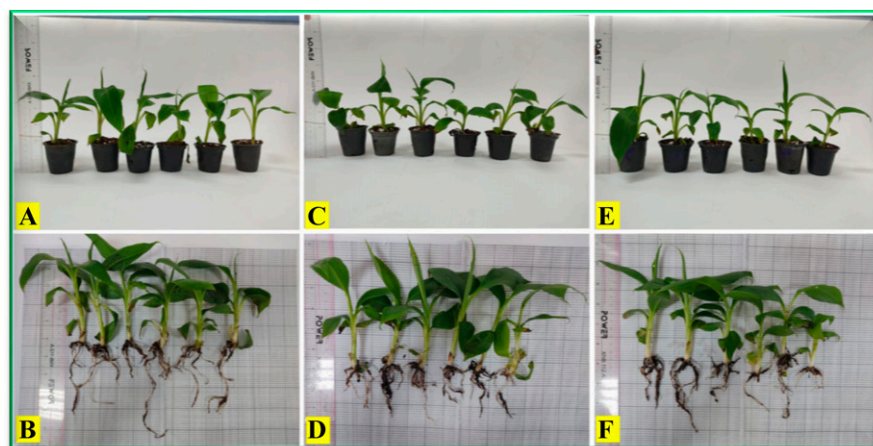


Fig. 10. Acclimatization of ‘Grand Nain’ banana in vitro plantlets treated with 0.0 mg·L⁻¹ paclobutrazol (PBZ; control) (A, B), 0.5 mg·L⁻¹ PBZ (C, D), and 1.0 mg·L⁻¹ PBZ (E, F). (A–F, left to right) Treatments in jars, round Magenta vessels (Magenta LLC, Chicago, IL, USA), and rectangular Magenta vessels with white, yellow, rose, and green filters, respectively.

Table 3. Effect of paclobutrazol (PBZ) and vessel type on photosynthesis-related parameters of 'Grand Nain' banana transplants after 40 d of acclimatization.ⁱ

Treatment		No. of leaves/ transplant	Leaf area (cm ²)	SPAD	Fv/Fm
PBZ (mg·L ⁻¹) and significance	Vessel type and interaction				
0.0 (control)	Jar	5.4 ± 0.29 b-e ⁱⁱ	127.8 ± 4.2 a	23.9 ± 1.9 gh	0.759 ± 0.0048 gh
0.0 (control)	Round Magenta	6.4 ± 0.33 a	114.6 ± 5.1 b	31.9 ± 2.1 b-e	0.782 ± 0.0047 a
0.0 (control)	White	5.6 ± 0.17 b-e	87.6 ± 2.5 ef	39.4 ± 1.5 a	0.768 ± 0.0043 b-g
0.0 (control)	Yellow	5.2 ± 0.36 c-e	114.4 ± 1.7 bc	32.6 ± 1.7 bc	0.768 ± 0.0025 c-g
0.0 (control)	Rose	5.3 ± 0.33 b-e	86.8 ± 4.9 ef	24.8 ± 1.8 gh	0.759 ± 0.0036 gh
0.0 (control)	Green	6.0 ± 0.0 ab	86.1 ± 2.7 ef	27.6 ± 1.3 e-g	0.775 ± 0.0041 a-d
0.5	Jar	4.3 ± 0.28 f	83.1 ± 0.89 f	22.7 ± 1.1 h	0.772 ± 0.0027 a-f
0.5	Round Magenta	5.1 ± 0.11 c-e	86.9 ± 1.8 ef	34.0 ± 1.0 b	0.773 ± 0.0014 a-e
0.5	White	5.8 ± 0.40 a-c	93.5 ± 3.5 e	31.7 ± 1.9 b-f	0.776 ± 0.0039 a-c
0.5	Yellow	5.1 ± 0.20 c-e	93.0 ± 1.7 e	31.3 ± 1.9 b-f	0.765 ± 0.0045 d-g
0.5	Rose	5.2 ± 0.27 c-e	87.2 ± 3.0 ef	27.7 ± 1.8 e-g	0.761 ± 0.0034 gh
0.5	Green	5.2 ± 0.36 c-e	89.8 ± 2.28 ef	32.0 ± 1.8 b-f	0.762 ± 0.0041 f-h
1.0	Jar	5.0 ± 0.16 d-f	71.8 ± 1.6 g	27.1 ± 1.6 f-h	0.773 ± 0.0046 a-e
1.0	Round Magenta	5.2 ± 0.14 c-e	108.1 ± 1.3 b-d	32.3 ± 2.2 b-d	0.778 ± 0.0027 ab
1.0	White	4.9 ± 0.11 ef	106.5 ± 1.1 cd	25.6 ± 1.2 gh	0.774 ± 0.0029 a-e
1.0	Yellow	5.0 ± 0.0 d-f	104.1 ± 1.8 d	28.3 ± 1.2 c-g	0.765 ± 0.0036 d-g
1.0	Rose	5.7 ± 0.28 a-d	109.2 ± 1.6 b-d	26.3 ± 1.1 gh	0.754 ± 0.0028 h
1.0	Green	5.6 ± 0.24 b-e	109.8 ± 3.2 b-d	27.7 ± 1.3 d-g	0.764 ± 0.0028 a-d
Significance	PBZ	**	***	*	NS
Significance	Vessel	**	***	***	***
Significance	PBZ × vessel	*	***	***	**

ⁱThe values represent the mean ± standard error of nine replicates.

ⁱⁱValues followed by the same letters in the same column are not significantly different.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively, according to analysis of variance followed by Duncan's multiple range tests.

Fm = maximum fluorescence; Fv = variable fluorescence; SPAD = soil plant analysis development.

thereby restricting xylem transport and leading to uneven or limited PBZ accumulation at its active site. Integrating this mechanistic framework explains why the interactive effects between vessel characteristics and chemical concentrations were so pronounced in our study, as the physical properties of the container modulated directly the biochemical efficacy of the growth regulator.

Increased antioxidant enzyme activity is not always beneficial and does not always indicate improved physiological quality or enhanced growth and development. It may also indicate stress induction causing severe physiological stress. If a spike in antioxidant enzyme activity happens, the tissues are subjected to an overproduction of destructive ROS (Kusvuran et al. 2016; Rajput et al. 2021). In this case, the plant will force a change in its metabolic pathway from growth and development to produce SOD and CAT just to survive under the oxidative stress (Mishra et al. 2023). Therefore, high enzyme activity can be a sign of a suboptimal, stressful microenvironment rather than improved acclimatization capacity. However, increased antioxidant activity could be translated directly to high survival rates and micropropagation success if it prepares the in vitro-raised plants for the next stage of development at ex vitro acclimatization. If a treatment increases the antioxidant slightly without stunting growth, it would be beneficial for giving the plant a greater survival rate during greenhouse transplantation (Mishra et al. 2023).

The antioxidant status of in vitro 'Grand Nain' banana plantlets was influenced significantly by the vessel type and PBZ concentration, showing enzyme-specific responses. Our results indicate that glass jars provide a suitable microenvironment for improving the

antioxidant status of in vitro plantlets, particularly when supplemented with 0.5 and 1.0 mg·L⁻¹ PBZ. Furthermore, the higher SPAD index observed in these treatments suggested enhanced photosynthetic and metabolic activity, which promoted superior growth and development, ultimately leading to high-quality banana transplants. Zero PBZ could be recommended for local cultivation of in vitro-propagated 'Grand Nain' banana transplants. However, in the case of exporting in vitro banana transplants, PBZ should be applied. It increases stem diameter, produces more compact transplants, and reduces leaf area, which decreases the transpiration rate and water loss. All these characteristics make the plantlets healthy and strong enough to survive until arrival. Magenta vessels are preferred for exportation to reduce shipping costs, whereas glass jars are fragile and not recommended in this case.

The distinctive contribution of our study lies in successfully decoupling the complex interaction between vessel-induced microenvironments and PBZ-mediated growth regulation—an approach previously undocumented for this specific cultivar. By proving that optimal transplant quality and a 100% survival rate can be achieved entirely without chemical retardants and simply through vessel selection, our protocol offers a highly original, standardized, and cost-effective framework for mass banana production.

Conclusion

Optimization of growth regulator concentrations and environmental conditions is essential to maximize the efficiency of the micropropagation protocol and ensure successful field establishment of tissue-cultured banana plants. Our

findings demonstrate that the interaction between culture vessel characteristics and chemical regulation is decisive, with round Magenta vessels without PBZ supplementation representing the most favorable treatment for the in vitro rooting stage using our experimental setup. This specific combination successfully bypassed the potential negative trade-offs of root suppression, supporting 100% survival and high-quality transplants during subsequent acclimatization. Furthermore, based on our results and previous investigations, we conclude that the interactive effect of PBZ and vessel type on morphological and physiological parameters related to transplant quality is highly dependent on the microenvironment and the cultivar. The results of our study offer insight into the cost-effective mass propagation of high-yielding and high-quality planting material for 'Grand Nain' bananas to enhance agricultural sustainability and help meet the increasing demands of a growing population.

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