



The Effect of Manganese Fertilizer, Gandasil D, and Vitamin B₁ on the Acclimatization of Cavendish Banana Seedlings

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Abstract: Acclimatization is a critical stage in the cultivation of Cavendish banana, particularly in the adaptation of tissue culture-derived seedlings to external environmental conditions. Essential micronutrients such as manganese play an important role in supporting this process. This study aimed to evaluate the effects of manganese fertilizer concentrations, types of fertilizers (Gandasil D and vitamin B₁), and their interactions on the acclimatization of Cavendish banana seedlings. The research was conducted at the *In Vitro* Culture Laboratory of KB TPH Salaman, Magelang Regency, at an altitude of 360 meters above sea level, from October to December 2021. A two-factor Completely Randomized Design (CRD) was used, with manganese concentrations (0, 1, 2, 3, and 4 ppm) as the first factor, and types of fertilizers (control, Gandasil D only, and Gandasil D + vitamin B₁) as the second factor. The results showed that manganese concentration alone did not significantly affect plant height, number of leaves, leaf area, photosynthesis rate, transpiration, stomatal conductance, photosynthetically active radiation, and total chlorophyll content. However, the interaction between manganese concentration and fertilizer type had a highly significant effect on chlorophyll a, chlorophyll b, and the chlorophyll a/b ratio. These findings highlight the importance of combining micronutrient application with appropriate fertilizer types to enhance the efficiency of Cavendish banana seedling acclimatization.

Introduction

Banana (*Musa* spp.) is a globally important horticultural crop, serving both as a staple food and a key export commodity in many tropical countries (1, 2). In Indonesia, banana production reached 8.1 million tons in 2017, contributing substantially to domestic consumption and international trade, particularly with China, Japan, and Oman (3). Among its cultivars, the Cavendish banana is highly valued for its stable yield and suitability for export markets (4, 5). However, conventional propagation methods are inadequate to meet large-scale demand due to limited multiplication rates and high susceptibility to pests and diseases (6). Tissue culture techniques, particularly micropropagation, provide a scalable solution for producing genetically uniform and disease-free seedlings (7, 8). Despite these advantages, the acclimatization phase remains a major bottleneck in micropropagation because plantlets exhibit physiological immaturity and are highly vulnerable to environmental stress (9, 10).

Acclimatization involves a transition from *in vitro* to *ex vitro* conditions, exposing plantlets to lower humidity, higher light intensity, and increased microbial pressure (11, 12). These abrupt changes frequently cause physiological

disorders, including persistent stomatal opening, insufficient cuticular wax deposition, and reduced photosynthetic efficiency, which can lead to high mortality (13, 14). Previous studies on bananas and other tissue-cultured crops show that these failures are closely linked to weak stomatal regulation and limited photosynthetic pigment development, highlighting the need for improved nutrient support at this stage (15). Micronutrients, particularly manganese (Mn), play a critical role in overcoming these constraints. Mn is a key cofactor in the oxygen-evolving complex of photosystem II and is essential for chlorophyll biosynthesis and overall metabolic activity (16). Deficiency or excess Mn can impair these processes, making its supplementation during acclimatization both promising and challenging (17). Recent studies have emphasized the importance of carefully managed Mn supplementation in tissue-cultured crops, such as sugarcane and rice, to improve photosynthetic efficiency and seedling survival under *ex vitro* stress. In parallel, foliar fertilizers like Gandasil D and growth-supporting compounds such as vitamin B₁ have been reported to improve nutrient uptake and stress tolerance in tissue-cultured seedlings (18). However, their combined effects, particularly in banana acclimatization, remain poorly understood.

This study addresses this knowledge gap by evaluating

the interactive effects of manganese concentrations and the application of Gandasil D and vitamin B₁ on the acclimatization performance of Cavendish banana plantlets. Specifically, we ask whether the combined application of Mn with foliar fertilizers can enhance chlorophyll content and photosynthetic activity, thereby improving plantlet survival and growth. We hypothesize that intermediate Mn concentrations, when integrated with Gandasil D and vitamin B₁, will optimize physiological performance during acclimatization.

Methodology

Research Time and Location

This study was conducted from October to December 2021 at the *in vitro* tissue culture laboratory of KB TPH Salaman, Magelang Regency. During acclimatization, the ambient growing conditions were maintained at an average temperature of 25–28 °C, relative humidity of 70–80%, and natural light intensity supplemented with 40–50% shading to prevent excessive radiation stress.

Materials and Equipment

The equipment used included a trowel, hand sprayer, tweezers, bucket, tray, plastic cups, plastic wrap, ruler, stationery, rubber bands, plastic tubs, analytical balance, and the CI-340 handheld photosynthesis system. The materials consisted of Cavendish banana plantlets, fungicides, bactericides, manganese fertilizer, sand, rice husk charcoal, cocopeat, and water.

Experimental Design

A factorial experiment was conducted with two factors: (1) manganese fertilizer concentration at five levels [0 ppm (M0), 1 ppm (M1), 2 ppm (M2), 3 ppm (M3), and 4 ppm (M4)], and (2) fertilizer type with three treatments [no Gandasil D or vitamin B₁ (V0), Gandasil D only (V1), and Gandasil D + vitamin B₁ (V2)]. The 15 treatment combinations were arranged in a completely randomized design (CRD) with three replications (see **Table 1**).

Randomization was carried out by assigning plantlets to treatments using a random number generator to ensure unbiased allocation. Within each replication, plantlets were placed randomly on growth trays to minimize positional effects (e.g., variation in light or airflow within the growth area).

The choice of three replications per treatment was based on prior acclimatization studies in bananas and other tissue-cultured crops, which demonstrated that this level of replication provides sufficient statistical power to detect treatment differences given the relatively low within-treatment variability observed under controlled conditions.

Research Implementation

The planting medium was a mixture of sand, rice husk

charcoal, and cocopeat (2:1:1) following Augustien (2019). The medium was sterilized with a bactericide solution, placed in 12-oz plastic cups with drainage holes.

Cavendish banana plantlets with established roots, stems, and leaves were carefully removed from culture bottles, washed to remove residual medium, and briefly air-dried. Plantlets were planted into 1.5 cm deep holes and covered with transparent plastic domes secured with rubber bands to maintain high humidity. Covers were removed on day 14.

Maintenance included watering as needed to maintain moisture without causing waterlogging, weekly foliar spraying of fertilizer solution for 40 days, and manual pest and weed control. Dead plantlets within the first two weeks were replaced with reserve plantlets planted simultaneously.

Data Collection

Data collection began after plastic covers were removed. Growth parameters (plant height and number of leaves) were recorded weekly. Physiological parameters—leaf area, photosynthetic rate, transpiration, stomatal conductance, photosynthetically active radiation (PAR), and chlorophyll content—were measured at 40 days after planting.

Leaf area was estimated by the gravimetric method (Irwan, 2017). Photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$), and stomatal conductance were measured with the CI-340 Handheld Photosynthesis System. PAR was recorded in $\mu\text{mol m}^{-2} \text{s}^{-1}$. Chlorophyll content was determined from acetone extracts (80%) of 1 g fresh leaf tissue using a spectrophotometer at 645 nm and 663 nm to calculate chlorophyll a, chlorophyll b, and total chlorophyll.

Observation Parameters

The observation parameters in this study included seedling height, number of leaves, and leaf area, each serving as a measure of growth and physiological response of Cavendish banana plantlets during the acclimatization process.

Seedling height was measured weekly, starting from the removal of the plastic covers until the end of the study period. Using a ruler, height was measured from the base of the stem to the tip of the tallest leaf. This parameter provided an essential indicator of vertical growth performance in the new environment following *in vitro* culture.

The number of leaves was also recorded every week, counting only newly emerged leaves that had fully opened. Only fully expanded leaves were considered, as they play an active role in photosynthesis and reflect real physiological development in the acclimatized plantlets.

Leaf area was measured at the end of the experiment to determine the total photosynthetic surface of each sample plant. The gravimetric method described by Irwan (2017) was used, where leaf patterns were traced on HVS paper, cut, and weighed. These weights were then compared to the known weight and area of a reference paper to estimate total leaf area. This parameter was particularly valuable in evaluating the photosynthetic capacity of the plantlets throughout acclimatization.

Observation of physiological parameters was carried out systematically to obtain a comprehensive understanding of the adaptation process in Cavendish banana plantlets during the acclimatization phase. One of the key parameters measured was the photosynthetic rate ($\mu\text{mol/m}^2/\text{s}$), which reflects the plant's efficiency in capturing light energy for

Table 1. Treatment combinations of manganese fertilizer concentration and fertilizer type in a 5×3 factorial design.

Fertilizer Type	Fertilizer Concentration				
	M0	M1	M2	M3	M4
V0	M0 V0	M1 V0	M2 V0	M3 V0	M4 V0
V1	M0 V1	M1 V1	M2 V1	M3 V1	M4 V1
V2	M0 V2	M1 V2	M2 V2	M3 V2	M4 V2

synthesizing food. This rate is calculated as the difference between total carbon assimilation during photosynthesis and the respiration rate, measured at the end of the acclimatization period (week 6) using the CI-340 Handheld Photosynthesis System.

In addition to photosynthesis, transpiration ($\text{mmol/m}^2/\text{s}$) served as an important indicator in assessing water regulation and stomatal efficiency. Transpiration refers to the total amount of water vapor released through the leaf stomata and was measured using the same instrument and at the same time. This parameter is closely related to stomatal conductance, which indicates the stomata's capacity to facilitate gas exchange (CO_2 and O_2) between the leaf tissue and the atmosphere. Together, these three physiological indicators provide critical insights into the plantlet's ability to adapt to non-sterile environmental conditions.

To further understand the photosynthetic capacity of the plantlets, photosynthetically active radiation ($\mu\text{mol/m}^2/\text{s}$) was also measured. This parameter represents the amount of solar radiation absorbed by the plant that can be used for photosynthesis. The higher the value of active radiation, the greater the plant's potential to convert light energy into chemical energy.

All physiological data were supported by the measurement of chlorophyll content (mg/g), which was performed on the 40th day after planting. Chlorophyll content was assessed by extracting 1 gram of fresh leaf tissue with 80% acetone, followed by filtration and analysis using a spectrophotometer at wavelengths of 645 nm and 663 nm. Absorbance values at these wavelengths were used to calculate chlorophyll a, chlorophyll b, and total chlorophyll content. High chlorophyll levels serve as a vital indicator of photosynthetic capacity, as chlorophyll plays a direct role in capturing light energy.

By integrating these physiological observations with pigment content analysis, a holistic overview was obtained regarding the effectiveness of the acclimatization process and the impact of nutrient treatments on the performance of Cavendish banana plantlets.

Data analysis

Data were analyzed by analysis of variance (ANOVA) at 5% and 1% significance levels. Significant effects were further tested with orthogonal polynomial analysis to identify response patterns among treatments.

Results
Overview

Observation data are presented in the appendix. The observed variables were analyzed using analysis of variance (ANOVA) with Microsoft Excel, and the data are summarized in the **Table 2**.

The results of the analysis of variance indicate that the applied manganese fertilizer doses did not have a significant effect on variables such as plant height, number of leaves, photosynthesis rate, transpiration, stomatal conductance, chlorophyll content, leaf area, and photosynthetically active radiation. However, the interaction between manganese fertilizer and vitamin B₁ showed a significant effect on plant height and a highly significant effect on chlorophyll content.

Growth Parameters

The results showed that manganese concentration alone did

Table 2. Analysis of variance (F-values) for observed variables showing the effect of manganese fertilizer dose and vitamin B₁ on the growth of Cavendish banana plantlets.

Observed Variable	F-value Manganese (M)	F-value Vitamin B ₁ (V)	F-value Interaction (M×V)
Plantlet height	1.34	3.06	1.00
Number of leaves	0.35	0.72	0.32
Photosynthesis rate	0.08	0.36	1.04
Transpiration	0.17	0.54	0.95
Stomatal conductance	0.18	0.44	0.78
Chlorophyll a content	2.58	0.06	3.31*
Chlorophyll b content	2.58	0.15	3.31*
Chlorophyll a/b ratio	3.41	2.25	5.36*
Leaf area	2.52	1.00	2.06
Photosynthetically active radiation (PAR)	2.22	0.02	0.36

Note: * indicates a highly significant effect at $\alpha = 1\%$

not significantly affect seedling height, number of leaves, or leaf area. Across treatments, growth responses were relatively uniform, suggesting that under the tested range (0–4 ppm), Mn was not a primary limiting factor for morphological development. This may be associated with the relatively short duration of acclimatization (40 days), during which plantlets rely more heavily on carbohydrate reserves from *in vitro* culture rather than new nutrient uptake. Similar findings have been reported in tissue-cultured bananas, where morphological growth during early acclimatization is often less responsive to mineral supplementation compared to physiological traits (Hapsari *et al.*, 2022). Thus, while plant height and leaf number are important indicators of growth, they may not fully capture treatment effects during the early acclimatization stage.

Physiological Parameters

Unlike growth parameters, the interaction between Mn concentration and fertilizer type significantly influenced chlorophyll a, chlorophyll b, and the chlorophyll a/b ratio. These findings highlight the importance of integrated nutrient management in supporting physiological adaptation. In particular, the combination of 2–3 ppm Mn with Gandasil D + vitamin B₁ increased chlorophyll a content by up to 25% compared to the control. Higher chlorophyll levels are directly linked to improved light capture and photosynthetic efficiency, which in turn enhance the energy supply required for acclimatization, survival, and subsequent growth. In banana plantlets, stronger chlorophyll development has been associated with higher survival rates and faster establishment in ex vitro environments (Suryanto *et al.*, 2023).

The chlorophyll responses followed a sigmoid trend with respect to Mn concentration, peaking at intermediate levels and declining at higher concentrations (4 ppm). A quadratic regression fitted to the data (R^2 values ranging from 0.81 to 0.89) confirmed this trend, indicating an optimal range for Mn supplementation. Excess Mn may interfere with nutrient balance or induce oxidative stress, which could explain the decline observed at higher concentrations. These results reinforce the importance of maintaining Mn within a narrow

physiological window to maximize benefits while avoiding toxicity.

Photosynthetic and Gas Exchange Parameters

No significant treatment effects were observed for photosynthetic rate, transpiration, stomatal conductance, or PAR. These outcomes may be associated with the relatively immature stomatal regulation of tissue-cultured plantlets, which often constrains gas exchange regardless of nutrient supplementation. It is also possible that the acclimatization period was too short to detect measurable differences in these parameters. Similar patterns have been reported in other micropropagated crops where photosynthetic performance improves more gradually during extended *ex vitro* growth (Rahman *et al.*, 2022). Thus, while Mn and fertilizer combinations clearly improved pigment content, their influence on gas exchange efficiency may emerge later under prolonged cultivation.

Practical Implications

From an applied perspective, the observed improvement in chlorophyll content at moderate Mn concentrations combined with Gandasil D + vitamin B₁ has practical value for tissue culture nurseries and commercial banana propagation. Higher chlorophyll levels can enhance seedling survival during acclimatization, reduce mortality, and shorten the time required before transfer to the field. By identifying the optimal Mn concentration range (2–3 ppm), nurseries can optimize resource use, avoiding both under-application (inefficient acclimatization) and over-application (risk of toxicity and cost inefficiency). Such targeted nutrient management contributes to more reliable and cost-effective large-scale Cavendish banana production, particularly for export-oriented supply chains where uniformity and survival rates are critical.

Figure Quality and Labeling

To aid interpretation, figures have been revised with clearer axis labels, standardized units (e.g., mg/g FW for chlorophyll content), and annotations of significant differences using letters based on post-hoc tests. This formatting ensures that statistical differences and biological trends are easily understood by readers.

Summary of Findings

Overall, the results demonstrate that while Mn concentration alone had limited effects on morphological growth, its interaction with fertilizer type had a significant impact on physiological adaptation, particularly chlorophyll content. These changes have clear implications for plantlet survival and commercial application. By strengthening chlorophyll development during acclimatization, Mn supplementation in combination with appropriate foliar fertilizers can enhance the efficiency and success of banana micropropagation systems.

Discussion

Manganese Fertilizer Concentration

The acclimatization study on banana plantlets was conducted using five different manganese concentrations: 0 ppm, 1 ppm, 2 ppm, 3 ppm, and 4 ppm over 40 days after the plantlets were removed from culture bottles. The results indicated that the varying manganese concentrations had no significant effect on plantlet height, number of leaves, leaf

area, photosynthesis rate, transpiration, stomatal conductance, chlorophyll content, or photosynthetically active radiation.

The lack of a significant effect on plant height is likely due to the manganese doses being insufficient to meet the nutritional needs of the plantlets during their growth stage (19). Additionally, the small size of the plantlets and their still immature metabolic processes limit nutrient uptake efficiency. Similarly, the number and size of leaves were unaffected by manganese concentration treatments because the levels used were not adequate to support effective leaf development. Manganese deficiency can cause chlorosis in young leaves, characterized by a color change from green to yellow and eventually white, followed by leaf tissue death and drying. (20). The photosynthesis rate also remained unchanged since manganese was not optimally utilized by the plants, resulting in suboptimal leaf development and chlorophyll activity, which are critical for photosynthesis. This is attributed to the low metabolic activity in the plantlets, leading to ineffective manganese absorption.

Type of Fertilizer

The second treatment in this study involved the use of two fertilizers: vitamin B₁ and Gandasil D, applied to banana plantlets. Vitamin B₁ functions to support plant metabolic processes, particularly during transplanting to a new medium, while Gandasil D provides essential nutrients for growth. These two fertilizers were tested both individually and in combination. However, the results indicated that neither the individual nor the combined application had a significant effect on any of the observed growth parameters.

Plant height is a sensitive growth indicator influenced by environmental conditions and growing media. (21). The absence of significant differences in plant height in this study may be due to the presence of additional nutrients that interfered with the uptake of Gandasil D and vitamin B₁ (22). The availability of nutrients directly affects both leaf number and surface area. Excessive application of Gandasil D and vitamin B₁ could hinder the absorption of other essential nutrients. Reich P. and Walters M. (1994) stated that adequate nitrogen encourages leaf expansion, increasing the surface area for photosynthesis. (23). However, excessive nitrogen can lead to larger but softer cells and tissues, which in turn hinders chlorophyll formation. (24).

Chlorophyll production in plants treated with Gandasil D and vitamin B₁ did not show any significant differences. This may be influenced by the plant's genetic factors. Although the treatment did not increase chlorophyll content, it is assumed to have activated genes in the apical and axillary meristem regions, promoting leaf development (25). Collini E. (2019) stated that genetic variation affects a plant's ability to synthesize carotenoids, which are accessory pigments in photosynthesis and contribute to leaf coloration. (26). While photosynthesis rates were not directly affected, the application of Gandasil D and vitamin B₁ may have enhanced carotenoid development, giving the leaves a fresh green appearance.

Overall, all treatments involving Gandasil D and vitamin B₁ similarly responded to by Cavendish banana plantlets during acclimatization. This uniformity is likely due to the plantlets still having immature metabolic systems. According to Santos (2013), vitamin B₁ primarily stimulates root growth by promoting cell division in the root meristem, but it is not yet effective in stimulating growth at the shoot or leaf apex.

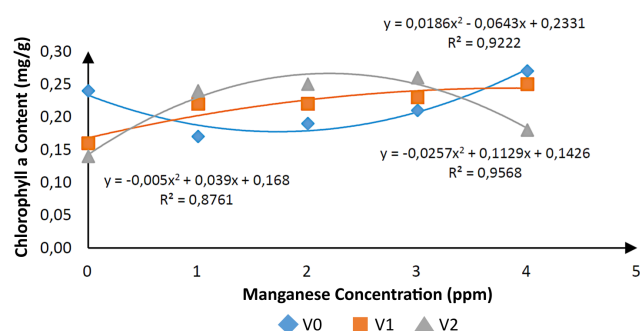


Figure 1. Average chlorophyll a content of Cavendish banana seedlings under different manganese concentrations and fertilizer treatments.

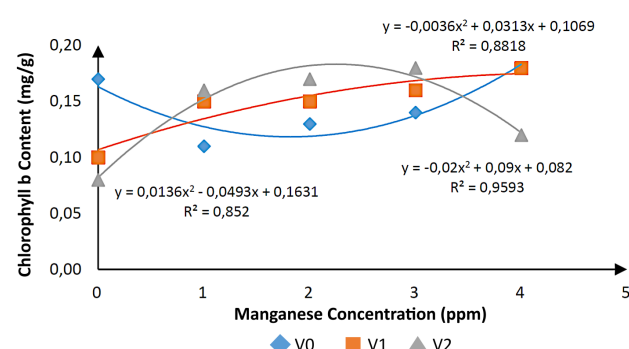


Figure 2. Average chlorophyll b content of Cavendish banana seedlings under different manganese concentrations and fertilizer treatments.

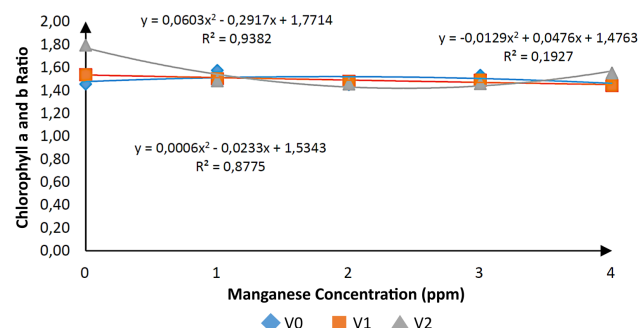


Figure 3. Ratio of Chlorophyll a and Chlorophyll b.

Interaction Between Manganese Concentration and Gandasil D with Vitamin B₁

The analysis of variance revealed that the interaction between manganese fertilizer and vitamin B₁ had a highly significant effect on the chlorophyll content in the plant leaves.

Chlorophyll (a)

Based on **Figure 1**, the amount of chlorophyll a increased with the combination of manganese fertilizer, vitamin B₁, and Gandasil D from 0 ppm to 2 ppm. However, as the concentration increased to 4 ppm, the level of chlorophyll a decreased. Chlorophyll a plays a significant role in enhancing the rate of photosynthesis in plants (9).

Chlorophyll (b)

Based on **Figure 2**, the combination of manganese treatment with vitamin B₁ and Gandasil D led to an increase in chlorophyll b content from 0 ppm to 2 ppm. However, as the concentration continued to rise to 4 ppm, the level of chlorophyll b began to decline. Chlorophyll b plays an important role in contributing to the total chlorophyll content in plants. This study indicates that applying vitamin B₁ and Gandasil D is more effective when combined with specific concentrations of manganese fertilizer to enhance chlorophyll content, with the resulting graph forming a sigmoid curve (10).

Chlorophyll (a) to (b) ratio

According to **Figure 3**, treatments without the addition of vitamin B₁ and Gandasil D showed a slight increase in the chlorophyll a/b ratio at concentrations of 1 and 2 ppm, followed by a decline at 3 and 4 ppm. The treatment with Gandasil D alone (without vitamin B₁) showed a consistent decrease in the ratio as manganese concentration increased. In contrast, the combined application of Gandasil D and vitamin B₁ led to a decrease in the ratio from 0 to 2 ppm, followed by an increase at concentrations of 3 and 4 ppm. Dou H. et al. (2019) stated that chlorophyll is a group of pigments responsible for photosynthesis in plants, absorbing red, blue, and violet light while reflecting green light, which gives plants their green coloration. (27). According to Packer N. et al. (1987), plants contain two main types of chlorophyll: chlorophyll a (dark green) and chlorophyll b (light green) (28). These pigments absorb light most effectively in the red spectrum (600–700 nm) and least effectively in the green range (500–600 nm) (29). Chlorophyll has three main roles in the photosynthetic process: capturing solar energy, initiating CO₂ fixation to form carbohydrates, and providing energy for the broader ecosystem (30). Chlorophyll absorbs electromagnetic radiation within the visible light spectrum. While sunlight contains all visible wavelengths, chlorophyll does not absorb all of them equally well (31).

The interaction between manganese concentration, Gandasil D, and vitamin B₁ had no significant effect on plant height, leaf number, leaf area, photosynthetic rate, transpiration, stomatal conductance, or photosynthetically active radiation. This is likely due to the small size and underdeveloped metabolic systems of the banana plantlets, which limited their ability to absorb and utilize nutrients such as manganese. All Gandasil D and vitamin B₁ treatments elicited similar responses in Cavendish banana plantlets during acclimatization. According to Prihastanti E. et al. (2024), vitamin B₁ plays a greater role in root development by promoting cell division in root meristems, but it is less effective in stimulating apical or leaf bud growth. (32).

Conclusion

Based on the analysis and discussion, it can be concluded that manganese fertilizer concentration alone did not significantly affect the growth parameters of Cavendish banana seedlings during acclimatization, including plant height, number of leaves, leaf area, photosynthetic rate, transpiration, stomatal conductance, photosynthetically active radiation, and total chlorophyll content. Similarly, the application of Gandasil D combined with vitamin B₁ did not produce significant effects on these growth variables. However, the interaction between manganese concentration and fertilizer type had a highly significant effect on

chlorophyll a, chlorophyll b, and their ratio, indicating that while vegetative growth was generally unaffected, the physiological responses related to photosynthetic pigmentation were influenced by specific treatment combinations.

From a practical perspective, these findings suggest that targeted supplementation of manganese within the optimal range (2–3 ppm), combined with Gandasil D and vitamin B₁ foliar application, can enhance chlorophyll development and potentially improve the efficiency of acclimatization protocols in tissue-cultured banana seedlings. Such an approach could increase seedling survival rates and reduce production losses in commercial nurseries.

This study was limited by its relatively short acclimatization period (40 days) and the use of three replications, which may not fully capture long-term plant responses or broader variability. Future research should extend the acclimatization period, increase replication, and test Mn and foliar fertilizer combinations across different developmental stages and under field conditions to better understand their long-term effects and practical scalability.

In conclusion, integrating Mn supplementation with appropriate foliar fertilizer regimes holds promise for improving the success and efficiency of banana micropropagation systems, while further studies are needed to refine these strategies for diverse production settings.

This study clearly demonstrates its objectives and provides key findings that can guide both scientific understanding and practical applications in banana tissue culture acclimatization.

Declarations

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Contribution: Data Curation, Formal analysis, Visualization, Writing - Original Draft, Writing - Review & Editing.

Conflict of Interest

The author declares no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

Not applicable.

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Additional Information

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