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Acclimatization of micropropagated seedlings of banana cv. 'Prata Catarina' under different environmental conditions

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ABSTRACT: Micropropagated seedlings need to be morphologically, anatomically and physiologically prepared for planting in the field, and the environmental conditions adopted during this acclimatization process are determinant for the successful establishment of seedlings. Height, pseudostem diameter, number of leaves, length of the largest root, fresh and dry matter weights, and accumulation of nutrients in micropropagated seedlings of banana cv. 'Prata Catarina' were evaluated in cultivation under greenhouse, air-conditioned room and shade net tunnel conditions. Micropropagated seedlings subjected to 14 days in a tunnel and 49 days in a greenhouse showed a trend of increase in height, pseudostem diameter, number of leaves and length of the largest root, although no significant differences were observed. On the other hand, for the fresh and dry matter weights and accumulation of nutrients, significant differences were observed between this treatment and the others. It is concluded that, for the acclimatization of micropropagated seedlings of banana cv. 'Prata Catarina', the most suitable condition is the combination of tunnel (with one layer of 50% shade net) for 14 days, followed by 49 days in a greenhouse. These conditions can be adopted by producers when acclimatizing micropropagated banana seedlings.

Key words: biometrics; mineral nutrition; *Musa* spp.; shade net tunnel

Aclimatização de mudas micropropagadas de bananeira cv. 'Prata Catarina' em diferentes condições ambientais

RESUMO: Mudas micropropagadas necessitam ser preparadas morfológica, anatômica e fisiologicamente para o plantio em campo, sendo as condições ambientais adotadas durante este processo de aclimatização determinantes para o sucesso do estabelecimento das mudas. A altura, o diâmetro do pseudocaule, o número de folhas, o comprimento da maior raiz, o peso da massas fresca e seca, e, o acúmulo de nutrientes nas mudas micropropagadas de bananeira cv. 'Prata Catarina' foram avaliados em cultivo em condição de casa de vegetação; sala climatizada e em túnel de sombrite. As mudas micropropagadas submetidas a 14 dias em túnel e 49 dias em casa de vegetação apresentaram tendência de aumento na altura, no diâmetro do pseudocaule, no número de folhas e no comprimento da maior raiz, apesar de não terem sido observadas diferenças significativas. Por outro lado, para o peso das massas fresca e seca e, acúmulo de nutrientes observou-se diferenças significativas entre este tratamento e os demais tratamentos. Conclui-se que, para a aclimatização de mudas micropropagadas de bananeira cv. 'Prata Catarina' a condição mais adequada é a combinação da utilização de túnel (com uma camada de sombrite 50%) por 14 dias, seguido de 49 dias em casa de vegetação. Estas condições são possíveis de serem adotadas por produtores na aclimatização de mudas micropropagadas de bananeira.

Palavras-chave: biometria; nutrição mineral; *Musa* spp.; túnel de sombrite

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Introduction

Banana (*Musa* spp.) is among the main fruit crops produced and consumed in Brazil. Brazil stands out as the fourth largest producer in the world, with 7.06 million tons (IBGE, 2023). The adoption of modern management techniques such as micropropagation, to obtain seedlings with high genetic and phytosanitary quality, is desirable by the producer market in order to ensure high yield (Cardoso et al., 2018; Gupta et al., 2020).

Micropropagated seedlings of banana, before being transplanted to the field, must go through a stage of adaptation known as acclimatization (El-Mahrouk et al., 2019; Sparta & Emilda, 2020). In this stage the seedlings can be grown in different containers, such as trays or tubes, in various types of substrates composed of coconut fiber or soil (Scaranari et al., 2008; Costa Cardoso et al., 2019; Rodrigues et al., 2019; Couto et al., 2022) and receive adequate nutritional supplementation through chemical, organic or biological inputs (Rodrigues et al., 2019; Silva et al., 2019; Couto et al., 2022; Hassan et al., 2022; Sanó et al., 2022). Controlling environmental variables such as radiation, temperature, air humidity, substrate moisture, and wind intensity in the acclimatization period is essential and generally requires greater care in the first 45 days (Rodrigues et al., 2022a; Rodrigues et al., 2023). In the control of environmental variables, the use of shade nets stands out, as they prevent direct incidence of sunlight on the seedlings, improving their quality, survival and development (Pereira et al., 2005; Scaranari et al., 2008; Selvakumar & Parasurama, 2020).

Proper management in the acclimatization stage of micropropagated banana seedlings increases their survival rate, improves variables related to their growth and results in more vigorous and more productive plants. In this context, the objective of the present study was to evaluate the impact of different environmental conditions on the development of micropropagated seedlings of banana cv. 'Prata Catarina', in the acclimatization stage. The selected conditions may be adopted by the production sector of micropropagated banana seedlings.

Materials and Methods

Description of the experimental site, treatments and experimental design

The experiment was conducted between February and June 2019, at the headquarters of Embrapa Tropical Agroindustry (3° 44' S and 38° 33' W), Fortaleza, CE, Brazil. Micropropagated seedlings of banana cv. 'Prata Catarina', completely elongated and rooted, were used in the study. The seedlings were washed in running water, their roots were standardized in size (1 cm), and they were planted in trays with 162 cells (50 mL volume), filled with commercial peat-based substrate.

The seedlings were subjected to the following treatments: T1) 63 days in a greenhouse (with two layers of 50% shade net and micro-sprinkler irrigation); T2) 7 days in a controlled environment (± 28 °C) + 56 days in a greenhouse; T3) 7 days in a controlled environment + 7 days in a tunnel (with one layer of 50% shade net) + 49 days in a greenhouse; T4) 14 days in a controlled environment + 49 days in a greenhouse; and T5) 14 days in a tunnel + 49 days in a greenhouse.

The greenhouse was built with two layers of 50% shade net, the controlled environment consisted of an air-conditioned masonry room, and the 50% shade net tunnel was kept in a greenhouse. After 6 days from the beginning of the experiment, for all treatments, the seedlings were fertilized with NPK 14-14-14 controlled-release fertilizer, at a dose of 5 kg per m^3 of commercial substrate (humic substances and organic carbon).

The experimental design was completely randomized, with 4 replicates, each replicate consisting of 15 seedlings. Irrigation was performed manually once a day in the controlled environment and automatically three times a day both in the greenhouse and in the tunnel. The climatic conditions in the controlled environment, greenhouse and shade net tunnel were monitored with a thermo-hygrometer (temperature and humidity) and a luxmeter (light intensity) (Table 1).

Variables evaluated and statistical analyses

Seedling height (cm), pseudostem diameter (mm) and number of leaves were evaluated at 14, 21, 28, 35, 42, 49, 56 and 63 days after transplantation (DAT). At 63 DAT, the seedlings were collected and evaluated for length of the largest root (cm) and fresh matter weight (g). The seedlings were dried in a forced air circulation oven at 65 °C for 72 h, weighed and ground in an analytical mill (IKA A11 Analytical Mill). To determine the nutrient contents in the aerial part, the samples were subjected to sulfuric digestion for N, nitric-perchloric digestion for P, K, Ca, Mg, S, Na, Cu, Fe, Mn and Zn, and incineration in muffle furnace for B (Rodrigues et al., 2022b).

Table 1. Climatic conditions (mean ± standard error) measured during the experimental period under different conditions, for acclimatization of micropropagated seedlings of banana cv. 'Prata Catarina', at Embrapa Tropical Agroindustry, Fortaleza, CE, Brazil.

Environments	Temperature (°C)		Relative humidity (%)		Light intensity (lux)	
	M	SE	M	SE	M	SE
Tunnel	27.8	1.36	86.7	1.14	5,945.3	1,121.5
Greenhouse	28.7	1.18	83.7	1.79	14,307.0	1,635.0
Controlled environment	28.0	0.33	66.2	2.18	563.0	158.0

M: Mean, SE: Standard error. Accumulated rainfall in the period from March to May 2019 = 1369.6 mm. Source: FUNCEME (2019).

The data were subjected to analysis of variance (ANOVA) and the means were compared by the Scott-Knott test (α = 0.05%) and regression models.

Results and Discussion

The growth variables of the micropropagated seedlings of banana cv. 'Prata Catarina' were influenced by the acclimatization time. However, the data of both number of leaves and pseudostem diameter were not described by first- and second-degree regression models. The height of the banana seedlings was influenced by the interaction between environment and time of evaluation. The heights of the seedlings in the treatments T1, T2 and T5 were described by the first-degree regression model (Figure 1), indicating constant growth rates throughout the evaluation period of 0.043, 0.025 and 0.042 cm day^{-1}, respectively.

The rate of T5 similar to that of T1 indicates that the change in light intensity from 5,945.3 to 14,307.0 lux (Table 1) did not negatively affect the growth during the acclimatization period of 'Prata Catarina' banana seedlings. However, the change in light intensity from 563.0 to 14,307.0 lux in T2 reduced the growth rate of banana seedlings.

The heights of banana seedlings in treatments T3 and T4 were described by the second-degree regression model, indicating a differentiated growth rate throughout the evaluation period. In the T3 treatment, the growth rates of seedling height were 0.047, 0.044, 0.042, 0.039, 0.036, 0.033 and 0.030 cm day^{-1}, and in T4 the values were 0.053, 0.047, 0.041, 0.036, 0.030, 0.025, 0.019 cm day^{-1} in the periods from 14 to 21, 21 to 28, 28 to 35, 35 to 42, 42 to 49, 49 to 56 and 56 to 63 days after transplantation, respectively.

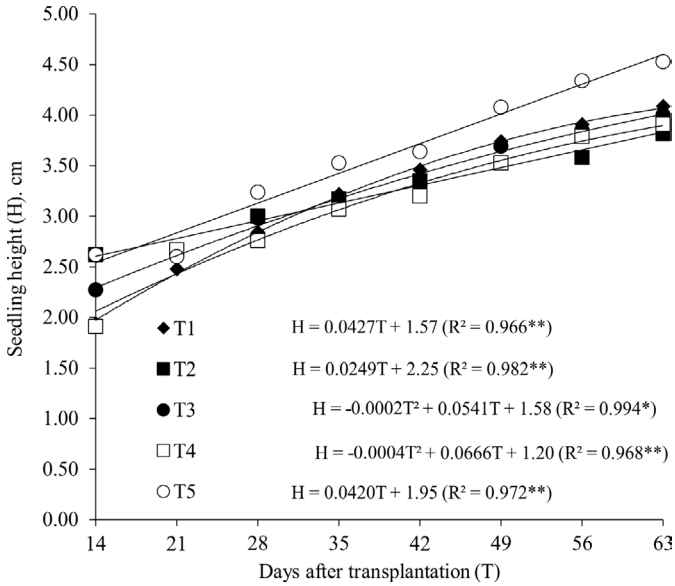


Figure 1. Height of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to different acclimatization conditions. T1) 63 days in a greenhouse; T2) 7 days in a controlled environment + 56 days in a greenhouse; T3) 7 days in a controlled environment + 7 days in a shade net tunnel + 49 days in a greenhouse; T4) 14 days in a controlled environment + 49 days in a greenhouse; and T5) 14 days in a shade net tunnel + 49 days in a greenhouse.

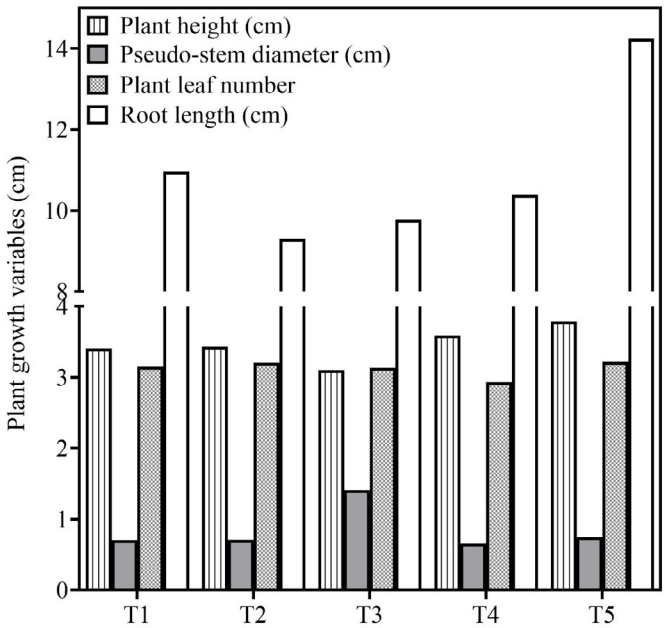
At 63 DAT, the T5 treatment had the best averages for the development of the micropropagated seedlings. However, for seedling height, pseudostem diameter, number of leaves and length of the largest root, no significant differences were observed between the other treatments (Figure 2).

Significant differences between the T5 treatment and the others were observed for the variables fresh and dry matter weights of micropropagated seedlings (Figure 3).

T5 also promoted greater accumulation of nitrogen, phosphorus, calcium and sulfur in the aerial part of the seedlings. However, for phosphorus accumulation, treatments T1 and T3 did not significantly differ from T5 (Table 2).

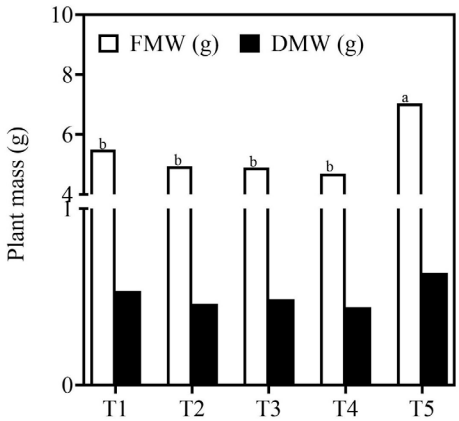
The highest levels of accumulation of boron, copper, iron, manganese and zinc in the aerial part of micropropagated seedlings of banana cv. 'Prata Catarina' were found in the T5 treatment (Table 3).

It was found that the different environmental conditions influenced the acclimatization of micropropagated seedlings of banana cv. 'Prata Catarina'. Seedlings initially subjected to 14 days under tunnel conditions (with one layer of 50% shade net) subsequently transferred to a greenhouse environment



DAT: days after transplantation, [1]T1) 63 days in a greenhouse (with two layers of 50% shade net and micro-sprinkler irrigation); T2) 7 days in a controlled environment (± 28 °C) + 56 days in a greenhouse; T3) 7 days in a controlled environment + 7 days in a tunnel (with one layer of 50% shade net) + 49 days in a greenhouse; T4) 14 days in a controlled environment + 49 days in a greenhouse; and T5) 14 days in a tunnel + 49 days in a greenhouse. [2] Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability level, respectively.

Figure 2. Plant height, pseudostem diameter, number of leaves and root length of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to different conditions of acclimatization environment[1], at 63 DAT. No significant differences were observed between the other treatments.



DAT: days after transplantation, FMW: fresh matter weight, DMW: dry matter weight, [1]T1) 63 days in a greenhouse (with two layers of 50% shade net and micro-sprinkler irrigation); T2) 7 days in a controlled environment (± 28 °C) + 56 days in a greenhouse; T3) 7 days in a controlled environment + 7 days in a tunnel (with one layer of 50% shade net) + 49 days in a greenhouse; T4) 14 days in a controlled environment + 49 days in a greenhouse; and T5) 14 days in a tunnel + 49 days in a greenhouse. [2] Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability level, respectively.

Figure 3. Fresh and dry matter weight of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to different conditions of acclimatization environment[1], at 63 DAT.

(with two layers of 50% shade net) (T5 treatment) showed considerable gains in the different variables related to growth: height, pseudostem diameter, number of leaves, length of the largest root, fresh, dry matter weights and accumulation of macro and micronutrients.

In all treatments, the survival rate reached values close to 100.0%, except for T3 (98.33%). Similar results were found in the acclimatization of seedlings of 'Prata Anã' banana cultivars (Pereira et al., 2005), and tree species (Bonamigo et al., 2016).

In each cultivation environment, depending on climatic conditions and genotypes, variations occur in the management of micropropagated seedlings during acclimatization (Holfmann, 2002; Scaranari et al., 2008; Pandino et al., 2022). This situation is not different for banana, since micropropagated seedlings are sensitive when they leave the *in vitro* conditions (environment with high humidity and restricted gas exchange) to an *ex vitro* condition, thus requiring a period to adapt to the new conditions (Grout, 1988; Scaranari et al., 2008).

Micropropagated banana seedlings in the initial periods of acclimatization require average temperature of 28 °C, relative air humidity above 60% and minimum shading of 80% (Sanó et al., 2022). In this acclimatization stage, photosynthetic stress occurs, as the seedlings need to move from the heterotrophic to the autotrophic phase (Grout, 1988). Thus, acclimatization plays a crucial role in promoting full development of micropropagated banana seedlings. The T5 treatment was the one that provided the micropropagated seedlings of banana cv. 'Prata Catarina' with the best adaptation to the acclimatization conditions.

Table 2. Accumulation of macronutrients and sodium in the aerial part of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to different environmental conditions[1], at 63 DAT during acclimatization.

Treatments	N	P	K	Ca	Mg	S	Na
	(mg per plant)						
T1	7.91 b[2]	0.75 a	7.08 a	7.08 b	5.05 a	0.73 b	0.59 a
T2	7.01 b	0.61 b	6.23 a	5.87 b	4.69 a	0.71 b	0.49 a
T3	7.24 b	0.75 a	6.82 a	6.59 b	5.25 a	0.73 b	0.57 a
T4	7.14 b	0.67 b	6.85 a	4.92 b	3.40 a	0.62 b	0.55 a
T5	9.82 a	0.84 a	8.44 a	8.98 a	6.71 a	1.08 a	0.74 a
				F test[3]			
Treatments	6.09**	5.49**	2.75^{ns}	7.41**	4.43*	7.22**	2.69^{ns}
CV (%)	12.08	10.71	13.97	16.68	22.42	16.97	18.82

DAT: days after transplantation, CV: coefficient of variation, [1]T1) 63 days in a greenhouse (with two layers of 50% shade net and micro-sprinkler irrigation); T2) 7 days in a controlled environment (± 28 °C) + 56 days in a greenhouse; T3) 7 days in a controlled environment + 7 days in a tunnel (with one layer of 50% shade net) + 49 days in a greenhouse; T4) 14 days in a controlled environment + 49 days in a greenhouse; and T5) 14 days in a tunnel + 49 days in a greenhouse. [2] Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability level. [ns]; ** and *: Not significant; significant at 1 and 5% probability levels, respectively.

Table 3. Accumulation of micronutrients in the aerial part of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to different environmental conditions[1], at 63 DAT during acclimatization.

Treatments	B	Cu	Fe	Mn	Zn
	(µg per plant)				
T1	7.21 b[2]	2.40 b	396.62 b	83.02 b	51.07 b
T2	6.23 b	2.60 b	375.25 b	64.43 b	41.42 b
T3	6.70 b	2.05 b	319.70 b	63.33 b	48.81 b
T4	5.84 b	2.30 b	306.42 b	64.06 b	38.04 b
T5	9.23 a	4.08 a	737.33 a	111.90 a	64.88 a
			F test[3]		
Treatments	12.26**	3.29*	12.38**	6.23**	6.04**
CV (%)	10.76	33.00	23.62	21.77	17.35

DAT: days after transplantation, CV: coefficient of variation, [1]T1) 63 days in a greenhouse (with two layers of 50% shade net and micro-sprinkler irrigation); T2) 7 days in a controlled environment (± 28 °C) + 56 days in a greenhouse; T3) 7 days in a controlled environment + 7 days in a tunnel (with one layer of 50% shade net) + 49 days in a greenhouse; T4) 14 days in a controlled environment + 49 days in a greenhouse; and T5) 14 days in a tunnel + 49 days in a greenhouse. [2]Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability level. [3ns]; ** and *: Not significant; significant at 1 and 5% probability levels, respectively.

In the present study, no marked variations in temperature (27.8 to 28.7 °C) were observed under the different environmental conditions for acclimatization of micropropagated seedlings of banana cv. 'Prata Catarina'. However, the relative humidity (66.2 to 86.7%) and luminosity (563 to 14,037 lux) conditions showed considerable variations in the environments, which may interfere in the development of seedlings. This statement can also be confirmed in some studies with micropropagated banana seedlings. Banana seedlings cv. 'Prata Anã' grown in nebulization chambers with temperature above 36 °C showed no temperature sensitivity, but the preponderant factor for their development with quality was the high relative air humidity, on average 90% (Pereira et al., 2005).

Micropropagated banana seedlings show the greatest anatomical changes at 42 days after *ex vitro* transplantation. In this stage, thickening of the chlorophyll parenchyma of the leaf blade and differentiation of most tissues are observed. Prior to this period, the seedlings show smaller epidermis thickness (Costa et al., 2009), small amounts of epicuticular wax and a slow mechanism of stomatal opening/closing. These characteristics favor the loss of water by banana leaves in this stage, thus highlighting the importance of using environmental conditions with high humidity in the early stages of acclimatization (Pinar et al., 2020).

These results mentioned in the literature make it possible to elucidate part of the information obtained in the present study. In T5 (14 days in a shade net tunnel + 49 days in a greenhouse), the micropropagated seedlings were kept in a tunnel (with one layer of 50% shade net) with relative humidity always above 80%, thus showing greater gains in the parameters height, number of leaves, pseudostem diameter, length of the largest root, and fresh and dry matter weights. In addition, they accumulated important macro and micronutrients for better development and reduction of stress when planted in the field. Nutrient accumulations are common in micropropagated banana seedlings when subjected to non-stressful conditions during acclimatization (Rodrigues et al., 2022b; Rodrigues et al., 2023). This treatment is the most suitable for acclimatizing micropropagated seedlings of banana cv. 'Prata Catarina'.

One of the ways to reduce leaf temperature and water losses from the aerial part of micropropagated banana seedlings is the use of shading (Holfmann, 2002; Mahendra et al., 2020; Rodrigues et al., 2022b), for instance with shade net tunnel systems (Scaranari et al., 2008). Low luminosity in the initial periods of development of the micropropagated seedlings followed by an increase of such luminosity promotes increments in aerial part and root dry matter weights and in the number of leaves (Bonamigo et al., 2016). This practice is recommended in the initial periods of development of seedlings during acclimatization, which was confirmed in the present study. Therefore, for the acclimatization of micropropagated seedlings of banana cv. 'Prata Catarina', the most suitable condition is the combination of tunnel (with one layer of 50% shade net) for 14 days, followed by 49 days in a greenhouse. These conditions can be adopted by producers when acclimatizing micropropagated banana seedlings.

Conclusions

Micropropagated seedlings of banana cv. 'Prata Catarina' initially kept for 14 days in a tunnel (with one layer of 50% shade net) and later for 49 days in a greenhouse, during acclimatization, showed better values of the variables related to growth (height, pseudostem diameter, number of leaves, length of the largest root, and fresh and dry matter weights) and accumulation of macro and micronutrients in the aerial part.

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Compliance with Ethical Standards

Author contributions: Conceptualization: PLP, ACPPC, CAKT, WLB, CFBS; Data curation: JNM; Formal analysis: JNM, PLP, ACPPC, CAKT, WLB; Funding acquisition: CFBS; Investigation: JNM; Methodology: CFBS; Project administration: CFBS; Resources: PLP, ACPPC, CAKT, WLB, CFBS; Supervision: CFBS; Writing – original draft: JNM, PLP, ACPPC, CAKT, WLB; Writing – review & editing: JNM, PLP, ACPPC, CAKT, WLB, CFBS.

Conflict of interest: The authors declare there to be no conflict of interest.

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