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ORIGINAL RESEARCH ARTICLE

Effects on the plantain proliferation in two propagation environments brought by corm size and benzylaminopurine

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ABSTRACT

Banana macropropagation in a thermal chamber is an economical technology, effective as a phytosanitary cleaning method, and efficient to enhance seedling production. The objective of this work was to evaluate the effects of corm size (CS) and benzylaminopurine (BAP) on plantain cv. Barraganete seedling proliferation in two propagation environments (PE). The treatments consisted of two levels of BAP (with and without BAP), three CS (2 ± 0.5, 4 ± 0.5 and 6 ± 0.5 kg) and two PE (thermal chamber and raised bed). The variables evaluated were sprouting time (days), multiplication rate (MT) per unit (seedlings per corm) and area (seedlings per m^2). Sprouting time was significantly influenced ($p < 0.05$) by the PE, where the thermal chamber advanced shoot emergence by 12 days, with respect to the raised bed. MT of seedlings per corm and m^2, were significantly influenced ($p < 0.05$) by BAP × AP and TC × AP interactions, where the highest seedling production per corm occurred inside thermal chamber with BAP and 6 ± 0.5 kg corms, while seedling production per m^2 was higher with 2 ± 0.5 kg corms under the same thermal chamber conditions and with BAP. The main effects results reported that with BAP there were 30 and 31% increases in MT per corm and per m^2, respectively, relative to the treatment without BAP. Within the thermal chamber the MT per corm and per m^2 increased by 44% relative to the raised bed. Regarding the effect of CS, larger corms achieved higher individual MT, while smaller corms achieved higher MT per area. The use of a thermal chamber and BAP is recommended for mass production of banana seedlings through macropropagation.

Keywords: Musa AAB; Macropropagation; Rhizome; Thermal Chamber; Phytoregulator

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1. Introduction

The cultivation of plantain (Musa AAB Simmonds) is of great transcendence for Ecuador, given its importance in the food, social and economic order, since 69% of the national production is destined for local consumption, contributing to the country's food security, while the remaining production is destined for export purposes, which generates sources of employment and foreign exchange for the state's coffers[1,2]. The propagation method most commonly used by small and medium producers is natural regeneration, where tillers are extracted directly from commercial plantations without any agronomic and phytosanitary selection criteria, which has as its main disadvantage—the easy spread of pests and diseases, which can significantly reduce the vigor and productive potential of the crop[3,4]. Another disadvantage of the traditional propagation method is the low proliferation rate, which has been related to the apical dominance exerted by mother plants over their offspring[5,6].

Mass production of plants through tissue culture is always the best propagation alternative, due to the high genetic, sanitary and physiological quality of the planting material obtained. Despite the above, most banana producers cannot access this type of planting material, mainly due to its high cost and the higher management requirements involved in the acclimatization of seedlings in nursery stages[7].

Given this scenario, macropropagation in a thermal chamber has been suggested as an alternative method for banana propagation, which consists of selecting tillers from healthy plants with high productive potential, which are then cleaned and disinfected to be placed in propagation chambers after removal of the apical meristem, with the aim of inhibiting apical dominance and accelerating the sprouting of axillary buds[8–10].

In addition, thermal chambers have been suggested as phytosanitary cleaning environments, due to the thermotherapy exerted by the high temperatures reached inside them, since, according to previous studies, pathogens degrade at temperatures below the thermal threshold supported by the vegetative material[11,12]. In this sense, according to research developed by Rodríguez *et al.*[13] and Cedeño *et al.*[9], temperatures between 50 to 70 °C can be reached inside thermal chambers, which contribute to the cleaning of the planting material, and to a rapid induction and proliferation of lateral buds. Therefore, in terms of multiplication rate, macropropagation in a thermal chamber has been considered as an intermediate propagation method between plant production by the traditional method and the tissue culture method[3,8,9].

According to several authors, in order to increase the multiplication rate of musaceae via macropropagation, it is important to consider the size of the rhizome. In this regard, Álvarez *et al.*[8] established that an adequate corm size for the propagation of plantain cv. Hartón in a thermal chamber is between 1 and 2 kg. Koné *et al.*[14] reported that corms of 600 g of the plantain cultivars Corne 1, Orishele and French 2 showed a higher multiplication rate in contrast to corms of lower weight. Likewise, Koné *et al.*[15] obtained higher multiplication rate in plantain (Musa AAB) corms weighing more than 750 g. The effectiveness of phytoregulators in banana macropropagation processes has been demonstrated in several investigations[9,16,17]. In this sense, research developed by Thiemele *et al.*[18] and Cedeño *et al.*[9] concluded that the use of benzylaminopurine (BAP) enhanced the multiplication rate of banana cultivars Corne 1, Orishele, FHIA-21, Pita-3 and banana cv. Williams, in relation to treatments without BAP. Similar results were achieved by Ramírez *et al.*[19] and Opata *et al.*[17] with BAP application on banana rhizomes cv. Saba and KTR subjected to macropropagation in growth chambers.

Previous research related to the use of BAP and the effect of corm size in the propagation of musaceae has been mostly directed to banana, which is why there is a lack of information on banana that does not allow adjusting the technology for small producers. This fact, added to the great demand for quality and low-cost planting material by banana growers, justifies the research. Therefore, the main objective of this work was to evaluate the effects of corm size and benzylaminopurine on the proliferation of plantain cv. Barraganete in two propagation environments.

2. Materials and methods

2.1 Location of research

The research was carried out during the dry season of 2021, at the experimental campus of the Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López, in Calceta, Manabí, Ecuador. The trial was geographically located at the coordinates 0°49′10″ S and 80°10′40″ W, at an altitude of 21 masl and an average ambient temperature of 27.6 °C.

2.2 Plant material

The planting material used were banana corms cv. Barraganete, which were cleaned with a knife in order to remove biological remains of pests and pathogens. They were then immersed in boiling water for 25 seconds for disinfection and sanitary purposes, as suggested by Coyne *et al.*[20]. Next, the apical meristem of the main corm and its second-

ary buds were removed in order to inhibit apical dominance and induce the activation and growth of axillary and multi-bud buds.

2.3 Thermal camera and flower beds

The structure of the thermal chamber and the raised bed were built with local materials (bamboo and wood), with dimensions of 2 × 6 × 1.5 m in width, length and height, respectively, where the thermal chamber was covered with transparent plastic of 0.6 mm thickness. Subsequently, both the plastic of the thermal chamber and the frame of the bed were covered with saran mesh at 50% shade, in order to reduce damage to the seedling tissue by photooxidation. In the interior of the thermal chamber and beds, 1.0 m wide and 0.25 m high beds were built above ground level, which were filled with a substrate composed of rice husks, compost and river sand in a 1:1:1 volumetric ratio. Prior to planting the corms, the substrates were sterilized in metal tanks placed over a fire for six hours, as recommended by Koné *et al.*[14].

2.4 Treatments, design and experimental unit

The experiment was established with a completely randomized design with a factorial arrangement of subdivided plots, where the large plots were assigned the propagation environmental factor (thermal chamber and raised beds), the small plots the hormonal factor (with BAP and without BAP) and the subplots the corm size factor (2 ± 0.5, 4 ± 0.5 and 6 ± 0.5 kg). In total, the experiment was developed with 12 treatments, three replications and 36 experimental units. The experimental unit consisted of plots of 1 m^2, where 12, 9 and 6 corms per m^2 of 2 ± 0.5, 4 ± 0.5 and 6 ± 0.5 kg, respectively, were placed according to the evaluated treatments. Sigma brand 6-benzylaminopurine, with a purity of over 99%, was used as a source of BAP. BAP was applied after disinfecting the corms and inhibiting their apical dominance, placing a solution of 4 mL of BAP per corm at a concentration of 40 mg L^{-1}. The BAP solution was applied in the cavity left in the center of the corm by the extirpation of the growth point. After this procedure, the corms were left for 24 hours in the shade in order to allow the BAP solution to be absorbed by the corm tissue and to avoid its decomposition by solar radiation. Finally, after this period, the corms were placed half-buried on the surface of the substrate of the thermal chamber and raised beds.

2.5 Response variables and data analysis

The variables recorded were days to sprouting, multiplication rate per corm (number of seedlings per corm) and multiplication rate per area (number of seedlings per m^2). Days to sprouting were recorded when 50% of the corms emitted shoots. The multiplication rate per corm was estimated with equation[1], 90 days after emergence of the first shoots. The number of total plants per m^2 was quantified by counting in each experimental unit at 90 days after bud break.

$$MR = \frac{Total\ number\ of\ plants}{Number\ of\ initial\ corms} \quad (1)$$

The data obtained were subjected to analysis of variance and separation of means with Tukey's test, both tests with 5% probability of error. The statistical package InfoStat version 2019 was used.

Specific handling of the experiment

In both trials, corms were fertilized 15 days after planting, placing a single dose of 20 g of a soluble compound fertilizer around each corm. The composition of the fertilizer was N (12%), P_2O_5 (11%), K_2O (18%), MgO (2.7%), S (8%), B (0.015%), Fe (0.2%), Mn (0.02%) and Zn (0.02%). Afterwards, the thermal chamber plastic was left open for 24 hours, in order to avoid the gasification of the fertilizer due to the effect of temperature. Irrigation was carried out manually with the help of a hose, applying water in a superficial drench around the corm until the soil was at field capacity. Finally, in order to avoid rotting of the center of the corm due to the fall of condensed water droplets from the plastic cover and high humidity generated in the thermal chamber, hydrated lime was applied on top of the corm.

3. Results and discussion

According to the analysis of variance, sprouting time was only significantly influenced (p = 0.0001) by the propagation environment (PE) factor,

while the factors benzylaminopurine (BAP), corm size (CS) and their respective interactions did not influence this variable (**Table 1**).

Table 1. Statistical significance of days to sprouting and multiplication rate of banana seedlings per corm and per m^2 of propagation environment.

Source of variation	p-value ANOVA		
	Days to sprouting	Multiplication rate	
		By corm	By area (m^2)
Propagation environment (PE)	0.0001**	0.0001**	0.0001**
Benzylaminopurine (BAP)	0.4737NS	0.0001**	0.0001**
Corm sizes (CS)	0.8142NS	0.0001**	0.0001**
AP × BAP	0.4737NS	0.0001**	0.0001**
AP × TC	0.9917NS	0.0226*	0.0214*
BAP × TC	0.8992NS	0.3144NS	0.2982NS
AP × BAP × TC	0.8141NS	0.567NS	0.8320NS
C.V. %	6.85	7.24	7.41

CV = coefficient of variation; *: significant at 0.05; **: significant at 0.01; NS: not significant at 0.05

This indicates that higher temperature reached inside the thermal chamber stimulated the activation and emergence of axillary buds from the corms, in relation to the raised bed that was kept at room temperature. Inside the thermal chamber, bud sprouting occurred in a shorter time, compared to the raised beds where sprouting was later, with a difference of 12 days (**Figure 1**).

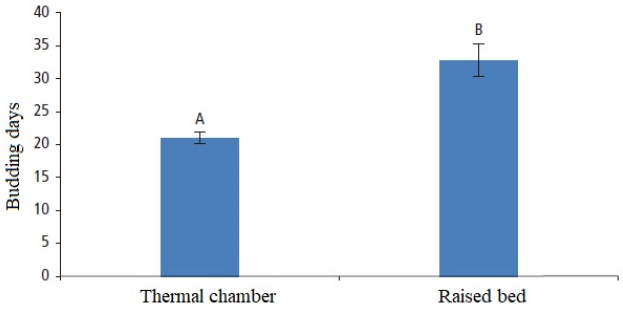

Figure 1. Effect of propagation environment on sprouting time of banana corms cv. Barraganete. Each bar represents the mean of three replications (± standard deviation). Different letters indicate statistical differences between means (Tukey, $p < 0.05$).

The results of sprouting time are close to those reported by Ramírez *et al.*[19], who obtained a sprouting time in banana cv. Saba of 17 days under humid chamber conditions, in contrast to the 20 days of sprouting that occurred in corms placed at ambient temperature and humidity. Similarly, Alvarez *et al.*[8] reported sprouting times in banana cv. Hartón of 18 days under thermal chamber conditions, in relation to the 29 days that sprouting took in raised beds.

Seedling multiplication rates per corm and per area were significantly affected ($p < 0.05$) by the factors propagation environments (PE), benzylaminopurine (BAP) and corm sizes (CS), in addition to PE × BAP and PE × CS interactions, while BAP × CS and PE × BAP × CS interactions were not significant ($p > 0.05$) for multiplication rates (**Table 1**).

Within the AP × BAP interaction, the treatment with BAP in a thermal chamber achieved the highest seedling production per corm, with an increase of 34, 48 and 59% in relation to the treatments without BAP in a thermal chamber, with BAP and without BAP in beds, respectively (**Table 2**). In the AP × TC interaction, the 6 ± 0.5 kg corm in thermal chamber achieved the highest seedling multiplication rate per corm, with a differential increase of 6, 10, 15, 15, 18 and 21 seedlings relative to the 4 ± 0.5 and 2 ± 0.5 kg corms in thermal chamber and the 6 ± 0.5, 4 ± 0.5 and 2 ± 0.5 kg corms in beds, respectively (**Table 2**).

Table 2. Interaction effect between propagation environment (PE) × benzylaminopurine (BAP) and propagation environment (PE) × corm size (CS) on banana seedling multiplication rate per corm at 90 days after sprouting.

AP × BAP interaction		
Benzylaminopurine	**Propagation environment (PE)**	
(BAP)	**Thermal camera**	**Raised bed**
With BAP	36.22 a1/	19.00 c
Without BAP	23.89 b	14.78 d
PE × CS Interaction		
Corm sizes (CS)	**Propagation environment (PE)**	
	Thermal camera	**Raised bed**
2 ± 0.5 kg	25.33 c1/	14.17 e
4 ± 0.5 kg	29.67 b	16.72 of
6 ± 0.5 kg	35.17 a	19.83 d

1/ means with different letters represent separation of means of the interaction between AP × BAP and AP × TC according to Tukey's test at 5% probability of error.

When comparing the main effect of BAP, in-

dependently of the effect of PE and CS, the application of BAP increased seedling production by 30%, with respect to the treatment without BAP (**Figure 2A**). On the other hand, independently of the effect of BAP and AP, with the larger corm size, seedling production increased by 16 and 28%, relative to the 4 ± 0.5 and 2 ± 0.5 kg corms (**Figure 2B**). Moreover, independently of the effect of BAP and TC, seedling production inside thermal chamber showed an increase of 13 seedlings, with respect to the raised bed (**Figure 2C**).

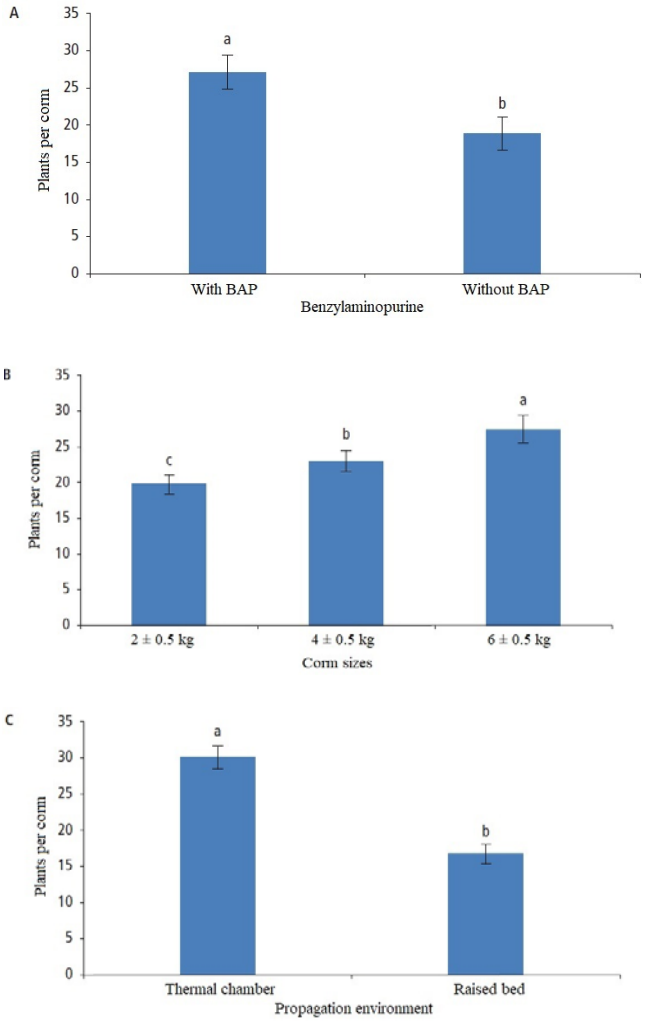

Figure 2. Main effect of benzylaminopurine **(A)**, corm size **(B)** and propagation environment **(C)** on corm multiplication rate.

Within the AP × BAP interaction analysis, the BAP treatment in thermal chamber achieved the highest multiplication rate per area, with 423 seedlings per m^2, which exceeded the thermal chamber treatments without BAP, raised beds with BAP and without BAP with 148, 201 and 255 seedlings per m^2, respectively (**Table 3**). Meanwhile, in the AP × TC interaction, the thermal chamber treatment with 2 ± 0.5 kg corms produced the highest rate of seedling production per m^2, outperforming the 4 ± 0.5 and 6 ± 0.5 corms planted in thermal chamber and the 2 ± 0.5, 4 ± 0.5 and 6 ± 0.5 kg corms established in beds by 6, 17, 44, 48 and 53 %, respectively (**Table 3**).

Table 3. Interaction effect between propagation environments (PE) × becilaminopurine (BAP) and propagation environments (PE) × corm sizes (CS) on seedling multiplication rate per m^2 at 90 days after sprouting.

AP × BAP interaction		
Benzylaminopurine	**Propagation environment (PE)**	
(BAP)	**Thermal camera**	**Raised bed**
With BAP	423.00 a[1/]	222.00 c
Without BAP	274.89 b	167.52 d
PE × CS Interaction		
Corm sizes (CS)	**Propagation environment (PE)**	
	Thermal camera	**Raised bed**
2 ± 0.5 kg	377.68 to[1/]	210.15 c
4 ± 0.5 kg	354.00 a	197.00 c
6 ± 0.5 kg	315.17 b	177.14 d

1/ means with different letters represent separation of means of the interaction between AP × BAP and AP × TC according to Tukey's test at 5% probability of error.

Regarding the main effect of BAP, independently of the effect of AP and TC, the production of seedlings per m^2 was higher with BAP application, with 31% increase, compared to the treatment without BAP (**Figure 3A**). For its part, the main effect of CS, independently of AP and BAP, the highest proliferation of seedlings per m^2 was achieved with the smaller size corms (2 ± 0.5 kg), which produced an additional 18 and 47 seedlings per m^2, with respect to corms of 4 ± 0.5 and 6 ± 0.5 kg, respectively (**Figure 3B**). Likewise, the main effect of AP, independently of the effect of BAP and TC, show that under thermal chamber conditions the multiplication rate per m^2 increased by 44 %, relative to the raised bed (**Figure 3C**).

The results also indicated a directly proportional increase in the seedling multiplication rate per corm with increasing CS, and a seedling multiplication rate per m^2 inversely proportional with increasing CS (**Figure** 4). The higher seedling production in large corms may be due to the fact that these rhizomes have more active buds and nutrient reserves than smaller corms. This may be related to a more advanced physiological state, which could

promote the formation of new tissues and organs[21,22].

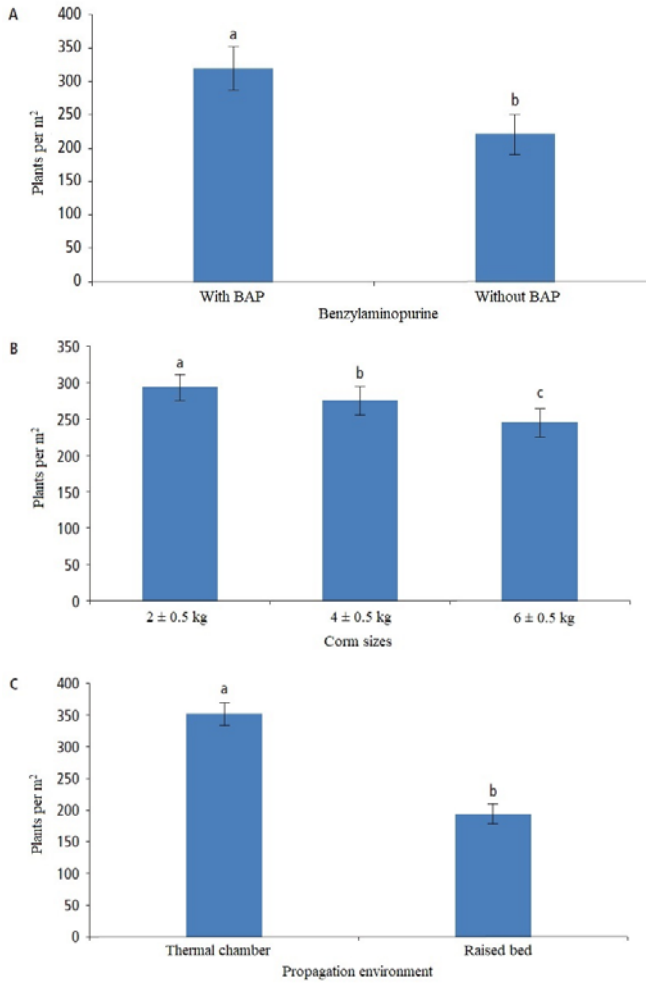

Figure 3. Main effect of benzylaminopurine **(A)**, corm sizes **(B)** and propagation environments **(C)** on the multiplication rate per m^2.

In relation to the above, several authors have concluded that, in several plant species, larger explants and seeds depend less on the nutrients of the environment where they develop, given the greater amount of nutrient reserves in their organs and tissues, which can be used for the growth of new structures[23].

On the other hand, the higher production of seedlings per m^2 obtained in smaller corms can be explained by the greater number of corms contained per thermal chamber and raised bed surface.

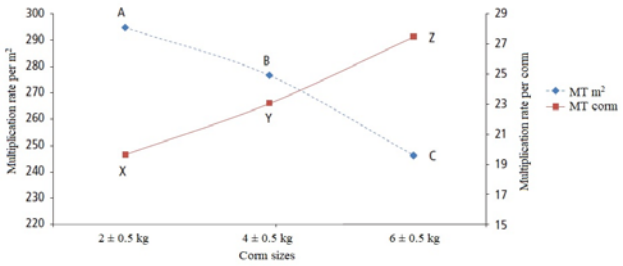

Figure 4. Trend of banana seedling production per corm and per m^2 of area, as a function of the corm sizes evaluated. Different letters indicate statistical differences between corm sizes within each variable (Tukey, $p < 0.05$).

The results achieved in relation to the effect of corm size (CS) are close to those obtained by Koné *et al.*[15] who reported higher seedling production in larger rhizomes. Similarly, the results reported by Ashango[24] concluded that greater seedling proliferation was achieved in 7 kg *Ensete ventricosum* corms, relative to smaller rhizomes. For their part, Baganta *et al.*[6] reported higher plantain seedling production per m^2 as corm size decreased, accounting for 942, 485 and 258 seedlings per m^2, in corms of 1–1.5, 1.6–2.0 and 2.1–4.0 kg, respectively.

On the other hand, the results obtained regarding the effect of BAP are similar to those reported by Thiemele *et al.*[18] and Cedeño *et al.*[9], who, with BAP application, obtained increases in multiplication rates in macropropagated banana of 50 and 80%, respectively, in relation to treatments without BAP. In this same context, similar results were also obtained by Ramírez *et al.*[19] and Opata *et al.*[17], who reported multiplication rates in banana and plantain of 73 and 33 seedlings per corm with BAP application, in relation to treatments without BAP, which obtained lower seedling production. López *et al.*[25] reported that in banana treated with 40 mg L^{-1} of BAP, the production of seedlings per corm and m^2 of thermal chamber increased by 44%, compared to the treatment without BAP.

The higher seedling production in BAP-treated corms may be due to the fact that this regulator modified the cytokinin/auxin hormone balance in favor of cytokinins, which contributed to a higher activation of axillary meristems. This theory was proposed by Skoog and Miller in 1957 and still holds true[26,27]. Furthermore, the application of exogenous cytokinins to organs lacking this hormone

has been shown to induce higher rates of cell division. Moreover, it has been confirmed that auxin-governed apical dominance of meristems is suppressed by high cytokinin concentrations, which favors organogenesis towards axillary bud formation[28,29].

In relation to the propagation environment (PE), the results obtained are similar to those obtained by Álvarez *et al.*[8], who reported a multiplication rate in banana of 90 shoots per m^2 per month inside a thermal chamber, in relation to the 35 shoots per m^2 per month obtained in raised beds. In the same context, results found by Ramírez *et al.*[19], reported a multiplication rate in banana of 54 shoots per corm inside a growth chamber with humidifier, in contrast to the 36 shoots per corm achieved without humidifier. For their part, Ntamwira *et al.*[10] reported on average a production of 13 seedlings per corm under growth chamber conditions, with respect to the 8 seedlings per corm achieved in a conventional bed at room temperature.

The higher seedling production inside thermal chambers compared to raised beds may be due to the fact that the high temperature caused by the plastic accelerated the cellular respiration of the corms, which promoted a higher rate of cell division and growth, leading to a rapid activation of dormant buds and formation of adventitious shoots and callus. In this regard, the effect of temperature on plant growth modification is widely documented[30,31].

4. Conclusions

The use of benzylaminopurine (BAP) was effective in increasing individual corm multiplication rate and increasing seedling production per m^2. The size of the corm influenced the multiplication rate in the thermal chamber and beds, where large corms produced more seedlings per corm, but fewer per m^2. The opposite effect was obtained with small corms. The heat and humidity generated by the thermal chamber was effective in accelerating corm sprouting and enhancing the multiplication rate of banana seedlings. The use of the thermal chamber is recommended as an effective method of banana macropropagation, whose effectiveness can be improved with the use of hormones such as BAP.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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