



## RESEARCH PAPER

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## Controlled environment system and method for rapid propagation of saba banana (*Musa balbisiana*) plantlets

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**Key words:** Growth enhancer, Humidifier, Mist system, Macropropagation, Banana, Benzyl amino purine, Napthalene acetic acid

### Abstract

Conventional propagation practices of banana challenge the production of disease-free planting materials. This study evaluates the use of misting system and different plant growth enhancers, Benzyl Amino Purine at 2mg/l and Napthalene Acetic Acid at 0.93g/L, on plantlet development of Saba banana (*Musa balbisiana*) macropropagated under glasshouse conditions. A total of 36 corms are equally distributed in three propagators. Four growth parameters are observed and analysed using factorial in Completely Randomized Design in first generation plantlets (GP1) and second generation plantlets (GP2). Results show that the use of misting system significantly increased ( $p < 0.01$ ) all the growth parameters tested during the first and second cycles. The growth enhancers significantly shortened the number of days to emergence ( $p < 0.01$ ), (GP1, GP2) and increased the number of shoots emerged ( $p < 0.01$ ) (GP1, GP2), shoot collar diameter ( $p < 0.01$ ) (GP1) ( $p < 0.05$ ) (GP2), and total leaf area ( $p < 0.05$ ) (GP1) ( $p < 0.01$ ) (GP2). The interaction of the two factors has significantly shortened the number of days to emergence ( $P \leq 0.05$ ), produced the most number of shoots ( $P \leq 0.01$ ) and the largest total leaf area ( $P \leq 0.05$ ) in GP2. The findings suggest that the combined use of misting system and plant growth enhancers accelerates the growth of macropropagated Saba banana.

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## Introduction

Banana stands out as the most important fruit crop in the Philippines, constituting a significant portion in the country's export revenue. It is one of the most important sources of food in the rural areas where Saba banana, in particular, is often used to extend, supplement or substitute staple food such as rice and corn.

The main production constraint of banana is the availability of disease-free and affordable banana seedlings. To establish new farms, farmers have relied on conventional suckers that are harvested from their existing farms. The traditional production of suckers in the field is inadequate to meet the demand, especially of large scale plantations.

Tissue culture propagation technique has been in the region for many years but it is yet to benefit majority of small-scale farmers due to its high cost and sophisticated skills requirement. To increase banana production in small-scale systems, there is a need for affordable and streamlined process for seedling production.

Macropropagation is a simple and low-cost technique that can boost banana production. The technology involves stimulation of lateral growth of multiple latent buds in a corm within a chamber where misting system is used to enhance ventilation and shading, reduce temperature, and increase humidity levels which can help avoid greenhouse overheating disasters. A single corm is capable of producing 10 to 30 plantlets in four months, but the productivity may vary depending on the cultivar and bud manipulation. This study was conducted to evaluate the effect of misting system and different growth enhancers on the proliferation and generation of Saba banana plantlets through macropropagation.

## Materials and methods

### *Preparing the Soil Media and Experimental Area*

Sandy loam soil and decomposed rice hull in 1:1 ratio were mixed thoroughly and was used as the soil media (Calvo, 2007). Three cemented propagators, each measuring 1.0 m wide, 6.0 m long, and 1.0m high, were constructed inside a glasshouse. The propagators were filled with the prepared soil media.

Then, the soil media was drenched with hot water for sterilization, and left for one day to cool down before planting with the prepared corms.

### *Source of planting materials*

Healthy maiden and sword suckers that were about to flower and from visibly suitable mother plants were selected for macropropagation. Corms of recently harvested banana plants that have good yielding characteristics were also selected.

### *Preparation of Corms*

Healthy sword suckers of Saba cultivar weighing 2.5 to 3.0kg of saba were used. The corms were washed in running water. The roots of the corms were removed and washed with soapy water. The corms were disinfected using 40% hypochlorite solution for 15 minutes. The outer sheaths were removed to expose axillary buds using sterilized sharp knives.

### *Decortication, Application of Growth hormones and Planting*

The exposed apical meristem and axillary buds of the mother corm were cut transversely 2cm above the rhizome collar region. The apical meristem was removed leaving a cavity of 2cm diameter and 4cm depth to suppress the apical dominance and induce sprouting, respectively (Macias, 2001 and Singh *et al.*, 2011). The corms were soaked in Benzyl Amino Purine, BAP (2.0mg/l) and Naphthalene acetic acid, NAA (0.93g/L) for 12 hours (Kindimba *et al.*, 2014 and Türkay, 2007). Thereafter, the corms were removed from the solution and were planted onto the prepared propagators. Shoots were allowed to develop at the collar of the rhizome. The first shoots to come out from the corm were allowed to grow for three weeks (Talengera *et al.*, 1994). These were the first generation plantlets (GP1). The suckers were dissected again using the procedure described above.

### *Cultural Management*

Corms under propagation were regularly monitored for sucker development and well-watered to maintain moisture.

To enhance the growth of the plantlets, the recommended fertilizer (46-0-0) was applied at 1.5 grams per plant once a month. Insecticides were sprayed as necessary. Mechanical weeding was done to maintain the sanitation of the experimental area (Calvo, 2007).

*Establishment of Misting System*

The misting system is controlled by a computerized irrigation controller. It was set to automatically turn on for 5 minutes to moisten the corms, then to turn off for the next 2 hours. The misting system operated for 8 hours a day with the nominal operating pressure of mister at 14.22 psi.

*Experimental Layout and Design*

All data were recorded, tabulated, and analyzed following the factorial in Completely Randomized Design (CRD). The gathered data were subjected to Analysis of Variance (ANOVA) using the software, Statistical Tool for Agricultural Research (STAR). The treatments with significant results were compared using the Least Significant Difference (LSD). The treatments were:

*Factor A : (Misting System or Humidifier)*

- A1 - with misting system
- A2 – without misting system

*Factor B : (Growth Enhancer)*

- B<sub>0</sub> - CONTROL
- B<sub>1</sub> – BAP at 2.0mg/l (Benzyl Amino Purine)
- B<sub>2</sub> – NAA at 0.93g/L (Naphthalene acetic acid)

*Collection of agro-climatic data*

The agro-climatic data were obtained directly from the DOST-PAGASA station at ISU, Echague, Isabela. Some supplementary meteorological data were generated from the Automatic Weather Station (AWS) installed near the area.

**Results and discussions**

*Climatic Data*

The climatic data during the implementation of the study in terms of temperature and relative humidity, as shown in the following table, was obtained from

the Agrometeorological Station-PAGASA<sup>1</sup>, ISU, Echague, Isabela. The temperature inside the glasshouse was around 34.03°C while outside was 29.6°C in the duration of the study. The relative humidity recorded was around 51.68% inside while outside humidity was at 77.18%.

**Table 1.** Climatic data during the study (2016).

Months	Inside glasshouse		Outside glasshouse	
	Tempe rature, °C	Relative humidity, %	Temp erature, °C	Relative humidity, %
April	30.6	66.0	28.5	72.3
May	35.5	49.5	30.8	67.7
June	35.5	45.6	30.3	79.1
July	36.2	45.9	29.7	77.9
August	35.5	54.4	29.0	83.5
Sept	36.9	48.7	29.3	82.6
Average	34.03	51.68	29.6	77.18

*Growth Parameters of Banana using Humidifier and Growth Enhancers to Produce First Generation Plantlets (GP1)*

Table 2 shows the growth parameters of banana using humidifier and growth enhancers to produce first generation Plantlets (GP1). It can be noted that the humidifier (Factor A) as a single factor has a highly significant effect ( $P \leq 0.01$ ) on all the parameters tested where the use of misting system and non-misting system have notable differences.

Growth enhancers as a single factor affect significantly the number of days to emergence ( $P \leq 0.01$ ), number of shoots emerged ( $P \leq 0.01$ ), shoots collar diameter (cm) ( $P \leq 0.01$ ), and total leaf area ( $\text{cm}^2$ ) ( $P \leq 0.05$ ).

Furthermore, the interaction of humidifier (Factor A) and growth enhancers (Factor B) has no significant effect on the growth parameters of banana. Results imply that humidifier and growth enhancers independently influence all the parameters tested.

*Growth Parameters of banana using Humidifier and Growth enhancers to Produce Second Generation Plantlets (GP2)*

Table 3 shows the growth parameters of banana using humidifier and growth enhancers to produce second generation Plantlets (GP2).

It can be noted that humidifier (Factor A) as a single factor has a highly significant effect ( $P \leq 0.01$ ) on number of days to emergence, number of shoots emerged, collar diameter (cm), and total leaf area ( $\text{cm}^2$ ) where the use of misting system and non-misting system have notable differences.

**Table 2.** Growth parameters of banana (*Musa spp.*) using humidifier and growth enhancers to produce first generation Plantlets (GP1) from corms.

Treatments	Days to emergence	Shoots emerged	Collar diameter (cm)	Total Leaf Area, $\text{cm}^2$
Factor A: Misting System				
A <sub>1</sub> - with	16.89 <sup>b</sup>	9.00 <sup>a</sup>	4.02 <sup>a</sup>	1948.92 <sup>a</sup>
A <sub>2</sub> - without	19.78 <sup>a</sup>	7.56 <sup>b</sup>	3.51 <sup>b</sup>	1459.42 <sup>b</sup>
Factor B: Growth Enhancers				
B <sub>0</sub> - CONTROL	22.33 <sup>a</sup>	6.00 <sup>c</sup>	3.40 <sup>c</sup>	1142.79 <sup>c</sup>
B <sub>1</sub> - BAP	15.00 <sup>c</sup>	11.33 <sup>a</sup>	4.17 <sup>a</sup>	2270.09 <sup>a</sup>
B <sub>2</sub> - NAA	17.67 <sup>b</sup>	7.50 <sup>b</sup>	3.73 <sup>b</sup>	1699.62 <sup>b</sup>
C.V%	7.4	11.4	4.3	21.9
F-Test				
A	**	**	**	**
B	**	**	**	*

ns = not significant, \* = significant, ( $p < 0.05$ ), \*\* = highly significant ( $p \leq 0.01$ )

Growth enhancers as a single factor affect significantly ( $P \leq 0.01$ ) the number of days to emergence, number of shoots emerged, collar diameter (cm), and total leaf area ( $\text{cm}^2$ ). Furthermore, the interaction of humidifier (Factor A) and growth enhancers (Factor B) shows significant effects on the number of days to emerge ( $P \leq 0.05$ ), number of shoots emerged ( $P \leq 0.01$ ) and total leaf area ( $P \leq 0.05$ ). On the other hand, the interaction of the factors did not significantly affect the shoot collar diameter.

The result also shows that using Benzylaminopurine (BAP) treatment at 2.0mg/l combined with misting system reduced the number of days from corm planting to first shoot emergence as compared with other treatments in the combined cycle of in vivo multiplication. The enhanced sucker emergence in corms treated with BAP at 2.0mg/l was probably due to the suppressed activity of apical meristem (Macias, 2001). Benzylaminopurine is a cytokinin based plant growth enhancer which stimulates the growth of lateral meristems.

**Table 3.** Growth parameters of banana (*Musa spp.*) using humidifier and growth enhancers to produce second generation Plantlets (GP2) from corms.

Treatments	Days to emergence	Shoots emerged	Collar diameter (cm)	Total Leaf Area, $\text{cm}^2$
Factor A: Misting System				
A <sub>1</sub> - with	24.89 <sup>b</sup>	55.44 <sup>a</sup>	3.66 <sup>a</sup>	2125.90 <sup>a</sup>
A <sub>2</sub> - without	31.78 <sup>a</sup>	36.00 <sup>b</sup>	3.32 <sup>b</sup>	1270.19 <sup>b</sup>
Factor B: Growth Enhancers				
B <sub>0</sub> - CONTROL	35.17 <sup>a</sup>	27.33 <sup>c</sup>	3.03 <sup>c</sup>	1044.05 <sup>c</sup>
B <sub>1</sub> - BAP	24.83 <sup>b</sup>	73.00 <sup>a</sup>	4.00 <sup>a</sup>	2490.35 <sup>a</sup>
B <sub>2</sub> - NAA	25.00 <sup>b</sup>	41.33 <sup>b</sup>	3.43 <sup>b</sup>	1559.75 <sup>b</sup>
AxB: Humidifier X Growth Enhancers				
With MSxControl	29.33 <sup>b</sup>	33.33 <sup>c</sup>	3.10	1228.89 <sup>c</sup>
With MSxBAP	21.33 <sup>c</sup>	94.33 <sup>a</sup>	4.30	3219.92 <sup>a</sup>
With MSxNAA	24.00 <sup>c</sup>	47.67 <sup>b</sup>	3.57	1928.90 <sup>b</sup>
Without MSxControl	41.00 <sup>a</sup>	21.33 <sup>d</sup>	2.97	859.19 <sup>c</sup>
Without MSxBAP	28.33 <sup>b</sup>	51.67 <sup>b</sup>	3.70	1760.78 <sup>b</sup>
Without MSxNAA	26.00 <sup>b</sup>	35.00 <sup>c</sup>	3.30	1190.60 <sup>c</sup>
C.V%	9.1	12.2	4.6	17.8
F-Test				
A	**	**	**	**
B	**	**	*	**
AXB	*	**	ns	*

ns = not significant, \* = significant, ( $p < 0.05$ ), \*\* = highly significant ( $p \leq 0.01$ )

The number of shoots emerged per corm in the combined cycles was highest in corms treated with BAP at 2.0mg/l compared with NAA at 0.93g/L and control. The significant increase in collar diameter and leaf area from corms treated with BAP at 2.0mg/l corresponds well with the enhanced shoot emergence. This confirms that injection of BAP in plantain enhance bud formation as well as the speed of shoot development (Osei, 2005).

### Conclusion

The findings of this research provide evidence that the combined use of misting system and Benzylaminopurine (BAP) concentration at 2.0mg/l as plant growth enhancers in macropropagation accelerates the growth and shoot emergence of banana.

### Footnote:

DOST-PAGASA- Department of Science and Technology - Philippine Atmospheric Geophysical and Astronomical Services Administration

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