

MICRONUTRIENT DELIVERY SYSTEM FOR INDUCTION OF ORGANOGENESIS IN BANANA *IN VITRO* CULTURES

Abstract: Micropropagation techniques represent one of the technologies, which allows the large-scale production of banana and the culture medium composition is one of the major factors affecting *in vitro* propagation of plants. The objective of this study was to evaluate the efficiency of a new micronutrient delivery system based on ionic Cu and Zn for inducing *in vitro* organogenesis in two banana cultivars in *in vitro* cultures, Grand Naine and Pysang Ceylon. The first experiment evaluated different concentrations of BAM-FX[®] (0.16, 0.32, 0.64, 1.28, and 2.56 $\mu\text{l ml}^{-1}$) added to the MS culture medium. The concentration of 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®] provided the best results for *in vitro* shoot and root growth and development. Therefore, a second experiment was performed to evaluate the potential of combining BAM-FX[®] with a reduced concentration of MS medium ($\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ strength), or the use of BAM-FX[®] alone without MS medium. Results indicate that MS at $\frac{3}{4}$ strength combined with 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®] provided proper *in vitro* shoot and root growth and development. The use of BAM-FX[®] *in vitro* requires additional studies to verify the feasibility of this product for efficient micropropagation of banana, as well as for other species.

Keywords: *Musa* species, Nutrient uptake, ionic copper and zinc

1. Introduction

Bananas and plantains (*Musa* sp.) constitute a major staple food crop for millions of people, being cultivated in over 100 countries in the tropical and subtropical regions of the world, as well as providing a valued source of income through local and international trade(1). Banana production has expanded in most countries for the last four decades, with worldwide production increased from 35 million tons in 2.6 million ha in 1978 to 114 million tons in 5.6 million ha in 2017, according to the FAOSTAT(2). This is because of more intensive use of technology and consequent increased productivity, including the use of *in vitro* propagation techniques for the production of disease-free bananas(3).

There are several factors that influence *in vitro* growth of tissues and plants, including plant growth regulators, temperature, and light quality(4, 5, 6). The culture medium greatly influences the success of *in vitro* techniques as a means of micropropagation and the optimization of the medium is genotype dependent (7,8,

9) Healthy and vigorous growth of plants requires efficient nutrient uptake by *in vitro* cultures and micronutrients have been shown to play important roles in plant regeneration (10). Metals, such as cobalt, iron, manganese, copper and zinc are essential for plants and required in very small or trace amounts(11).

Among micronutrients, copper is essential for normal growth and development of plants, holding important physiological and biochemical functions. It is involved in the processes of photosynthesis, respiration, conversion of nitrogen compounds, transport of carbohydrates and also regulates the process of DNA formation(12). In addition, Cu is a constituent of protein component of several enzymes in plants, mainly those participating in electron flow, catalyzing redox reactions in mitochondria, chloroplasts, cell wall and cytoplasm of plant cells(13, 14, 15). Zinc is another essential plant micronutrient, as a component of thousands of proteins in plants, and also involved in the biosynthesis of chlorophyll and carotenoids. Zinc is also the most common crop micronutrient

deficiency(16). Improved Zn uptake can result in increased crop yields (17, 18). Under *in vitro* conditions, optimum Cu and Zn concentrations in the culture medium positively affect plant development (19, 10). However, studies on *in vitro* mineral nutrition, specifically with micronutrients, are still limited (4, 20, 21).

The objective of this study is to evaluate the efficiency of a new micronutrient delivery system based on ionic Cu and Zn for inducing *in vitro* organogenesis in two banana cultivars, Grand Naine and Pysang Ceylon. Growth and development of banana *in vitro* shoots and roots was assessed.

2. Material and Methods

2.1. First experiment

2.1.1. Plant material and treatments

The study was performed at the Ornamental Horticulture and Biotechnology Laboratory of the Tropical Research and Education Center (TREC), Institute of Food and Agricultural Sciences (IFAS), University of Florida (UF), Homestead, Florida, USA.

Three-week old banana *in vitro* plantlets of the cultivars Grand Naine and Pisang Ceylon were obtained from AgriStarts, Inc. (Apopka, FL), a micropropagation commercial company. The sterile *in vitro* plantlets were subcultured onto baby-food glass jars containing 50 ml of MS (22) medium supplemented with 117 mM sucrose (pH 5.7) and solidified with 7% agar (Fisher[®], Chicago, IL, USA). Jars containing MS culture medium were autoclaved 121 °C and 15 psi for 30 min, prior to subculturing. Cultures were maintained under controlled environmental conditions at 27 ± 2 °C; 520 μmol m⁻² s⁻¹; 16/8 light/dark using Philips[®] LED top lighting. After 20 days, seedlings (12 cm height) with 4 buds were subdivided into 3-cm sections and used as explants. Explants were placed on the same culture medium as described above supplemented with different concentrations of micronutrient delivery system containing Zn and Cu. According to the manufacturer's description, the micronutrient delivery system,

Bio-Available Mineral Formula-X (BAM-FX[®], BAM Agricultural Solutions, Inc., Boca Raton, Florida, USA) enables the systemic uptake of specific, targeted minerals and other nutrients. The product consists of a highly positively charged solution of ionic copper and zinc in an ammonia ligand that contains 2.1% Cu sulfate pentahydrate and 6.9% Zn sulfate as active ingredients, and 91% of inert carriers such as water (86%) and other sulfates (5%). The manufacturer recommends BAM-FX[®] applications in the range of 100-128 ml l⁻¹ in field crops. Because this is the first study with this product for use *in vitro*, treatments with the addition of BAM-FX[®] to the culture medium were adapted from the manufacturer's recommendations for field application:

- MS basal; no BAM-FX[®] (control)
- MS basal + 0.16 μl ml⁻¹ BAM-FX[®]
- MS basal + 0.32 μl ml⁻¹ BAM-FX[®]
- MS basal + 0.64 μl ml⁻¹ BAM-FX[®]
- MS basal + 1.28 μl ml⁻¹ BAM-FX[®]
- MS basal + 2.56 μl ml⁻¹ BAM-FX[®]

All treatments were maintained under controlled environmental conditions at 27±2 °C; 120 μmol l⁻² s⁻¹; 16/8 light/dark; 2×9A Philips[®] fluorescent bulbs for 4 weeks. After 30 days the experiment was evaluated for shoot growth and development.

2.2. Second experiment

2.2.1. Plant material and treatments

Nine-week old banana *in vitro* plantlets of the same cultivars Grand Naine and Pisang Ceylon from AgriStarts, Inc. (Apopka, FL) were used for the second experiment. The same procedures used for the first experiment were used for the second experiment. Briefly, sterile *in vitro* plantlets were subcultured onto baby-food glass jars containing 50 ml of MS medium supplemented with 117 mM sucrose (pH 5.7) and solidified with 7% agar. Cultures were maintained under controlled environmental conditions; 27 ± 2 °C; 520 μmol m⁻² s⁻¹; 16/8 light/dark using Philips[®] LED top lighting. After 20 days, seedlings (12 cm height) with 4 buds

were subdivided into 3-cm sections and used as explants.

The best treatment (concentration of BAM-FX[®]) showing highest growth based on results from the first experiment was selected for the second experiment. According to manufacturer's description of product, BAM-FX[®] may reduce the need for regular fertilization. Therefore, the second experiment focused on the evaluation of the best concentration of BAM-FX[®] from the first experiment combined with MS medium at different concentrations (full strength, 3/4 strength, 1/2 strength, and 1/4 strength), as follows:

- MS basal; no BAM-FX[®] (Control)
- MS Vitamins +117 mM sucrose + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®]
- 3/4 MS+ 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®]
- 1/2 MS+ 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®]
- 1/4 MS+ 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®]

Cultures were maintained under controlled environmental conditions 27 ± 2 °C; $120 \mu\text{mol}^{-2} \text{s}^{-1}$; 16/8 light/dark; 2×9A Philips[®] fluorescent bulbs for 4 weeks. After 30 days, cultures were evaluated for growth and development

2.3. Experimental design

2.3.1. First experiment

The experimental design was conducted in a factorial 2x6 (variety x treatment) in a completely randomized design and consisted of 5 treatments plus a control, with 4 replicates of 3 shoots per treatment/control, with a total of 144 shoots used per experiment, being 72 of Grand Naine and 72 of Pisang Ceylon. The entire experiment was repeated once. Shoot/root formation and elongation were calculated by their number and length, respectively. Data was transformed using and analyzed using analysis of variance (ANOVA). Means were compared using the Tukey range test at $\alpha=0.01$.

2.3.2. Second experiment

The experimental design was conducted in a factorial 2x5 (variety x treatment) in a completely randomized design, with 10 replicates of 3 shoots per treatment/control, with a total of 300 shoots used per experiment, being

150 of Grand Naine and 150 of Pisang Ceylon. The entire experiment was repeated once. Shoot formation and elongation and root multiplication and elongation were calculated by their number and length, respectively. Data was transformed using and analyzed using analysis of variance (ANOVA). Means were compared using the Tukey range test at $\alpha=0.01$.

3. Results and discussion

3.1. First experiment

There was a significant effect of the interaction between cultivar and treatment for *in vitro* shoot and root multiplication and elongation (Table 1). After 30 days of *in vitro* establishment, shoots in the MS medium without BAM-FX[®] (control) showed the best growth and development for both cultivars (Plates 1 and 2).

Table 1. Analysis of variance for *in vitro* shoot multiplication (SM), shoot elongation (SE), root multiplication (RM) and root elongation (RE) of banana plantlets cv. Grand Naine and Pisang Ceylon under different treatments with BAM-FX®.

Source of Variation	DF	Mean Square			
		SM	SE	RM	RE
Cultivar	1	0.32*	0.15*	1.32*	0.03 ^{ns}
Treatment	5	1.58**	4.14**	3.14**	8.28**
Cultivar*Treatment	5	0.11*	0.12*	0.25*	0.19*
Error	36	0.06	0.11	0.27	0.25
CV (%)		21.22	32.60	85.06	70.69
General Mean		1.19	1.04	0.62	0.71

** Significant at P = 0.01 by the F test

* Significant at P = 0.05 by the F test

^{ns} No significant at P = 0.05

DF = degrees of freedom; CV = coefficient of variation



Plate 1. Banana in vitro plantlets cv. Grand Naine developed after BAM-FX[®] treatments: (A) MS basal, (B) MS + 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®], (C) MS + 0.32 $\mu\text{L ml}^{-1}$ BAM-FX[®], (D) MS + 0.64 $\mu\text{L ml}^{-1}$ BAM-FX[®], (E) MS + 1.28 $\mu\text{L ml}^{-1}$ BAM-FX[®], (F) MS + 2.56 $\mu\text{L ml}^{-1}$ BAM-FX[®].



Plates 2. Banana in vitro plantlets cv. Pisang Ceylon developed after BAM-FX[®] treatments: (A) MS basal, (B) MS + 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®], (C) MS + 0.32 $\mu\text{L ml}^{-1}$ BAM-FX[®], (D) MS + 0.64 $\mu\text{L ml}^{-1}$ BAM-FX[®], (E) MS + 1.28 $\mu\text{L ml}^{-1}$ BAM-FX[®], (F) MS + 2.56 $\mu\text{L ml}^{-1}$ BAM-FX[®].

Shoots from Grand Naine (GN) showed an average of 3 shoots per explant, while shoots from Pisang Ceylon (PC) formed an average of 4.25 shoots per explant, with an average shoot length of 2.08 cm for both cultivars (Table 2).

Among the treatments with BAM-FX[®] added to MS medium, the concentration of 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®] was the most responsive to multiplication and elongation of shoots,

producing an average of 2.5 shoots per explant for both cultivars and average shoot lengths of 1.84 cm for GN and 1.96 cm for PC (Table 2). However, compared to the control, shoots under 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®] were smaller, with shorter leaves and roots (Plates 1 and 2), but had significant elongation, similar to the control (Table 2). Shoot and root formation and elongation in *in vitro* cultures of banana can be enhanced when Cu is applied in lower concentrations, as shown by (4). However, when BAM-FX[®] concentrations above 0.64 $\mu\text{L ml}^{-1}$ were added to MS medium, oxidation of the explants was observed for both cultivars (Plates 1 and 2). Oxidation is most likely the result of excessive Cu, causing toxicity on leaves and reduced growth. Copper toxicity can affect essential physiological processes in plants, leading to disruption in growth and development, including inhibition of root growth (14). Zinc toxicity is not common in plants, while Zn is the most common micronutrient deficiency in plants (16). However, excess zinc can result in stunted growth, which in combination with excess Cu could also have

Table 2. Number of shoots and shoot elongation (length) of *in vitro* banana plantlets cv. Grand Naine (GN) and Pisang Ceylon (PC) in MS basal medium without (control) and with different concentrations of BAM-FX[®].

Treatments	Number of Shoots		Shoot Elongation (cm)	
	GN	PC	GN	PC
MS Basal (Control)	3.00 ^a	4.25 ^a	2.08 ^a	2.08 ^a
MS + 0.16 $\mu\text{L ml}^{-1}$ BAM-FX [®]	2.50 ^b	2.50 ^b	1.84 ^a	1.96 ^a
MS + 0.32 $\mu\text{L ml}^{-1}$ BAM-FX [®]	1.25 ^c	1.00 ^c	0.70 ^b	0.50 ^b
MS + 0.64 $\mu\text{L ml}^{-1}$ BAM-FX [®]	1.00 ^c	1.00 ^c	0.64 ^b	0.48 ^b
MS + 1.28 $\mu\text{L ml}^{-1}$ BAM-FX [®]	0.75 ^c	1.00 ^c	0.60 ^c	0.48 ^b
MS + 2.56 $\mu\text{L ml}^{-1}$ BAM-FX [®]	0.50 ^c	1.00 ^c	0.41 ^c	0.40 ^b

^{abc} Means followed by the same **superscripts** along columns are not significantly different by Tukey test (P = 0.05).

MS – Murashige and Skoog culture medium

accounted for reduced growth of *in vitro* banana plants.

For the multiplication and elongation of the roots, a similar response was observed. The MS basal medium without BAM-FX[®] (control) resulted in larger number of roots per explant and longer roots on average (Plates 1 and 2), with 1.88 roots per explant measuring 2.48 cm in length for GN, and 1.47 roots per explant measuring 2.75 cm in length for PC (Table 3). The use of BAM-FX[®] at 0.16 $\mu\text{l ml}^{-1}$ provided similar results for root multiplication, with 0.93 and 1.21 roots per explant for GN and CN cultivars, respectively (Table 3). However, root length was significantly lower when BAM-FX[®] was used at 0.16 $\mu\text{l ml}^{-1}$, with average of 0.93 cm and 1.35 cm for GN and CN cultivars, respectively (Table 3). Although root multiplication and elongation still occurred under the treatments varying from 0.32 $\mu\text{l ml}^{-1}$ to 2.56 $\mu\text{l ml}^{-1}$ BAM-FX[®] for cultivar GN, no root multiplication or elongation was observed for the cultivar PC (Table 3). Higher levels of Cu can result in toxicity symptoms and inhibit root growth (5), which is likely an effect of either reduced cell division or cell elongation, or yet a combination of both factors (23, 24). In an attempt to verify the benefits of BAM-FX[®] for *in vitro* cultures of banana, the MS medium was not altered to reduce the content of Cu or Zn. Therefore, the additional Cu and Zn in the BAM-FX[®] formulation could also have accounted for toxicity symptoms and consequent reduced growth. High concentrations of Cu and Zn have been shown to decrease growth in wheat (11). However, in banana only Cu toxicity has been reported (4), while no information is available on Zn toxicity. The use of BAM-FX[®] for *in vitro* cultures is novel and based on these initial results, it still requires additional studies to verify the potential benefits of the product for *in vitro* shoot and root formation and multiplication. Optimum Cu and Zn concentrations can have positive effects on *in vitro* growth (15). Therefore, a second experiment was implemented to verify whether changes in the composition of the MS medium would provide a more balanced combination with BAM-FX[®].

Table 3. Number of roots and root elongation (length) of *in vitro* banana plantlets cv. Grand Naine (GN) and Pisang Ceylon (PC) in MS basal medium without (control) and with different concentrations of BAM-FX®.

Treatments	Number of Roots		Root Elongation (cm)	
	GN	PC	GN	PC
MS Basal (Control)	1.88 ^a	1.47 ^a	2.48 ^a	2.75 ^a
MS + 0.16µL ml ⁻¹ BAM-FX®	0.93 ^{ab}	1.21 ^a	0.93 ^b	1.35 ^b
MS + 0.32 µL ml ⁻¹ BAM-FX®	0.75 ^c	0.00 ^b	0.36 ^b	0.00 ^c
MS + 0.64 µL ml ⁻¹ BAM-FX®	0.60 ^c	0.00 ^b	0.16 ^b	0.00 ^c
MS + 1.28 µL ml ⁻¹ BAM-FX®	0.25 ^c	0.00 ^b	0.30 ^b	0.00 ^c
MS + 2.56 µL ml ⁻¹ BAM-FX®	0.25 ^c	0.00 ^b	0.18 ^b	0.00 ^c

^{abc} Means followed by the same **superscripts** along columns are not significantly different by Tukey test ($P = 0.05$).

MS – Murashige and Skoog culture medium

3.2. Second experiment

There was a significant effect of the interaction between cultivar and treatment for *in vitro* shoot and root multiplication and elongation (Table 4). Shoot multiplication was low for all treatments, including the control and both varieties, varying from 1.21 to 1.66 shoots per explant. However, shoot length was highest for cultivar GN under $\frac{3}{4}$ MS + $0.16 \mu\text{l ml}^{-1}$ BAM-FX[®], with an average of 2.36 cm in height and 1.62 shoots per explant (Figure 3A). In comparison under the same treatment, cultivar PC showed an average length of 1.21 cm and 1.35 shoots per explant (Table 5). In contrast, the best treatment for cultivar PC was under $\frac{1}{2}$ MS + $0.16 \mu\text{l ml}^{-1}$ BAM-FX[®], with an average of 1.66 shoots per explant and a length of 1.80 cm (Plate 4A), compared to GN under the same treatment with an average 1.55 shoots per explant and a length of 1.72 cm (Table 5). The different results between cultivars could be attributed to their potential genotypic differences. Nutrient uptake, translocation, accumulation, and use has been long demonstrated to differ among genotypes, cultivars, and varieties within species (25, 26, 27).

The treatment with no MS medium, but with the addition of MS vitamins + 117 mM sucrose + $0.16 \mu\text{l ml}^{-1}$ BAM-FX[®] showed results that were similar to treatments containing either $\frac{1}{2}$ MS or $\frac{1}{4}$ MS, with an average of 1.21 and 1.30 shoots per explant and length of 1.47 cm and 1.45 cm for cultivars GN and PC, respectively (Table 5). However, $\frac{3}{4}$ MS medium had higher shoot multiplication and elongation, specifically for cultivar GN. Shoot development for the treatment with no MS + BAM-FX[®] was reduced when compared to MS Basal with no BAM-FX[®], particularly for cultivar GN (Plate 3B). For cultivar PC, shoot development under treatment with no MS medium (MS Vitamins + 117 mM sucrose + $0.16 \mu\text{l ml}^{-1}$ BAM-FX[®]) was slightly reduced compared to the best treatment ($\frac{1}{2}$ MS + $0.16 \mu\text{l ml}^{-1}$ BAM-FX[®]), but not significantly different (Table 5; Plate 4B). These results confirm the need for a balanced nutrient composition in the culture medium for *in vitro* growth of plants, as well as proper mineral nutrient uptake, as described by (8, 27). In addition, although nutrient levels in most *in*

vitro culture media are based on levels established for tobacco by (22) and considered standard for most plant species cultured *in vitro*, micronutrient levels have not been studied in depth and may differ for banana, as compared to tobacco (26).

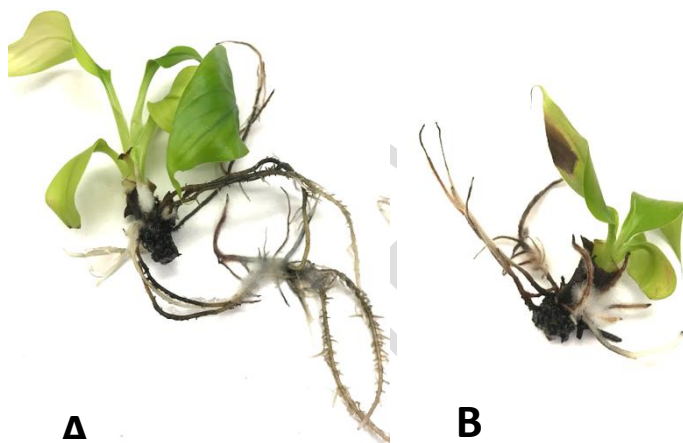


Plate 3. Banana *in vitro* plantlets cv. Grand Naine developed after BAM-FX[®] + $0.16 \mu\text{L ml}^{-1}$ BAM-FX[®]: (A) $\frac{3}{4}$ MS + $0.16 \mu\text{L ml}^{-1}$ BAM-FX[®]; (B) MS Vitamins + 117 mM sucrose + $0.16 \mu\text{l ml}^{-1}$ BAM-FX[®]

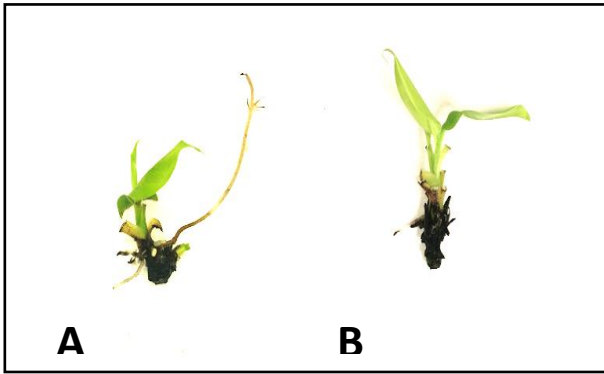


Plate 4. Banana *in vitro* plantlets cv. Pisang Ceylon developed after BAM-FX[®] + 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®]: (A) $\frac{3}{4}$ MS + 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®]; (B) MS Vitamins + 117 mM sucrose + 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®]

Similar results were observed for root multiplication and root elongation. Root multiplication was low for all treatments and both varieties. Highest root multiplication (3.03 roots per explant) and root elongation (2.75 cm) for cultivar GN was observed with $\frac{3}{4}$ MS + 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®]. However, for cultivar PC, highest root multiplication (3.24 roots per explant) and root elongation (2.95 cm) was observed with $\frac{1}{2}$ MS + 0.16 $\mu\text{L ml}^{-1}$ BAM-FX[®] (Table 6).

Table 4. Analysis of variance for *in vitro* shoot multiplication (SM), shoot elongation (SE), root multiplication (RM) and root elongation (RE) of banana plantlets cv. Grand Naine and Pisang Ceylon under different concentrations of MS medium with 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®].

Source of Variation	DF	Mean Square			
		SM	SE	RM	RE
Cultivar	1	2.56**	4.44*	4.74*	0.18 ^{ns}
Treatment	4	2.63*	1.45*	3.25*	1.82*
Cultivar*Treatment	4	1.63**	0.39**	2.14*	1.87**
Error	90	0.65	0.16	0.62	0.55
CV (%)		19.81	23.66	33.94	32.23
General Mean		1.43	1.72	2.31	2.29

** Significant at P = 0.01 by the F test

* Significant at P = 0.05 by the F test

Table 5. Number of shoots and shoot elongation (length) of *in vitro* banana plantlets cv. Grand Naine (GN) and Pisang Ceylon (PC) in MS basal medium (control, no BAM-FX[®]), medium without MS (MS vitamins + 117 mM sucrose + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®]) and 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®] combined with different concentrations of MS medium.

Treatments	Number of Shoots		Shoot Elongation (cm)	
	GN	PC	GN	PC
MS Basal (no BAM-FX [®])	1.53 ^{ab}	1.31 ^{ab}	1.83 ^{ab}	1.54 ^{ab}
No MS + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX [®]	1.21 ^b	1.30 ^{ab}	1.47 ^b	1.45 ^{ab}
$\frac{3}{4}$ MS + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX [®]	1.62 ^a	1.35 ^{ab}	2.36 ^a	1.21 ^b
$\frac{1}{2}$ MS + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX [®]	1.55 ^{ab}	1.66 ^a	1.72 ^b	1.80 ^a
$\frac{1}{4}$ MS + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX [®]	1.54 ^{ab}	1.28 ^b	2.24 ^{ab}	1.50 ^{ab}

^{abc} Means followed by the same **superscripts** along columns are not significantly different by Tukey test (P = 0.05).

MS – Murashige and Skoog culture medium

Table 6. Number of roots and root elongation (length) of *in vitro* banana plantlets cv. Grand Naine (GN) and Pisang Ceylon (PC) in MS basal medium (control, no BAM-FX[®]), medium without MS (MS vitamins + 117 mM sucrose + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®]) and 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®] combined with different concentrations of MS medium.

Treatments	Number of Roots		Root Elongation (cm)	
	GN	PC	GN	PC
MS Basal (no BAM-FX [®])	2.14 ^b	1.64 ^b	2.62 ^{ab}	1.82 ^b
No MS + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX [®]	2.42 ^b	2.01 ^{ab}	1.84 ^{bc}	1.94 ^b
$\frac{3}{4}$ MS + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX [®]	3.03 ^a	1.98 ^b	2.75 ^a	2.48 ^{ab}
$\frac{1}{2}$ MS + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX [®]	2.47 ^{ab}	3.24 ^a	2.49 ^b	2.95 ^a
$\frac{1}{4}$ MS + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX [®]	2.58 ^{ab}	1.61 ^b	1.98 ^{bc}	2.08 ^{ab}

^{abc}Means followed by the same **supercrrips** along columns are not significantly different by Tukey test (P = 0.05).

MS – Murashige and Skoog culture medium

Although results for root production and elongation under the treatment with no MS (MS Vitamins + 117 mM sucrose + 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®]) were inferior compared to other treatments, root development was still observed for both cultivars (Plates 3B; 4B).

These results provide some evidence that the addition of 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®] to the culture medium still allows for proper shoot and root growth development under reduced amount of nutrients (i.e., $\frac{3}{4}$ MS medium). However, adjustments in the culture medium combined with adjustments in the concentration of BAM-FX[®] are necessary, therefore warranting further studies, not only specifically for banana, but also for different species. Because this is the first study with BAM-FX[®] use *in vitro*, the feasibility of this product for efficient micropropagation of banana needs additional studies and validation. Yet, this initial study provides some promising applications for products that enhance nutrient uptake in *in vitro* cultures.

4. Conclusion

In the first experiment the concentration of 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®] provided the best results for *in vitro* shoot and root growth and development while in the second experiment the $\frac{3}{4}$ strength combined with 0.16 $\mu\text{l ml}^{-1}$ BAM-FX[®] provided proper *in vitro* shoot and root growth and development.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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