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Phloroglucinol is Effective for *in vitro* Growth and Multiplication of *Musa accuminata* Cv. Grand Naine Shoots and Roots

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Authors' contributions

This work was carried out in collaboration between all authors. Author LCNL designed the study, performed the statistical analysis, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. Authors WAV and ABDO performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Despite being a major staple food in the world, banana production in the United States is still limited, with about 500 acres under cultivation. Micropropagation has been an effective method for the large-scale production of bananas to meet both domestic and international markets. However, the efficiency of micropropagation protocols depends on several factors, particularly on the types, combinations, and levels of plant growth regulators used in the culture media. Phloroglucinol is a growth regulator that acts synergistically with auxins and cytokinins. The use of phloroglucinol for

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the production and development of *in vitro* plantlets of *Musa* spp. cv. Grande Naine were investigated. Multiplication and elongation of shoots and roots *in vitro* was enhanced by the addition of 200 µM phloroglucinol to MS medium, as compared to the control with 13.2 µM BA. Higher concentrations (400 to 1000 µM phloroglucinol) resulted in reduced growth and development of shoots and roots *in vitro*.

Keywords: Musa; organogenesis; plant growth regulators.

1. INTRODUCTION

Bananas (*Musa* sp.) are cultivated in over 100 countries in the tropical and subtropical regions of the world, where they constitute a major staple food crop for millions of people and fodder for animals, 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 three decades, from 35 million tons in 1978 to 107 million tons in 2011, ranking first in fruits [2,3]. This was due to more intensive use and development of modern technology, which resulted in increased productivity.

Banana production in the United States is very limited. Florida is estimated to have about 500 acres of banana, valued at approximately $2 million. Recently, there has been a renewed interest in expanding US banana production to satisfy various niche markets, including the market for organic and processed bananas [4].

Conventional propagation of bananas is done by suckers, which are known to perpetuate the spread of diseases and pests and the potential of variety mix-ups [5,6]. In contrast, *in vitro* propagation or micropropagation is a superior technique providing plants with vigorous growth, precocity and higher yields. Micropropagated banana plants also have high field establishment rates, uniformity in growth ensuring synchronized harvesting, and better-quality fruits [7,8,9,10]. Micropropagation allows the mass production of young plants, with subsequent greater distribution in shorter time, therefore attending the global demand.

The efficiency of micropropagation systems is determined by the rate of *in vitro* shoot production, which is directly influenced by plant growth regulators and numerous other factors [11]. Phloroglucinol (1,3,5–trihydroxybenzene) or phloroglucin (PG tautomer) is a benzenetriol that has growth regulating properties [12]. It is a phenolic compound known for its properties as a promoter of plant growth [13,14]. Studies focusing on the effects of phloroglucinol on *in vitro* cultures have shown to enhance growth and rate of axillary shoots in several woody plants, to initiate adventitious roots on *in vitro* shoots of different woody species, to enhance survival of meristems and/or shoot tips *in vitro* [15,16,17], to enhance shoot multiplication and elongation [18,19,20,21,22], and root proliferation [23,24,25, 26,27,28], to embryogenesis induction [29,30] and to improve recovery of cryopreserved protocorms [31]. A synergistic effect with auxin during root initiation has also been reported [17,32,13,33,14].

Often *in vitro* plants are submitted to MS medium [34] with different concentrations of BAP and/or NAA. In this work, only the floroglucinol was used to observe the development behavior of the *in vitro* banana plant, because in the literature was observed that we can evidence responses to the formation of shoots in several species.

Therefore, the aim of the present study was to evaluate the effects of phloroglucinol on growth and multiplication of banana cv. Grand Naine (*Musa acuminata* Colla group AAA) *in vitro* shoots, as well as root multiplication and elongation.

2. MATERIALS AND METHODS

2.1 Plant Material and Experimental Location

The experiments for this study were performed in the Ornamental Horticulture`s Laboratory of the University of Florida, at the Tropical Research and Education Center, in Homestead, Florida, USA.

Three-week old banana *in vitro* plantlets of the cultivar Grand Naine were obtained from AgriStarts, Inc. (Apopka, FL). The *in vitro* plants were approximately 20 cm long. The aseptic *in vitro* plantlets were subcultured onto baby-food glass jars containing 50 ml of MS [34] medium supplemented with 117 mM sucrose, pH was adjusted to 5.7 prior to autoclaving and solidified with 0.7% agar (Fisher®, Chicago, IL, USA). The initial medium had no growth regulator. Cultures

were maintained under controlled environmental conditions; 27 ± 2ºC; an average 120 µmol m^{-2} s^{-1}; 16/8 light/dark using Philips® LED toplighting. After 20 days, shoots or plantlets (12 cm height) with four axillary buds were subdivided into 3-cm sections and used as explants.

2.2 Treatments

Explants were placed on the same culture medium as described above, including a control, a treatment with 6-benzylaminopurine (6-BA) as previously reported by [1,35], and five phloroglucinol treatments, as follows:

T1 – Control: MS Basal (no plant growth regulators)
T2 – MS + 13.2 µM 6-BA
T3 – MS + 200 µM of phloroglucinol
T4 – MS + 400 µM of phloroglucinol
T5 – MS + 600 µM of phloroglucinol
T6 – MS + 800 µM of phloroglucinol
T7 – MS + 1000 µM of phloroglucinol

Cultures were maintained under the same controlled environmental conditions as described above. After 4 weeks cultures were evaluated for *in vitro* shoot multiplication and elongation, and root induction and elongation.

2.3 Experimental Design

The experimental design consisted of 6 treatments plus a control, with 8 replicates of 3 shoots per treatment/control, with a total of 144 shoots used in the experiment. The entire experiment was repeated once. Shoot and root multiplication (SM, RM) were calculated by counting the number of shoots and roots per plant, respectively. Shoot and root elongation (SE, RE) were calculated by measuring the length of shoots and roots, respectively using a pachymeter. The length of shoots was measured from the leaf base of the rhizome. Survival percentage was calculated by the growth and development of normal plants that successfully survived after 30 days on *in vitro* conditions. The total number of inoculated explants was considered 100%. Data were transformed using $\sqrt{x} + 0.5$ and analyzed using analysis of variance (ANOVA). Means were compared using the Scott-Knott range test at α= 0.01.

3. RESULTS

After 30 days of *in vitro* culture, the number of banana *in vitro* shoots formed was higher for concentrations of 200 µM phloroglucinol and 13.2 µM 6-BA as compared to the control and other treatments with phloroglucinol (Table 1).

There were no significant differences for shoot multiplication between the treatments with 200 µM phloroglucinol and 13.2 µM 6-BA, with production of about two shoots in each treatment.

However, significant differences were observed for shoot elongation (2.84 cm), number of roots (4.17) and root elongation (4.04 cm) under 200 µM phloroglucinol, which were significantly higher than the control and other treatments (Fig. 1).

For the number of shoots, no significant differences were observed between the control and levels of phloroglucinol varying from 400 to 1000 µM. For the number of roots, 400 and 600 µM phloroglucinol were similar to the control, and significantly higher than 13.2 µM 6-BA and 800 and 1000 µM phloroglucinol. Root length showed the highest variation, with best results for 200 µM phloroglucinol (4.04 cm) followed by the control (3.24 cm). There were no differences in root length between 400 and 600 µM phloroglucinol (1.77 cm for both), and for 13.2 µM 6-BA (1.17 cm), and 800 µM (1.14 cm) and 1000 µM (0.84 cm) phloroglucinol (Table 1).

For root multiplication and elongation, 200 µM phloroglucinol proved to be more responsive to root induction, as higher concentrations inhibited root growth and multiplication (Table 1). This establishes a threshold concentration for organogenesis *in vitro* in banana plantlets cv. Grand Naine, whereby concentrations of phloroglucinol higher than 400 µM can lead to a negative effect on plantlet morphogenetic responses.

Therefore, 200 µM phloroglucinol appears to be the most responsive for direct organogenesis of banana's *in vitro* explants (Table 1).

4. DISCUSSION

We observed that among the phloroglucinol concentrations evaluated, the optimal concentration was 200 µM, whereby organogenesis responses were equal to or higher than those observed in the control.

The role of 6-BA has been recently reported for efficient *in vitro* shoot production in different

Fig. 1. Shoot and root development of *in vitro* banana plantlets cv. Grand Naine (bar = 1 cm) in Murashige and Skoog (MS) basal medium (T1), MS medium with 6-benzylaminopurine (T2), and MS medium with different concentrations of phloroglucinol (T3-T7)

T1 – MS Basal; T2 – MS + 13.2 µM 6-BA; T3 – MS + 200 µM PG; T4 – MS + 400 µM PG; T5 – MS + 600 µM PG; T6 – MS + 800 µM PG; T7 – MS + 1000 µM PG

Table 1. Number of shoots, shoot elongation (length) (cm), number of roots, and root elongation (length) (cm) of *in vitro* banana plantlets cv. Grand Naine in Murashige and Skoog (MS) basal medium (T1), MS medium with 6-benzylaminopurine (T2), and MS medium with different concentrations of phloroglucinol (T3-T7), after 30 days cultivation

Treatments	Number of shoots per plant	Shoot length (cm)	Number of roots per plant	Root length (cm)
T1 – MS Basal	1.68[b]	2.32[b]	3.22[b]	3.24[b]
T2 – MS + 13.2 µM 6-BA	2.01[a]	1.89[d]	1.26[c]	1.17[d]
T3 – MS + 200 µM PG	2.32[a]	2.45[a]	4.18[a]	4.04[a]
T4 – MS + 400 µM PG	1.60[b]	2.13[c]	2.44[b]	1.77[c]
T5 – MS + 600 µM PG	1.87[b]	2.44[b]	2.67[b]	1.77[c]
T6 – MS + 800 µM PG	1.78[b]	2.10[c]	1.73[c]	1.14[d]
T7 – MS + 1000 µM PG	1.66[b]	1.66[d]	1.33[c]	0.84[d]

Means followed by the same letter are not significantly different by Scott-Knott test (P = 0.01).
MS – Murashige and Skoog medium, 6-BA – 6-benzylaminopurine, PG – Phloroglucinol

banana cultivars, such as Prata-Anã clone Gorutuba [1], and Amritasagar and Sabri [35]. Therefore, in our study we included a treatment with 6-BA for comparison with the different concentrations of phloroglucinol. Phloroglucinol showed superior results as compared to 6-BA, with better shoot and root elongation, and number of roots. Similarly, [13] reported that phloroglucinol has a similar effect to that of 6-BA in promoting the induction and multiplication of *in vitro* shoots in others species of plant. Therefore the use of phloroglucinol in protocols for *in vitro* multiplication of banana cv. Grand Naine provides a positive impact for shoot multiplication and elongation, and consequently mass production of plants.

However, concentrations of phloroglucinol might need to be adjusted according to different banana cultivars, as well as for other crops or plant species. This is evidenced by several studies. [13] reported that phloroglucinol

promoted growth and development of *in vitro* shoots of potato using concentrations between 80 to 1600 µM. [21] improved multiplication in *Prunus armeniaca* L. by using 800 µM of phloroglucinol. [36] demonstratedthat 400 µM of phloroglucinol enhanced axillary shoot proliferation in *Wrightia tomentosa* Roem. et Schult. Contrasting to the higher concentrations of phloroglucinol reported in the studies above, [22] observed that 39.64 µM was the most responsive concentration for shoot bud elongation in *Capsicum annum* L.. Similarly, [37] verified that 3.9 µM was sufficient to improve shoot elongation in *Wrightia tomentosa*. Therefore, the concentration range selected for this study was based on the existing literature.

We observed that higher concentrations of phloroglucinol (400-1000 µM) showed little positive effect on in vitro multiplication of banana cv. Grand Naine. However, results similar to our study were observed with *Decalepis hamiltonii*, whereby 200 µM of phloroglucinol promoted elongation of shoots [19].

In our study, phloroglucinol had a major role on *in vitro* rooting of banana cv. Grand Naine. Because it did not use auxin in the medium, phloroglucinol may have acted along with the plant's endogenous auxin and may have promoted root induction, elongation, and increasing root numbers. When comparing a study performed by [38] using 1.0 mg L^{-1} of 6-BA with 1.0 mg L^{-1} IAA for *in vitro* root formation in banana, 4 roots were obtained per explant. This number was similar to the number of roots obtained in our study with 200 µM phloroglucinol.

Although root induction was observed in the control, composed of basal MS medium with no plant growth regulators, root number and length were inferior to those found under the concentration of 200 µM phloroglucinol. The root characteristics observed for *in vitro* plantlets within 50 days of culture establishment under 200 µM phloroglucinol were similar to the roots observed for *in vitro* plantlets after 150 days of culture establishment in previous studies without phloroglucinol (Londe, personal communication). Therefore, phloroglucinol appears to enhance root number and length by promoting early root induction and elongation.

In similar studies, [39] observed enhancement of rooting frequency using 79.3–198.25 µM phloroglucinol in *Asparagus racemosus* Willd., whereas induction and longer roots were observed [40] using 3.97 µM phloroglucinol in *Bacopa monnieri* (L.) Wettst. [14] used the 79 µM phloroglucinol combined with 9.8 µM IBA and zeolite, with a positive effect on *in vitro* rooting and acclimatization of shoots of papaya var. Marado/Roja. [28] recommended 80 µM phloroglucinol for *Citrus* rootstocks, while [29] confirmed best results for *Crataeva magna* Hook. et Thomson using 198.25 µM phloroglucinol.

The studies reported above used lower concentrations compared to our study. This suggests that rooting responses are also specific to each species. Similar to our study, [27] used 200 µM phloroglucinol in *Jatropha curcas* L. In contrast, [25] reported that 634.4 µM phloroglucinol was best for enhancing rooting in *Carthamus tinctorius* L. Higher concentrations of phloroglucinol were also evaluated in different studies. [41] evaluated concentrations of phloroglucinol between 1,982.5 and 2,379 µM for *Garcinia indica* (Thouars) Choisy. [42] reported concentrations of 158.6 – 317.2µM in *Prunus salicina* Lindl., and [43] reported 793µM as the best concentration for *Prunus domestica* L.. However, specifically for banana cv. Grand Naine, we demonstrated that higher concentrations of phloroglucinol varying from 400-1,000 µM would not be favorable for *in vitro* root induction and elongation.

Therefore, 200 µM phloroglucinol proved to return better responses for *in vitro* root multiplication and elongation in banana cv. Grand naine. Longer roots can be induced in a short time; therefore, validating that phloroglucinol can accelerate the organogenic process *in* vitro. These results suggest that phloroglucinol can be used in the production of *in vitro* banana plants, and that it can be adapted to a large-scale *in vitro* mass production system, such as bioreactors.

This is the first study reporting the use of phloroglucinol for *in vitro* multiplication of banana. Additional studies should address the effects of phloroglucinol in plant morphogenesis, as well as for other banana cultivars. We showed that phloroglucinol successfully induced the development of shoots and roots *in vitro* in banana cv. Grand Naine. These results also provide valuable data that can serve as preliminary information for the continued improvement of *in vitro* micropropagation systems for banana. This is particularly true for the adaptation of protocols with phloroglucinol for large-scale mass production of *in vitro* banana

plantlets using bioreactors, as suggested above. Additional studies with temporary immersion bioreactors are therefore warranted to improve multiplication rates.

5. CONCLUSION

The concentration of 200 µM phloroglucinol promoted *in vitro* root multiplication and elongation of banana cv. Grand naine.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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