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Phloroglucinol is effective for *in vitro* growth and multiplication of banana shoots and roots

Floroglucinol é efetivo no crescimento e multiplicação *in vitro* de brotos e raízes em bananeira

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ABSTRACT

Despite being a major staple food in the world, banana production is still limited, with about 500 acres under cultivation. Micropropagation has been an effective alternative for 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 the production and development of *in vitro* plantlets of banana cv. Grande Naine was 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*.

Index terms: Micropropagation; *Musa*; Organogenesis; Plant growth regulator.

RESUMO

Apesar de ser um alimento básico no mundo, a produção de banana nos Estados Unidos ainda é limitada, com cerca de 500 acres sob cultivo. A micropropagação tem sido uma alternativa eficaz para a produção em grande escala de bananas para atender mercados domésticos e internacionais. Entretanto, a eficiência dos protocolos de micropropagação depende de vários fatores, particularmente sobre os tipos, combinações e níveis de reguladores de crescimento de plantas usados nos meios de cultura. O floroglucinol é um regulador de crescimento que age sinergicamente com auxinas e citocininas. A utilização de floroglucinol para a produção e desenvolvimento de plântulas *in vitro* de cv. Grande Naine foi investigado. A multiplicação e alongamento de brotos e raízes *in vitro* foi aumentada pela adição de 200 µM de floroglucinol ao meio MS, em comparação com o controle com 13,2 µM de BA. Concentrações mais elevadas (400 a 1000 µM de floroglucinol) resultaram em redução do crescimento e desenvolvimento de brotos e raízes *in vitro*.

Termos para indexação: micropropagação; Musa; organogenese; regulador de crescimento de planta.

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, as well as providing a valued source of income through local and international trade (Sharrock; Frisson, 1998). 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 (FAO - Food and Agriculture Organization of the United Nations, 2012; IBGE - Instituto Brasileiro de Geografia e Estatística, 2014). This was due to more intensive use of 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 (Evans; Ballen, 2012).

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 (Karembu et al., 2009; Mustaffa, 2011; Tiruchirapalli et al., 2012), among other potential hazards. In contrast, *in vitro* propagation or micropropagation is a superior alternative providing plants with vigorous growth, precocity and higher yields. Micropropagated banana plants also have high field establishment rates, uniformity in growth ensuring

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synchronized harvesting, and better-quality fruits (Nguth et al., 2009; Bhanusree et al., 2015). Micropropagation allows the mass production of seedlings, 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. Phloroglucinol (1,3,5–trihydroxybenzene) or phloroglucin (PG tautomer) is a benzenetriol that has growth regulating properties (Sarkar; Naik, 2000). It is a phenolic compound known for its properties as a promoter of plant growth (Teixeira da Silva et al., 2013, Perez et al., 2016). 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 in in vitro shoots of different woody species, to enhance survival of meristems and/or shoot tips in vitro (Jones; Hatfield, 1976; James; Thurbon, 1979, 1981), to enhance shoot multiplication and elongation (Gururaj et al., 2004; Giridhar et al., 2005; Siwach; Gill, 2011; Wang et al., 2011; Bairwa et al., 2012), and root proliferation (Romais et al., 2000; Buthuc-Keul; Deliu, 2001; Sujatha; Kumar, 2007; Bopana; Saxena, 2009; Kumar et al., 2010; Tallon et al., 2012), embryogenesis induction (Find et al., 2002; Reis et al., 2008) and improved recovery of cryopreserved protocorms (Vendrame; Faria, 2011). A synergistic effect with auxin during root initiation has also been reported (James; Thurbon, 1981; Sharifian et al., 2009; Teixeira da Silva, 2013; Daud et al., 2013, Perez et al., 2016).

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* group AAA) *in vitro* shoots, as well as root multiplication and elongation.

MATERIAL AND METHODS

Plant material and 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 sterile *in vitro* plantlets were subcultured onto baby-food glass jars containing 50 ml of MS (Murashige; Skoog, 1962) medium supplemented with 117 mM sucrose (pH 5.7) and solidified with 7% agar (Fisher®, Chicago, IL, USA). 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.

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 Faria et al. (2014) and Ferdous et al. (2015), 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 multiplication and elongation.

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, respectively. Shoot and root elongation (SE, RE) were calculated by measuring the length of shoots and roots, respectively. Survival percentage was calculated by the growth and development of normal plants that successfully survived after 30 days. Data was 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.

RESULTS AND DISCUSSION

There was a significant effect of the different treatments for *in vitro* shoot and root multiplication and elongation (Table 1). The variation coefficients were between 15.55% and 34.26%, whereby root multiplication showed the highest value as compared to other variables. This variation may be due to large differences in root elongation response within the treatments.

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 under different treatments.

Source of Variation	DF	Mean Square			
		SM	SE	RM	RE
Treatment	6	0.52**	1.17**	9.06**	11.39**
Error	49	0.16	0.12	0.62	0.47
CV (%)		21.34	15.55	32.61	34.26
General Mean		1.85	2.20	2.41	1.99

** Significant at P = 0.01 by the F test.
DF = degrees of freedom; CV = coefficient of variation.

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 2).

The results obtained in this study show that there is great potential in the utilization of phloroglucinol in *in vitro* organogenesis of banana cv Grand Naine. We observed that among the phloroglucinol concentrations evaluated, the optimal concentration was 200µM, whereby organogenesis responses were equal to or higher than those observed in the control.

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 (Figure 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.

The role of 6- BA has been recently reported for efficient *in vitro* shoot production in different banana cultivars, such as Prata-Anã clone Gorutuba (Faria et al., 2014), and Amritasagar and Sabri (Ferdous et al., 2015). Therefore, in our study we included a treatment with 6-BA for comparison with the different concentrations of

Table 2 – Number of shoots, shoot elongation (length), number of roots, and root elongation (length) 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).

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

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

phloroglucinol. Phloroglucinol showed superior results as compared to 6-BA, with better shoot and root elongation, and number of roots. Similarly, Silva et al. (2013) reported that phloroglucinol has a similar effect to that of 6-BA in promoting the induction and multiplication of *in vitro* shoots. 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.

Figure 1 – Shoot and root development of *in vitro* banana plantlets cv. Grand Naine in Murashige & 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.

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. Sarkar and Naik (2000) reported that phloroglucinol promoted growth and development of *in vitro* shoots of potato using concentrations between 80 to 1600 µM. Wang et al. (2011) improved multiplication in *Prunus armeniaca* L. by using 800 µM of phloroglucinol. Purohit et al. (2005) demonstrated that 400 µM of phloroglucinol enhanced axillary shoot proliferation in *Wrightia tomentosa*. Contrasting to the higher concentrations of phloroglucinol reported in the studies above, Bairwa et al. (2012) observed that 39.64 µM was the most responsive concentration for shoot bud elongation in *Capsicum annum*. Similarly, Jain et al. (2009) 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 (Giridhar et al., 2005).

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 2).

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 2). 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 2).

In our study, phloroglucinol had a major role on *in vitro* rooting of banana cv. Grand Naine. Because no auxins were added to the medium, it is evident that phloroglucinol had a similar effect to that of auxin *in vitro* by promoting root induction, elongation, and increasing root numbers. When comparing a study performed by Al-Amin et al. (2009) 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, Bopana and Saxena (2008) observed enhancement of rooting frequency using 79.3 – 198.25 µM phloroglucinol in *Asparagus racemosus*, whereas induction and longer roots were observed by Ceasar et al. (2010) using 3.97 µM phloroglucinol in *Bacopa monnieri*. Perez et al. (2016) 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. Tallon et al. (2012) recommended 80 µM phloroglucinol for *Citrus* rootstocks, while Bopana and Saxena (2009) confirmed best results for *Crataeva magna* 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, Kumar et al. (2010) used 200 µM phloroglucinol in *Jatropha curcas*. In contrast, Sujatha and Kumar (2007) reported that 634.4 µM phloroglucinol was best for enhancing rooting in *Carthamus tinctorius*. Higher concentrations of phloroglucinol were also evaluated in different studies. Chabukswar and Deodhar (2006) evaluated concentrations of phloroglucinol between 1,982.5 and 2,379 µM for *Garcinia indica*. Zou (2010) reported concentrations of 158.6 – 317.2 µM in *Prunus salicina*, and Petri and Scorza (2010) reported 793 µM as the best concentration for *Prunus domestica*. 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 period of time; therefore validating that phloroglucinol can accelerate the organogenic process *in vitro*. These results suggest that phloroglucinol can be used in the production of banana seedlings *in vitro*, 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 varieties. We showed that phloroglucinol has an effect similar to that of cytokinins and auxins combined, as we observed the successful induction of shoots and roots in vitro in banana cv. Grand Naine.

CONCLUSIONS

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.

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