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In vitro multiplication of woody bamboo in the Southwestern Amazon, Acre State, Brazil

Multiplicação in vitro de bamboo lenhoso na Amazônia Sul-Ocidental, Acre, Brasil

Multiplicación in vitro de bambú leñoso en la Amazonía Sudoccidental, Acre, Brasil

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Abstract
Bamboos from the genus *Guadua* in the Southwestern Amazon basin are difficult to propagate because of their recalcitrant characteristics. This study aimed to evaluate the action of 6-benzylaminopurine (BA) during in vitro multiplication of *G. latifolia* in a laboratory for clonal seedling production. In vitro multiplication was performed using the cytokinin 6-benzylaminopurine at concentrations of 0, 2, 4, 6, 8 mg L^{-1} in glass tubes containing liquid medium, incubated for 38 days. The analyzed variables were shoot height, number of shoots, multiplication rate, and the presence of callus and roots in two consecutive subcultures. The use of 6-benzylaminopurine during in vitro multiplication was effective at increasing the number of shoots in the first and second subculture. Shoot height was not influenced by the cytokinin in the culture with a maximum multiplication rate of 4.44 shoots. Therefore, the use of 6 mg L^{-1} of BA is recommended during in vitro multiplication and to obtain a high multipication rate.
Keywords: *Guadua latifolia*; Micropropagation; 6-benzilaminopurine; Cytokinin.

Resumo
Os bambus do gênero *Guadua* da Amazônia Sul-Ocidental são de difícil propagação devido suas características recalcitrantes. O objetivo deste estudo foi avaliar a ação da 6-benzilaminopurina (BAP) na multiplicação do bambu lenhoso *G. latifolia* em laboratório visando a produção de mudas clonais. A multiplicação *in vitro* foi realizada com 6-benzilaminopurina nas concentrações de 0, 2, 4, 6 e 8 mg L^{-1} em tubos de ensaio contendo meio líquido durante 38 dias de incubação. As variáveis analisadas foram número e altura de brotos, taxa de multiplicação, presença de calos e de raízes em dois subcultivos consecutivos. O uso de 6-benzilaminopurina foi eficiente durante a multiplicação *in vitro* influenciando no incremento do número de brotos durante o primeiro e o segundo subcultivo. A altura de brotos não foi influenciada pela adição da citocinina no cultivo e a taxa de multiplicação foi de 4,44 brotos. Portanto, recomenda-se o uso de 6 mg L^{-1} de BAP durante a multiplicação *in vitro* e também para se alcançar uma alta taxa de multiplicação de *G. latifolia*.
Palavras-chave: *Guadua latifolia*; Micropropagação; 6-benzilaminopurina; Citocinina.

Resumen
Los bambús del género Guadua de la Amazonía Sudoccidental son difíciles de propagar debido a sus características recalcitrantes. El objetivo de este estudio fue evaluar la acción de la 6-bencilaminopurina (BAP) sobre la multiplicación de bambú leñoso *G. latifolia* en el laboratorio con vistas a la producción de plántulas clonales. La multiplicación in vitro se realizó con 6-bencilaminopurina a concentraciones de 0, 2, 4, 6 y 8 mg L^{-1} en probetas con medio líquido durante 38 días de incubación. Las variables analizadas fueron número y altura de brotes, tasa de multiplicación, presencia de callo y raíz en dos subcultivos consecutivos. El uso de 6-bencilaminopurina fue eficiente durante la multiplicación in vitro para aumentar el número de brotes durante el primer y segundo subcultivo. La altura de los brotes no fue influenciada

por la adición de citoquinina en el cultivo y la tasa de multiplicación fue de 4.44 brotes. Por lo tanto, se recomienda utilizar 6 mg L^{-1} de BAP durante la multiplicación in vitro y también para lograr una alta tasa de multiplicación.
Palabras clave: *Guadua latifolia*; Micropropagación; 6-bencilaminopurina; Citoquinina.

1. Introduction

Bamboos from the genus *Guadua* (Poaceae: Bambusoideae) are important and widely used in civil construction, coal production, pharmaceutical industry, paper and cellulose production, and show potential for architectural applications (Drumond; Wiedman, 2017).

Their main environmentally-based function is mitigating environmental issues, acting in the recovery of degraded areas via rhizomes that helps to stabilize the effects of soil degradation, reducing erosion by up to 75% (Barbosa; Diniz, 2010).

In addition to controlling soil erosion, bamboos are efficient carbon scavengers, that makes them a renewable and eco-friendly crop (Osse & Meireles, 2011).

Bamboo cultivation is becoming important in environmental development and eradication of poverty. More than 2.5 billion people worldwide are economically dependent on this plant (Singh et al., 2013).

Bamboo is a monocarpous plant, which death occurs after flowering. The seeds exhibit low viability due to the minimal levels of auxins and endogenous abscisic acid, and the fact that species bloom in prolonged intervals, making sexual propagation extremely difficult (Richa & Nerru, 2006).

Asexual reproduction can be carried out via the vegetative parts of the plant, such as branches, buds, stems and rhizomes. Each species has a preferred form of propagation depending on its ecological characteristics (Castaño Nieto; Orjuela, 2004).

According to Pereira and Beraldo (2010), the main advantage of vegetative propagation is the possibility of obtaining clonal plants with genetic and phenotypic uniformity.

The Brazilian Amazon is home of an extensive natural bamboo forest that occupies an area of approximately 161,500 km^2 in the state of Acre (Brazil), extending to the border with Peru and Bolivia (Carvalho et al., 2013).

Further research is needed to determine the most appropriate propagation method and a seedling production system with high survival rates for *G. latifolia* in this region (Leão et al., 2020).

The production of bamboo seedlings on an industrial scale using micropropagation is recent. However, microbial contamination is still the main problem when establishing in vitro cultures (Nadha et al., 2012; Leão et al., 2020; Silva et al., 2021).

As such, several bamboo species were multiplied on a large scale using different concentrations of 6-benzylaminopurine (BA), including *Bambusa nutans* (Mehta et al., 2011), *Dracaena sanderiana* (Gradaille et al., 2010), *Bambusa vulgaris* (Ribeiro et al., 2016), *Dendrocalamus asper* (Araújo et al., 2015), *Bambusa arundinacea* (Kalaiarasi et al., 2014); *Dendrocalamus strictus* (Goyal et al., 2015) and *Drepanostachyum falcatum* (Saini et al., 2016).

Several studies were conducted involving in vitro multiplication of *G. angustifolia* using BA in shoot propagation, but there is still no consensus on the ideal concentration of 6-benzylaminopurine: 2 mg L^{-1} (Nadha et al., 2012), 3 mg L^{-1} (Gutiérrez et al., 2016), 5 mg L^{-1} (Jiménez et al., 2006) or 6 mg L^{-1} (Mendoza et al., 2010). The objective of this study was to evaluate the action of cytokinin 6-benzylaminopurine in the in vitro multiplication of *G. latifolia* (Bonpl.) Kunth.

2. Methodology

This study was carried out in the morphogenesis and molecular biology laboratory of the Brazilian Agricultural Research Corporation (Embrapa) in Rio Branco, Acre State, Brazil, from January to March 2017. The seedlings were collected in the Chico Mendes Extractive Reserve in the municipality of Assis Brasil (Acre State), at 10°43'02.2"S 69°24'06.5"W, and

planted in soil, commercial substrate and organic material (1:1:1) in a greenhouse.

The pH of the culture medium used was adjusted to 5.8 using sodium hydroxide (NaOH) and chloridric acid (HCl) solutions and autoclaved for 15 minutes. After inoculation, the cultures were stored in a growth chamber at a controlled temperature of 25 ± 2°C, under a 16-hour photoperiod.

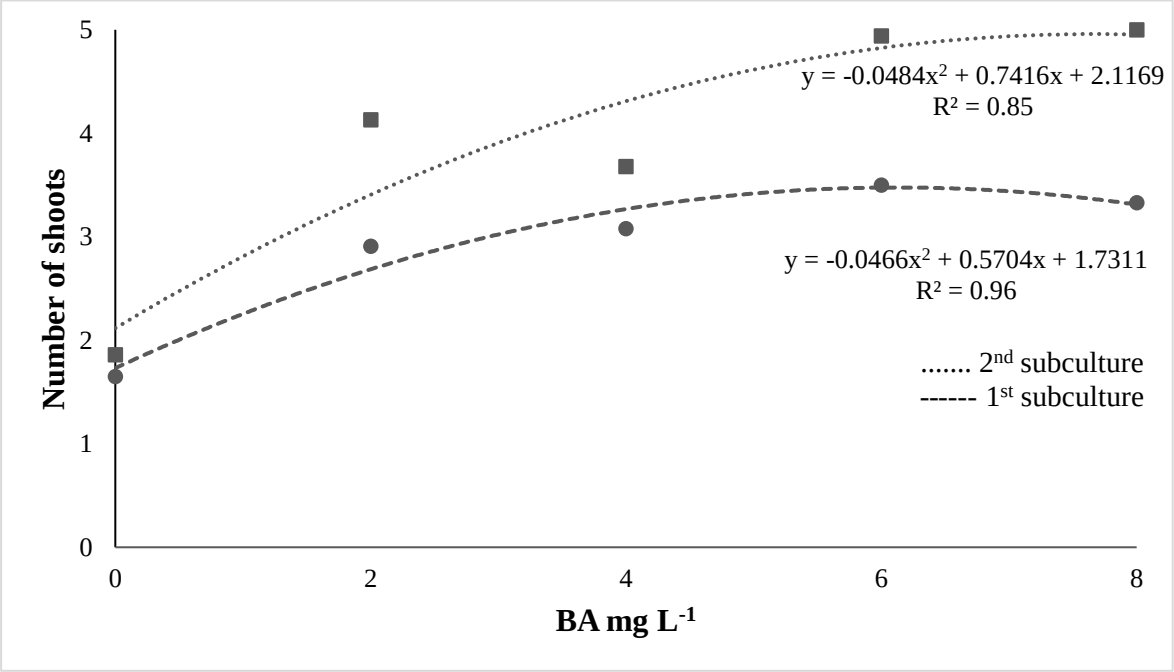
Following selection of the optimal sterilization treatment (Leão et al., 2020), the explants were inoculated in glass tubes containing 10 mL of MS liquid medium (Murashige & Skoog, 1962), supplemented with sucrose (30 g L^{-1}), 2 mL L^{-1} of Plant Preservative Mixture - PPM® and 6-benzylaminopurine (BA) concentrations of 0, 2, 4, 6 and 8 mg L^{-1}. The analyzed variables after 2 subcultures were number of shoots, shoot height, multiplication rate and the presence of callus and roots. A completely randomized design was used with 24 replications per treatment and 1 shoot per glass tube.

The results were evaluated by analysis of variance (ANOVA) and by means Tukey's test at 5% probability and quadratic regression. Statistical analysis was performed using Assistat 7.7 software (Silva & Azevedo, 2016).

3. Results and Discussion

In vitro multiplication was performed in two consecutive subcultures and the multiple adventitious sprouts were evaluated. There was a significant increase in the average number of shoots from subculture 1 to 2 in all treatments. In vitro conditions influenced the final result with the lowest values recorded in the control treatment at only 1.65 (first subculture) and 1.86 (second subculture) new shoots (Figure 1).

Figure 1. Effect of 6-benzylaminopurine (BA) on the number of *Guadua latifolia* shoots after 21 and 38 days of in vitro culture in Murashige and Skoog (1962) liquid medium.

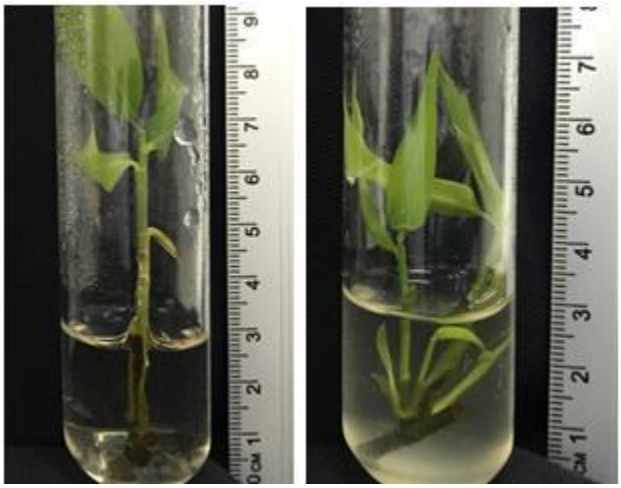


Source: Authors.

The absence of BA significantly influenced the number of shoots in the control treatment, which exhibited the smallest number of regenerated shoots (Figure 2 A and B). The subculturing time was also recorded with the first subculture measuring 21 days and the second 38 days. The remaining in vitro material and short incubation period with a flask change could serve as a strategy to control any contaminants not eliminated in the establishment phase. However, these questions have yet to be

elucidated.

Figure 2. *Guadua latifolia* shoots after 21 days. No shoots in 0 mg L^{-1} of BA (A) and shoot multiplication in 6 mg L^{-1} of BA (B).

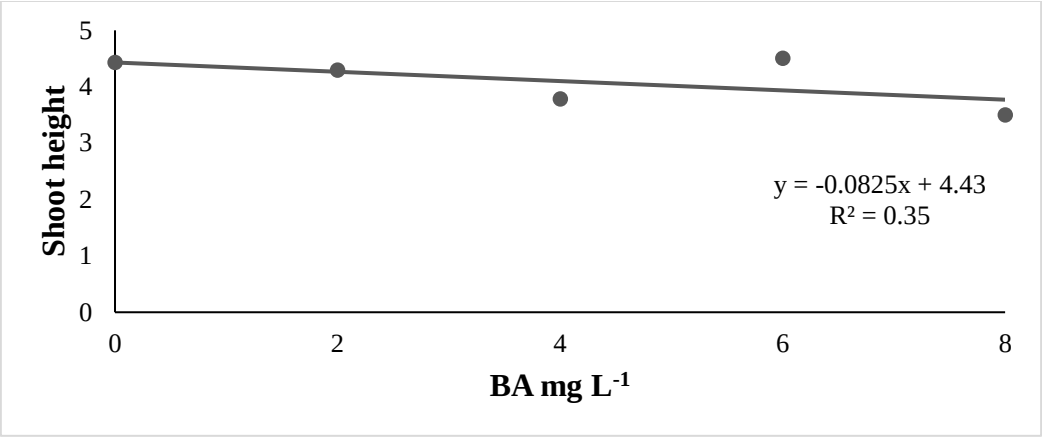


(A) (B)

Source: Authors.

In addition, there was no statistical difference in shoot height in BA concentrations for this culture, but the highest means recorded for treatments with 6 mg L^{-1} (Figure 3 and 4). However, despite its importance, this is not a decisive factor in the multiplication phase because shoot elongation is carried out in the rooting phase if necessary.

Figure 3. Effect of 6-benzylaminopurine (BA) on the height of *Guadua latifolia* shoots after 38 days of in vitro culture in Murashige and Skoog (1962) liquid medium.



Source: Authors.

Figure 4. *Guadua latifolia* shoot height in 6 mg L^{-1} of BA after 38 days of in vitro culture in Murashige and Skoog (1962) liquid medium.

Source: Authors.

According to Gutiérrez et al. (2016) in vitro multiplication of *G. angustifolia* with 3 mg L^{-1} of BA produced the best results in terms of the number and height of shoots. Similar findings were reported by Muthukumaran et al. (2018), who observed effective shoot proliferation in *Bambusa bambos* using 3 mg L^{-1} of BA. In the present study, the effect of BA was observed from a concentration of 2 mg L^{-1} onwards (Figure 1). However, Ornellas et al. (2019), reported that the shoot height was negatively affected by the culture medium supplemented with BA for *G. chacoensis.*

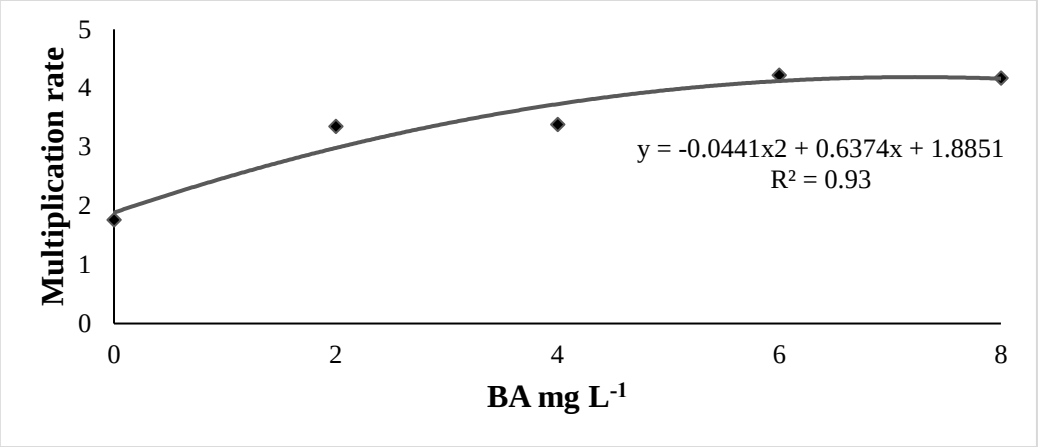
Shoot multiplication is an important criterion in successful commercial micropropagation. The cytokinin BA plays an important role in the proliferation of bamboo shoots, stimulating growth and multiple shoots, as observed by Jiménez et al. (2006) in *G. angustifolia* with 2 mg L^{-1} of BA. Bamboo multiplication with BA is widely used for shoot proliferation, likely due to its cost effectiveness and autoclavable nature (Ray & Ali, 2016).

The multiplication phase of *Bambusa balcooa* was reported by Gantait et al. (2018), with the best results obtained from 4 mg L^{-1} of BA onwards. The use of liquid medium was also found to be ideal in the multiplication of *Drepanostachyum falcatum* (Saini et al., 2016) and *Dendrocalamus strictus* (Goyal et al., 2015), similarly to the results obtained here. Nogueira et al. (2019) found that the liquid medium was more efficient than the semi-solid for the multiplication of *G. magna* and *G. angustifolia* for a greater plant production.

Moreover, Raju and Roy (2016) studied *Bambusa bambos* and found high shoot induction in MS liquid medium supplemented with 2.0 mg L^{-1} BA and 1.0 mg L^{-1} TDZ, as well as a high average number of shoots (3.14) per explant, shoot multiplication rate and average shoot length using the same MS medium.

Multiplication rate showed an increasing trend with a slight drop at the last tested concentration, indicating the positive influence of BA on this characteristic (Figure 5).

Figure 5. Effect of 6-benzylaminopurine (BA) on the multiplication rate of *Guadua latifolia* after 38 days of in vitro culture in Murashige and Skoog (1962) liquid medium.



Source: Authors.

Ornellas et al. (2019) observed that the culture medium supplemented with BA significantly increased the in vitro multiplication rate of *G. chacoensis* clumps. While Waikhom and Louis (2014) studied *Bambusa tulda* and *Melocanna baccifera* and reported that the highest shoot multiplication rate was achieved with the synergistic interaction between different cytokines. In the present study a single dose of BA increased the multiplication rate, but further research is needed to understand the effect of other plant growth regulators and concentrations in terms of increasing the multiplication rate and number of shoots. Then, with the multiplication rate obtained in this study was possible to estimate a cycle of 6 subcultures in 180 days to induce more than 5,000 *G. latifolia* shoots in a scenario with no microbial contamination.

4. Conclusion

Bamboo multiplication using 6.0 mg L^{-1} of 6-benzylaminopurine is more efficient and beneficial in terms of the number and height of shoots than other concentrations tested in this study. The same dose is recommended to obtain a multiplication rate of 4.22 shoots in 38 days of inoculation. In this experiment was not observed presence of callus and roots as well.

Recommendations for future research are proposed to evaluate the efficacy of different cytokinins and their concentrations in in vitro multiplication focus on kinetin (KIN), thidiazuron (TDZ) and isopentenyl adenine (2iP).

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