Concentrations of 6-Benzylaminopurine (BAP) in micropropagation of banana 'Farta Velhaco' (AAB)

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Abstract

Propagation methods such as in vitro micropropagation have been developed and improved to increase the multiplication rate in a short time and improve the seedlings quality. The objective of this study was to evaluate the efficiency of the different BAP concentrations on micropropagation of 'Farta Velhaco' banana during in vitro multiplication phase. The treatments were: T1 – control (0 mg L^{-1}); T2 - 0.5 mg L^{-1}; T3 - 1 mg L^{-1}; T4 - 1.5 mg L^{-1}; T5 - 2 mg L^{-1}; T6 - 2.5 mg L^{-1}; T7 - 3.0 mg L^{-1}; T8 - 3.5 mg L^{-1} and T9 - 4.0 mg L^{-1} of BAP added to the MS culture medium. The total number of shoots produced per explant initial subculture every 30 days was evaluated. The highest number of shoots is recorded 2.5 mg L^{-1} BAP concentration, which promotes 3 shoots per explant in the 4th subculture. The 4.0 mg L^{-1} BAP concentration reduces the number of shoots.

Keywords: *Musa sp*, biotechnology, citokinin

Introduction

Brazil is one of the world's leading banana (*Musa sp.*) producers. In 2015 there was a planted area of 484,430 ha with a yield of 6,844,491 tons. In Mato Grosso state, banana is gown in a small area, presenting 6,426 ha and production of 70,046 tons, accounting for only 0.8% of total Brazilian production (IBGE, 2015).

Banana can be propagated by seeds (Plant breeding) or vegetative by rhizome seedlings and *in vitro* seedlings. In the vegetative method, even using excellent quality materials, the process is slow and promotes the dissemination of diseases and pests such as Panama disease, black Sigatoka, yellow Sigatoka and rhizome borer (Souza et al., 2006).

The banana micropropagation, or *in vitro* propagation, consists of growing explants in artificial medium under specific conditions of luminosity, temperature and photoperiod. The main advantages of this method are: high multiplication rate compared to traditional methods and high phytosanitary quality of seedlings (Costa et al., 2009).

A micropropagation usually involves six steps: preparation, establishment, multiplication, stretching, rooting and, finally, acclimatization. The initial phase of the *in vitro* culture is considered the preparation of the matrix plants, destined to the supply of the primary explants for the culture.

The step involving multiplication is when the explants are multiplied in scale during successive subcultures in their own medium, so that the formed parts are subdivided into smaller parts or are individualized to form new explants (Costa et al., 2008).

In micropropagation, plant growth regulators are components in determining the development of plant cells. Cytokinins e auxins are the classes of plant growth regulators most used in tissue culture, with great variety of these regulators, both natural and synthetic, and great diversity of responses depending on used type. The cytokinins effect on differentiation of photosynthetic tissues, promoting amplification of the photosynthetic apparatus of dappled plants, leading to chloroplasts with larger granular system and greater accumulation of chlorophylls and photosynthetic enzymes, suggesting that the cytokinins, along with other factors such as light and plant nutrition, regulate the synthesis of pigments and proteins (Taiz & Zeiger, 2004).

Cytokinins play an important role during leaf ontogeny and, together with auxins, regulate the cell division rate. During the early stages of foliar ontogeny, there is cytokinin activity predominance, determining the maximum rate of chloroplast and cell division, membrane formation and proteins synthesis. After this linear growth phase, there is reduction on cytokinin activity and, in contrast, an increase in auxin activity, thus stimulating the lengthening of the mesophyll cells (Chernyad'ev, 2000).

Cytokinins such as 6-benzylaminopurine (BAP) and kinetin are usually used to reduce apical dominance and induce the meristem to form and stimulate sprouts in banana meristematic explants (Madhulatha et al., 2004).

BAP strongly stimulates growth of axillary, adventitious and foliar buds (Buah et al., 2010). Although BAP stimulates the shoot proliferation in bananas, if used in high concentrations it can cause high somaclonal variation rates becoming unviable its use for in vitro seedlings production (Bairus et al., 2008).

Micropropagation of the different banana genotypes was established using BAP and the culture media known as and Murashige and Skoog (MS) (Murashige & Skoog, 1962). The effectiveness of BAP on other cytokinins in the induction of meristem multiplication has also been reported in different banana cultivars (Farahani et al., 2008).

The 'Farta velhaco' banana is the most planted cultivar in Mato Grosso state among the small banana growers, especially in family agriculture, becoming an income source and promoting family sustainability in field. However, the lack of studies for the micropropagation of this banana cultivar make impossible its use, so most banana growers use banana seedlings propagated by the traditional method, in most cases, infested with borer and nematodes and contaminating non-infested areas.

The objective of this work was to evaluate the efficiency of the different BAP concentrations on micropropagation of 'Farta Velhaco' banana during in vitro multiplication phase.

Material and Methods

The experiment was carried out in the tissue culture laboratory of the Matogrossense Research, Assistance and Rural Extension Company (EMPAER-MT), located in the Regional Center for Research and Technology Transfer (CRPTT), Várzea Grande County, Mato Grosso State, Brazil. The explants used were from parent plants originated from *Musa* sp. 'Farta Velhaco' grown in the CRPTT of EMPAER-MT in Cáceres County, Matro Grosso State, Brazil, at 16°43'42 S and 57°40'51" W, from February 11 to July 11 2014. The rhizomes were washed to remove excess soil and root, and then the sheaths were sectioned with a sterilized knife, thereby reducing their size.

The explants were immersed in active chlorine 2% during twenty minutes, as also performed in other studies such as Carneiro et al. (2008), Pereira et al. (2009), Souza et al. (2010) and Pereira et al (2011).

The establishment phase was performed in a growth room with temperature 25 ± 2 °C and photoperiod of 16 hours of light at a luminous intensity of 30 µmol m^{-2} s^{-1}. The treatments used were: T1 – control (0 mg L^{-1}); T2 - 0.5 mg L^{-1}; T3 - 1 mg L^{-1}; T4 - 1.5 mg L^{-1}; T5 - 2 mg L^{-1}; T6 - 2.5 mg L^{-1}; T7 - 3.0 mg L^{-1}; T8 - 3.5 mg L^{-1} and T9 - 4.0 mg L^{-1} of BAP added to the MS culture medium.

The meristems were extracted under

aseptic conditions and incubated in MS medium, supplemented with sucrose at 30 g L^{-1} and the phytagel solidifier at 2.5 g L^{-1}, pH adjusted to 5.8 ± 1 before autoclaving (sterilization) at 120 ° C with 1 Kgf cm^{-2} for twenty minutes. In the multiplication process, four subcultures were performed, at 30 (first subculture), 60 (second subculture), 90 (third subculture) and 120 (fourth subculture) days after inoculation, realizing the cultivation of the lateral buds from the explant longitudinal subdivision and/or from the shoot isolation, with the leaves cut whenever possible.

At the end of each subculture, the shoots were isolated and re-inoculated in culture medium. The number of shoots obtained per initial explant in each 30-day subculture was evaluated. The experimental design was a completely randomized design with nine treatments, five replicates, each replicate represented by five explants representing a total of 225 explants. Data were submitted to analysis of variance using the SISVAR program (Ferreira, 2010), and regression analysis for BAP concentrations.

Results and Discussion

According to the variance analysis, there was significant difference among 6-Benzylaminopurine (BAP) regulator concentrations (Table 1) for the shoot number obtained per initial explant in each 30-day subculture (P <0.05). Regression analysis indicated second degree equations as the best fit for all variables.

It was found that there was a significant difference among the 6-Benzylaminopurine (BAP) regulator concentrations for shoot multiplication rate (P <0.05). Regression analysis indicated second degree equations as the best fit for all variables.

In the 1st subculture until 30 days after inoculation the maximum estimated peak was recorded at 2.78 mg L^{-1}, corresponding to 1.28 shoots. A reduction was observed in the number of shoots with BAP doses increase to 4.0 mg L^{-1}, presenting an average value of 1 shoot, with formation of vitrified tissues and callus (Figure 1).

In the second subculture until 60 days after inoculation, the derivative was calculated and indicated a maximum peak at 2.69 corresponding to 1.4 shoots and at the lowest concentration of 0.5 mg L^{-1}, which promoted 0.6 shoots. At the 1.0 mg L^{-1} concentration it is observed a 0.9 shoots average value, followed by a shoot number reduction with BAP dose up to 4.0 mg L^{-1}, with an average value of 1 shoot (Figure 2).

Table 1. Average squares of variance analysis and significance levels for the percentage of shoots emitted by explants under different 6-Benzylaminopurine (BAP) concentrations during subcultures of banana 'Farta Velhaco' multiplication. Várzea Grande, Brazil, 2014.

	Square means/Characteristics			
	1°	2°	3°	4°
BAP concentrations	2.50*	3.05*	4.50*	5.00*
C.V. (%)	13.73	10.00	15.70	20.70

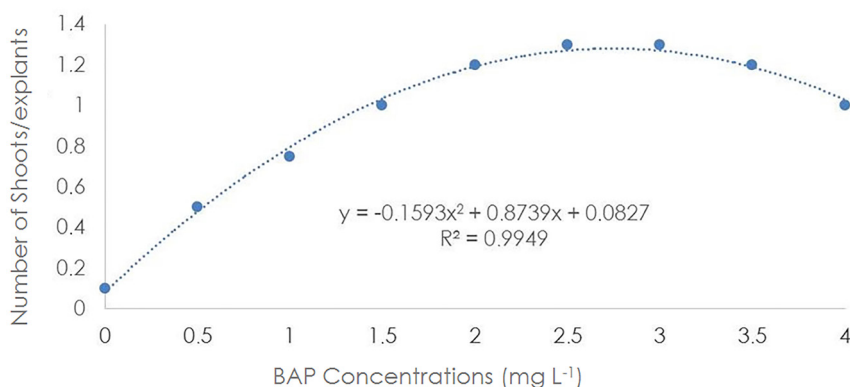*Significant at 5% of probability by 'F' Test. C.V. (%)= Coefficient of variation

Figure 1. Number of shoots emitted by 'Farta Velhaco' banana explant in the 1st subculture in culture medium containing different 6-Benzylaminopurine (BAP)concentrations. Várzea Grande, Brazil, 2014.

Several authors report the effect of cytokinins for apical dominance breakdown and emission of new shoots, but in contrast, effects on elongation inhibition due to the accumulation of these regulators in tissues are also observed (Costa et al., 2006).

The results demonstrated that the BAP concentration increase during the initial phase increased the shoot number, because it is a plant regulator of cytokinin group and physiologically it stimulates the totipotential cells responsible for multiplication, being of great importance the study to define which ideal BAP concentration to be used, and similar results were obtained by Subramaniam et al. (2008) for Cavendish (AAA) subgroup. Other authors indicated the concentration of 5.0 mg L^{-1} of BAP as optimal for Williams and Grande naine banana cultivars (Venkatachalam et al., 2007; Bairus et al., 2008)

In the 3rd subculture, the derivative calculation indicated the maximum peak at 3.0 mg L^{-1} corresponding to 2.4 shoots. At 2.0 mg L^{-1} BAP concentration it was observed 2.2 shoots. Then the number of shoots decreased with BAP dose increasing until 4.0 mg L^{-1}, presenting 2.2 shoots (Figure 3).

Oliveira & Pereira (2011) observed better multiplication rates using 4.0 mg L^{-1} of BAP in floral propagules with 5.3 shoots for Preciosa banana cultivar and 6.1 shoots for FHIA 02 cultivar, in the third subculture, thus in disagreement with present data. However after this subculture, the clones of both cultivars remained with a similar multiplication rate until the seventh subculture, with an average of 2.1 shoots per explant.

Lima and Moraes (2006) evaluated the absolute multiplication rate for FHIA 01 and verified the highest rate at 4.0 mg L^{-1} BAP concentration in the third subculture, thus different results of this work.

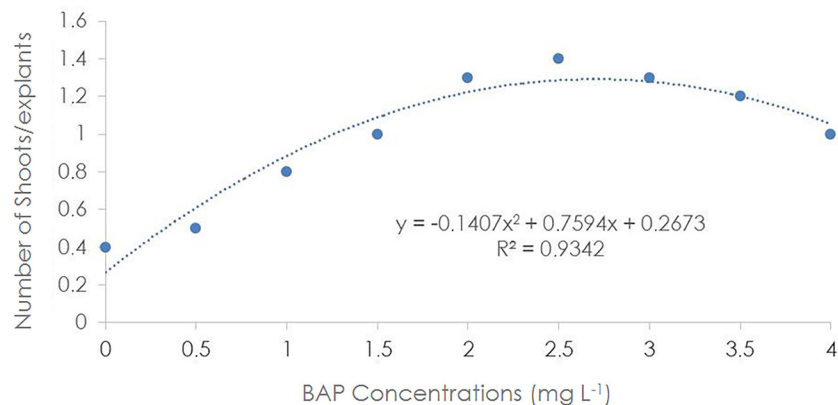The equation shown: $y = -0.1407x^2 + 0.7594x + 0.2673$, $R^2 = 0.9342$

Figure 2. Number of shoots emitted by 'Farta Velhaco' banana explant in the 2nd subculture in culture medium containing different 6-Benzylaminopurine (BAP)concentrations. Várzea Grande, Brazil, 2014.
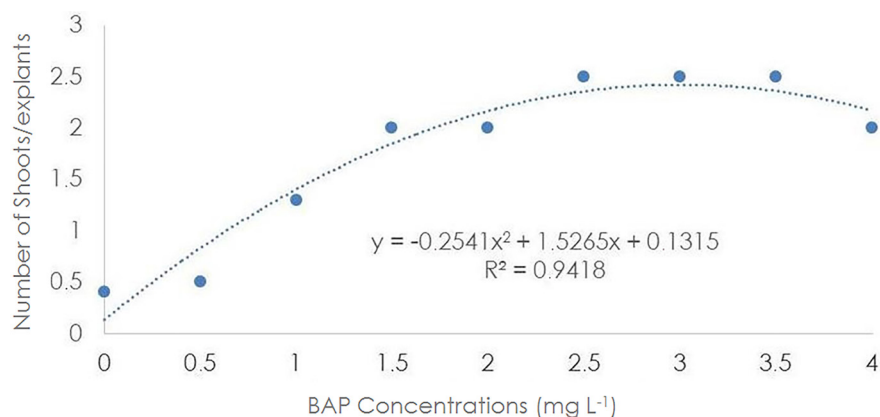

The equation shown: $y = -0.2541x^2 + 1.5265x + 0.1315$, $R^2 = 0.9418$

Figure 3. Number of shoots emitted by 'Farta Velhaco' banana explant in the 3rd subculture in culture medium containing different 6-Benzylaminopurine (BAP)concentrations. Várzea Grande, Brazil, 2014.

In the 4th subculture it was verified, a calculated peak at 2.47 mg L^{-1} BAP, corresponding to 2.9 shoots. From the 2.5 mg L^{-1} BAP concentration, the number of shoots decreased reaching 1.82 shoots at 4.0 mg L^{-1} BAP concentration (Figure 4).

Another study published by Oliveira & Silva (1997) showed that the highest *in vitro* multiplication rates for banana triploid cultivars Nanicão and Grande Naine, were obtained in the third and fourth subcultures. According to the authors, the multiplication rate is one of the main indicators to evaluate the performance of a company that produces micropropagated banana seedlings.

Similar results were obtained by Costa et al. (2006) with Grand naine banana cultivar who obtained a 2.0 shoots multiplication rate using 2.3 mg L^{-1} of BAP in 5 subcultures.

Differently from the present data, Ngomuo et al. (2013) studied the Yagami banana cultivar and obtained better results with 6.0 mg L^{-1} of BAP concentration obtaining an average of 9.55 shoots per initial explant; Urazibara et al. (2015) obtained a higher number of shoots (8.8) using the 4 mg L^{-1} BAP concentration for red banana micropropagation.

The different results quoted in the scientific literature can be explained by the influence of genotype, number of subcultures, concentrations and types of cytokinins on multiplication rate (Oliveira et al., 2001).
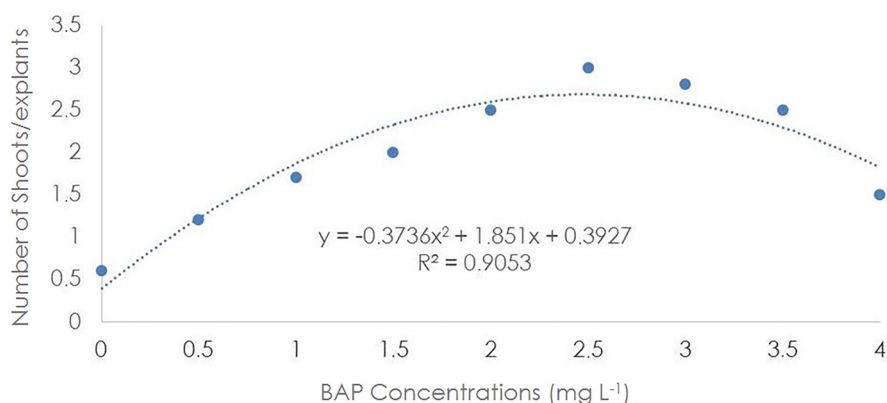

Figure 4. Number of shoots emitted by 'Farta Velhaco' banana explant in the 4th subculture in culture medium containing different 6-Benzylaminopurine (BAP)concentrations. Várzea Grande, Brazil, 2014.

Conclusions

- The highest number of shoots is recorded 2.5 mg L^{-1} BAP concentration, which promotes 3 shoots per explant in the 4th subculture;

- The 4.0 mg L^{-1} BAP concentration reduces the number of shoots.

Acknowledgements

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