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Cytokinins induce the development of *Campomanesia pubescens* root cuttings

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Abstract

The gabirobeira (*Campomanesia pubescens*) is a species native to Brazilian Cerrado with great potential but is threatened to extinction and remains wild. The nursery plant production is an alternative to solve those problems and the use of plant growth regulators can support vegetative propagation. The aim of the present study was to evaluate the effect of cytokinin concentrations on the growth of *C. pubescens* root cuttings. The concentrations of the cytokinin 6-Benzylaminopurine were: 0.0 (Control), 1.0; 2.0 and 4.0 mg L^{-1} diluted in NaOH solution (6 mol L^{-1}). The cuttings with a standard size of 5 cm were dipped in the concentrations for 15 seconds and transplanted in bags containing the substrate Bioplant. After 140 days the cuttings were evaluated for: the number of shoots, length of the shoots, number of leaves, length of the leaves, the diameter of the main stem, the diameter of the main root, the total number of roots, length of the main root, total fresh matter and total dry matter. The experiment was conducted in a completely randomized design, with fifteen replicates per treatment. The data were submitted to analysis of variance and the effects of the concentrations tested and adjusted by regression equations. The lower concentrations of cytokinin were beneficial to the development of the cuttings, while higher concentrations inhibited their development.

Keywords: 6-Benzylaminopurine, plant growth regulator, root propagation

Introduction

The gabirobeira (*Campomanesia pubescens*) is a fruit species native to the Brazilian Cerrado that belongs to the Myrtaceae family and has a high potential for cultivation. Can be found in several states, where fruits are appreciated for fresh consumption or in the form of processed such as juices, ice creams, popsicles, jellies, and liqueurs (Periotto & Gualtieri, 2017).

The absence of adequate techniques for the propagation of this species, aiming commercial production contributes to extractivism and predation. Associated with this, the advance of the agricultural frontiers has been causing exhaustion of this natural resource. Therefore, the domain of gabirobeira cultivation would reduce the scarcity of this natural resource and could enable its propagation (Rossato et al., 2015).

The success in seedlings production is determined by its method of propagation, which includes reproduction vegetative and by seed. Many plant species are usually propagated through seeds but it causes high genetic diversity among plants, long juvenile periods, and nonuniform production, besides, some seeds are considered recalcitrant, which means that they do not tolerate long periods of storage (Periotto & Gualtieri, 2017).

The nursery plant production by cuttings can be an alternative to obtain plants with desirable agronomic characteristics. The use of good quality propagation material is essential for success in agricultural activities and the rooting can be increased when associated with plant growth regulators (Timm et al., 2015; Paranatinga et al., 2018).

Despite being common the use of stem cuttings, the propagation with root cuttings can be an efficient technique, as observed by Tiberti et al. (2015), in which raspberry root cuttings treated with cytokinin were more

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viable when compared to stem cuttings. These authors also recommended other studies associating root cuttings with different concentrations of phytohormones since it is a scarce content in literature.

Cytokinin promotes growth and vegetative development through meristematic elongation and cell division, which are the main responsible for the formation of axillary gems and the development of shoots (Silva et al., 2017). This hormone also regulates the source-drain relationship between tissues, influencing the flow of assimilates and nutrients (Lima et al., 2016).

The use of plant growth regulators is common in the agronomic context and can promote early plant growth, quick recovery after water stress, resistance to pests, and a uniform establishment of plants improving nutrient absorption and yield (Dantas et al., 2012). In fruit species, cytokinin has different responses and functions, depending on the culture and time of supplementation of the concentrations (Giovanaz et al., 2014).

Given the potential use of gabirobeira and the importance of the use of plant growth regulators in the field of cultivation of native species, the present work aimed to evaluate the effect of different concentrations of cytokinin on the growth of *C. pubescens* root cuttings.

Material and Methods

For the evaluation of the growth of root cuttings of *C. pubescens*, the vegetal material was obtained on the summer in seedlings produced from seeds that were collected in the field of evaluation and conservation of genetic resources and grown in greenhouse conditions at the Federal University of Jataí. The cuttings with 1 year old were selected and standardized in 5 cm of length. The treatments consisted of four concentrations of cytokinin 6-benzylaminopurine (BAP): 0.0 (Control); 1.0; 2.0 and 4.0 mg L^{-1}.

The cuttings were dipped into distilled water for 15 seconds for the control treatment, and for the other treatments, the concentrations of BAP were diluted in NaOH (6 mol L^{-1}), in which the cuttings were dipped into the solution for 15 seconds. The treated cuttings were placed in polyethylene bags of 1.0 dm^3, filled with the commercial substrate Bioplant and maintained in a greenhouse for 140 days, where daily irrigations were carried out by nebulization twice a day for ten minutes with a flow rate of 48L h^{-1}.

At the end of the experiment, the number of shoots (NS), length of shoots (LS), the number of leaves (NL), length of leaves (LL), the diameter of the main stem (DMS), the diameter of the main root (DMR), the total number of roots (TNR), length of the main root (LMR), fresh matter (FM) and dry matter (DM) were evaluated.

The features NS, NL, and TNR were quantified by direct counting, and the length and diameter (mm) were performed with a digital caliper, in which the data of LMR was expressed in cm. To obtain the FM, the shoots and roots were separated and weighed individually in a weighing machine. Then, the plants were placed into an oven with a temperature of 60°C for 72 hours, to obtain the data of DM, according to the methodology proposed by Nakagawa (1999).

A completely randomized design with fifteen replicates per treatment was used, where each bag was considered as an experimental unit. The data were submitted to analysis of variance and the effects of the concentrations tested and adjusted in regression equations. The models were chosen based on the significance of the regression coefficients using the t-test ($p < 0.05$), in the coefficient of determination (R^2), and by the potential to explain the biological phenomenon. The data was obtained in the software SAS (STATISTICAL ANALYSIS SYSTEM, 2002) and the graphs were plotted by the SigmaPlot version 12.0 program.

Results and Discussion

The number of shoots presented a linear and descending behavior for the supplementation of the cytokinin 6-Benzylaminopurine (BAP). The values estimated by the model indicate that the increase of cytokinin concentration led to a reduction in the number of shoots, in which at the concentration 0.0 mg L^{-1} of BAP were formed 0.44 shoots and at 3.99 mg L^{-1} of BAP, a number of 0.08 shoots was produced (Figure 1A).

The reduction in the number of shoots may be due to the inhibition of meristem adventitial elongation promoted by the use of higher concentrations of BAP (Gonbad et al., 2014). Rossato et al. (2015), also reported a decrease in the number of shoots when the concentration increased to 2.0 mg L^{-1} on nodal segments of *Campomanesia adamantium* propagated *in vitro* and a greater number of shoots at the concentration of 1.0 mg L^{-1}.

There was also a reduction in the length of shoots with the increase of the concentrations, which was adjusted by the exponential decay model. At the absence of the 6-Benzylaminopurine, the shoots presented 7.77 mm, and the increase of the cytokinin concentrations led to a reduction in the length until reach 0.09 mm at the concentration 4.0 mg L^{-1} of BAP (Figure 1B).

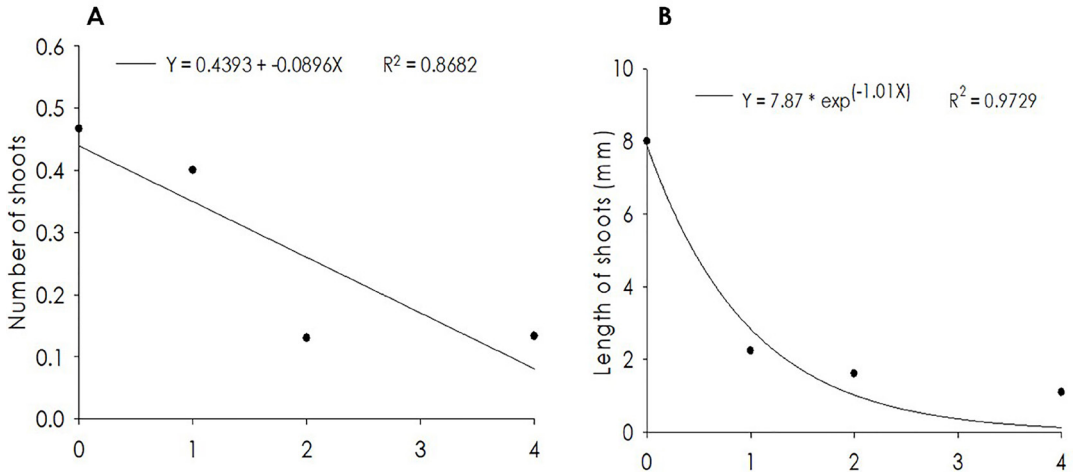

Figure 1. Number of shoots (A) and Length of shoots (mm) (B) from *Campomanesia pubescens* root cuttings treated with four concentrations of the cytokinin 6-benzylaminopurine.

The emanation of shoots is one of the fundamental characteristics in the formation of quality plants because they are a source of nutrition through photosynthesis after the exhaustion of the reserves of the cuttings (Raines et al., 2016), and its formation can be an indicator that the exogenous supplementation of BAP at reduced concentrations may be effective to promote the development of shoots since the action of this plant growth regulator is closely related to the modulation of shoots in cuttings (Dias et al., 2012).

High levels of BAP may cause phytotoxicity in root cuttings leading to a decrease in shoots emission, as observed at the higher concentrations in the present study, and according to Pereira et al. (2017), the number of shoots is influenced by several factors which range between the species, like seasons of the year and especially the concentration of plant regulators.

Cytokinin induces responsive cells to multiply and emit shoots, but it does not regulate shoot elongation, explaining the reduction of this variable when supplemented with BAP. This result is under accordance with Dias et al. (2012), which obtained a higher shoot length in mulberry (*Rubus* spp.) root cuttings in lower concentrations or the absence of BAP, and with Rossato et al. (2015), in which the highest concentrations of the 6-Benzylaminopurine in lighted conditions led to a decrease on the length of shoots from nodal segments of *C. adamantium* propagated *in vitro*. Goelzer et al. (2019), also reported a decrease in the length of shoots from *C. adamantium* propagated in vitro with the increase of the BAP concentrations and the higher length at the absence of the phytohormone.

There was a reduction in the number and length of leaves of the cuttings as the concentrations of BAP increased. Both characteristics were adjusted by the exponential decay model, in which the highest number of leaves was estimated at the absence of the use of cytokinin (0.48 leaves) and the lowest number of leaves was estimated at the concentration 4.0 mg L^{-1} of BAP (0.02 leaves), at the same way, was estimated that the concentration 0.0 mg L^{-1} led to the highest length (4.64 mm) and the concentration 4.0 mg L^{-1} resulted in the lowest length (0.12 mm) (Figure 2A and B).

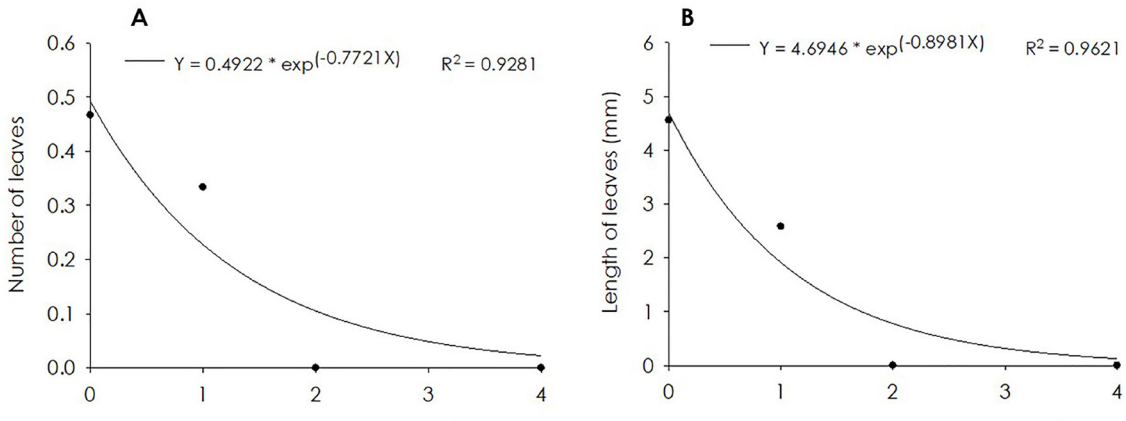

Figure 2. Number of leaves (A) and Length of leaves (mm) (B) from *Campomanesia pubescens* root cuttings treated with four concentrations of the cytokinin 6-benzylaminopurine.

Similar behavior was observed by Rossato et al. (2015), in which high concentrations of BAP exhibited the inhibitory action of the regulator on the induction and the leaf formation of gabirobeira explants. The BAP stimulates the morphogenetic processes, resulting in a significant number of shoots but reduced in size and number of leaves, with little development (Skalák et al., 2019).

The presence of leaves has been recognized as one of the most important characteristics for the survival and development of the cuttings since they are the center of production of auxin and other beneficial co-factors to the maintenance of the cuttings (Bischoff et al., 2017).

Regarding the length of leaves, it was observed that the exogenous supplementation of BAP at high concentrations caused a decrease in leaf length. Cytokinin is responsible for the modulation of shoot members, however, when in extreme levels it can cause tissue damage and reduction in the size of structures due to the phytotoxicity effect, varying as a function of cellular capacity (Neumann et al., 2018).

Was observed a linear and ascending behavior of the concentrations of cytokinin for the diameter of the main stem and a linear and descending effect was observed for the diameter of the main root. The diameter of the main stem increased linearly with the concentrations, which was estimated that 4.0 mg L^{-1} of BAP provided the highest stem diameter (1.37 mm) and the absence of the use of the cytokinin 6-Benzylaminopurine promoted the lowest diameter (1.18 mm), for the diameter of the main root, was observed that the values decreased linearly with the increase of the concentration, in which the lowest diameter observed was 0.66 mm at 4.0 mg L^{-1} and 0.88 mm when were no cytokinin supply (Figure 3A and B).

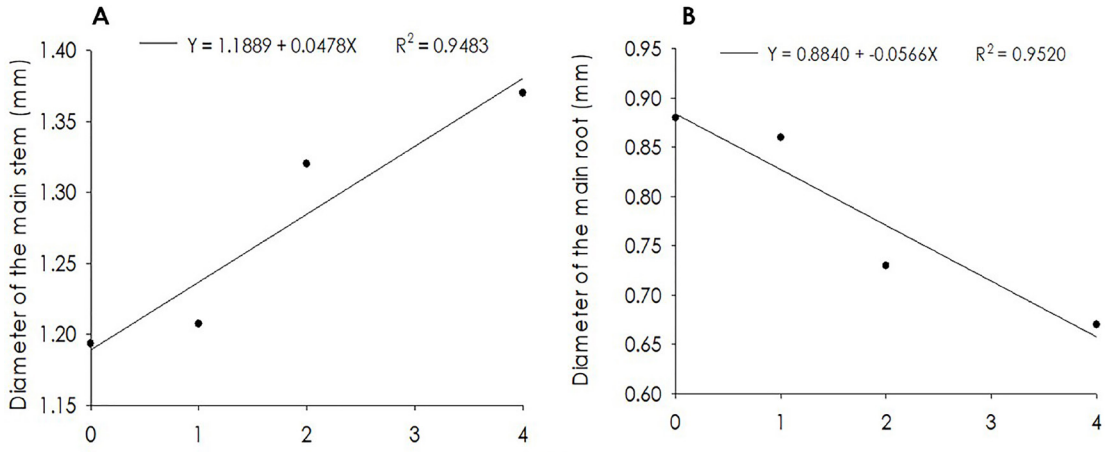

Figure 3. diameter of the main stem (mm) (A) and diameter of the main root (mm) (B) from 1-year old *Campomanesia pubescens* root cuttings treated with four concentrations of the cytokinin 6-benzylaminopurine.

Cytokinin promotes cell differentiation and elongation of the apical meristem, which leads to the expansion of plant tissues and consequently, to vegetative growth, however, large doses may cause opposite effects. The behavior observed in Figure 3B was described by Botin & Carvalho (2015), where high concentrations of 6-benzylaminopurine reduced meristematic activity and acted as inhibitors.

For the number of roots, was observed a linear and descending model, as a function of cytokinin concentrations, in which the adjustment of the equation indicates that the concentration of 4.0 mg L^{-1} led to a reduction to 0.93 roots and the highest number of roots was observed in the absence of cytokinin, which was 1.37 roots (Figure 4).

The reduction in the number of roots through BAP supplementation in roots cuttings demonstrates

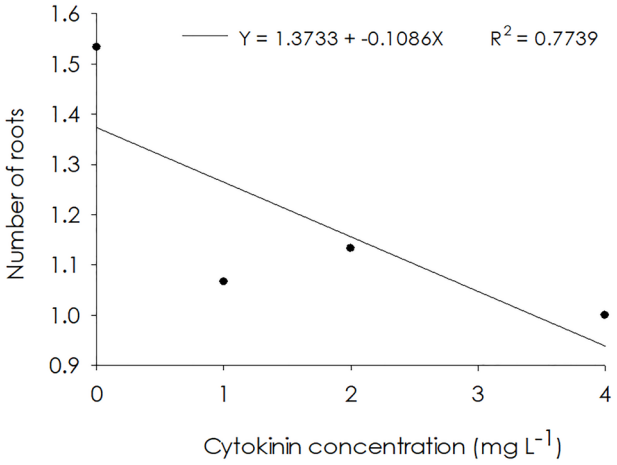

Figure 4. Number of roots from *Campomanesia pubescens* root cuttings treated with four concentrations of the cytokinin 6-benzylaminopurine.

the antagonistic action of the cytokinin, inhibiting the formation and development of the roots (Marhavy et al., 2014). The number of roots can influence the formation of seedlings since this material is used in the propagation to obtain new plants (Nogueira et al., 2017).

There was an influence of the cytokinin concentration on the length of the main root, whose equation was adjusted by the quadratic model as a function of the cytokinin concentrations and presented ascending behavior. Based on the adjustment, it can be observed that the supply of 3.89 mg L^{-1} of BAP promoted the highest root length, 5.34 cm (Figure 5).

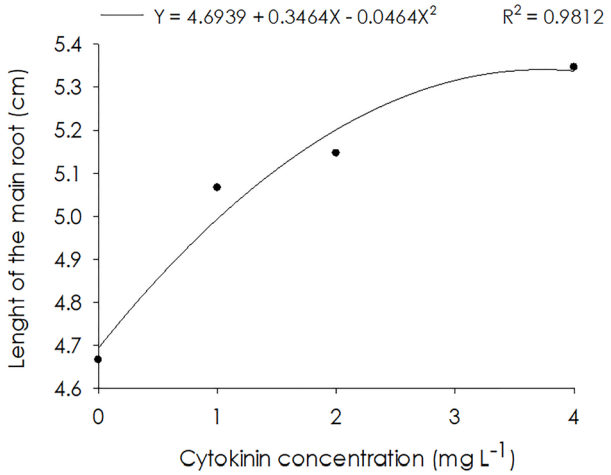

Figure 5. Length of the main root (cm) from 1-year old *Campomanesia pubescens* root cuttings treated with four concentrations of the cytokinin 6-benzylaminopurine.

The supply of exogenous cytokinin in the present work did not have an influence on the root elongation and a distinctive behavior of the cytokinin was observed, who depending on its specificity, acts on the regulation of shoots and inhibits the initiation of roots. Cytokinin is also an essential regulator of the break of apical dominance and proliferation of axillary gems, and the BAP is related to cell division and development of adventitious shoots (Jing & Strader, 2019).

The fresh and dry matter of the root cuttings presented a quadratic and ascending behavior, as a function of BAP concentrations. The fitting of the equation indicates that the highest fresh matter production was obtained in the concentration of 3.18 mg L^{-1} of BAP, in which produced cuttings with 0.14 g and the highest dry matter production was provided by the concentration of 2.96 mg L^{-1}, which was 0.049 g (Figure 6A and B).

Asmar et al. (2011) described similar behavior in the propagation of peppermint *in vitro*, in which the increase in the fresh matter and dry matter was observed in the higher concentrations, considering the beneficial effect on the mass accumulation, given the use of the plant growth regulator.

The seedlings that present a greater increment in the fresh matter are more suitable in case of transplanting, and better anchored in situations of a prolonged permanence in the field. The dry matter reflects directly the total increment accumulated and made available by the plant, being a parameter of great importance in terms of the productivity gain of the species. In general, the cytokinin at reduced concentrations is physiologically relevant, acting in the organism to promote inhibition of lateral roots and stimulate the formation of shoot limbs, being its use extremely favorable to the development of plants (Zem et al., 2016).

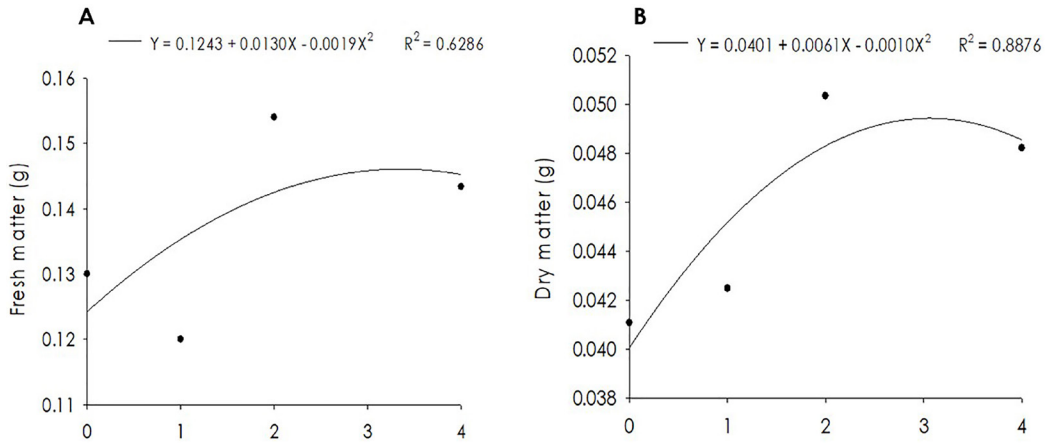

Figure 6. Fresh matter (g) (A) and dry matter (g) (B) from *Campomanesia pubescens* root cuttings treated with four concentrations of the cytokinin 6-benzylaminopurine.

The cytokinins are capable of promoting buds differentiation and root growth, besides influence the plant metabolical rate, enzymatic activity and the break of apical dominance, they are endogenously produced in the roots but it contributes to complete vegetal development, inducing the production of stem

buds (Peres et al., 1997; Soares et al., 2011). However, the excess of cytokinin concentration has been reported as a cause for the formation of non-normal plants, mainly with leaves of reduced size (Sousa & Miranda, 2006). The results obtained in the present study indicate that the mode of action of the 6-Benzylaminopurine on *C. pubescens* growth is similar to the responses obtained by other plant species, in which low concentrations induces the vegetal development and greater concentrations promote a reduction in the number and size of the structures.

Conclusions

The lower concentrations of BAP were efficient to promote the development of cuttings since the plant endogenously produces this hormone.

The higher concentration may cause a decrease in some growth parameters of the cuttings.

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