



## Cytokinin induces the development of gabirobeira root cuttings

Lasara Kamila Ferreira de Souza<sup>1</sup>  Alejandro Hurtado-Salazar<sup>2\*</sup>  Francielly Rodrigues Gomes<sup>1</sup>   
Laísse Danielle Pereira<sup>1</sup>  Diego Ismael Rocha<sup>1</sup>  Danielle Fabíola Pereira da Silva<sup>1</sup> 

<sup>1</sup>Universidade Federal de Jataí (UFJ), Jataí, GO, Brasil.

<sup>2</sup>Universidad de Caldas, 170004, Manizales, Caldas, Colombia. E-mail: alhuza@gmail.com. \*Corresponding author.

**ABSTRACT:** *The gabirobeira is a species native to the Brazilian Cerrado with potential for use in cropping systems. This study evaluated the effect of the cytokinin 6-benzylaminopurine (BAP) on root cuttings of gabirobeira (Campomanesia adamantium). The plant material was obtained from gabirobeira progenies of one and two-years-old. The cuttings were segmented in 5 cm length and 1.90 to 3.22 mm diameter, immersed in the following BAP concentrations: 0.0; 1.0; 2.0 and 4.0 mg L<sup>-1</sup> for 15 seconds and planted in trays containing the substrate Bioplant®. A complete randomized experimental design was adopted in a factorial scheme 2x4, (cuttings age x BAP concentrations) with fifteen replicates per treatment. After 140 days the number of cuttings with shoots, number of shoots, number of leaves, and diameter of the main root were evaluated. The better development of the cuttings was observed on progenies of two-years-old. The lowest cytokinin concentrations promoted the better emission and number of shoots of the progenies from both ages.*

**Key words:** *Campomanesia adamantium, plant growth regulator, shoots, native species.*

## Citocininas induzem o desenvolvimento de estacas de raiz de gabirobeira

**RESUMO:** *A gabirobeira é uma espécie nativa do Cerrado brasileiro com potencial para uso em sistemas de cultivo. O objetivo deste estudo foi avaliar o efeito da 6-benzilaminopurina (BAP) em estacas radiculares de progênies de Gabirobeira (Campomanesia adamantium). As estacas radiculares foram obtidas de progênies de Gabirobeira de um e dois anos de idade. Estes foram segmentados em 5 cm de comprimento e apresentavam entre 1,90 a 3,22 mm de diâmetro, imersos nas concentrações: 0.0; 1.0; 2.0 e 4.0 mg L<sup>-1</sup> de BAP por 15 segundos e plantados em bandejas contendo Bioplant®. O delineamento experimental foi o inteiramente casualizado, em esquema fatorial 2x4 (idades das estacas x concentrações de BAP), com quinze repetições por tratamento. Após 140 dias, foram avaliados o número de estacas com brotações, número de brotações, número de folhas e diâmetro da raiz principal. Estacas de raízes de progênies de dois anos de idade apresentaram melhor desenvolvimento. Menores concentrações de citocinina trouxeram melhores resultados de emissão e número de brotações das progênies de ambas as idades.*

**Palavras-chave:** *Campomanesia adamantium, regulador de crescimento, brotos, espécies nativas.*

The species from the genus *Campomanesia* (*Myrtaceae*) are native to the Brazilian Cerrado and some parts of Argentina and Paraguay, they have good market acceptance due to their specific characteristics, such as sweet flavor and pleasant smell. This species produces a high number of fruits per plant and has great genetic variability that can be used on cultivar development. Its fruits can be consumed *in natura* or processed to compose desserts, ice-creams or juices, and this diversity of use gives to *Campomanesia* plants a high economic potential who needs to be better exploited and characterized to its probable domestication (DAMIANI et al., 2016;

ARAÚJO & SOUZA, 2018; ZUNTINI et al., 2017; ARANCON et al., 2019).

The predatory exploitation combined with the expansion of new agricultural frontiers causes genetic erosion and contribute to environmental degradation, making it difficult to continue the evolutionary process and implementation of initiatives aiming species pre-breeding. Thus, actions aiming to plant propagation may allow the expansion of the genetic basis, conservation and reproduction of plants (ALMEIDA et al., 2019).

The species from the genus *Campomanesia* are allogamous and usually are propagated by seeds;

however, it presents high genetic variability by sexual propagation and has recalcitrant seeds that do not tolerate storage, which makes the uniform reproduction of progenies difficult (EMER et al., 2018a). An alternative is the employ of vegetative propagation techniques, it includes air layering (TELEGINSKI et al., 2018), cuttings (MARTINS et al., 2015; EMER et al., 2016; EMER et al., 2018b) or *in vitro* (ROSSATO et al., 2015; GOELZER et al., 2019; ROSSATO et al., 2019; MACHADO et al., 2020), always associated with plant growth regulators.

Therefore, vegetative propagation through root cuttings is one of the alternatives that allow the anticipation of the reproductive stage, reduces the juvenile period of the plants and ensures that their characteristics will be maintained (BIAZATTI et al., 2018). Few researches are using *in vivo* propagation of *Campomanesia* in the literature, but its use may be an alternative for the production of clonal nursery trees since great results have been obtained for other species and contributed to fast reproductions of new progenies (KOUAKOU et al., 2016). Besides, the use of cytokinin may be helpful to induce adventitious shoots when used exogenously favoring the development of vegetative organs (AZIZ & AL-TAWEEL, 2019).

It is known that the age of the cuttings influences the propagation due to the variation in the hormonal concentration, in the carbohydrate reserve and the degree of lignification of the vegetative material (ROSA et al., 2017), thus, this study aimed to evaluate the effect of the cytokinin 6-Benzylaminopurine (BAP) in root cuttings of *C. adamantium* obtained from progenies of one and two-years-old.

The vegetative material of *C. adamantium* was obtained from progenies of 1 and 2-years-old propagated by seeds collected in the municipality of Jataí – GO. The branches were collected in the morning and standardized with 5 cm length and a diameter ranging from 1.90 3.22 mm. After the preparation of the solution, the cuttings were immersed in 6-benzylaminopurine (BAP [Sigma Aldrich]) solution at the concentrations: 0; 1.0; 2.0 and 4 mg L<sup>-1</sup> diluted in NaOH (6 mol L<sup>-1</sup>) for 15 seconds. In the control, the cuttings were treated with distilled water. Subsequently, the planting was carried out in polypropylene tubes (55 cm<sup>3</sup>) containing Bioplant<sup>®</sup>, which is composed of pine bark, manure, sawdust, coconut fiber, vermiculite, rice husk, ashes, gypsum, calcium carbonate, magnesium, magnesium thermophosphate (yoorin<sup>®</sup>), and additives (fertilizers). During the experimental period, intermittent mist irrigation was programmed to be activated for one

minute every hour for 12 hours (6:00 am - 6:00 pm). The cuttings were kept on a greenhouse covered with polyethylene tarp and anti-aphid screen at the Federal University of Jataí – GO (17.8828° S, 51.7264° W).

After 140 days the cuttings were evaluated for the number of cuttings with adventitious shoots (NCS), obtained by direct counting, the number of shoots (NS), the length of shoots (LS), the number of leaves (NL), the length of leaves (LL) and the diameter of the main root (DMR), measured with a digital caliper and given in mm.

The experimental design was completely randomized in a factorial scheme 2x4, (two ages of root cuttings x four concentrations of BAP) with fifteen replicates per treatment, in which each tube was considered an experimental unit.

The data were subjected to analysis of variance, and the effects of the concentrations were tested and adjusted in regression equations. The models were chosen based on the significance of the regression coefficients using the t-test ( $p < 0.05$ ), on the coefficient of determination ( $R^2$ ) and on the potential to explain the biological phenomenon. The averages from the BAP concentrations were compared by the Duncan test ( $p < 0.05$ ). The data analysis was performed with the aid of the statistical program Sas (STATISTICAL ANALYSIS SYSTEM, 2002).

Results observed for the number of cuttings with adventitious shoots (NCS) for the progenies of one and two-years-old had a quadratic adjustment. The two-year-old root cuttings emitted 0.93 shoots at the concentration of 0.15 mg L<sup>-1</sup>, and the one-year-old progenies emitted 0.21 shoots at 1.0 mg L<sup>-1</sup> of BAP (Figure 1A). The increase in BAP concentrations is responsible for the reduction in the number and size of the plant structures since high concentrations can repress vegetative growth (MEGHWAL et al., 2010), this behavior could be observed in figure 2, in which the highest concentrations did not allow the development of the root cuttings.

The exogenous supplementation of lowest concentrations of BAP evidences its decisive impact on the growth regulation and the photosynthetic mechanism stabilization, modulating the responses according to its levels on the tissues (PREROSTOVA et al., 2018). The cytokinin (BAP) is responsible for breaking the apical dominance inducing the proliferation of axillary shoots, and when associated with other plant growth regulators it stimulates cell division and differentiation, transforming the cells into plant organs through morphogenesis (MÁXIMO et al., 2018).

The number of shoots on root cuttings from the progenies of both ages was adjusted by the

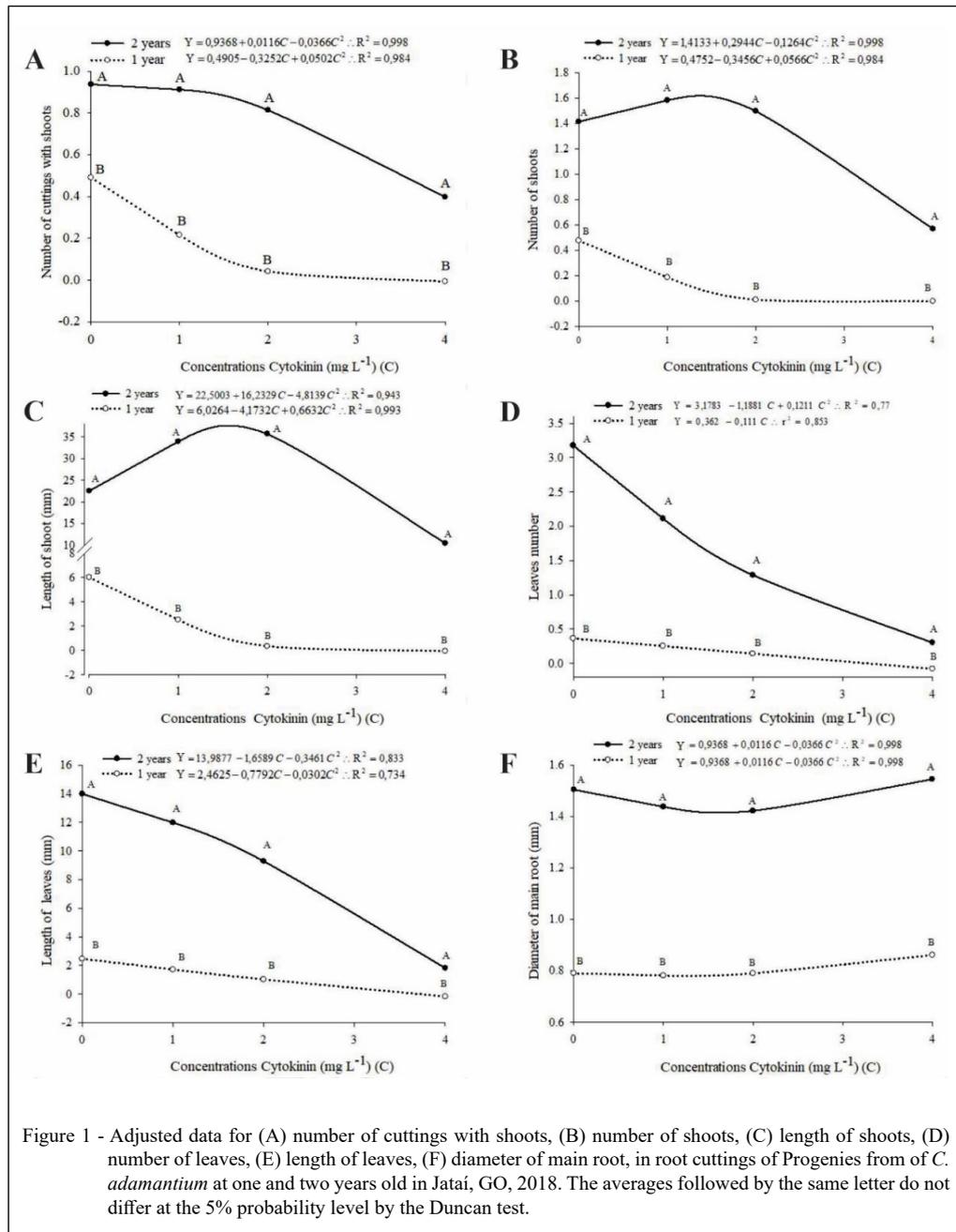


Figure 1 - Adjusted data for (A) number of cuttings with shoots, (B) number of shoots, (C) length of shoots, (D) number of leaves, (E) length of leaves, (F) diameter of main root, in root cuttings of Progenies from of *C. adamantium* at one and two years old in Jataí, GO, 2018. The averages followed by the same letter do not differ at the 5% probability level by the Duncan test.

quadratic equation. The concentration of  $1.68 \text{ mg L}^{-1}$  promoted the maximum development of two-year-old progenies, which was 1.58 shoots. In one-year-old progenies, the concentration of  $2.0 \text{ mg L}^{-1}$  developed a total of 0.01 shoots. (Figure 1B). ROSSATO et al. (2015), evaluating the effect of the BAP on the multiplication and rooting of *C. adamantium in vitro*, reported that there were a great number of shoots in the absence of the plant growth regulator,

probable due to endogenous levels of cytokinin on the vegetative material.

The length of shoots from *C. adamantium* root cuttings of one and two-years-old was adjusted by the quadratic model as a function of BAP concentrations. The maximum length of the shoots on two-years-old progenies was 36 mm at the concentration of  $1.68 \text{ mg L}^{-1}$ . The concentration of  $2.0 \text{ mg L}^{-1}$  promoted shoots with a minimum of 0.33 mm length in one-year-old

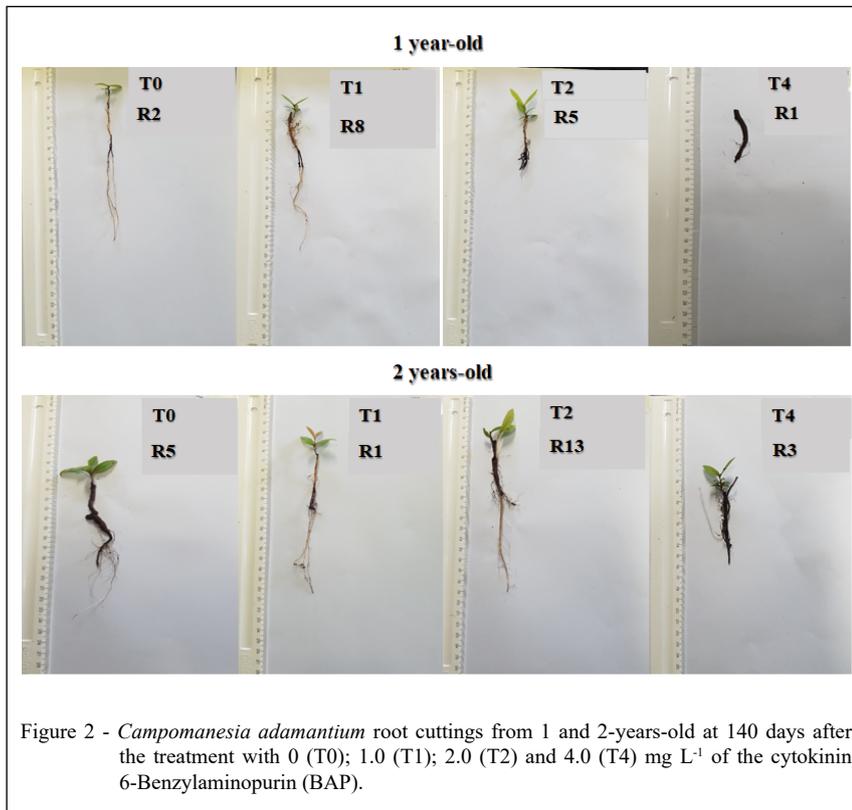


Figure 2 - *Campomanesia adamantium* root cuttings from 1 and 2-years-old at 140 days after the treatment with 0 (T0); 1.0 (T1); 2.0 (T2) and 4.0 (T4) mg L<sup>-1</sup> of the cytokinin 6-Benzylaminopurin (BAP).

progenies (Figure 1C), indicating that the responses to the cytokinin varied according to the genetic potential of the species, which is a process widely controlled (CHENG et al., 2013).

The BAP plays an important role in the multiplication of axillary gems, modulating the size, the length, and the number of leaves. When supplemented exogenously, depending on the concentration, it may cause a decrease in the growth of structures for being biologically active (KIEBER & SCHALLER, 2018). Similar results were obtained by GOELZER et al. (2019), in which the increase of BAP concentrations led to a reduction in the length of the shoots of *C. adamantium* explants grown in the murashige and skoog medium (MS).

The number of leaves from one-year-old root cuttings had a linear adjustment and the two-years-old progenies had a quadratic adjustment. The cuttings from both ages produced a higher number of leaves in the absence of the cytokinin supplementation. At the concentration of 4.0 mg L<sup>-1</sup>, there was an emission of 0.36 leaves in progenies of two-years-old, and 0.14 leaves in progenies of one-year-old (Figure 1D).

The number of leaves is extremely important for the development of the cuttings, due to the accomplishment of photosynthesis with the availability of photoassimilates or by the essential dynamics between the shoots and the root system (PEREIRA et al., 2017). ASMAR et al. (2012), evaluating BAP concentrations on *Lippia alba* *in vitro* propagation observed a decrease in the number of leaves at higher concentrations of cytokinin, BHALLA et al. (2009), also reported that the addition of BAP on the MS in the propagation of *Hibiscus Rosa-sinensis* led to a decrease in the number of leaves.

The length of leaves from progenies of one and two-years-old of *C. adamantium* supplemented with BAP had a quadratic adjustment. The absence of exogenous BAP supplementation provided a greater length on progenies from both ages; however, at the concentration 2.0 mg L<sup>-1</sup>, the length of leaves from two-years-old progenies was 0.78 mm, and at the concentration 1.0 mg L<sup>-1</sup> on one-year-old progenies, the leaves presented 1.65 mm length (Figure 1E). TIBERTI et al. (2015), observed that *Rubus idaeus* root cuttings treated with different concentrations of BAP presented a reduction in the size of its structures as the concentrations

increased and were bigger at the absence of plant growth regulators, probably caused by phytotoxicity.

The diameter of the main root of progenies from *C. adamantium* with one and two-years-old supplemented at different concentrations of the cytokinin 6-Benzylaminopurine had a quadratic adjustment. The diameter of the roots was not influenced by the exogenous BAP supplementation in cuttings of progenies of one and two-years-old and they presented similar behavior (Figure 1F).

The consistency in the root diameter demonstrated that cytokinin does not act in the modulation and regulation of this structure (KIEBER & SCHALLER, 2018). Commonly, the exogenous supplementation of cytokinin induces root diameter due to the swelling in the root elongation zone (BERTELL & ELIASSON, 1992).

Concerning the evaluated parameters of *C. adamantium*, the progenies of two-years-old had a greater development, which suggested the potential of this material for its conservation and reproduction. The use of cytokinin at reduced concentrations can favor the growth and development processes of gabirobeira root cuttings, and the use of this plant growth regulator can help in the process of rapid mass propagation of true-to-type vegetative material of *C. adamantium*.

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## DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

Conceptualization and material collection: Souza LKF, Gomes FR, Pereira LD. Design of methodology and data acquisition: Souza LKF, Salazar AH, Silva DFP. Data analysis: Souza LKF, Salazar AH, Pereira LD. Writing and editing: Souza LKF. Review of the manuscript: Salazar AH, Rocha DI, Silva, DFP. Orientation: Silva DFP.

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