

# Effect of *trans*-zeatin riboside application on growth of banana (*Musa* AAA Simmonds) cv. Williams in the juvenile phase

Efecto de la aplicación de *trans*-zeatina ribósido sobre el crecimiento de banano (*Musa* AAA Simmonds) cv. Williams en etapa juvenil



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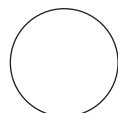
**Banana plantlets grown in nursery before field experiment with cytokinin applications.**

Photo: L.G. Schiller

## ABSTRACT

Cytokinins are physiologically active adenine derivatives that are vital to the regulation of various developmental processes in plants, such as vegetative growth and flower induction. The objective of this study was to understand how the application of cytokinin *trans*-zeatin riboside affects growth in banana (*Musa* AAA Simmonds) cv. Williams plants during the vegetative phase on two farms located in the production zone of the Magdalena province, Colombia. The effect of *trans*-zeatin riboside applications at doses of 0.00, 0.05, 0.25, or 0.45 mg L<sup>-1</sup> via foliar spraying was evaluated, with 15 days between the applications, starting with transplant to the field and lasting until 10 weeks of growth. The treatment 0.05 mg L<sup>-1</sup> of cytokinin resulted in increases in the plant growth variables, such as height in V, pseudostem diameter, leaf width, and leaf area.

**Additional key words:** hormone; cytokinin; meristem; Musaceae.



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## RESUMEN

Las citoquininas son compuestos fisiológicamente activos derivados de adenina, vitales para la regulación de varios procesos de desarrollo en plantas, tal como el crecimiento vegetativo y la inducción floral. El objetivo de este trabajo fue conocer como la aplicación de citoquinina *trans*-zeatina ribósido afecta el crecimiento vegetativo de plantas de banano (*Musa* AAA Simmonds) cv. Williams en dos fincas ubicadas en la zona bananera del departamento de Magdalena, Colombia. Se evaluó el efecto de la aplicación de *trans*-zeatina ribósido en dosis de 0,00; 0,05; 0,25 o 0,45 mg L<sup>-1</sup> vía foliar, con espacio entre aplicación de 15 días iniciando en el momento de trasplante de plantas al sitio definitivo y hasta 10 semanas de crecimiento. El tratamiento de plantas con 0,05 mg L<sup>-1</sup> de citoquinina resultó en incrementos en variables del crecimiento, tales como altura a la V, diámetro del seudotallo, ancho foliar y área foliar.

**Palabras clave adicionales:** hormona; citoquinina; meristemo; Musaceae.

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## INTRODUCTION

In Colombia, banana cultivation occupied 85,700 ha in 2016, of which 8,635 ha were in the municipalities of the banana production zone, Magdalena province, which generated 290,428 t, corresponding to 14.2% of domestic production. This production had an approximate value of 2,093,985 million US dollars (Agronet, 2018). One of the tasks necessary for a better yield is adequate edaphic and foliar fertilization of the crop that is done properly. Phytohormones could be used as a complement to mineral nutrition, acting on the metabolic functions of plants (Albán, 2014). Among the main phytohormones, cytokinins stimulate plant growth and development (Mok, 2018). Within the group of cytokinins, agricultural applications in the field exist for kinetin, zeatin, zeatin riboside, *trans*-zeatin, *cis*-zeatin, dihydrozeatin, 6-benzylaminopurine, and other natural and synthetic compounds (Sánchez and Mira, 2013). The physiological benefits of cytokinin applications to plants include stimulation of leaf growth, regulation of shoot and root development, stimulation of respiration and photosynthesis, flower induction, and fruit growth (Jordán and Casaretto, 2006; Bar and Ori, 2014; Mok, 2018).

In Musaceae, according to the BBCH scale of development, the vegetative meristem has a characteristic arrangement of leaves with a spiral form, absence of lateral shoots and absence of internode growth (Nalina *et al.*, 2006). The reproductive phase in this crop begins with changes occurring in the vegetative meristem, which undergoes metabolic shifts that transform it into the floral bud (Sánchez and Mira, 2013; Landrein *et al.*, 2018). In banana, flower induction

depends on the number of emerged leaves (Rodríguez *et al.*, 2012), which, in turn, relays on external factors and internal regulators, such as hormone activity (Chaurasia *et al.*, 2017).

An important phase in the life cycle of banana plants is inflorescence development, where climatic conditions including temperature, wind, and precipitation can delay flower differentiation and affect bunch formation (Galán *et al.*, 2012). Air temperature influences the transpiration rate, as well as duration of life cycle and bunch weight in banana plants through the control that it exerts on leaf metabolism (Robinson and Galán, 2012). Precipitation is one of the main climatic factors that determine banana crop development (Santos *et al.*, 2018); plants can quickly adapt to a water deficit in soil, which would further severely reduce the rates of leaf emission and bunch development (Galán *et al.*, 2012). The effects of edaphoclimatic conditions on flower induction and differentiation in Musaceae could be explained, at least in part, by the hormonal interactions in plants (Robinson and Galán, 2012).

According to previous studies, applications of plant growth regulators to Musaceae might shorten the juvenile phase of growth and accelerate flower differentiation. Thus, the application of auxins at a rate 100 mg L<sup>-1</sup> have stimulated floral differentiation in banana; however, treatments with naphthalene acetic acid with doses exceeding 500 mg L<sup>-1</sup> have caused fruit malformations (Lima *et al.*, 2016). At the same time, applying gibberellins at doses of 500 to 1,000 mg

L<sup>-1</sup> has caused elongation of the pseudostem (Lima *et al.*, 2016). Likewise, during vegetative growth, spraying brassinolide at 3–6 g L<sup>-1</sup> on banana (*Musa* sp. cv. Berangan) plantlets significantly has increased plant height, pseudostem diameter, leaf number, leaf area, and fresh and dry weight of plants (Zakaria *et al.*, 2018). Applications of cytokinins, such as benzyladenine, at doses of 20 to 100 mg L<sup>-1</sup> have increased the growth of corm in banana plants (Lahav and Gottreich, 1984). According to Muriel (2012), cytokinins influenced cell division, retarded senescence, and increased growth, fruit weight, and number of exportable hands of banana.

In general, the effects of cytokinin applications on banana growth in the field are poorly studied. López (2014) evaluated applications of cytokinins, comparing two fertilizer alternatives injected into the pseudostem of *Musa* sp. These treatments had no positive effect on the plant growth; one of the treatments contained kinetin at 1 g L<sup>-1</sup> (López, 2014). In the vegetative propagation of *Musa* sp. with 6-benzylaminopurine (BAP) and indoleacetic acid (IAA), the best treatment was 30 mg L<sup>-1</sup> BAP, which positively influenced shoot formation in each studied variety, followed by the concentration 30 mg L<sup>-1</sup> BAP + 10 mg L<sup>-1</sup> IAA (Canchignia *et al.*, 2008). Albán (2014) found positive effects from cytokinin applications on leaf emission, increasing the pseudostem diameter in cv. Grand Naine; in addition, foliar applications of algae extracts rich in cytokinins were a source of hormones and essential carbohydrates, which resulted in better yield and harvest quality (Albán, 2014). Aspiazu (2014) compared the applications of various hormones, concluding that gibberellins (20 mg L<sup>-1</sup>) and brassinosteroids (2 mg L<sup>-1</sup>) positively influenced plant growth and increased leaf length in banana. However, when 20 mg L<sup>-1</sup> gibberellin, 20 mg L<sup>-1</sup> cytokinin, and 2 mg L<sup>-1</sup> brassinosteroids were jointly applied, no significant increases were found in the pseudostem diameter, number/weight of roots or leaf width (Aspiazu, 2014). Langford *et al.* (2017) studied the macropropagation of banana plants in a nursery with BAP at two concentrations: 10<sup>-2</sup> M and 5 × 10<sup>-3</sup> M, with the following treatments: immersion of corms in BAP solutions for 30 min, immersion of corms in coconut water for 30 min, and placing the corms in a substrate of rice husk. The treatments with BAP and coconut water (a natural source of cytokinins) induced sprouting in the corms and a loss of the apical dominance (Langford *et al.*, 2017).

The use of phytohormones gibberellins, brassinosteroids, and cytokinins, individually or mixed, is mainly studied in the propagation of *Musa* sp., such as in the asexual multiplication of corms planted in the field (Canchignia *et al.*, 2008). However, no reports were found in the literature on the use of *trans*-zeatin riboside as a possible factor increasing vegetative growth in banana. The objective of the present research was to evaluate the effect of applications of *trans*-zeatin riboside (cytokinin) on the vegetative growth of banana cv. Williams in the Magdalena Province of Colombia.

## MATERIALS AND METHODS

### Experiment locations

This field study was carried out on two farms: El Polo located at 10°53'39.905" N and 74°11'58.214" W and La Paz 1 located at 10°53'37.17" N and 74°11'54.623" W, both at an altitude of 20 m a.s.l. in the town El Mamey (Colombia), and characterized with a tropical dry climate according to Holdridge (Aguirre, 2012). This study was conducted between October 2016 and January 2017. The duration of the field experiment was 13 weeks.

The climatic data in the field were obtained from the meteorological station El Enano of the Colombian meteorological institute INAT (El Mamey). The average air temperature was 26.8°C, with monthly precipitation of 12.8 mm and an average wind speed of 14.54 km h<sup>-1</sup>. In general, the climatic conditions were typical for areas of commercial banana production on the Atlantic coast of Colombia, which are adequate for the development of banana plants (Sánchez and Mira, 2013). The two farms, where the plants were established, differed in physical and chemical characteristics of the soil. The soil type on both was Inceptisoles (Soil Survey Staff, 2010). The marked differences between the two locations were in soil texture (La Paz 1: Loamy, El Polo: Sandy Loam); pH (La Paz 1: 5.8, El Polo: 7.7), C/N ratio (La Paz 1: 11, El Polo: 12.2), and cation exchange capacity (La Paz 1: 16.9, El Polo: 9.4 meq 100 g<sup>-1</sup>) of the soil arable layer.

### Field crop management

Banana (*Musa* AAA Simmonds) cv. Williams plants, propagated *in vitro*, were employed. Prior to transplant to the field, the plants were hardened in a

shade house, in which water and mineral nutrients were supplied as edaphic and foliar applications for 6 weeks.

The plants were established in the field at week zero with 4 leaves and 25 cm maximum height at the time of transplant; the planting density was 2.40 m between the plants and 2.40 m between the rows. In the sowing sites, 60 g per plant of Rafos® (Yara, Colombia) edaphic fertilizer were applied. The plant management practices employed by Torres (2016) for conventional banana produced for export in Colombia were used.

The irrigation with sprinkling was done between weeks 0 and 13 according to the needs of the crop. Between weeks 0 and 6 after planting, 6 mm ha<sup>-1</sup> of irrigation water were applied daily, divided in two irrigation periods. Starting from week 7 after planting, 5 mm ha<sup>-1</sup> water were applied per day; for this, a sub-foliar spray system was used, with 2014 Senninger® sprinklers spaced at 10 m by 10 m.

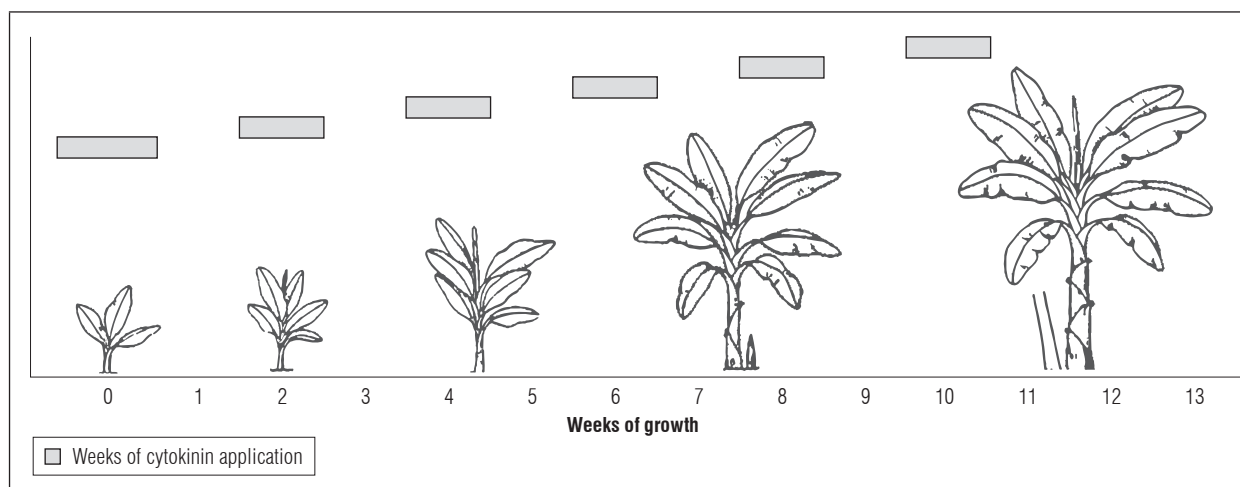
Every 15 d during weeks 0-12, edaphic fertilizers were applied at a rate of 60-120 g/plant. On the La Paz 1 farm, Rafos® was applied at 60 g/plant (week 0), Ammonium sulfate at 90 g/plant (week 2), Amidas® (Yara, Colombia) at 90 g/plant (week 4), Calcium Nitrate at 90 g/plant (week 6), and Abotek® (Yara, Colombia) at 90 g/plant (weeks 8 and 10) and 120 g/plant (week 12). On the El Polo farm, the same products were used but the doses were different: 60 g/plant (week 2), 60 g/plant (week 4), and 90 g/plant (week 12). Every 15 d starting from week 1, foliar

fertilizers (Wuxal®, Bayer, Colombia) were applied using a 20 L mechanical back pump, prepared with 100 cm<sup>3</sup> of molasse water.

The mechanical weed control (Quintero-Pertúz and Carbonó-Delahoz, 2015) was done every 15 d. Every week, the lower leaves affected by Black Sigatoka (*Mycosphaerella fijiensis*) were eliminated until week 5. Starting from week 6, only the fraction of the leaf affected by Black Sigatoka was removed. The products applied to control Black Sigatoka were Sico-Dithane® (3<sup>rd</sup> week), Siganax-Dithane® (5<sup>th</sup> week), Opus-Dithane® (8<sup>th</sup> week), and Voley-Dithane® (12<sup>th</sup> week). The suckers were cut off the plants every 6<sup>th</sup> week.

### Applications of *trans*-zeatin riboside

The hormone *trans*-zeatin riboside (t-ZR), 95% purity, was obtained from Sigma® laboratories (Sigma-Aldrich, St. Louis, MO), stored according to the manufacturer recommendations at -20°C, and diluted in type 1 water before the applications to reach the dose required in each treatment. Four treatments were tested on the plants via foliar spraying: water (control, 0 mg L<sup>-1</sup> *trans*-zeatin riboside), 0.05 mg L<sup>-1</sup>, 0.25 mg L<sup>-1</sup>, or 0.45 mg L<sup>-1</sup> of *trans*-zeatin riboside. Applications were made every 15 d (Fig. 1) using a manual back pump with a 21 pound pressure regulator and yellow nozzle (GEF-REPCar, 2011), spraying the plant leaves and pseudostem. The treatments started at planting in the field and continued until completing 6 applications of the hormone in each plant. The applications of cytokinin ended at week 10 after transplant (Fig. 1).



**Figure 1. Development of banana plants and the time of cytokinin applications.**

## Data collection in the field

The experiment had a bi-factorial randomized block design with six replicates (one plant per replicate) on the La Paz 1 and El Polo farms. A total of 360 plants were planted; in each location, 20 plots were arranged, of which, 15 plots had 6 plants and the other five plots had 18 plants.

From week 1 and up to week 13, in each research plot, biometric data were collected weekly on each plant: leaf length (in each leaf), leaf width (in each leaf), pseudostem diameter, and plant height. From the ground to the bifurcation point of the pseudostem, the height of the plant (cm) denominated as "height in V" was measured with a tape measure. The diameter of the pseudostem was measured with a caliper, 5 cm from the ground. The area of each leaf was calculated with the following equation (1) (Martínez *et al.*, 2015):

$$LA = \Sigma L \times A \times 0.80 \quad (1)$$

where  $LA$  is leaf area ( $\text{cm}^2$ ),  $L$  is length of the leaf blade from the apex of the leaf to its base (cm), and  $A$  is width of the leaf blade in the middle part (cm).

## Statistical analysis

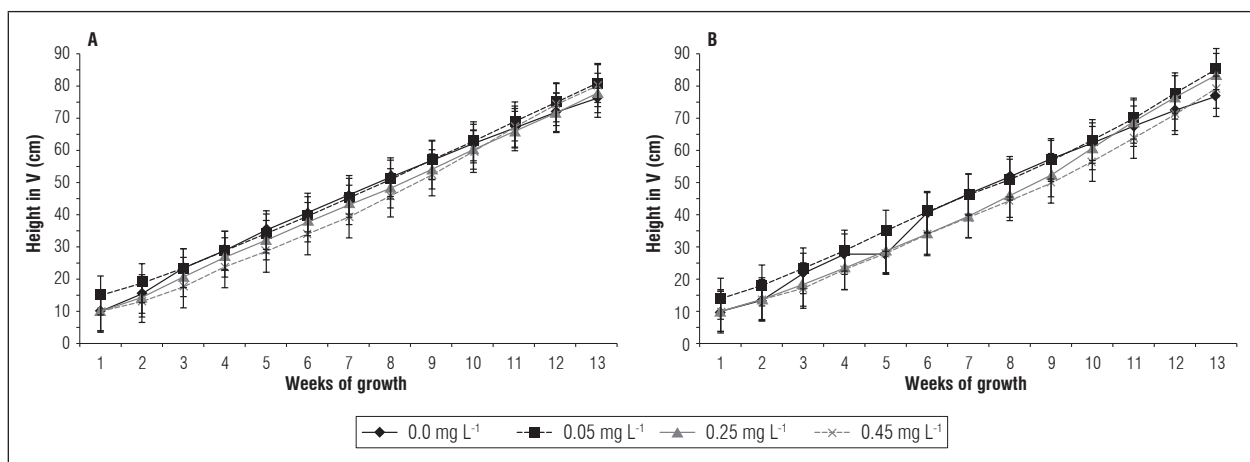
The statistical analysis used one-way analysis of variance, with a Tukey test at a confidence level of 95%. For all cases, the assumption of normality of the Shapiro-Wilk residuals and Bartlett's variance equality was tested. The area under the curve was calculated using the AUDPS function of the Agricolae® package

(Simko and Piepho, 2012). To identify statistically significant differences between the means of the variables, the Student t test ( $P < 0.05$ ) was used.

## RESULTS AND DISCUSSION

### Plant height

On the farm La Paz 1, treatments with 0 and 0.05  $\text{mg L}^{-1}$  *trans*-zeatin riboside resulted in a significantly larger plant height in V (height of the plant from the ground level up to point of the pseudostem bifurcation), as compared to the treatments with 0.25 and 0.45  $\text{mg L}^{-1}$  cytokinin (Fig. 2A). The tallest plants were obtained in the treatment with 0.05  $\text{mg L}^{-1}$  cytokinin, with a 46.3 cm average height (average value of measurements between weeks 1 and 13) (Fig. 2A). On El Polo, the four treatments had different heights, with the tallest plants, 46.9 cm average height, obtained after applications of 0.05  $\text{mg L}^{-1}$  cytokinin (Fig. 2B). Apparently, this indicated that *trans*-zeatin riboside, at a rate of 0.05  $\text{mg L}^{-1}$ , stimulated cell growth (cell division or/and cell elongation), which resulted in an increased height. These results are consistent with the research of Ortiz *et al.* (2013), in which cytokinin applications generated a faster growth in *Musa* sp. In banana, the pseudostem diameter and height-to-circumference ratio (HCR) for tall cultivars as well as HCR for medium-height cultivars are known as good predictors of inflorescence emergence since these variables have exhibited linear or quadratic



**Figure 2.** Height in V of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean  $\pm$  standard error.



relationships with the number of days from planting to inflorescence emergence (Vinson *et al.*, 2018).

### Pseudostem diameter

The diameter of the pseudostem was assessed as a variable of plant development; all treatments increased the pseudostem diameter on the farms La Paz 1 and El Polo over time. However, the plants grown on La Paz 1 (Fig. 3A) differed in the confidence intervals for the treatments 0 and 0.05 mg L<sup>-1</sup>, as compared to the treatments 0.25 and 0.45 mg L<sup>-1</sup>; the treatment 0.05 mg L<sup>-1</sup> yielded the highest average pseudostem diameter, 4.6 cm (average value of measurements between 1 and 13 weeks). On the El Polo farm, the plants treated with 0.05 mg L<sup>-1</sup> cytokinin had the largest average pseudostem diameter, 4.6 cm (Fig. 3B). These results indicate that the best treatment in both locations was 0.05 mg L<sup>-1</sup> *trans*-zeatin riboside, which was the lowest dose. The increase in pseudostem diameter could indicate a higher cellular activity, which implies increases in dry mass, higher demand for nutrients, and a higher meristematic activity (Sánchez and Mira, 2013). In *Musa* sp., a large pseudostem diameter correlates with a higher plant resistance to breakage by wind and reflects a plant's ability to sustain the bunch (Gonçalves *et al.*, 2018), as well as correlates with a higher storage capacity in the pseudostem for water and carbohydrates (Sánchez and Mira, 2013; Shivashankar *et al.*, 2016).

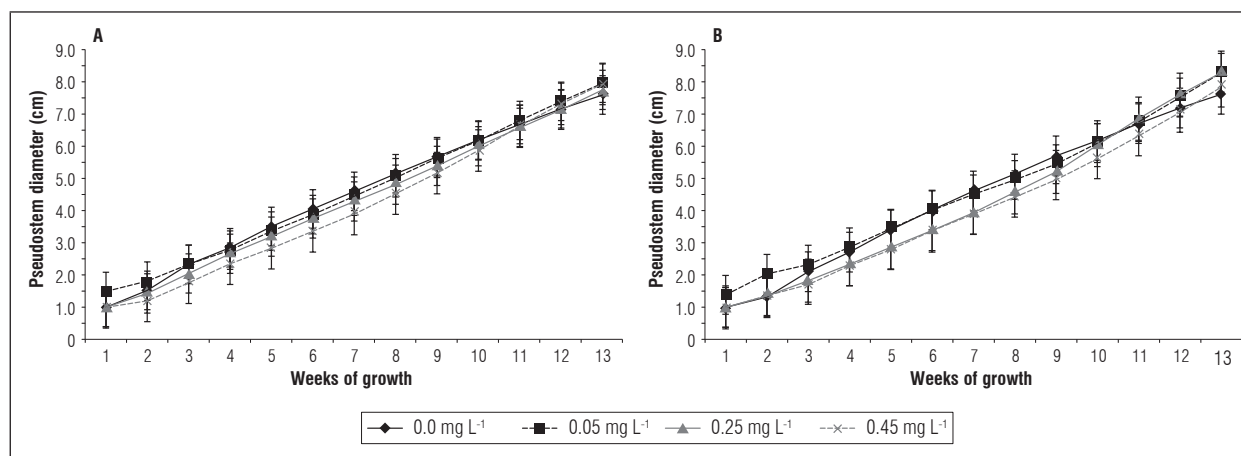
### Leaf growth variables

The leaf length did not present significant differences between the treatments (Fig. 4), while on La Paz

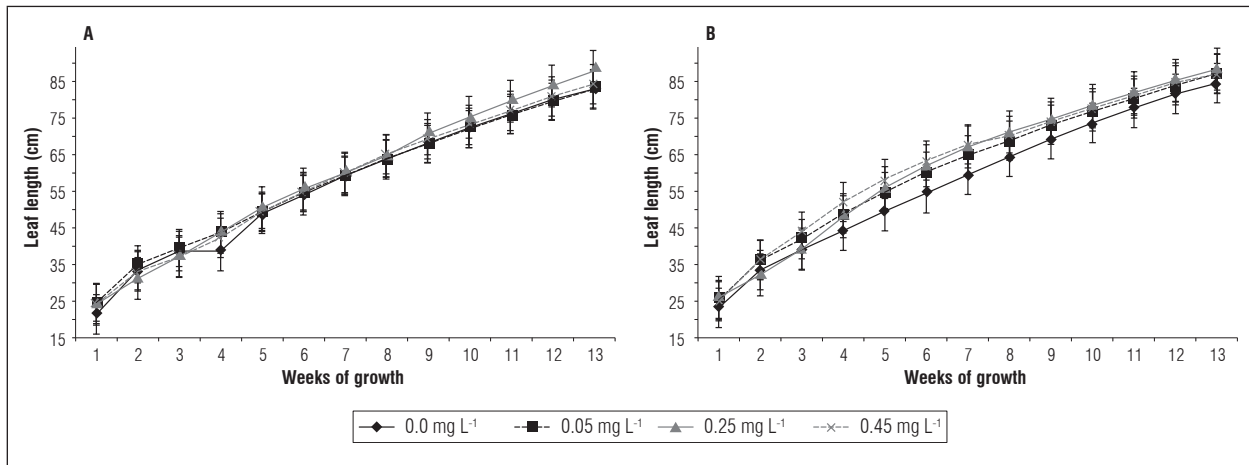
1, the leaves tended to be the longest with the 0.25 mg L<sup>-1</sup> cytokinin application, with an average length value of 58.5 cm (average value of measurements between 1 and 13 weeks) and, on El Polo, the best treatment was 0.45 mg L<sup>-1</sup>, with a length value of 63.1 cm (Fig. 4 A and B).

At the same time, the leaf width significantly differed between the treatments (Fig. 5). On La Paz 1, the widest leaves were obtained in the treatment with 0.25 mg L<sup>-1</sup> cytokinin, where the average leaf width reached 28.3 cm (average value of the measurements between 1 and 13 weeks) (Fig. 5A). On El Polo, the largest leaf width was recorded in the 0.05 mg L<sup>-1</sup> treatment, with a 29.9 cm average value (Fig. 5B). As a result, the plants on the El Polo farm presented the widest leaves. This effect from the cytokinins was consistent with the results of Bar and Ori (2014), who indicated that leaf blade development depends on light and cytokinins for maintaining balance with auxins and stimulation of meristem activity. Cytokinins are known to stimulate cell division and cell expansion in leaves (Mok, 2018). In a review carried out by Landrein *et al.* (2018) on *Arabidopsis*, expression of the *CYCD3* gene is required for development of new leaves until reaching the leaf size typical of this species.

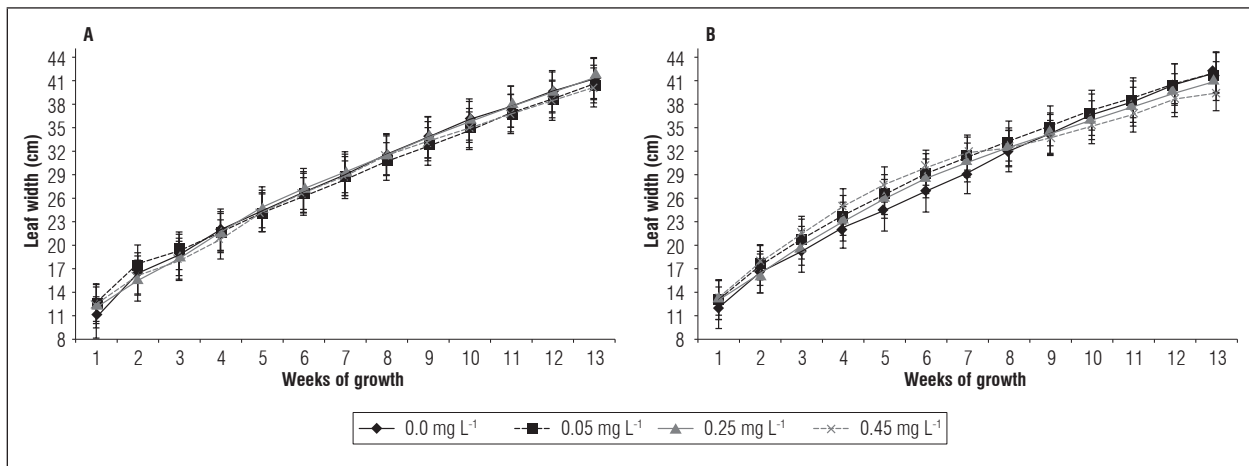
The leaf area reflected a relationship between leaf length and width, presenting no significant differences between the locations or between the treatments on La Paz 1 and El Polo. The highest average leaf area on La Paz 1 was reached with 0.25 mg L<sup>-1</sup> cytokinin, 1473 cm<sup>2</sup> (average value of measurements between weeks 1 and 13) and, on the El Polo farm, the 0.05 mg L<sup>-1</sup> cytokinin treatment resulted in the



**Figure 3.** Pseudostem diameter of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean  $\pm$  standard error.



**Figure 4.** Leaf length of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean  $\pm$  standard error.

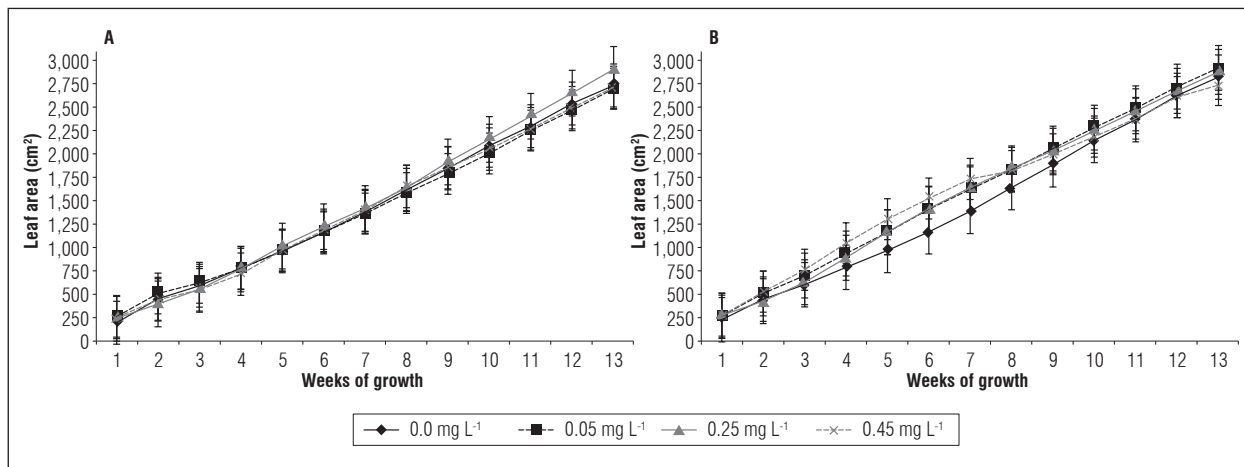


**Figure 5.** Leaf width of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean  $\pm$  standard error.

highest average leaf area, 1616 cm<sup>2</sup> (Fig. 6A and B). It can be speculated that the phytohormone spraying on the leaves/pseudostem affected expression of the *LOG* genes in the apical meristem region of the stem, where the stem cells reside. In *Arabidopsis*, the *LOG4* gene is expressed in the L1 layer of the vegetative meristem; *LOG* genes encode enzymes that convert inactive cytokinin ribosides into active forms, providing a localized source of active cytokinin in the vegetative meristem (Landrein *et al.*, 2018). Cytokinins could increase leaf size because of the high rate of cell expansion, yielding a higher shoot biomass (Skalák *et al.*, 2019) and delayed leaf senescence (Gan, 2014). In banana, direct relationships between leaf

area and yield were previously established (Robinson and Galán, 2012); these data are important since the growth and formation of banana bunches depends on the number and physiological activity of functional leaves (Rodríguez *et al.*, 2012).

The results of the present study indicated that the application of *trans*-zeatin riboside positively influenced the development of banana plants without a reduction in growth or presenting deformations in plant morphology, such as the ones reported by Albán (2014). In our research, the treatment that generated, on average, the highest leaf expansion was 0.05 mg L<sup>-1</sup> *trans*-zeatin riboside, as compared with



**Figure 6.** Leaf area of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean  $\pm$  standard error.

the other evaluated treatments. These findings agree with the data reported for applications of other rates/formulae of cytokinins, which tended to increase the growth rate of plants, resulting in increases in height, pseudostem diameter, leaf length and leaf width in *Musa* sp. plants (Albán, 2014; Aremu *et al.*, 2014; Aspiazu, 2014).

The practical importance of faster vegetative growth in Musaceae at the commercial level includes a reduction of the juvenile phase of growth, which favors a change from vegetative to reproductive growth, accelerates flower differentiation, and reduces the number of weeks required for flowering and initiation of bunch formation. The applications of *trans*-zeatin riboside on *Musa* sp. plants during vegetative development could be explored for possibly counteracting the effect of stress caused by biotic and abiotic factors (Schäfer *et al.*, 2015; Miller *et al.*, 2017), which might further increase production levels on commercial plantations.

## CONCLUSIONS

The growth variables height in V, leaf width, leaf area, and pseudostem diameter presented statistically significant differences, depending on the level of *trans*-zeatin riboside sprayed on the banana plants cv. Williams during 10 weeks of growth in the field. The low doses of *trans*-zeatin riboside, such as 0.05 mg L<sup>-1</sup>, generated the best results for plant height at V, pseudostem diameter, leaf width and leaf area.

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**Conflict of interests:** this manuscript was prepared and reviewed with the participation of the authors, who declare that there is no exists conflict of interest that puts the validity of the presented results at risk.

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