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Stimulus to rooting of *Saccharum* sp. mini-cuttings with talc indole-butyric acid

Estímulo ao enraizamento de minirrebolos de *Saccharum* sp. com ácido indolbutítico na forma de talco

Fomentando el enraizamiento de minirrebolos de *Saccharum* sp. con ácido indolbutítico en forma de talco

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Abstract
Sugarcane is a commercially important species for sugar and biofuel production and because of the high demand for plant material in planting, new means of propagation are being developed and require improvements. Therefore, the objective of this study was to evaluate the effect of different concentrations of indole-butyric acid (IBA) applied in the form of talc on the initial growth and emergence of sugarcane mini-cuttings used for the system of pre-sprouted plantlets (PSP). Mini-cuttings were treated with concentrations of 0, 500, 1000, 1500 and 2000 mg kg^{-1} of IBA in the form of talc and evaluated for emergence and initial growth. There was no difference between IBA concentrations for the physiological, biometric and dry mass variables analyzed. The sugarcane plantlets formed had emergence above 89%, functional photosynthetic apparatus, 4.4 leaves on average, stem diameter of 11.90 mm, shoot length of 12.18 cm, leaf area of 108.81 cm^2 and root area of 142.17 cm^2. Shoot dry mass and root dry mass were equal to 2.27 and 1.20 grams, respectively. Thus, concentrations of up to 2000 mg kg^{-1} of IBA applied in the form of talc in sugarcane mini-cuttings had no effect on rooting and initial growth of plantlets.
Keywords: Sugarcane; Propagation; IBA; Growth regulator; Rooting.

Resumo
A cana-de-açúcar é uma espécie comercialmente importante para a produção de açúcar e biocombustíveis e, devido à alta demanda de material vegetal no plantio, novos meios de propagação estão sendo desenvolvidos e necessitam de melhorias. Portanto, o objetivo deste trabalho foi avaliar o efeito de diferentes concentrações de ácido indolbutírico (AIB) aplicado na forma de talco sobre o crescimento inicial e a emergência de minirrebolos de cana-de-açúcar utilizadas no sistema de mudas pré-brotadas (MPB). Minirrebolos foram tratadas com concentrações de 0, 500, 1000, 1500 e 2000 mg kg^{-1} de AIB na forma de talco e avaliados quanto à emergência e crescimento inicial. Não houve diferença entre as concentrações de AIB para as variáveis fisiológicas, biométricas e de massa seca analisadas. As mudas de cana-de-açúcar formadas apresentaram emergência acima de 89%, aparato fotossintético funcional, 4,4 folhas em média, diâmetro do caule de 11,90 mm, comprimento da parte aérea de 12,18 cm, área foliar de 108,81 cm^2 e área radicular de 142,17 cm^2. A massa seca da parte aérea e a massa seca da raiz foram iguais a 2,27 e 1,20 gramas,

respectivamente. Assim, concentrações de até 2.000 mg kg-1 de AIB aplicadas na forma de talco em minirrebolos de cana-de-açúcar não afetaram o enraizamento e o crescimento inicial das mudas.
Palavras-chave: Cana-de-açúcar, Propagação, AIB, Regulador de crescimento, Enraizamento.

Resumen
La caña de azúcar es una especie de importancia comercial para la producción de azúcar y biocombustibles y, debido a la alta demanda de material vegetal en la siembra, se están desarrollando nuevos medios de propagación que necesitan mejoras. Por lo tanto, el objetivo de este trabajo fue evaluar el efecto de diferentes concentraciones de ácido indolbutírico (AIB) aplicado en forma de talco sobre el crecimiento inicial y la emergencia de las miniruelas de caña de azúcar utilizadas en el sistema de plántula prebroteda (MPB). Las miniruelas se trataron con concentraciones de 0, 500, 1000, 1500 y 2000 mg kg^{-1} de AIB en forma de talco y se evaluaron para determinar la emergencia y el crecimiento inicial. No hubo diferencia entre las concentraciones de IBA para las variables fisiológicas, biométricas y de masa seca analizadas. Las plántulas de caña de azúcar formadas presentaron emergencia superior al 89%, aparato fotosintético funcional, 4.4 hojas en promedio, diámetro del tallo de 11.90 mm, largo de brote de 12.18 cm, área foliar de 108, 81 cm^2 y área de raíces de 142.17 cm^2. La masa seca de la parte aérea y la masa seca de la raíz fueron de 2,27 y 1,20 gramos, respectivamente. Así, concentraciones de hasta 2.000 mg kg-1 de AIB aplicadas en forma de talco en miniruelas de caña de azúcar no afectaron el enraizamiento y crecimiento inicial de las plántulas.
Palabras clave: Caña de azúcar, Propagación, AIB, Regulador de crecimiento, Enraizamiento.

1. Introduction

Sugarcane has proven to be a crop of global interest due to its capacity for bioenergy production (Guo et al. 2015). Sugar is another product that stands out among sugarcane derivatives, as it is consumed worldwide and its demand increases every day (Conab 2017). The processing of sugarcane for ethanol and sugar production also generates several by-products that can be used as fertilizers, textile fiber, bioenergy, animal feed, among others (Sindhu et al. 2016). Thus, it is clear the importance of optimizing sugarcane yield in order to meet the demands for products, and this increase in yield can be achieved with proper management of the crop.

The system of pre-sprouted plantlets (PSP) of sugarcane is a new planting technique that proposes the formation of healthy plantlets that will be taken to the field with a high standard of health and homogeneity. The technique proposes the use of mini-cuttings, a region of the stem with an approximately 3-cm-long bud, for plantlet formation. Moreover, it is feasible, because it reduces the planting material from on average 20 t ha^{-1} to 2 t ha^{-1} and contemplates the uniformity and use of smaller machines for planting (Landell et al. 2013). For being a recent technique, few studies have been conducted to improve this plantlet production system, so research aimed at reducing nursery time and optimizing this technique is needed.

Auxins are a group of phytohormones responsible for various plant growth processes (Finet and Jaillais 2012). They can be applied in the form of powder at different concentrations, immersion for approximately 5 seconds in concentrated solution (200 to 10000 mg L^{-1}) or immersion for approximately 24 hours in diluted solution (20 to 200 mg L^{-1}). Indole-butyric acid (IBA) is considered to be more efficient for the cutting process for being more stable to the action of light and resistant to the action of enzymes. When applied in the form of talc, it outperforms the application in liquid form, because the talc easily adheres to the propagule, is easy to identify in the treated cuttings and the period of exposure of the cutting to the regulator is longer and the solution is more durable (Fachinello et al. 2005; Yamamoto et al. 2010).

Sugarcane cultivars have genetic characteristics of high rooting, and the use of agricultural techniques such as fertilization with vinasse, which is rich in nitrogen, has already been shown to favor sugarcane root growth in the surface soil layer (Otto et al. 2009). The rooting capacity of plants is a crucial factor that ensures the uptake of water and nutrients and their consequent survival and performance in the field (Chopart et al. 2010). Thus, increasing rooting during the phase of plantlets can ensure their successful growth in the field.

Rooting can occur more rapidly if exogenous auxin is applied to stimulate root growth, a method widely used in the propagation by cuttings (Kesari et al. 2009). Ideal concentrations should be studied for each species since the endogenous

levels of this growth regulator are variable (Ljung 2013). Thus, optimizing the growth of the root system of sugarcane plantlets can lead to a reduction in the time for their production and ensure their survival and growth in the field. Although research is being conducted to increase the yield of the PSP system, no study has addressed the use of growth regulators such as auxin to improve plantlet growth. Thus, the use of indole-butyric acid can promote greater rooting, increasing the production of plantlets. Given the above, the objective of this study was to evaluate the effect of different concentrations of IBA applied in the form of talc on the initial growth of sugarcane plantlets.

2. Methodology

Study location and plant material origin

The study was carried out in the greenhouse of the Plant Tissue Culture Laboratory of the Federal Institute Goiano – Campus of Rio Verde, Goiás, Brazil (17° 48' 17.137" S; 50° 54' 21.693" W). The sugarcane multiplication system was adapted from Landell et al. (2013). The cultivar CTC 04 was used because it is one of the most planted in Brazil, adapted to the edaphoclimatic conditions of Goiás, maintains yield along the cuts, is drought tolerant and has high tillering (Braga Junior et al. 2017). This cultivar was provided by the São Martinho plant, located in Quirinópolis, Goiás.

IBA preparation, treatment of mini-cuttings and biometric evaluations

IBA solutions at concentrations of 500, 1000, 1500 and 2000 mg kg^{-1} were obtained after dissolution in 100 mL of 50% alcohol; then, the solutions were mixed in a matrix of 100 grams of inert talc and placed in an oven for solvent evaporation at 35 ºC for 24 hours with semi-open door (Yamamoto et al. 2010). The control consisted of only 50% alcohol mixed with talc. After being washed, the mini-cuttings were subjected to antifungal treatment with the commercial fungicide Frowncide®, 0.25% for 5 minutes, and placed on paper towel for 5 minutes to remove excess moisture. The mini-cuttings were covered with talc and planted in 290cm^3 tubes with the commercial substrate Bioplant® (Table 1). These were kept in a greenhouse, with 10 mm of daily irrigation, split into four intervals for 30 days. At 30 days, gas exchange and photosynthetic pigments were evaluated. Subsequently, the plants were pruned to avoid losses through evapotranspiration and taken to benches under full sun with irrigation as described above.

Table 1. Chemical analysis of the Bioplant® substrate used for planting the plantlets.

Substrate	Chemical Characteristics											
	pH	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		.....g kg^{-1}.....					.....mg kg^{-1}.....					
Bioplant®	5.0	5.5	4.5	2.0	8.6	5.4	8.3	22	20	18212	291	60

Source: Authors.

Along the experiment (Feb-2017 to Apr-2017), emergence percentage and emergence speed index (ESI) were evaluated daily. ESI was calculated according to the formula of Maguire (1962). Biometric analyses were performed 60 days after planting to monitor the initial growth. The variables analyzed were the number of leaves, stem diameter, shoot length, leaf area and root area. Stem diameter was measured with a caliper and shoot length with a tape measure from the substrate surface to the insertion of the Leaf +1 (Kuijper numbering system). Leaf area was calculated based on the measurements of length and width according to the formula:

$$LA = L \times W \times 0.75 \times N$$

Where L is leaf length in cm, W is the width of the leaf central region in cm, 0.75 is the correction factor used for sugarcane (Hermann & Câmara 1999), and N is the number of leaves with at least 20% green area. To calculate root area, roots were washed, removed from the mini-cutting, separated and stretched on a black background, then photographed with identifications and a scale. Subsequently, root area was calculated by processing the images in the SAFIRA software (Jorge & Rodrigues 2008).

At 60 days after planting, the plants were also collected and separated to determine shoot dry mass (SDM) and root dry mass (RDM). Then, they were dried at 65 °C in a forced air circulation oven until reaching constant weight. The mean maximum temperature along the experiment was 38.2 ºC, with mean minimum temperature of 19.0 °C and relative humidity of 58.2%. These data were monitored with a thermo-hygrometer (INCOTERM-7663).

Evaluation of gas exchanges and photosynthetic pigments

Gas exchange evaluations were performed after 30 days of planting. Gas exchanges were evaluated to record the photosynthetic rate [A, μmol (CO_2) m^{-2} s^{-1}], stomatal conductance [g_s, mol (H_2O) m^{-2} s^{-1}], transpiration rate [E, mmol (H_2O) m^{-2} s^{-1}], and water use efficiency, obtained by the following formula:

$$WUE = A/E$$

Where A is the photosynthetic rate and E is the transpiration rate.

These evaluations were performed using a portable infrared gas analyzer (IRGA), LI6400 model (Li-Cor, Nebraska, USA), with photon flux density of 1500 μmol m^{-2} s^{-1}, between 08h and 12h, in the leaf +1.

The concentration of photosynthetic pigments was determined by extraction with dimethyl sulfoxide (DMSO). Three leaf discs were collected from each repetition using a metal hole puncher with 5 mm in diameter. The discs were incubated in 10-mL amber vials, containing 5 mL of DMSO saturated with 50 g L^{-1} of calcium carbonate ($CaCO_3$) for a period of 48 hours. The samples were heated to 65 °C in water bath for 24 hours. After reaching room temperature, sample readings were performed in UV-VIS spectrophotometer, Evolution 60S model (Thermo Fisher Scientific, Madison - USA). Wavelengths, equations and calculations for determining the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were obtained according to the methodology described by Wellburn (1994). For evaluations of gas exchange and photosynthetic pigments, 5 replicates per treatment were used.

Statistical analysis

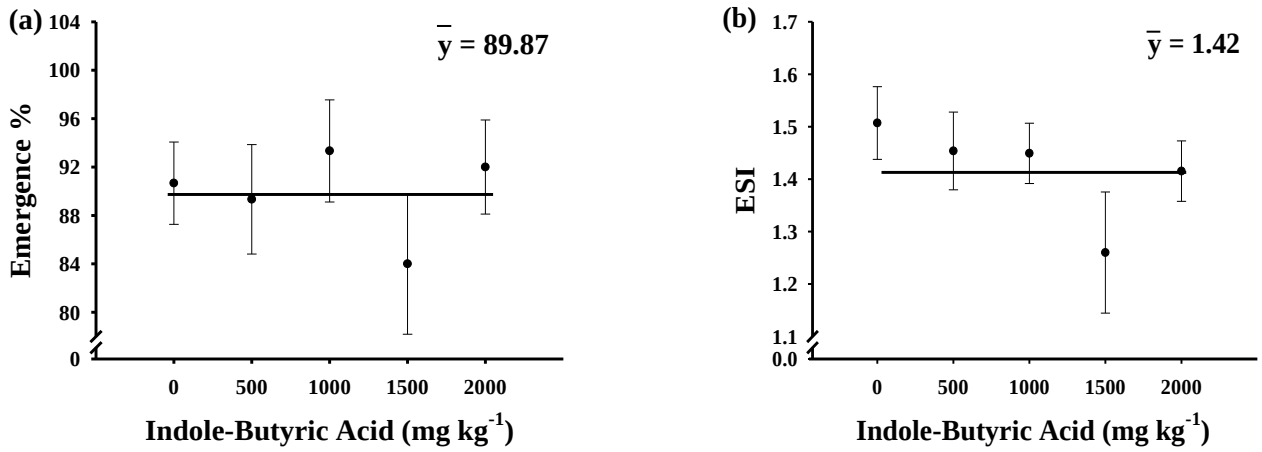
The experimental design was completely randomized with five treatments and five replicates of 15 plants each. The data were subjected to the Shapiro-Wilk normality test and then to analyses of variance and regression with the programs Sisvar 5.6 (Ferreira 2011) and SigmaPlot 11.0, at 5% probability level.

3. Results and Discussion

There was no difference between IBA concentrations for emergence percentage and ESI, which had averages of 89.9% and 1.4, respectively (Figure 1). Gírio et al. (2015) evaluated growth-promoting bacteria and nitrogen fertilization on the initial growth of sugarcane plantlets and found values of 100% for emergence percentage and 1.17 for mean ESI. Synthetic auxins have been widely used for propagation of plants by cutting. After application, it penetrates through the cut and in the cell can either undergo changes such as degradation, which irreversibly inactivates these molecules, or be conjugated with

other molecules, which temporarily inactivate them. As auxins act at low concentrations, usually the remaining auxin content is sufficient to ensure its effect on plant growth (Ludwig-Müller, 2011).

Figure 1. Emergence Percentage (Emergence %, a) and Emergence Speed Index (ESI, b) of mini-cuttings of *Saccharum* sp. L., cultivar CTC 4, treated with different concentrations of Indole-Butyric Acid in the form of talc.



Source: Authors.

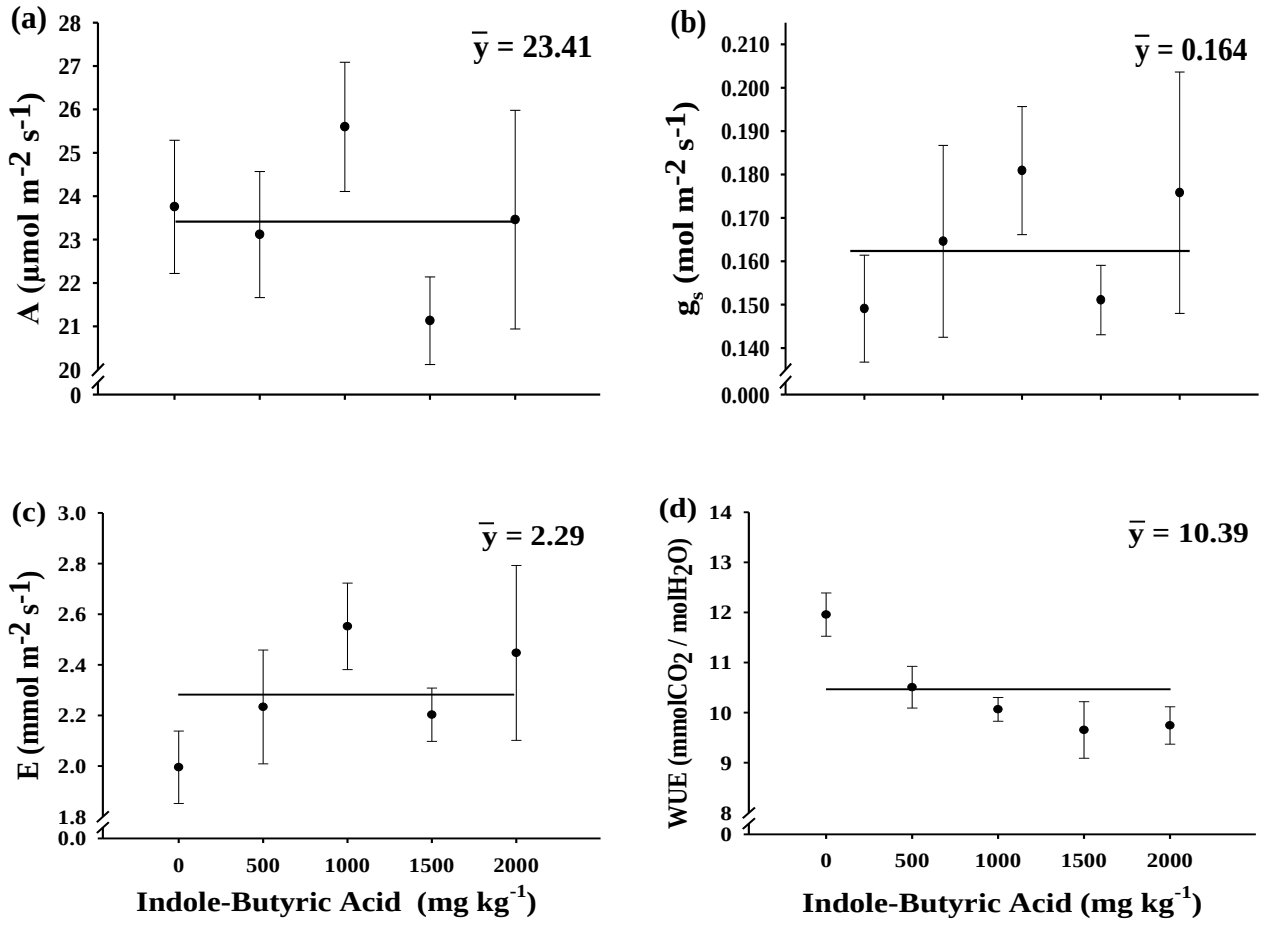
Emergence percentage evaluates the potential of propagules to form plants, while ESI measures the average daily emergence as a function of the days after planting, and these parameters are important to verify the initial growth of the plants (Guedes et al. 2013). As it stimulates the growth of lateral roots and adventitious roots, IBA can lead to an increase in these parameters (Kumari et al. 2010). However, this growth regulator is commonly used in cuttings which have been half buried into the soil, unlike those in the present study, which were completely buried, so ESI evaluation is important to infer about the plant's ability to emerge and initiate photosynthetic processes necessary for its growth. Emergence percentage in this study is related to the percentage of rooting, because all the emerged plants had roots.

The effects of IBA to improve plant rooting have been widely studied (Kesari et al. 2009; Salvador et al. 2014; Neto et al. 2017). However, the use of indole-butyric acid applied in the form of talc for the rooting of sugarcane mini-cuttings has not yet been reported in the literature. Tesfa et al. (2016), when studying ex vitro rooting of sugarcane plants derived from micropropagation, observed that the treatment of shoots with solution containing 20 mg L^{-1} of naphthalene-acetic acid for 24 hours increased rooting by 40% when compared to the control without regulator.

There was no difference for the evaluations of photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E) and water use efficiency (WUE), whose averages were 23.41 μmol m^{-2} s^{-1}, 0.164 mol m^{-2} s^{-1}, 2.29 mmol m^{-2} s^{-1} and 10.39 mmolCO_2/molH_2O, respectively (Figure 2). For having C4 metabolism, sugarcane has high photosynthetic rates with light intensity from 1500 μmol m^{-2} s^{-1} and ambient CO_2 concentration and temperature. Photosynthetic rates range from 16 to 61 μmol m^{-2} s^{-1}, according to cultivar, environmental conditions, cultivation time and nutritional status of the plant (Moore and Botha 2013).

IBA is a growth regulator that acts on root growth, so its action is related to the absorption of water and nutrients. Stomatal opening occurs with the osmotic change inside the guard cells, which leads to water absorption, and with the turgor pressure, which causes the ostiole to open. Thus, the water status of plants is directly related to photosynthetic processes (Silva et al. 2015).

Figure 2. Photosynthetic rate (A, a), stomatal conductance (gs, b), transpiration rate (E, c) and water use efficiency (WUE, d) of mini-cuttings of *Saccharum* sp. L., cultivar CTC 4, treated with different concentrations of Indole-Butyric Acid in the form of talc, after 30 days of cultivation.



Source: Authors.

The literature has no data on physiological evaluations of sugarcane plantlets at 30 days after planting. These evaluations are important to verify the performance of CO_2 assimilation and directly influence plant growth. According to the data obtained in this study, sugarcane plantlets showed a functional photosynthetic apparatus, because these values are consistent with gas exchanges of healthy sugarcane plants (Marchiori et al. 2014). Stokes et al. (2016) evaluated the effects of CO_2 on sugarcane growth and found similar results with photosynthetic rate of 25.4 µmol m^{-2} s^{-1} and stomatal conductance of 0.179 mol m^{-2} s^{-1} in control plants at 9 months of age. Souza et al. (2008), when working with high concentration of CO_2 in sugarcane, verified photosynthetic rate of 26 µmol m^{-2} s^{-1} and stomatal conductance of 0.260 mol m^{-2} s^{-1} in plants with 4 months of age. These data corroborate those of the present study regarding the photosynthetic rate and stomatal conductance of healthy sugarcane plants. This confirms that the sugarcane plantlets produced had functional photosynthetic apparatus.

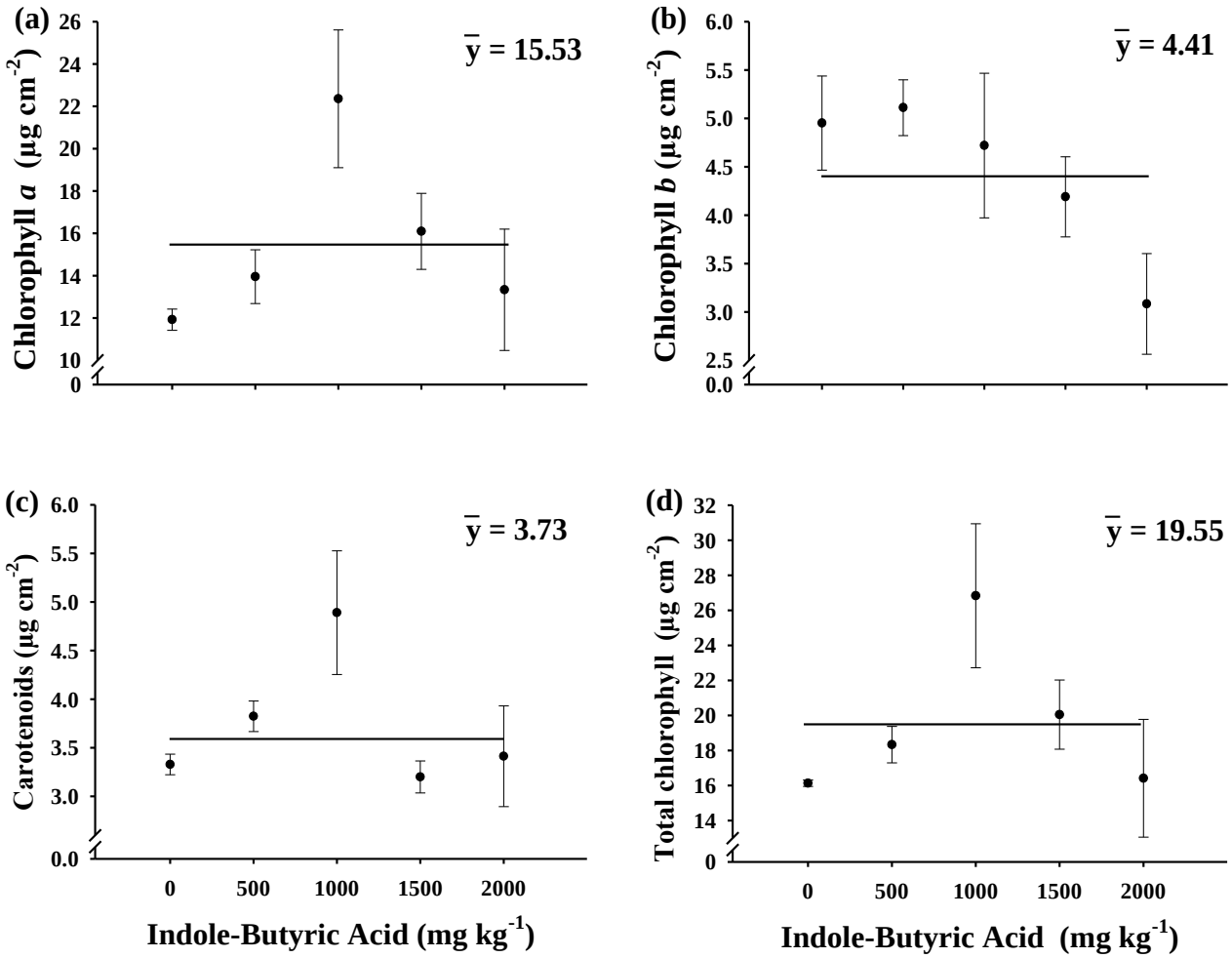
The transpiration rate (E) measures the loss of water through the stomata. The physiological response of different sugarcane cultivars under three distinct doses of nitrogen after five months of planting ranged from 5.05 to 7.37 mmol m^{-2} s^{-1} (Izquierdo-Hernández et al. 2016). The transpiration rate of sugarcane plants at different stages of leaf development ranged

from 1.8 to 2.5 mmol m^{-2} s^{-1} (Barbosa et al. 2015), which corroborates the data found in the present study, in which the average was 2.29 mmol m^{-2} s^{-1}.

Water use efficiency (WUE) is defined as the amount of water lost through transpiration, while a certain amount of CO_2 is absorbed for dry mass accumulation. Water use efficiency is directly associated with photosynthetic rate, as well as with stomatal conductance and transpiration rate, because at the same time as the plant absorbs CO_2 it loses H_2O molecules to the atmosphere (Galon et al. 2013). Thus, it can be noted that IBA had no effect on the growth of sugarcane mini-cuttings, and all plants had functional photosynthetic apparatus.

It was not possible to fit a regression model that explained the behavior of the photosynthetic pigments with the application of the different IBA concentrations used (Figure 3). The following mean values were observed: 16.24 µg cm^{-2} for chlorophyll *a*, 4.20 µg cm^{-2} for chlorophyll *b*, 3.97 µg cm^{-2} for carotenoids and 20.45 µg cm^{-2} for total chlorophyll. Chlorophylls and carotenoids are groups of photosynthetic pigments that act in the capture of light and induction of photosynthetic processes, being directly or indirectly a usual tool for nutritional and photosynthetic diagnosis (Prior et al. 2003). The production of these pigments can be stimulated with a more vigorous root system, responsible for absorbing nutrients that are constituents of these molecules (Baldotto et al. 2009).

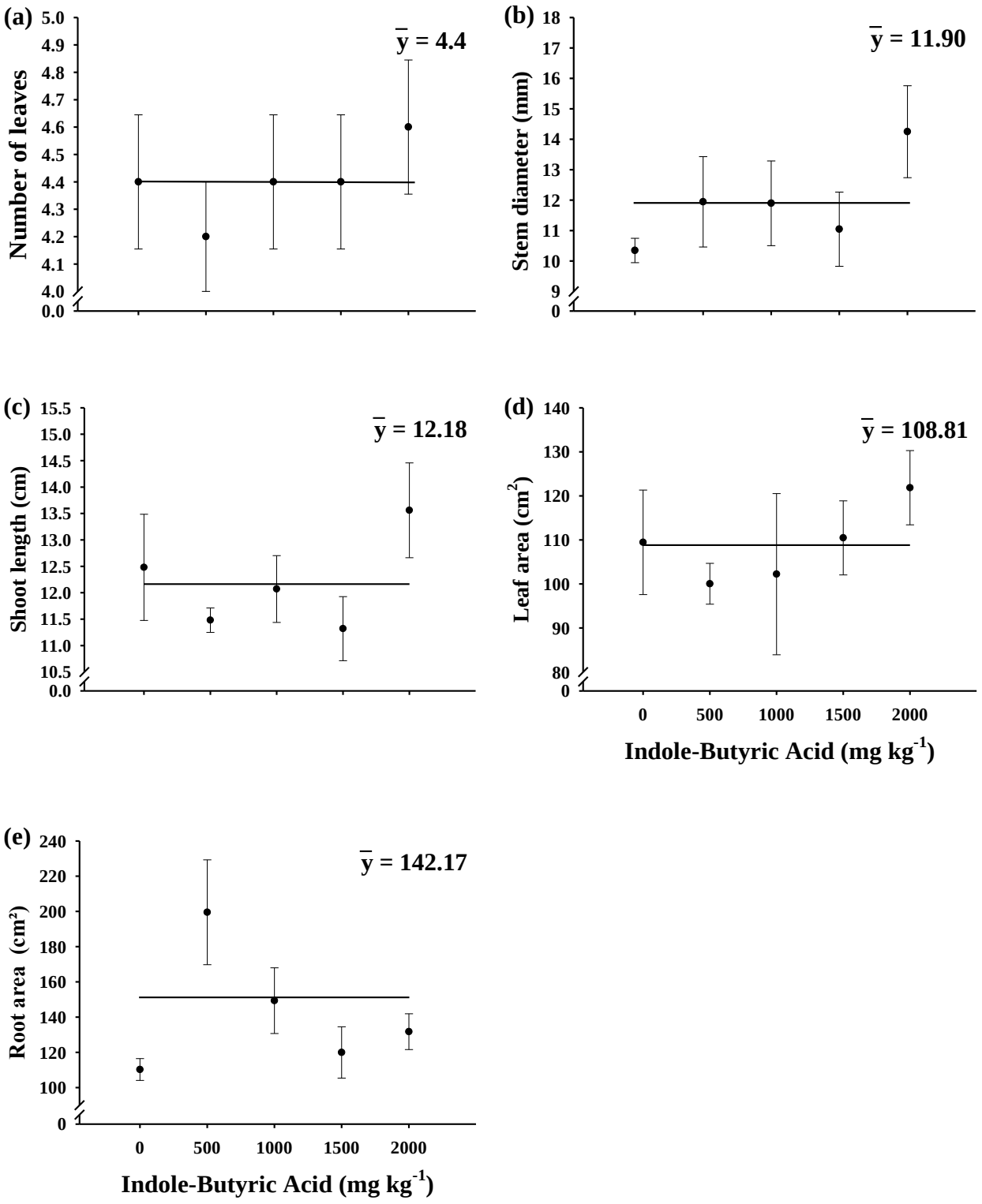
Figure 3. Chlorophyll a (a), chlorophyll b (b), carotenoids (c) and total chlorophyll (d) of mini-cuttings of *Saccharum* sp. L., cultivar CTC 4, treated with different concentrations of Indole-Butyric Acid in the form of talc, after 30 days of cultivation.



Source: Authors.

The biometric and dry mass parameters evaluated did not differ between the IBA concentrations used (Figure 4 and 5). Sugarcane plantlets with 60 days after planting had an average of 4.4 leaves, stem diameter of 11.90 mm, shoot length of 12.18 cm, leaf area of 108.81 cm² and root area of 142.17 cm². SDM and RDM were equal to 2.27 and 1.20 grams, respectively. IBA is a growth regulator that plays a key role in plant rooting. For being a precursor of indoleacetic acid (IAA), it influences several processes such as cell division, differentiation and elongation, hence acting as a rapid inducer of roots. However, at high concentrations, auxins can inhibit root growth, with the production of ethylene (Muday et al. 2012).

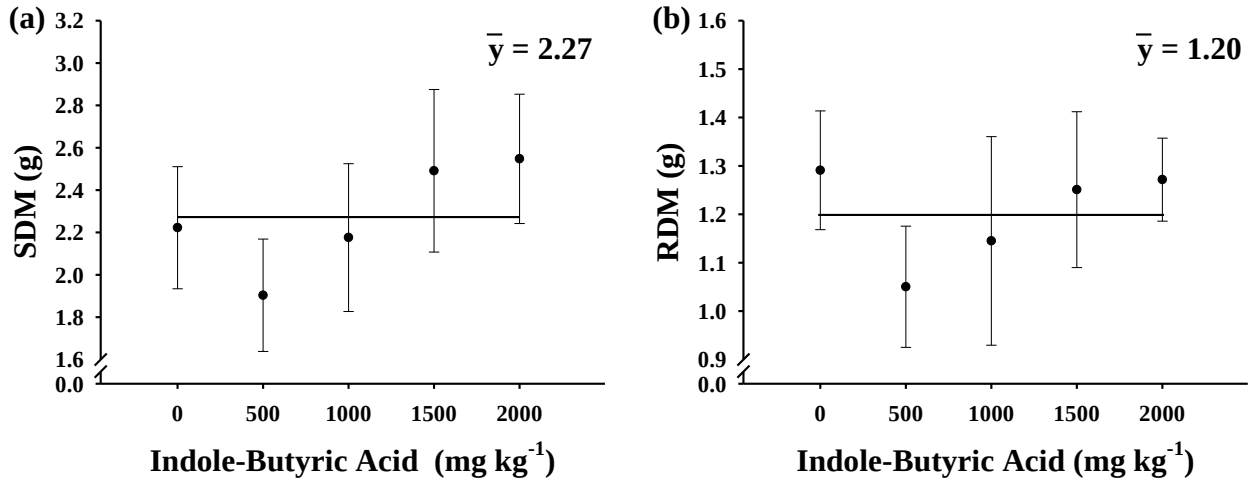
Figure 4. Number of leaves (a), stem diameter (b), shoot length (c), leaf area (d) and root area (e) of mini-cuttings of *Saccharum* sp. L., cultivar CTC 4, treated with different concentrations of Indole-Butyric Acid in the form of talc, after 60 days of cultivation.



Source: Authors.

Roots derived from the sugarcane propagation are initially originated from root primordia located in the structure called node. Under adequate temperature and moisture, the roots grow from these primordia. These roots are functional for anchoring and absorbing water and nutrients until the aerial part develops enough to produce permanent roots (Moore and Botha 2013). The good development of roots determines the potential of the plant to absorb water and nutrients and affects its growth and development (Azevedo et al. 2011; Matsuoka and Garcia 2011).

Figure 5. Shoot dry mass (SDM, a) and root dry mass (RDM, b) of mini-cuttings of *Saccharum* sp. L., cultivar CTC 4, treated with different concentrations of Indole-Butyric Acid in the form of talc, after 60 days of cultivation.



Source: Authors.

Mustafa and Khan (2016) worked with in vitro and in vivo propagation of sugarcane and found that, although in the absence of IBA micropropagated plants produce roots, this effect is increased at increasing concentrations up to 5 mg L^{-1}. Neto et al. (2017) observed the rooting of bamboo cuttings is optimized with the use of 500 mg L^{-1} of IAA. Indole-butyric acid in the form of talc has already been shown to be viable for rooting and production of several species through the cutting method (Bortolini et al. 2008; Kareem et al. 2013). The advantages of using talc include practicality, greater adherence and greater reliability in the treatment of the material (Yamamoto et al. 2010).

The use of IBA to optimize the growth of sugarcane roots has not yet been reported. However, the results of this study indicate that the internal levels of auxins present in sugarcane mini-cuttings guarantee the growth of plantlets with no need for the application of exogenous regulators up to 2000 mg kg^{-1}, which leads to saving in plantlet production. However, new technologies must be tested to optimize the time of production of plantlets and increase their quality and vigor.

The morphological aspects of sugarcane mini-cuttings represent a barrier for IBA absorption. The waxy stem can prevent auxin permeability, which compromises IBA absorption. This may be an obstacle for IBA to arrive at its site of action. In addition, when exogenous auxin is absorbed by cells, it undergoes processes of degradation and conjugation, which reduces the concentration of the exogenous regulator (Simon and Petrášek 2011). The response of plants to the application of growth regulators is dependent on the genotype and leads to inference that this sugarcane genotype also does not respond to the application of exogenous auxins (Ferreira et al. 2017).

Thus, investigating different sugarcane genotypes and their responses to IBA is necessary to clarify these processes. The forms of application should also be tested, as in vitro sugarcane rooting has already been successfully reported. Perhaps in

the ex vitro environment, there should be an initial growth of the roots for subsequent application of auxin, which would enable an appropriate pathway for the absorption and action of this regulator.

4. Conclusion

There was no effect of the concentrations of up to 2000 mg kg^{-1} of IBA in the form of talc applied in mini-cuttings on the growth of sugarcane plantlets, cultivar CTC 4, so the endogenous levels of this growth regulator present in mini-cuttings are sufficient to supply the growth of the plantlets. Therefore, future strategies to improve the rooting of pre-sprouted sugarcane seedlings should consider above 2000 mg kg-1 of IBA and also different forms of application, that is, hydroalcoholic solution or solid solution with talc.

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