

Full Length Research Paper

## Influence of reducing sugar contents on sprout growth of blackberry cuttings

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The objective of this study was to analyze the use of different doses of indolebutyric acid (IBA) and its influence on reducing sugar contents and the relationship of root and leaf development of mulberry cuttings, using commercial substrates and sand. The experimental design was a completely randomized design, with 3 replicates per plot and 5 cuttings per replicate, in a 3 × 3 factorial scheme, consisting of three IBA doses and three substrates: commercial substrate, sand and 50% substrate with 50% of sand. The cuttings planted on substrate presented a higher percentage of rooting in the treatments with 0 and 1000 mg L<sup>-1</sup>, differing from the other substrates, which represented increases of 16.68 and 44.45% in relation to the substrate of sand and sand + substrate, respectively. In the control treatment, estimates of the reducing sugar content of the leaves showed interaction between the substrates and doses of IBA, presenting an increasing quadratic effect for the treatment. It is concluded that the use of commercial substrate provides greater development of primary leaves and root length; the concentration of 1000 mg L<sup>-1</sup> of IBA was higher among the analyzed doses. The reducing sugar content of the cuttings influenced the growth of the shoots.

**Key words:** Reducing sugars, fit regulator, multiplication, *Rubus*, substrates.

### INTRODUCTION

The mulberry tree is a plant belonging to the family, Moraceae, which produces plants of the genus *Rubus* and Rosaceae, in which there are other important genera

(*Malus*, *Prunus*, *Pyrus* among others) for Brazilian fruit growing (Antunes, 2002).

The mulberry tree (*Rubus* sp.) is one of the most

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promising fruit species in the market, having a very promising market due to its palatability, good cultivation and commercialization perspectives (Silva et al., 2016). Due to the low cost of plantation, orchard maintenance and especially, the reduction of pesticide use, the crop is presented as an option in family agriculture (Antunes et al., 2014). This fruit also shows good adaptation in a subtropical climate, where some cultivars have higher yields as compared to cold regions and even has superior fruit quality (Guedes et al., 2013). However, because it is a fruit tree, it is very susceptible to damage mainly after its harvest.

In order to have a structured chain for the cultivation of mulberry, it is necessary firstly to have plantations installed in order to supply the demand of fruits. But for the installation of new plantations, the production of seedlings with good quality is necessary. For the production of quality seedlings, the seminiferous route and vegetative propagation are the most used techniques for the propagation of this plant.

Seminiferous propagation has long been used for production, depending on the species that can arrive for 30 years. Seeking uniformity and speed in the production of seedlings, vegetative propagation is an alternative that may be viable due to the use of cuttings, roots or shoots. The use of cuttings is a widely used alternative to obtain shoots of woody plants with long periods of development through sowing (Antunes and Rasseira, 2004).

However, in order to increase the chances of success of the use of vegetative propagation, the use of rooting promoters is important in increasing rooting rates. Among these promoters are cell growth, root and leaf emission stimulators. It is known that the most used form is the exogenous application of phytohormones, such as indolebutyric acid (IBA), which increases the auxin content in the tissue (Pasqual et al., 2001).

Although, the performance of propagated cuttings is related to genetic, physiological and environmental factors (Fachinello et al., 2005), it is essential to use substrates that promote root formation and shoot (Kämpf et al., 2006; Yamamoto et al., 2013; Hussain et al., 2014). In general, black mulberry tolerates a wide range of soils, but it develops best in sandy soils with good organic matter content (Grandall 1995; Gazda and Kochmanska-bednarz, 2010).

The objective of this study was to analyze the use of different doses of IBA in reducing sugar contents and in the promotion of root and leaf development of blackberry cuttings, using commercial substrates and sand.

## MATERIALS AND METHODS

The experiment was conducted between October and November 2016, for 40 days, at the School Farm of Dom Bosco Catholic University, Campo Grande, MS. Herbaceous branches of mulberry plants were collected from the orchard of the farm. The propagation material was kept in a protected environment (agricultural greenhouse) under automatic intermittent irrigation system by micro

sprinkler, in order to maintain the relative humidity close to 90%, avoiding the dehydration of the cuttings.

After the collection, the branches were prepared in cuttings with 10 to 15 cm length and diameter of 3 to 5 mm. The cuttings were treated with 0, 1000 and 2000 mg L<sup>-1</sup> IBA for 15 s (concentrated solutions of the immersion method). After the application of IBA, the cuttings were placed in PVC tubes using 3 types of substrates (commercial substrate, sand and 50% substrate + 50% sand). For the control concentration, only distilled water was used.

The experimental design was a completely randomized one, with 3 replications per plot and 5 cuttings per replication, in a 3 × 3 factorial scheme, consisting of the three IBA doses and the three substrates. In order to obtain the results in each IBA dose, the average of the three concentrations and substrate types was considered, considering the basic principles of experimentation: repetition, randomization and local control (Gomes, 1990).

The variables analyzed were the percentages of rooted cuttings, the number of rooted cuttings, the average length of the rooted cuttings, the weight of the fresh biomass of the rooted cuttings, the weight of the fresh root biomass per cutting, dry matter of the biomass of the primary leaves of the rooted cuttings and weight of the dry biomass of the roots per cutting. Dry matter (DM), calculated according to the methodologies described by Silva and Queiroz (2002) were used to determine the dry biomass of leaves and roots.

The methodology described by Somogy (1945) and Nelson (1944) was used to determine the reducing sugar contents. The apparatus used was the spectrophotometer, and the reading was performed at 535 nm. Data were subjected to analysis of variance (ANOVA, P <0.05), linear regression, ACP and Chi-square, according to Levine (2000), using the statistical program SAS version 9.1 (2004).

## RESULTS AND DISCUSSION

Analysis of the number of cuttings with sprouting of mulberry was significant (P <0.05) between the substrates (Table 1). There was no interaction between the different concentrations of IBA and substrates, nor effect between IBA doses.

The cuttings planted in substrate presented a higher percentage of rooting between treatments of 0 and 1000 mg L<sup>-1</sup>, differing from the other substrates, which presented an increase of 16.68 and 44.45% in relation to the substrate of sand and sand + substrate respectively, with the control treatment. For the treatment with 2000 mg L<sup>-1</sup>, lower amount of rooted cuttings was observed in sand substrate with 53.3% of rooting. Possibly, the stakes that did not root and did not increase in the other variables, ended up reducing their reserves, thus leading to cell death. Porosity of the substrate influences the amount of retained water and aeration, having an effect on the percentage of rooting and the number of roots formed (Fachinello et al., 2005). A good substrate should have low density, good water absorption and retention capacity, good aeration and drainage to avoid excessive moisture accumulation (Kämpf, 2005). Yamamoto et al. (2013) reported that IBA has no influence on the survival of mulberry saplings. An effect (P <0.05) was observed between the substrates for root length (Table 2). For the number of leaves, there was no difference between the substrates.

**Table 1.** Number of cuttings of mulberry sprout treated with different concentrations of IBA and different substrates.

Treatment (mg L <sup>-1</sup> )	Commercial substrate	Sand	Sand+ Substrate	P
0	10 <sup>a</sup>	9 <sup>a</sup>	6 <sup>b</sup>	0.01
1000	13 <sup>a</sup>	8 <sup>b</sup>	9 <sup>b</sup>	0.01
2000	10 <sup>a</sup>	8 <sup>b</sup>	10 <sup>a</sup>	0.01

P- Effect of treatment; mean of the same line with different lowercase letters are significant by the Kruskal-Wallis test (P <0.05) effect of hormonal treatment.

**Table 2.** Number of leaves and root length of mulberry cuttings on different substrates.

Variables	Substrate	Sand	Sand + substrate	EP	P
Number of leaves	3.71	3.6	4.04	0.212	0.372
Root length	8.08 <sup>a</sup>	6.10 <sup>b</sup>	5.91 <sup>b</sup>	0.172	0.001

Standard error (EP); P- treatment effect; averages of the same line with different lowercase letters are significant by the Waller-Duncan test (P <0.05) substrate effect.

**Table 3.** Weight of the biomass and reducing sugar of mulberry botanical components subjected to different substrates.

Variables	Substrate	Sand	Sand + Substrate	EP	P
Wet mass of leaves (mg)	337	295	354	0.021	0.281
Wet mass of roots (mg)	253	252	186	0.026	0.096
Wet mass of cuttings (mg)	1.084	1.078	1.119	0.078	0.834
Dry mass of leaves (mg)	87 <sup>a</sup>	66 <sup>b</sup>	84 <sup>a</sup>	0.005	0.041
Dry mass of roots (mg)	63 <sup>a</sup>	78 <sup>a</sup>	47 <sup>b</sup>	0.006	0.047
Dry mass of cuttings (mg)	419	556	405	0.029	0.028
Reducing sugar of leaves (g 100 g <sup>-1</sup> )	14.51 <sup>b</sup>	14.48 <sup>b</sup>	19.44 <sup>a</sup>	0.389	0.001
Reducing sugar of cuttings (g 100 g <sup>-1</sup> )	16.04 <sup>a</sup>	6.63 <sup>b</sup>	16.94 <sup>a</sup>	0.835	0.001

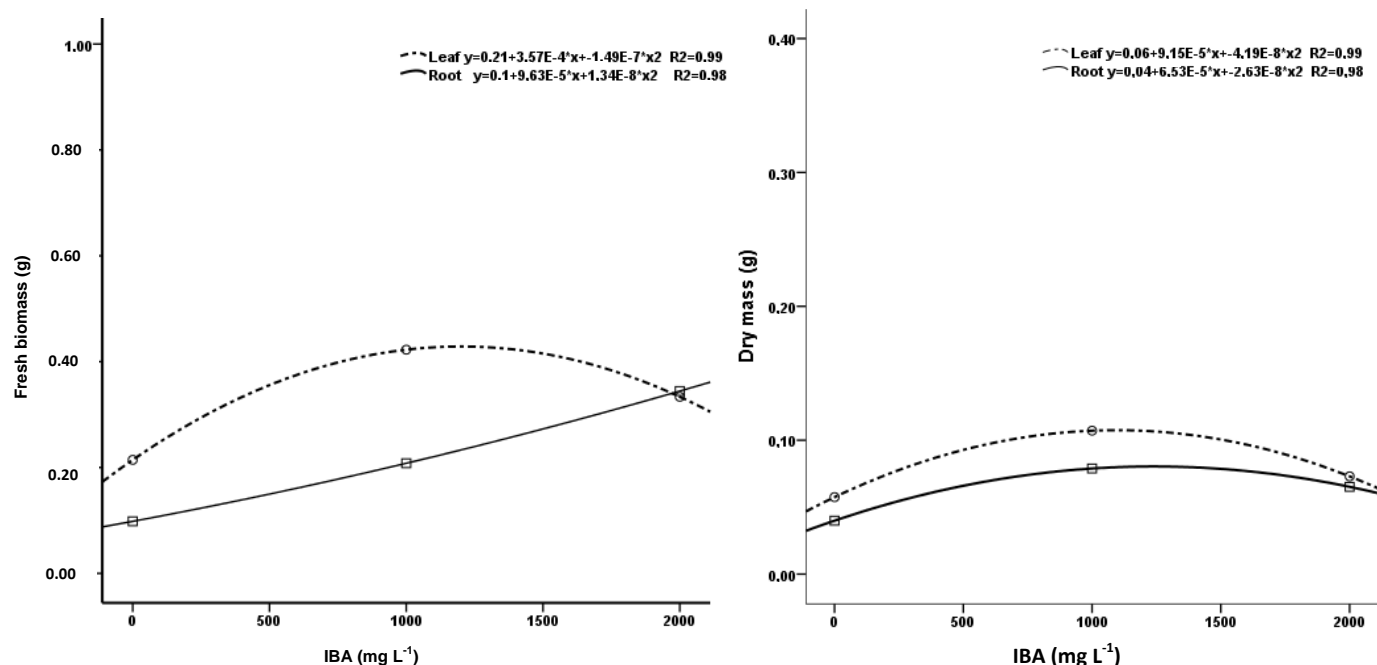
Standard error (EP); P- treatment effect; Same line averages with different lowercase letters are significant by the Waller-Duncan test (P <0.05) substrate effect.

The cuttings that rooted with commercial substrate presented greater amount of primary leaves, in relation to the other substrates (Mendonça et al., 2010). The leaves obtained carbohydrate through photosynthesis and also a small amount of natural auxins present in the leaves, helping in the rooting and sprouting of cuttings (Hartmann et al., 1990). The dry biomass values of the leaves and roots obtained in this study had an effect (P <0.05) between the substrates (Table 3). However, no significant difference was observed for the values of natural biomass of the leaves and roots.

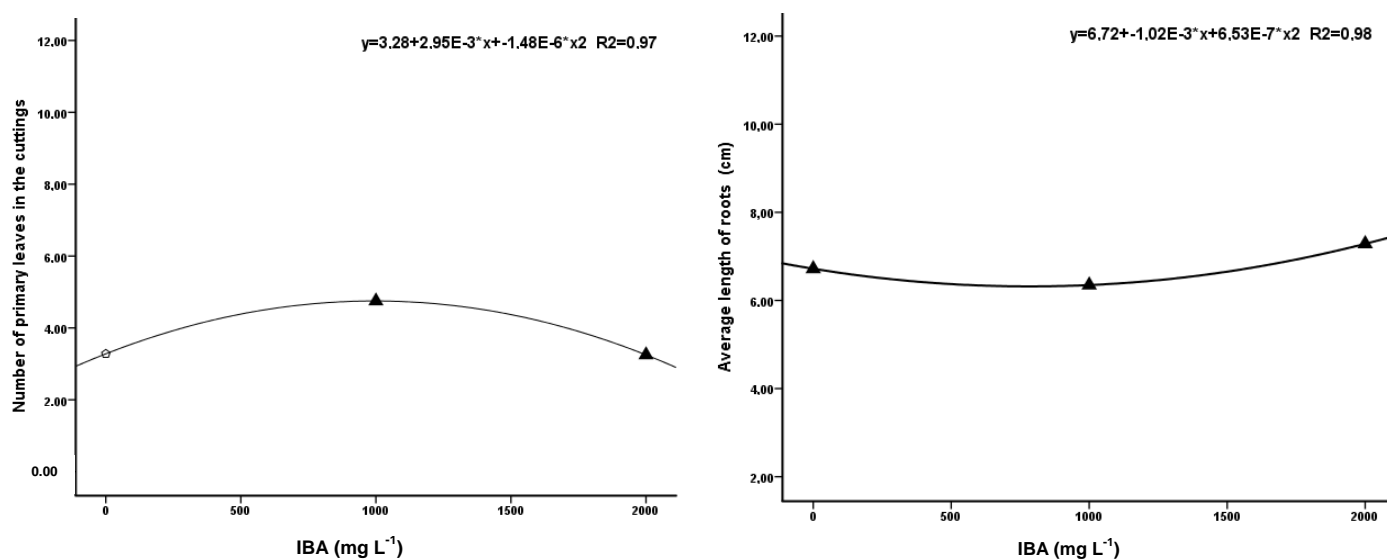
The commercial substrate and substrate with 50% sand + 50% substrate did not differ from each other, showing a yield of 87 and 84 mg kg<sup>-1</sup> dry leaf biomass, respectively, and 22.8% more efficient than the substrate sand. This result is related to the physical characteristics of sand, which is considered as an inert material with a low water retention capacity (Pasqual et al., 2001; Mendonça et al., 2010).

According to the analysis, there was a quadratic effect (P <0.05) of treatment for fresh leaf biomass weight. The absence of IBA in the culture medium resulted in lower biomass of the leaves, although higher production was observed with the use of 1000 mg L<sup>-1</sup> (Figure 1). Figure 1 shows that there was an increasing linear effect (P <0.05) for the roots with increase of IBA concentrations applied on the cuttings. This fact is possibly associated with auxin stimulation. Silva et al. (2012) reported that the best IBA concentrations for rooting promotion is about 1634 mg L<sup>-1</sup> and Hussain et al. (2014) reported that 2,000 mg L<sup>-1</sup> of IBA is considered the best option for the increase of roots in cut of blackberry. Dias et al. (2011) stated that IBA concentrations of 250 to 500 mg L<sup>-1</sup> provide a higher volume of root shoots.

However, when dry biomass yield is evaluated, quadratic effect (P <0.05) is observed for leaf and root weight per cutting (Figure 1). Therefore, in the absence of auxin, there is a reduction in the dry biomass values of



**Figure 1.** Weight of fresh biomass (left) and dry mass (right) of leaves and roots of mulberry cuttings subjected to different doses of IBA.

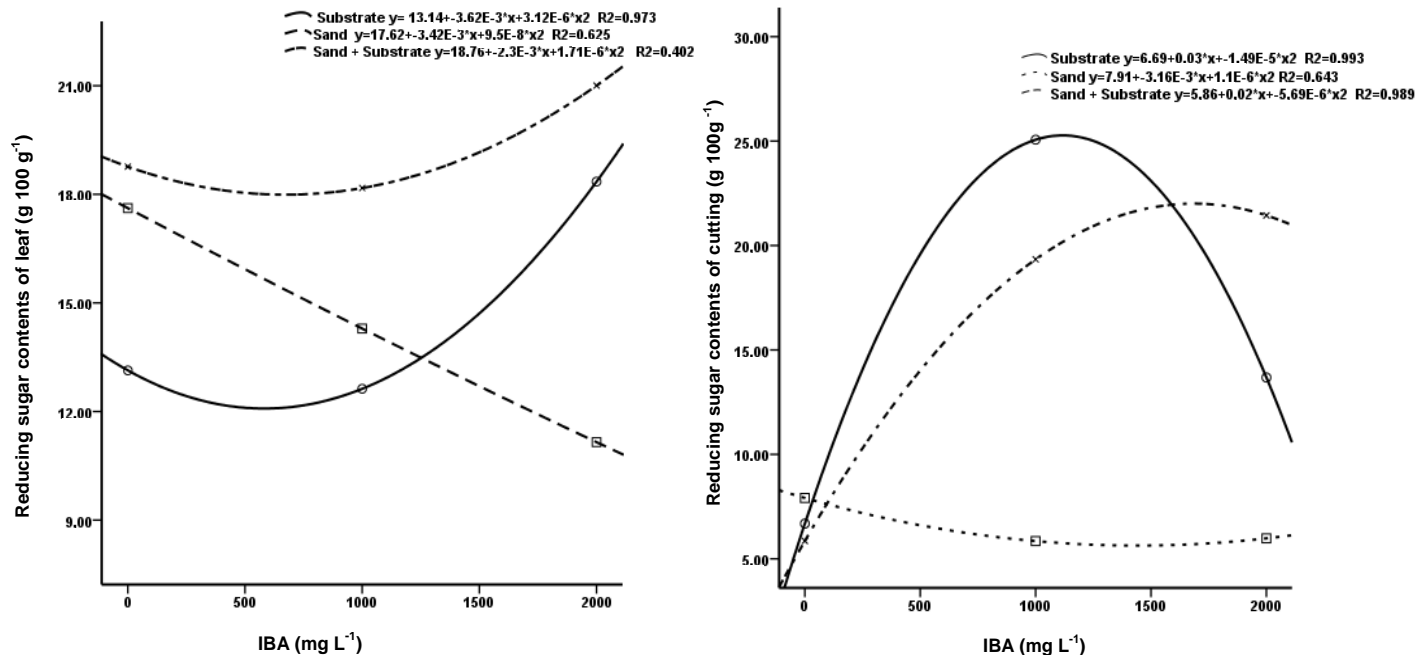


**Figure 2.** Number of primary leaves in the cuttings (left) and average length of roots per mulberry cutting subjected to different doses of IBA.

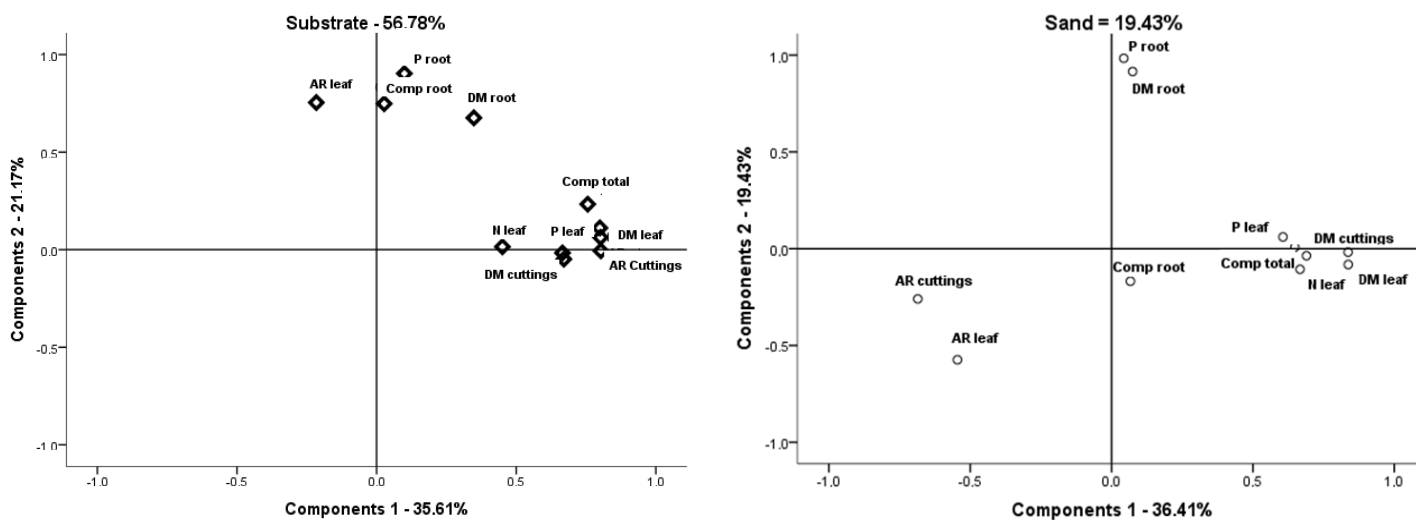
leaves and roots, and the same behavior can be observed after the use of 1400 mg L<sup>-1</sup> of IBA, the plant did not respond.

As the IBA concentration increased, there was an increase in rooting, consequently, roots had longer length (Figure 2). However, the exogenous supply of AIB, above 1200 mg L<sup>-1</sup>, may not favor the number of primary leaves (Figure 2). The estimates of the reducing sugar contents

(AR) of the leaves showed interaction between the substrates and doses of IBA (Table 3), presenting an increasing quadratic effect for the treatment with 50% sand and 50% commercial substrate, which showed 18.76 g 100 g<sup>-1</sup> reducing sugar content with the AIB dose of 672 mg L<sup>-1</sup> (Figure 3). The same behavior was observed with 100% commercial substratum, however, with lower contents. When only sand was used as the



**Figure 3.** Reducing sugar contents of leaf and cuttings in relation to the substrates used for mulberry cuttings subjected to different doses of IBA.



**Figure 4.** Variation graph for the main components between the levels of reducing sugar in the leaf (AR leaf), stem (AR cuttings), and fresh leaf mass (P leaf), fresh mass of roots (P root), dry leaf mass (DM leaf), root dry mass (MS root), root length (Comp root), number of leaves (N leaf) and total length of the plant (Comp total) in relation to the commercial substrates and sand used for mulberry cuttings subjected to different doses of IBA.

substrate, the doses of IBA did not provide an increase in the levels of RA.

In relation to the AR content of the stakes, interaction between IBA and substrate doses was observed (Figure 3); it can be observed that the commercial substrate use had the highest efficiency of the IBA hormone with a dose of 1,006 mg L<sup>-1</sup> with respect to the contents (21.79 g

100g<sup>-1</sup>) present in the cuttings. However, with the use of sand as a substrate, there was a greater demand for hormone for the maintenance of RA in the system. This fact is related to water retention and dark environment at the base of the stem obtained by the use of the substrate influencing the rooting (Figure 4) as well as the type of roots formed (Hoffmann et al., 1996).

Correlations of the factors related to development of the botanical components of the cuttings and their characteristics studied (Figure 4), are indicated in cluster 1 with commercial substrate, and the reducing sugar levels of the cuttings influenced the morphological development of the aerial part of the plant.

On the other hand, in group 2, the root growth of cuttings with substrate use is positively correlated with the amount of carbohydrates metabolized in the leaves. In the sand substrate treatment, development of the aerial part is related to the greater biomass of the stake. It is necessary to increase the amount of endogenous reserves of the cuttings, because the leaves that will be formed during the rooting process may act as a drain, decreasing the reducing sugar content of the cuttings (Dias et al., 2011).

Mendonça et al. (2010) emphasized that the substrate is fundamental for the rhizogenesis of cuttings; in addition to being a support for the cuttings, it retains water supplied via a longer period of time.

## Conclusion

The results of this work showed that the use of commercial substrate provided greater development of primary leaves and root length; the concentration of 1000 mg L<sup>-1</sup> of IBA was effective among the analyzed doses. The reducing sugar content of the cuttings influenced the growth of the shoots.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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