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MINI-STUMPS PRODUCTIVITY AND ROOTING OF *Khaya ivorensis* A. CHEV MINI-CUTTINGS TREATED WITH IBA

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HIGHLIGHTS

It's possible to propagate *Khaya ivorensis* by mini-cuttings without exogenous auxin.

It's possible to propagate *Khaya ivorensis* by mini-cuttings from youthful stock plants.

Khaya ivorensis mini-stump were tolerant to the successive mini-cuttings harvests.

More vigorous root system were obtained with increasing IBA concentration.

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ABSTRACT

Khaya ivorensis stands out because of the quality of the wood and high economic value. Its propagation has been carried out by seeds, an obstacle in the production in large scale seedlings, since the seeds present high value and they result in heterogenous stands. The vegetative propagation is an alternative to overcome these problems. The aim of this work was to verify the feasibility of mini-cutting for african mahogany, evaluating the productivity of the mini-stumps, the rooting of mini-cuttings using different concentrations of IBA and the clonal seedlings quality. The mini-stumps were produced from seeds in tubes, pruned at 8 cm, at 60 days. At 100 days after apical pruning, the first sprouts collection was performed, and the mini-cuttings were submitted to concentrations of IBA (0; 2,000; 4,000 and 6,000 mg·L^{-1}) for rooting evaluation. The seedlings were transplanted to clonal mini-garden, in which four collections of mini-cuttings were performed. Clonal seedlings quality was evaluated at 120 days after staking. The species showed tolerance to apical pruning and successive collections of mini-cuttings, with 96.4% survival and more than 92% of the mini-stumps producing mini-cuttings. It was observed 100% survival and rooting of mini-cuttings. There was no effect of the IBA on the rooting percentage of the mini-cuttings, however, the higher concentration of IBA increase the thinner root production. The final clonal seedling quality has similar growth for all treatments.

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INTRODUCTION

The forest species *Khaya ivorensis* A.Chev. is usually known as african mahogany and belongs to the Meliaceae family in which the first plantations in Brazil were in north region in 1976. Its adapted to the Brazilian weather, where found suitable conditions for development, highlighting for the wood quality, used by the furniture and naval industries, also presenting high economic value in the national and international trade (Ribeiro et al., 2017).

K. ivorensis reaches the harvest age between 15 and 20 years and presents tolerance to *Hypsipyla grandella*, known as the Meliaceae drill, which causes damages mainly in the growing parts (buds and shoots), occurring formation of lateral shoots, making it impossible commercial production, especially in pure plantations (Lunz et al., 2009; Dias et al., 2012; França et al., 2015).

The *K. ivorensis* has been propagated by seeds, resulting in heterogenous stands. Moreover, the seeds have a high germination rate as soon as they are removed from the fruits (Carvalho et al., 2016) however there is a rapid decrease in their viability, 95% of reduction after three months of seeds processing (Pinheiro et al., 2011).

According to the Brazilian Forest Institute, in 2015 the value of one kilogram of seeds corresponds to approximately US$ 815.00 raising the price of seedlings production. According to Dias et al. (2012), one kilogram had approximately 3,000 seeds. In this context, the vegetative propagation represents an alternative to improve seedlings production and standardization of plants quality as well as allow the selection of more productive genotypes.

The mini-cutting consists in use sprouts from plants propagated by cuttings or seeds (Alfenas et al., 2004). This technique was successful for several species propagation such as *Eucalyptus benthamii* (Cunha et al., 2005), *Pinus taeda* (Alcantara et al., 2007), *Erythrina falcata* (Wendling et al., 2005), *Ilex paraguariensis* (Wendling et al., 2007), *Gravillea robusta* (Souza Junior et al., 2008), *Calophyllum brasiliense* (Silva et al., 2010), *Handroanthus heptaphyllus* (Oliveira et al., 2015; Oliveira et al., 2016), *Toona ciliata* (Silva et al., 2012) and *Swietenia macrophylla* (Fernandes, 2015).

The success of mini-cutting technique depends mainly of propagules rooting. For species that have low or non-rooting, the application of growth regulators may be necessary (Xavier et al., 2013). Among them, auxins have the best effect on the formation of adventitious roots and their use, in most species, provides a higher survival percentage, speed, quality and rotting uniformity (Hartmann et al., 2002). Studies with auxins effects on *K. ivorensis* mini-cuttings rooting only tested 0 and 2000 mg L^{-1} of IBA doses (Tchoundjeu and Leakey, 2,000).

The indole-3-butyric acid (IBA) is found that the most well-know and employed auxin on rooting because of the higher chemical stability inside the plants (Xavier et al., 2013). However, studies are needed to verify the best concentration of the regulator which varies as the species among other factors like genotype, physiology, mineral nutrition, plant age, phytosanitary, lightness, temperature and humidity (Xavier et al., 2013).

The aim of this study was to verify the viability of mini-cutting technique for the african mahogany (*Khaya ivorensis* A. Chev.), evaluating the productivity of the mini-stumps, rooting of mini-cuttings and clonal seedlings quality with different concentrations of indole-3-butyric acid.

MATERIAL AND METHODS

Mini-stumps productivity

The work was performed from august to december, 2012 in a greenhouse covered with polypropylene plastic (density = 150 μm) and shading screen (30%), at the State University of Northern Fluminense Darcy Ribeiro, Campos dos Goytacazes - RJ, Brazil; 21°19'23 (S) and 41°19'41" (W). The average temperature and relative humidity was 26.2°C ± 15.96 and 73.7% ± 50.7, respectively. The greenhouse was irrigated by microsprinkler with 4 mm·day^{-1} of water sheet three times a day.

The seedlings used for the multiclonal mini-garden were produced from seeds, in 280 cm^3 polyethylene tubes, with commercial substrate (Basaplant florestal®), and slow release fertilizer N-P-K (Osmocote® 14-14-14), at 8 g·Kg^{-1} of substrate. To form the mini-stumps the seedlings were pruned at 8 cm in height after 60 days of sowing.

The first sprouts collection was made 100 days after seedlings pruning, when most of them had suitable size for cutting (at least 5 cm). After the first mini-cuttings collection, the mini-stumps were transplanted to two suspended sand beds, at 0.15 x 0.15 m array, with 41 plants in each one to extend the mini-stumps life time.

The suspended sand beds were made with polyvinyl chloride, with length = 1.13, width = 1.05 and height = 0.28, respectively. Moreover, nine equidistant holes of two cm were placed in base for drainage, lined with 150 μm shading screen, in which was added a crushed stone layer (5 cm). On the crushed stone, was added washed sand layer (8 cm) and 15 cm of the commercial substrate, coconut fiber and filter cake (2:1:1 v/v mixture, respectively), with 2.2 kg·m^{-3} of coated urea and 1.5 kg·m^{-3} of simple superphosphate (Silva et al., 2012).

After the first, four collections still were performed at 200, 245, 290 and 335 days with the number of mini-cuttings produced by mini-stump was quantified in each one of them. The sequential collections aim check if *K. ivorensis* sprouts production will keep on overtime.

Mini-cuttings rooting and clonal seedlings production

The sprouts were cut with pruning shears and mini-cuttings were cut with at least 5 cm and one axillary bud and one or two leaves left with two leaflets pairs. The leaf area was reduced by half, to reduce water loss by transpiration and ensure that nebulization water reaches the substrate.

The mini-cuttings of first sprouts collection had the base immersed for 10 seconds in the indole-3-butyric acid (IBA) solution. The following concentrations were tested: 0, 2,000, 4,000 and 6,000 mg·L^{-1}. The IBA solutions were prepared before immersion, with ethyl alcohol P.A. (50%) and deionized water (50%) to dilute the stock solution of 10,000 mg·L^{-1} of IBA. The treatments were arranged in a completely randomized design, with four replicates and plots with nine mini-cuttings of different positions in the sprouts, homogeneously distributed in each plot.

After immersion, staking was carried out in polyethylene tubes (180 cm^3), with commercial substrate (Basaplant Florestal®), fertilized with slow release fertilizer N-P-K (Osmocote® 14-14-14) at 8 g·Kg^{-1} of substrate. The mini-cuttings was taken in nebulization chamber, with temperature and air humidity averages of 28°C and 89%, respectively.

After thirty days in nebulization chamber, all mini-cuttings were evaluated for survival and rooting and then transferred to greenhouse where they remained for 90 days. To determine if mini-cuttings has rooted we looked for roots on polyethylene tubes base or by the removal resistence of the mini-cuttings from substrate (Freitas et al., 2006).

Three mini-cuttings were randomly selected from each plot and the roots was washed for substrate removal to evaluate the first order roots number and second order root number, root length and root dry mass. First order roots were considered those rise on mini-cuttings basis and second order was those rise from the first order. The root number was determined by optical observation and the length measured with a millimeter rule. The mini-cuttings root dry mass was dried in stove at 65°C for 72 hours. After drying it was weighed in a precision analytical balance (0.001g).

At 120 days after staking the clonal seedlings were evaluated for height, stem diameter, leaf area, shoot and root dry mass. All remaining clonal seedlings in each plot were evaluated in height and diameter. The height was determined with a millimeter ruler from stem ground level to apical bud and the stem diameter with a digital caliper.

For the leaf area, shoot and root dry mass evaluations, three clonal seedlings per plot were randomly selected. The leaves were detached from the stem and the leaf area was determined with a leaf area meter (LI-COR® 3100). The shoot was separated from the root system and the roots were washed for substrate removal. Subsequently the parts were dried in stove at 65°C for 72 hours. After drying it was weighed in a precision analytical balance (0.001g) to obtain shoot and root dry mass.

Statistical analysis

The mini-stumps production productivity were submitted to descriptive analysis and compared by Confidence Interval ($p < 5\%$) considering that data has no statistical design and no fill requirements of parametrical analysis.

The rooting data were previously submitted to a maximum likehood function (boxcox) to apply the suitable data transformation Log(x+1) (R Core Team, 2017) and the percentage data transformed for (X+1)1/2 (Zimmermann, 2004).

The data resulting from quantitative fixed variables were submitted to regression analysis and rooting and the final clonal seedling quality data were submitted to ANOVA and the means were compared by Tukey test ($p < 5\%$).

RESULTS

Mini-stumps productivity

More than 92% of the mini-stumps have produced mini-cuttings in all collections, showing tolerance to apical pruning, with 96.4% of survival, therefore, it's possible to use this propagation method to *K. ivorensis* sprout production over time.

The first sprouts collection was possible 100 days after prunning of the seedlings, with 2.06 mini-cuttings for mini-stumps, when plants were still in polyethylene tubes, not differing in relation to the second collection, when mini-stumps had already been transplanted to the suspend sand beds, without restriction of the root system (Figure 1).

After the transfer and adaptation of the mini-stumps to the suspended sand beds it was possible, over five collections, increases in sprouts productivity.

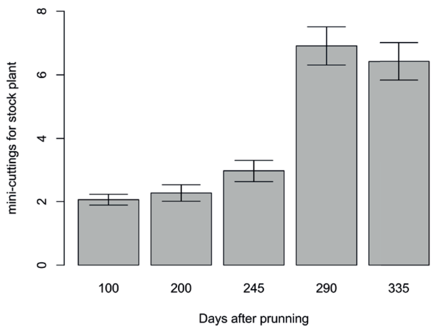

FIGURE 1 Average productivity of *Khaya ivorensis* mini-stumps, started 100 days after apical prunning. Vertical bars indicated the Confidence Interval – CI(p < 5%), n=82.

Mini-cuttings Rooting

In all IBA doses tested and without IBA, there was 100% of mini-cuttings survival and rooting at 30 days after staking. Rooted mini-cuttings of *K. ivorensis* can be seen in Figure 2.

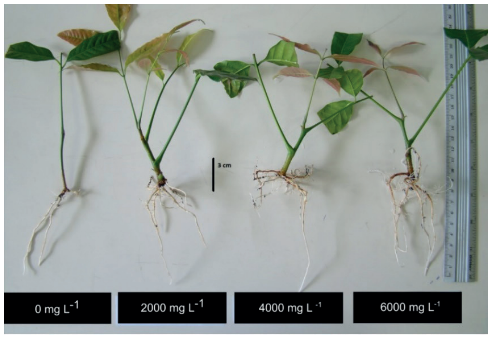

FIGURE 2 *Khaya ivorensis* mini-cuttings rooted, 30 days after staking, with the different indole-3-butyric acid (IBA) doses tested.

There was no effect of the IBA doses on the first order root number, with average of 4.6, however, the second order root number was increased as a result of the IBA concentrations increase, with a linear correlation (Figure 3).

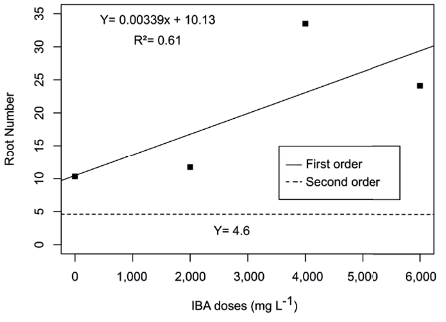

FIGURE 3 Effect of indole-3-butyric acid (IBA) doses on first and second order root number average in *Khaya ivorensis* mini-cuttings at 30 days after staking (N=12).

Root length and dry mass of the root system also showed a positive linear correlation with the application of IBA (Figure 4a and 4b), confirming the positive effect on the thinner root system development with the use of this growth regulator.

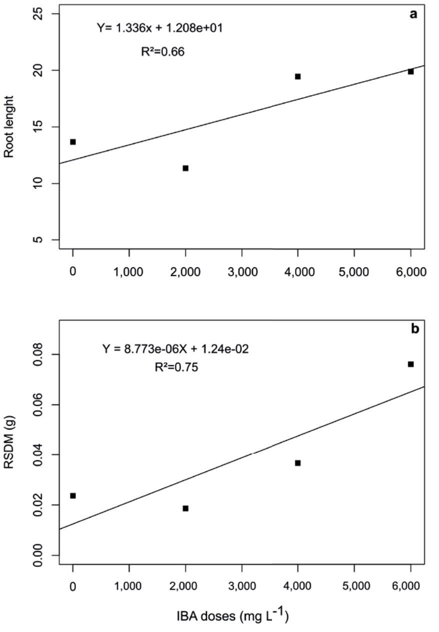

FIGURE 4 Effect of indole-3-butyric acid (IBA) doses on root length (a) and root system dry mass (RSDM) (b) average of *Khaya ivorensis* mini-cuttings at 30 days after staking, (N=12).

Clonal seedlings production

There was no effect of treatments on stem diameter, leaf area, shoot and root dry mass of *K. ivorensis* clonal seedlings. Mini-cuttings immersed in 4,000 mg·L^{-1} of IBA solution resulted in clonal seedlings with higher height in relation to those immersed in 2,000 mg·L^{-1} of IBA solution (Table 1).

TABLE 1 Height (H), stem diameter (SD), leaf area (LA), shoot dry mass (SDM) and root system dry mass (RSDM) of Khaya ivorensis clonal seedlings, 120 days after staking.

IBA doses	H	SD	LA	SDM	RSDM
mg·L^{-1}	cm	mm	cm²	---------- g	-----------
0	17.45 AB*	6.53 A	264.07 A	3.01 A	1.57 A
2,000	15.12 B	6.49 A	272.98 A	2.88 A	1.73 A
4,000	18.87 A	6.70 A	285.92 A	3.19 A	1.86 A
6,000	17.29 AB	6.58 A	265.67 A	2.87 A	1.70 A
VC (%)	17.55	12.93	26.88	23.55	25.31

*Means with same letters in columns, not differ in Tukey test (p < 5%). VC - Variation coefficient.

DISCUSSION

Mini-stumps productivity

The high survival of *K. ivorensis* mini-stumps were similar as observed for *Khaya anthotheca* which grown in polyethylene tubes, with 97% of survival (Barbosa Filho et al., 2018), *Grevillea robusta* conducted in sand beds was 95% of survival, with fifteen mini-cuttings collections (Souza Junior et al., 2008) and in *Toona ciliata* mini-stumps, in suspended sand beds and polyethylene tubes with 100% of survival in both systems (Silva et al., 2012).

The increase in the mini-stumps productivity from the third collection may result from no root restriction and a higher substrate volume exploited by the mini-stumps, provided by suspend sand bed. Although from the second collection the mini-stumps were already in suspended sand beds, the productivity was still smaller due to the transplant stress and adaptation of mini-stumps to the sand beds.

The consecutive sprouts collection also stimulates productivity increases in different species. After pruning, the plants had no proper arrangement of their canopy yet, an implication of the loss of apical dominance increases the number of axillary buds for sprouts producing (Barbosa Filho et al., 2018) that can promote benefits in sprouts productivity over collections and probably may reduce the interval between collections without losses to productivity.

The mini-stumps productivity of *Khaya anthotheca* was 1.8 mini-cuttings for mini-stump with increases over the collections (Barbosa Filho et al., 2018). Silva et al. (2012) found in *Toona ciliata* mini-stumps that the first sprout collection can only be made at 115 days after prunning, with 0.68 mini-cuttings for mini-stump productivity. They explain this result to the seedlings stress due the apical pruning and the root restriction by tubes.

Cunha et al. (2008) observed in *Erythrina falcata* an average of 2.9 mini-cuttings for mini-stump, conducted in suspend sand beds in biweekly collections. The productivity is variable according to the mini-garden system adopted. For *Eucalyptus*, the average found was 5.6 mini-cuttings for mini-stump in each collection, every 5-10 days, in a hydroponic system in sand beds (Wendling, 2002).

Mini-cuttings rooting and clonal seedlings production

The high *K. ivorensis* mini-cuttings survival and rooting was similar than other species such as *Toona ciliata* (Souza et al., 2009), *Handroanthus heptaphyllus* (Oliveira et al., 2016) and *Eucalyptus benthamii* and *Eucalyptus dunnii* clones (Brondani et al., 2008), all presenting more than 80% survival of mini-cuttings.

The high survival and rooting point that the high degree of juvenility (Oliveira et al., 2016) and the high endogenous auxin concentration, thereafter, auxin/cytokinin balance in the mini-cuttings of *K. ivorensis* allows the rooting of these without growth regulators. Although there have been increases in thinner roots development of mini-cuttings with rise IBA concentrations (Figure 3). This same behavior in *Handroanthus heptaphyllus* seedlings was found by Oliveira et al. (2016), in a experiment which the seedlings rooted without IBA addition. However, there was increase in root vigor with growth regulator application.

Vasconcelos et al. (2016) found that the *Khaya senegalensis* propagation also can be performed without IBA, however, the authors tested fast (five seconds) and slow (twelve hours) immersion of mini-cuttings in the growth regulator, concluding the slow immersion make benefits on rooting than the fast. In fast immersion, the IBA doses tested were 0, 3,000, 6,000, 9,000 and 12,000 $mg \cdot L^{-1}$, whereas in slow, the doses were 0, 100, 200 and 400 $mg \cdot L^{-1}$.

Opuni-Frimpong et al. (2008) found that *Khaya anthotheca* can root without IBA immersion. However, there were positive effects for the number and length roots mini-cuttings, and root percentage of those submitted to IBA. Like this work, the authors attributed the rooting without IBA to the endogenous auxin. Barbosa Filho et al. (2018) found that IBA application in *Khaya anthotheca* mini-cuttings was associated with greater rooting percentage and the mini-cuttings whitout IBA application had higher callus percentage.

For the *Toona ciliata* mini-cuttings rooting, the use of the IBA is also dispensable (Souza et al., 2009). However, Pereira et al. (2015), studying the vegetative rescue of trees of the same species, found that the cuttings from sprouts collected after tree semi-girdling increased in 55% the rooting with IBA (6,000 $mg \cdot L^{-1}$). For clearcut and total girdling, the rooting was smaller, 32 and 35%, respectively. In all vegetative rescue methods, the cuttings treated with IBA has a greater root number.

The second order roots are responsible for water and nutrient absorption (Eissenstat, 1992).The IBA application provided increases in thinner roots formation of the *K. ivorensis* which may be advantageous for plantations in sites with water and nutritional limitation.

The survival, establishment and good productivity of forest stands are higher with the robust root system, regardless of the shoot height (Gomes and Paiva, 2011). Therefore, the positive linear increase in mini-cuttings root length and root dry mass are important for clonal seedlings quality, indicating that *K. ivorensis* submitted to the higher IBA concentration perharps can accelerate seedling growth, with possible greater chances to survive under stress conditions. However, these aspects were not evaluated.

At 120 days after staking, the height of the clonal seedlings did not have a similar trend under the treatments, moreover, the means has a small variation (Figure 5). The stem diameter, leaf area, shoot and root dry mass of the clonal seedlings did not present variation as a function of the IBA concentrations in the mini-cuttings. After the mini-cuttings rooting, probably the nutrient supply by substrate were sufficient to leverage all clonal seedlings growth and development over 120 days.

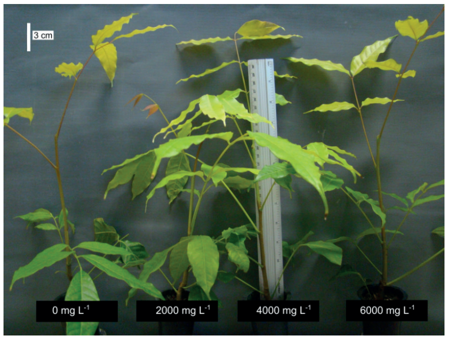

FIGURE 5 Height variation of the *Khaya ivorensis* clonal seedlings under IBA concentrations, 120 days after staking.

Oliveira et al. (2016) evaluating the IBA concentrations and mini-cutting type on *Handroanthus heptaphyllus* vegetative propagation also found that the height, stem diameter, shoot and root dry mass of the clonal seedling were no effect by the auxin, similar results were found in this work.

Although the mini-cuttings length and number of second order roots were higher with the application of IBA at 30 days after staking, these differences were not observed on root dry mass of the clonal seedlings probably due to the roots thickness with lower dry mass, not differing on total root dry mass (Figure 6).

Although the production cycle of the clonal seedlings was not influenced by the treatments, based on clonal seedlings growth characteristics, new tests should

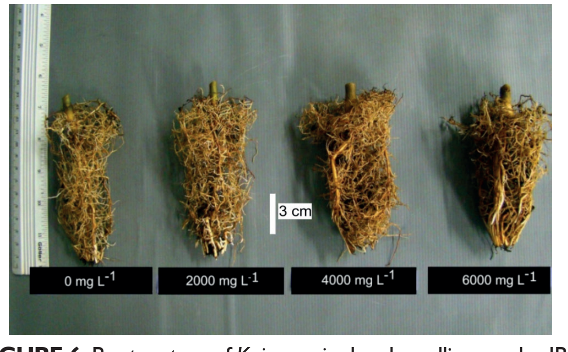

FIGURE 6 Root system of *K. ivorensis* clonal seedlings under IBA concentration, 120 days after staking.

evaluate the possibility of reducing the mini-cuttings period permanency in the nebulization chamber.

CONCLUSIONS

Khaya ivorensis mini-stumps were tolerant to the successive mini-cuttings collection, with increasing productivity throughout the collections.

It's possible to propagate *Khaya ivorensis* by mini-cuttings from seminal mini-stumps, without exogenous IBA, however, the higher auxin doses increases thinner roots.

The final clonal seedling quality has similar growth for all treatments.

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