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Rooting of African mahogany (*Khaya senegalensis* A. Juss.) leafy stem cuttings under different concentrations of indole-3-butyric acid

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Vegetative propagation were studied in order to implement *Khaya senegalensis* A. Juss. wood production, conservation and genetic improvement programs. The objective of this research work was to establish the requirement as well the appropriated concentration of indolbutiric acid (IBA) in the *K. senegalensis* leafy stem cuttings to produce new plants. The basal end of the leafy stem cuttings were immersed, at first subjected to the so called slow method, in a 5% ethanol solution with 0, 100, 200 and 400 mg L⁻¹ of IBA for 12 h and, as another procedure, the so called quick method, to a 50% ethanol solution with 0, 3000, 6000, 9000 and 12000 mg L⁻¹ of IBA for 5. The leafy stem cuttings were transferred to plastic trays filled with 9.5 L of medium texture expanded vermiculite in which the cuttings had their basal end immersed to a depth of 3 cm in an 8.0 x 8.0 cm spacing. The experimental units were distributed according to a completely random design with five replications. Each experimental unit consisted of twelve cuttings. The experiment was conducted in a greenhouse under a 50% of shade condition during the period from January to June of 2012. The quick method, in comparison with the slow one, gave the best results in terms of percentage of rooted stem cuttings and height of the sprouted plantlets. In the quick method, no differences between IBA concentrations were observed. The slow and the quick methods did not differ as to stem cuttings survival, total root length per leafy stem cutting, number of roots per stem cutting, plantlets survival and percentage of sprouted plantlets. It was concluded that *K. senegalensis* may be propagated by leafy stem cuttings taken from plantlets of seminal origin without the use of IBA.

Key words: *Khaya*, Senegal mahogany, vegetative propagation, auxin, seedlings.

INTRODUCTION

The genus *Khaya*, a member of the Meliaceae family, is composed of four important species of commercial wood-

Khaya ivorensis, *Khaya grandifolia*, *Khaya anthotheca* and *Khaya senegalensis*; all of them are commonly

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known as African mahogany. None of those species are substantially different from mahogany species of Tropical America (*Swietenia macrophylla*, *Swietenia mahagoni*) either as to physiognomic aspects or wood quality (Lamprecht, 1990).

K. senegalensis natural distribution is verified to occur from Mauritania and Senegal east region up to Uganda northern region, where rain precipitation varies from 650 to 1300 mm up to 1800 mm, a year (Nikiema and Pasternak, 2008), where, under conditions of fertile soils, trees may reach up to 35 m of height, diameter of 1.5 m, and trunks 8 to 16 m long (Joker and Gaméné, 2003). The species was introduced in Australia, China, Vietnam, Malaysia, Indonesia, Sri Lanka and Tropical America for wood production (Arnold et al., 2004).

K. senegalensis wood is hard, heavy, durable and displays very nice graphic delineations. It is used in furniture manufacture and in internal decoration of civil engineering constructions (Lamprecht, 1990). Reforestation with *K. senegalensis* in Africa is restricted to areas where the species is of natural occurrence since it is susceptible to cedar tip moth (*Hypsipyla robusta*). In northern Australia, the species was well succeeded due to the trees growing rapidly and because of the absence of *H. robusta* (Arnold et al., 2004; Nikles et al., 2008). In Brazil, the *K. senegalensis* plantations are young (they started in 2000), *H. robusta* does not occur and they are resistant to the cedar tip moth occurring in the Tropical America (*Hypsipyla grandella* Zeller) and they grow more than the mahogany of the genus *Swietenia*. Cultivating mahogany trees of the *Swietenia* genus in the American continent is economically unviable since they are susceptible to cedar tip moth (*H. grandella*). Thus, cultivating *K. senegalensis* in the Tropical America is a promising investment considering periods between 12 and 20 years. Studies on the vegetative propagation of *K. senegalensis* are just beginning thus demanding further work to reach a desirable level of efficiency in the production of seedlings (Pinheiro et al., 2011).

The sexual propagation of *K. senegalensis* is limited by the long time (between 15 and 20 years) it takes for a tree to be formed and to produce commercially satisfactory volumes of wood and also because its seeds loose viability in a very short period of time— under natural conditions, 2 to 3 weeks after harvest (Arnold et al., 2004). The International Union for the Conservation of Natural Resources (IUCN) has classified the species as a vulnerable one due to the loss of its natural habitat, to the selective cutting of its wood, and the large scale harvest of its bark and branches for medicinal purposes and of the leaves as fodder (Nikiema and Pasternak, 2008). The high costs and bureaucratic problems for the importation of seeds as well as the inconveniences of becoming dependent on international seed stocks make sexual propagation of *K. senegalensis* a very difficult option. These are reasons important enough to justify opting for vegetative propagation in order to implement *K.*

senegalensis wood production, conservation and genetic improvement programs (Opuni-frimpong et al., 2008).

Although, a protocol for the in vitro propagation of *K. senegalensis* has already been established (Hung and Trueman, 2011), the use of stem cuttings is still one of the most important techniques for the vegetative propagation of selected clones viewing silvicultural purposes due to its technical applicability, operationality and competitive cost as compared to other asexual propagation techniques.

Indole-3-butyric acid (IBA) is one of the common growth regulators used to stimulate rooting cuttings, it is a localized action photostable substance, but less sensitive to biological degradation as compared to other synthetic auxin. Increasing the concentration of exogenous auxin stimulates the rooting of stem cuttings to a maximum value, from which any concentration can increase inhibitory effect. The appropriate content of exogenous auxin that stimulates rooting depends on the species and the auxin concentration in the tissue (Fachinello et al., 2005).

The objective of this research work was establish the requirement as well the appropriated concentration of IBA in the *K. senegalensis* A. Juss. stem cuttings to produce new plants.

MATERIALS AND METHODS

The stem cuttings used in this experiment were taken from seedlings developed from seeds imported from the African village of Tiakane, in Burkina Faso (11° 11' 10" N and 1° 12' 14" W). The seedlings were produced at the 'São Gabriel' nursery in Monte Alto, state of São Paulo, Brazil (21° 15' 39" S and 48° 29' 45" W), during the period of November of 2011 to January of 2012. The seedlings grew in plastic containers with a volume of 120 cm³ filled with Plantmax® substratum whose structural composition was of 60% of pine bark, 15% of granulometrically fine vermiculite, 15% of granulometrically superfine vermiculite, and 10% of humus. To the substratum in each container, 1.2 g of Osmocote Plus® (slow action fertilizer with a NPK composition of 15-09-12 and 1.0% of Mg, 2.3% of S, 0.012% of B, 0.05% of Cu, 0.45% of Fe, 0.06% of Mn, 0.02% of Mo, and 0.05% of Zn) were added.

The seedlings were 80 days old when the leafy stem cuttings were taken from them. The basic plant material was formed by stem cuttings taken from 540 seedling sprouts. From each one of the 540 seedlings, a stem cutting was taken from the apical branch. The preparing of the leafy stem cuttings consisted in the discarding of the first pair of apical leaves to avoid dehydration keeping three whole subsequent leaves.

The leafy stem cuttings had a mean diameter of 4 mm, 8 cm of length and three whole leaves with a leaf area of 48 cm², using the LI-3100C Area Meter - LI-COR Biosciences. The leafy stem cutting base was shaped to a bevel just below the bud so as to increase the contact surface between the plant tissue and the ethanol/indole-3-butyric acid- IBA solutions.

At first, the leafy stem cuttings were submitted to the quick immersion method meaning that they were immersed for 5 s in hydroalcoholic (50% of ethanol) solutions containing 0, 3000, 6000, 9000, and 12000 mg L⁻¹ of IBA. In the other procedure (the slow method), the stem cuttings were immersed for 12 h in a 5% ethanol solution containing 0, 100, 200 and 400 mg L⁻¹ of IBA. After those periods, the stem cuttings were transferred to plastic trays (internal

dimensions of 49.5 cm of length, 30.5 cm of width and 11 cm of height) filled with 9.5 L of medium texture expanded vermiculite in which the cuttings had their basal end immersed to a depth of 3 cm in an 8.0 x 8.0 cm spacing.

The experimental units were distributed according to a completely random design with five replications. Each experimental unit consisted of twelve cuttings. The experimental units were placed in a greenhouse under a 50% of shade condition made possible by a screen, during the period of January to June of 2012. The cuttings were irrigated by means of a nebulization system which provided the plants with water for 20 s at 40 s intervals. A thermo hygrometer was set in the greenhouse near the experimental units. The mean low and the maximum temperatures were, respectively 17 (night) and 35°C (day). The mean low and maximum relative humidity were, respectively, 45 and 95%, using a digital thermometer-hygrometer.

45 days after the cuttings had been planted, non-destructive evaluations of cutting survival, number of rooting cuttings, adventitious roots total length using a graduated ruler, and number of adventitious roots per cutting were evaluated. Following those measurements, the rooting cuttings were transferred to plastic vases with 1500 cm³ of the substratum Plantimax®. To each vase, 15 g of Osmocote Plus® were added and the vases taken to the growth area where illumination underwent a reduction of 30%. The vases stayed in that area for additional 120 days. During that period, the plants were irrigated by micro aspersion for 15 min at 45 min intervals. Percentage of surviving plants, sprouted plants and sprout height were evaluated 165 days after the cuttings had been planted.

The statistical analyses of the data were performed considering two factors: forms by which the cuttings were immersed in the solution (slow and quick) and IBA concentrations in each form of immersion, in a hierarchical scheme. The effects of IBA concentration in each form of immersion were determined by a polynomial regression analysis, using a program AgroEstat (Barbosa and Maldonado Júnior, 2015).

RESULTS AND DISCUSSION

Survival

Leafy stem cuttings survival 45 days after rooting started was of 100%. The treatments had no effect on plant survival. This is thought to be due to the presence of three whole leaves in the cuttings. Leaves contain carbohydrates, growth regulators, and other components essential for rhizogenesis (Alfenas et al., 2004).

Ky-Dembele et al. (2011) verified that leafless *K. senegalensis* cuttings taken from 1 year old plants showed mortality larger than those where leaves were present. These results indicate that leaves are of fundamental importance for *K. senegalensis* cuttings survival. Results published by Faria et al. (2007) indicated that the survival of cuttings of grape IAC 572-Jales was highly dependent on the presence of leaves.

Work by Ferriani et al. (2008) with stem cuttings of *Piptocarpha angustifolia* Dusén showed that the presence of leaves represents the photosynthetic apparatus needed for root initiation.

Rooting

Rooted cuttings 45 days after planting was 95.74%. This

good rooting rate is partially due to the presence of three whole leaves attached to the cuttings. Leaves represent an essential stimulus for root initiation (Hartmann et al., 2002). This is an effect related to the translocation of carbohydrates to the cutting basal end, in addition to auxin and other important rooting cofactors. Data published by Ky-Dembele et al. (2011) showed that the rooting of *K. senegalensis* cuttings were of 80, 50 and 57% when the cuttings at planting had leaf areas of 22-28, 12-16, and 6-8 cm², respectively.

The age (80 days) of the plantlets at which the cuttings were taken is also considered an important factor for the rooting percentage presented by them. The successful rooting of the cuttings may be ascribed to the juvenile characteristics of the mother plant. When a plant passes from the juvenile to the adult phase it undergoes several changes the main ones being related to the growth habit, the shape and retention of leaves, leaf and stem anatomy, cuttings rooting capacity and formation of roots, and growth vigor (Wendling and Xavier, 2001). Among the factors affecting root formation in cuttings, age of the mother plant is of high importance. Cuttings taken from young plants root is more easy as a result of a higher number of rooting cofactors and lower inhibitors content (Fachinello et al., 2005).

Ky-Dembele et al. (2011) evaluated the effects of mother plants age and IBA concentration (0, 2500, 5000 and 10000 mg L⁻¹) using the quick method on the rooting of cuttings taken from 3 month old seedlings, 100 year old trees and 5 to 15 year old trees. Cuttings taken from the seedlings rooted significantly more (90%) than those taken from the 100 year old trees (11%) and from those 5 to 15 year old trees. The authors concluded that the age of the mother plant affects the vegetative propagation of *K. senegalensis*.

The cuttings whose basal end was treated by the quick method gave the best results as to rooting percentage in comparison with the slow method (Table 1). In the slow method, the amount of absorbed phytohormones depends on the environmental conditions surrounding the place where the treatment is applied, the type of cutting and the species (Fachinello et al., 2005). Due to the long period of time the cuttings remain in the solution, results become dependent on those environmental conditions (Hartmann et al., 2002). On the other hand, some species, such as *Caesalpinia echinata*, respond better to the slow method in comparison with the quick one (Valeri et al., 2012).

Tofanelli et al. (2003) verified that the quick method gave better results to root stem cuttings of peach cultivars. According to these authors, a possible explanation for these results is that the absence of an artificial irrigation by nebulization system may have forced the cuttings to absorb too high an amount of the IBA solution which, instead of a stimulatory, had an inhibitory effect on root development.

Number of roots per cutting did not differ when both

Table 1. *Khaya senegalensis* rooting percentage, number of adventitious roots per leafy steam cutting, and total length of adventitious roots per cutting 45 days after planting as influenced by the way of immersion and indole-3-butyric acid (IBA) concentrations.

Sources of variation	Means		
	Rooting percentage	Root number	Root length (cm)
Quick Immersion	98.33 ^a	5.96 ^a	57.24 ^a
Slow Immersion	92.50 ^b	6.41 ^a	53.09 ^a

Sources of variation	Significance F-Test		
	Rooting percentage	Root number	Root length (cm)
Immersion Forms	9.80**	2.05 ^{ns}	2.07 ^{ns}
[IBA]/Quick Immersion	0.54 ^{ns}	3.68*	2.12 ^{ns}
[IBA]/ Slow Immersion	1.56 ^{ns}	8.37**	5.42**
Coefficient of variation (%)	6.49	17.05	17.37

Means, in the same column, followed by the same small case letter, are not significantly different ($P > 0.05$). (^{ns}) = non significant; (* and **) = Significant differences, respectively, at $P < 0.05$ and $P < 0.01$.

Table 2. *Khaya senegalensis* rooting percentage, number of adventitious roots per leafy steam cutting, and total length of adventitious roots per cutting 45 days after planting as influenced by IBA concentrations applied by the quick immersion method.

IBA concentrations mg L ⁻¹	Means		
	Rooting percentage	Root number	Root length (cm)
0	100.00	6.28	63.87
3000	98.33	6.96	63.78
6000	95.00	4.89	50.93
9000	98.33	5.15	51.92
12000	100.00	6.53	55.70

Sources of variation	Significance F-Test		
	Rooting percentage	Root number	Root length (cm)
Linear Regression	0.00 ^{ns}	0.78 ^{ns}	4.29*
Quadratic Regression	1.64 ^{ns}	4.56*	1.80 ^{ns}
Cubic Regression	0.00 ^{ns}	6.82*	1.30 ^{ns}

(^{ns}) = Non-significant; (*) = Significant differences at $P < 0.05$.

immersion methods were compared (Table 1). In the quick immersion method, the number of roots per cutting varied with growing doses of IBA, this variation being more clearly described by a third degree equation (Table 2). This variation results from small differences between the means, which varied between 4.89 and 6.96 and that close to a straight line with a mean number of 5.96 roots per cutting.

Ky-Dembele et al. (2011), using the quick method (the IBA concentrations were 0, 2500, 5000 and 10000 mg L⁻¹) to root *K. senegalensis* cuttings verified that when the cuttings were taken from 100 year old trees, the higher doses of IBA caused the number of secondary roots to increase. On the other hand, when the cuttings were taken from 3 month old seedlings, IBA had no effect on the number of roots per cutting. The results found for the quick immersion method may be explained by the degree

of juvenility of the plant material– in this case, the endogenous hormone balance is favorable to rooting. This material may show negative response to exogenous applications of plant hormones (Souza Júnior et al., 2008).

Increasing IBA concentration in the slow immersion method caused a linear increment in the number of roots per cutting (Table 3 and Figure 1). Similar results were reported by Vale et al. (2008) when increasing doses of IBA (0, 100, 200, and 300 mg L⁻¹) were applied to guava 'Paluma' cultivar cuttings- the higher the dose, the larger the number of roots per cutting. Similar results were also reported by Pinto and Franco (2009), when *Lippia alba* cuttings were immersed in IBA solutions with 500 and 1000 mg L⁻¹ in the slow immersion method– the larger IBA concentration resulted in higher numbers of roots per cutting.

Table 3. *Khaya senegalensis* rooting percentage, number of adventitious roots per leafy stem cutting, and total length of adventitious roots per cutting 45 days after planting as influenced by indole-3-butyric acid (IBA) concentrations applied by the slow immersion method.

IBA concentrations mg L ⁻¹	Means		
	Rooting percentage	Root number	Root length (cm)
0	91.67	5.27	41.43
100	88.33	5.54	51.65
200	96.67	6.57	53.41
400	93.33	8.27	65.86

Sources of variation	Significance F-Test		
	Rooting percentage	Root number	Root length (cm)
Linear Regression	0.83 ^{ns}	24.48 ^{**}	15.61 ^{**}
Quadratic Regression	0.28 ^{ns}	0.35 ^{ns}	0.07 ^{ns}
Cubic Regression	3.57 ^{ns}	0.29 ^{ns}	0.58 ^{ns}

(^{ns}) = Non-significant; (^{**}) = Significant difference at $P < 0.01$.

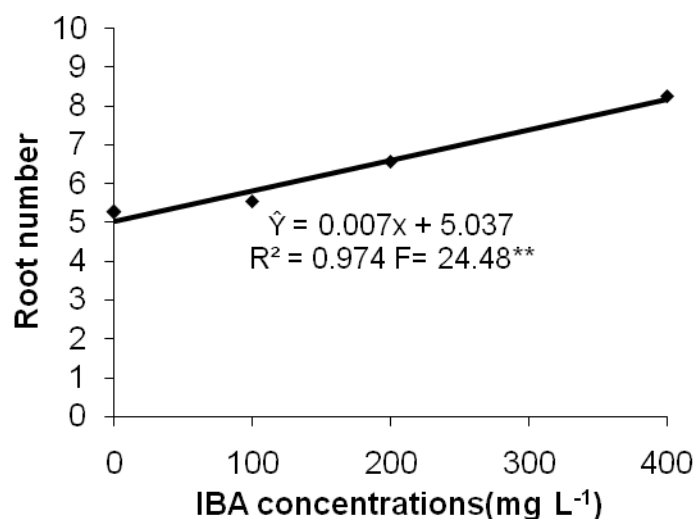


Figure 1. Number of adventitious roots per leafy stem cutting of *Khaya senegalensis* 45 days after planting as influenced by indole-3-butyric acid concentrations applied by the slow immersion method.

The cuttings whose basal end were treated with the quick immersion method showed a linear reduction in the roots total length as the IBA concentration increased (Table 2 and Figure 2). Souza Júnior et al. (2008), evaluating the effects of IBA concentration on the rooting of *Grevillea robusta* cuttings, and verified that roots total length was reduced with IBA doses higher than 2000 mg L⁻¹. IBA exogenously applied may stimulate root initiation but it may be toxic to certain types of cuttings (Hartmann et al., 2002).

Ky-Dembele et al. (2011), applying growing doses of IBA (0, 2500, 5000 and 10000 mg L⁻¹) to *K. senegalensis* cuttings verified that the length of the longest root in

cuttings taken from 3 month old seedlings did not differ from that of cuttings taken from 100 year old trees, although the length of the longest root of both types of cutting was larger than that of cuttings taken from 5 year old trees. These results show that young propagules, including sprouts taken after pruning 100 year old trees, are favorable for the rooting of *K. senegalensis* cuttings.

In the cuttings whose basal end had been treated by the slow immersion method, the roots total length showed a linear increment as IBA concentration was increased (Table 3 and Figure 3). Tonietto et al. (2001), working with two cultivars (Reubennel and Puma 7) of *Prunus domestica* verified that IBA increases rooting, the number

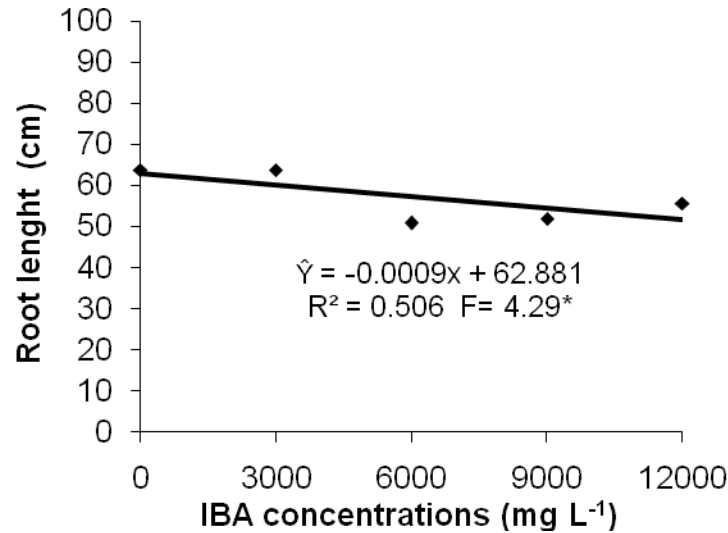


Figure 2. Total length of adventitious roots per leafy steam cutting of *Khaya senegalensis* 45 days after planting as influenced by IBA concentrations applied by the quick immersion method.

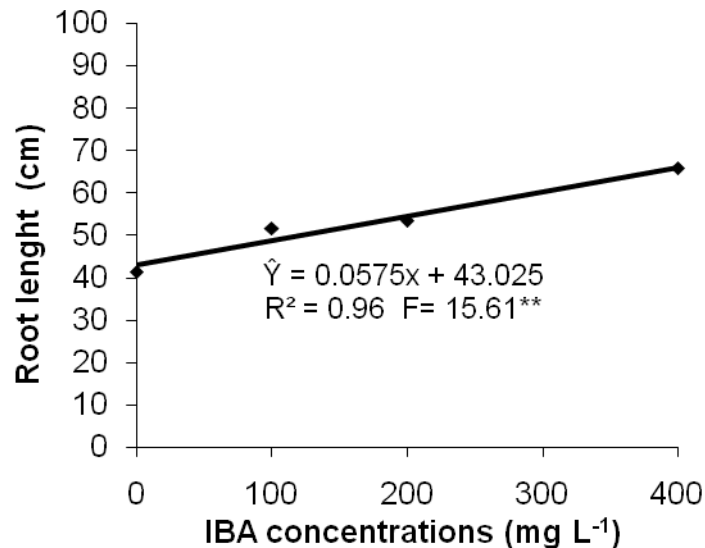


Figure 3. Total length of adventitious roots per leafy steam cutting of *Khaya senegalensis* 45 days after planting as influenced by IBA concentrations applied by the slow immersion method.

and length of roots of both cultivars. IBA, according to the authors, because it facilitates root formation, gave the roots more time to grow thus reaching higher lengths which is an indirect effect of IBA. Similar observations were reported by Cunha et al. (2004) in a research work with *Sapium glandulatus* cuttings- their length increased with IBA doses.

Seedling survival

New plants survival was 100% 165 days after the cuttings had been planted. The presence of leaves in the cuttings is thought to be the main factor determining such high survival percentage. In addition to that, optimal temperature and humidity conditions to which the

Table 4. Percentage of *Khaya senegalensis* plants bearing sprouts and sprouts height 165 days after planting as influenced by the way of immersion and indole-3-butyric acid (IBA) concentrations.

Sources of variation	Means	
	Plants bearing sprouts (%)	sprouts height (cm)
Quick Immersion	96.94 ^a	8.04 ^a
Slow Immersion	95.88 ^a	6.47 ^b
Significance F-Test		
Immersion Forms	0.44 ^{ns}	8.02 ^{**}
[IBA]/ Quick Immersion	0.89 ^{ns}	0.36 ^{ns}
[IBA]/ Slow Immersion	0.52 ^{ns}	0.73 ^{ns}
Coefficient of variation (%)	5.45	25.27

Means, in the same column, followed by the same small case letter, are not significantly different ($P > 0.05$). (^{ns}) = non-significant; (^{**}) = Significant difference at $P < 0.01$.

seedlings were exposed are also considered as very important for new plants survival. Xavier et al. (2003) ascribed the survival of vegetative *Cedar fissilis* propagules to the adequate control of greenhouse environmental conditions. Wendling and Xavier (2003) attributed the high survival percentages observed in their experiment to good environmental conditions under which *Eucalyptus* clones were submitted. They also pointed the importance of the genetic makeup of their plant material.

New plants characteristics

There was no difference between the immersion methods as to percentage of sprout bearing plants (Table 4). Even when the data were submitted to regression analysis, no differences were found between IBA concentrations in both immersion methods.

Dias et al. (2011), in a study with *Prunus cerrulata* cuttings, verified that IBA concentrations of 0, 1000 and 2000 mg L⁻¹ did not influence the results of number of plants bearing sprouts. In *Prunus mume*, IBA concentrations of 0 and 2000 had no significant effect on the percentage of plants bearing sprouts (Mayer et al., 2001).

The quick method resulted in higher sprout growth (Table 4). Even when the data were submitted to the regression study, no significant difference between IBA concentrations was observed either in the quick or in the slow immersion method. In *Sapium glandulatum*, Cunha et al. (2004) found no significant effect of IBA concentration on sprout height in rooted cuttings.

The quick immersion method, in comparison with the slow one, promoted the highest results of rooting percentage and sprouts height. The immersion methods did not differ with regards to cuttings survival percentage, total root length per cutting, number of roots per cutting, new plants survival percentage, and percentage of plants

showing sprouts.

Plants which easily root may justify the non use of using auxins. Auxins are more efficient in promoting rooting when the species is of low to difficult rooting. Although auxins are useful for plant propagation, and may increase the time and production efficiency, the final size and vigor of such plants are not larger than when no auxin is applied (Hartmann et al., 2002).

Conclusion

K. senegalensis may be propagated by means of leafy stem cuttings taken from seedling branches without the use of IBA.

Conflict of Interests

The authors have not declared any conflict of interest.

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