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Study of the effect of indole-3-butyric acid (IBA) on the rooting of kola tree cuttings (*Cola nitida* [Vent.] Schott and Endlicher.)

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Abstract

The vegetative propagation of the kola tree constitutes a real problem because the plants obtained by cuttings suffer from an imperfect root system, without pivoting axis and sensitive to drought. It is therefore necessary to propose methods to accelerate the growth and root development of kola plants. The objective of this study is to determine the effect of Indole 3-Butyric Acid (IBA) on the rooting of kola tree cuttings and to identify the concentrations favorable to rooting. *Cola nitida* cuttings from two clones: 313 and D3/2L3A1 were treated with four concentrations of IBA: 0 mg/L, 7500 mg/L; 10,000 mg/L and 12,500 mg/L and transplanted onto a substrate composed of topsoil according to a split-splot device. This study highlighted the significant interaction between IBA and clone on the rhizogenesis of kola tree cuttings. Indeed, the IBA stimulated the rooting while increasing the number and length of the roots of the treated cuttings and the number of new leaves. However, this effect remains clone-dependent. The D3/2L3A1 clone reacts better to IBA for doses of 10,000 mg/L and 12,500 mg/L than clone 313. It should be noted that IBA did not influence the survival rate, the collar diameter, height and biomass. It also appears that the D3/2L3A1 (82.78±12.86%) and 313 (88.34±7.58%) genotypes are suitable for tunnel cuttings. With a view to improving the rooting of kola plants independently of the clone, it would be interesting to test in the nursery Indole 3-Butyric Acid in combination with Indole-3 Acetic Acid and Naphthalene Acetic Acid.

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Introduction

Non-Timber Forest Products (NTFPs) occupy an important and vital place in the socio-economic life of rural and peri-urban populations in developing countries (Moupela *et al.*, 2011; Tieguhong *et al.*, 2009). The abusive and uncontrolled exploitation of these species contributes to the progressive depletion of their genetic resources. Also the majority of species have a low capacity for natural regeneration. These include *Cola nitida*, a perennial, allogamous and monoecious plant belonging to the Malvaceae family (Whitlock *et al.*, 2001).

The kola tree grows in the regions of tropical and equatorial Africa where it can reach more than 25 meters (Ouattara *et al.*, 2018). Côte d'Ivoire is the world's leading producer of kola nuts ahead of Nigeria, with an estimated production of 260,000 tonnes per year of fresh nuts (MINADER, 2018), for a turnover of around 100 billion CFA francs (Aloko-n'guessan, 2000). The kola nut therefore remains the leading agricultural product exported by Côte d'Ivoire to other African countries. Characterized by its richness in caffeine, it is a tonic for the heart, nervous and muscular stimulant. The kola nut is used in socio-cultural rites such as weddings and baptisms (Asogwa *et al.*, 2008). As a result, the kola tree is subject to uncontrolled exploitation of the fruits, bark, roots as well as the wood which is suitable for furniture and interior carpentry. This pressure is at the origin of the drastic reduction in the number of individuals in community forests and national parks.

Work carried out on the generative reproduction of *Cola nitida* has shown that the tree has a slow germination and a late entry into production (Mbete *et al.*, 2011) moreover, the use of seeds poses the problems of the non-reproducibility of characters from the mother plant to the daughter plant due to the allogamy of the tree (Odutayo *et al.*, 2018). Consequently, vegetative reproduction, long used for the reproduction of several forest species, constitutes an alternative to the reproduction of this species, in particular cuttings (Degrande and Facheux, 2002; Paluku *et al.*, 2018). Research has shown that a large number of factors influence the success rate and rooting of cuttings. These factors are, among others, the genotype, the number of leaves, the type of substrate, the length of the cuttings, the type of branch, the number and position of nodes as well as the concentration of plant hormones (Paluku *et al.*, 2018). In addition, the survival and rooting rates obtained from kola tree cuttings were relatively low (Sery *et al.*, 2019). It is therefore necessary to propose methods to accelerate the growth and root development of kola tree cuttings. Several authors have mentioned the effectiveness of indole-3-byturic acid (IBA) in the process of cuttings. These products are known for their rhyzogenic power. They would generally act on the physiology of the plant and would promote the development of root biomass. However, the concentrations used vary greatly and depend of the species (Jeruto *et al.*, 2008). However, the response of plants to auxin is not universal, which requires research to define the most suitable concentration for each plant species for large-scale vegetative propagation. It will be a question of testing the effect of three concentrations of IBA on the rooting of semi-woody cuttings of two clones of *Cola nitida* in order to identify the treatments that can be recommended for the success of the cuttings of the kola tree.

Materials and methods

Presentation of the test area and environmental conditions

The trial was set up in June 2020, at the start of the rainy season, on the nursery site of the National Center for Agronomic Research (CNRA) in Man. The nursery site is in the west of Côte d'Ivoire (7° 19.130' N; 8° 19.452' W). Rainfall in the Man area is monomodal. The dry season generally extends from October to March and the rainy season from April to September. The site has received an average annual rainfall of 1703.71 mm over the past ten years (2007-2017). The soils of the Man zone are of the ferralitic type. There are also soils developed on basic rocks (potentially rich), hydromorphic soils (lowlands) and soils rich in minerals (Iron, Manganese, Gold, etc.).

Plant material

The plant material used was made up of 360 kola tree cuttings. These cuttings were taken from two trees planted in 2016, in the nursery of the CNRA station in Man. These two trees are identified by the following codes: 313 and D3/2L3A1.

Technical material

The technical equipment used for the realization of this study consists of pruning shears for the removal and dressing of the cuttings, plastic bags for the conservation of the kola tree cuttings during transport, a stapler for closing the plastic bag, a decameter to measure the correct size of cutting for transplanting, a foam to keep the cuttings and maintain the humidity inside the bags, a PH meter to check the solution acidity and the soil as substrate.

Chemical material

The chemical material used consists of IBA (Indole-3-Butyric Acid; Manufacturer: Glentham Life Sciences, GK 7772), a plant hormone (phytohormone) that stimulates the rooting of cuttings. There is also potassium hydroxide (KOH) for dissolving IBA, IVORY* 80% WP fungicide (active ingredient: Maneb, Manufacturer: ARYSTA Life science) for preventive treatment before transplanting cuttings. Hydrochloric acid (HCL) was also used to adjust the pH of the IBA solution. TRICEL 480 EC insecticide (active ingredient: chlorpyrifos-ethyl) was used to treat termite attacks on cuttings.

Experimental device

Two factors were studied in this experiment. These are phytohormones with 4 modalities (0 mg/L; 7500 mg/L; 10,000 mg/L and 12,500 mg/L) and the clone with 2 levels. The different modalities of "Phytohormones" are as follows: To "Cuttings transplanted into a substrate without treatment with phytohormones (control treatment 0 mg/L)"; T1 "Cuttings soaked in a 7500 mg/L solution of Idol-3-Butyric Acid (IBA) then planted in a substrate without fertilizer"; T2 "Cuttings soaked in a 10,000 mg/L solution of IBA in a substrate without fertilizer"; T3 "Cuttings soaked in a 12,500 mg/L solution of IBA in a substrate without fertilizer". For the "Genotype" factor, clones 313 and D3/2L3A1 are noted.

The experimental device was a factorial device in split plot with two factors which are the phytohormones and the genotype (clone). The main factor was the clone and the subsidiary factor was the phytohormone. We had 8 treatments (2 clones × 4 concentrations) per block. Fifteen pots (nursery bags) each containing a 12 cm cutting with four (4) leaves and a severed terminal bud are used per treatment. The different treatments were repeated three (03) times. We had 120 cuttings per replicate, each block being one replicate. A total of 180 cuttings from the same clone were used for this test. This gave us a number of 360 cuttings for the two clones. The experiment was conducted at the CNRA research station in Man.

Preparation of IBA liquid solution

Preparation of the alkaline solution of Potassium hydroxide 1 mol/L

For the preparation of 1000 ml of a 1 mol/L potassium hydroxide solution, 56.11 g of potassium hydroxide were dissolved in 200 ml of distilled water using a magnetic bar.

Preparation of the alkaline solution of hydrochloric acid 1 mol/L

For the preparation of 200 ml of a 1 mol hydrochloric acid solution, dissolve 16.7 ml of a commercial 37% HCL solution in distilled water until reaching 200 ml.

Preparation of a stock solution of 12,500 mg/L of IBA

A stock solution with a concentration of 12,500 mg/L was prepared from powdered IBA with very low water solubility. To dissolve it, an alkaline solution of Potassium hydroxide 1 mol/L will be added to 12.5 g of IBA in a 500 mL beaker, with a magnetic stirrer. The pH was adjusted to 6.5 by adding drops of 1 mol/L hydrochloric acid. Ultra-pure water was added to complete the volume to one liter. To better preserve the auxin and avoid its degradation, we kept this solution at low temperature and in a bottle covered with aluminum foil to protect it from light.

Preparation of 10,000 mg/L and 7,500 mg/L solutions of IBA

The 10,000 mg/L solution was prepared just by diluting 240 mL of the stock solution with distilled water. The 7500 mg/L solution was prepared by diluting 180 mL of the stock solution with distilled water.

Making cuttings

The semi-augured cuttings were made from the terminal branches of the year, taken from the adult trees. Cuttings 10 to 12 cm in length and 3 to 5 mm in diameter were cut 1 cm below the terminal bud, then packaged in labeled packets, before treating their bases with indole butyric acid (IBA) (7500 mg/L, 10,000 mg/L and 12,500 mg/L). The bases of the cuttings were immersed in this solution to a height of 1.5 cm for 30 seconds. The cuttings were left to dry for 10 minutes to ensure absorption of the hormone. After drying, a treatment with a fungicide was applied by soaking in a solution of IVORY* 80% WP fungicide (active ingredient : Manèbe, Manufacturer: ARYSTA Life science) for 15 seconds, due to 5g, to avoid any rots due to fungi at the level of the basal cut. The cuttings were then planted in pots, one genotype per pot. The substrate used was composed of topsoil. The pots were filled up to 25 cm in height. The cuttings were buried over a quarter of their length.

After potting, the cuttings were watered regularly (3 times a week) with two towers of 15-litre watering cans, the plants under the tunnel were continuously monitored to ensure the absence of diseases and to schedule an intervention if necessary.

Measurements and observations

The notations relating to the collar diameter, to the plant height were carried out on the 10 plants in each treatment in each repetition at 6 months after transplanting. The notations relating to the level of rooting (Number of roots and length of the roots) and to the dry biomass of the roots were carried out on 03 plants per treatment. This dry biomass was evaluated using an electronic scale after drying in the open air for two weeks. Indeed, for each treatment and each repetition, three (03) seedlings were removed at 6 months after transplanting.

Statistical analysis

All data have been statistically analyzed using Statistica 7.1 software. The comparison of the means has been made with the Newman-Keuls test at the probability threshold of 5%.

Results

Effect of Clone interaction and Indole-3-Butyric Acid (IBA) application on the cuttings survival rate

Table 1. Effect of clone and Indole-3-Butyric Acid (IBA) on the survival rate of kola tree cuttings at 6 months

Factors	Sum of squares	Degrees of freedom	Mean square	F*	p*
Clone	185.2	1	185.2	1.51	0.236
Indole-3-Butyric Acid (IBA)	133.3	3	44.4	0.36	0.780
Clone* IBA	363.0	3	121.0	0.99	0.422
Error	1955.6	16	122.2		

Table 2. Survival rate according to the clone and the dose of Indole-3-Butyric Acid (IBA) of kola tree cuttings at 6 months

Clones	Indole-3-Butyric Acid (IBA)	Average survival rate per treatment (%)	Average survival rate per clone (%)
313	T0= 0 mg/L	88.9±3.8	88.34±7.58
	T1= 7500 mg/L	80±6.7	
	T2=10 000 mg/L	95.5±3.8	
	T3= 12 500 mg/L	88.9±7.7	
D3/2L3A1	T0= 0 mg/L	84.4±7.7	82.78±12.86
	T1= 7500 mg/L	86.7±6.7	
	T2=10 000 mg/L	82.2±16.7	
	T3= 12 500 mg/L	77.8±21.4	

Table 3. Effect of clone and Indole-3-Butyric Acid (IBA) treatment on root development of kola tree cuttings at 6 months

Factors	Test	Value	F	Effect	Error	p
Clone	Wilk	0,389666	11,74726	2	15	0,000
Indole-3-Butyric Acid (IBA)	Wilk	0,369480	3,22573	6	30	0,014
Clone* IBA	Wilk	0,369480	3,22573	6	30	0,014

*F: Fischer; p: Probability

Table 4. Length and roots cuttings average number from 6-month-old kola trees according to clone and Indole-3-Butyric Acid (IBA) treatment

Treatments	Average root length (cm)	Average number of roots
313T0	0±0[b]	0±0[b]
313T1	0±0[b]	0±0[b]
313T2	0±0[b]	0±0[b]
313T3	0±0[b]	0±0[b]
D3/2L3A1T0	0±0[b]	0±0[b]
D3/2L3A1T1	0±0[b]	0±0[b]
D3/2L3A1T2	4.67±4.16[a]	0.67±0.57[a]
D3/2L3A1T3	6.67±2.11[a]	1±0[a]

The multivariate analysis of variance revealed that there is no significant interaction ($p > 0.05$) between the two factors studied (Clone and Indole-3-Butyric Acid (IBA)) on the survival rate of kola tree cuttings. The clone factor and Indole-3-Butyric Acid (IBA) did not significantly influence the survival rate during kola tree cuttings (Table 1).

Survival rate according to clone and IBA dose

Survival rates vary from 80 to 95.5% for clone 313 and from 77.8 to 86.7% for clone D3/2L3A1 on average (Table 2). Hormonal application had no significant effect on the cuttings survival rate. There was therefore no significant difference between treatments compared to the control. The cuttings average success rate was 88.34% for clone 313 and 82.78% for clone D3/2L3A1.

Effect of Clone and Indole-3-Butyric Acid (IBA) factors on root development

The multivariate analysis shows that there is an effect of the two factors (Clone and IBA) and their interaction on root development, namely the average number of roots emitted and their length. The indole-3-butyric acid (IBA) application significantly influenced the cuttings rooting of young *cola nitida* plants ($p<0.05$). However, this effect was clone dependent. In fact, there is an interaction between the IBA dose applied and the response of the clone (Table 3). Clone D3/2L3A1 reacted favorably to IBA unlike clone 313. It produced more roots than the untreated control D3/2L3A1T0 for IBA doses of 10,000 mg/L and 12,500 mg/L (Table 4). Callus formation was observed on living cuttings of other treatments that did not root.

Table 5. Effect of Indole-3-Butyric Acid (IBA) hormone application and clone on collar diameter, average height and dry matter of kola tree cuttings at 6 months

Treatments	Average collar diameter at (mm)	Average height (cm)	Average dry matter (g)
313	4.04±0.57[b]	6.48±0.5	1.15±0.12[c]
D3/2L3A1	4.7±0.51[a]	6.6±1.3	1.77±0.5[c]
Clone	*p=0.003	p=0.656	p=0.000
Indole-3-Butyric Acid (IBA)	p=0.06	p=0.296	p=0.406
Clone* IBA	p=0.33	p=0.382	p=0.243

*p: Probability

Effect of Clone and Indole-3-Butyric Acid (IBA) factors on plant growth at 6 months

The analysis reveals that only the clone factor had an effect on the growth parameters, in particular the dry matter (Table 5). Collar diameter and dry biomass were influenced by the clone factor (Table 6).

Clone D3/2L3A1 has a greater vegetative development than clone 313. The collar diameter of Clone D3/2L3A1 was 4.7±0.51 mm against 4.04±0.57 mm for clone 313. It the same is true for dry biomass with 1.77±0.5 g for clone D3/2L3A1 against 1.15±0.12 g for clone 313.

On the contrary, no effect of factors on growth in height was noted. Indole-3-Butyric Acid (IBA) had no effect on these variables during these six months of testing (Table 5). The number of new leaves emitted by the cuttings was impacted by the clone, the dose of IBA and the Clone*IBA interaction (Table 6). The effectiveness of IBA on the number of new leaves emitted was however a function of the genotype.

Table 6. Effect of Indole-3-Butyric Acid (IBA) and the clone on the number of new leaves of kola tree cuttings at 6 months

Treatments	Average number of new leaves
313T0	0 ± 0^{b}
313T1	0 ± 0^{b}
313T2	0 ± 0^{b}
313T3	0.0 ± 0^{b}
D3/2L3A1T0	0 ± 0^{b}
D3/2L3A1T1	0 ± 0^{b}
D3/2L3A1T2	3.33 ± 3^{a}
D3/2L3A1T3	2.6 ± 0.57^{a}
Clone	*p=0.004
Indole-3-Butyric Acid (IBA)	p=0.03
Clone* IBA	p=0.03

Discussion

The tunnel cuttings carried out at the nursery during this experiment recorded a high survival rate (88.34% for clone 313 and 82.78% for clone D3/2L3A1). However, the results showed no effect of genotype and Indole-3-Butyric Acid (IBA) on *cola nitida* cuttings. These results are in contrast to the results of Séry and collaborators in 2019, which highlighted the effect of genotype. This result is explained by the optimal conditions in which the cuttings were carried out. Indeed, the cuttings were carried out during the rainy period with good relative humidity which favored its success unlike the trials in the dry season. According to the work carried out by Ricez in 2008, periods of dry seasons should be avoided because they would promote greater dehydration of the cuttings despite watering and would increase the mortality rate. This dehydration can modify the response of the genotype to the cuttings. In addition, the two genotypes tested reveal to us through the results that they have the same aptitudes for cuttings in ideal conditions. There is therefore no significant difference between treatments compared to the control for the survival rate.

After analyzing the results, it appears that the application of IBA had an influence on the rooting of *cola nitida* cuttings. However, the response at the level of the kola tree depends on the genotype. According to Auclair (2009), there are great differences between species and even between individuals of the same species, some take root in less than three weeks while others may require more than 8 to 10 weeks. This experiment demonstrated an interaction between the IBA dose applied and the clone response. Clone D3/2L3A1 reacted favorably to IBA unlike clone 313. It produced more roots than the untreated control D3/2L3A1T0 for IBA doses of 10,000 mg/L and 12,500 mg/L. This result shows that IBA can stimulate the rhizogenesis of young *cola nitida* plants. These behavioral differences between the genotypes would be due either to the intrinsic properties of each genotype, or to their physiological state. Similar effects have been reported in the argan tree propagated by cuttings (Metougui *et al.*, 2017) and in the cocoa tree under the same growing conditions (Koko *et al.*, 2011). According to these authors, even if auxin can have an influence, rooting ability is more related to genotype than to other factors.

Significant effects of IBA were also obtained in *Vitellaria paradoxa* C. F. Gaertn (Akakpo *et al.*, 2014), jojoba (Zahra *et al.*, 2014). For these authors, the application of IBA promotes the increase of nutrient reserves at the base of the cuttings. For (Leakey, 2004), the application of IBA accelerates the transformation process and the transport of reserve substances at the base of the cuttings. Similarly (Hartmann *et al.*, 1990) argue that IBA activates cell division and differentiation in the root primordium to later give roots.

Plant hormone (Auxin) plays a crucial role in the process of inducing new root formation (Jackson, 1987). In some species, cuttings root easily without auxin treatment; on the other hand, in others, a suitable supply of exogenous auxin is necessary for successful rooting (Gaspar *et al.*, 2016). In our experiment, relatively high doses of 10,000 mg/L and 12,500 mg/L of IBA were required. Several studies have shown that with a low concentration of AlB, roots appear late and in low numbers (Alsup and Cole, 2000).

During our experiment we observed in addition to the effect on rooting, an effect on the number of new leaves for the same concentrations. However, it always depended on the genotype. The D3/2L3A1 clone had the greatest number of new leaves at the concentrations of 10,000 mg/L and 12,500 mg/L. This shows the existence of a correlation between the root volume and the leaf area materialized by the number of leaves. Rooting is linked to the presence of leaves and their surface (Atangana *et al.*, 2006; Oboho and Iyadi 2013). In a similar study Nyansi (2004) showed that the leaf area of leaves influences the rooting of young *G. kola* cuttings.

For other agronomic parameters such as collar diameter (mm) and height (cm), no effect of IBA was noted. The dry matter is influenced by the genotype. The short duration of experimentation could explain this.

After this study we can retain that the genotype significantly influences the cuttings of *cola nitida*. High-producing clones with good aptitude for cuttings should therefore be selected to improve the success rate of cuttings. The application of exogenous auxin (IBA) stimulates the rooting of young kola plants by increasing the number and length of roots but also the number of new leaves at concentrations of 10,000 mg/L and 12,500 mg/L. However, this effect was genotype and concentration dependent, even within the same species.

Conclusion

This study made it possible to show the aptitude of *Cola nitida* for vegetative propagation, in particular for cuttings. The aim was to test the effect of three concentrations of indole-3-butyric acid on the rooting of young kola plants in the nursery in order to determine the concentration favorable to the cuttings rooting. The results obtained provide an answer to the specific objectives that were the subject of this study. This study highlighted the significant interaction between indole butyric acid (IBA) and genotype (Clone) on the rhizogenesis of kola tree cuttings. In fact, IBA stimulated the rooting of kola tree cuttings while increasing the number and length of the roots of the treated cuttings and the number of new leaves. However, this effect remains clone-dependent (genotype). The D3/2L3A1 clone reacts better to IBA than the 313 clone. For this clone, the doses of 10,000 mg/L and 12,500 mg/L have been shown to be very effective. It should be noted that IBA did not influence survival rate, collar diameter, height and biomass. It also appears that the D3/2L3A1 (82.78±12.86%) and 313 (88.34±7.58%) genotypes are suitable for tunnel cuttings. The results of this study constitute an important step in the process of improving kola productivity in Côte d'Ivoire. With a view to improving the rooting of all young *cola nitida* plants, it would be interesting to test in the nursery over a longer period (9 to 12 months) Indole 3-Butyric Acid (IBA) in combination with Indole-3 Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA). The objective is to eventually propose an effective dose of auxin for all clones of kola trees that can be popularized.

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