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Propagation of *Garcinia kola* (Heckel) by stem and root cuttings

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Highlights

- Roots and branches cuttings from mature forest trees are difficult to sprout.
- The highest mean numbers of roots and buds produced were obtained with softwood cuttings.
- The aqueous IBA treatment was more effective than the powder in promoting rooting and root development.
- Non-mist poly-propagator gave the best propagation results.
- Seedling stockplants cut above node 1 promoted most vigorous shoots production.

Abstract

The availability of appropriate propagation techniques is a major constraint to the domestication of the forest trees widely used by rural communities; such as *Garcinia kola* (Heckel). This study tested the ability of root and stem cuttings to regenerate vegetatively when treated with indole-3-butyric acid (IBA), and set in non-mist poly-propagator within a shaded nursery. It found that *G. kola* is amenable to propagation by softwood stem cuttings. Attention was given to the effect of cutting age (softwood, semi hardwood) and IBA application with regard to the sprouting and rooting efficiency. Bud and leaf emergence time were also investigated, as was the coppicing ability of the stump. Results revealed that roots and branches from mature forest trees did not sprout under any culture conditions. The highest percentages of rooting (70–85%) were obtained with softwood cuttings set in non-mist poly-propagator, regardless of hormonal treatment. The mean numbers of buds (2.9 ± 0.4) and roots (2.6 ± 0.1) produced by softwood cuttings was significantly greater than those obtained in the Control. The best average emergence time of buds (25.1 days ± 9.3) and leaves (36.8days ± 8.4) was obtained with cuttings treated with IBA and set in non-mist poly-propagator against, 54.4 ± 12.5 and 72.6 ± 3.4 days respectively for the Control. In general, non-mist poly-propagator gave the best propagation results. When coppiced, the shoots emerging from stumps with one node were the most vigorous.

Keywords bitter kola; non-mist polypropagators; vegetative propagation; coppicing
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1 Introduction

In addition to a few well-known major crops, there are many other underutilized species which nevertheless have vital socioeconomic importance in the economies of developing countries. Many of these plants produce common property Non Timber Forest Products (NTFPs) in forests and woodlands (Howthorne 1990; Sunderland 1999, 2001; Zoro Bi and Kouakou 2004). These are now recognized as new crops producing Agroforestry Tree Products (AFTPs) from farmland (Leakey et al. 2005) playing an important role in food security and the well-being of rural populations due to their contribution to the income and nutrition of many local populations. Harvest and sale of these resources are usually performed by women and children. Therefore, NTFPs/AFTPs are particularly important for the well-being of the poorest social strata (Sunderland 2001; Tchatat et al. 2006; Tieguhong et al. 2009). Unfortunately, in most of these countries, timber/wood has always been considered to be the main forest product while the other products are designated to be secondary products. Overexploitation of these species combined with deforestation/land clearance have caused a steady depletion of their natural populations and there are no efficient conservation strategies at either the community or national level, apart from their presence in national reserves (N'Danikou et al. 2011; Dako et al. 2014).

Garcinia kola (Heckel) in the Clusiaceae or Guttiferae family, known as Bitter Kola, occurs in west and central Africa forests and its products have multiple uses thanks to their medicinal and nutritional properties.The seeds are chewed and have a bitter taste, followed by a slight sweetness. They are also used in the manufacture of traditional soft drinks. They have also many uses of a ceremonial nature and in traditional medicine (Adu-Tutu et al. 1979; Koffi et al. 2015). Indeed, *G. kola* seeds extract can prevent or cure bacterial infection (Stanley et al. 2014; Denloye et al. 2009). The seed/kernels also contain a chemical called 'kolaviron', a biflavonoide which has anti-malarial properties (Murray et al. 2012; Oluwatosin et al. 2014). Farombi and Owoeye (2011) reported antiviral, antihepatotoxic and antidiabetic properties of *G. kola*, while anti-sickle cell disease (Antisickling) activities of leaf extracts were revealed by Adejumo et al. (2011).

To promote the cultivation of *G. kola*, most work has investigated seed germination (Agyili et al. 2006; Eyog-Matig et al. 2007; Yakubu et al. 2014a) aimed at the domestication of this species. In addition, vegetative propagation studies have investigated the grafting of juvenile seedlings (Yakubu et al. 2014b), but there has been little work on the rooting of stem and root cuttings in this species. However, many other West African indigenous tree species producing AFTPs have been found to be easily propagated by leafy stem cuttings (Leakey 2014a):*Vitex madiensis* (Mapongmetsem 2006); *Vitellaria paradoxa* and *Parkia biglobosa* (Teklehaimanot 2004); *Dacryodes edulis* and *Irvingia gabonensis* (Shiembo et al.1996); *Vitex doniana* (Achigan-Dako et al. 2011) etc.

Studies of the vegetative propagation of tree by stem and root cuttings is extensive but the results are often contradictory. However, Leakey (2004, 2014a) has developed some principles to aid the development of propagation techniques for tree species. Stem cuttings can come in many forms, but the two major groups are leafy softwood cuttings from juvenile shoots (which are dependent of current photosynthates for rooting) and leafless hardwood cuttings from older and more lignified shoots (which are dependent on the mobilization of stored carbohydrate reserves) (Leakey 2014a). Concerning root cuttings, the ability of sprouting seem to be limited to certain species and depends of the length and the diameter of the root (Ede et al. 1997; Nguyen et al. 1990; Leakey 2014) and polarity (Ofori et al. 2015) although thick roots may regenerate slowly (Del Tredici 1995).

According to Leakey (2014a), successful rooting of stem cuttings depends on optimising both pre-severance and post-severance factors. Post-severance factors include both those within and between shoot, which are affected by stockplant age and size, soil nutrition and environmental factors like light and temperature. Post-severance, the important factors mediated by photosynthesis, transpiration, respiration and carbohydrate mobilisation, as well as the effect of growth regulators like auxins, are cutting size, leaf area, the minimization of physiological water stress such as drought and anoxia due to medium saturation (both aerial and in the rooting medium). To reduce these stresses, non-mist polypropagators which ensure a high environmental uniformity of both the aerial and rooting medium is used in several techniques of macropropagation (Leakey 1990; Newton and Jones 1993). In general, rooting normally occurs before the sprouting of new shoots.

The aim of the current study was to develop efficient methods of clonally propagating of *G. kola*, which are easily transferable to the rural populations. The principal question was, whether the nature and the age of cuttings and nursery conditions might limit or promote propagation success and so contribute to the food security and livelihoods of farmer through the improved silviculture or agroforestry systems.

2 Materials and methods

2.1 Sites of study

The experiments were carried out from September to December 2014 in Abidjan, at University Nangui Abrogoua (former University Abobo-Adjame) research station (05°23´N, 04°00´W). Plant material was collected in natural vegetation near Ahouabo, (Adzope Department) situated 100 km north-east of Abidjan. This site has similar climate to that of the research station. Both sites are located in the forest zone where the rainfall pattern is bimodal with two dry seasons (from December to March and from July to August), and two rainy seasons (from April to June and from September to November). Mean annual rainfall varies between 1800–2000 mm. Mean monthly temperature varies between 27 and 30 °C, whereas the mean relative humidity ranges from 70 to 84%.

2.2 Plant material

Two types of plant materials were used (stems and roots). Firstly, leafy stem cuttings were harvested from lateral branches (3 to 6 m above the ground) of 10 to 15 years old *G. kola* trees in Ahouabo forest. Additionally stem cuttings were collected from young seedlings (12 months old and 25 to 30 cm in height) in the nursery, at the research station. Secondly, root samples were collected from the same mature trees in Ahouabo, but not from the seedlings which were too small. Whatever their origin, stem cuttings were divided into two categories: the semi hardwood or lignified portion (Fig. 1A) and the softwood (Fig. 1B) or non-lignified. Once collected (between 7 and 8 am), branch cuttings from forest trees, with intact leaves, were stored in a wet jute bag and transported back to the nursery for vegetative regeneration the next day (juvenile cuttings from the nursery were set the same day).

2.3 Methods

2.3.1 Propagation by stem cuttings

Stems, whatever their origin, were excised from plants with a disinfected pair of secateurs. The average length of softwood and semi hardwood cuttings was 10.2 ± 2.2 cm and 9.7 ± 1.8 cm, respectively. The average diameters of softwood and semi hardwood stems were 1.2 ± 0.2 cm and 1.0 ± 0.5 cm, respectively. Two leaves, trimmed to half were left on each softwood cuttings while semi hardwood cuttings were leafless.

Fig. 1. Stem cuttings used for propagation: A = leafless semi-hardwood cuttings; B = leafy softwood cuttings of *G. kola*.

Fig. 2. Unrooted cuttings of *G. kola* set in plastic bags filled with substrate (soil) and arranged in a shaded nursery.

Trials were established on two methods of propagation:

The first method was in individual pots in an open nursery shaded by palm fronds and canes of bamboo with an average air temperature of 26±2 °C and average relative humidity of 87% (Fig. 2). Temperature and relative humidity were collected every two days at 2 pm. In this nursery, cuttings (45 per treatment) were set in perforated black plastic bags (750 cm^3), filled with substrate and arranged in three different blocks. The substrate consisted of soil taken from a fallow with vegetation mainly composed of *Chromolaena odorata* (L.) and *Panicum maximum* (Jacq.) and a sandy soil from a river in a proportion 1:1 (V/V). The mixed substrates (pH 5.5) were treated with a fungicide (mancozeb Callivoire-Abidjan 880 W in water at 15 g l^{-1}).

The second propagation method was a non-mist poly-propagator set in the same nursery, measuring 200 × 120 × 150 cm modified from the design of Leakey et al. (1990) and Leakey (2014a). This propagator was a wooden frame enclosed in clear polythene. The base, covered with a thin layer of fine sea sand to prevent the polythene from being perforated by the stones followed by successive layers of small stones, was airtight (Fig. 3).This culture condition reduces the post-severance physiological stress resulting from water loss through transpiration by keeping the cuttings cool, moist, and turgid and facilitates rooting evaluation (Leakey et al.1990; Leakey 2014a). Air temperature and relative humidity registered under this nursery were 28±3 °C and 93%, respectively.

In order to stimulate root formation, stem cuttings were treated with a commercial 'rooting' hormone; indole-3-butyric acid (IBA, Sigma-Aldrich Co, St Louis, USA) or left as an untreated (Control). The basal end of each treated cutting was sprinkled with IBA powder or was dipped briefly into 2.5 g of IBA dissolved in 1 litre of distilled water, before planting. Control cuttings were not treated with IBA. Cuttings were then planted out vertically, with the apical end up. The cuttings were watered 2 to 3 times per week in the shaded nursery in order to keep the substrate moist. In the non-mist poly-propagator, watering frequency was once per week because of the high moisture content in this propagator.

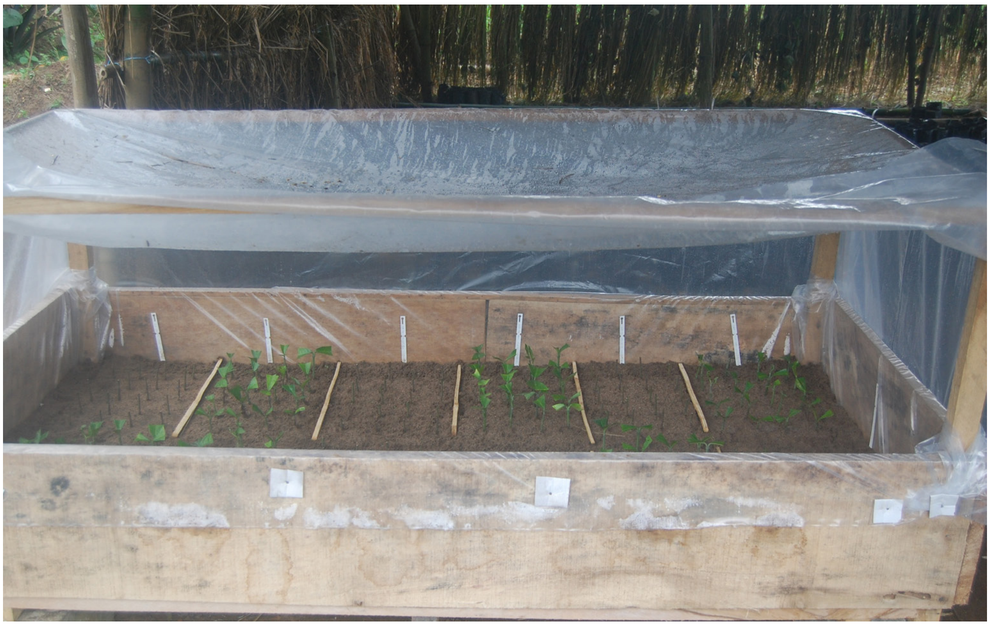

Fig. 3. Unrooted cuttings of *G. kola* in a modified non-mist poly-propagator.

Seven quantitative traits, generally studied in vegetative propagation were investigated: (1) the viability (cuttings remained green and alive, or dead); final percentage estimated 120 days after planting (DAP); (2) and (3) the percentage of sprouting and rooting, (4) and (5) the mean numbers of sprouts and roots produced per cutting, (6) and (7) the mean emergence time of sprouts and first leaves. The cutting was said to be rooted or sprouted when they had one root or bud exceeding 0.5 cm in length. The root counting was essentially based on principal roots. Secondary roots (most numerous) were not registered. Sprouting was assessed every two days while rooting was assessed at weekly interval throughout the trials, and final percentages were calculated 120 days after planting.

The poly-propagator rooting experiment was laid out in a randomized block design with two treatments: the cutting type (softwood/semi-hardwood), and IBA application (sprinkled/dipped) with 45 cuttings arranged in three replicates (15 cuttings per replicate block) were planted out directly on the substrate.

In the shaded nursery experiment, 60 plastic bags containing substrate and cuttings were arranged in three replicate blocks (20 cuttings per block).

Once rooted, cuttings from the non-mist poly-propagator were transferred into plastic bags and placed under the shaded nursery conditions for acclimatization.

2.3.2 Propagation by root cuttings

The root cuttings, with average length 10.2 ± 2.2 cm, were cut from the distal parts of root system of field trees. Their average diameter was 1.3 ± 0.1 cm. Root cuttings were also treated with IBA or left untreated (Control), as previously, before setting them in the two propagation methods. Totally 45 root cuttings arranged in three replicate (15 cuttings per replicate block) were set in the same perforated black plastic bags (750 cm^3), filled with the same substrate for the shaded nursery, or planted out vertically in the substrate for non-mist poly-propagator.

2.3.3 Coppicing of tree stumps

G. kola seedlings possess two buds per node, in the axils of two opposite leaves.To test the coppicing ability of stumps in the nursery, plants were cut down to 1, 2 or 3 nodes above the collar region of 12 month old seedlings (25 to 30 cm in height). These stumps were in 90 plastic bags (30 stumps × 3 replicates) each containing one of each level of coppicing. These stumps were arranged in three replicate blocks under palm fronds shade in the nursery. Stumps were watered 2 to 3 times per week, under shaded nursery. Traits such as: shoot emergence, number of shoots, height growth of shoots, collar diameter of shoots and mean leaf length, were assessed regularly every three days.

2.4 Statistical analysis

Mean values and standard deviations were calculated for each of the 7 characters with respect to nursery type, cutting and hormonal treatment. Combined multivariate analysis of variance (MANOVA) appropriate to three factors was performed to compare nursery type, cutting and hormonal treatment effect, as well as their interaction. This allowed the identification of significant factors based on a vector of dependent variables. The General Linear Models (GLM) procedure of the SAS statistical package version 9.1 (SAS, 2004) was used to identify traits contributing to difference when MANOVA revealed significant difference for a factor. Least Significant Difference (LSD) multiple range-tests were used to identify differences between means. One way analysis of variance (ANOVA 1) was performed for the comparison of the different levels of coppicing.

3 Results

3.1 Propagation by stem cuttings

3.1.1 Effect of treatments on the rooting ability of stem cuttings

The type of cuttings, method of propagation, and the use of IBA rooting hormone, all had significant effects on the success of vegetative propagation ($P<0.05$), as did the 'cutting × nursery × hormone interaction (Tables 1–3).

3.1.1.1 Effect of the method of propagation on rooting ability of juvenile, leafy stem cuttings

The method of propagation greatly affected rooting ability of stem cuttings. The highest percentages of rooting (85.0 ± 9.2 % and 70.3 ± 4.2 %) were obtained in a non-mist poly-propagator for softwood cuttings treated with aqueous or powdered IBA, respectively, compared with 69.7 ± 32.2 and 62.5 ± 17.5 % for shaded nursery bed. The highest mean numbers of roots produced: 2.5 ± 0.1 to 2.6 ± 0.1 roots were obtained in non-mist poly-propagator (with softwood cuttings) against 2.1 ± 1.0 to 2.3 ± 1.6 in shaded nursery bed with the same type of cutting (Table 1).

3.1.1.2 Effect of cutting type on rooting ability of stem cuttings

The cutting type affected significantly rooting ability. Softwood cuttings rooted better, with more roots, than semi-hardwood cuttings. Indeed, the highest percentages of rooting: 70.3 ± 4.2 % to 85.0 ± 9.2 % (non-mist poly-propagator) and 62.5 ± 17.5 % to 69.7 ± 32.2 % (shaded nursery bed) were obtained with softwood cuttings treated with IBA. The mean numbers of roots produced (2.1 to 2.6 roots) by softwood cuttings were higher than those obtained with semi-hardwood (1.2 to 1.7 roots) and control (1.0 to 1.6 roots), regardless the method of propagation (Table 1).

3.1.1.3 Effect of IBA application method on rooting ability of stem cuttings

Hormonal application affected significantly rooting ability of stem cuttings. The percentage of rooting (10.0 ± 0.0 to 85.0 ± 9.2 %) and the mean number of roots (1.2 ± 0.5 to 2.6 ± 0.1 roots) produced by the two types of cutting treated were higher than those obtained with Control (5.0 ± 1.1 to 17.5 ± 7.7) and (1.0 ± 0.0 to 1.6 ± 0.5 roots) for percentage of rooting and number of roots, respectively and regardless of the method of propagation. However, the aqueous IBA treatment was more effective than the powder in promoting rooting and root development in softwood cuttings planted in non-mist poly-propagator than in semi-hardwood cuttings (Table 1). IBA was generally more effective in the non-mist propagator than in a shaded nursery bed. Cutting mortality was lower when cuttings rooted well.

3.1.1.4 Effect treatment interaction on rooting ability of stem cuttings

With regard to cutting mortality, there were no significant interactions between the treatments (Tables 1 and 3). However, there were significant interactions between rooting and other treatments. Softwood cuttings treated with IBA powder or dipped in 2.5 g l^{-1} of IBA solution greatly improved rooting success and root development (Fig. 4). The percentage of rooted cuttings and the mean number of roots per cutting were higher with softwood cuttings, regardless of the method of IBA application.

Table 1. Combined effect of methods of propagation on rooting ability and death of juvenile stem cuttings of *G. kola.*

Traits	Methods of propagation												Stat
	Non-mist Poly-propagator						Shaded nursery bed						
	Semi-hardwood			Softwood			Semi-hardwood			Softwood			
	Powder (IBA)	Dipped in IBA solution (2.5 g l^{-1})	Control	Powder (IBA)	Dipped in IBA solution (2.5 g l^{-1})	Control	Powder (IBA)	Dipped in IBA solution (2.5 g l^{-1})	Control	Powder (IBA)	Dipped in IBA solution (2.5 g l^{-1})	Control	
Rooted (%)	22.5 ± 3.5^{c}	55.0 ± 24.4^{b}	12.5 ± 1.5^{d}	70.3 ± 4.2^{a}	85.0 ± 9.2^{a}	17.5 ± 7.7^{cd}	10.0 ± 0.0^{d}	27.5 ± 14.7^{c}	5.0 ± 1.1^{d}	62.5 ± 17.5^{ab}	69.7 ± 32.2^{a}	12.5 ± 3.5^{d}	$P<0.05$
Death (%)	25.5^{a}	22.5^{a}	15.8^{a}	14.8^{a}	17.8^{a}	16.9^{a}	19.9^{a}	22.8^{a}	14.8^{a}	19.2^{a}	20.5^{a}	25.5^{a}	$P<0.05$
Nb roots	1.2 ± 0.5^{de}	2.5 ± 0.1^{a}	1.0 ± 0.0^{e}	2.6 ± 0.1^{a}	2.5 ± 0.1^{a}	1.1 ± 0.3^{e}	1.7 ± 0.8^{c}	1.3 ± 0.6^{d}	1.0 ± 0.0^{e}	2.3 ± 1.6^{b}	2.1 ± 1.0^{b}	1.6 ± 0.5^{c}	$P<0.05$

Mean values in a line followed by the different letters are significantly different at $P < 0.05$ (Newman-keuls -test)
Nb = number; IBA = indole-3-butyric acid

Table 2. Combined effect of methods of propagation on sprouting and leaves emergence ability of juvenile stem cuttings of *G. kola.*

Traits	Methods of propagation												Stat
	Non-mist Poly-propagator						Shaded nursery bed						
	Semi-hardwood			Softwood			Semi-hardwood			Softwood			
	Powder (IBA)	Dipped in IBA solution (2.5 g l^{-1})	Control	Powder (IBA)	Dipped in IBA solution (2.5 g l^{-1})	Control	Powder (IBA)	Dipped in IBA solution (2.5 g l^{-1})	Control	Powder (IBA)	Dipped in IBA solution (2.5 g l^{-1})	Control	
Sprouting (%)	79.2 ± 7.1^{a}	80.5 ± 11.8^{a}	63.9 ± 11.1^{b}	72.0 ± 15.0^{a}	77.0 ± 11.2^{a}	74.1 ± 17.4^{a}	37.2 ± 17.1^{d}	47.5 ± 11.8^{c}	65.0 ± 7.1^{b}	54.0 ± 1.1^{bc}	29.0 ± 4.1^{d}	28.2 ± 2.0^{d}	$P<0.05$
Nb sprout	1.0 ± 0.0^{c}	1.0 ± 0.0^{c}	1.2 ± 0.4^{c}	2.9 ± 0.4^{a}	2.8 ± 0.4^{a}	1.1 ± 0.2^{c}	1.1 ± 0.9^{c}	1.4 ± 0.5^{bc}	1.2 ± 0.5^{c}	1.8 ± 0.4^{b}	1.7 ± 0.4^{b}	1.0 ± 0.0^{c}	$P<0.05$
Sprouting (day)	68.8 ± 14.8^{c}	54.9 ± 13.0^{b}	57.1 ± 13.0^{b}	27.7 ± 9.7^{a}	25.1 ± 9.3^{a}	54.4 ± 12.5^{b}	63.3 ± 1.0^{c}	53.8 ± 20.2^{b}	51.3 ± 14.8^{b}	37.5 ± 16.7^{a}	44.0 ± 10.4^{a}	81.3 ± 3.3^{d}	P<0.05
Leafing (day)	77.5 ± 7.1^{de}	83.2 ± 7.4^{e}	87.7 ± 8.4^{e}	55.5 ± 7.0^{bc}	36.8 ± 8.4^{a}	72.6 ± 3.4^{d}	78.4 ± 11.3^{de}	76.7 ± 12.2^{de}	74.5 ± 5.3^{d}	49.4 ± 15.9^{a}	63.9 ± 11.1^{c}	84.2 ± 8.7^{e}	$P<0.05$

Mean values in a line followed by the different letters are significantly different at $P < 0.05$ (Newman-keuls -test)
Nb = number; IBA = indole-3-butyric acid

Table 3. Multivariate analysis of variance (AMOVA) performed to test the overall effect of cutting type, hormonal treatment and nursery on vegetative propagation of *G. kola.*

Sources	Statistical	
	F	*P*
NT	2,66	<0,001
CT	96,74	<0,001
HT	3,58	<0,012
NT × CT	64.44	<0,001
NT × HT	12.34	<0,001
CT × HT	5.95	<0,001
NT × CT × HT	0.54	<0,001

NT = Nursery type; CT = Cutting types; HT = Hormonal treatment

Fig. 4. Rooted softwood cuttings of *G. kola* treated with indole-3-butyric acid rooting powder (90 days after potting).

Fig. 5. Rooted cuttings (9 months old) of *G. kola* regenerated in non-mist poly-propagator and acclimatized in shaded nursery.

3.1.1.5 Effect of treatment on sprouting and subsequent shoot growth

Treatment affected significantly sprouting, sprouts and leaves emergence, regardless of the method of propagation (Table 2). The shortest time to sprouting was 27.7±9.7 days and for leaf emergence was 55.5±7.0 days from IBA-treated softwood cuttings in a non-mist poly-propagator versus 54.4±12.5 and 72.6±3.4 days for bud and leaf emergence in control cuttings in a non-mist propagator. Similar results were obtained in a shaded nursery bed.

The mean numbers of sprouts per cutting were higher from softwood cuttings (2.8±0.4 to 2.9±0.4 buds treated with aqueous or powdered IBA, respectively) for non-mist poly-propagator than in a shaded nursery bed (1.7±0.4 to 1.8±0.4 buds treated with aqueous or powdered IBA, respectively).

When rooted cuttings from the non-mist poly-propagators were potted in plastic bags and transferred out into the shaded nursery, they all (100%) survived (Fig. 5). It is the same for rooted cuttings from the nursery bed.

Table 4. Impact of coppicing height on shoot growth of *G. kola* seedlings.

Level of coppicing	Traits				
	Shoot emergence time (Days)	Mean number of shoots per strain	Height growth of shoots (cm/month)	Mean collar diameter (cm) of Shoots	Mean length of leaves (cm)
1 node	80.33±2.88[a]	1.19±0.035[b]	4.27±0.77[a]	0.56±0.085[a]	13.55±0.47[a]
2 nodes	74.02±15.09[a]	1.59±0.06[b]	3.82±0.73[a]	0.34±0.09[b]	11.93±1.29[a]
3 nodes	69.66±11.06[a]	2.56±0.06[a]	2.35±0.21[b]	0.29±0.14[b]	10.72±1.17[b]
F	4.15	17.59	7.69	15.70	5.53
P	0.073	0.003	0.022	0.004	0.043

Mean values in a column followed by the different letters are significantly different at $P < 0.05$ (Newman-keuls -test)

3.2 Propagation by root cuttings

None of the root cuttings collected from old trees in the forest regenerated. All of them died regardless of the nursery type and hormonal treatment.

3.3 Effect of coppicing seedlings at 3 node positions (1, 2 or 3)

The time for coppice shoot formation was not influenced significantly by the position of coppicing ($P > 0.05$). However, the mean number of shoots per stump, the length of shoots, the mean diameter of collar region and the mean length of leaves were significantly influenced ($P < 0.05$) by the level of coppicing (Table 4). Plants cut above node 3 produced an average of 3 shoots per stump instead of 2 at other two positions (Fig. 6). However, shoots produced by plants cut above node 1 and 2 were the longest and had the greatest mean length of leaves. In addition, the greatest stem diameter was obtained with plants cut above node 1.

Fig. 6. Two vigorous shoots induced from a *G. kola* stump after cutting back to node one.

4 Discussion

The chronological age differences in cutting morphology between softwood, semi-hardwood and hardwood significantly affected propagation in terms of rooting ability, numbers of roots, sprouting and leaf emergence. Successful vegetative regeneration was best with leafy, softwood cuttings from young seedlings. This emphasises the importance of the physiological state of the stockplant (Leakey 2014a) and while this is well known, it is still often overlooked by people starting to propagate tree species by cuttings.

The failure of cuttings gathered from lateral branches of mature trees to root, especially when leafless, is a common phenomenon attributable to several factors like the age-related physiology and morphology of donor plants and their phenology (Stenvall et al. 2004, 2009; Snedden et al. 2010; Teklehaimanot et al. 2012); including factors such as plant carbohydrate and nutrient contents and the content of growth regulators (Maile and Nieuwenhuis 2010; Ky-Dembele et al. 2010; Dick and Leakey 2006).

Experience with regeneration from root cuttings is much more limited, and appears to be species specific. However, when it has been reported, thick roots near the collar region have better regeneration ability than the thin roots in distal parts of the root system (Stenvall et al. 2006). Indeed, according to Ede et al. (1997); Nguyen et al. (1990), Ofori et al. (1996) and Stenvall et al. (2006), the diameter of the root defines the carbohydrate content of the cutting.

The time between the collection of cuttings in field and planting in nursery could induce physiological stresses arising from the loss of water and carbohydrate reserves from the tissues due to transpiration and respiration during this period (Aminah et al. 1997; Sanoussi et al. 2012; Leakey 2014a). However, in the present study this is unlikely to explain the poor performance of the cuttings brought in from the field as they were stored in the dark in a sealed damp sack for less than 24 hours.

The results on *G. kola* stem cuttings from young plants reveal that it is possible to regenerate entire plant of this species from organs other than the seeds, opening up the possibility of developing superior cultivars for cultivation by African farmers (Leakey 2014b). Stem cuttings of *G. kola* exhibited a good capacity to produce new shoots and roots, especially in non-mist polypropagators, although a greater number of roots would be beneficial. The increasing regeneration rates and quicker emergence of buds and leaves in non-mist poly-propagators obtained in this study may be explained by the uniform and relative high temperature and humidity that characterized this propagation system (Leakey 2014a).The greatest mean values of rooting with softwood stem cuttings, whatever hormone application and nursery type, could be explained by the presence of leaves. Generally, the rooting of stem cuttings depends on the physiological processes of the leaf such as photosynthesis and transpiration (Leakey and Storeton-West 1992; Oboho and Iyadi 2013; Takoutsing et al. 2014). Rooting is maximized when the cutting is photosynthetically active and producing assimilates for the development and elongation of the root primordia (Hartmann et al. 2002; George et al. 2007; Leakey 2014a).

Indole-3-butyric acid treatments are well known to improve rooting, especially, as in the case of *G. kola*, when the cuttings have been gathered from the most appropriate shoots. Many studies have shown that dipping cuttings in auxin solutions ranging from concentrations of 2.5 to 5 g l^{-1} or sprinkled with rooting powder stimulate the rhizogenesis of forest trees (Tchoundjeu et al. 2002; Teklehaimanot et al. 2004; Sanoussi et al. 2012). Furthermore, the interaction between auxin and carbohydrates has been recognised as vital for root formation (Sorinet al. 2005; Georges et al. 2007). Indeed, root formation is stimulated by IBA which hydrolyses the polysaccharides, thus increasing the metabolic activities required to provide vital energy for the formation and elongation of meristematic tissues responsible for root production and sprouting (Georges et al. 2007; Husen et al. 2007).

The relatively low number (2–3) of shoots produced in this study may be related to the small diameter of the young plants used as cuttings. Indeed, according to Leakey (1983), Dick et al. (2004), sometimes, there is a great interaction between species and stem diameter on total shoot and root number and length. Sulaiman et al. (2005) revealed for Veronese (*Populus deltoides x nigra*) and Willow (*Salix matsudana x alba*) that thick stem diameter produced higher total root length and shoot number. Leakey (1983) and Leakey and Longman (1986) mentioned other parameters such as environmental and stockplant factors which affect greatly root and shoot initiation in cutting. Thick stems produce a larger number of roots which would promote water and nutrients uptake, necessary for plant development. Similar results were obtained in hybrid aspen clones regeneration (Stenvall et al. 2009) and in clonal propagation of *Detarium microcarpum* (Guill. & Perr.) (Ky-Dembele et al. 2010).

Regarding the level of coppicing, heavily cut-back stumps with only one or two nodes produced fewer shoots, but these shoots were stronger and made rapid growth. This could be explained by the fact that the low number of buds produced by coppicing stumps with one node caused low competition for the resources needed for growth and development (Bory et al. 1991; Luostarinen and Kauppi 2005).

5 Conclusion

This study has shown that *G. kola* is amenable to vegetative propagation using stem cuttings and so can be domesticated for wider cultivation. IBA treatments promoted shoot and root production and accelerated the emergence of shoots and leaves. A non-mist poly-propagator was found to give the greatest rates of success, especially when softwood cuttings were propaged with an aqueous application of IBA. Seedling stockplants cut above node 1 promoted the most vigorous shoots production. The development of an efficient propagation method for *G. kola* is an essential step towards growing *G. kola* in forestry or agroforestry systems.

Further research is needed to determine the optimal conditions for robust and larger scale propagation of *G. kola*. Tissues culture, notably, micropropagation, could be investigated for large scale commercial production, but this approach would not be practical for use by small-scale farmers.

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