European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 27-07-2020; Accepted: 13-07-2020; Published: 28-08-2020
www.biosciencejournals.com
Volume 8; Issue 3; 2020; Page No. 34-40

Improving propagation methods of *Garcinia kola* Heckel (Clusiaceae) for rapid multiplication, domestication, and conservation in the tropical moist forests

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Abstract

The establishment of an efficient protocol for vegetative propagation is still a challenge for *Garcinia kola*, a priority target for domestication and conservation in the humid tropics of Africa. The present study addressed the effects of three ranges of leaf area and six concentrations of indole-3-butyric acid (IBA) on the propagation of stem cuttings. Results showed that, when considering all auxin concentrations taken together, leafless cuttings resulted in the average mortality rate of 76.12 ± 2.22%, which was higher than 10.62 ± 2.22% and 12.22 ± 2.22 % recorded with 35-40 cm^2 and 70-80 cm^2 respectively. Auxin concentration had no significant individual effect on the mortality rate. Combining 35-40 cm^2 leaf area with 4% IBA resulted in the overall lowest mortality rate (6.62 ± 4.62%). Leafless cuttings failed to root. When considering all ranges of leaf area together, the percentage of rooted cuttings and the number of roots per rooted cutting both increased with increasing IBA concentrations and reached their highest values (79.31 ± 7.42% and 8.15 ± 0.17 respectively) with 4% IBA. The overall highest percentage of rooted cuttings (92.91 ± 6.24%) and highest roots count (8.25 ± 0.23) were recorded with [35-40 cm^2 X 4% IBA] combination. These results make progress towards the establishment the clonal propagation protocol for commercial and conservation planting of this high valued threatened species.

Keywords: auxin concentration; *Garcinia kola*; leaf area; rooting; stem cuttings; vegetative propagation

1. Introduction

In the African humid tropics, most of the forest natural resources with direct relevance to the well-being of the people are subject to strong pressures, which are exerted through overexploitation coupled with lack of appropriate management strategy [1]. These pressures have led to the progressive decline of many high valued tree species indigenous to the area [2]. In order to prevent the extinction and derive maximum benefits from these indigenous trees, it is necessary to promote their conservation in the environment. One important approach for the conservation of plant species is cultivation. However, most of these forest tree species remain essentially wild and information on propagation techniques is scantily available [3]. Therefore, there is a huge need for developing methods for growing and propagating such indigenous species [4].

Garcinia kola (Heckel), a member of the Clusiaceae family [5], is a medium size tree growing up to 12 m high in 8 years in moist forest throughout tropical Africa [6]. The economic and social importance of *G. kola* tree to the local populations in many areas of sub-Saharan Africa cannot be emphasized enough. Indeed, it is one of the most high valued forest tree species of West and Central Africa where it is extensively used in traditional medicine, the manufacture of traditional soft drinks, and traditional ceremonies [7]. Various parts of the plant are used for the treatment of various diseases including laryngitis, mouth infections, cough, heart burn, liver disorder, chest colds hoarseness, throat troubles, post partum hemorrhage, urinary tract infections and emesis [7-12]. Farombi and Owoeye [13] reported antiviral, antihepatotoxic and antidiabetic properties of *G. kola*, while anti-sickle cell disease activities of leaf extracts were revealed by Adejumo *et al.* [14]. The pharmacological properties of different *G. kola* plant parts have been attributed to numerous benzophenones and flavanones they contain and which exhibit a wide range of biological effects [15-17]. *G. kola*'s seed, commonly referred to as "bitter kola" owing to its bitter astringent taste, is chewed as stimulant and may also have potential use in the brewing industry as an alternative to hops [7, 18, 19]. *G. kola*'s seed is one of the many important non timber forest products (NTFPs) of Western and Central African countries where its commercialization in both domestic and national markets raises the standard of living of those involved in its trade [20]. A study by Fondoun and Tiki-Manga [21] revealed that bitter kola can generate up to US$ 1,167.6 annual revenue per household in South Cameroon.

In spite of great demand for *G. kola* seeds, the cultivation of the species is not popular owing to poor seed germination [20]. Indeed, *G. kola* seed exhibit dormancy which hinders its germination. The trees are still exploited in the wild. Because of its overexploitation which results from its high interest, the species is now close to commercial extinction [22]. *G. kola* has been identified through priority setting exercises as one of the priority targets for domestication in humid lowlands of Southern, Western and Central Africa [23, 24]. With the aim to promote the cultivation of *G. kola*, there have been some attempts to overcome seed dormancy and enhance germination in some Nigerian, Cameroonian, Ivorian and Ghanaian collections [25-27], but the results are often contradictory, making it difficult to prescribe a standard procedure for enhancing seed germination. Therefore, the development of alternative means of propagation is needed for the domestication of the species. An alternative means of propagating this species, other than by its seed, would be the vegetative approach. Vegetative propagation techniques are extremely useful as a tool to capture and mass propagate the superior phenotype of selected individual trees. The recent advances in the development of a low-technology propagation system have resulted in successful propagation of a wide range of timber and multipurpose tropical tree species through stem cuttings [28]. However, the success in using stem cuttings for propagation varies from one species to another. A previous study has been done on vegetative propagation of *G. kola* through rooting of stem cuttings [29]. It examined the influence of propagation environment (non-mist poly-propagator vs. shaded nursery bed), type of cuttings (softwood vs. semi hardwood) and exogenous hormone application (untreated control vs. indole-3-butyric acid at the single concentration of 2.5 g/l) on the rooting ability of stem cuttings. The results of this study revealed that (1) *G. kola* is amenable to this approach of vegetative propagation; (2) non-mist poly-propagator gave the best propagation results; (3) softwood cuttings had the highest rooting ability and (4) application of IBA at the single concentration of 2.5 g/l significantly increased the rooting potential of cuttings. Nevertheless, when optimal treatments of the above mentioned factors were achieved, although the rooting percentage (85%) was acceptable, it was, together with the maximum number of roots per rooted cutting (2.6), still fewer than really desirable and less than reported for other tropical tree species [30-32]. Moreover, the mortality rate of cuttings (25%) was higher than desirable. This indicates the need for further research, to improve rooting success in *G. kola* stem cuttings. Investigating the effects of other factors including leaf area and concentrations of IBA applied to cuttings would contribute to achieve this goal. Indeed, the vegetative propagation literature indicates that in stem cutting rooting experiments, high mortality and low rooting efficiency may be attributed among others to inappropriate leaf area [33-35] and/or inappropriate auxin concentration [36, 37].

With the aim at improving the rooting success in *G. kola* stem cuttings, the present study examined the effects of different ranges of leaf area and different concentrations of IBA on the rooting of softwood stem cuttings in non-mist propagator.

2. Materials and Methods

2.1 Plant material

Coppice shoots developing from pollarded mature mother trees were used as cuttings' donors for the experiment. Mature *G. kola* trees growing in Penja, a location situated in the monomodal rainfall moist forest zone of Cameroon, were pollarded in June 2018 for coppicing, and shoots were allowed to grow for one year before cutting collection. Very early in the morning (between 6 and 6.30 am), orthotropic coppice shoots were cut 30 cm below the top and then decapitated 4 cm below the top. Each of the resulting 26 cm shoot represented the softwood portion which has been reported more responsive to the induction of rooting [29]. These shoots were enclosed in polythene bags to check moisture loss and were transported quickly to the propagation site, at the nursery of the Department of Plant Biology, University of Dschang (5°27'N, 10°3'E, altitude 1400 m) where cuttings preparation and planting were done the same day.

2.2 Preparation of cuttings

Each shoot of 26 cm length was cut into 2-3 cuttings. Cuttings were 8-10 cm length, 0.6-0.8 cm diameter and comprised 2-4 nodes (depending on the length of internodes). For each cutting, all the leaves were cut off and discarded, except those attached to the upper node, which were trimmed so as to retain the desired leaf area. Three ranges of leaf area were investigated; 0 cm^2, 35-40 cm^2 and 70-80 cm^2. Cuttings were drenched in Metalaxyl-M fungicidal solution (3 $g.l^{-1}$) and allowed to dry for approximately 10 minutes before hormone application.

To evaluate the efficiency of hormone treatments in promoting root initiation from stem cuttings, six concentrations [i.e. 0, 0.25, 0.5, 1, 2 and 4 % (w/w)] of indole-3- butyric acid (IBA) powder were tested. IBA powders were commercial formulations supplied by the manufacturer (RHIZOPON® BV, Holland). Auxin treatments were applied by sprinkling the basal end of the cutting with the respective IBA powder.

2.3 Propagation environment

Rooting of cuttings was conducted in non-mist propagators, with moist fine river sand as substrate. The non-mist propagator was constructed following the design described by Leakey *et al.* [38]. The relative humidity inside the propagator remained between 75 and 80% throughout the propagation period. The propagator was placed in the nursery at 25 ± 3°C with a mean irradiance of 540 $\mu mol\ m^{-2}\ s^{-1}$ during the day.

2.4 Experimental design

To evaluate the effects of leaf area and auxin treatment on the propagation of *G. kola* stem cuttings, a split-plot experimental design was used. Each main plot contained three leaf areas (0 cm^2, 35-40 cm^2 and 70-80 cm^2), whereas six concentrations of IBA powder [0, 0.25, 0.5, 1, 2 and 4% (w/w)] were tested at the subplot level. A total of 18 treatment combinations (3 leaf areas X 6 IBA concentrations) were tested. Three replications each of 20 cuttings were used per treatment combination; this made a total of 1080 cuttings used for the study.

Cuttings were planted vertically in the propagation substrate, and were regularly watered to avoid desiccation. The experiment ran for six months after which each cutting was assessed for mortality, rooting and root count.

2.6 Data analysis

Data were analyzed using the SPSS 17.0 software package. The dependent variables were mortality rate, percentage of rooted cuttings and number of roots per rooted cutting. Prior to analysis, all percentage data (mortality rate and percentage of rooted cuttings) were subject to arcsine transformation as follows: $y = arcsin\sqrt{p/100}$ where y is the transformed data and p is percentage [39]. Transformed data were subjected to Analysis of variance (ANOVA). Significant different means were separated using Duncan's Multiple Range tests ($p \leq 0.05$).

3. Results

3.1 Morphological differentiation patterns

Cuttings which were still alive at six months after insertion in non-mist propagator had developed different morphological differentiation patterns. Independently to treatment, an overall 35% of cuttings developed at their basal cut end a mass of undifferentiated tissue (callus) which was white in color and with variable size (Fig. 1a).

Some cuttings developed one or many roots at their base. The roots were simple at their juvenile stage (Fig. 1b) and then developed secondary roots as they matured (Fig. 1C). The number of roots per rooted cutting varied from one (Fig. 1c) to twelve (Fig. 1d).

Fig 1: Different morphological differentiation patterns from *G. kola* stem cuttings at six months after insertion in non-mist propagator: a) development of callus at the basal cut end; b) root without ramification at its early stage of development; c) cutting with a single root bearing many secondary roots at maturity; d) cutting with maximum number of roots (Twelve).

3.2 Mortality rate

Results of analysis of variance (Table 1) showed that leaf area had significant effects ($p < 0.001$) on the mortality rate but auxin concentration and interaction of leaf area with auxin concentration had no significant effect. The mean mortality rate recorded with cuttings having 35-40 cm^2 leaf area (10.62 ± 2.22%) and that recorded with cutting having 70-80 cm^2 leaf area (12.22 ± 2.22%) were not different from each other, but were significantly lower than the 76.12 ± 2.22% mortality rate recorded with leafless cuttings. The lowest mortality rate (6.62 ± 4.62%) was recorded with 35-40 cm^2 x 4% IBA combination (Table 2).

Table 1: Result of the analysis of variance showing the degree of freedom (*df*), and the level of significance (*F* and *p* values) at which each of the factors [leaf area (LA) and auxin concentration (AC)] and their interaction (LA X AC) affected each of the dependent variable (mortality rate, percentage of rooted cuttings and number of roots per rooted cutting) investigated.

Source of variation	Dependent variables								
	Mortality rate			Percentage of rooted cuttings			Number of roots/rooted cutting		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
LA	2	194.14	< 0.001	2	23.98	< 0.001	1	0.98	0.322
AC	5	1.01	0.40	5	5.89	< 0.001	5	178.62	< 0.001
LA X AC	10	0,39	0.95	10	1.06	0.39	5	1.77	0.12

Table 2: Mortality rate (%) of *G. kola* stem cuttings as influenced by auxin concentration and leaf area at six months after insertion in non-mist propagator

Auxin concentration	Leaf area			Average
	0 cm^2	35-40 cm^2	70-80 cm^2	
0%	80 ± 4.62	10 ± 4.62	13.33 ± 4.62	34.40 ± 3.8^{ns}
0.25%	80 ± 4.62	13.30 ± 4.62	13.33 ± 4.62	35.62 ± 3.8^{ns}
0.5%	76.70 ± 4.62	10 ± 4.62	10 ± 4.62	32.20 ± 3.8^{ns}

1%	60 ± 4.62	10 ± 4.62	10 ± 4.62	26.66 ± 3.8^{ns}
2%	76.71 ± 4.62	13.33 ± 4.62	13.33 ± 4.62	34.46 ± 3.8^{ns}
4%	83.30 ± 4.62	6.62 ± 4.62	13.33± 4.62	34.41 ± 3.8^{ns}
Average	76.12 ± 2.22^{b}	10.62 ± 2.22^{a}	12.22 ± 2.22^{a}	

Note: Within the last row, means ± SEs followed by same letter are not significantly different at 5% level; ns: no significant effect of auxin concentration.

3.3 Percentage of rooted cuttings

There were highly significant ($p < 0.001$) effects of leaf area and auxin concentration on the percentage of rooted cuttings, but there was no significant interaction between the two factors (Table 1). The mean rooting percentage recorded with cuttings having 35-40 cm^2 leaf area (48.45 ± 3.33%) and that recorded with cuttings having 70-80 cm^2 leaf area (50 ± 3.40%) were not different from each other, but were significantly higher than the 0% which was recorded with leafless (0 cm^2) cuttings. The mean percentage of rooted cuttings increased with increasing auxin concentrations and reached 79.31 ± 7.42% at 4% IBA. The mean percentage of rooted cuttings recorded with 4% IBA was higher than the rooting percentage recorded with each of the other IBA concentrations. The highest percentage of rooted cuttings (92.91 ± 6.24%) was recorded with cuttings having 35-40 cm^2 leaf area and treated with 4% IBA (Table 3).

Table 3: Rooting percentage of *G. kola* stem cuttings as influenced by auxin concentration and leaf area at six months after insertion in non-mist propagator

Auxin concentration	Leaf area			Average
	0 cm^2	35-40 cm^2	70-80 cm^2	
0%	0 ± 0	11.12 ± 2.1	7.72 ± 2.60	8.47 ± 2.80^{d}
0.25%	0 ± 0	38.50 ± 3.3	44.46 ± 4.60	37.70 ± 6.60^{c}
0.5%	0 ± 0	40.72 ± 4.3	46.20 ± 4.40	37.93 ± 7.32^{c}
1%	0 ± 0	50 ± 4.3	48.12 ± 6.17	40.30 ± 5.60^{c}
2%	0 ± 0	61.5 ± 4.6	69.24 ± 8.31	57.63 ± 6.64^{b}
4%	0 ± 0	92.91 ± 6.24	84.62 ± 8.34	79.31 ± 7.42^{a}
Average	0 ± 0*	48.45 ± 3.33^{ns}	50 ± 3.40^{ns}	

Note: Within the last column, means ± SEs followed by same letter are not significantly different at 5% level; ns: no significant difference between 35-40 cm^2 and 70-80 cm^2 leaf areas; *: 0 cm^2 significantly different from 35-40 cm^2 and 70-80 cm^2.

3.4 Number of roots per rooted cutting

Auxin concentration had highly significant effects ($p < 0.001$) on the mean number of roots per rooted cutting, but the individual effect of leaf area as well as interaction of leaf area and auxin concentration had no significant effect (Table 1). When considering all ranges of leaf area taken together, the average mean number of roots per rooted cutting increased with increasing auxin concentration and reached the value of 8.15 ± 0.17 at 4% IBA. This average mean roots count which was recorded with 4% IBA was higher than each of those obtained with other auxin concentrations. The highest mean number of roots per rooted cutting (8.25 ± 0.23) was recorded with cuttings treated with 4% IBA and having 35-40 cm^2 leaf area (Table 4).

Table 4: Number of roots per rooted *G. kola* stem cutting as influenced by auxin concentration and leaf area at six months after insertion in non-mist propagator

Auxin concentration	Leaf area			Average
	0 cm^2	35-40 cm^2	70-80 cm^2	
0%	-	1 ± 0.66	1 ± 0.4	1± 0.52^{d}
0.25%	-	1.50 ± 0.36	1.58 ± 0.33	1.54 ± 0.24^{cd}
0.5%	-	1.54 ± 0.35	1.58 ± 0.33	1.56 ± 0.24^{cd}
1%	-	2 ± 0.31	2.23 ± 0.32	2.12 ± 0.22^{c}
2%	-	3.93 ± 0.29	5.22 ± 0.27	4.61 ± 0.19^{b}
4%	-	8.25 ± 0.23	8.04 ± 0.24	8.15 ± 0.17^{a}
Average	-	3.03 ± 0.16^{ns}	3.27± 0.17^{ns}	

Note: Within the last column, means ± SEs followed by same letter(s) are not significantly different at 5% level; ns: no significant difference between 35-40 cm^2 and 70-80 cm^2 leaf areas.

4. Discussion

The influence of leaf area on the rooting efficiency of stem cuttings is widely recognized [36, 37] but to date no study has been carried out on *G. kola*. The results of the present study showed that leaf area significantly influenced rooting of *G. kola* stem cutting by affecting mortality rate and rooting percentage. Most (76.12%) of leafless cuttings had died within six months, and rooting was restricted to leafy cuttings. This trend has been reported with other tropical tree species such as *Lovoa trichilioides* [33] and *Prunus Africana* [34], and has been associated with the rapid depletion of carbohydrates in stem tissues; in contrast, the concentrations in leafy cuttings tend to increase due to photosynthesis [40]. The results of the present study appear to indicate that survival and rooting of stem cuttings depend on carbohydrates formed and utilized after cuttings have been excised from the donor plant. Cuttings have to produce assimilates faster than they are losing them through respiration to survive and to root [41]. Leaf surface also influences rooting of stem cuttings through its effect on the rate of transpiration, which determines the water status of the cutting. Successful rooting therefore requires an optimal leaf area which balances the positive effect of photosynthesis and the negative effect of transpiration [36, 42]. The leaf area associated with this balance varies from a species to another. The results of the present study showed that cuttings with 35-40 cm^2 leaf area were similar to those with 70-80 cm^2 leaf area for their mortality rate as well as for their rooting percentage and roots count. This indicates that within the range of leaf areas investigated in this experiment, water stress was not a major problem.

Auxins are reported to play a significant role in stimulating root initiation in stem cuttings of woody plants [30, 34]. Indeed, in addition to its effects on cell differentiation, auxins promote starch hydrolysis and the mobilization of sugars and nutrients to the cutting base, all of which influence rooting [43]. The present study reported that the average percentage of rooted cuttings as well as the average number of roots per rooted cutting increased with increasing IBA concentrations and reached their highest values (i.e. 79.31% and 8.15 respectively) at the IBA concentration of 4% (w/w). However, previous researches reported IBA concentrations lower than 4% to be optimal for rooting stem cuttings of many other tropical tree species such as *Cordia alliodora* [44], *Khaya ivorensis* [45] and *Litsea monopetala* [46]. Furthermore, successful rooting without applied auxin has been reported in a number of tropical species such as *Nauclea diderrichii* [47], *Allanblackia floribunda* [48] and *Balanites aegyptiaca* [49]. Such contrasting results indicate that there exist between-species differences in the responses of cuttings to exogenously applied auxins. These differences may be due to variation in endogenous auxin contents at time of severance, as previously reported by Dick and Leakey [50].

Combining the optimal leaf surface with the optimal auxin concentration resulted in a synergistic effect on all rooting traits investigated. These results are consistent with the hypothesis developed by Atangana *et al.* [48] according to which higher rooting percentages can be achieved when factors affecting rooting are optimized. The highest rooting percentage (92.91 ± 6.24%) and the highest number of roots per rooted cutting (8.25 ± 0.23) which were obtained with the optimal treatment combination [35-40 cm^2 leaf area x 4% IBA] greatly exceeded the previously reported maxima of 85% of rooted cuttings and 2.6 roots per rooted cutting [29]. Moreover, the same treatment combination resulted in a mortality rate of 6.62 ± 4.62%, which represents an improvement on the minimum of 25% previously reported [29]. This provides evidence for practical recommendation of this treatment combination for *G. kola* vegetative propagation protocol.

5. Conclusion

The present study provides new valuable information to optimize the clonal propagation protocol for *G. kola*. This is an incentive for farmers developing nurseries for the domestication of high-value multipurpose agroforestry tree species indigenous to the humid tropics of Africa and institutes in charge of reforestation programs, as *G. kola* is an important agroforestry tree species in the region [28]. Propagation through stem cuttings would be a means for rapid multiplication and *in situ* conservation of the tree species. It would serve as alternative to the use of seeds whose germination is poor. The study adds to the increasing number of indigenous fruit and nut species of tropical Africa that are being domesticated using techniques of vegetative propagation.

6. References

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