Journal of Applied Biosciences 181: 18891– 18900
ISSN 1997-5902

Vegetative propagation of *Xylopia parviflora*, a wild medicinal spice tree species in the tropics: insights for domestication

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Submission 1st November 2022. Published online at https://www.m.elewa.org/Journals/ on 31st January 2023
https://doi.org/10.35759/JABs.181.2

ABSTRACT

Objective: Xylopia parviflora (A. Rich.) Benth. (commonly known as “striped African pepper”) is one of the most socio-economically high-valued tree species of the Central and Western African humid forests. Its propagation is hindered by poor seed germination. The present study aimed at examining its potential for vegetative propagation through the rooting of stem cuttings.

Methodology and Results: In a split-plot experimental design, three leaf areas (0, 15 and 30 cm^2) and six concentrations [(0, 0.25, 0.5, 1, 2 and 4% (w/w)] of indole-3-butyric acid (IBA) were tested for their effects on stem cutting parameters after five months in a non-mist propagator. Maximum rooting percentage (100%) was recorded with 15 cm^2 x 4% IBA, 30 cm^2 x 2% IBA and 30 cm^2 x 4% IBA treatment combinations. The highest mean root count (9.1) was recorded with 30 cm^2 x 4% IBA. The mortality rate of cutting was significantly affected by leaf area only. Leafless cuttings (0 cm^2) resulted in 73.5% mortality, which was higher than 11.8% and 10.7% recorded with cuttings having 15 and 30 cm^2 leaf areas respectively.

Conclusion and application of findings: X. parviflora is amenable to vegetative propagation through rooting of leafy stem cutting within five months. For an efficient propagation of *X. parviflora* through stem cuttings, the use of cuttings with 30 cm^2 leaf area and application of 4% (w/w) IBA are recommended. This is valuable insight into the domestication and *in situ* conservation of this species that is of direct relevance to the well-being of millions of people in sub-Saharan Africa. Propagation of *X. parviflora* using robust and low-cost procedure as described in this study would stand as an alternative to the sexual propagation which is hindered by poor seed germination.

Keywords: auxin, leaf area, rooting, stem cuttings, striped African pepper.

INTRODUCTION

Xylopia parviflora (A. Rich.) Benth., a member of the Annonaceae family (APG III, 2009), is a socioeconomically high-valued tree species in tropical Africa. *X. parviflora* uses in African cuisine, construction, production of various household utensils and traditional medicine have been abundantly documented (Orwa *et al.*, 2009; Nishiyama *et al.*, 2010; Tsabang *et al.*, 2012; Bukar *et al.*, 2014; Woguem *et al.*, 2014). Commonly known as “striped African pepper”, it is one of the indigenous tree species, which significantly contributes to the well-being of the population in central and western Africa by providing numerous exosystemic services and substantial income (Orwa *et al*., 2009). Despite its socioeconomic importance, *X. parviflora* has not been domesticated yet. Previous attempts to sexually propagate *X. parviflora* revealed coat-imposed seed dormancy, which hinders the regeneration of the species. Pre-treatment through chemical scarification using concentrated hydrochloric acid and sulfuric acid enhanced seeds' germination (Kanmegne *et al*., 2018). Nevertheless, such chemical pre-treatment using concentrated acid is extremely dangerous if not carefully handled. Therefore, the development of alternative means of propagation that do not require the application of such corrosive chemicals is fundamental. Vegetative propagation is presumed a better technology for the use of seeds in propagating the species and will give more chances to tree growers to increase the number of seedlings produced. Rooting of stem cutting is among the most suitable means of vegetative propagation. The desired phenotype of the selected plant is obtained by rooting of stem cutting and mass propagation. The development of an efficient protocol for vegetative propagation through rooting of stem cuttings would be an incentive for smallholder farmers and agro-foresters to adopt the cultivation of this species whose exploitation enhances the living standard of people in many areas around tropical Africa. Protocols for vegetative propagation through stem cuttings have been successfully developed for many tropical forest tree species including *Allanblackia floribunda*, *Prunus africana*, *Annona muricata*, *Cola* spp, and *Ricinodendron heudelotii* (Leakey, 2014; Leakey *et al*., 2022). However, the success in using stem cuttings for propagation varies from one species to another. For *Xylopia parviflora*, there has been no systematic investigation into raising plantlets through stem cuttings. Success in propagating a plant species through stem cuttings is influenced by a wide range of pre and post-severance factors such as stock plant management, exogenous hormone application, leaf area, cuttings length, propagation environment, (Leakey, 2004; Leakey, 2014). The objective of this research was to establish an efficient protocol for *X. parviflora* propagation by the rooting of stem cuttings in a non-mist propagator. The present study aimed to determine 1) The effects of leaf area and IBA concentration on the percentage of root cuttings, 2) the effects of leaf area and IBA concentration on the mean number of roots per rooted cutting and 3) the effects of leaf area and IBA concentration on cuttings' mortality rate.

MATERIALS AND METHODS

Plant Material and Study Site: Coppice shoots developing from pruned mother trees were used as cuttings' donors for the experiment. Twelve *X. parviflora* trees that had already fruited at least once were selected in Bangoua, a location situated in the Western Highlands of Cameroon (5°12'N, 10°29'E, altitude 1500 m) and used for the study. These trees were pruned in June 2018 for coppicing, and shoots were allowed to grow for one year before cutting collection. After one year of growth, twenty-five orthotropic coppice shoots each 36 cm in length were cut from each mother plant very early in the morning (between 6 and 6.30 am). Shoots that were collected from the twelve mother trees were mixed in a single batch, enclosed in moistened bags, and transported to 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 on the same day.

Preparation of Cuttings: At the nursery, each shoot was cut into four cuttings. Cuttings were 6.5-7.5 cm long, 0.6-0.8 cm in diameter and comprised 2-4 nodes (depending on the length of internodes) to each of which a secondary axis bearing two rows of leaves was attached. For each cutting, all secondary axis was cut off, except that attached to the upper node, which was trimmed using a pair of scissors, to retain the left leaf area defined for the trial. Leaf area was measured using an AM100 256 scanner brand leaf area meter (Analytical Development Co. Ltd, Hoddesdon, England). Cuttings were disinfected in Metalaxyl-M solution (4 g/L) before hormone application. The hormone was applied by sprinkling the base of the cutting with the respective IBA powder. Hormone powders were commercial formulations supplied by the manufacturer (RHIZOPON® BV, Holland). After treatment of cuttings, their basal 3 cm were vertically inserted into the rooting medium.

Propagation Environment: Rooting of cuttings was conducted in non-mist polythene propagators, which were constructed following the design by Leakey (2014), with fine river sand as the rooting medium. The relative humidity within the propagator ranged from 70 to 80% during the experiment. The propagator was placed in the nursery at 23 ± 4°C with a mean irradiance of 540 µmoL m^{-2} s^{-1} during the day.

Experimental Design: A split-plot experimental design was used. Each main plot contained three leaf areas (0, 15 and 30 cm^2, each measured with a margin of error of approximately 2 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 thus tested. There were three replications each of 20 cuttings per treatment combination, making a total of 1080 cuttings for the trial. Cuttings were planted vertically in the rooting medium (Fig. 1) and were sprinkled at 2-day intervals with water before 7.00 am to avoid desiccation.

Figure 1: Inside view of a non-mist propagator after cuttings' planting

At one-month intervals, cuttings were inspected for evidence of rooting. The experiment ended after five months when there was no further rooting for two consecutive months. At the end of the experiment, each cutting was assessed for mortality, rooting success and roots count.

Data Analysis: IBM SPSS 23.0 software package was used for data analysis. The variables were the percentage of rooted cuttings, the number of roots per rooted cutting and the mortality rate of cuttings. Before 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 (Stroup, 2018). Transformed data were submitted to Analysis of Variance (ANOVA) using the General Linear Model. A comparison of means was done using Duncan's tests ($p \leq 0.05$).

RESULTS

Effects of Leaf Area and IBA Concentration on the Percentage of Rooted Cuttings: Leaf area and IBA concentration had significant ($p < 0.001$) individual effects on the percentage of rooted cuttings. The interaction effect of leaf area and IBA concentration was also significant (Table 1). Untreated (0% IBA) leafless (0 cm^2) cuttings failed to root (0% rooting). With each of the leaf areas investigated, the rooting percentage increased with increasing IBA concentration and reached its highest value, which was 17.4 ± 3.7% with leafless cuttings and 100% with each of the other leaf areas (15 and 30 cm^2). At each IBA concentration except 4%, the percentage of rooted cutting increased with increasing leave area. At 4% IBA, 15 and 30 cm^2 leaf areas recorded a similar percentage of rooted cuttings (100%) which was higher than the 17.4 ± 3.7% recorded with leafless cuttings. Maximum rooting (100%) cuttings were achieved at both 2% and 4% IBA with 30 cm^2 leaf area, whilst with 15 cm^2 leaf areas 100% rooting was achieved only at 4% IBA (Fig. 2).

Table 1: ANOVA's significance and factors affecting each of the variables investigated in *X. parviflora* stem cutting rooting.

Source of variation	**Percentage of rooted cuttings**			**Number of roots/rooted cutting**			**Mortality rate**		
	df	***F***	***p***	***df***	***F***	***p***	***df***	***F***	***p***
LA	2	184.844	< 0.001	2	143.118	< 0.001	2	588.490	< 0.001
AC	5	37.300	< 0.001	5	165.190	< 0.001	5	0.557	0.733
LA x AC	10	4.375	< 0.001	9	43.402	< 0.001	10	1.161	0.312

Footnote: [leaf area (LA), auxin concentration (AC) and their interaction (LA x AC)], degree of freedom (*df*) and the level of significance (*F* and *p* values)

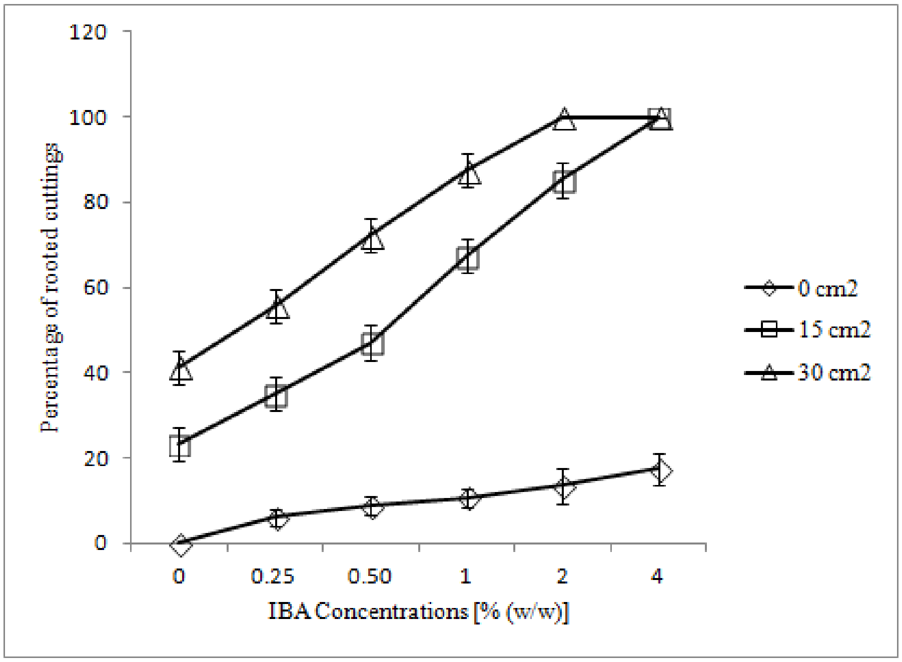

Figure 2: Interaction effect of leaf area and IBA concentration on rooting percentage of *X. parviflora* cuttings at five months after planting

Footnote: [n = 60 cuttings/treatment combination; Error bars = ± S.E.]

Effects of Leaf Area and IBA Concentration on the Number of Roots per Rooted Cutting: Analysis of variance (Table 1) indicated that leaf area, IBA concentration and interaction of both factors had significant effects ($p < 0.001$) on mean roots count. With each of the leaf areas investigated, the mean number of roots per rooted cutting increased with increasing IBA concentration (Fig. 3). The mean number of roots per rooted cutting was lowest with leafless (0 cm^2) cuttings whatever the concentration of IBA. The highest mean number of roots per rooted cutting recorded with 0, 15 and 30 cm^2 leaf areas were 2.5 ± 0.4, 6 ± 0.1 and 9 ± 0.1 respectively, which were different from each other and all of which were achieved with 4% IBA (Fig. 3). Roots were ramified and distributed at the base of cutting (Fig. 4).

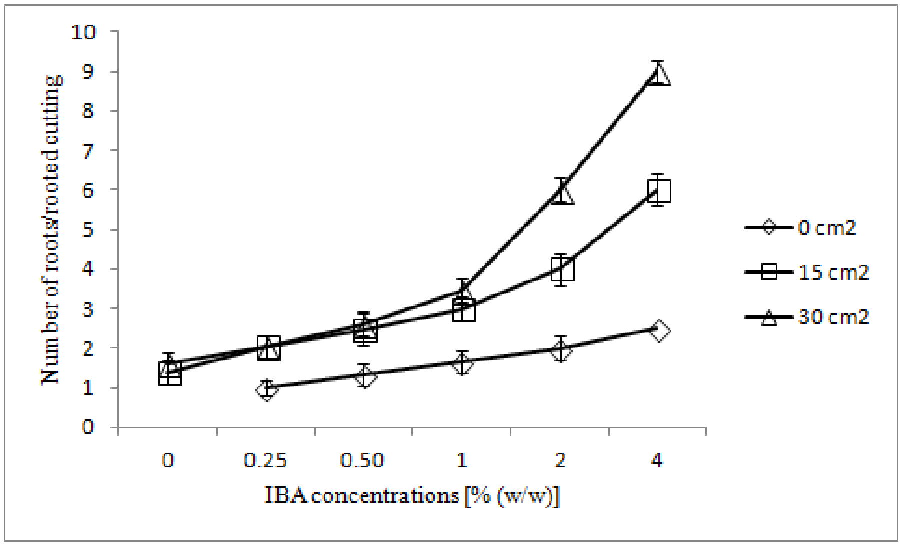

Figure 3: Interaction effect of leaf area and IBA concentration on the mean number of roots per rooted *X. parviflora* cuttings at five months of planting

Footnote: [n = 60 cuttings/treatment combination; Error bars = ± S.E.]

Figure 4: Rooted *X. parviflora* leafy cutting at five months after planting.

Footnote: The cutting had 30 cm^2 leaf area and was treated with 4% IBA

Effects of Leaf Area and IBA Concentration on the Mortality Rate of Cuttings: Results of the ANOVA (Table 1) indicated that neither IBA concentration nor interaction of leaf area and IBA concentration affected the mortality rate. On the contrary, leaf area significantly ($p < 0.001$) affected the cuttings' mortality rate. Leafless cuttings (0 cm^2) resulted in 73.3 ± 1.5% mortality, which was significantly higher than 11.8 ± 1.5 and 10.7 ± 1.5% recorded with 15 and 30 cm^2 leaf areas respectively (Fig. 5).

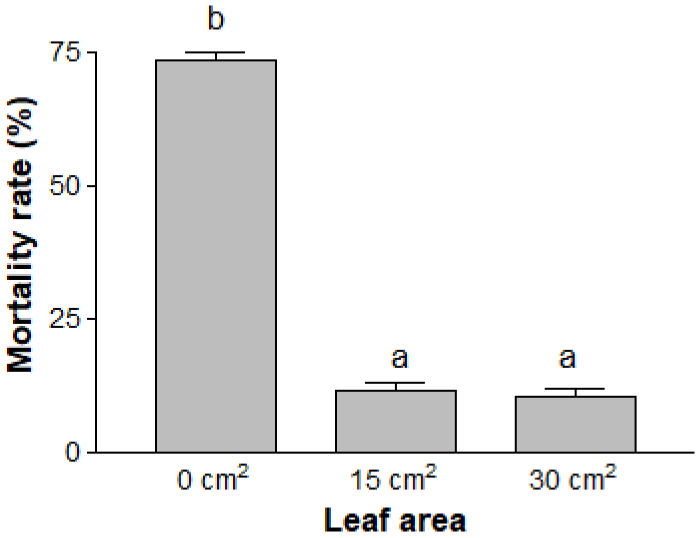

Figure 5: Mortality rate of *X. parviflora* stem cuttings with different leaf areas at five months of planting Footnote: [Bars with different letters are significantly different at $p < 0.05$; n = 360 cuttings/treatment; Error bars = ± S.E.]

DISCUSSION

This study showed that whatever \the concentration of hormone applied to cuttings, increase the leaf area resulted in a decrease in the mortality rate and an increase in the percentage of rooted cuttings. Similar results have been reported with other tree species such as *Lovoa trichilioides* (Tchoundjeu and Leakey, 2001) and *Prunus Africana* (Tchoundjeu *et al*., 2002). The high mortality rate and low rooting ability of leafless cuttings may have resulted from the depletion of carbohydrates in the stem tissues; in contrast, the concentrations of carbohydrates in leafy cuttings tend to increase due to photosynthesis (Leakey *et al*., 1982). This indicated that the survival and rooting of stem cuttings depend on photo-assimilates that were synthesized by the cutting after its excision from the stock plant. Indeed, cuttings have to produce assimilates faster than they are losing them through respiration to survive and root (Leakey and Coutts, 1989). The leaf area also influences rooting by affecting the rate of transpiration, which determines the water status of the cutting (Aminah *et al*., 1997). For an efficient rooting of stem cutting, the leaf attached to the cutting should have an optimal area, which balances the positive effect of photosynthesis and the negative effect of respiration (Leakey, 2004; Tchoundjeu *et al*., 2004). This optimal area varies from one species to another. Within the range of leaf areas tested in the present study, there was neither increase in cuttings' mortality nor a decrease in rooting ability as response to an increase in leaf area. This is an indication that within the ranges of leaf area investigated, water stress was not a major problem. In the present study, increasing IBA concentration resulted in an increase of rooting percentage as well as an increase of the mean roots count. This was consistent with numerous reports, which indicated the role of auxin in enhancing root initiation and cutting of stem (Tchoundjeu *et al*., 2002; Tchoundjeu *et al*., 2004). Indeed, auxins affect cell differentiation and promote starch hydrolysis and the mobilization of sugars and nutrients to the cutting base, all of which enhance root development (Shao *et al*., 2018). Nevertheless, it is well established that there exist between-species differences in the optimal auxin concentration needed for maximum rooting of stem cuttings (Leakey, 2004; Dick and Leakey, 2006; Leakey, 2014). These differences are generally attributed to variations in the amount of endogenous auxin already present in the plant tissues at the moment of severance (Shao *et al*., 2018). This study reported that an IBA concentration of 4% was needed for cuttings with 15 cm^2 leaf area to achieve maximum (100%) rooting, whilst maximum rooting could be achieved at 2% IBA with cutting having a 30 cm^2 leaf area. These results indicated that the hormone concentration needed for inducing maximum rooting of *X. parviflora* cuttings depended on the leaf area. This was consistent with many previous works reporting that in stem cuttings rooting experiment, auxin is interactive with many other rooting factors including leaf area (Atangana *et al*., 2006; Leakey, 2014; Kanmegne *et al*., 2017). The results of the present study clearly indicate that the use of cuttings with 30 cm^2 leaf area and application of 4% (w/w) IBA are recommended for an efficient propagation of *X. parviflora* through stem cuttings.

CONCLUSION AND APPLICATION OF RESULTS

X. parviflora is amenable to vegetative propagation through rooting of stem cuttings. Leaf area, the concentration of exogenously applied IBA and their interaction affect the rooting ability of stem cuttings. Combining 30 cm^2 leaf area and 4% IBA resulted in the overall highest rooting percentage associated with the highest mean number of roots per rooted cutting and lowest mortality rate. This provides evidence for the practical recommendation of this treatment combination for rooting *X. parviflora* stem cuttings. Propagation through rooting of cuttings would stand as a means for rapid multiplication and *in situ* conservation of this important tree species for which sexual propagation requires dangerous chemical product and is hindered by poor seed germination.

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