

**RESEARCH ARTICLE** 

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# Vegetative propagation of *Sclerocarya birrea* (A. Rich.) Hochst. from root segments cuttings: effect of substrate and root diameter

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# ABSTRACT

Sclerocarya birrea (A. Rich.) Hochst. (Anacardiaceae) is one of the most prized multipurpose tree species of the Sudano-sahelian zone of Africa for its food, medicinal and artisanal uses. Unfortunately, it is still exploited from the wild and there has been little or no focused effort to domesticate and cultivate. To contribute to its domestication, a study was conducted on root segments cuttings (RSC) in view to assess the effect of substrate (sand, sawdust, top dark soil, sand/sawdust, top dark soil /sand, top dark soil /sawdust, top dark soil/sand/sawdust) and root diameter (0.5-1.1cm, 1.2-1.7cm, 1.8-3.7cm) on the aptitude to produce adventitious buds and roots. The root system of 17 genotypes was carefully and partially excavated to a depth of 20 cm. RSC of 15 cm long were carefully cut and arranged horizontally in a non-mist propagator, in seven rooting media. The experimental design used was a splitplot with four replications. The main treatment consisted of seven substrates while the secondary treatment was represented by three diameter size classes. For the rate of budding, the effect of substrate was significant (0.020<0.05). Sawdust performed the most with the rate of 28.33%. Concerning the diameter effect, the best class size was 1.8-3.5cm with a rate of 12.14%. The effect of substrate on rooting ranged significant (0.003<0.01). Rooting happened only in sawdust (26.75%) while for diameter, the rate of rooting ranged significantly (0.027< 0.05) from 3.99% in RSC of 0.5-1.1 cm to 23.33 % in those of 1.8-3.7 cm diameter. These results demonstrate that S. birrea presents ability to vegetative propagation. It is possible to regenerate superior trees by root segments cuttings.

Keywords: Cameroon, *Sclerocarya birrea*, Vegetative propagation, Domestication, Rooting ability, Root segments cuttings.

Abbreviations: RSC = root segments cuttings; Guinea savannah Highlands = GSH

# **INTRODUCTION**

Sclerocarya birrea (Synoyme: Spondia birrea A. Rich.; Poupartia birrea (A.Rich) Aubrév.), known in fufulde as «ede», is among the most local plants valued by the populations of the Sudano-sahelian zone of Africa. This plant species presents three sub - species: S. birrea subsp. birrea common in north of the equator, S. birrea subsp. multifoliolata (Engl.) Kokwaro found mainly in Tanzania and S. birrea subsp.caffra (Sond.) Kokwaro abundant in South Africa where it is known locally as « marula » [1].Deciduous tree, it is found in Sahelo-sudanian to Sudanian savannah mainly in sandy soils. Generally, it is found in the ninetieth parallel in the north of West Africa, from Senegal to Cameroon, up to Uganda and Ethiopia [2]. It is 12 m height and 80 cm in diameter with a relatively dense and round canopy. The slice of the bark is fibrous and reddish. It is one of the "Top sixteen species" having priority in the Guinean Savannah Highlands (GSH) of Cameroon [3-6]. Yellow at maturity, fruits are consumed and commercialized. They content an acidulous fibrous pulp which test suitable and contains a stone with 1 to 3 seeds. The pulp can be transformed to fermented win or not. It is rich in divers nutriments among which the most abundant is vitamin C [6-9]. In South Africa, the pulp is used in the manufacture of liquor «Marula» appreciated and

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commercialized in local, regional and international markets [10,11]. The stone of the fruits is consumed as nuts by many tribes in Cameroon (Tupuri, Guiziga, Mundang, etc.) [6]. In Togo, the species is among the fruits trees having priority [12]. In Mali, leaves of the tree are used as fodder [13]. In South Africa, *S.birrea* is among the species retained for agroforestry development and domestication [14]. In Botswana, it is reported that, the tree brings annually an amount of 39 000 FCFA to farmers [15]. In West and Central Africa, fruits, barks and leaves are diversely valued for food, medicine, handicraft, etc. by the local populations [16-18]. All the parts of the species are used in the treatment of various affections: schistosomiasis, snake bite, etc. [2]; diabetis [19,20], malaria, VIH [21], Blood pressure [22], etc.

Despite the importance of the species in rural area, it is still exploited from the wild. In its natural distribution zone, numerous studies have been realized on taxonomy [2], demography [21-25], ethnobotany [18,8] and ecology [26]. However researches on its domestication are rare or inexistent except those of authors who tackled few aspects concerning seed germination, grafting, root suckering and stem cutting of the species[27,28,8,11,28,29]. In fact, these studies should end to the master of technical itinerates leading to the introduction of the species in the existing farmer production systems and then contributed to add value to it.

Getting control of vegetative propagation of *S.birrea* by root segments cuttings can bring a real satisfaction to local populations to stabilize important selected characters. This technique requires very few tools and almost no training for rural people [30-35]. RSC is currently the most suitable tool to propagate elite trees with advantageous phenotypic and genotypic characters [36-38]. Lack of data on factors controlling RSC aptitude to neoform aerial shoots and roots, is a handicap for the promotion of this technique.

Objective of the work is to contribute to the domestication of the species in the prospect to introduce it in farmer existing production systems. Specifically, it is to assess the effects of substrate and root diameter on the ability of RSC to produce leafy shoots and roots.

### MATERIALS AND METHODS

### Study area

Root segments used in the study were sampled in Guinea Savannah Highlands (GSH) of Cameroon precisely in Toumbéré-Boumdjéré. This zone is characterized by a Guinean climate, with two seasons: a rainy season from April to October and a dry season from November to March. For the period 2000-2012, the average annual rainfall was 1447 mm, the average annual temperature 22.3 °C, the average annual relative humidity 67% and the average annual evaporation 1645 mm. The GSH is delimited by two boundaries: the sudanian avannahs in the north and the semi-deciduous guinean vegetation in the south. The area is covered with shrubland and / or woody savannah dominated by *Daniellia oliveri* and *Lophira lanceolata* [39]. The evolution of the vegetation is severely hampered by human impacts [40].

### Preparation and cultivation of root segments cuttings

The experiment was conducted at the nursery of the University of Ngaoundere during 23 weeks, from February 17 to August 25, 2013. Plant material used in the experiment came from superficial roots of 17 adult *S. birrea* trees. These genotypes were selected in the Toumbéré-Boumdjéré upper guinean savannahs (Alt: 1098m; LN: 7°5'002''; LE: 13°66'30''). Preferred characteristics of local populations were considered in the selection of the parent trees: good sanitary condition, large fruits, regular production and low branching. After partial excavation to a depth of 20 cm, the root segments were collected using a pruner, then wrapped in a moistened newsprint paper and transported in a cooler to the nursery. Upon arrival at the nursery, the root segments were cut into 15 cm long root segments cuttings (RSC) and divided into three size classes: 0.5 - 1cm, 1.1 – 1.7cm and 1.8 – 3.7cm. A 1 cm notch was made at the distal end of each RSC [35].

A total of 420 RSC were obtained and arranged horizontally within 3 cm of substrate in a non-mist propagator [41]. The RSC were watered twice a day (morning and evening) using a hand sprayer. Seven types of substrates were tested: sawdust (Sd), sand (S), black soil (Bs), and three 50/50% homogeneous mixtures: black soil-sawdust (Bs-Sd), sand-sawdust (S-Sc) and black soil-sand (Bs-S), and black soil-sawdust-sand (Bs-Sc-S) (33.3/33.3/33.3%). Starting from the end of the eighth week, corresponding to the first appearance of leafy shoots, a systematic inventory was conducted weekly to assess the number of RSC that emit one or more leafy shoots, the number of leafy shoots and the number of leaves per shoot. Regarding rooting, the RSC with one or more leafy shoots were meticulously excavated each week and the presence of new roots noted. Unrooted cuttings were reintroduced into the substrate. A cutting was considered as rooted if the length of the root was greater than 1 cm [42]. Rooted cuttings were inserted horizontally in large polythene bags containing the black soil-sawdust which was found performant by the above authors. These bags were introduced in acclimatization propagators and watered in the mornings and evenings.

During the acclimatization phase, the propagators were left open each night during a month. Watering was then reduced to once a day. After this acclimatization phase, the plants were transferred to the field.

### **Experimental Design and data analysis**

The experimental design was a split-plot design with three replications. The substrate was the main treatment with seven modalities, while the diameter with three classes represented the secondary treatment. The experimental unit consisted of 10 cuttings and the 17 genotypes were not tested separately. The following parameters were determined: a) The rate of budding, the number of leafy shoots per RSC, the height and number of leaves of each leafy shoot. Budding corresponds to bud burst of latent buds that emits one or more leafy shoots on the RSC;

b) The rate of rooting (equated to the success rate of the RSC), the pole on which the leafy shoots have developed (distal or proximal pole), the number and length of the roots. The distal pole corresponds to the extremity of the RSC which was originally located farthest from the base of the parent tree.

Quantitative data were subjected to an analysis of variance (ANOVA) and Post-hoc comparisons were done with the Duncan's Multiple Range Test (DMRT) when significant main effects were detected. All analysis have been performed using the Statgraphics 5.0 software.

### **RESULTS AND DISCUSSION**

# Effect of the substrate and the length of RSC on leafy shoots formation Rate of budding

The first leafy shoots were observed after 4 weeks for cuttings inserted in sand (1.67%) and sawdust (1.67%) substrates. From the fourth to the 23 weeks, the sawdust medium performed the most (28.33%) (Fig.1a). According to Duncan Multiple Range, the budding rate shows four groups of substrates among which Sawdust is the best whereas black soil is the least.

Concerning the root diameter, first leafy shoots were observed in root segment cuttings from 0.5-1cm and 1.1-1.7cm root diameter (Fig. 1b). This result confirms the above time within a month (4 weeks). In the Guinea Savannah Highlands (GSH) of Cameroon, the first buds were observed in *V.doniana* after 8 weeks in RSC of 1.1-2.5cm diameter while 11 weeks for those of 0.5-1cm [42]. In Burkina Faso, a duration of 5 weeks was reported in *Detarium microcarpum*[35] while in *Acacia albida,* 8 weeks were registered [43]. The precocity of root segments cuttings of *S.birrea* could be explained by different factors: ecologic, pedoclimatic conditions of the habitat and genotypes. The latency time of RSC varies according to species.



Figure 1. Effect of the substrate (a) and the RSC diameter (b) on the RSC budding rate during 23 weeks

On the eighth week, the budding rate registered varied from 0 (black soil, black soil -sawdust, black soil-sawdust – sand to 15% (sawdust) (Table 1). There was a significant difference between the substrates (0.0001 < 0.001). In fifteenth week, it varied significantly from 0 (black soil, black soil-sand; black soil-sand-sawdust) to 23.33% (sawdust) (0.0004 < 0.001). This significant differences were maintained until 23<sup>th</sup> week (0.0005 < 0.001). They suggested that the physico-chemical properties of the seven substrates were different. Bud formation in tropical plant species varies with the rooting media. However, performing substrates are porous and light [44]. At the end of the experiment, sawdust presented the best success rate (28.33%) followed by sand-sawdust mixture (13.67%).

SUBSTRATES	8WAP	15WAP	23 WAP	Mean
Sand	5 <sup>a</sup>	8,33ª	10 <sup>ab</sup>	7.78
Sawdust	15 <sup>b</sup>	23,33 <sup>b</sup>	28,33°	22.22
Black soil	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$	0
Sand-Sawdust	5 <sup>a</sup>	8,33ª	13,33 <sup>bc</sup>	8.89
Black soil-Sand	$0^{\mathbf{a}}$	$0^{a}$	$0^{\mathbf{a}}$	0
Black soil -Sawdust	1,66 <sup>a</sup>	3,33ª	3,33 <sup>ab</sup>	2.77
Black soil-Sand-Sawdust	$0^{\mathbf{a}}$	1,66 <sup>a</sup>	5 <sup>ab</sup>	2.22

Table 1. Rate of Budding of RSC in substrates at different times

In column, means followed by different letter are statistically different (P < 0.05). WAP: Week after planting

Concerning the root diameter, the budding rate was observed in the 8<sup>th</sup> week ranged from 2.14% (0.5-1cm) to 5.71% (1.8-3.7cm). This variability is not significant among the diameter class size (0.2310>0.05). In the fifteenth week, the budding rate ranged from 2.14% for cuttings of 0.5-1cm to 10.5% for those of 1.8-3.7cm diameter. There was a significant difference between diameter classes (0.0377<0.05). At the end of the trial (23 <sup>th</sup> week), the most important rate (25.77%) was recorded in the class diameter of 1.8-3.7cm (Tableau 2) despite the absence of significance between the diameter classes (0.0673>0.05). Such situation suggests that at this date, root segment cuttings showed identical physiological characteristics. Nutrient concentrations in RSC do not constitute a limiting factor for the budding [38].

RSC diameter class 1.8-3.7cm has aptitude to form buds. This result can be explained by the high concentration of carbohydrates, minerals and other endogenous hormones reserves of RSC of large diameter. According to Ede *et al.* (1997), Root segments cuttings of large diameter contain important quantity of carbohydrates [45]. The more the reserves, the more their mobilization for the survival of RSC and the rate of success become important [43]. These reserves are useful for the development of buds until the beginning of the leaf photosynthetic activity [38].

Table 2. rate of Budding according root diameter at different times

Time (weeks)	0.5-1.2cm	1.2-1.7cm	1.8-3.7cm
8WAP	2.14a	3.57a	5.71a
15WAP	2.14a	5.71b	10.50c
23WAP	5.57a	18.97a	25.77a
Mean	3.28	9.42	13.99

In lines, means followed by the same letters are statistically similar. WAP: Week after planting.

The rate of budding recorded in *Acacia albida*, in RSC of 1.5-2 cm was 47% [43] while in *Vitex doniana*, it ranged from 21% in RSC of 0.5-1cm to 86% for those of 1.1-2.5cm [42]. A rate of 19% was reported in *Detarium microcarpum* for 2.1-4cm of diameter [15]. In Finland, a rate of 34% for 0.15-0.30cm and 12% for those of 0.61-1cm was recorded in populous[38]. From these various studies, it appears that neoformation of buds varied according species and ecological habitat. However, large diameters appear suitable in many species.

In the 8<sup>th</sup> week (0.5072>0.05) as well as in  $15^{th}$  (0.7782>0.05) or in the  $23^{th}$  (0.7290>0.05) weeks, there was no significant interaction between rooting medium and root diameter. This result indicates that the effect of substrate combined to that of root diameter didn't affect significantly the budding rate of the leafy shoots of the RSC in *S.birrea*. Growth and development of leafy buds lead to leafy shoots.

### Location of the leafy shoots formation

The leafy shoots were developed at different locations on the RSC: 87.57% on the proximal pole, 3.14% on the middle section and 9.29% on the distal pole (Fig. 2). Furthermore, the morphology of the first leaves (large jagged folioles) was different from the mature leaves. Analogous observations were reported in *Vitex doniana* in the Guinea savannah highlands of Cameroon [46].

The maximum percentage of leafy shoots registered in the proximal position suggests that neoformation of buds follows the channel of the distribution of elaborate sap. As reported by others, polarity of the mother root is conserved in RSC [43]. Results obtained in this study corroborate those reported in Burkina Faso (90% for proximal pole) in *Acacia albida, Lannea microcarpa and Detarium macrocarpum*[43,35]. In addition, Stenvall *et al.* (2006) registered a rate of 92% concerning proximal pole in populous hybridus in Finland [38]. These are against those reported on *Vitex doniana* for which majority of leafy shoots were formed in distal pole of the species in Cameroon [42]. The location of the leafy shoots formation is apparently species specific.



Figure 2. RSC showing leafy shoot in proximal (a) and in median poles (b)

# Number of leafy shoots per RSC

Concerning the applied treatments, the effect of substrates as well as that of root diameter was significant at different times. By the end of the experiment (after 23 weeks), the average number of leafy shoots ranged from 0 in black soil, black soil-sand mixture to 1.45 in Sawdust (Table 3). Porosity and lightness of the Sawdust favor the supply of RSC in water and oxygen on the RSC base.

Table 3. Average	able 3. Average number of leafy shoots per RSC			
SUBSTRATES	8WAP	15WAP	23WAP	Μ

SUBSTRATES	8WAP	15WAP	23WAP	Mean
Sand	0.41 <sup>a</sup>	0.5 <sup>a</sup>	1 <sup>bcd</sup>	0.64
Sawdust	1.45 <sup>b</sup>	1.45 <sup>b</sup>	1.58 <sup>d</sup>	1.49
Black soil	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$	0
Sand-Sawdust	0.58 <sup>a</sup>	0.62 <sup>a</sup>	1.16 <sub>cd</sub>	0.79
Black soil-Sand	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$	0
Black soil-Sawdust	0.16 <sup>a</sup>	0.25 <sup>a</sup>	0.25 <sup>ab</sup>	0.22
Black soil-Sand-Sawdust	$0^{\mathrm{a}}$	$0.16^{a}$	0.33 <sup>abc</sup>	0.16

Means followed by different letters are statistically different in column. WAP: Week after planting

There are significant differences between substrates at  $8^{th}$  (0.0003<0.001),  $15^{th}$  (0.0007<0.01) and  $23^{th}$  weeks (0.0021<0.001). The average number of leafy shoots registered is similar to that obtained in *Acacia albida* in Burkina Faso [43].

From the 4 <sup>th</sup> to 23 <sup>th</sup> weeks, class diameter size of 1.8-3.7 cm appeared the most performing (Table 4). The average number of leafy shoots formed at the end of the experiment was 6.75. In *Vitex doniana*, the number of leafy shoots per class diameter recorded was 4.57 in RSC of 1.1-2.5cm class diameter [46]. In Burkina Faso, an average of 2 leafy shoots was obtained in *Acacia albida* [43]. Analysis of variance showed significant differences between class diameters at 8<sup>th</sup> (0.0263>0.05) and 15<sup>th</sup> weeks (0.0007<0.01). In the contrary to these previous times, no significant difference at the 23<sup>th</sup> week (0.114>0.005) as well as for the substrate by root diameter interaction (0.5243>0.05).

Time (Weeks)	0.5-1.2cm	1.2-1.7cm	1.8-3.7cm
8WAP	0.11 <sup>a</sup>	0.28 <sup>a</sup>	0.71 <sup>a</sup>
15WAP	0.12 <sup>a</sup>	0.42 <sup>a</sup>	0.75 <sup>b</sup>
23WAP	2.50 <sup>a</sup>	3.50 <sup>a</sup>	6.75a
Mean	0.91	1.4	2.74

Means followed by different letters are statistically different in column. WAP: Week after planting

### Height of the leafy shoots and number of leaves per leafy shoot

By the end of the experiment, the average height of leafy shoots ranged from 0 cm in the black soil and mixture black soil-sand to 6.59 cm in sawdust (Table 5). This result suggested that sawdust contains nutritive reserves that leafy shoots need for their growth and development. In *Vitex doniana*, the average height of the leafy shoot after 23 weeks is 3.45 cm [42].

SUBSTRATS	8WAP	15WAP	23WAP
Sand	1.04 <sup>ab</sup>	1.33 <sup>e</sup>	2.34 <sup>bcd</sup>
Sawdust	2.66 °	3.10 <sup>d</sup>	6.59 <sup>d</sup>
Black soil	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$
Sand/Sawdust	1.28 <sup>b</sup>	0.79 <sup>ab</sup>	2.92 <sup>cd</sup>
Black soil/Sand	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$	$0^{\mathbf{a}}$
Black soil/Sawdust	0.22 <sup>ab</sup>	0.5 <sup>ab</sup>	0.62 <sup>ab</sup>
Black soil /Sand / Sawdust	$0^{\mathbf{a}}$	0.41 <sup>ab</sup>	0.9 <sup>abc</sup>
Mean	0.74	0.88	1.91

Table 5. Average height (cm) of leafy shoots in substrates at different times

Means followed by different letters are statistically different. WAP –Week after planting

A height of 8 cm after 8 weeks was reported in *Acacia albida* in Burkina Faso [43]. Significant differences existed between substrates at  $8^{th}$  (0.0001<0.001), 15 <sup>th</sup> (0.0000<0.001) and 23 <sup>th</sup> weeks (0.0000<0.001) after planting (Table 5). Substrates are important factors for the growth of the leafy shoots.

Concerning the class diameter, the average height of leafy ranged from 0.6 cm in 0.5-1.1 cm class diameter to 3.79 cm in large diameter (1.8-3.7 cm) at the  $23^{\text{th}}$  week (Table 6). Analysis of variance showed significant differences between class diameter during the experiment:  $8^{\text{th}}$  (0.0087<0.05),  $15^{\text{th}}$  (0.0001<0.001) and  $23^{\text{th}}$  (0.0004<0.001) weeks. The growth of leaf shoots depend on the availability of reserves accumulated in RSC waiting establishment of photosynthetic apparatus. RSC from class diameter size 1.8-3.7cm have nutritive reserves at their disposal favoring rapid growth of adventitious buds [38] (Stenvall *et al.*, 2006). Values obtained were superior to that reported in *Vitex doniana* in the Guinean Savannah highlands of Cameroon [42]. After 24 weeks of planting, authors registered an average height of 0.53cm for RSC of 0.5-1cm class diameter and 1.7cm for those of 1.1-2.5cm class diameter.

TIME (Weeks)	0.5-1.1cm	1.2-1.7cm	1.8-3.7cm
8WAP	0.15 <sup>a</sup>	0.65 <sup>a</sup>	1.39 <sup>b</sup>
15WAP	0.18 <sup>a</sup>	0.68 <sup>a</sup>	1.79 <sup>ь</sup>
23WAP	$0.60^{a}$	1.23 <sup>a</sup>	3.79 <sup>b</sup>
Mean	0.31	0.85	2.33

In line, means followed by different letters are statistically different. WAP: Week after planting

By the end of the experiment  $(23^{th} \text{ week})$ , the substrate by RSC diameter interaction was significant (0.02 < 0.05) on the height of the leafy shoots. This result indicated that the combined effect of substrate and root diameter had an impact on the growth of the leafy shoots of *S. birrea*.

For the average number of leaves per leafy shoots, it ranged from 0 in black soil and black soil-sand mixture to 9.9 for those from RSC in sawdust at the  $23^{th}$  week (Table 7). The general mean height is 3.33. There are significant differences at different dates:  $8^{th}$  (0.0001<0.001),  $15^{th}$  (0.0001<0.001) and  $23^{th}$  (0.0022<0.001) weeks. Sawdust appeared to be the powerful substrate according to Duncan Multiple Range Test classification and black soil, sand the least. Potentialities of the sawdust will be due to the following characteristics: porosity, lightness, richness in nutritious elements easily to move.

Table 7. Mean	number	of leaves	leafy	shoot
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Substrats	8WAP	15WAP	23WAP	Mean
Sand	1,08 <sup>ab</sup>	1,91°	3,83 <sup>ab</sup>	2.27
Sawdust	2,66 <sup>c</sup>	3,90 <sup>d</sup>	9,91°	5.49
Black soil	$0.00^{a}$	$0.00^{a}$	$0.00^{a}$	0.00
Sand-Sawdust	1,33 <sup>b</sup>	1,40 <sup>ab</sup>	6,66 <sup>bc</sup>	3.13
Black soil-Sand	$0.00^{a}$	$0.00^{a}$	$0.00^{a}$	0.00
Black soil-Sawdust	0,25 <sup>ab</sup>	0,66 <sup>ab</sup>	1,25 <sup>ab</sup>	0.72
Black soil - Sand – Sawdust	$0^{\mathbf{a}}$	0,5 <sup>ab</sup>	1,66 <sup>a</sup>	0.72

In Column Means followed by different letters are statistically different. WAP – Week after planting

At the end of the trial, the average number of leaves per leafy shoot ranged from 0.89 in RSC of 0.5-1.1cm to 7.14 in those of 1.8-3.7cm diameter (Table 8). This variation was important since the analysis of variance showed significant differences between class diameters along the experiment: 8th (0.0058<0.05), 15 <sup>th</sup> (0.0001<0.05) and  $23^{th}$  (0.0011<0.001) weeks.

Growth of leafy shoots was affected by the diameter size of the RSC. This could be explained by the fact that large RSC contain important carbohydrate reserves to supply adventitious leafy buds, favoring their development.

TIME (Weeks)	0.5-1.1cm	1.2-1.7cm	1.8-3.7cm
8WAP	0.19 <sup>a</sup>	0.62 <sup>a</sup>	1.46 <sup>b</sup>
15WAP	0.23 <sup>a</sup>	0.81 <sup>a</sup>	2.54 <sup>b</sup>
23WAP	0.89 <sup>a</sup>	1.96 <sup>a</sup>	7.14 <sup>b</sup>
Mean	0.46	1.13	3.72

Table 8. Average number leaves/leafy shoot according root class diameter

Means followed by different letters are statistically different. WAP –Week after planting

The interaction substrate-RSC diameter was not significant on the number of leaves per leafy shoot (0.229>0.05). This behavior indicates absence of synergy between substrate and root diameter. However between the height of leafy shoots and number of leaves, there is positive and significant correlation (r = 0.98; 0.0000< 0.001). This correlation suggests that the more the height of leafy shoot, the more the leafy shoot hold leaves. This relation can be translated by the following mathematical regression model: number of leaves = 0.03(height of the leafy shoot) +3.25

# Effect of the substrate and the length of RSC on roots formation Neoformation of roots

The rate of rooting at the end of the experiment was 26.75% in the sawdust. The analysis of variance showed a significant difference among the substrates (0.020<0.05). For the RSC diameter, the rate of rooting ranged significantly (0.027< 0.05) from 3.99% in RSC of 0.5-1.1 cm to 23.33% in those of 1.8-3.7 cm diameter. In *Vitex doniana*, a rooting rate of 76.7% was reported in sand and 59.33  $\pm$  4.67% on RSC of 1.1-2.5 cm [42]. The poor rate of rooting of *Sclerocarya birrea* could be due to the cuttings' age. To optimize the rate of rooting rate registered in Benin was 10% [28]. The rate of rooting of *S. birrea* has been improved in the current experiment. The latency time for the initiation of new adventitious roots in this Anacardiaceae is 13 weeks after planting. Leafy shoots appeared in the 4<sup>th</sup> weeks after planting, enhanced the rooting process. Carbohydrates supplied by the leaves favor the roots development of RSC [38].

At the end of the experiment (week 23), the average number of roots per RSC varied from  $1.33 \pm 1.41$  in the black soil-sand mixture to 6.17 in the sawdust. There was however no significant difference between substrates (0.058 > 0.05).

The diameter of the RSC had a significant effect on the number of roots formed (0.0006 <0.001) and the maximum number of roots ( $6.46 \pm 1.46$ ) was recorded in RSC of 1.8-3.7 cm diameter (Table 9). The average length of roots was 2.79 cm. This is low compared with that obtained in *Vitex doniana* after 28 weeks (30.16 cm) [46].

### Tableau 9. Growth characteristics of roots according root class diameter.8.91

TIME (Weeks)	0.5-1.1cm	1.2-1.7cm	1.8-3.7cm	Mean
Rooting rate (%)	3.99	18.94	23.33	15.42
Average number of roots	1.12	2.87	6.46	3.48
Average length of root (cm)	0.74	4.27	3.36	2.79

Means followed by different letters are statistically different. WAP -Week after planting

Adventitious roots of *S. birrea* were formed at the distal pole of the RSC (Fig.3). Similar results were reported in *Acacia albida* in Burkina Faso [43].



Figure 3. Rooted RSC presenting leafy shoot

Substrate quality is an important parameter for the success of the RSC rooting. Exigencies of different species in connection with rooting media depend on their hydromorphic or exomorphic characters [47,44]. The best substrate in this experiment was sawdust characterized by good water capacity retention facilitating both water and oxygen circulations which are essential conditions for the rooting of cuttings. The ability of a plant to propagate through RSC is regulated by several endogenous and exogenous factors. It is at maximum when the optimal combination of these factors is achieved. The formation of adventitious roots is more important than the development of aerial shoots, as the later could fade after a few days or weeks, if there is no newly formed root to feed the leafy shoots.

In line with our results, a rooting rate of 8% was reported in *Detarium microcarpum's* cuttings of 1.1-2 cm diameter [35] and 19% for cuttings of 2.1-4 cm diameter. Similarly, for *Faidherbia albida*, a rooting rate of 0% for 0.5 cm cuttings, and 30% and 47% for those of 1 cm and 1.5-2 cm cuttings was registered respectively [43].

In our experiment, the average percentage (15.66%) of RSC that formed roots was high compared with 10.0% obtained in the same species in Benin [28]. This result is low compared to those obtained in other species: hybrid poplar [38], *Vitex doniana* [46]. But among all, the experimental conditions were very different (diameter classes, soil temperature, species, season, genotypes, etc.). Unfortunately, there are very few trials with RSC in Africa to effectively compare these results (but see [43,35,48,46].

The diameter of the RSC influenced significantly the formation of leafy shoots as well as roots. Overall, the success rate in our experiment was improved for larger diameters and this was particularly true for the number of leafy shoots, as well as their height and the number of leaves formed.

Regarding the substrates, significant difference was found in this experiment regarding leafy shoots and roots formation of the RSC. These observations are consistent with results for other species: *Vitex doniana* in Benin [45] and *Brosimum gaudichaudii* in Brazil [47].

The RSC diameter determines the amount of nutrients and the availability of carbohydrates in the cutting. It is for this reason that the RSC were harvested shortly before the end of the dry season. Some authors argue that the carbohydrate content is high in the roots during the rainy season [47], but these reserves are transported to the collar or the surface roots at the beginning of periods of stress [48].

### CONCLUSION

The results of this study illustrate for the first time that *S.birrea* is amenable to vegetative propagation, opening up opportunities for domestication and the development of cultivars with desirable traits for fruits to supply the brewery industry. This opens a new window of opportunity both for researchers and farmers in future genetic improvement research and large-scale cultivation of *S.birrea* using vegetative propagation techniques such as RSC harvested in February (i.e. near the end of the dry season). Rooting media and diameter of the RSC are important factors that influence the formation of leafy shoots and adventitious roots. The lower rate of budding and rooting obtained, compared to those of other works suggest the necessity to develop supplementary research in order to optimize RSC budding and rooting of *S.birrea*.

For future work, it is envisaged to assess the effects of (i) the harvest season of the RSC (beginning of the dry season vs. end of the dry season), (ii) the length of the cuttings, (iii) the horizontal vs. vertical positioning of RSC in the substrate and (iv) the effect of origin of RSC on the ability to form new leafy shoots and a dense root system. Possibly, tests with growth substances could be undertaken to improve the success rate, but this will lead away from the main objective of this research, namely the development of a low cost vegetative propagation technic.

Likewise, it will be judicious to analyze the survival of the RSC after two or three years from planting and observe the architecture of the root system to assess whether the plants have developed taproots and oblique roots.

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