Discoveries in Agriculture and Food Sciences - Vol. 12, No. 06
Publication Date: December 25, 2024
DOI:10.14738/tnc.1206.17839.
De Dieu, W. J., Yougouda, H., Guidawa, F., Jean-Baptiste, B., Rodrigue, M. A. D., Hervé, A. E. J., Parfait, W. S. D., Zéphirin, O. H., Herbert, A., & Marie, M. P. (2024). Influence of Substrate and Length on The Ability of Root Segments Cuttings of *Amblygonocarpus andongensis* (Welw. ex Oliv.) Exell & Torre to Regenerate. Discoveries in Agriculture and Food Sciences, 12(06). 56-71.

Influence of Substrate and Length on The Ability of Root Segments Cuttings of *Amblygonocarpus andongensis* (Welw. ex Oliv.) Exell & Torre to Regenerate

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ABSTRACT

Background: The Guinean Savanah Highlands of Adamawa is replete with multipurpose tree species, among which *Ambligonocarpus andongensis* is particularly noteworthy. This species is widely known and appreciated by the local population. Despite its importance, it remains in the wild and is subjected to overexploitation. The objective of the present study is to evaluate the effect of substrate and length of cuttings on the budding and rooting capacity of this species. Methods/The experimental design was a split-plot with three replications. The main treatment comprised three substrates (sand/sawdust, black soil/sawdust, black soil), while the sub-treatments were represented by three lengths of root segments cuttings (RSC) (10,15,20cm). The experimental unit consisted of 10 cuttings. Following 37 weeks of monitoring, the budding rates demonstrated fluctuations, with the rates of 12.22 ± 10.92% observed in black soil and 32.22 ± 18.55% in sand/sawdust mixture. The analysis of variance indicated a significant difference between the substrate (0,005 < 0.01). For the length of cuttings, the budding rates exhibited significant fluctuations (0.001< 0.01), with the lowest rate observed for cutting of 10 cm (11.11 ± 10.86%), and the highest observed for those of 20 cm (42.22 ± 25.87%). The height of the leafy shoots revealed variations, with a mean value of 3.38 ± 3.09 cm in the black soil and a maximum value of 9.2 ± 7.10 cm in the black soil/sawdust substrate. The analysis of variance indicated a significant difference (0.03 > 0.05). The rooting rate varied significantly from 1.11±0.96% in the black soil to 12.22±12.01% in the sand/sawdust mixture (0.005 < 0.01) and for the length it also significantly fluctuated from 2.22±1.96% for cuttings of 10 cm to 15.55±13.33% for those of 20 cm (0.001 < 0.01). All these informations are important to develop scales and strategies toward the domestication of this species.

Keywords: *Amblygonocarpus andongensis,* Bud*,* Guinea savannah highlands, RSC, Root, vegetative propagation.

INTRODUCTION

Amblygonocarpus andongensis, known as yaké in Fulani, belongs to the Fabaceae family, is a widespread species in Tropical Africa. The wood is durable and termite-resistant, charcoal made from it is considered excellent for iron-forge work [1]. Roasted seeds are eaten. Boiled and fermented seeds are used as a spice locally known as ''Dadawa'' [2]. The inner bark and roots are poisonous [3], but medicinal uses have been recorded in them. Nwinyi *et al*. (2006) [4] reported that *A. andongensis* is ethnomedicinally used in Northern Nigeria for the relief of pain. According to [5], a decoction prepared from roots is used to treat food poisoning and against colic, cough and as a vermifuge. Anthropic pressures are causing the rapid spread of many forest species, resulting in the depletion of timber and non-timber forest products [6]. It is therefore essential to preserve local species of socio-economic interest not only in forest reserves but even more in agropastoral systems close to users [7]. To keep off pressure on useful natural resources, domestication and promotion of Agroforestry have been practiced by local communities as an immediate and affordable option in developing countries. Sexual reproduction is the main technique used for the propagation of timber, and the richness of the biodiversity [8,7]. Germination conditions and development of trees are very poor in dry tropical Savannah [9,10]. Subsequently, seed multiplication remains uncertain for many rural communities in Africa that do not have sufficient seed resources or financial means and sometimes because of ants, birds and animal attacks [7]. Vegetative propagation, which is faster and less costly [11], appears to be an adaptive strategy of these species to environmental disturbances and climatic contingencies [12,13], by capturing germplasm from the wild for purposes of its domestication, conservation and continuous availability to the community. It copies parental genes and has the following advantages: rapid plant production, precocious fructification and reduction of the size of individuals [14,7 ,15]. The main objective of this work is to contribute to the domestication of this species in order to protect and introduce them in existing farmer's production systems. Specifically

- evaluate the effect of substrate on the budding and rooting ability;
- assess the effect of cutting length on the budding and rooting ability

MATERIELS AND METHOD

Study Site Description

The study was conducted in the Guinean savannah highlands of Adamawa, situated between 6° and 8° North latitude and 11° 30' and 15° 30' East longitude. The zone is demarcated by two boundaries. The northern boundary is formed by the Sudanese savannah, while the southern boundary is demarcated by the semi-deciduous guinean forest. The area is characterised by shrubland and/or tree savannah, with a dominant presence of *Daniellia oliveri* and *Lophira lanceolata* [16]. The evolution of the vegetation is significantly influenced by human activities [17]. The region is characterised by a guinean climate, comprising two distinct seasons: a rainy season from April to October and a dry season from November to March (MINEF, 1994) [18]. The area is home to numerous ethnic groups, including the Fulani, Mboums, Pères, Koutines, Haoussas, Niza'as, and Dourous [18]. The soil is characterized by a red ferritic structure developed on old basalt [19].

Description of the Nursery and The Non-Mist Propagator

The cutting trials were conducted in a non-mist propagator situated at the nursery of the Laboratory of Ecology and Sustainable Development of the University of Ngaoundere, located in close proximity to the Bini River. The non-mist propagators are situated beneath a sheet shed, which provides shade. Six transparent sheet sheds are positioned within the roof to filter the light. Each non-mist propagator is constructed from local materials and is shaped like a parallelepiped, subdivided into three compartments. The frame is made of wood and covered with transparent polyethylene, which ensures favourable conditions for the development of cuttings. The relative humidity in the non-mist propagators is between 80 and 100%, while the temperature varies from 23 to 28 °C [20]. The internal configuration of the non-mist propagators comprises the following layers, from the bottom to the top : a thin layer of fine sand, large pebbles, medium pebbles, gravel, sand and finally rooting substrates [2]. A PVC pipe is fixed to the corner of the non-mist propagators to facilitate regular gauging of the water level.

Methodology

The preliminary phase of the study involved the partial and safety excavation of the root systems of 15 adult trees in the first hour of the morning, a period when the cells are still turgid.

The excavation process was conducted in Nyambaka. Following the excavation process, the cuttings segments were placed in a cooler containing an ice block in order to reduce the risk of deshydration [21,20]. Upon arrival at the nursery of the Laboratory of Ecology and Sustainable Development of the University of Ngaoundere, the cuttings segments were trimmed to three distinct lengths: 10, 15, and 20 cm (Fig. 1) and marked at the proximal end, then inserted vertically in the non-mist propagator. The different substrates had already been prepared and were of three kinds (sand/sawdust, black soil/sawdust and black soil).

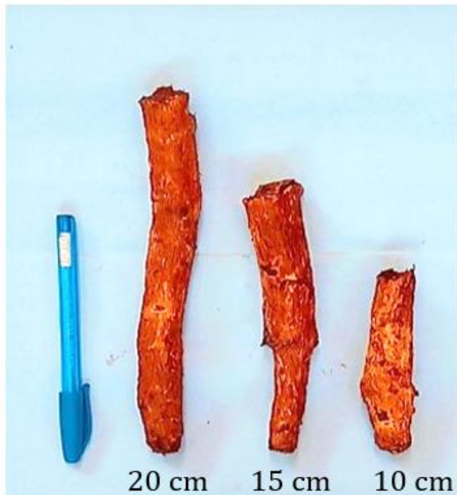


Figure 1: different length of cuttings

For the mixture of sand and sawdust and the mixture of black soil and sawdust, the substrates were prepared in a 50:50%. Approximately 1 cm of the proximal end of the cuttings was left exposed beyond the substrate. The sawdust was sourced from a local sawmill and allowed to decompose. The black soil was obtained from the nursery, while the sand was collected from the river in the vicinity of Ndom, in close proximity to the cliffs of Ngaoundere. The experimental design was a split plot of three replications, with the primary treatment being the substrate and the secondary treatment, the length of root segments cuttings. The experimental unit was composed of 10 cuttings.

Collection and Data Analysis

Data were collected on a weekly basis in order to monitor the budding process (from the date of appearance of the first bud) and at the end of the experiment in order to assess the rooting success. The data set comprised the following variables: the number of cuttings that exhibited budding, the number of leafy axes, the height of the leafy axes, the number of leaves per shoot, the number of rooted cuttings, the number of roots per cutting, and the length of adventitious roots. Furthermore, the aforementioned growth parameters were evaluated at the end of the experimental period. A root segment cutting (RSC) is deemed to have successfully rooted if the length of the root is equal to or greater than 1 cm. In the event that this criterion is not met, the cutting is returned to the substrate [22]. The rooted cuttings were transferred to pots for acclimatitation. The data collected were subjected to an analysis of variance. Significant means were separated using the Duncan multiple range test. The statistical programme used for the analysis of variance was Statgraphics Plus 5.0. Excel was used to generate graphs.

RESULTS

Budding Rate

The sprouting time for this species is eight weeks, which is the period between the placement of the cutting into cultivation and the initial appearance of the bud (Fig.2).

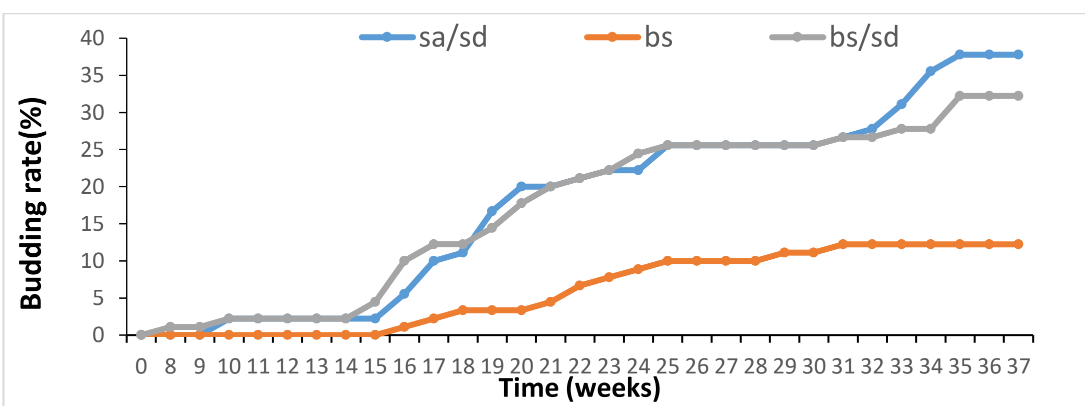
Figure 2: Budded cuttings

Effect of Substrate:

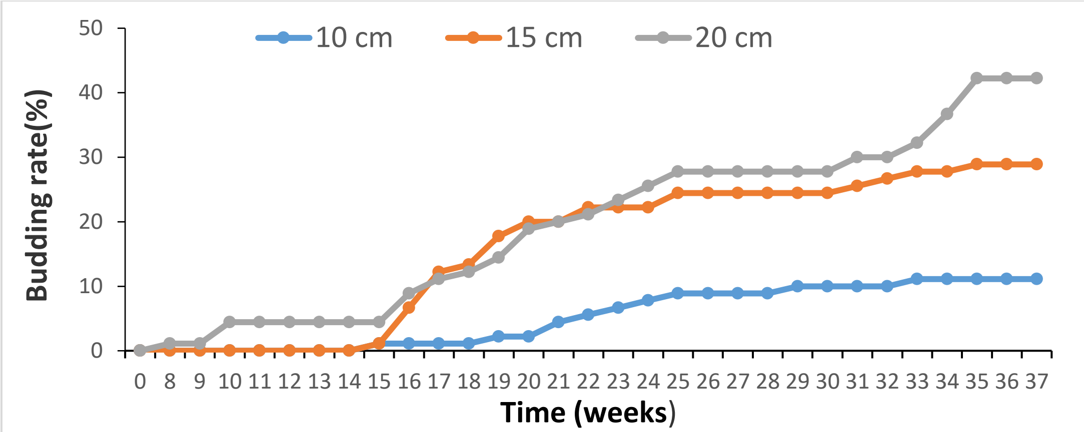
Following 37 weeks of monitoring, the budding rates for *Amblygonocarpus andongensis* demonstrated fluctuations, with the rates of 12.22 ± 10.92% observed in black soil and 37.77 ± 18.55% in sand/sawdust mixture (Fig. 3a). The analysis of variance indicated a significant difference between substrates (0.005 < 0.01).

Effect of Length

For the effect of the length of RSC the budding rates exhibited also fluctuations (0.001< 0.01), with the lowest rate observed for cuttings of 10 cm (11.11 ± 10.86%) and the highest observed for those of 20 cm (42.22 ± 25.87%) (Fig.3b).



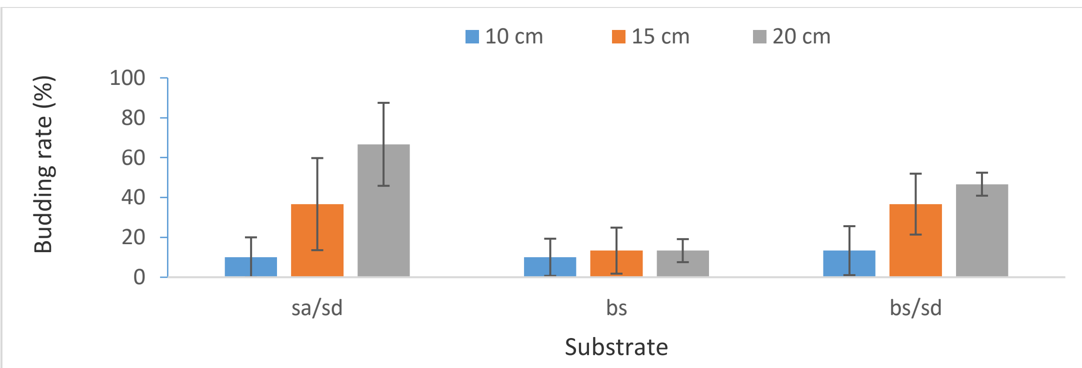
a = effect of substrate



b=effect of length of RSC, Sa=sand, sd= sawdust, bs= blacksoil
Figure 3:Budding rate

Effect of the Interaction Substrate*Length

The interaction substrate*length of RSC demonstrated a non-significant effect with variability observed in the response. The budding varied from 10 ±10% for cuttings of 10cm placed in the mixture of sand/sawdust substrate to 66.66 ± 20.81% for those of 20cm inserted in the mixture of sand/sawdust substrate (Fig. 4).



Sa=sand, sd= sawdust, bs= blacksoil
Figure 4:Budding rate following the interaction substrate*length of RSC

Growth Parameters

Effect of Substrate

The number of leafy shoots exhibited fluctuation (0.10 > 0.05) across different substrate types, ranging from 2.88 ± 2.14 in black soil (bs) to 9.55 ± 8.06 in sand/sawdust (Table 1). The height of the leafy shoots exhibited fluctuations, with a mean value of 3.38 ± 3.09 in the black soil and a maximum value of 9.2 ± 7.10 in the black soil/sawdust substrate (Table 1). The analysis of variance indicated a significant difference between substrates (0.03 > 0.05). The height of shoots varied from 3.38± 3.09 cm in black soil to 9.2± 7.10cm in the mixture black soil /sawdust. Analisis of variance demonstrated a significant difference among the substrates (0.03<0.05).

The number of leaves exhibited variations, with a mean of 2.33 ± 2.06 in the black soil and a mean of 6.11 ± 5.58 in the black soil/sawdust substrate (Table 1). The analysis of variance did not indicate a significant difference (0.07 > 0.05).

Table 1 : effect of substrates on growht parameters

Substrates	Number of shoots	Height of shoots (cm)	Number of leaves
Sand/Sawdust	9.55 ± 8.06a	7.03 ± 3.66ab	5.58 ± 4.48a
Black soil	2.88 ± 3.14a	3.38 ± 3.09a	2.33 ± 2.06a
Black/Sawdust	8.55 ± 8.40a	9.2 ± 7.10b	6.11 ± 5.58a
Probability	0.10	0.03	0.07

Means followed by the same letter are statically identical at the 5% probability threshold

Effect of Length

The number of leafy shoots showed significant fluctuation (0.01 < 0.05), ranging from 1.55 ± 1.46 for cuttings of 10 cm to 12 ± 8.52 for those of 20 cm (Table 2). The height of the leafy shoot exhibited considerable variation for *Amblygonocarpus andongensis*, with values ranging from 3.93 ± 3.67cm for cuttings of 10 cm to 10.17 ± 6.14cm for those of 20 cm (Table 2). The analysis of variance indicated a significant difference (0.02 < 0.05). Concerning the height of shoots, it fluctuates cuttings of 10 cm (3.93 ± 3.67 cm) and those of 20 cm (10.17 ± 6.14) (0.02<0.05). The number of leaves ranged from 1.55 ± 1.48 for cuttings of 10 cm to 7.88 ± 4.59 for those of 20 cm (Table 2). The analysis of variance did not indicate a statistically significant difference (0.006 < 0.01). It was observed that the number of leaves was relatively higher for longer cuttings.

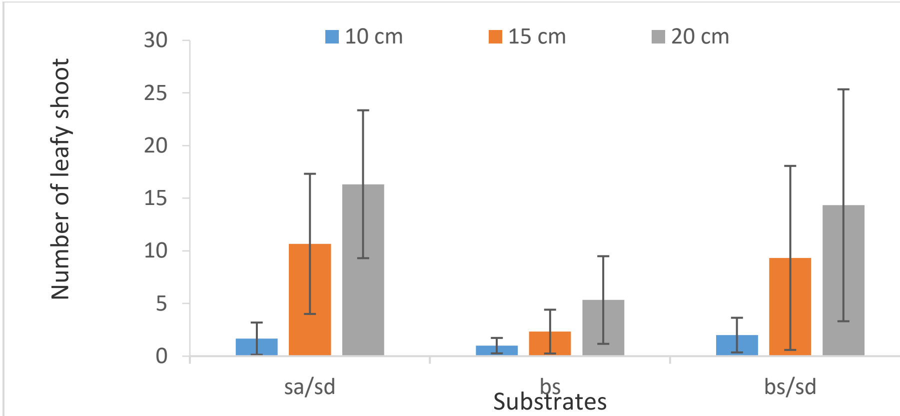
Table 2 : effect of length on growth parameters

Length (cm)	Number of shoot	Height of shoot (cm)	Number of leaves
10	1.55 ± 1.46a	3.93 ± 3.67a	1.55 ± 1.48a
15	7.44 ± 7.32ab	5.51 ± 2.54a	4.88 ± 4.53ab
20	12 ± 8.52b	10.17 ± 6.14b	7.88 ± 4.59b
Probability	0.01	0.02	0.006

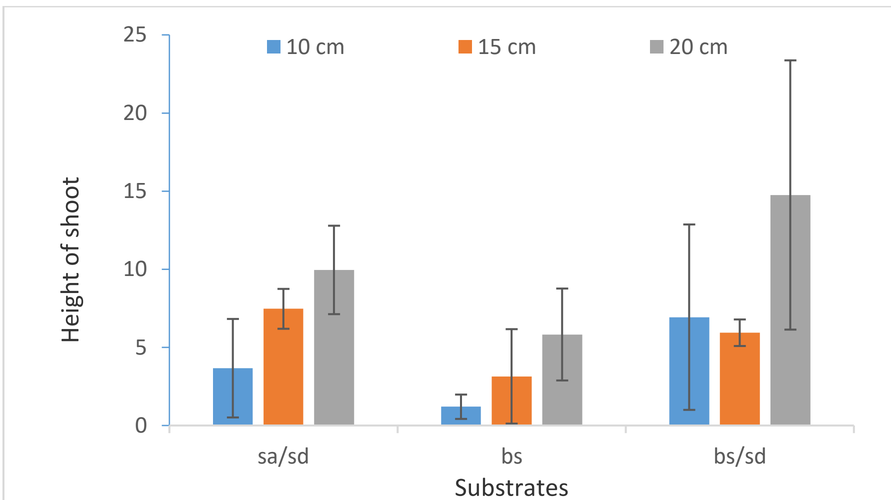
Means followed by the same letter are statically identical at the 5% probability threshold

Effect of Substrate*Length Interaction:

The number of leafy shoots exhibited considerable variations, with values ranging from 1 ± 0.6 for the 10 cm cuttings planted in black soil to 14.33 ± 11.01 for those of 20cm cutting growing in a mixture black soil / sawdust (Fig. 5a). The analysis of variance did not indicate a significant variation (0.72 > 0.05). The height of leafy shoots demonstrated a considerable range, from a mean of 1.2 ±0.77 cm for cuttings of 10 cm inserted in black soil to a peak mean of 14.75 ±8.61 for those of 20 cm cultured in the mixture black soil/sawdust (Fig .5b). However, the analysis of variance indicated no statistically significant variation (0.70 > 0.05). The number of leaves observed exhibited considerable variation. Those of the 10cm cuttings, inserted in black soil, averaged 1 ± 0.73, while those of the 20cm cuttings in sand/sawdust mixture displayed a higher average of 10.33 ± 3.51(Fig. 5c). The analysis of variance did not indicate a significant variation (0.69 > 0.05).



a



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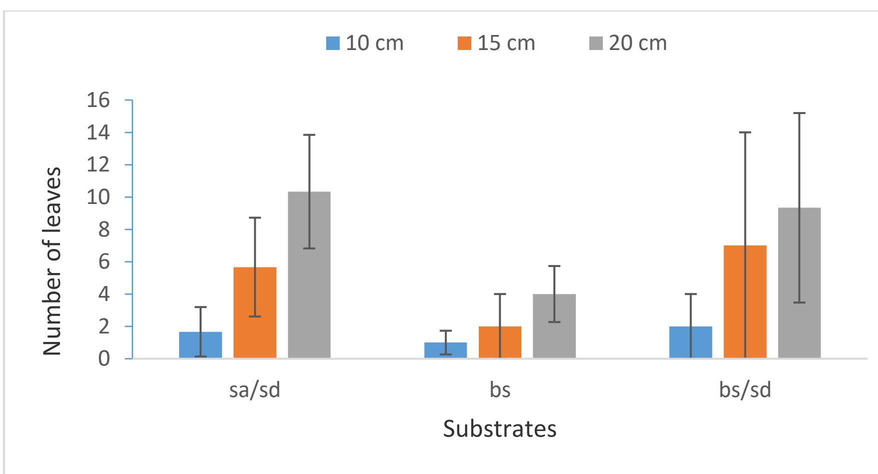


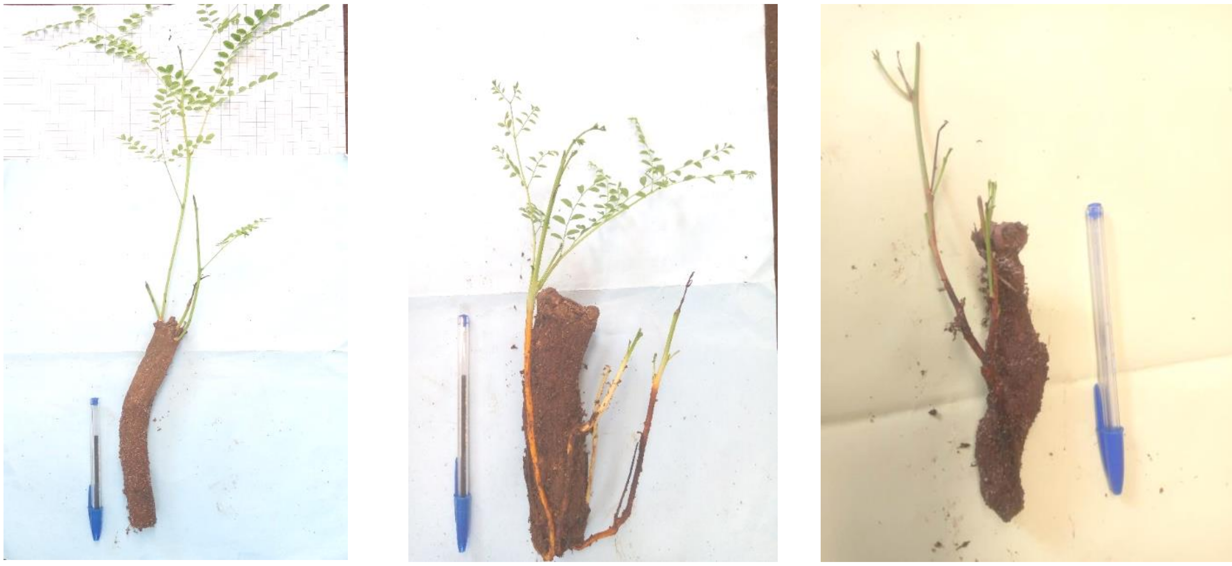
c

Sa=sand; sd= sawdust; bs= blacksoil; a= Number of shoots; b=Height of shoots; c=Numbers of leaves

Figure 5: interaction substrate*length on growth parameters

Shoots Polarity:

Following 37-week survey, it was observed that the majority of the shoots (93.94%) emerged from the proximal end, with a smaller proportion (4.05%) emerging from the distal end and a further 1.35% from the median and proximo-median end (Fig.6), of the root segment cuttings.



a **b** **c**

a=proximal, b=distal, c=median

Figure 6: Shoot positions on cuttings

Rooting Rate

Effect of Substrate:

The processes of budding and rooting are separated in time and joint-on space, occurring at different stages of the cultivation cycle. Upon completion of each trial, the rooting capacity was evaluated. The rooting rate exhibited significant fluctuations across the different substrates, with values ranging from 1.11 ± 0.96% in the black soil to 12.22 ± 11.84% in the sand/sawdust substrate (0.005 < 0.01).

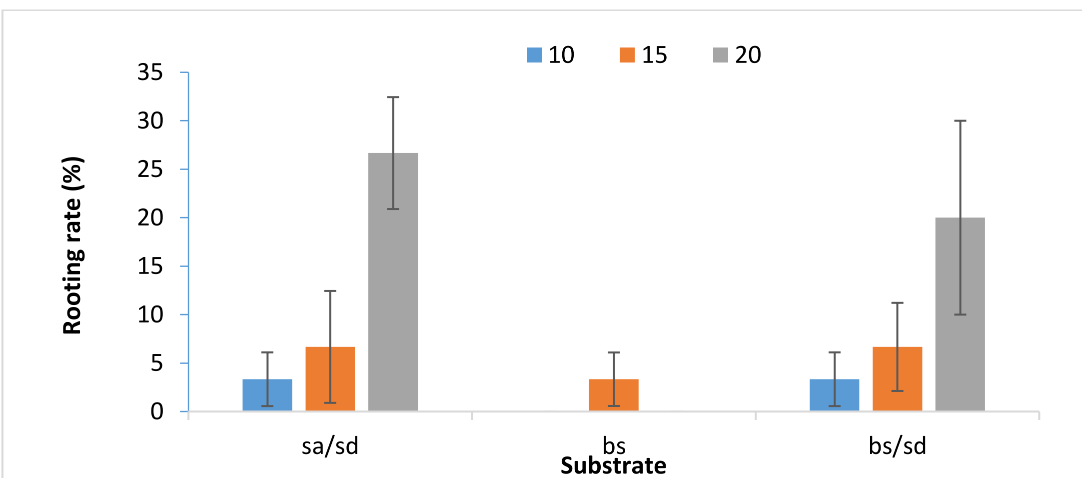
Effect of Length of Root Segments Cuttings

With regard to the length of root segments cuttings (RSC), the rooting rate was found to vary significantly from 2.22 ± 1.67% for cuttings of 10 cm to 15.55 ± 13.33% for those of 20 cm. The analysis of variance demonstrated a statistically significant difference (0.03 < 0.05).

Figure 7 : rooted cuttings

Effect of Substrate*Length Interaction:

The rooting rate manifested considerable variation, with values ranging from 0 ± 0% for the 10 and 20 cm cuttings inserted in the black soil to 26.66 ± 5.77% for the 20 cm cuttings grown in the black soil/sawdust mixture (Fig.8). The analysis of variance revealed a statistically significant difference (0.03 < 0.05).



Sa=sand, sd= sawdust, bs= blacksoil

Figure 8 : Rooting percentage according the interaction substrate*length

Rooting Parameters

Effect of Substrate:

The number of roots showed considerable variation, with values ranging from 1 ± 0.6 for cuttings planted in black soil to 2.77 ± 1.79 (Table 3) for those cultivated in a mixture of black soil and sawdust. The analysis of variance did not indicate a significant variation (0.07 > 0.05).

The length of roots observed exhibited considerable variation, with values starting from 0.31 ± 0.16 cm for cuttings planted in black soil to 2.89 ± 2.48 cm (Table 3) for those cultivated in sand and sawdust mixture. The analysis of variance revealed a statistically significant variation (0.03< 0.05).

Tableau 3: effect of substrate on rooting parameters

Substrates	Number of roots	Length of roots (cm)
Sand /Sawdust	2.55 ± 2.01a	2.89 ± 2.48b
Black soil	0.33 ± 0.11a	0.31 ± 0.16a
Black soil /Sawdust	2.77 ± 1.79a	1.84± 1.70b
Probability	0.07	0.03

Means followed by the same letter are statically identical at the 5% probability threshold

Effect of Length:

The number of roots observed in *A. andongensis,* exhibited considerable variation, with values fluctuating from 0.22 ± 0.10 for the 10 cm cuttings to 2.77 ± 1.79 for those 20 cm (Table 4). The analysis of variance revealed a statistically significant difference (0.03<0.05).

The length of roots oscillated from 0.83 ± 0.67 for RSC of 10cm to 2.42 ± 2.21 for those of 20 cm (Table 4). The analysis of variance did not indicate a significant variation (0.22 > 0.05)

Tableau 4: effect of substrate on rooting parameters

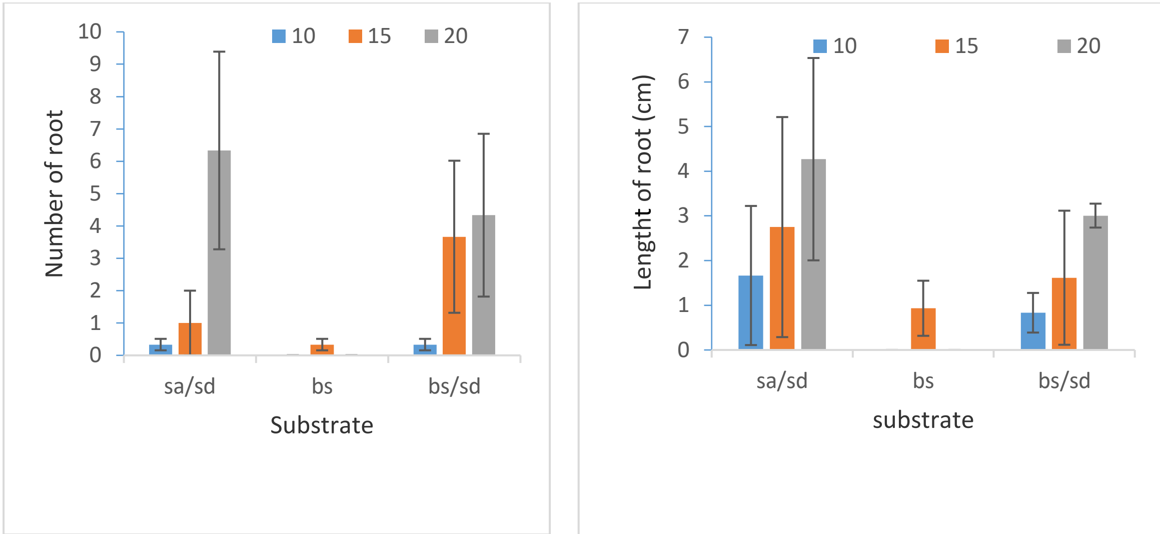
Length of roots (cm)	Number of root	Length of roots (cm)
10	0.22 ± 0.10a	0.83 ± 0.67a
15	1.66 ± 1.32a	1.76 ± 1.54a
20	3.55± 3.43b	2.42 ± 2.21a
Probability	0.03	0.22

Means followed by the same letter are statically identical at the 5% probability threshold

Effect of Substrate*Length Interaction:

The number of roots observed exhibited considerable variation. In the black soil treatment, the cuttings of 10 and 20 cm exhibited a mean of 0 ± 0, while the 20 cm cutting in the sand/sawdust treatment demonstrated a mean of 6.33 ± 3.05 (Fig.9a). The analysis of variance revealed no statistically significant variation (0.20 > 0.05).

The length of root exhibited ranged from 0 ± 0 cm for the cuttings of 10 and 20 cm inserted in the black soil to 4.27 ± 2.26 cm (Fig.9b) for those of 20 cm cultivated in the sand/sawdust mixture. The analysis of variance did not indicate a significant variation (0.70 > 0.05)



a **b**

Sa=sand, sd= sawdust, bs= black soil; a=Number of roots, b=length of roots

Figure 9 : interaction substrate*length of RSC on the rootings parameters

DISCUSSION

Budding

The sprouting time was found to be 8 weeks, which is consistent with the findings of [23,24]. The former study concentrated on *Vitex doniana,* whereas the latter examined *Bombax costatum*. This result differs from those reported by [25,21,15,26] on *Bombax costatum, Detarium microcarpum*, *Vitex doniana* and *Securidaca longepedunculata*, respectively. The respective sprouting times were four, five, six and seven weeks. The sprouting time may be linked to the species type, the period during which the cuttings were collected, and even whether or not growth hormones were used [27,21,15].

Similar results to those presented here have been obtained in the same agroecological conditions, as reported by [26,15]. The optimal substrate for *Securidaca longepedunculata* and *Vitex doniana,* respectively, was identified as a mixture of sand and sawdust for the former and black soil and sawdust for the latter. In contrast, [28] observed that the substrate had no significant effect on the growth of *Daniellia oliveri* in Benin. The use of porous substrates allows the circulation of water and air around the cuttings, which in turn improves the budding process [22].

The processes of budding and rooting are separated in time while joint in space. The distinction between these two processes enables leafy shoots to provide adventitious roots with carbohydrates. This separation necessitates that cuttings prioritize the allocation of their resources toward the development of the leafy stem. The findings align with those previously reported by [15,22], who conducted research on *Vitex doniana* and *Detarium microcarpum*, respectively. The authors demonstrated that cuttings measuring 20 cm exhibited a fivefold higher budding rate than those measuring 10 cm. Furthermore, the 15 and 20 cm cuttings were identified as the most effective in terms of budding. These findings are in accordance with those of [29,7], who conducted research on *Faldheidia albida* and *Spathodea camparulata*, respectively. The discrepancy in root length may be associated with the concentration of sugars or endogenous phytohormones. Prior research has demonstrated that an increase in root length is accompanied by a rise in sugar concentration [30,21].

In regard to the interaction substrate*length, our findings are inconsistent with those of [15] on *Vitex doniana* in the same region. The aforementioned authors observed a high budding rate (80.33 ± 20.38%) in 20 cm RSC cultivated in black soil/sawdust substrate. During the course of these experiments, the buds underwent development, resulting in the emergence of leafy axes.

The discrepancy in the length of cuttings in relation to the number of shoots observed in this study is also reported by [21] in their investigation of *Detarium microcarpum*, and by [15,26] in their research on *Vitex doniana* and *Securidaca longepedunculata* in the guinean savannah highlands. Similarly, the research team observed a positive correlation between cutting size and the number of aerial shoots in *Vitex doniana* and *Securidaca longepedunculata*. This phenomenon may be attributed to the presence of carbohydrates in the cuttings, which could potentially exert an influence on the growth and development of the aerial shoots. This finding is inconsistent with the results reported by [21] for *Detarium microcarpum* in Burkina Faso. In the aforementioned species, the authors observed a considerable size of the leafy shoot (10.70 ± 3.22 cm) in cuttings of 10 cm.

The maximum percentage of leafy shoots recorded in the proximal position indicates that bud neoformation follows the distribution channel of elaborated sap. As other authors have previously reported, the polarity of the mother root is preserved by RSC [29]. Moreover, the polarity of the root system results in the initiation of new roots at the distal end of root segment cuttings and the emergence of aerial shoots near the proximal end [31]. These findings are consistent with those previously reported in Burkina Faso for the proximal pole [22]. The authors observed a budding rate of 90% in *Detarium microcarpum*.

Rooting

Following the development of young leaves, which are produced by photosynthesis, auxins and carbohydrates are transferred via an intricate network of sap in the basal part of the RSC. This process is responsible for the induction of rhizogenesis. The rooting process was found to be longer than the budding process, which is in accordance with the findings of previous studies [32,27] that have demonstrated that rooting time is often longer than sprouting time. The sand/sawdust proved to be the optimal substrate for rooting rate. These findings are in accordance with those of [33] on *Ximenia americana* and also those of [26] on *Securidaca longepedunculata.* This result can be attributed to the physical structure of porous substrates, which provide a supportive environment for roots to penetrate and expand. The utilisation of porous substrates has been evidenced to diminish soil compaction, thereby facilitating root growth [34]. Moreover, porous substrates possess the ability to retain moisture while simultaneously enabling the drainage of excess water, thus maintaining consistent moisture levels for roots. The findings of [22] on *Detarium microcarpum* in Burkina Faso exhibited a similar range to that observed in the present study. The authors observed that the length of cuttings had a significant effect on the rooting rate. Furthermore, they observed that the longest root segments (20 cm) produced more new roots than the 10 cm root segments. The present findings align with those of numerous preceding studies, where in comparable lengths have resulted in the successful sprouting of *Faidherbia albida* [29], *Spathodea campanulata* and *Vitex doniana* (Mapongmetsem [7,15]. Additionally, the findings align with those of previous studies on other species, including *Maerua crassifolia* [35], *Prunus avium* [36]. The combination of sand and sawdust resulted in the longest root length per plant, in comparison to the other substrates. This may be attributed to the low bulk density of the sand/sawdust medium, which facilitates greater root penetration and the formation of longer roots. This is consistent with the findings of [37], who reported that soil or other materials are prepared and mixed to create an optimal rooting environment, free from pests, diseases and with adequate air-filled porosity, available water and suitable bulk density.

CONCLUSION

The findings of this study demonstrate that the Fabaceae is amenable to vegetative propagation through root segments cuttings, which represents a significant contribution to the domestication programme for *Amblygonocarpus andongensis*. The sand/sawdust substrate appears to be the best of all the culture media tested for budding (46.67 ± 20%) and number of aerial axes (2.16 ± 0.84). The 20 cm length proved to be the best for budding rate (47.77 ± 9.71%), and the height of leafy shoot (10.17 ± 6.14 cm). The best rooting rate was observed in the sand/sawdust 12.22 ± 11.84% and the best length for the rooting was 20 cm (15.55 ± 13.33%). These investigations could help in the development of the domestication programme of the species in order to provide farmers with improved seedlings.

AKNOWLEDGEMENTS

The authors are in dept to anonymous reviewers who improve the manuscript with they valuable comments.

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