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Ability of *Carissa edulis* Guill. & Perr., 1832 to reproduce vegetatively by aerial layering in the high savannas of Guinea (Adamaoua, Cameroon)

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Abstract

The Sudano-Sahelian zone of Cameroon is rich in many species of high socio-economic value. Carissa edulis is one of the fruit species highly valued by local populations. Despite its importance in the farming community, it still grows in the wild. The objective of this study is to contribute to the domestication of this species by aerial layering. Two trials were conducted. The first aimed to test three substrates (black soil, sawdust, and a mixture of black soil and sawdust in equal proportions) on branches ranging in diameter from 0-1.5cm and 1.6-3cm. The experimental design used was a split-plot with three replicates. The substrate was the primary treatment and the diameter was the secondary treatment. The second trial focused on the influence of covering with aluminum foil and the orientation of the branches on the rooting of the layers. The experimental design used was a split-plot with three replicates. The first factor was covering and the second factor was branch orientation. The results showed 67% callogenesis with 53.33+13.66% rooting in black soil substrate, 73.33±16.32% in sawdust, and 75±18.40% in a mixture of black soil and decomposed sawdust. Diameter significantly influences the rooting of layering. This rooting rate ranges from 55.55+10.13% for the range 0-1.5cm to 78.88±17.63% for the range 1.6-3cm. Uncovered layering rooted at a rate of 73.33±8.16% compared to 93.33±8.16% for covered layering. East and west orientations of the layering resulted in rooting rates of 88.33±9.83% and 78.33±14.71%, respectively. During acclimatization, a survival rate of 99% and a recovery rate of 88% were observed. These results are important for the domestication process of this species of socioeconomic interest.

Keywords: Layering, substrate, diameters, covering, east-west, acclimatization.

Introduction

Savanna ecosystems are diverse and rich in species of socio-economic interest[1]. These species contribute to climate regulation and also play an important role in carbon sequestration[2]. The importance of these local species in food, traditional medicine, and income diversification for local populations is well established[1]. However, their plant formations are threatened by a series of human and natural factors. *Carissa edulis*, from the Apocynaceae family and locally known as Dadiré, is one of these threatened species. It is a highly branched shrub that can reach 5 m in height, with white sap. Its bark is gray and smooth when young. The leaves of *Carissa. edulis* are grazed by goats. This species is also a source of firewood in some regions and the fruits are edible[3]. The round berries, about 1 cm in size and purple-black when ripe, are eaten raw both unripe and ripe[4]. *Carissa edulis* is one of the most commonly used plants in the traditional treatment of several diseases. It should be promoted because it is recognized as non-toxic to humans, even in high doses[5]. The roots contain an active ingredient, carissin, which may be useful in the treatment of cancer[3]. The roots of this plant are highly sought after by local populations for the production of a popular local drink. The harvesting of roots does not take regeneration into account[6]. The harvested roots are sold in bundles at local markets. The twigs contain quebrachytol and cardioglycosides, which are useful as anthelmintics against tapeworms[7]. Despite its importance to local communities, *Carissa edulis* is poorly managed. Its overexploitation threatens its existence in the Sudano-Sahelian zone. In addition, its natural habitat is disappearing at an alarming rate, leading to the loss of these tree specie[8]. The process of domesticating wild fruit trees of known interest and subject to high pressure, including their cultivation and integration into agroforestry systems, often focuses on sexual propagation. The latter is the preferred mode of propagation for biodiversity conservation[9]. The high pressure exerted on seed resources makes their regeneration in the natural environment difficult [10].

In the case of *Carissa edulis*, the overexploitation of roots for medicinal purposes is accelerating its disappearance. Vegetative regeneration, which is faster and less costly, appears to be an adaptive strategy for this Apocynaceae[11,12]. The lack of information on the ability to reproduce vegetative, particularly by aerial layering, is a major obstacle to the domestication of this species in the Guinean high savannahs of Cameroon. The main objective of the study is to contribute to the domestication of this species by aerial layering. The aim is to evaluate the influence of the substrate and branch diameter on the rooting potential of aerial layers: to test the effect of coverage and east-west orientation of branches on the rooting of layers of this species.

Materials and Methods

Study site: The study was conducted in the Guinean high savannahs of Cameroon, in the Department of Vina, specifically at the Mbé escarpment (°3715"S, 13°34'58"E) (Figure-1). The average annual rainfall is 1962.4 mm[13]. The average annual temperature is approximately 25.7°C. Temperature peaks occur from February to April with optimums (30.1°C), while minimums are observed from July to August (21.3°C). The dominant soils are ferralitic with a yellow facies, where all elements of the parent rock are hydrolyzed and most of the bases are exported[14]. The vegetation cover consists mainly of savannas ranging from grasslands to wooded savannas dominated by *Daniellia olivieri* and *Lophira lanceolata*[15].

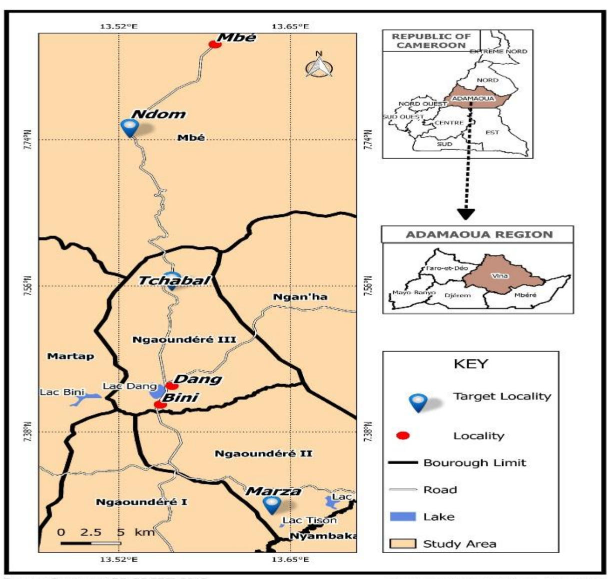

Figure-1: Location map of the study area.

Methodology: The aerial layering of this plant was evaluated through two trials, the first of which focused on the effect of the substrate and diameter, while the second looked at the effect of aluminum foil and the east-west orientation of the branch. The layering technique used was that described by several authors[9,16,17].

Influence of substrate and branch diameter on the rooting potential of *Carissa edulis* layers (trial 1): Three substrates were tested: black soil, decomposed sawdust, and a mixture of decomposed sawdust (50%) and black soil (50%). Two diameter ranges were used 0-1.5cm and 1.6-3cm. A caliper was used to determine the aerial diameter of the branches. The experimental design used was a split plot with three replicates. The substrate represented the main treatment, while the branch diameter corresponded to the secondary treatment. The experimental unit consisted of 20 layering cuttings. The 360 layering cuttings produced were divided into 120 cuttings for each substrate. The trial took place over a period of seven months, from November 2023 to May 2024. Six-centimeter rings were made on the middle parts of the branches using a sharp knife. The ringing of the branch consisted of removing the bark over a length of 6 to 7 cm until the wood appeared (Figure-2a). Complete annular removal of the bark down to the cambium level destroys the phloem at this point and prevents the downward flow of sap. The cambium exposed by the cortical annulation is immediately covered with a transparent polyethylene sleeve containing the chosen substrate, which has been moistened beforehand. The sleeve is positioned correctly and held in place by tying it at the top and bottom with polyethylene string to prevent the substrate from drying out (Figure-2b). This sleeve is left in place for as long as necessary for the adventitious roots to form. Each sleeve was labeled with information to facilitate identification, namely: type of substrate, branch diameter, and sleeve number. The substrates were moistened during the trial using a 60 ml syringe, taking care to plug the hole left by the needle with a piece of tape. The layering was checked twice a month.

Effect of coverage and east-west orientation of branches on the rooting of *C. edulis* layering (trial 2): This trial took place from May to November 2024. The layering was carried out as before. However, the substrate with high success rates and the most effective diameter range from the previous experiment were used. Some of the sleeves were covered with aluminum foil to prevent the roots from overheating (Figure-2c). Only branches oriented east and west were selected. Every 30 days, water was added to the substrate using a medical syringe to prevent it from drying out. As soon as roots appeared below the surface of the plastic film, the branch was cut 4 or 5 cm below the notched section using a horticultural saw. The experimental design used was a split-plot design with three replicates. Covering the layering was the main treatment, while the orientation of the branches was the secondary treatment. The experimental unit consisted of 20 layers, for a total of 240 layers.

Weaning: Acclimatization process: The layering cuttings were pruned to remove certain branches in order to facilitate their recovery and strengthen their root system. They were then placed individually in a rehabilitation propagator (in the shade) in bags containing potting soil and watered regularly before being transplanted into the field.

Data collection and analysis: Data collection began as soon as the first roots were observed through the plastic film and continued on a monthly basis. Data analysis focused on the number of rooted layers, the recovery rate, and the survival rate after weaning. The collected data were analyzed using variance analysis with Statgraphic 5.0 software. Significant averages were separated using the Duncan multiple range test. Microsoft Word (Excel) was used to plot the graphs.

Results and Discussion

The ability of *Carissa edulis* to form aerial layers was assessed at the end of trial 1, seven months after it was set up. It should be noted that callus formation occurred in almost all the sheaths that were placed, with 98% of them forming callus (Figure-3a). 67% of the layers that formed callus rooted (Figure-3b), 31% formed only callus that did not develop during the trial period, and 2% were damaged (dried out or died naturally). In this trial, the rooting rate is similar to the success rate of the layering, as almost all of the rooted sleeves reached the weaning phase.

The presence of callus can be encouraging for the future, as over time cell differentiation may occur, allowing root organogenesis on these same calluses.

Effect of substrate and branch diameter on layering rooting (trial 1): Effect of substrate: The average rooting rate of layering at the end of the trial (7 months) after planting ranged from 53.33±13.66% in black soil substrate to 75±18.40% for the black soil-sawdust mixture (Figure-5). The analysis of variance indicates a significant difference between the different substrates (0.0066<0.01). The best substrate for rooting layering cuttings has several important properties: a mixture of black soil and decomposed sawdust has a granular structure, facilitates good circulation of water and essential nutrients to the roots of the layering cuttings, and has a suitable pH, facilitating water drainage. All these properties make the mixture of black soil and decomposed sawdust a suitable substrate for the growth of layering in *Carissa edulis.* One author in Burkina Faso obtained 71.7% rooting in the substrate composed of 3/5 sawdust and 2/5 black soil for the aerial layering of *Balanites aegyptiaca*[18]. In Cameroon, in the Guinean high savannahs we obtained a rooting rate of over 80% on *Lophira lanceolata* using the same substrate[19]. In the Sahelian zone authors obtain a rooting rate of 100% on *Anacardium occidentale*[20]. Mixed substrates are generally chemically stable provided that the sawdust is carefully decomposed to prevent organic matter from evolving during layering, which would then disrupt nitrogen dynamics[21].

Figure-2: a) complete annulation; b) layer not covered by aluminum foil; c) layers covered by aluminum foil.

Figure-3: a) rooted layering; b) root system after weaning and cleaning.

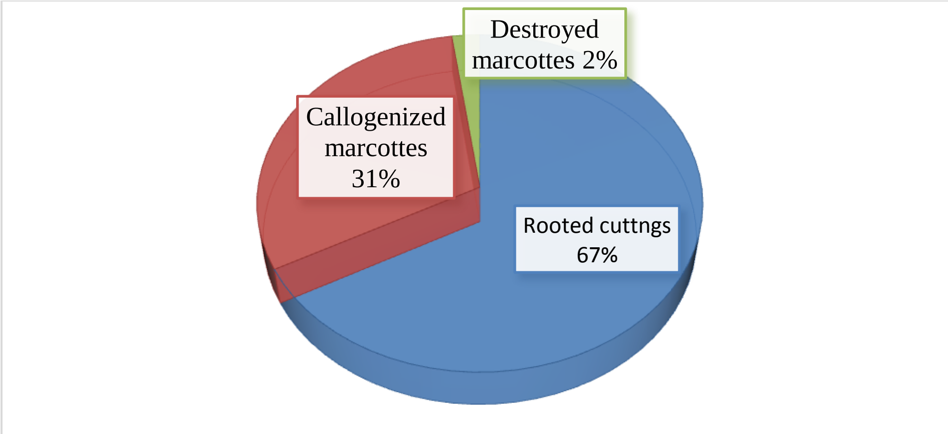

Figure-4: Proportional distribution of the types of responses observed for aerial layering in *Carissa edulis.*

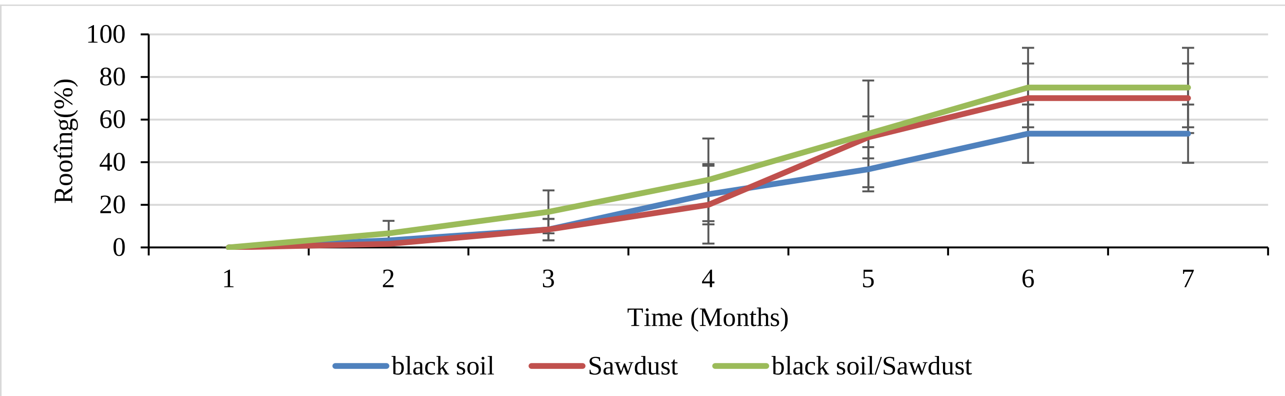

Figure-5: Evolution of the rooting rate of layering cuttings according to the substrates.

Influence of diameter: In terms of branch diameter, the rooting rate ranged from 55.55±10.13% for the 0-1.5cm diameter range to 78.88+17.63% for the 1.6-3cm diameter range (Figure-6). Analysis of variance shows a very significant difference between the different diameters (0.0005 < 0.001). The rooting of aerial layering in *Carissa edulis* depends on the diameter of the branches, with larger diameters producing the best results 1.6-3. These results are similar to those obtained in Congo[22]. These authors obtained a rooting rate of 76.7% for branches with larger diameters in *Cola acuminata*. The thicker a branch is, the more likely it is to root. This trend has already been observed in other plants, mainly the safou tree, *Dacryodes edulis*[23,24]. The latter authors recommend branches with a diameter greater than 4 cm and between 3 and 5 cm for this species. The vigor of the branch has a positive influence on the rooting of layering[23,16]. This is because large branches accumulate more carbohydrates and phytohormones (1994). The rooting of layering cuttings of each diameter range varies from one substrate to another. The chemical composition of the substrates has a significant impact on this phenomenon[25].

Influence of substrate-diameter interaction on the rooting of *C. edulis* layering cuttings: In terms of the substrate-diameter interaction of the branches, the rooting rate of layering varies between 46.66±5.77% for layering with a diameter between 0-1.5cm in black soil and 90±10% for layering with a diameter between 1.6-3cm in the black soil/sawdust mixture (Table-1). Analysis of variance shows that the substrate-diameter interaction is not significant (0.38>0.05).

Influence of covering and east-west orientation on layering rooting: Influence of covering: Seven months after planting, the rooting rate of layers varies from 80±8.16% for unprotected layers to 93.33±8.16% for those covered with aluminum foil. Statistical analysis shows that the exposure effect of the sleeves is significant (0.008 <0.01). A large root system is observed in layering based on black soil/sawdust and wrapped in aluminum foil. The latter has good thermal conductivity, which helps regulate the temperature inside the sleeves. This treatment is very necessary in hot regions where plants are prone to dehydration. In the high Guinean savannahs, authors obtain 69.44% rooting on *Lophipra lanceolata*, and on *Vitex doniana*[19]. In the sahelian zone of Cameroon, they obtain a rooting rate of 98.88% on *Anacardium occidentale*[20]. Aluminum foil maintains moisture in the sheaths, preventing them from drying out, while making water available for absorption by the newly developed roots. Unlike aerial organs, plant roots avoid light during their development; this phenomenon is called negative phototropism. Low exposure of the roots to light causes their growth to accelerate in order to escape the light radiation[26].

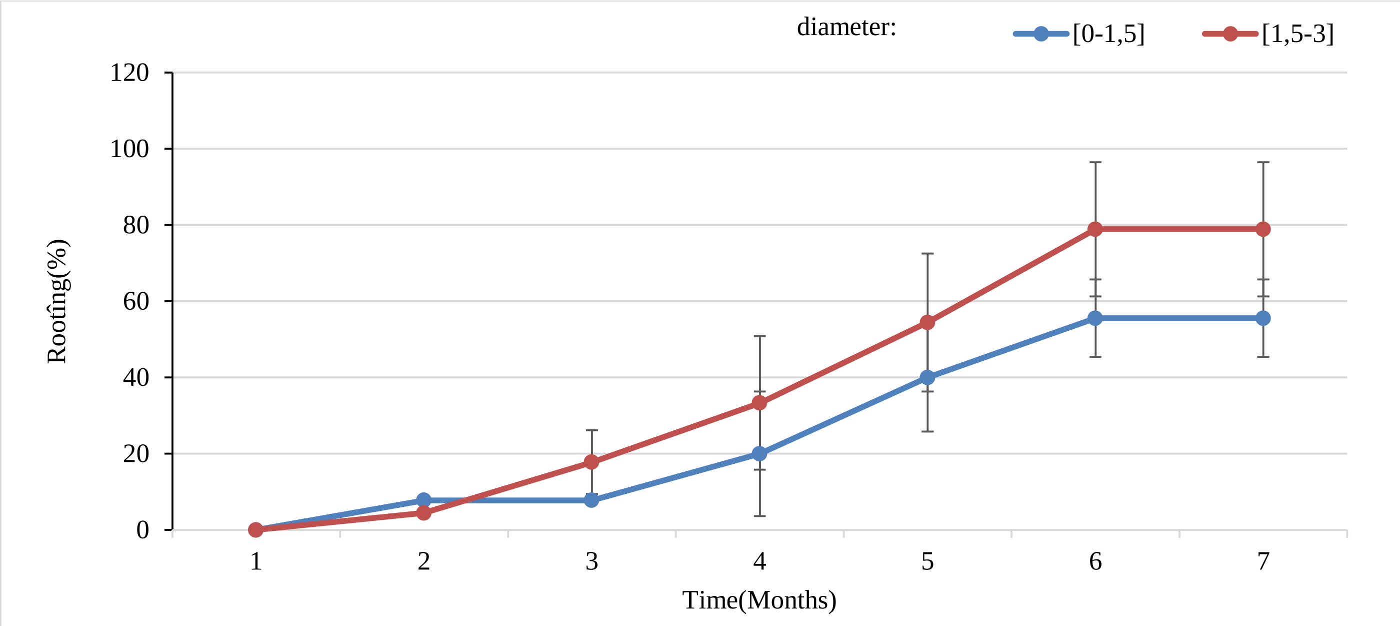

Figure-6: Evolution of the rooting rate of layering cuttings according to branch diameter.

Table-1: Substrate*diameter interaction on the rooting of *Carissa edulis* cuttings.

Diameter (cm)	Substrate			
	Black soil	Saw dust	Black soil/Saw dust mixture	Average
0-1,5	46,66±5,77[a]	60±10[b]	60±10[b]	55,55±10,13[a]
1,6-3	60±17,32[a]	86,66±5,77[b]	90±10[b]	78,88±17,63[b]
Average	53,33±13,66[a]	73,33±16,32[b]	75±18,40[b]	67,22±18,40[b]

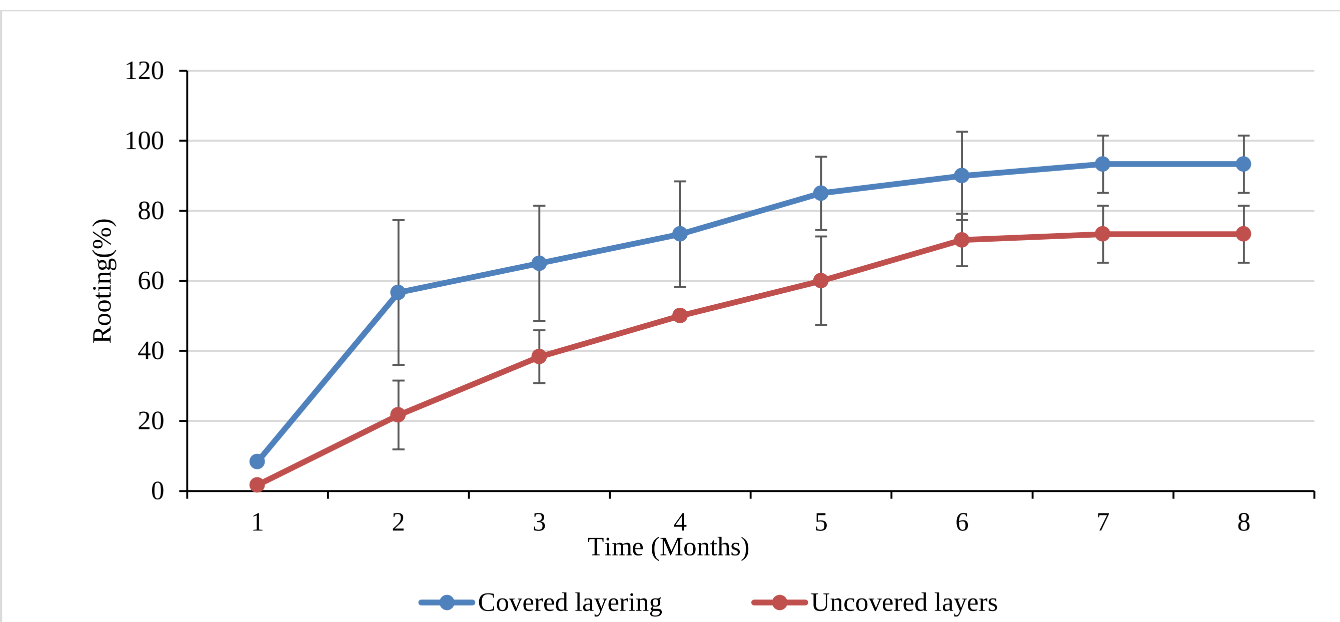

Figure-7: Evolution of the rooting rate of layering according to the exposure effect.

Influence of east-west orientation on the rooting of layering: The rooting rate of layering cuttings according to branch orientation eight months after planting varies from 78.33±14.71% for cuttings placed in a westward orientation to 88.33±9.83% for those placed in an eastward orientation (Figure-8). Statistical analysis shows that the effect of branch orientation is not significant (0.057 >0.05). The layering began to produce roots in the first month for the layering placed in the east and west orientations. The orientation of the layering did not have a significant effect on rooting because the branches on either side receive almost the same light intensity. Light can have an impact on the rooting of layering, but its effect depends on several factors, including the quality and quantity of light and the environmental conditions in which the layering is placed.

Effect of the interaction between covering and branch orientation: With regard to the interaction between exposure and sleeve orientation, it should be noted that the layering covered in the east position had the highest rate, 96.66±5.77%, compared to 66.66±5.77% for the layering not covered in the west position. The analysis of variance did not reveal a significant difference (0.07>0.05).

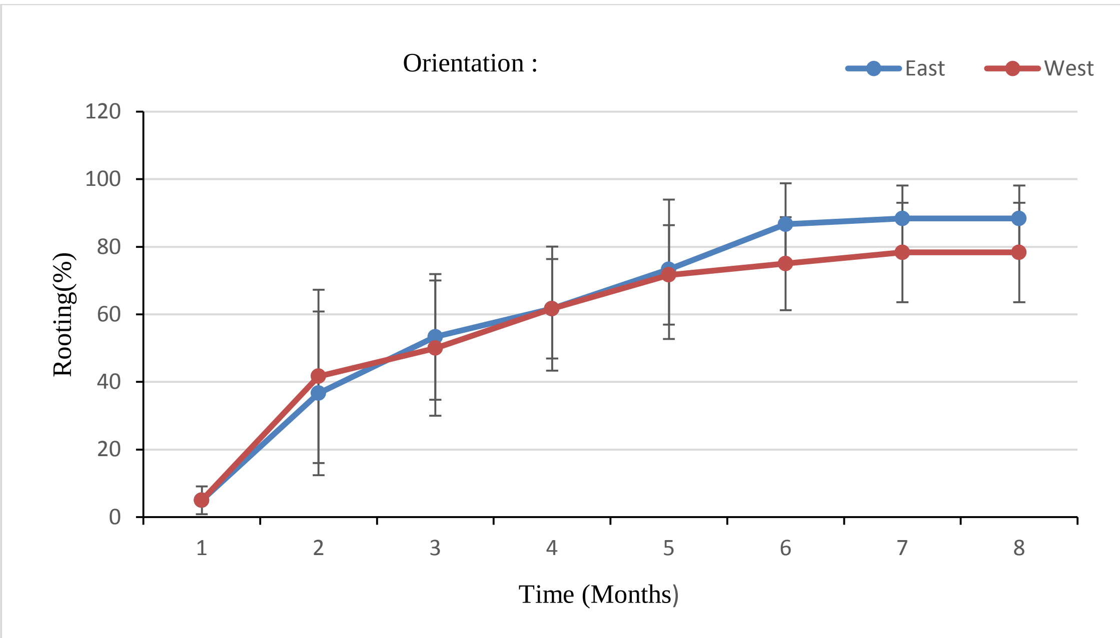

Figure-8: Evolution of the rooting rate of layering according to the east-west orientation of the branches.

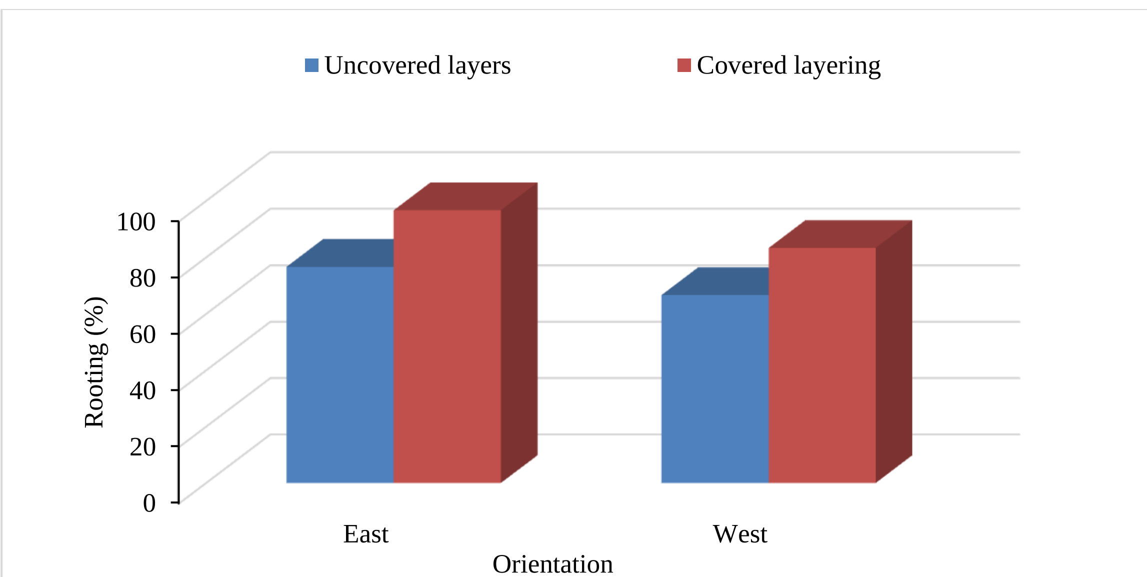

Figure-9: Percentage of rooting according to the interaction between covering and branch orientation.

Acclimatization: At the end of the first experiment, 100 layering cuttings were weaned and cleaned, placed in 30x10cm pots containing potting soil, and then introduced into the polypropagator for rehabilitation. All the layering of different diameters sprouted after one week and after three weeks all the layering had produced new leafy shoots, i.e. a 100% survival rate. Eight weeks after weaning, a gradual drying out of all the layering was observed and after 12 weeks all the layering had dried out. For the cuttings in trial 2, at the end of the trial, the cuttings with a diameter of between 1.6-3cm were weaned and placed individually in pots containing potting soil mixed with compost made from household waste and stored in the rehabilitation propagator. Watering was carried out regularly throughout the process. During acclimatization, the appearance of buds. The substrate used allowed for good aeration of the roots, promoting good water and air circulation between the roots. It was also noted that all layered cuttings that had been weaned and contained at least one new aerial axis were more resistant than those without any new aerial axis, as the former still perform photosynthesis and supply the newly formed roots with the organic matter essential for their survival. In Cameroon, 100% of aerial layering was successful on *Dacryo desedulis* if defoliation was partial or if all leaves were retained[24].

He reports that complete defoliation of the layering resulted in a 58.4% failure rate. Furthermore, in Ghana several authors obtain a 72% success rate on *Allanblackia parviflora*[27,28]. In Benin, 84.6% success rate on *Englerophytum oblanceolatum*[29], and in India, we obtained a survival rate of 90.82% on *Carissa carandas*[30]. The quantity of roots formed and the number of leaves present are also believed to be responsible for the increase in the survival rate of layering.

Figure-10: Layers in the rehabilitation propagator: a) Appearance of leafy axes, b) Young plant 2 months old after acclimatization.

Conclusion

This study shows that *Carissa edulis* is suitable for vegetative propagation by air layering. The mixture of black soil and decomposed sawdust was recorded as the best rooting substrate with a rate of 75+18.40%, and the diameter range 1.6-3cm recorded a rooting rate of 78.88+17.63%, higher than other diameter ranges. Similarly, covering with aluminum foil resulted in a rooting rate of 93.33+8.16%. Regarding the orientation of the layered branches, those facing east performed best with a rate of 88.33+9.83%. The recovery and follow-up rate of the layering during the acclimatization period yielded promising and favorable results for the introduction of this species into farmers' production systems. Future research could focus on in situ monitoring of the growth and development of the layering after acclimatization.

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