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(RESEARCH ARTICLE)

Vegetative propagation trial of *Carapa procera* CD (Meliaceae), a spontaneous species with multiple uses (Daloa, Central-West, Côte d'Ivoire)

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Abstract

Carapa procera, a species with numerous cosmetic and therapeutic virtues, is plagued by excessive use of its seeds for the extraction of its oil, considerably limiting its seminal regeneration. In order to contribute to its preservation, trials were conducted using three modes of vegetative propagation for 60 days. Stem cuttings included three types, 6 cm, 12 cm and 20 cm cuttings. Stump propagation included three sizes, 8 cm, 14 cm and 20 cm. Layering was carried out at two levels, basal (1.5-2 cm from the ground) and median. The trial design was a completely randomized block design with three replications for each mode. Stem cuttings were inconclusive, with only a 3.33% survival rate for 20 cm long cuttings, but no viable offsets. The 6 cm and 12 cm long cuttings did not survive long. Similarly, the aerial cuttings did not produce any roots. In contrast, the stumps vigorously rejected the three lengths tested (8, 14 and 20 cm), with success rates ranging from 70 to 87% with no significant difference between treatments ($p > 0.05$). At the present stage of the study, stump cuttings remain the best way of vegetative propagation for the domestication of *Carapa procera*.

Keywords: Côte d'Ivoire; Domestication; *Carapa procera*; Cutting; Stump; Marcots

1. Introduction

In Africa, rural populations derive a large part of their income from the exploitation of wood and non-wood forest products (wood, fodder) and non-wood products (fruits, sap, gum, honey, etc.). However, some species that are very useful to the community are finding it increasingly difficult to regenerate due to environmental and anthropogenic constraints [1]. This leads to their rapid regression or even disappearance. This is the case of *Carapa procera*, a specie with many medicinal, cosmetic and timber properties, etc.

Today, in Côte d'Ivoire, *Carapa procera* is only represented by old plants whose fruit production is low and irregular. It is thus confined to the wooded areas of the northern zone and rarely in coffee and cocoa stands in west of the country. All parts of the tree are useful to humans to satisfy their needs [2], [3]. From its seed, oil is extracted, the content of which in the kernel is estimated at 55% [4]. This oil is used in the manufacture of cosmetics and traditional soap [5], [6], [7]. It is also used for the treatment of several diseases in traditional veterinary and human medicine [8], [9].

Despite its multiple virtues, seminal regeneration of *Carapa procera* remains very limited due to increasingly harsh climatic conditions and anthropogenic pressures that deteriorate the forest gallery habitats that still harbor the few isolated feet of this species [10]. The natural regeneration of *C. procera* is confronted with multiple environmental constraints, including recurrent drought and agroforestry practices using slash-and-burn agriculture, over exploitation of timber and non-timber forest products, overgrazing, etc. According to [2], the lack of natural regeneration of the species is caused by intense consumption of seeds by rodents and infestation of seeds by the larvae of an insect,

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Hipsiphyla grandella. This is compounded by the regular collection of seeds for oil extraction [11]. In addition, experiments have shown that *C. procera* seeds do not tolerate dehydration and, as a result, this poses a crucial problem for seed conservation [12].

Moreover, little scientific work has been done on the domestication of *Carapa procera*in sub-Saharan Africa, despite its many uses. It is therefore necessary to investigate artificial regeneration techniques, particularly those of vegetative propagation. This would contribute to the maintenance and pre-domestication of this species of interest to rural populations. The objective of this study is to domesticate *Carapa procera*in in order to improve its production and thus generate additional income. More specifically, the aim is

- To evaluate the recovery and growth of *Carapa procera* stem cuttings and
- To assess the rooting and survival of marcots of this species

2. Material and methods

2.1. Study area

The study was carried out on the site of the University Jean Lorougnon Guédé (Daloa, Centre-West of Côte d'Ivoire). It is located at 6°54'32" North Latitude and 6°54'15" West Longitude (Figure 1). The study area belongs to the mesophilic forest domain [13], [14]. It is characterized by dense forest vegetation with regressive evolution and a high rainfall exceeding 1500 mm. The average annual temperature is 26°C [15]. The relief is made up of plateaus with an altitude of200 to 400 m [16]. Hydrographically, the region is influenced by the Sassandra river and its tributaries (the Lobo and Davo) and the Buyo dam lake [15]. The soil is ferralitic of granitic origin with low denaturation [17], [18]. The dense semi-deciduous forest vegetation is dominated by *Celtis* sp and *Triplochiton scleroxylon* [19]. The climate is sub-equatorial attian. The average annual temperature and rainfall are 20.3°C and 223 mm respectively [20].

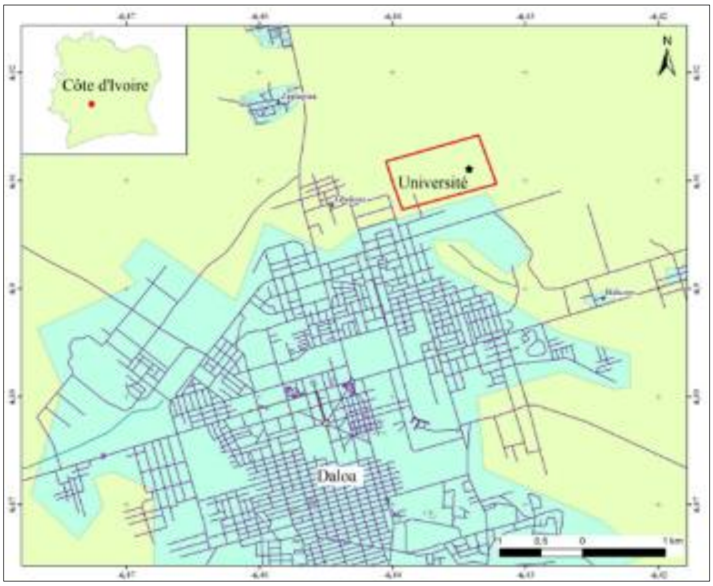


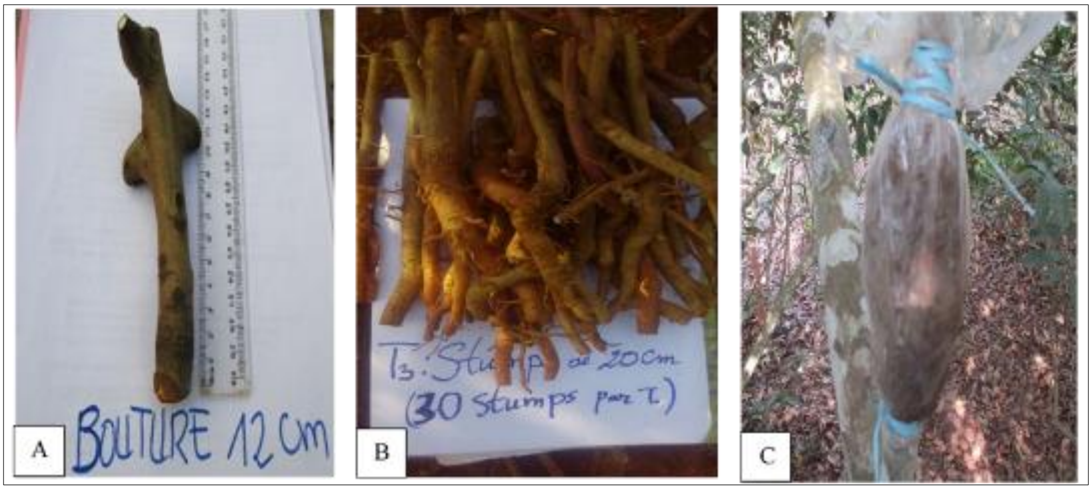
Figure 1 Carte de la zone d'étude

2.2. Plan for setting up the trial

2.2.1. Experimental device

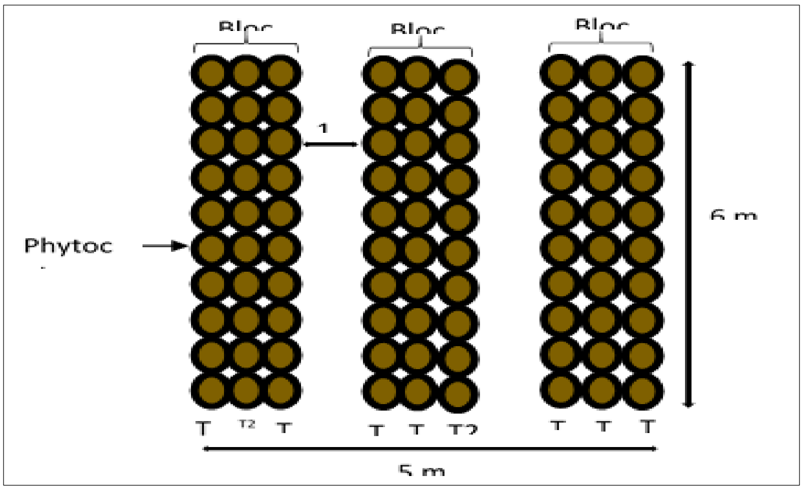
Stem cuttings, stumps and marcots of *Carapa procera* were taken from mother plants (Figure 2) from the coffee plantations of Kéibla, a rural area of Daloa. The trial was conducted under a 6 m x 5 m shade house (30 m^2). The substrate was taken from the trial site and then stored on a tarpaulin to extract the coarse elements. The cuttings and stumps trials were conducted in a completely randomized block design with three replications (Figure 3). This design consisted of three blocks (replicates) per type of plant material. Each elementary block of cuttings contained 60 nursery phytocels divided into three treatments of 20 phytocels each, for a total of 180 phytocels. The stumps were arranged in blocks of

30, for a total of 90 phytocels for the three treatments. In total, 270 phytocels for the two types of plant material used. The blocks were separated by one meter from each other.



A-stumps of 20 cm; B- young cutting of 12 cm; C- half-height marcot

Figure 2 Plant material used to conduct the trials



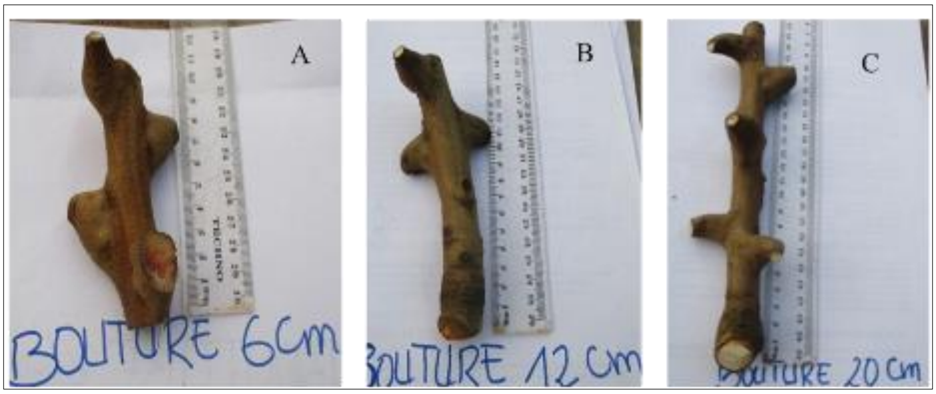
T1- treatment 1; T2-treatment 2; T3-treatment 3

Figure 3 Experimental scheme adopted

2.2.2. Stem fragment cuttings trials

Removal and planting of stem cuttings

Stem fragments of 2 to 3 cm in diameter were removed from mother trees with pruning shears. The leaves were then trimmed to avoid excessive transpiration of the plants. Three treatments related to the size of the cuttings were made up (Figure 4): T1 (6 cm cuttings), T2 (12 cm cuttings) and T3 (20 cm cuttings). The collected cuttings were planted in the phytocels, one cutting per bag (Figure 5). Transport and planting were done on the same day to avoid drying out. All the plants received regular and constant watering for 60 days. Regular weeding was done to increase the chance of the plants recovering.



A- Treatment T1; B- Treatment T2; C- Treatment T3

Figure 4 Cuttings of different lengths (treatments)

A-Arrangement of the culture bags; B-Planted cuttings

Figure 5 Setting up *Carapa procera* nursery beds in the nursery

Collection and processing of data on stem cuttings

The data collected on the cuttings concerned the survival rate of the cuttings (Tsb), the number of dried out cuttings (Nbd) and the assessment of anomalies during the trial. The survival rate was used to determine the fragility or resistance of the cuttings tested. This rate was noted regularly until the end of the experiment (60 days), according to the following calculation method [21]:

$$\mathrm{Ts} = \frac{\mathrm{nb}}{\mathrm{Nt}} \times 100$$

With nb-number of live cuttings and Nt-total number of cuttings planted.

The drying out of the cuttings was observed during the trial on the above-ground part. The time of appearance of the signs of dieback of the cuttings was noted, as well as the number of cuttings affected, until the end of the experiment (60 days of culture).

2.2.3. Stump cultivation trials

Removal, planting and maintenance of stumps

*Carapa procer a*wildings were dug out of the ground in the wake of the mother plants with a hoe and then dressed into stumps of different sizes from the crown at 4 cm, 7 cm and 10 cm. The stem was also cut to the same dimensions from the collar. Three stump length treatments were tested (Figure 6): T1 (8 cm stumps), T2 (14 cm stumps) and T3 (20 cm stumps). The collected stumps were planted on the same day, vertically with a slight inclination to facilitate the observation of possible sprouts. Regular watering and maintenance were carried out to ensure good plant recovery.

Figure 6 Stumps of different lengths taken

Stump data collection and processing

The data collected at the stump level covered four parameters: stump survival rate (Ts), average number of shoots per stump (Nmrs), shoot growth dynamics (Dcr) and assessment of abnormalities during the stump trial.

The survival rate of the stumps was assessed over a period of 60 days after planting. This rate was equated with the budding rate and the differentiation of these buds into seedlings. The rate was recorded every three days until the end of the trial. Thus, it was obtained by dividing the number of living stumps (Sv) at a given time after planting by the number of stumps initially planted (Ns), all multiplied by 100. The calculation method used is as follows:

$$\mathrm{Tp} = \frac{\mathrm{Sv}}{\mathrm{Ns}} \times 100$$

The formation of shoots started as soon as the buds appeared on the viable stumps. The time of bud appearance and the number of shoots developed from these buds were recorded every 3 days until the end of the experiment (60 days of culture). The shoot count was done on all plants to avoid any ambiguity. Thus, an average of the counts was made to obtain the average number of shoots per plant. According to [22], the calculation method is expressed as follows:

$$Nmrp = \frac{N_{tr}}{N_{sv}}$$

With Ntr-Total number of discards; Nsv-Number of living stumps.

The growth dynamics of the stumps were assessed for each treatment. On each stump, the height of the shoot was measured with a 40 cm ruler. The average height of the shoots (Hm) is the ratio of the sum of the heights (Hi) to the number of living stumps (Sv). It was given according to [22]:

$$Hm = \frac{H_i}{S_w}$$

2.2.4. Layering trials

Setting up and monitoring the rooting of the layering process

Aerial layering was carried out from a stand of *Carapa procera* mother plants. Preferably lignified young orthotropic stems or easily accessible low branches were used. Thus, two treatments were carried out following the recommendations of [23]. Layering of the basal part (MAB) from 1.5 to 2 m above the ground and of the middle part (MAM) at mid-height of the tree were tested to determine which part of the stem was more favorable for layering (Figure 7). A total of 24 aerial marcots (AM) were placed, three marcots for each of the eight *Carapa procera* trees considered. The cores were determined to be rooted when the neoformed roots at the ring are observable through the transparent sleeve. When this was observed, the time of rooting was recorded from the date of marcotting. The observation was made every seven days.

A- Portion of corrugated rod; B- plastic sleeve with soil inside

Figure 7 Different stages in the production of *Carapa procera* marcots

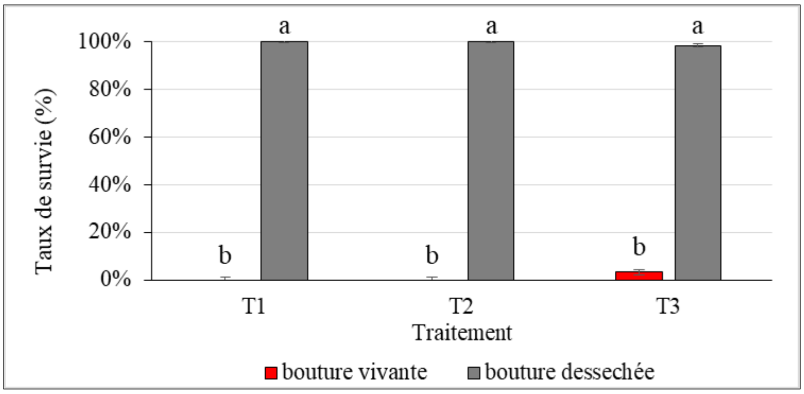
2.3. Statistical analysis of data

The data collected was entered using EXCEL 2013 software, which enabled us to create dynamic cross-tabulations in order to synthesize them according to the treatments. These curves allowed us to compare the different treatments over time. To statistically compare the means of the different treatments, STATISTICA version 7.1 software was used for the analysis of variance (ANOVA). Also, the Student-Newman-Keuls (S-N-K) multiple comparison test was used to classify the different treatments into homogeneous groups when the ANOVA revealed a significant difference in the treatments. This difference is affirmed when the probability is lower than 5% (significance level adopted). Thus, when the probability is less than 0.05, the difference is said to be significant. If the probability is less than 0.01, it is said to be highly significant and when the probability is less than 0.001, it is said to be very highly significant

3. Results

3.1. Survival rate of stem cuttings

Cuttings from aerial fragments (BFA) of *Carapa procera* were not rooted, regardless of the treatment applied. After 60 days, the cuttings from treatments T1 and T2 were completely dried out (Figure 8). In treatment T3, a few live, but not rooted cuttings (2 out of 60, i.e. 3.33%) were observed with the beginning of budding (Figure 9). However, the KrisKal Wallis test showed no significant difference between the different treatments for the survival of cuttings ($P > 0.05$; Table 1).



T1-cutting of 6 cm; T2- cutting of 12 cm; T3- cutting of 20 cm (For the same parameter, the means assigned the same letter are not significantly different at the 5% threshold)

Figure 8 Survival rate of *Carapa procera* cuttings after 60 days of cultivation

Figure 9 Live cuttings from T3 treatment

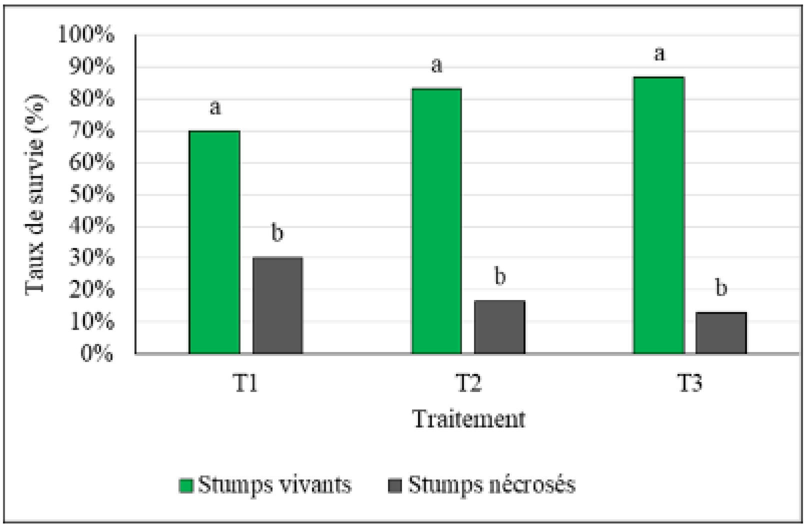
Table 1 Parameters of *Carapa procera* stem cuttings after 60 days of culture

Trait.	Number of cuttings installed	Rooted cuttings		Live cuttings		Dead cuttings	
		Number	Proportion (%)	Number	Proportion	Number	Proportion (%)
T1	60	0	0	0	0	60	100
T2	60	0	0	0	0	60	100
T3	60	0	0	2	3.33	58	96.66
LSD		NS	-	NS	-	NS	-

NS-non significant difference at 5% level; T1-6 cm cuttings; T1-12 cm cuttings; T3-20 cm cuttings, LSD-test for smallest significant difference; Trait.-Treatment

3.2. Survival rate of stumps

Figure 10 shows the survival rate (SR) of *Carapa procera* stumps during 60 days of culture, depending on the treatments. During the 60 days of observation, the survival rate obtained varied from 70 to 87%. The best survival rate (87%) was obtained from treatment T3 (20 cm stumps). Stumps from T1 treatment had the lowest survival rate with 70%. There was no significant difference between treatments for stump survival at the 5% threshold (Figure 10).

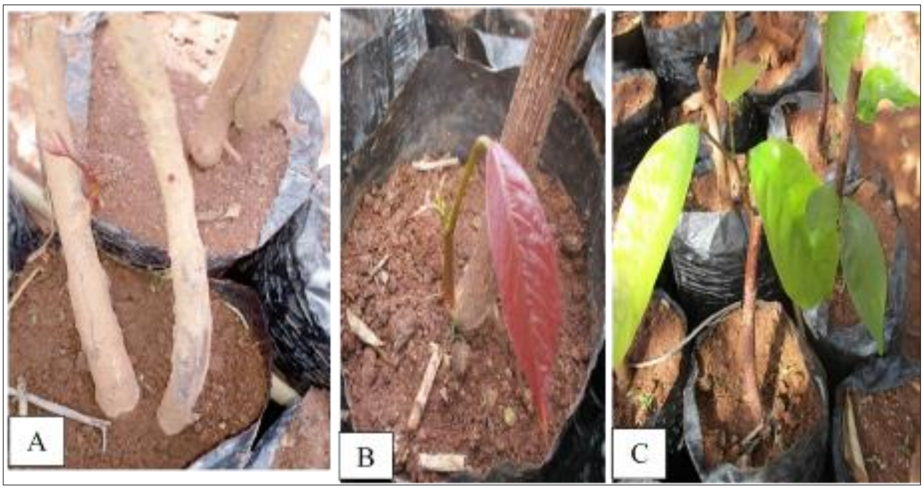


T1-Stump of 8 cm; T2-Stump of 12 cm; T3-Stump of 20 cm Stumps vivants-Live stumps; Stumps necroses-Necrotic stumps (For the same parameter, the means assigned the same letter are not significantly different at the 5% threshold)

Figure 10 Survival rate of *Carapa procera* stumps for 60 days

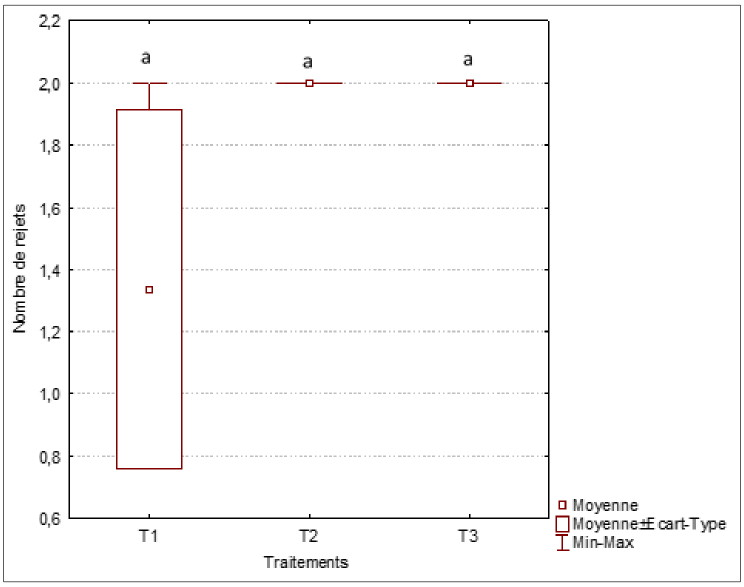
3.3. Morphological characteristics and number of stump releases produced

The appearance of buds on the stumps was observed from 11 days after planting. The buds appeared with a reddish color and were differentiated into simple or compound leaves during the second week (Figure 11). After 60 days of cultivation, *C. procera* stumps bore at least two shoots per stump. The highest number of shoots was observed in treatments T2 and T3 (two shoots per stump). There was no significant difference between the treatments for shoot production (Figure 12).



A-bud ; B-young shoot (one week) ; C-aged shoot (two weeks)

Figure 11 Transformation modes of stump-formed seedlings

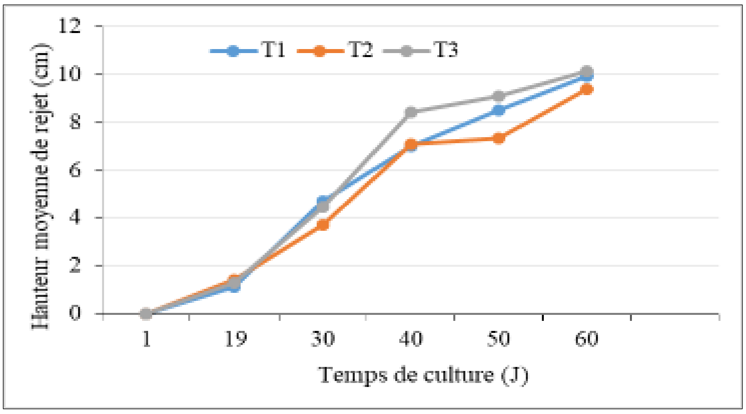


T1-stumps of 8 cm; T2-stumps of 14 cm, T3-stumps of 20 cm (The same alphabetical letter indicates that the values are statistically identical)

Figure 12 Average number of shoots per stump per treatment

3.4. Shoots of Stumps evolution

Figure 12 shows the evolution of the average height growth of the stump sprouts after 60 days of observation. This growth takes place in two phases. The first phase, with a slower evolution, showed a weak growth of the shoots between the first and the thirtieth day, whatever the treatment. The sizes obtained in this case did not reach 5 cm in height. The second phase, with exponential growth, took place between days 30 and 60. During this phase, the plants from treatments T2 and T3 had relatively larger shoots (10 cm) than the other shoots, which reached 9 cm in height (Figures 13; 14).



T1-stumps of 8 cm; T2-stumps of 14 cm ; T3-stumps of 20 cm ; Temps de culture- Cultivation time; hauteur moyenne des rejets- average shoots height

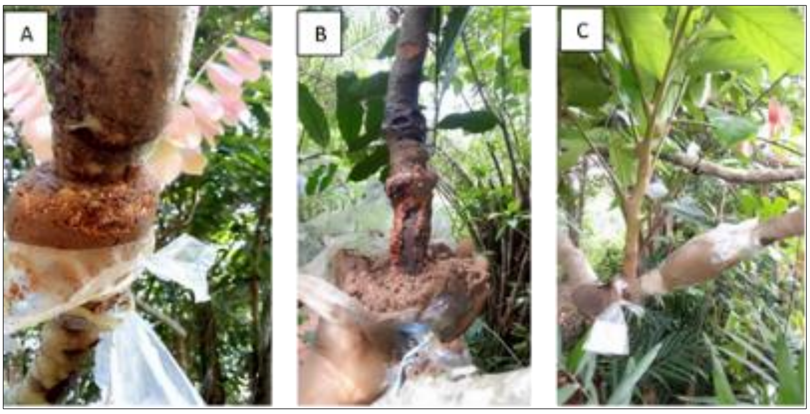
Figure 13 Evolution of the average height of *Carapa procera* stumps after 60 days of cultivation

A-Treatment T3; B-Treatment T2; C-Treatment T1

Figure 14 Pruning of plants from stumps

3.5. Evolution of *Carapa procera* marcots

Aerial layering trials on *Carapa procera* were inconclusive at less than three months (Figure 15). No seedlings were rooted during the first 90 days of monitoring. The seedlings also showed no signs of mortality. However, there were signs of new cells setting up to cover the incised part (bark) on all marcots. In addition, upstream of the ring and sleeve, the appearance of new shoots was observed on all marcots.



A- formation of new cells ; B : reconstruction of the bark ; C : emission of a new shoot just before the marcot

Figure 15 Adaptive behaviour of *Carapa procera* marcots

4. Discussion

This study focused on the vegetative propagation of *Carapa procera*, specifically on the recovery of stem fragments and stumps and the rooting of marcots. The results showed that not all three vegetative propagation techniques used for the low-cost domestication of *Carapa procera* were fully successful. Regarding survival rate, the stump sprouts were the most resistant, with a survival rate of 70-90%, regardless of the treatment. Stumps produce more roots, and it is thanks to the roots and especially to their number that there is a significant absorption of nutrients, thus allowing the good development of the plants. These results are in agreement with those of [24] and [22] on planting techniques and planting method on the survival of *Tectona grandis*. The latter had already reported good recovery and a survival rate of more than 80% of shoots from teak stumps. This is because the shoots develop directly on the stumps from the biochemical reserves they contain. The stump thus offers more reserves and space for the development of a large number of shoots. The basal part of the plant is chronologically older, but physiologically younger, because of its proximity to the root system. Also, [25] adds that each shoot observed on this part of the plant has a better nutritional and hormonal status. Another justification for the success of stumps is the high production of auxins by the roots, allowing the release of more buds, whose development favours a large number of shoots [26]. The different survival rates are high (above 50%), because the stumps used have a root part, giving them the capacity to absorb water and some minerals from the soil. This results in a low mortality rate.

Plants from cuttings develop at the nodes using available biochemicals to guide their development [27]. According to our results, stem segments grown in culture had a very poor survival rate (1.11%), although these segments have at least two nodes each. Similar results were obtained on stem cuttings of *Guiera senegalensis* in Burkina Faso [28]. This author reports that cuttings without a root system are kept alive by accumulated reserves, but gradually die back as these reserves are depleted. The time of year when the trials were conducted (December) could also explain the poor results obtained.

The results of the layering trials confirm those of [29], carried out at the end of the rainy season (October), who also noted a high suitability of *Balanites aegyptiaca* for aerial layering with a success rate of around 95%. Their results differ from ours, however, with regard to *Carapa procera*, where no rooted seedlings were observed after three months of observation. In view of our results, the time of year and the time factor seem to influence the ability of *C. procera* marcots to emit roots. The long follow-up time (3 months) could however be a discouraging factor for the adoption of this technique on this species. In Burkina Faso, it is recommended to initiate aerial layering at the beginning of the rainy season (June to August-September) and to wean them at the end of August, beginning of September after a rain [30]. Numerous studies show that the success of layering depends on the season, suggesting that tests should be spread out over the year to identify the most favourable periods. In Benin, the first half of April seems to be the most favourable time for aerial layering of *Englerophytum oblanceolatum* [31], while for safflower (*Dacryodes edulis*) in Cameroon, the best time is late November when it is in the pre-flowering stage [32]. In Nigeria, the rainy season from June to October coincides with the layering period of *Irvingia gabonensis* and *Dacryodes edulis* [33]. In south-eastern Gabon, in an equatorial climate, aerial layering of *Coula edulis* works well if laid in November [34] during the main rainy season (September to December).

In view of these results, propagation by stumps appears to be economically more interesting (high leaf mass) and also easier to carry out, although certain conditions must be met. This method of propagation does not affect the physical integrity of the mother plant. However, one of these difficulties is the construction of the nursery, which, if not properly maintained, can lead to the death of the stumps or plants. Therefore, to avoid damage to the plants and seedlings in the nursery by insects and fungi, it is advisable to treat the soil with fungicide and the seedlings with insecticide in case insects appear. This propagation method can therefore be a source of genetic variability.

5. Conclusion

Better knowledge of the genetic resources of useful spontaneous plants is a major challenge for their rational management. With this in mind, we investigated the possibilities of vegetative propagation of *Carapa procera* in the face of constraints related to the availability and conservation of seeds. To this end, plant material (stem cuttings, stumpts, marcots) was collected in the department of Daloa in the centre-west of Côte d'Ivoire. The tests carried out showed that it was possible to propagate *Carapa procera* by stumps. The best way to use stumps for efficient multiplication is to use old organs with a size of 14 to 20 cm. With regard to propagation by layering and cuttings, the results obtained show a low suitability for aerial layering and cutting of *Carapa procra* stem segments. However, further seasonal trials are needed to determine the effect of weather on the layering and cutting of this species. With the aim of sustainable management of *Carapa procra*, it seems important to conserve the populations of the plant, particularly those in the surveyed area, with the support of local populations. Investigations on *Carapa procra* should be deepened and supported because of the threat to the species due to the pressure of human activity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest related to the publication of this article

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