GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(Research Article)

Comparative analysis of three vegetative propagation techniques of *Rhizophora racemosa* G.F.W. Meyer for ex-situ conservation

Chantal Pasua Nzambi TSHIABA [1,*], Alexandre Ntumbula MBAYA [1,2], Assumani Zabo IDRISSA [1], Antoine Djamba MUMBA [3], Sébatien Ndiku LUYINDULA [2] and Raphaël Kalima Nkoma MWANGE [1]

[1] *Department of Biology, National Pedagogical University (UPN), Kinshasa, Democratic Republic of Congo.*
[2] *General Commission for Atomic Energy/ Regional Center for Nuclear Studies of Kinshasa,*
[3] *Faculty of Agronomic Sciences National Pedagogical University (UPN), Kinshasa, Democratic Republic of Congo.*

GSC Advanced Research and Reviews, 2023, 17(01), 072–087

Publication history: Received on 16 August 2023; revised on 27 September 2023; accepted on 29 September 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.17.1.0374

Abstract

Context: Mangroves are threatened by human activities and climate change which affect their biotopes. For the sake of their preservation, this prospective study of the adaptation of *Rhizophora racemosa* from the Marine Park of Mangroves (PMM) in the Democratic Republic of Congo was conducted on the sandy soils of Kinshasa, more than 400 km from its natural site.

Methods: Three vegetative propagation techniques, including transplanting wildlings, germinating propagules and sowing propagule cuttings, were tested in pots. The experimental trial lasted six months.

Results: The transplanted wildlings barely survived the end of the trial (20%). The immature and mature propagules recorded the germination rates of 99.5% and 100% respectively. The seedlings resulting from this vegetative propagation experienced good growth and development after 6 months of cultivation. The use of propagules and their cuttings is thus the most recommended technique for transplanting *R. racemosa* G.F.W. Meyer into the soil of Kinshasa.

Conclusion: The domestication of *R. racemosa* G.F.W. Meyer *ex situ* based on propagule cuttings offers the possibility of ecologically relocating this plant species potentially threatened with extinction and thus ensuring its conservation.

Keywords: Mangroves; *Rhizophora racemosa* G.F.W. Meyer; Domestication; Wildlings; Cuttings; Propagules

1. Introduction

Mangroves, communities of tropical plants evolving in the intertidal zones located between the sea and the continental lands, are of great ecological and economic importance. They serve as buffers against flooding and climate change linked to rising sea levels [1]; [2].

Among the plant species of mangroves, mangroves play an essential role in the functioning of the ecosystem, the balance of our planet and the maintenance of marine biodiversity. Exposed to almost permanent immersion in marine areas, these species develop rhizophores allowing the plant to breathe [3].

In the Democratic Republic of Congo (DRC), mangroves are part of the protected area of “Parc Marin des Mangroves” (PMM). Located on the Atlantic coast in the west of the country, they constitute the country's marine ecosystem. Six plant species are mainly inventoried there: *Rhizophora racemosa* G.F.W. Meyer or Red mangrove, *R. mangle* or Red

* Corresponding author: Chantal Pasua Nzambi TSHIABA.

mangrove, *Avicennia germinans* or Black mangrove, *Laguncularia racemosa* or White mangrove, *Conocarpus erectus* or Gray mangrove, and *Acrostichum aureum* L. or false mangrove [4].

Every year, this area is frequented by various species of migratory birds. It hosts breeding grounds for fish, crustaceans, turtles and marine mammals. Mangroves support a variety of fish and shellfish resources [5].

The exploitation of mangroves results in exacerbating deforestation. Mangrove forests have suffered a significant reduction of at least 25% of their natural extent [6].

In addition, oil exploitation concentrated on the coast is responsible for the emission of liquid, solid and gaseous effluents. It constitutes not only an additional source of destruction of habitats and species, but also a danger to human health [7]; [8].

Also, the acceleration of urbanization all along the coastal fringe leads not only to the erosion of the coastline, but above all to the destruction of most of the fragile sites, which are nevertheless important from an ecological point of view, in particular the spawning grounds of sea turtles and migratory birds.

The numerous human activities in the PMM have caused the retreat of the mangrove forest, the reduction of the population of turtles and manatees, the destruction of the natural habitat and the deterioration of the quality of the natural resources [9].

Actions in favor of the restoration of mangroves are developed through individual or collective initiatives, studies and scientific programs and specific and life-saving activities of various national and international NGOs [9].

To date, the ex-situ conservation of plant genetic resources is mentioned among the mechanisms for combating climate change.

However, mangrove plant species are hardly cultivated outside their natural salinized biotopes.

Notwithstanding the assertions of the aforementioned, the present study aims to explore the possibility of ex-situ domestication of *R. racemosa* G.F.W. Meyer in the pedoclimatic conditions of the City-Province of Kinshasa by the transplantation of wildlings, the germination of mature and immature propagules and propagules cuttings.

2. Material and methods

2.1. Experimental site

This test was entirely conducted on the site of the Regional Center for Nuclear Studies of Kinshasa (CREN-K) at the General Atomic Energy Commission of DRC between January and July 2018. The site is located 430 m altitude, 15°18'N longitude and 4°30'S latitude. The average temperature that prevailed during the experimental period was 26.06°C against 76.7% relative humidity.

2.2. Soil collection and potting

The potting soil came from the CREN-K undergrowth. It was taken between 0 and 30 cm deep. Of ortho type arenoferralsols, its physico-chemical characteristics are as follows:

- pH (H20) 5.49;
- C = 1.39%;
- N = 0.08%;
- Ca = 1200 meq/100g;
- Mg++ = 0.390meq/100g;
- C/N = 17.375;
- P= 4.1 ppm.

Although sandy, the soil of CREN-K is sufficiently rich in organic matter with good water retention capacity.

The soil sampled was transferred directly into 10 L plastic pots and/or bags. The manual filling of the pots and bags was carried out in such a way as to ensure a regular compaction of the soil avoiding, on one hand, a strong compaction of the soil and, on another hand, the creation of large air pockets in the substrate used. Large pebbles and other bulky residues were removed manually. A small space has been left above each pot to facilitate watering.

For the whole experiment, a total of 1,155 pots were required. These were stored next to each other in an open, sunny space under ambient weather conditions. The distance between the rows of pots was 20 cm, and that between 2 pots in a row was 10 cm. The water for the seedlings came either from the tap or from the rain.

2.3. Origin, harvest and packaging of plant material

The plant material used was collected at the parking point called "Kilometer 5", on the Atlantic coast of the Kongo Central Province in the DRC PMM (Fig. 1), about 400 km away from Kinshasa.

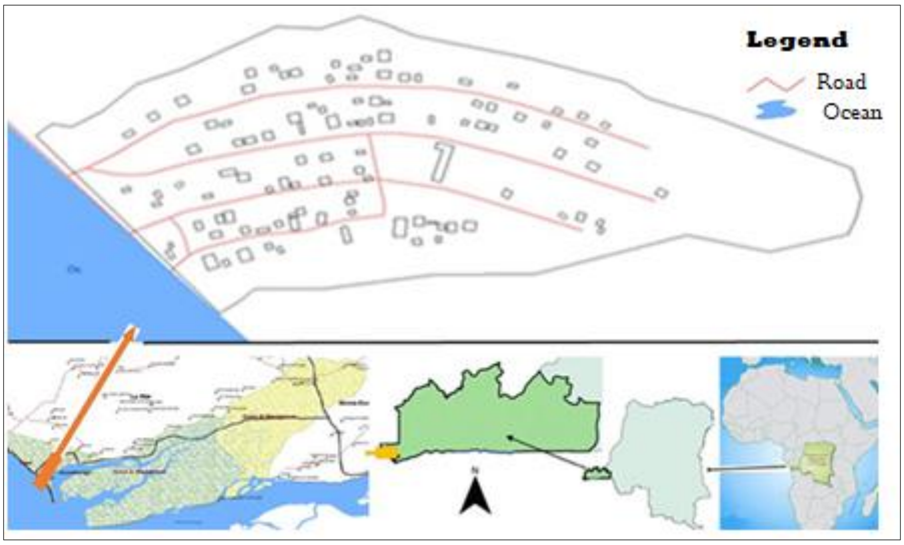

Figure 1 Sampling site for *R. racemosa* wildlings and propagules at Kilometer 5 in Kongo Central Province

The plant material (Fig. 2) consisted mainly of wildlings (seedlings from the natural regeneration of propagules fallen to the ground and growing around the mother plants of *R. racemosa* G.F.W. Meyer) and propagules (mature and immature) harvested manually under or on the feet of *R. racemosa* G.F.W. Meyer.

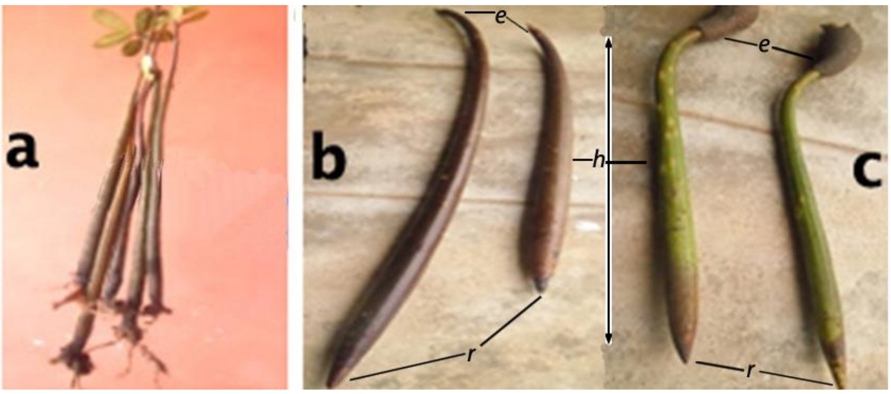

Figure 2 *R. racemosa* G.F.W. Meyer plant material used

Legend: (a) Wildlings; (b) mature propagules; (c) immature propagules [e: Propagule epicotyl, h: Propagule hypocotyl; and r: propagule radicle.

The physico-chemical parameters, taken from each harvest site using a multi-parameter probe (Combo Hanna HI 98130 type), showed the following average values:

- pH 7.54.

- Temperature 29.1°C;
- Dissolved oxygen 2.24 mg/L and
- Conductivity 43.3 Ms/cm.

The wildlings were carefully pulled out of the ground to minimize stress on the roots and stems of the seedlings. Only plants that had a straight habit, a rigid stem, without wounds or necrosis, were harvested.

Particular attention was thus focused on the aspects of good seedling growth:

- Green leaves, testifying ~~to~~ good photosynthetic activity;
- Apical buds in good condition, likely to guarantee good growth in height, and
- Abundant root system, a sign of good mineral and water nutrition of the seedlings.

Mature and immature propagules were selected based on their stiffness and size (at least 50 cm long).

Precautions were taken to cause as little physical damage as possible to all plant material harvested throughout its transportation to Kinshasa. Thus, to avoid bruising and drying out along the way, root parts of wildlings and propagules were first immersed in water at room temperature for 48 hours before moving. The seedlings were then tied in bunches, with the root parts placed in the black plastic bags and the aerial parts kept in the open air. The trip took about 12 hours. As for the propagules, they were entirely packaged in the black bags.

Upon arrival in Kinshasa, the boots were untied; the seedlings separated and spread on the ground under the shade, then carefully sorted. Root moisture was checked. Damaged, twisted or very long seedlings were cut with scissors. The wildlings were then submerged in tap water.

In total, the *R. racemosa* G.F.W. Meyer plant material selected for the experiment after sorting consisted of 200 wildlings and 680 propagules (including 340 mature and 340 immature).

2.4. Treatment, potting and growth of wildlings

The 200 wildlings selected were, 12 hours after their sorting, transferred to pots containing the CREN-K soil and prepared as indicated above. Each pot received a wildling. Its stem was set well vertically, the collar left uncovered at ground level, and the roots neatly spread out in the soil of the pot. The seedlings planted in this way were placed on a sunny and well-drained platform [10].

This trial of acclimatization of *R. racemosa* G.F.W. Meyer wildlings on Kinshasa soil was conducted for 6 months. The following parameters were analyzed on the wildlings:

- Size ;
- Root-collar diameter;
- Number of leaves;
- Stem branching;

2.5. Treatment, potting and monitoring of propagules germination and seedlings growth

The batch of 680 propagules (mature and immature) was germinated in pots, the soil of which was previously moistened with tap water. Direct sowing was carried out respecting the polarity of the propagule (Fig. 3).

Figure 3 Sowing propagules (mature and immature) of *R. racemosa* **G.F.W. Meyer** in pots

Pots were watered daily until the propagules germinated. Germination monitoring consisted of monthly sampling of the number of propagules that had germinated. To do this, 20 randomly selected propagules were regularly sacrificed to assess the elongation of the propagule radicle and thus estimate the germination rate of the propagules. At the same time, the growth in height and thickness of seedlings from germination was determined based on the root-collar diameter and the size of the stem since the appearance of the first leaf on the hypocotyl of the propagule. Seedling development was also assessed by periodically counting leaf and node number as well as the gradual appearance of branches on the growing plant stem.

Other parameters were taken into account to evaluate the growth and development of seedlings in this trial, such as the:

- Latency time [11]
- Velocity coefficient (Cv) [12];
- Average germination time[13];
- Germination index [14].

Faded plants were regularly eliminated from the experiment. Manual and regular weeding of the pots served to control weeds invasion.

2.6. Treatment, potting and growth of propagule cuttings

Fifty-five additional propagules of *R. racemosa* G.F.W. Meyer were used for the preparation of the cuttings. Each propagule was sectioned into 5 fragments (Fig. 4) of 10 cm each, including:

- 1 apical (epicotyl) cutting;
- 3 medial (hypocotyl) cuttings, and
- 1 basal (root) cutting.

A total of 275 cuttings for the set of propagules were, thus, recorded, including 55 apical, 165 medial and 55 basal.

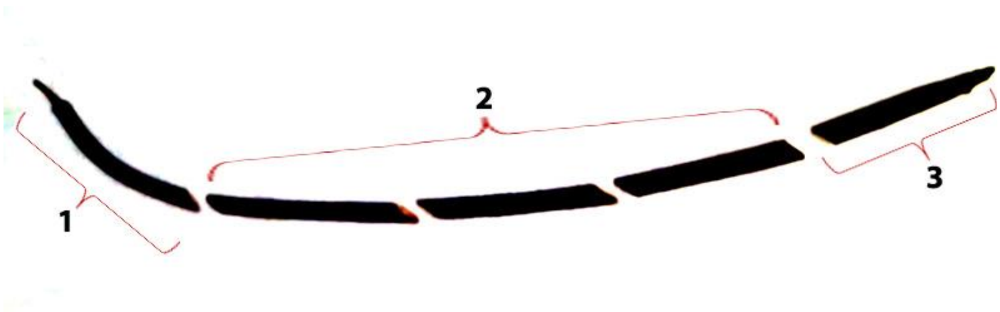

Figure 4 Cuttings from one propagule

Legend: 1. Apical cutting; 2. Medial cuttings; 3. Basal cutting

Before potting (Fig. 5), respecting the polarity, all the cuttings were cleaned with running water and "Nobacter" liquid to possibly sanitize the cuttings.

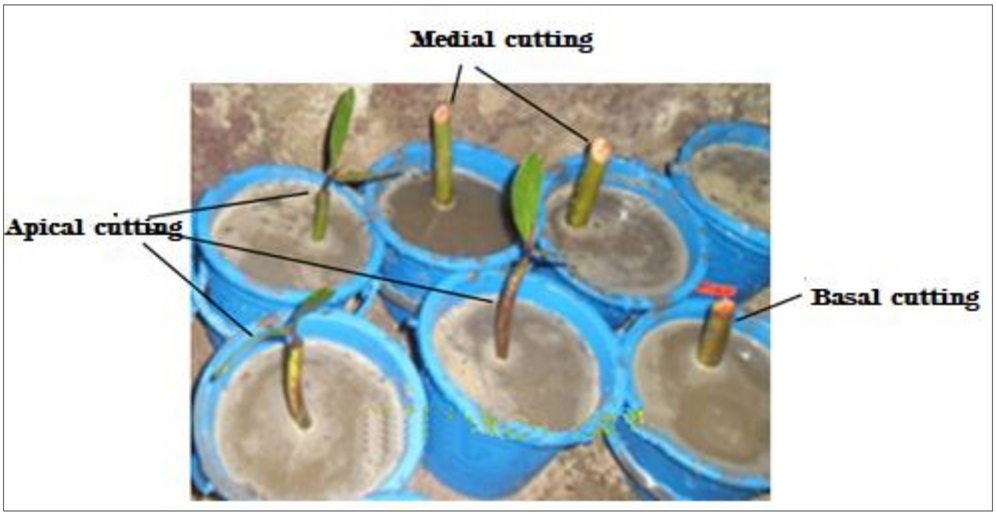

Figure 5 Potting of *R. racemosa* G.F.W. Meyer propagules cuttings

Monitoring and evaluation of root and stem release from cuttings were conducted for both mature and immature propagules.

3. Results

3.1. Transplantation and acclimation of wildlings

Table 1 Acclimation (in %) of wildlings of *R. racemosa* G.F.W. Meyer in the nursery

	Number	%
Transplantation	200	100
Acclimation	40	20

Table-1 shows that, out of a total of 200 transplanted wilding plants, only 40 plants (or 20%) barely survived in Kinshasa soil (CREN-K) for 6 months after planting. Throughout the trial, the wildings exhibited poor growth and poor development.

In addition to the reduced size observed, these wildlings remained stunted. The leaves showed necrosis in several places and obvious signs of nutrient deficiencies (yellowish color of the leaves). Internodes were short on the stems and leaf loss was recurrent, regularly leading to the death of affected seedlings. The wilting rate was evaluated at 80%. Figure 6 clearly shows that if the experiment had continued beyond 6 months, all the seedlings would probably not have survived.

Figure 6 Growing *R. racemosa* wildlings in Kinshasa Soil

3.2. Germination of mature and immature propagules

The propagule germination results are shown in Table 2.

Table 2 Germination rate of *R. racemosa* propagules

Propagules	**Number**	**Germination**	**%**
Matures	340	340	100
Immatures	340	338	99.5
Total	*680*	*678*	

This table shows that, out of a sample of 680 propagules (mature and immature), 678 germinated, giving a germination rate of 100% for mature propagules and 99.5% for immature propagules.

Figure 7 illustrates the results on the germination rate through the germination curve of mature and immature propagules.

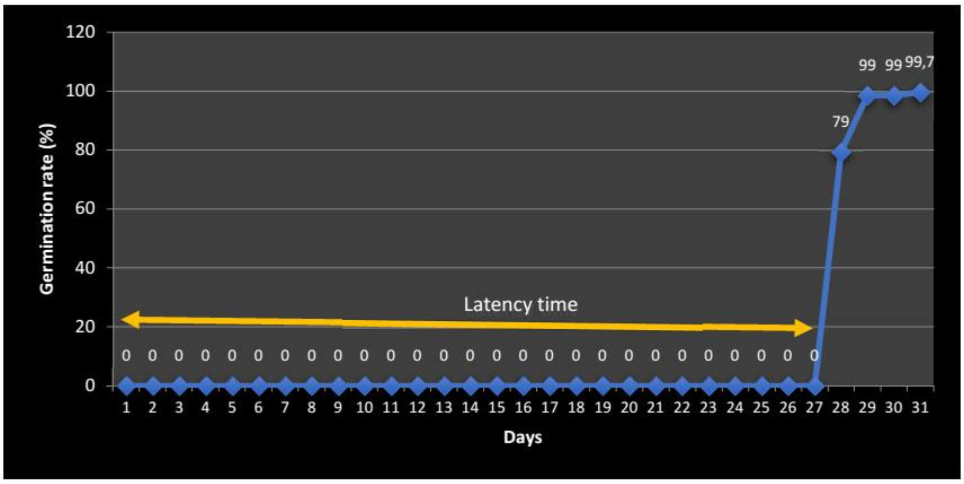

Figure 7 Propagule germination curve

Figure 7 shows that propagules experienced a latency time of 27 days in Kinshasa soil. Once started, germination reached a rate of 79% (540 propagules germinated) on the 28th day since their planting. On the 29th and 30th day, the germination rate is estimated at 99% before reaching 99.7% on the 31st day. The velocity coefficient is 3.54 while the germination index is 25.

3.3. Growth and development of seedlings from propagules

The monitoring of propagules growth was conducted over 6 months and focused on the measurements of the stem size and the root-collar diameter as well as on the counting of leaves and internodes. The related results are presented in Figures 8 and 9. The seedlings resulting from the germination of the propagules (mature and immature) continued their growth until the branching stage, which occurred 5 to 6 months after planting.

The growth and development of the seedling from the germination of propagules in Kinshasa soil (CREN-K) began, as for most seeds, with rhizogenesis followed by caulogenesis, respectively on the side of the radicle (basal pole) and that of the epicotyl (apical pole) of the propagule. The aerial part of the seedling formed into a vertical axis, at the top of the propagule (Fig. 8a). At the level of the cauline terminal meristem, the beginning of organogenesis was characterized by the appearance of two simple and opposite leaves.

The process evolved by the establishment of an internode as well as two buds with opposite leaves. The initial phase of axial growth was followed by the bud burst of axillary buds in the leaf axils. The modification of the seedling stem continued with the branching of the main axis from the axillary buds (Fig. 8c and d). This branching was observed at the end of the 5th and the beginning of the 6th month after propagules sowing.

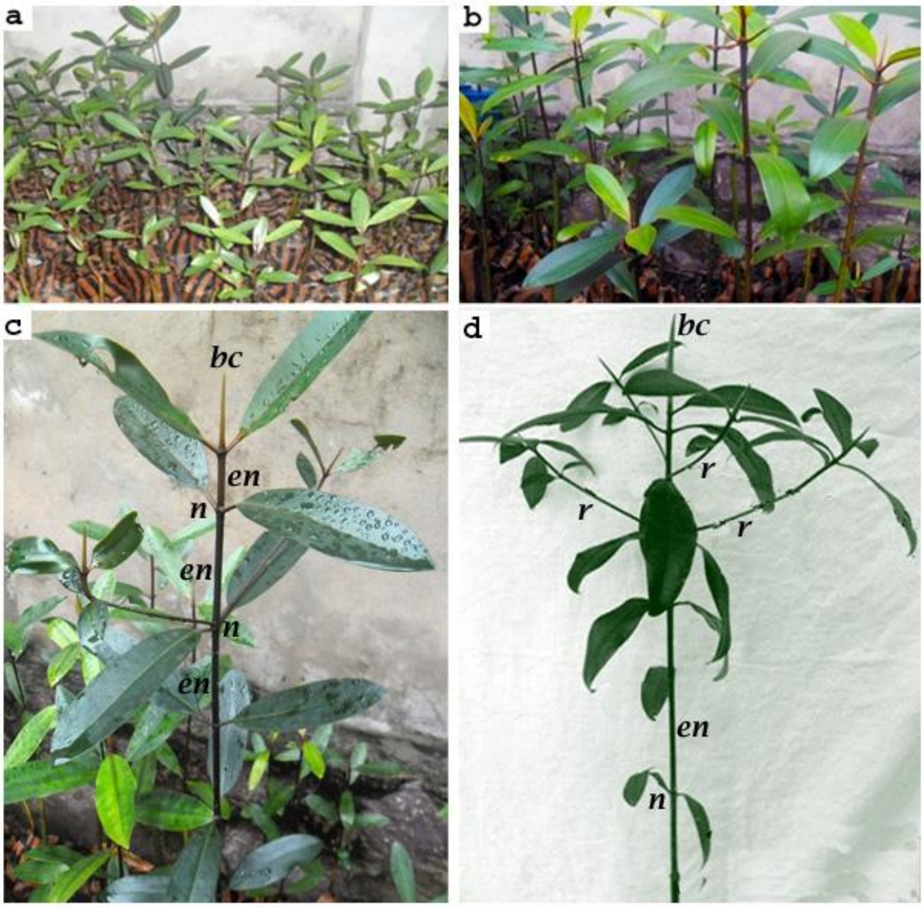

Figure 8 Seedlings resulting from the germination of mature and immature propagules

Legend: (a) 2 months; (b) 4 months; (c) 5 months (bc) cauline buds; (en) internodes; (n) nodes; (r) early branching of seedlings of *R. racemosa*.

Ultimately, the young plant of *R. racemosa* G.F.W. Meyer presented an arborescent stem. The cauline bud, narrow and pointed, is located at the apex; the lateral and dormant bud is found in the axil of each leaf in the form of a tiny button.

It is likely to initiate a branch from the nodes. The internodes are about 10 cm long and roughly equal to each other. The monocaule phase, lasting 5 months, consisted of building-up the internodes. Stem branching occurred six months later, thanks to the axillary buds developed throughout the seedlings growth (Fig. 8d). Twigs or branches are monopodial, with plagiotropic growth direction (hypotonia) and alternate distichous phyllotaxis.

The size of propagules-derived seedlings (Fig. 8a) increased gradually from the 1st to the 4th month, with a regular rate of about 6 cm each month, thus increasing from 6.98 to 25.62 cm long. The size practically doubled between the 4th and 5th month, reaching 47 cm in height. At the end of the experiment, seedlings reached an average height of 51.45 cm.

Height increase was proportional to that of the root-collar diameter (Fig. 8b), as well as to the number of leaves (Fig. 8c) and nodes (Fig. 8d) of *R. racemosa* G.F.W. Meyer seedlings. The evolution of the collar diameter was on average 0.65 cm each month. After 6 months, the seedlings have reached a diameter of about 4 cm. The number of leaves increased over time, reaching the value of 14 leaves, which remained constant until the 6th month. The number of pairs of nodes also increased with the age of the plant and reached its peak in the 5th month, averaging 7 nodes per seedling.

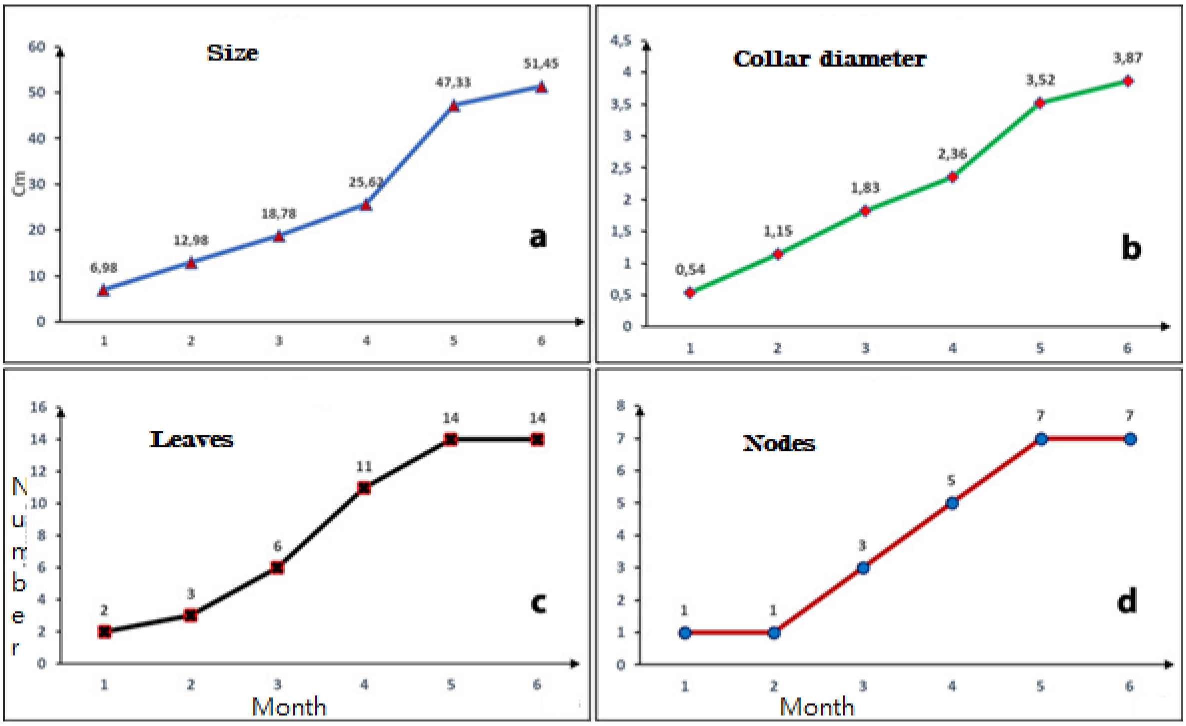

Figure 9 Growth of seedlings from *R. racemosa* propagules

The growth and development of *R. racemosa* G.F.W. Meyer thus followed a precise and ordered sequence of differentiations, during which the repetition of homologous elementary entities (nodes and internodes) was accompanied by a coordinated evolution of their morphological characteristics.

3.4. Growth and development of seedlings from propagule cuttings

The cuttings of the propagules were cultured in pots in order to study their capacity for regeneration and to consider their use as seeds for the dissemination and ex-situ conservation of *R. racemosa* G.F.W. Meyer.

3.4.1. Morphogenesis (rhizogenesis and caulogenesis)

The sequential change of sprouts on the different cuttings was followed over time since their planting. Organogenesis began with rhizogenesis, followed by caulogenesis, respectively at the basal pole and at the apical pole of each cutting (Fig. 10).

For the apical and median cuttings of the propagules, rhizogenesis was observed at the edge of the cut parts embedded in the soil (Fig. 10A and Fig. 10B). This involved the dedifferentiation of propagule cells in contact with the soil through the process of plant cell totipotency. For the basal cuttings, the roots appeared in the normal root regions (Fig. 10C).

The roots obtained from the basal cuttings of the propagules (Fig. 10C) were more abundant than those that appeared from the apical and middle cuttings (Fig. 10A and Fig. 10B).

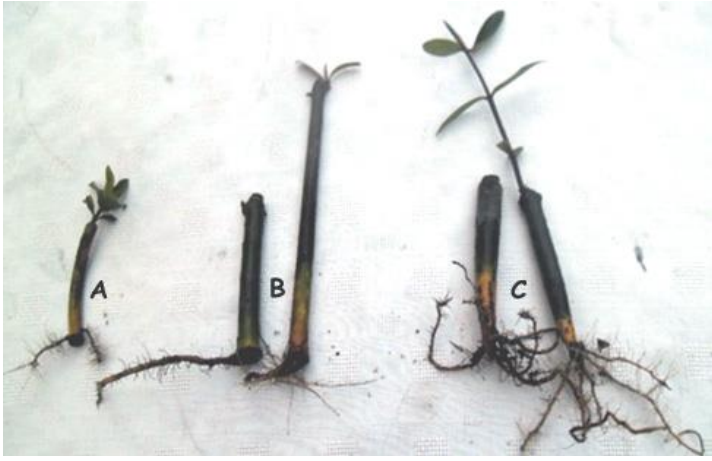

Figure 10 Rejection of *R. racemosa* propagule cuttings

Legend : (A) Apical cutting; (B) Middle cuttings; and (C) Basal cuttings

The aerial part of *R. racemosa* G.F.W. Meyer seedling formed in the form of a vertical axis from the epicotyl tip of the apical cutting, on the one hand, and all around the healed part at the top of the middle and basal cuttings propagules, on the other hand (Fig. 11A).

The caulogenesis of the median and basal cuttings was characterized by the appearance of a multitude of calluses on the healed part (Fig. 11b), some of which subsequently transformed into shoot buds or meristems with two leaves simple and opposite (Fig. 11c). The process of growth and development continued with the development of buds and the establishment of the first internodes (Fig. 11d), then, over time, several internodes (Fig. 11e) appear with the stem elongation. The axial growth phase continued with the budburst of axillary buds in the leaf axils. These will cause a significant change in the habit of the seedling, as they will be the basis of the branching of the main axis observed from the end of the 5th month of growth.

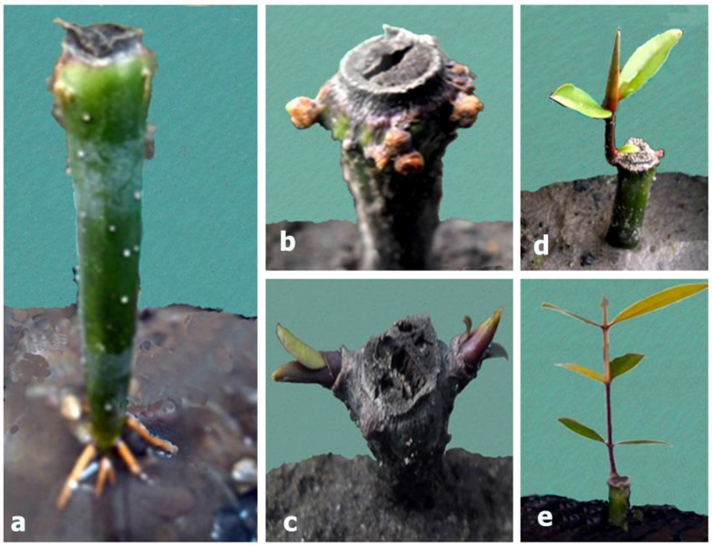

Figure 11 Sequential growth and development of the architectural unit of seedlings from basal cuttings of *R. racemosa* G.F.W. Meyer propagules

Legend: a: Rooting; b: callus budding; c: appearance of stem buds; d: cauline rejection; e: elongation of the seedling.

3.5. Propagule Cuttings Rejection Parameters

The results for the different cutting rejection parameters are summarized in Table 3.

Table 3 Latency time and quantitative evaluation of releases of propagule cuttings of *R. racemosa*

Fragments or cuttings	Days after sowing	Average time for rejection of cuttings (days)	Number of cuttings having rejected	Total number of rejections	Number of cuttings planted	Rejection rate
Apicals	26	33	11	21	55	38.2%
	35		6			
	45		4			
Medians	30	48	34	98	165	59.4%
	45		21			
	60		29			
	65		9			
	70		5			
Basals	45	61	3	37	55	67.3%
	60		24			
	65		10			
			Total	156	275	56.7%

Overall, the success rate of germination of propagule cuttings is quite low, about 56.7%. Quantitative evaluation of the germination of cuttings indicates that fragments from the basal part of the propagule recorded a higher rejection rate (67.3%), followed by the middle part (59.4%). The germination of the apical part was the weakest (38.2%).

Table 3 also shows that the first appearance of cauline bud shoots on propagule cuttings was noted respectively at 26 days after potting the cuttings for the apical fragments, 30 days for the middle fragments and 45 days for the basal fragments.

The most active period of rejection of the apical cuttings was observed on the 26th day after their planting with 11 cuttings germinated (20%) out of the 55 fragments used. For the middle cuttings, 84 out of 165 (about 51%) rejected between 30 and 60 days after sowing. A very low frequency of sprouts was recorded in general on the basal cuttings. Indeed, the first appearance of shoots, which occurred 45 days after sowing, only concerned 3 cuttings (5.5%). About fifteen days were required to obtain the highest rejection rate (43%) from basal cuttings.

Table 4 The rejection indices and the velocity coefficients of different cuttings of the propagules.

Fragments	Apicals	Medians	Basals
Rejection index	7.84	24.36	8.84
Velocity coefficient (%)	3.1	2.11	1.66

Table 4 shows that the middle cuttings have the highest rejection index (24.36). They are followed by basal (8.84) and apical (7.84) cuttings. As for the velocity coefficient, the highest percentage is that of apical cuttings (3.1%) followed by median (2.11%) and basal (1.66%) cuttings.

The rejection trend of cuttings from *R. racemosa* G.F.W. Meyer propagules is best illustrated in Figure 12.

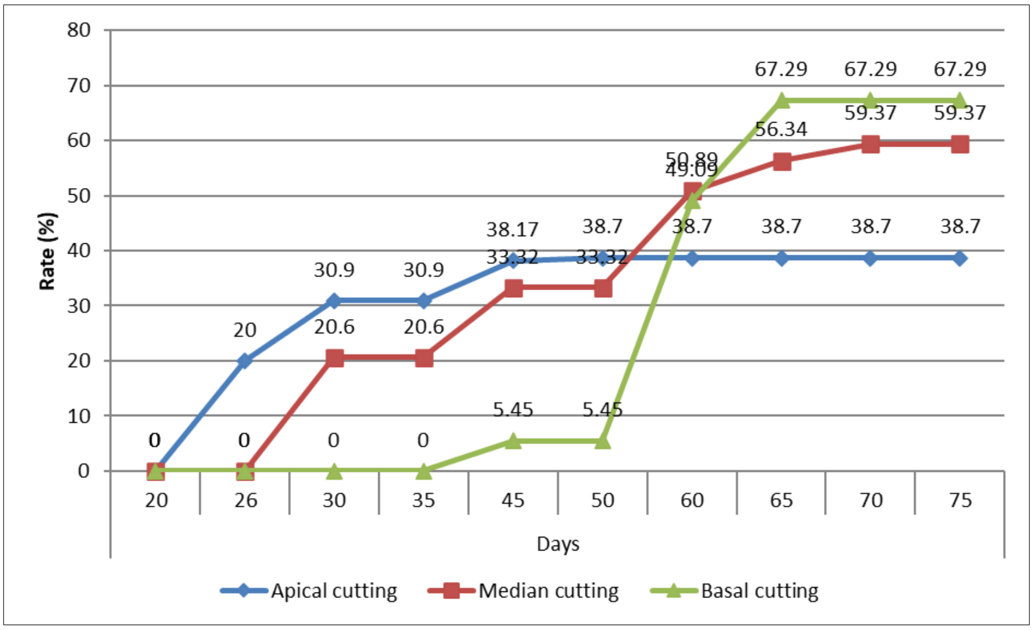

Figure 12 Resumption rate of mitotic activities of different propagules cuttings

The analysis of Figure 12 reveals that the resumption of mitotic activities observed on propagule cuttings varies according to the cutting type:

• **Apical cuttings** initiate buds early (20th day). The percentage of cases increases until reaching the maximum level of 38.7% on the 45th day.

• Bud initiations on the **median cuttings** appear on the 26th day. On the 70th day, the maximum number of recorded cases is estimated at 59.37%.

• **Basal level cuttings** start up late (35th day), but the buds continue to be initiated until reaching 67.29% of cases on the 65th day.

4. Discussion

4.1. Domestication of *R. racemosa* in Kinshasa environments using wildlings

The present study shows that, out of 200 transplanted wilding plants, 80% withered after 6 months in pots. Thus, the attempt to transplant wildlings of *R. racemosa* G.F.W. Meyer from Moanda did not give satisfactory results. After 6 months of growth, the wildlings showed a necrotic appearance, the leaves dried out and fell easily over time. Only 20% of them survived the pedoclimatic conditions of Kinshasa. Most of the wildings eventually withered away and the few that were able to survive did not show good growth.

At the time *R. racemosa* G.F.W. Meyer wildlings were pulled out from the soil in their natural biotope (PMM), they have already begun the physiological process of growth and installation in the pedoclimatic conditions of the mangroves. Moved to Kinshasa's soil, they could not withstand the post-harvest traumas (root incision, long-distance transportation, water and nutrients stress, ...) they faced and easily adapt to the new environmental conditions of Kinshasa.

This confirms and completes findings of Rakotondrazafy [15]. Considering the transplantation of wildlings as a second technique for the reforestation of the mangrove, this author suggests that roots must not be destroyed or incised. He suggests transporting them with clods of earth or balls of mud. A prospecting effort and special attention must be provided at the time of collection when it comes to wildlings.

Thus, the use of wildings of *R. racemosa*, taken directly from their growth media in the PMM mangroves for their transplantation into new media in the purpose of conservation of this species, is a procedure, not to be avoided, but to be carried out with enormous precautions, in order to prevent causing the transplanted seedlings both physical and physiological trauma.

4.2. Domestication of *R. racemosa* in Kinshasa environments using mature and immature propagules

In terms of propagule germination and seedling growth from these seeds, the quantitative assessment demonstrated that the propagules were, in general, viable in Kinshasa's soil. After breaking dormancy, the results show that:

- Propagules record a high germination capacity (99.7%);
- Shoot height gradually increases in the 1st 4-month period from 7 to 25.62 cm, then reaches 51.45 cm after 6 months of growth;
- Seedlings root-collar diameter increases from the first to the sixth month. After 6 months, it is about 3.9 cm;
- Leaves number increases with time, from 2 the first month to 14 after 5 months;
- Axillary buds organogenesis of in the axils of leaves is evident through the budburst which initiates young shoots. The number of pairs of nodes increases with the age of the plant and reaches its peak in the 5th month.

The architectural expression of *R. racemosa* G.F.W. Meyer in the nursery shows that its development is punctuated by a succession of phases which reflect modifications due to the functioning of meristems during ontogeny [16]; [17].

The plant goes through several phases during its life [18]. Growth of seedlings in the nursery is continuous. The meristems function permanently. Seedlings continually create new organs. The seedlings grew in an unusual environment. This agrees with Ameziane and Persoons [19] who stipulate that the growth of an entire plant (or of a plant cover) involves two concomitant phenomena:

- The growth *stricto sensu* in dimension of each of the organs after their initiation:
- The multiplication of the number of these organs in connection with development.

Seedlings grow in length, as their apical meristems create and develop growth units. They ramify following the lateral axes which develop [20].

Growth of *R. racemosa* G.F.W. Meyer is normal under totally desalinated conditions. The species is described as an optional halophyte [21].

The growth of *R. racemosa* G.F.W. Meyer in the nursery is normal since the plant adapts well to it. Good quality seedlings from the nursery are critical to obtaining quality trees in the field for afforestation [22].

4.3. Domestication of *R. racemosa* G.F.W. Meyer in Kinshasa environments using propagule cuttings

Experiments on the multiplication of *R. racemosa* G.F.W. Meyer by cuttings showed that the ability to shoot (stems and roots) was better expressed in basal fragments (67.27%) than in median fragments (59.39%). The apical fragments recorded the lowest rate (38.18%) of caulogenesis and rhizogenesis. The maturity of the shoots and the physiological condition of the cuttings affect root formation. The lower part of the propagule contains a large store of nutrients. Therefore, it can easily form roots and resist the longest.

The propagule has the ability to produce buds. Its cuttings developed leafy axes and new roots. These results are in line with those obtained on other woody species where a woody fragment is enough to generate another foot [23].

There is then a cellular dedifferentiation at the level of a meristem, beginning with a somatic embryogenesis then a regeneration of certain organs of the plant. This phenomenon, involving the primary meristem, also applies to natural propagules.

Propagule cuttings resumed mitotic activities. Their resistance capacity is related to their location on the propagule. Basal cuttings are more resistant than middle and apical ones. As for many other woody plants, Poirier [24] confirmed that starting from a segment of the plant, one can regenerate the whole plant; R. racemosa also offer this possibility. Fragments of its propagules can constitute a seed lot for afforestation.

The latency time of the apical and median fragments approaches that (26 days) of the whole propagules. Basal cuttings took almost twice as long as other fragments to sprout.

Some propagule fragments successfully regenerate new seedlings under ambient conditions. The results of this work complete the list of root cuttings work in greenhouses or outdoors, as carried out by Vanstone et al. [25] in the vegetative propagation of woody plants.

Rooting of cuttings in this study shows that *R. racemosa* propagules have genetic predispositions for this regeneration pathway. This study completes the results of other researchers on the different modes of propagation of *R. racemosa.*

Vegetative propagation demonstrates that *R. racemosa* possesses the ability to regenerate through cuttings. This confirms the hypothesis of Tchoundjeu *et al.*: "*Vegetative propagation can be useful to researchers working on domestication to assess the potential of some trees from selected populations*" [26].

5. Conclusion

The exploitation of the mangrove has become a worrying problem due to its scale. There is an urgent need to carry out afforestation/reforestation in order to preserve the floristic biodiversity of marine plants, including *Rhizophora racemosa* G.F.W. Meyer. Whether for reforestation or for agroforestry, the nursery is the basis of successful tree planting.

The application of vegetative propagation techniques (transplantation of wildlings, sowing and cuttings of propagules, somatic micropropagation of *Rhizophora racemosa* G.F.W. Meyer) made it possible to obtain in the nursery, for six months, a good number of seedlings, germinated and transplanted into pots and observe them for a quantitative assessment of adaptation rate as well as that of natural off-site growth.

Through various attempts undertaken to analyze growth and development by mathematical models, it is possible to predict the nature of a process that could be verified experimentally. These simple models could allow to describe the plant growth process, but can not account for the qualitative modifications and explain the differentiation. Nevertheless, the mathematical analysis provided information on the nature of the growth process and made it possible to follow the evolution of the seedlings under unusual conditions.

The strong germination capacity of the propagules, the architectural physical expression of seedlings of *Rhizophora racemosa* G.F.W. Meyer as well as its evolution in an ex-situ environment for six months, in the nursery, gave good yields. The cuttings of the propagules have sufficiently demonstrated their capacity for organogenesis. The studied species reacts favorably to all the vegetative propagation techniques tested to varying degrees. Taking this observation into account, it can be stated that *R. racemosa* G.F.W. Meyer domestication outside its natural biotope is possible.

Thus, given the rapid decline of mangroves due various reasons (sea pollutions, climate change, ...), human action must be directed towards:

- Reforestation for the recolonization of spaces;
- Afforestation by creating new seed-providing plantations, in areas far from the Mangrove Marine Park.

For a successful transfer of R. racemose in Kinshasa's soil, promising vegetative propagation techniques include the use of :

- mature propagules (100%)
- immature propagules (99.5%)
- propagule basal fragments (67.3%)
- propagule middle fragments (59.4%)
- propagule apical fragments (38.18%)

Compliance with ethical standards

Acknowledgments

We thank everyone who participated in this study, particularly the agents of the Congolese Institute for Nature Conservation (ICCN) for their collaboration in the collection of cuttings and propagules. We would like to thank the Chemical Laboratories of the Regional Center for Nuclear Studies of Kinshasa (CREN-K) at the General Atomic Energy Commission of DRC for supporting us in carrying out this study.

Disclosure of conflict of interest

There is no conflict of interest be the authors of this manuscript. Then, authors have declared that no competing interests exist.

References

[1] FAO. *The World's mangroves 1980-2005*, FAO Forestry Paper 153, Food and Agriculture Organization of the United Nations, Rome (Italy), (2007) 89p.

[2] Tomlinson, P.B. *The Botany of mangroves*. Cambridge University Press, Cambridge (UK), (2016) 413 pp

[3] Feuillassier L et Rognant A. *Les Mangroves.* Dossier Pédagogique 2019-2020. Oceannopolis, Brest. Ecosystems& Co, (2020) pp 60

[4] Gordon C. *Principles for a Code of Conduct for the Sustainable Management of Mangrove Ecosystems*". Centre for African Wetlands. (2005) http://mit.biology.au.dk/cenTER/MCB_Files/Ramsar/2005_Ramsar_Arusha_V2.

[5] Japanese Forest Technology Association (JAFTA). *Study for a sustainable management of the mangrove of the small coast and delta of the Saloum of the Republic of Senegal plan of sustainable management of the mangrove.* Final report, Japan Agency for International Cooperation (JICA)(2015).

[6] Journal Le Monde, *Marine ecosystems at the mercy of human activities* (2019) (https://www.lemonde.fr/planete/article/2019/05/06/les-ecosystemes-marins-a-la-merci-des-activites-humaines_5458820_3244.html#:~:text=Estuaires%2C%20 deltas%2C%20marais%20salants%2C,lieux%20expos%C3%a9s%20aux%20vagues%20submersives), consulted le 30 juillet 2022.

[7] Usha Mukunda. In: *Conservation Of Mangrove Forest Genetic Resources* A Training Manual Edited By Sanjay V. Deshmukh And V. Balaji © Crsard (1994); https://unfccc.int/files/adaptation/napas/application/.../10_demorc_pp.pdf

[8] MECNEF. . *DRC Coastal Area Profile, Kinshasa* (2000)

[9] MECNEF. *Profile of the Coastal Zone of the Democratic Republic of Congo.* Ministry of Environment, Nature Conservation, Water and Forests. National Project Management (GEM – CG/ GCLME) / RDC. Kinshasa (RDC)(2007),

[10] Fogefor A. *Selected species, environmental conditions, relief and topography, infrastructure.* The Plantation Session of 21/10/2005 Planting Techniques; technique to be retained is based on 5 Criteria: Planting Techniques, area to be planted (2005).

[11] Meyer S, Reeb C et Bosdeveix R. *Botany, biology and plant physiology. Moline (Ed.), Paris (France), (2004) 461pp.*

[12] Kotowski F. Temperature Relations to Germination of Vegetable Seeds. *American Society of Horticulture Science Proceedings* (1926) **23**: 176–184.

[13] Timson J. New method of recording germination data. *Nature* (1965) 207:216-217.

[14] Abbott DL. Temperature and the dormancy of apple seeds. *14th Intern. Horticult. Congo* (La Haye Sceveningen), (1955) 1, 746-753.

[15] Rakotondrazafy J. *Practical guide for community actions to restore mangroves: case of the tsiribihina delta,*, Manambolo Tsiribihina landscape (2022)

[16] Barthélémy D. *Architecture and sexuality in some tropical plants: the concept of automatic flowering. PhD thesis, University of Sciences and Techniques of Languedoc* -Montpellier II, Montpellier (France), (1988) 262 p.

[17] Barthélémy D, Caraglio Y, DrénouCh, Figureau C. *Architecture and senescence of trees*. Bulletin de Vulgarisation forestière. Forest Company (1992) 83 : 15-35

[18] Chopard J, Godin C, and Traas J. *Towards a formal expression of morphogenesis: a mechanics based integration of cell growth at tissue scale*. Proceedings of the 7th International workshop on information processing in cells and tissues, (2007) pages 387–399.

[19] Ameziane El Hassani T and Persoons E. (Eds) *Modern Agronomy: Agronomic and physiological bases of plant production.* Collection Universités Francophones, Edition Hatier, Paris (1995)

[20] Rey M, Rodriguez R, Cuozzo L and Ancora G. In vitro propagation of caper (Capparis spinosa L.). *In vitro cell Biol* (1990) 26:531-536.

[21] Stern WL and Voigt GK. Effect of salt concentration on growth of red mangrove in culture. *Biol Gazette* (1959) 9:36–39.

[22] Wightman KE, *Technical Manual No. 2: Good Growing Practices in Forest Nurseries: Practical Guidelines for Community Nurseries*, World Agroforestery Centre (ICRAF), Nairobi (Kenya) (1999).

[23] Zida, W. A., Bationo, B. A., Somé, A. N. and Bellefontaine, R. *Vegetative propagation by cuttings and aerial layering of three agroforestry species in Burkina Faso*. *VertigO*, (2018) 18(2).

[24] Poirier, J. *The multiplication of remarkable trees*, Botanical Garden of Montreal (2006),

[25] Vanstone, D. E. and Ronald W. G. Seed germination of American basswood in relation to seed maturity. *Can. J. Plant Sci*. (1982) 62: 709-713.

[26] Tchoundjeu, Z. De Wolf J. and Jaenicke H., Vegetative propagation for domestication of agroforestry trees. *AgroforestryToday*,(1997) 9 (2): 10 -12