Net Journal of Agricultural Science
Vol. 11(1), pp. 1-12, Februuary 2023
DOI: 10.30918/NJAS.111.23.003
ISSN: 2315-9766
Full Length Research Paper

Stem cuttings assessment for domesticating the medicinal melliferous plant, *Combretum micranthum* G. Don. in the Republic of Benin

Felicien Amakpe[1]*, Dirk C. de Graaf[2], Brice Sinsin[3] and Honore S. Biaou[4]

[1]Ministère du Cadre de Vie et du Développement Durable, Republic of Benin.
[2]Laboratory of Molecular Entomology and Bee Pathology, Gent University, Belgium.
[3]Laboratory of Applied Ecology, University of Abomey-Calavi, Cotonou, Republic of Benin.
[4]Laboratory of Forestry and Ecology, University of Parakou, Republic of Benin.

Accepted 6 February, 2023

ABSTRACT

Combretum micranthum is a medicinal and melliferous plant from the dry tropical regions where it is threatened with extinction and it urges to develop suitable methods for its sustainable domestication. The challenges of producing seedlings from the seeds of the species make stem cutting, a potential approach for its domestication and this research evaluated the possibility to use such cuttings for seedlings production at a nursery located in the northern part of the Republic of Benin by determining the most effective season to harvest the stem cuttings, the parts of the branch which could yield the best rate of vigorous seedlings and the optimal transplantation time. An experimental dispositive of 4 plots of 35 cuttings of 25 cm long of the categories named A, B, C and D from the base to the apex of the branch was repeated in two seasons. The first trial consisted of cuttings harvested during the end of the dry season and the second trial was carried out with cuttings that were harvested at the heart of the rainy season. The results showed that the dry season cuttings yielded the best vigorous seedlings rate (46% against only 6% for those harvested in the rainy season). Basal cuttings of the dry season had the best survival rate at 49% while apical cuttings from the same season had the best vigorous seedlings rate at 43% versus 23% for the basal cuttings. Intermediate cuttings yielded low vigorous plantlet rates. The optimum nursery time was 90 days regardless of the harvest season. Dry season stem cuttings, based on basal and apical cuttings were the best approach for domesticating *C. micranthum*. The technology did not require any root activator and the vigorous seedlings were available during the relevant reforestation rain season.

Keywords: Forestry, medicinal plant, melliferous plant, non-timber product, nursery, seedling, stem cutting.

*Corresponding author. E-mail: famakpem@hotmail.com. Tel: +22995062997.

INTRODUCTION

In most developing countries, where the ratio of endogen healers to population is far better than the ratio of conventional medical doctors to population, the community relies on traditional medicines for many diseases, including the ones that already have proven remedies. The dependence on traditional medicine is even more crucial that many national and international organisations, institutions, and universities include traditional and complementary medicines in their curricula to ensure, the safe uses of medicinal plants in Africa (WHO, 2011). A survey conducted in the Republic of Benin by Kouchade et al. (2016) proved that 272 plant species were used for healing 24 diseases and Tene et al. (2016) found that 46 plant species were used against malaria in Cameroon. Despite the tangible contribution of traditional medicine to the improvement of health standards in most African countries, side effects, overdoses, presence of allergens and toxic components, are usually reported to negatively impact vital organs such as the kidney, spleen, liver and even the brain and heart of many users. Such challenges which deserve great care compromise the adoption and wide use of medicinal plants in the world (Pal, 2002). Fortunately, many plants have proved to be side effect free, with limited toxicity. This is the case of *Combretum micranthum* which is widely used in Africa as a medicinal plant. In fact, according to Kpemissi et al. (2020), the plant extract is free of cyanide group compounds, and it is widely adopted to combat infectious diseases, diabetes, urinary retention, liver cancer and to prevent heart attack. All the plant organs, including green and dried leaves, roots, bark, flowers, and fruits are intensively collected from the wild and this leads to high pressure on the different ecological areas from where the plant is collected (Compaore et al., 2020; Tine et al., 2021).

Besides the medicinal value of *C. micranthum,* it is a melliferous plant that issues pollen and nectar to honeybees and many other pollinators in the entire tropical area. It is particularly appreciated by beekeepers as it blossoms during the dry season after the recurrent bushfires (Amakpe et al., 2015), and produces valued honey which is also highly solicited for the numerous medicinal values the community attributes to it. As such, promoting this plant will improve the community's livelihoods and contribute to the local economy and to the sustainable diversity and conservation of pollinators on which the whole food chain is based on earth (Klein et al., 2007; Le Conte and Navajas, 2008).

While *C. micranthum* is highly solicited for its medico-social and economic values and contributes to key ecological functions, it is not domesticated in West Africa. On the other hand, the natural regeneration of the plant is strongly limited by bushfires, drought, grazing and harvesting stress (Bellefontaine, 2005; Bationo et al., 2005; Dayamba et al., 2008). The plant is then likely to disappear in the near future with the numerous scientific, magico-social, and cultural knowledge that is associated with it if effective domestication methods are not developed. As far as the artificial production of the plant is concerned, Thiombiano (2003) and Amani et al. (2015) found that the germination rate of well-selected seeds may be good in a nursery. But in the entire Republic of Benin, the seeds are very rare, and difficult to harvest and most of them lose their germination capacity when it comes the time of sowing in a nursery. The reforestation of the species is then uncertain through seed germination which also requires too much time before transplantation (Amani et al., 2015). The seedlings obtained from seed germination are also too much disparate and this constitutes a great challenge for the genetic values and identity required for the effectiveness of the medicinal uses of the species (Thirunanvoukkarasu et al., 2004; Mohamed et al., 2014). It is then crucial to develop more effective vegetative propagation methods at the nursery that could also conserve the genetic traits of the species and help obtain uniform vigorous plants in a reasonable time for the domestication of this multipurpose plant.

Meyer et al. (2011), Pereira et al. (2017) and Pigato et al. (2018) found that most tropical plants may be reproduced through vegetative propagation using stems or roots, and the success may be improved by the use of specific hormones or root activators such as auxin, indolbutyric or indolacetic acid. But in the entire Republic of Benin where gardeners have limited access to root activators and are used to working on natural reproduction methods, it is not worth working with such additional inputs which will increase the seedling production cost at the nursery. As the branch collection also has a lesser impact on the plant survival than the root digging, these investigations set out to evaluate the possibility to reproduce without any root activator, *C. micranthum* by vegetative multiplication of stem cuttings at a nursery located in the department of Alibori (North Benin) as a contribution to the domestication and conservation of the species. Specifically, this research aimed at determining the most appropriate parts of the branch on which the stem cutting could be successful, the parts of the branches that were more likely to yield vigorous plantlets for reforestation and the required transplantation time for the cutting production at the nursery.

MATERIALS AND METHODS

Study area and vegetal material collection

The study area was the department of Alibori, located in the northern part of the Republic of Benin. The climate is dry tropical, with one rainy season of 500 mm from May to October. The hottest month is May when the temperature may reach 40°C while August is the coldest month of the year with 25°C at night (ASECNA, 2021). The vegetation is made up of degraded savannahs dominated by crop and fallow mosaics. The original vegetation is very rare and can mainly be found in the W National and Niger Parc or mosaics of rocky areas outside the Parc.

C. micranthum is a Eudicot Angiosperm of the Myrtale order, Rosid clade and the Combretaceae family. It is a small tree, shrub or liana that can reach 12 to 20 m in height distributed in the savannah areas of tropical Africa (Thiombiano et al., 2003). In the department of Alibori, it is a deciduous gregarious plant that can be found in pure and dense colonies on laterite and concretion soils. They establish on uncultivated lands with a preference for termite muds. Most plants bear scars from frequent harvests of bark, roots, and branches for medicinal uses.

For the investigation, a colony of five well-developed trees was selected in the village of Koutakroukou which lies 10 km East, away from the city of Kandi where the experimental nursery was located. A total of 35 branches measuring 105 to 110 cm long and a basal diameter of 1.95 to 2.2 cm were collected twice during the year for making the stem cuttings. After the branches were harvested, their base was immediately sealed in plastics to prevent desiccation during transportation to the experimental nursery. The first branch collection was carried out on 1st May 2021 corresponding to the end of the dry season while the second branch harvest occurred on 1st July of the same year which corresponded to the full rainy season. The harvested branches were brought to the Nursery of the General Directorate of Forest and Natural Resources of Benin, located in the city of Kandi in the department of Alibori. The geographic coordinates of the nursery in the World Geographic System (WGS 1984) were 2,94329°E and 11,134°N. Figure 1 presents the location of the experimental nursery in the department of Alibori.

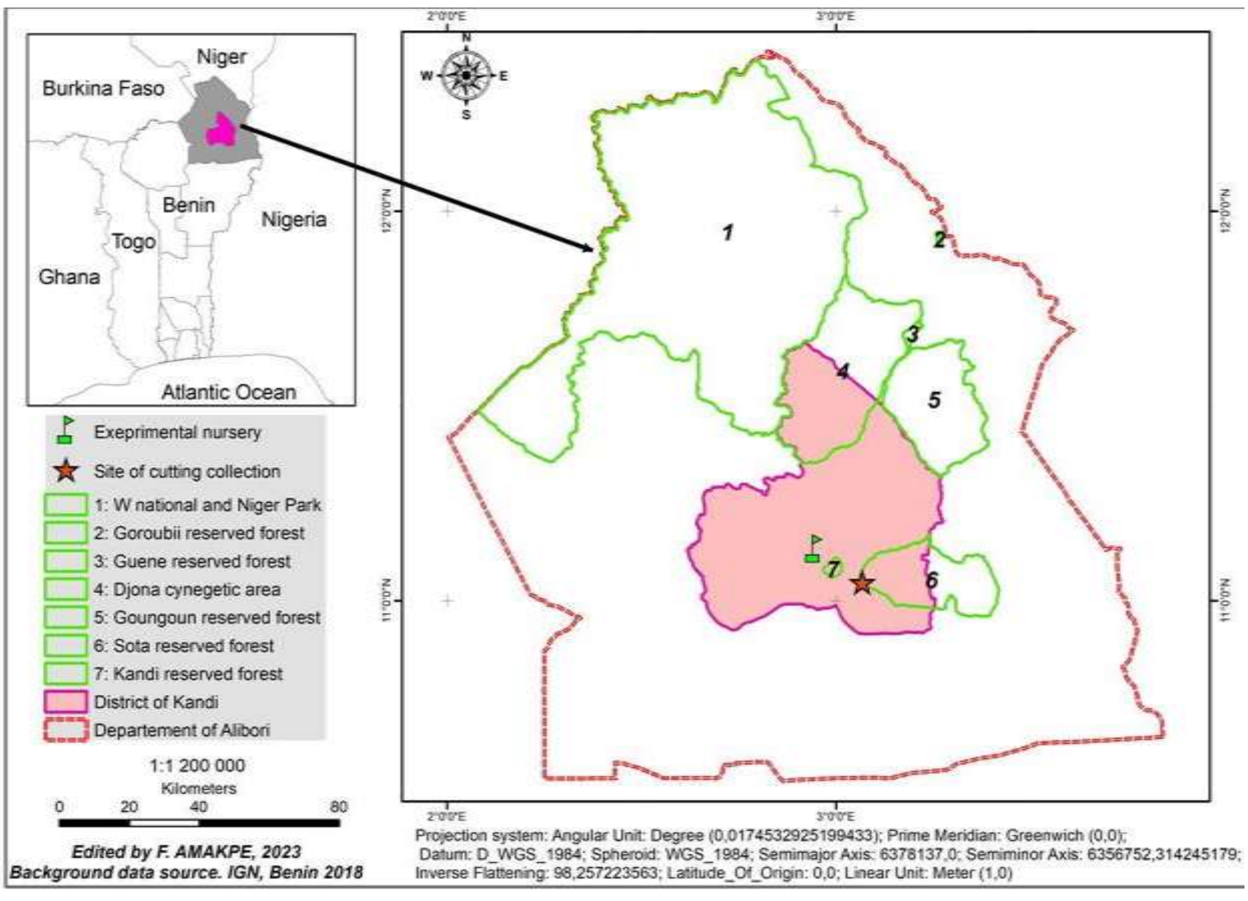

Figure 1. Location of the experimental nursery and the cutting collection site in the department of Alibori (Republic of Benin).

Nursery design and follow up

One week before the cutting plantation occurred in each season, a total of 297 plant pots of 5 cm diameter and 20 cm deep were filled with sand. The sand was collected in February from the superficial dry ground layer near the site, sieved with a 2 mm diameter sieve and stocked at the granary of the nursery (protected against rain). The filled plots were set in a dispositive of 27×11 plant pots and the space between each consecutive pot was also filled with sand to prevent water runoff and leaking when watering the bloc. The established pots were properly watered each day for 7 consecutive days prior to the cutting plantation of each season. The base of each pot was perforated to evacuate the excess water during the watering process. This small perforation also served to check the root emission of the cuttings during the cutting assessment.

On the day of the branch collection and transportation to the nursery, each branch was cut in 4 parts (cuttings) of 25 cm each and grouped into 4 categories or cutting types, named A, B, C and D from the base to the apex of the branch as follow:

1: A for the first 35 cuttings of 25 cm long from the base of the branch (35 basal cutting);
2: B for the second 35 cuttings of 25 cm long starting from 25 cm of the branch (first 35 intermediary cuttings) ;
3: C for the third 35 cuttings of 25 cm long starting from 50 cm of the branch (second 35 intermediary cuttings) ;
4: D for the fourth 35 cuttings of 25 cm long starting from 75 cm of the branch (35 apical cuttings).

The experimental site was set in a dispositive of 4 plots of each of the four cutting categories A, B, C and D which was repeated in the two harvesting seasons. The dry season trial was conducted with the 140 dry season cuttings while the rain season trial was conducted with the 140 cuttings of the rainy season. In order to reduce the border effect, each cutting plot was surrounded by a line of pots that were planted with any type of cuttings. Such planted pots were also used to separate each consecutive plot and the entire bloc of each season was finally surrounded by a line of empty pots. The bordering or separation of planted pots and empty plant pots was not considered for the cutting assessment.

After the cutting plantation, the site was properly watered every day in the morning from 9 o'clock during the entire investigation period of each season. From the 7th day after plantation, any emerging weed was carefully removed by hand every day till the 120th day to keep the site clean. From the 30th day after plantation, started the checking of the whole site, and each cutting was classified as follows:

1: The dead cuttings. They were counted and discarded from the bloc and the total cumulated number of dead cuttings of each date and each plot was recorded.
2: The remaining live cuttings. They were counted and maintained on the site.
3: The vigorous plants. These were the live plantlets that showed well-developed and green leaves compared to the other seedlings which seemed vulnerable.

After this first counting, further assessments of the cutting statues occurred every 15 days, from 25 June to 25 September for the dry season trial and from 25 August to 11 November for the rain season trial. The site was designed as indicated by Figure 2 and Figure 3 illustrates the bloc view after 45 days of cutting plantation.

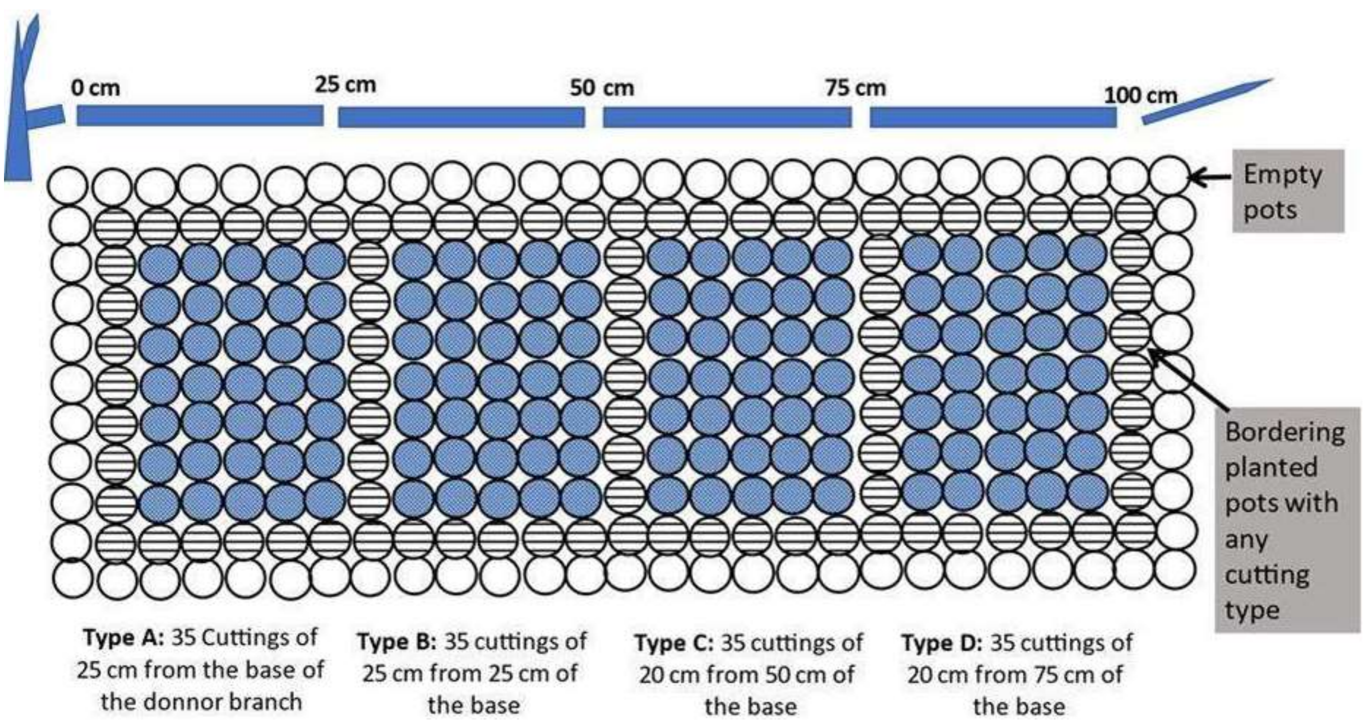

Figure 2. Experimental dispositive of the plant's bloc at the nursery.

Figure 3. View of the nursery site indicating live and dead cuttings.

Data collection and analysis

The number of live, vigorous, and dead cuttings or plantlets per cutting type were noted at each assessment date of each season and recorded in a database. These parameters were analysed with StatistiXL application®, which is a Microsoft Excel Add-in. The data were submitted for analysis of variance to determine how the collection season and the cutting category influenced the stem-cutting production of *C. micranthum* at a nursery. The performance of the cuttings was determined by the clustering analysis, by submitting the number of live and vigorous plantlets to the hierarchical analysis, using the Euclidean distance and the nearest neighbourhood method (Cottam and Curtis, 1956; Bray and Curtis, 1957). This also helped to identify key cutting groups that emerged at the nursery. For each identified cutting group, the cutting survival rate (CS %) and plantlet vigour rate (PV %) at any relevant time was calculated. There were respectively, the percentage of remaining surviving cuttings and the percentage of vigorous plantlets available at the nursery in each cutting category and harvest season as indicated by the following equations.

$$CS\% = \frac{nt}{Ng} \quad \text{and} \quad PV\% = \frac{nv}{Ng}$$

CS % is the survival rate of the considered group, PV % is the vigour rate of the cuttings of the considered group, nt is the number of remaining live cuttings in the group at the considered time; nv is the number of vigorous plantlets at the considered time and Ng is the total number of planted cuttings in the group at the beginning.

RESULTS

Ecological determinants on vegetative multiplication of *Combretum micranthum*

The multivariate analysis indicated that the difference between the number of surviving cuttings of each cutting category and each harvesting season was significant ($P < 0.001$) and the season was highly correlated to the survival rate of the cutting (correlation coefficient $R^2 = 0.95$ and $P = 0.000$). On the other hand, the difference between the cutting longevity and the required time for obtaining vigorous cuttings was not significant between the cutting harvesting season ($P > 0.3$), indicating that the harvest season did not influence the transplantation time or the required time for obtaining vigorous plantlets at the nursery. In fact, after 90 days, there was no more change in the survival and vigour rates at the site regardless of the harvest season and the cutting type. Furthermore, the entire live and vigorous plantlets showed roots at the bottom of each plant pot on the 90th days after the cutting plantation. Ninety (90) days represented then the optimum nursery time of the *C. micranthum* stem-cutting production (Figure 4). The established root systems proved that these plants have acquired the necessary autonomy to perform their vital functions as fully independent autotrophs which no longer rely on the primary reserves of the cut stem.

The dynamic in the cutting survival and vigour over the cutting production period indicated that the dry season cuttings had the best success compared to the ones collected during the rainy season. More than 50% of the rain season cuttings were dead on the 45th day after plantation (half of the optimal transplantation time) and only 3% reached the end of the 120 days of investigation. Surprisingly, the entire 3% of live plantlets of the rain season cuttings were all vigorous from the 90th day after the cutting plantation. As far as the dry season cuttings were concerned, 36% of them could reach the 120 days that the investigation lasted at the nursery and yielded 25% of vigorous plantlets with well-developed root systems at the bottom of their plant pot. Figure 5 summarises the characteristics of the different cutting types at the nursery.

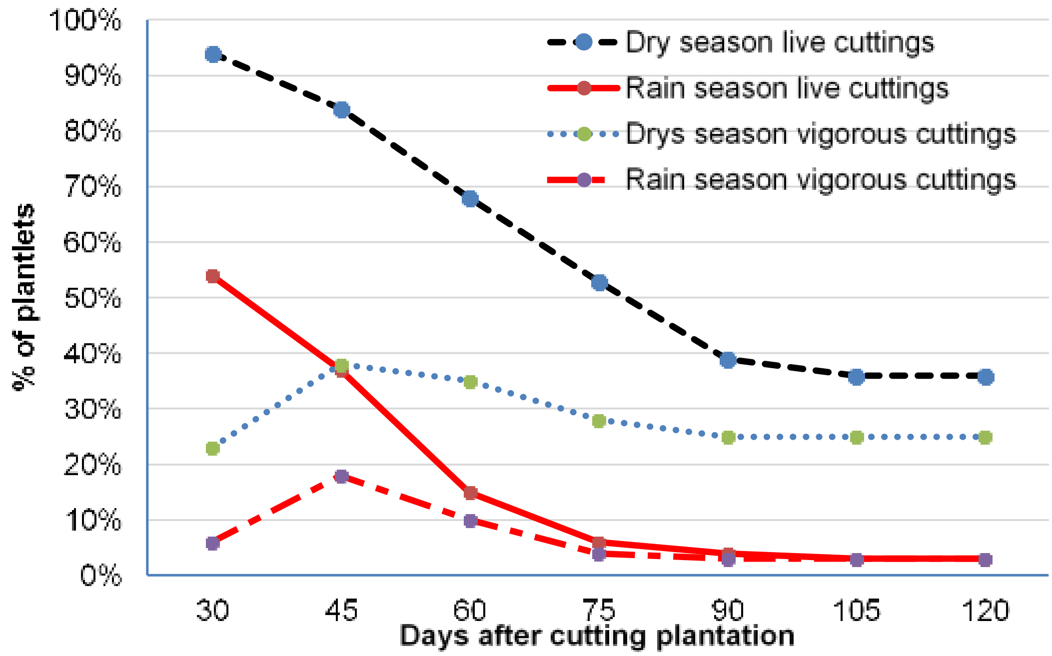

Figure 4. Cutting survival and vigour dynamics of each season.

Seasonality and cutting performance

The hierarchical clustering analysis, based on the cutting survival and the cutting vigour indicated that the success in stem-cutting production of *C. micranthum* at the nursery was determined by both the harvest season and the cutting type. But the cutting survival and the cutting vigour were differently impacted by the cutting harvest season or the cutting type (A, B, C and D).

The cutting survival at the nursery was specifically, highly determined by the harvest season alone (P = 0.000 and the cophenetic correlation value R = 0.827), leading to two groups of cuttings in which the dry season cuttings were separated from the rain season ones (Figure 6a). The cutting vigour at the nursery on the other hand was determined by both the harvest season and the cutting type, leading to the following cuttings groups from the dendrogram (P = 0.000 and the cophenetic correlation value R = 0.87) as indicated in Figure 6b:

1. **The entire rain season cuttings:** They made up a clear distinct group with only 3% of vigorous cuttings after 90 days. In this group, the C (second intermediary cutting) and the D (apical cuttings) made up a particular subgroup by being the only one that yielded each, 6% of vigorous plantlets in 90 days.
2. **The D (apical) cuttings of the dry season**: They bore the highest number of vigorous plantlets (43 %) in 90 days. Though they bore fewer surviving plantlets than the A (basal) cuttings of the same season, most of the surviving plantlets worked out in vigorous plantlets at the nursery.
3. **The A, B and C dry season cuttings**: they made up the third branch of cuttings on the dendrogram with 14 % of vigorous plantlets on 90 days. In this group, the B and

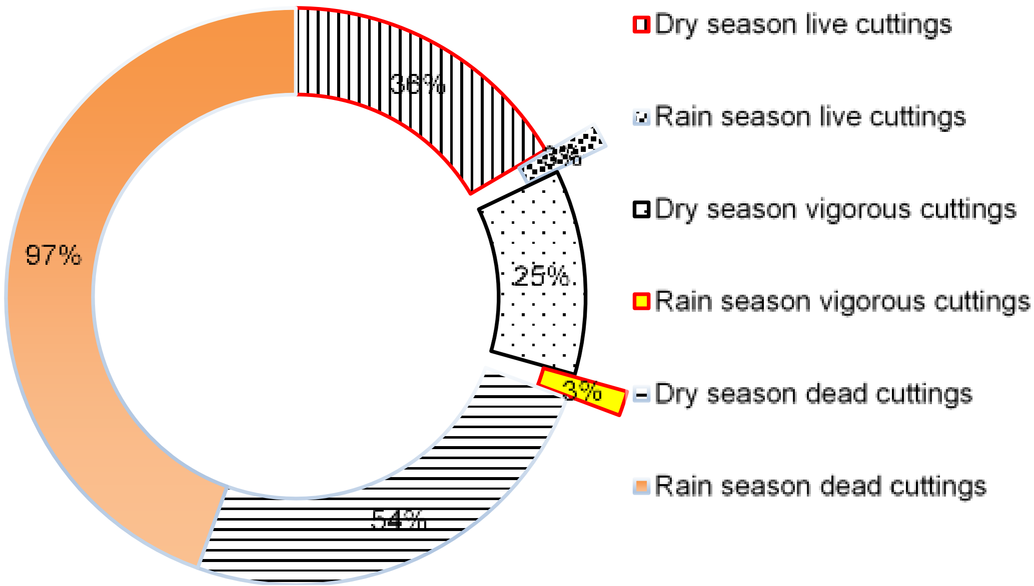

Figure 5. Survival and vigour status of the cuttings per harvest season.

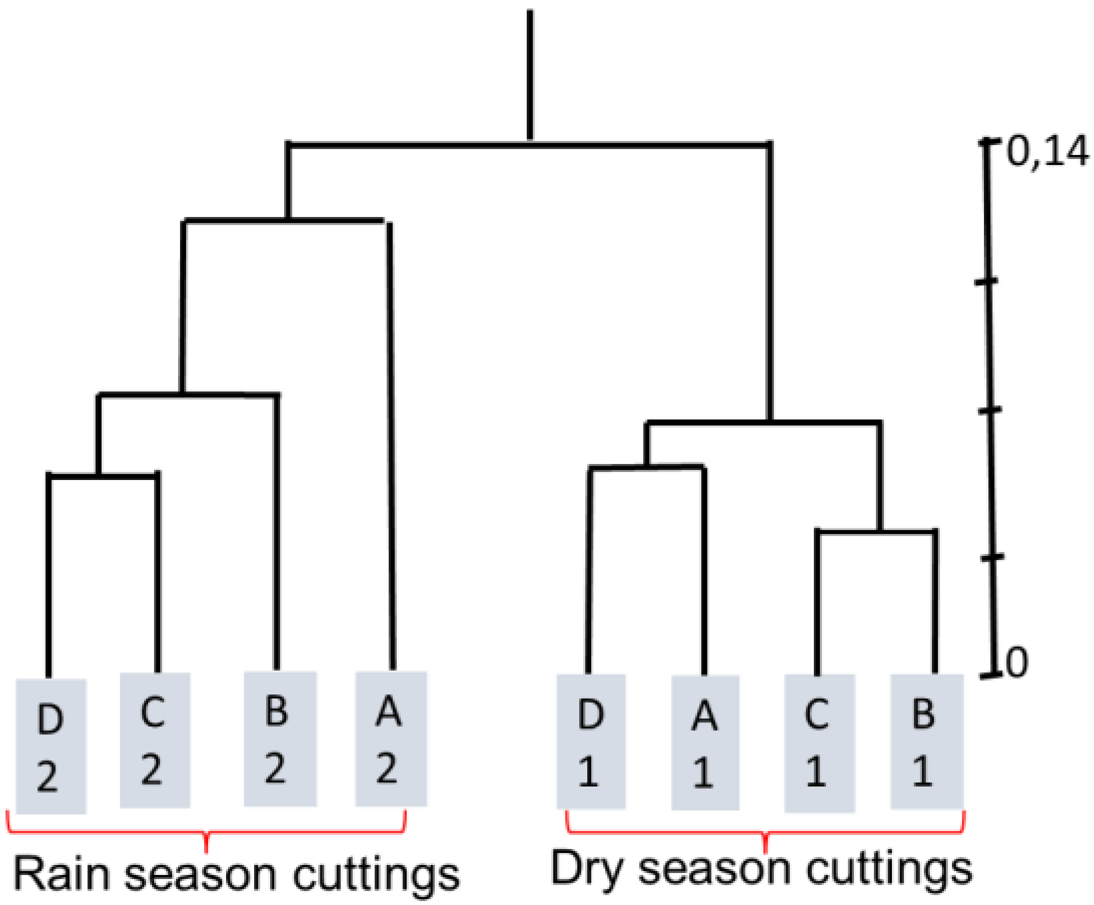

Figure 6a. Hierarchical analysis of the cuttings, based on their survival. A, B, C and D are the type of cuttings. 1 for the dry season cuttings, 2 for the rain season cuttings.

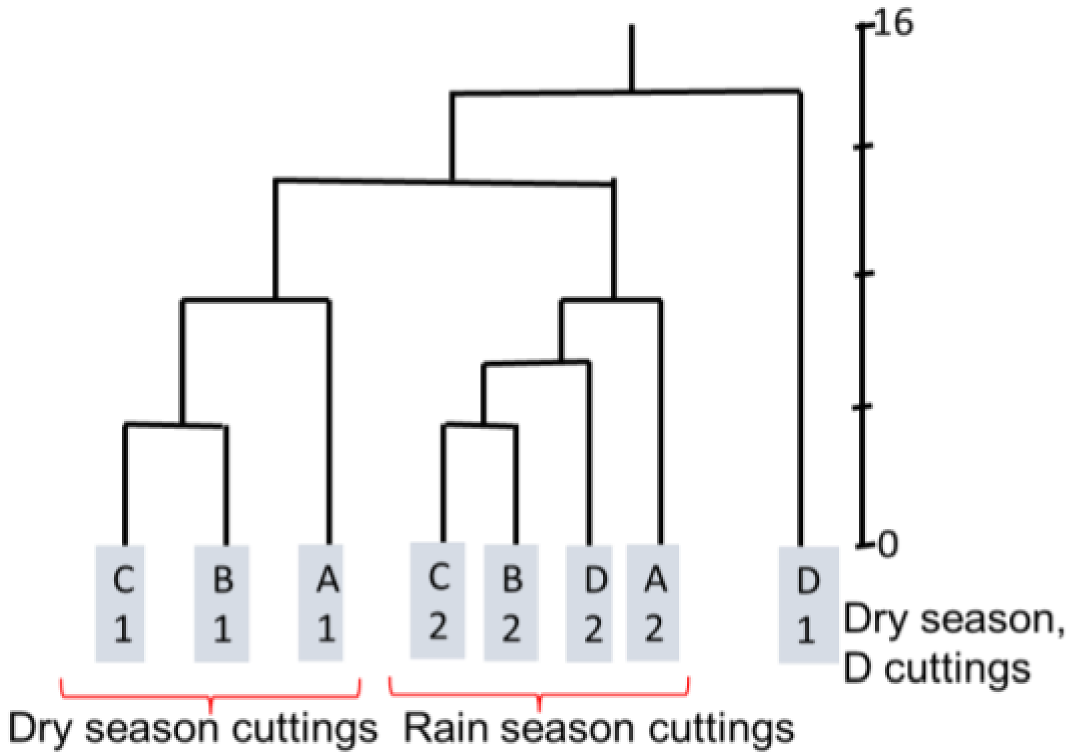

Figure 6b. Hierarchical analysis of the cuttings, based on their vigour. A, B, C and D are the type of cuttings. 1 for the dry season cuttings, 2 for the rain season cuttings.

C cuttings (intermediaries) showed the same survival rate as the D (apical) cutting and when it came to the vigour, they behaved as the A cuttings of the dry season.

Figure 7a summarises the survival dynamic of the different identified cutting groups and Figure 7b analyses the vigour dynamic of these cutting groups.

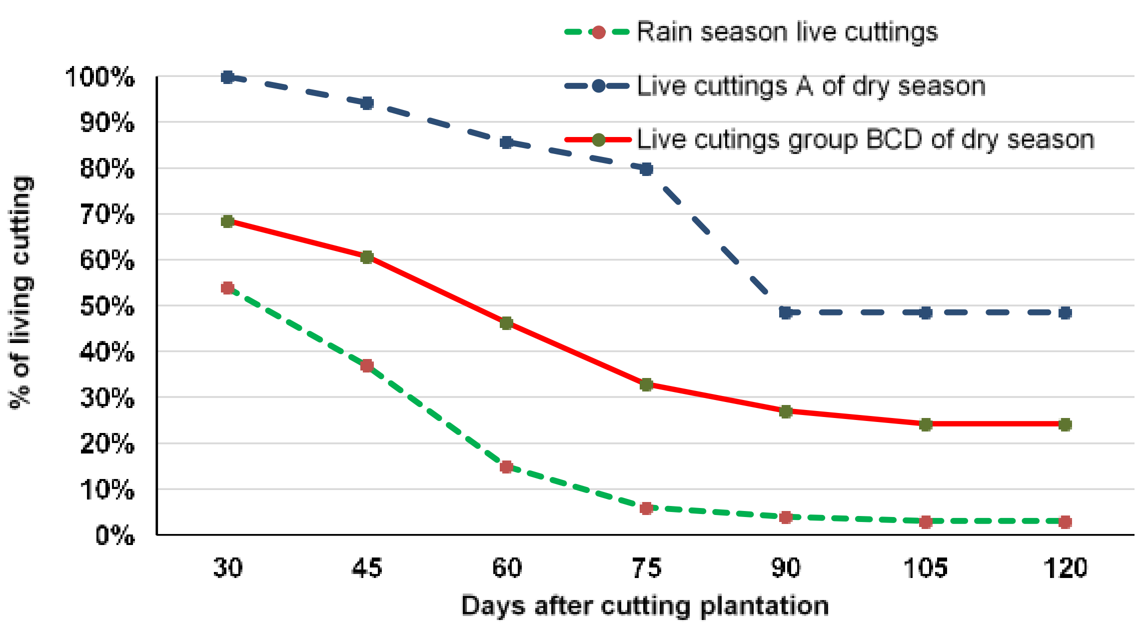

Figure 7a. Survival dynamic of the three cutting groups.

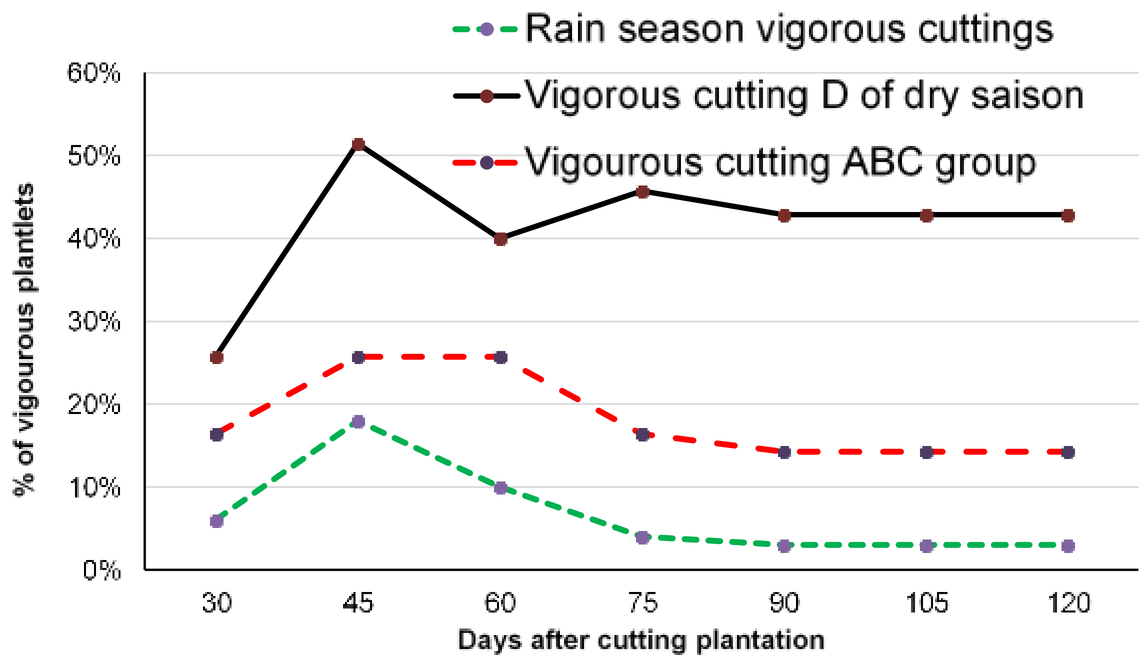

Figure 7b. Vigour dynamics of the three cutting groups.

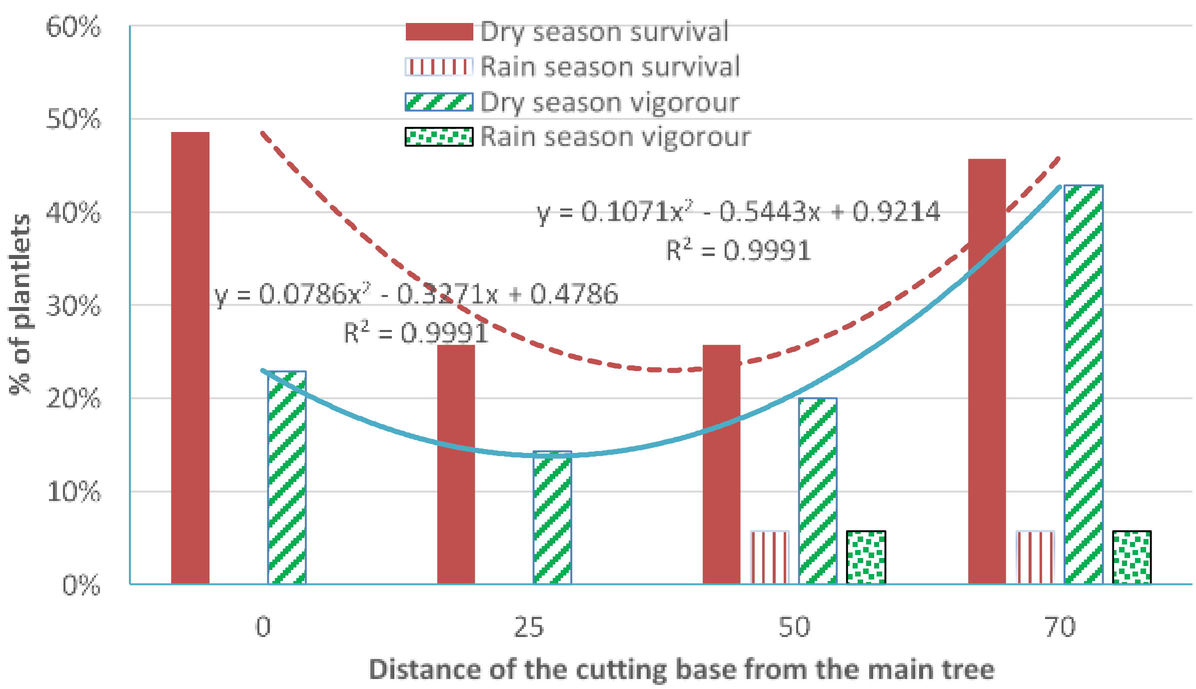

Figure 8. Survival and vigour dynamic of the gradient cutting along the branch.

The cutting position on the branch

The vigour and the survival of the cuttings were also determined by the position of the cutting base on the branch. As indicated in Figure 8, almost 95 % of the rain season cuttings were dead and only the C and D cuttings survived till the end of the experimentation. For the dry season cuttings which yielded the best surviving and vigorous plantlets, the results showed that:

1. The dry season cutting survival was determined by a polynomial gradient curve shape along the branch with an equation of $y = 0.1071x^2 - 0.5443x + 0.9214$ (correlation coefficient $R^2 = 0.9991$). In this system, the basal cutting (A) had the highest survival rate (49%), followed by the D (apical) cuttings (46%). The B and C (intermediary cuttings) had the same low survival value of 16%, giving a “reversed J” shape for the gradient along the branch. The minimum survival rate of this modelized trendline of the gradient, determined by the point where the first derivative function $y' = 0.2142x-0.5443$ equalled zero was obtained at 2.5, corresponding to a cutting starting from 38 cm on the branch.
2. The dry season cutting vigour was also determined by a polynomial shape of the gradient along the branch with an equation of $y = 0.0786x^2-0.3271x+0.4786$ (correlation coefficient $R^2 = 0.9991$). In opposition to the survival, the apical cutting (D) yielded the best vigorous plantlets rate (43%) which is almost twice the vigour rate of the basal cuttings. The C cuttings here had higher vigorous plantlets compared to the B, giving the curve, a “normal J” shape for the gradient along the branch. The minimum vigour rate value of this modelized trendline of the gradient, determined by the point where the first derivative function $y' = 0.1572x - 0.3271$ equalled zero was obtained at 2.0, corresponding to a cutting starting from 25 cm on the branch.

DISCUSSION

Harvest season and vegetative multiplication of *Combretum micranthum* at a nursery

The survival and vigour of the cuttings were highly determined by the cutting harvest season. Almost 97% of the rain season cutting was dead while up to 45% of the dry season cuttings yielded vigorous plantlets at the nursery. This tendance was also found by Teklehaimanot et al. (2004) for *Osyris lanceolata* in Tanzania where the basal cuttings that were harvested in September (dry season) had the best rooting systems. By the end of the dry season, the plants are under maximum hydric stress and efficiently shoot as soon as the limiting factor which is water shortage is removed (Adomou, 2005). In the department of Alibori, the plants are all at maximum drought stress in May, after 6 consecutive dry months (ASECNA, 2021). This high stress is also associated with the higher dry matter or food accumulation in the deciduous plant species stems following the active photosynthesis of the rainy season. In the heart of the rainy season, the phenology of *C. micranthum* and most savannah deciduous plants is characterised by the initiation of floral muds in July which corresponds to the summertime in the northern hemisphere when the days are longer (Ern, 1988). This process mobilises a lot of energy and nutrients to the future floral organs that inhibit the vegetative shooting functions of the plant (Thiombiano et al., 2003, Ceccon et al., 2006). In addition to that, the water concentration of the stem is maximum in the heart of the rain season and the stems, and the cuttings that were obtained from them were too much hydrated and were then not prone to emit roots and leaves. They die from starvation and desiccation after the exhaustion of the poor and diluted food and water reserve they contained. The best performance of the dry season cuttings was then due to their higher water absorbance potential, and to the higher food stock accumulated in the plant stem before the leaves fall to support the interactive stresses from drought, fire and grazing (Biaou, 2009).

The plantlets that were obtained from the dry season cuttings became also vigorous in July and August, which is the full plantation period in the entire Republic of Benin (ASECNA, 2021). They can then efficiently be transplanted and benefit from the required rain for their establishment in the same year. On the other hand, the plantlets of the rain season cuttings became vigorous by November which corresponds to the beginning of the dry season in the entire Republic of Benin. Such rare plantlets could then not be planted before June of the second year, because of the following 6 months of the dry season. They should then be kept at the nursery for a long time with additional watering and other production costs. It is then more realistic and economically sound to base the stem cutting of *C. micranthum* on the dry season cuttings.

Implication for *Combretum micranthum* vegetative production at nursery

With no root activator, the dry season cuttings yielded 25% of vigorous plantlets with well-developed root systems in 90 days after the cutting plantation. This performance was far better than the one of *Peterocarpus erynaceus* and other plant species which do not emit root without the use of indolbutyric or indolacetic acid at the appropriate dose and soaking time prior to cutting plantation (Bodjrenou et al., 2018, Pigatto et al., 2018; Ouinsavi et al., 2019). The results indicated that It took only 90 days after plantation to obtain vigorous plants for the dry season cuttings, ready for transplantation while the optimum high of 20 cm required for the transplantation of the same species through seed production was not reached after 100 days at nursery according to Amani et al. (2015). There was then a higher susceptibility of the dry season cuttings of *C. micranthum* to stem cutting production when compared to other tropical species (Koko et al., 2011) and the finding supported that the natural dry season stem cuttings were the best approach for the restoration and mass production of *C. micranthum* in the tropical areas.

The cutting survival and vigour were determined by a polynomial gradient along the branch and the intermediary cuttings were disadvantaged compared to the apical and basal ones (Figure 8). Such positive impacts of both the base and apex of the branch are sustained by the strong stimulating influence of buds and auxin sources of the base and apical areas of the branch which boost root initiation and nutrient assimilation as found by Edmond et al. (1994). In this process, the basal cuttings were first to emit roots that help compensate for the cutting desiccation, leading to a higher life span of these cuttings. The findings indicated that the maximum acceptable distance that could separate the base of the cutting from the main tree to benefit from a good survival rate was 37 cm. The vigour on the other hand was sustained by the apical cells which cumulatively boosted the leaves and roots emission of the apical branches where they were more concentrated (Tsobeng et al., 2011), and the results showed that the optimal distance of the base of the cutting to benefit from good vigorous plant rate should not be shorter than 25 cm. In other words, a *C. micranthum* stem-cutting should be at least 25 cm long for success. The specificity of the lower performance of intermediary cuttings of *C. micranthum* was due to the fact that they bore fewer buds, had limited activating hormones, and easily exhausted their nutrient stock after they were cut from the branch as found by Sery et al. (2019) for *Cola nitida*. Better performance of basal cuttings was also found by Okunlola (2013) for *Duranta Repens* production at the nursery. But, as found by Naidu and Jones (2009) for other plant species, the possibility of root emission is determined by the reserve in the cutting and the apical cuttings yield poor plantlets. With the positions of the minimum survival and vigour rates on the branch, the results supported that the combination of the basal and apical effects on the same cutting could give the best result. Then, shorter branches of 50 cm maximum should be targeted in order to obtain no more than two cuttings per cut branch as the intermediary cuttings were not relevant. But the shorter the branch, the smaller the cutting base diameter is for the species. It is then important to consider, the minimum required basal diameter of 2 to 3 cm of the cutting when harvesting such short branches as recommended by Ouinsavi et al. (2019) for better success.

As far as the nursery time or the transplantation time of the cuttings was concerned for the dry season cuttings, a longer optimal nursery time of 120 days was obtained for *Pterocarpus Erynaceus* stem cuttings using indolacetic acid as root stimulator by Ouinsavi et al. (2019) in the same climatic areas of the Republic of Benin. A shorter time of ninety days found here clearly supported that the dry season stem cutting was a better approach for the propagation of the *C. micranthum* in tropical areas where water shortage is a great challenge for nurseries and plantations. In fact, for the financial feasibility of the production of the cutting at the nursery, the transplantation time which is linked to the production cost (Abadi Ghadim and Pannel, 1999) is one of the critical decision-making factors to consider (Amoah, 2006). In the department of Alibori where the reforestation season lasts from June to August, the stem-cutting production at the nursery should then start no later than April to May, so that the seedlings could be planted in July or August in order to benefit from enough rain for the establishment of the newly created plantation.

CONCLUSIONS

C. micranthum stem cutting is successful at the nursery with no root activator. The best season for collecting the cuttings was the end of the dry season and 90 days were required to obtain 43% of vigorous plantlets for reforestation when targeted on apical stem cuttings. In order to benefit from the synergy of the basal and apical impacts at the nursery, short branches of 50 cm long maximum could help benefit from the best survival and vigour rates in natural conditions. This research was aimed at national gardeners with limited access to root-activating inputs which are likely to improve the yield in vigorous plantlets. But the use of such root activators for *C. micranthum* stem-cutting production requires specific conditions that have to be assessed and mastered for the technical, economic, and financial feasibility of the project.

RECOMMENDATION

This research elucidated the possibility of mass production of *C. micranthum* in any ecological area. Deeper investigations should then be conducted on the relevant agronomic, socio-economic and financial factors that determine the adoption and success of the establishment of the species gardens or plantations in the relevant ecological areas of the country and entire Africa.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered a potential conflict of interest.

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Citation: Amakpe F, de Graaf DC, Sinsin B, Biaou HS, 2023. Stem cuttings assessment for domesticating the medicinal melliferous plant, *Combretum micranthum* G. Don. in the Republic of Benin. Net J Agric Sci, 11(1): 1-12.