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Ex-situ propagation by cutting of *Flemingea faginea* (Guill.& Perr.) (Burkina Faso, West Africa)

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Medicinal plants are especially used by vulnerable populations in Burkina Faso to solve their health problems. *Flemingia faginea* is a medicinal plant overexploited and its wetland habitat is threatened by climate change. Thus, this study aims to promote the *ex-situ* reproduction of *F. faginea*, and make it accessible as a remedy for people suffering from blood pressure disease. The capacity of reproduction by cutting has been tested by using two types of substrates and four types of cuttings. The results showed that the composite substrate is performing better than the sterilized sand for height growth (P<0.001), leaves production (P<0.006) and number of branching (P<0.001). As the types of cutting is concerned, the apical cuttings recorded better growth for two parameters than others in the nursery about the height growth in centimetre (14.44 ± 9.37a), the number of leaves (5.11 ± 3.18a) and the median cuttings recorded the greatest number of branching (2.30 ± 2.03a). For each growth parameter the results are different between the types of cuttings (P<0.001). This survey allows the cutting reproduction of *F. faginea*.

Key words: *Flemingia faginea*, cuttings, nursery, growth parameters, Burkina Faso.

INTRODUCTION

There is a renewed interest in medicinal plants research, nowadays (Gbekley et al., 2018) herbal drugs are used

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for the treatment and prevention of many diseases. The WHO (2001) has stated that herbal drugs trade is growing. About 25% of the world pharmaceutical market in 1996, approximately 250 billion dollars is allocated to drugs derived from plants (Mashayekhan et al. 2016). The contribution of medicinal plants to human livelihood is unlimited (Hamilton, 2004). Traditional medicine remains the main source of primary health care for 70% of the population, in Burkina Faso (Zerbo et al., 2011).

However, plants species are declining drastically as a result of the combined action of human pressure and climate change. In addition, researcher's work has also demonstrated particularly the vulnerability and rarity of many medicinal species (Traoré et al., 2011; Koadima, 2008; Ouédraogo et al., 2006). This situation of declining does not seem to worry populations because, until now rural as well as urban populations seem to be reluctant to the domestication of medicinal wild species. Their interest is mainly focused on edible plants, food-producing and ornamental plants. Efforts are being made to reproduce the other useful plants and improve their variety, but medicinal plants cultivation are not focus their interest. However, cultivating these medicinal plants could contribute to their conservation and sustainable management. Low-cost techniques for vegetative propagation must be used to make available nursery to practitioners of traditional medicine and other person who need it. Vegetative propagation allows the production of progeny composed by individuals genetically similar to the parent and identical to each other. It permits effectively and inexpensively remedies the inadequacies of seed regeneration (Meunier et al., 2008), allows also plants to reproduce rapidly and shortens the time it takes to reach maturity (Bellefontaine et al., 2018). That why in this study, an interest is focus on *Flemingia faginea* a high value species in traditional medicine.

This plant is preferred in the care traditional of many diseases. For example, freeze-dried leaf extracts of *F. faginea* have bradycardic and hypotensive effects (Coulibaly, 2006). The leaves in decoction are used against high blood pressure to eliminate excess salt (Dembelé, 1988; Cissé et al., 2016) and to regulate blood glucose levels for diabetics who reach the age of 40 (Dembelé, 1988). Macerating bark and leaves are prescribed for pregnant women during pregnancy. For internal use, bark or roots in decoctions are used to treat haemorrhoids and gonorrhoea (Dembelé, 1988). The plant is also used as a component of an anti-gonococcal preparation (Kerharo and Adam, 1964). The biochemical studies have shown that extracts from the leaves contain isoflavones (Sidibé, 2003). *F. faginea* is harvested by people in its natural habitats. Furthermore, its distribution is limited alongside some permanent rivers in protected areas where it is growing. These wet lands are also threatened by climate changed. The main goal of the conservation of those wild plant species and their cultivating medicinal plants for this matter, is to ensure sustainable use. As the reproduction of medical plants is scare in the country, this research is important because it consists at enhancing the conservation of *F. faginea* and will make it available and accessible to everyone.

MATERIALS AND METHODS

Geographical location of cuttings collection sites

The cuttings of *F. faginea* come from National Park of Kaboré Tambi (PNKT) and the test has been done at the National Centre of Forest Seed located in Ouagadougou. The both sites are located in the north-Sudanian climate (Fontès and Guinko, 1995). Figure 1 shows the location of the cuttings collection (PNKT) and testing site (Ouagadougou).

Methods of harvesting cuttings

The cuttings have been taken early in the morning before periods of high temperature, to limit dehydration while taking into account their physiological and sanitary state (Dembelé, 2012). They have been taken from the same site and from a representative number of lignified individuals (minimum 30). Stem cuttings (about 200) have been taken *in situ* from stem shoots about 60 cm long and then stripped of leaves. These twigs have been cut with a three-stage (basal, median and apical) (Zida et al., 2018). The removal of leaves has been done and the cuttings inserted in small bags to prevent evapotranspiration. However, leaf removal is done by avoiding damage to the buds (Asseh et al., 2017). Root fragments have been collected from an adult and healthy rooted mother tree after partial excavation of superficial roots with a metallic tool (Meunier et al., 2008). To avoid dehydration, they were labelled, collected by type and placed in previously wet jute bags, placed in a cooler and brought back as quickly as possible (Harivel et al., 2006) to the laboratory of the National Center of Forest Seed (CNSF). They were then kept in an air-conditioned room with the cooler for 24 hours before cutting.

Preparation and sowing of cuttings

The preparation and sowing of cuttings was carried out in May 2019, twenty-four hours after the harvest due to the distance (115 km) separating the PNKT from Ouagadougou (CNSF). Thus, the preparation of the cuttings consisted of sizing the stem cuttings (basal, median and apical) and roots (Figure 2). The sizes of the cutting have been chosen based on some successful studies on savannah trees and shrubs (Sanogo et al., 2008; Bellefontaine et al., 2018). In fact, the root cuttings were 5 cm long and with an average diameter of 8.33 ± 3.26 mm. As for the stem cuttings, they were 15 cm long with the following average diameters:

(i) basal stem cuttings 6.37 ± 1.23 mm
(ii) median stem cuttings 5.33 ± 0.90 mm
(iii) apical stem cuttings 4.31 ± 0.79 mm

After the sectioning of each fragment, a mark was made on the basal part to facilitate the identification of the direction of polarity

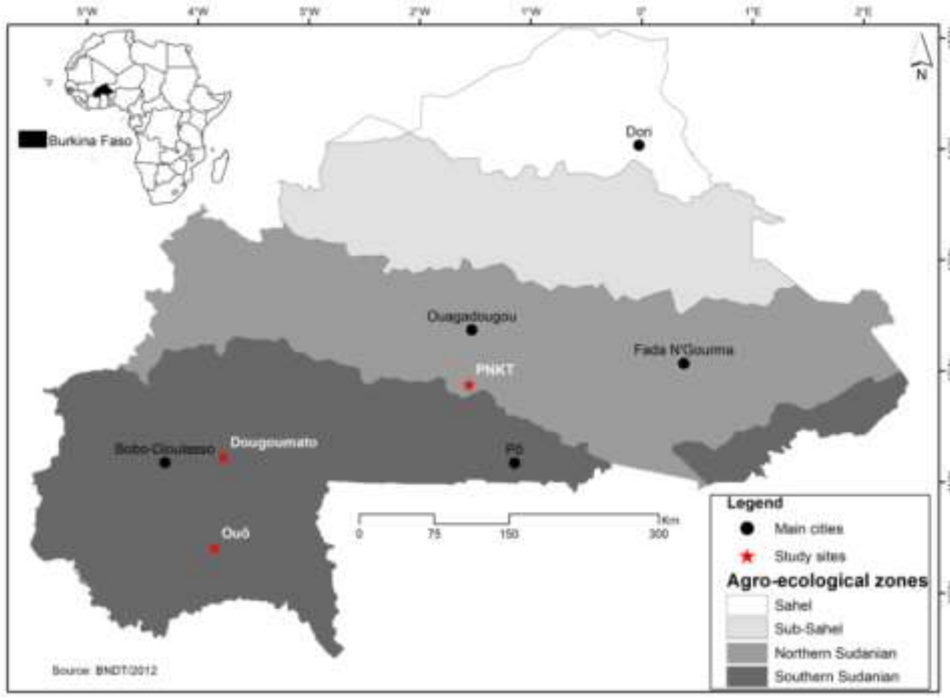

Figure 1. Map showing the location of PNKT and Ouagadougou.

Figure 2. Stem cuttings (a) and root cuttings (b).

during sowing (Meunier et al., 2008). Sowing of the cuttings was carried out just after the preparation of the cuttings. For this purpose, the stem cuttings were sown vertically with respect to polarity and horizontally for the root cuttings (Meunier et al., 2008) in small plastic pots. After making a hole in the substrate, the stem cuttings were inserted vertically and individually with at least two visible buds, as it is from these buds regrowth will start. The root cuttings were arranged horizontally and they were completely covered with a layer of soil of about 1 cm. Thus for each block and for each substrate, twenty cuttings were sown per type of cuttings.

Substrate preparation

Two types of substrates were used for the cuttings trials to determine the influence of the substrate on bud re-growth and seedling growth. These were:

(i) sterilised sand: it has been sterilised with a steriliser for two hours at 100°C. It has been cooled in the open air before use.
(ii) composite substrate consisting of a mixture of three volumes of soil, one volume of sand and one volume of homogenised manure.

Figure 3. Cuttings after weaning in the glasshouse (a) and outside the glasshouse (b).

Both types of substrates were used to fill small plastic bagged pots, 250 mm high and 70 mm in diameter, before being placed in the experimental device.

Experimental device

The experimental device is a split plot device allowing to compare the two types of substrates with the four types of cuttings, in four repetitions. Indeed, the cuttings tests were carried out in small greenhouses to maintain a high relative air humidity and an adequate temperature, in order to favour the appearance of buds. In each greenhouse or block, the pots were arranged in 10 ×16 pots, one part of which was filled with composite substrate and the other with sterilised sand. Each block contains the four types of cuttings (basal, median and apical stem and root cuttings) per substrate. The pots were filled, graded with 20 pots per substrate and per type of cuttings in the greenhouse two days before the cuttings were placed.

Weaning of the seedlings

After sowing, the plants were raised in mini greenhouses for 45 days and then weaned. After the 45 days, the recovery of the root cuttings was too weak. So they were moved in a single block to encourage their recovery. Indeed, weaning consisted of a progressive opening of the different blocks containing the stem cuttings to allow them to adapt to the external conditions. After 15 days of weaning, the stem cuttings were transferred to the natural environment (under the shade of a tree) (Figure 3). The transfer of the cuttings was done by respecting the initial arrangement.

Measured agronomic parameters

The following parameters were measured for each cutting: the time of bud appearance was noted, then the next step was to count the number of buds that had appeared. The growth parameters were noted, the height of the buds, as well as the number of leaves on 16 individuals of each type of cuttings contained in each substrate. The resumption of a cutting is characterised by the appearance of a stem bud (Diatta et al., 2007). The average temperature and humidity inside the glasshouse were 34.60 ± 0.46°C and 70.57 ± 1.31% respectively. The same parameters outside the glasshouse environment were 29.72 ± 2.22°C and 70.36 ± 5.16%. The following parameters have been calculated:

(i) the latency time: the time that elapses between the planting of the cutting and the appearance of the first bud;
(ii) the rate of budding: ratio between the number of cuttings that have produced buds and the total number of cuttings planted. The method Asseh et al. (2017) was used for the calculation.
(iii) leafy shoots survival rate is obtained by dividing the number of surviving leafy shoots by the number of cuttings initially transplanted, multiplied by 100 (Bekker et al., 2004). In this case, this parameter was taken into account 2 months after cutting (at the end of weaning).

The watering was done every two days in the greenhouse, and every day (morning) after weaning except in case of rain.

Data analysis

The data were recorded and calculated using the Excel 2013 spreadsheet. Minitab. 18 software was used for the descriptive and analysis of variance (ANOVA) calculations. Then a comparison of the mean values was made by applying the Tukey test to the 5% significant probability threshold using the same software.

RESULTS

Latency time or budding time

Table 1 shows the budding times in the two substrates (sterilised sand and composite substrate) for all cuttings used. For each type of stem cuttings in both substrates, budburst started 3 days after sowing. For the root cuttings, they recorded the longest latency time, which

Table 1. Latency time and rate of budding.

Substrate	Type of cutting	Latency time (day)	Rate of budding (%)
Sterilised sand	Basal	3	97.5 ± 0.02
	Median	3	96.25 ± 0.02
	Apical	3	83.75 ± 0.13
	Root	79	8.75 ± 0.07
Composite substrate	Basal	3	73.75 ± 0.25
	Median	3	92.5 ± 0.06
	Apical	3	83.75 ± 0.06
	Root	13	6.25 ± 0.02

Figure 4. Shows the budburst of stem (A) and root cuttings (B).

was 13 days in the sand substrate and 79 days in the composite substrate.

Rate of budding

The rates of regrowth are shown in Table 1 for each type of cutting in the different substrates (sterilised sand and composite substrate). The highest rate was obtained in the sand substrate with basal stem cuttings (97.5 ± 0.02%) followed by median stem cuttings of which 96.25 ± 0.02% for the sterilised sand substrate and 92.5 ± 0.06% for the composite substrate. As for apical stem cuttings, a rate of 83.75 ± 0.06% was recorded in both substrates while the rate for basal stem cuttings in the composite substrate was 73.75 ± 0.25%. However, the lowest rate was recorded with root cuttings at 8.75 ± 0.07% and 6.25 ± 0.02% respectively for the sterilized sand substrate and the composite substrate. Figure 4 shows the budburst of stem and root cuttings. The

Table 2. Two-by-two comparison of Tukey for height versus type of substrate at the 5% threshold.

Substrate	Mean of height (cm)
Composite substrate	10.93 ± 10.87[a]
Sterilised sand	8.90 ± 8.07[b]

Averages that do not share any letters are significantly different.

Table 3. Two-by-two comparison of Tukey for height versus type of cuttings at the 5% threshold.

Type of cuttings	Mean height (cm)
Apical	14.44 ± 9.37[a]
Median	13.20 ± 8.68[a]
Basal	11.10 ± 9.08[b]
Root	0.91 ± 3.44[c]

Averages that do not share any letters are significantly different.

maximum number of buds obtained with stem cuttings in the sand substrate is 11 and 12 for the composite substrate. The maximum number of root cuttings is 1 bud for the sand substrate and 7 buds for the composite substrate.

Survival rate after weaning

For the sterilized sand substrate, the basal stem cuttings (90%) are the cuttings that survived best, followed by the median stem cuttings (82.5%) and then the apical stem cuttings. Then the apical stem cuttings (71.25%) from the sand substrate, followed by the median stem cuttings (78.33%) from the composite substrate, the apical stem cuttings (60%) from the composite substrate and the basal stem cuttings (50%). As for the root cuttings, they remain the weakest with (5%) and (3.75%) for the sand and composite substrate respectively.

Effect of substrate and type of cutting on seedling growth

Height of the leafy shoots

According to the descriptive analysis, the maximum heights in the composite substrate were: 51 cm for apical cuttings, 35 cm for basal cuttings, 45 cm for median cuttings and 24.5 cm for root cuttings. In the sand substrate, the maximum heights for apical cuttings were 35 and 28 cm for basal stem cuttings and 22.5 cm for median stem cuttings. As the analysis of variance is concerned, it revealed that the difference between the substrates was very highly significant ($P<0.001$) for plant height. Thus, Tukey's test at the 5% threshold for the mean height value shows that the composite substrate is higher than the sterilised sand substrate (Table 2). The effect of the type of cuttings on height showed a very highly significant difference ($P<0.001$). Thus, the mean value of the height in the Tukey test at the 5% threshold shows that the apical and median cuttings have the same heights. These two cuttings have the highest heights followed by the basal cuttings and finally the root cuttings (Table 3).

Leaves production per leafy shoot

The maximum leaf production for the composite substrate is 15 leaves for apical cuttings, 17 leaves for stem median cuttings, 16 leaves for basal cuttings, and 10 leaves for root cuttings. In the sand substrate, basal cuttings produced a maximum of 12 leaves, stem medians produced 17 leaves and 11 leaves for the basal stem cuttings, while root cuttings produced no individuals in the sand substrate. ANOVA on leaf production revealed a very highly significant difference ($P<0.001$) for the types of cuttings. Thus the mean value of leaves in Tukey's test at the 5% threshold showed that it is the apical stem cuttings which are superior followed by the median cuttings and the basal cuttings which are identical to each other and finally the root cuttings (Table 5). As for the effect of substrate on leaf production, statistical

Table 4. Tukey's two-by-two comparison of leaves production between the type of substrate at the 5% threshold.

Substrate	Mean of leaves production
Composite substrate	3.79 ± 3.68^{a}
Sterilised Sand	3.29 ± 3.11^{b}

Averages that do not share any letters are significantly different.

Table 5. Tukey's two-by-two comparison of leaves production between the type of cuttings at the 5% threshold.

Type of cuttings	Mean of leaves production
Apical	5.11 ± 3.18^{a}
Median	4.48 ± 2.98^{b}
Basal	4.17 ± 3.47^{b}
Root	0.39 ± 1.44^{c}

Averages that do not share any letters are significantly different.

Table 6. Tukey's two-by-two comparison of the number of branches between the types of substrate at the 5% threshold.

Substrate	Mean number of branches
Sterilised sand	2.49 ± 1.95^{a}
Composite substrate	1.64 ± 1.82^{b}

analysis revealed a significant difference (P<0.006). Tukey's 5% threshold test (Table 4) for the mean value of leaves revealed that the composite substrate produced more leaves than the sterilised sand substrate.

Number of branching per leafy shoot

The ramification represents the new buds that develop on the cutting. Thus the maximum number of branches in the sand substrate is 8 for apical and median cuttings and 7 for basal cuttings. In the composite substrate, 8 branches were also obtained for the apical and median cuttings and 7 branches for the basal cuttings. As for the effect of substrate and type of cuttings on the number of branches, it was revealed to be highly significant with P<0.001. Table 6 shows the results of the mean Tukey test values at the 5% threshold. For the substrate, these results showed that the sterilised sand substrate is higher than the composite substrate. And for the types of cuttings, the Tukey test showed that the middle cuttings produced more branches than the basal and apical cuttings (Table 7). Basal and apical stem cuttings, on the other hand, produce the same number of branches.

DISCUSSION

As far as vegetative propagation by cuttings is concerned, the results showed that stem cuttings (basal, median and apical) are appropriate for reproducing the species. The main characteristics of cuttings (stem cuttings) of the species indicated that: firstly, the latency time is low, uniform everywhere and does not depend on the type of cutting or the type of substrate; secondly, the recovery time varies according to the substrate and the type of cuttings; and thirdly, the ability to take cuttings depends on the position of the fragment on the branch. These two substrates would be adapted to water saturation conditions. This is the case for *F. faginea* whose preferred ecological environment is the forest gallery. Indeed, the recovery rate increases from the base of the branch towards the top (basal towards apical). These results do not corroborate those of Yameogo (2018), which obtained a low latency time on *Securidaca longepedunculata* Fres budburst. The study on *Lawsonia*

Table 7. Tukey's two-by-two comparison of the number of branches between the types of cuttings at the 5% threshold.

Type of cuttings	Mean number of branches
Median	2.30 ± 2.03[a]
Basal	2.02 ± 1.84[b]
Apical	1.88 ± 1.91[b]
Root	0.03 ± 0.21[c]

Averages that do not share any letters are significantly different.

inermis L. (Sanogo et al., 2008) also showed that basal cuttings gave better results compared to apical cuttings. Similarly, the results of Diatta et al. (2007) on the bud break of *Maerua crassifolia* Forssk. showed that the recovery time increases from the base of the branch towards the top, while the recovery capacity evolves in the opposite direction. Thus, the height rate of basal cuttings budburst could be explained by the fact that these cuttings are the youngest. According to Bellefontaine et al (2018), it is recognised that the basal part of the plants is chronologically the oldest, but physiologically the youngest because of its proximity to the root system. But the low recovery rate of basal cuttings in the composite substrate is due to the fact that in our first block, these basal cuttings were close to the glass and the most exposed to the rising sun. So the sunburn associated with the heat of the glass (greenhouse) caused these cuttings to dry out before they budded. This resulted in a very low recovery rate in this block. In fact, there was a relationship between the moisture content of the substrate and that of the cuttings. Martin and Quillet (1974) suggested that sunburn and high temperatures should be avoided and that humidity should always be maintained at 100% around the cuttings in order to reduce losses by evapotranspiration as much as possible. As far as root cuttings are concerned, the results of the recovery rates obtained are very low (5 and 3.75% for sand and composite substrates respectively) whatever the substrate used. These results are similar to those of Zida et al. (2018) who found a low budding rate with *Sclerocarya birrea* (A. RICH.) HOCHST roots. These low rates could be due to a lack of nutrient reserves in these cuttings due to the small size of the cuttings. According to Agbogan et al. (2014) a lack of nutrient reserves can prevent new organ appearance. Similarly, Mapongmetsem et al. (2012b) obtained 86.0±7.8% for cuttings with a diameter between 1.1-2.5 cm and 21±1.8% for cuttings with a diameter between 0.5-1.0 cm by cutting root segments of *Vitex doniana* Sweet. In terms of survival rates after weaning, the sand substrate has the best rate compared to the composite substrate. In the same substrate, basal cuttings recorded the best survival rate.

However, the high mortality of some cuttings (23.75% mortality for basal and apical cuttings of the composite substrate) recorded could be explained by: the heat of the greenhouse due to the respiration of these plants which increased the temperature following the excessive release of carbon dioxide; or the exhaustion of the reserves of these plants before rooting. Similarly, the substrate could be unfavourable for the survival of the species. Indeed, Agbogan et al. (2014) explained the dieback of stem segments of *Scleroclarya birrea*, *Lannea microparca* and *Haematostaphis barteri* by a lack of nutrient reserves before the roots take over and also by fungal attacks. Moreover, the cuttings that survived show that they have successfully completed the healing stage. It is this scarring which favours the emission of their roots (Asseh et al., 2017). At this level, the sandy substratum proved to be better, favouring the rooting of cuttings. Indeed, the difference between these substrates can be linked to aeration and their air/water ratio. A similar result was found with *Coula acuminata* Baill. showing that sand is the best rooting substrate (Paluku et al., 2018) then with Mapongmetsem et al., (2016) on *V. doniana* Sweet. These results show a good aptitude of the species for vegetative propagation with stem cuttings.

Concerning the growth of cuttings, the analysis of variance revealed that the composite substrate was superior to the sterilized sand substrate for height and leaf production while the sterilized sand substrate produced more branching. This could be explained by the fact that the sand does not contain any combined nutrient due to the high number of active buds that need more nutrients to keep them alive. These results do not corroborate those of Asseh et al. (2017) showing in the study of *Thunbergia atacorensis* Akoegninou & Lisowski that plant height and leaf production are not influenced by substrate type. As for the types of cuttings, it revealed that stem apical cuttings were better for height growth and leaves production. This could be explained by the fact that these cuttings have fewer branches than other types of cuttings. This requires less nutrient expenditure for these branches and promotes height growth and leaf production. For Sanogo et al. (2008) basal cuttings grow better than other types of cuttings. However, Obulbiga et al. (2018) did not find any significant difference between the types of cuttings.

Conclusion

This survey allows the cutting reproduction of *F. faginea* easily with lost cost by population. For vegetative propagation by cuttings the apical and basal stem cuttings have a good capacity for vegetative recovery of the species. The median stem cuttings are the best in the production of branches. As the substrate is concerned, the two substrates also give better results for regrowth with a short delay. For better growth and vigour of the species, the composite substrate as well as the apical stem cuttings performs good result. On the other hand, the sandy substrate and median stem cuttings are the best in the production of branches. Based on the literature this reproduction by cutting of *F. faginea* held for the first time in Burkina Faso. In addition, the success of the species reproduction is very important for the plant user's; traditional healers, stakeholders, and scientists. It will permit the conservation and management of this species *ex situ*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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