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Tests of propagation of *Pseudospondias microcarpa* A. Rich. under the climatic conditions of Franceville in the southeastern of Gabon

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Abstract

Pseudospondias microcarpa A. Rich is a tropical tree species, which is not domesticated despite its food and pharmacological potentials. This study aims at introducing its domestication using two types of substrate. The first substrate (S1) is a mixture of compost and local soil while the second substrate (S2) is only made of local soil. The goal is to determine the best method of propagating P. microcarpa, among the following three techniques: direct sowing of seeds, cutting and layering. For the direct sowing, seeds germinated in 41.67 % and 29.16 %, respectively in the substrates S1 and S2. Besides, the axillary and foliar growth of the plantlets was identical in both substrates. These results are due to the composition of the substrates and to the phenological stage occurring at the end of the observations. Out of the 55 % of cuttings which recovered, all the plantlets withered then dried out. The insufficiency of the photosynthetic reserves and the lack of formation of calluses introducing the roots formation explain these phenomena. About 83 % of marcotts formed adventitious roots regardless the substrate, which explains a predisposition of the substrates in the induction of the roots formation. Of the three techniques tested, layering was found to be the best method of propagation of *P. microcarpa*.

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Introduction

The tropical forests are very rich and useful ecosystems. They play an important role in the regulation of the greenhouse gas emission, the establishment of climatic great balances, and they constitute an important biodiversity reservoir for the planet (Tchatat and Ndoye, 2006). The flora provides food products, construction materials, fuel, and drugs to rural populations. According to many authors (Breteler, 1990; Bourobou and Posso, 1995), Gabon possesses one of the richest forests in the Congo basin. However forest trees in Gabon, like in many African countries, remain unexploited to their full potential beside other non-timber forest products (de Jong *et al.*, 2000; Ndoye and Ruiz-Pérez, 2004). In addition to the resources it offers, forest cover regulates local microclimate, prevents soil erosion, and safeguards soil fertility according to Simons *et al.* (2000) and Verheij (2005).

The woody species found in the humid tropical regions meet most of the needs of the local populations (Doucet, 2003; Biloso and Lejoly, 2006; Loubelo, 2012). But this anthropic action contributes to exhaust resources because of the increasing demand of the arable lands. This phenomenon can be evidenced by a pronounced deforestation, an excessive overgrazing and collection of firewood. Moreover, species biodiversity continues to shrink and the genetic base of the tropical forest erodes including resources which are essential for the survival of the populations (Russell and Franzel, 2004; Belcher, 2005).

It becomes thus necessary to nurture forest species whose products have a high value added to populations (Simons 1996 &1997; Simons and Leakey, 2004; Tchoundjeu *et al.*, 2004). It is an artificial selection process leading to the preservation of interesting characters and to the diminution of others, undesired and present in wild plants. The tropical fruit trees are species which play an essential role in the diversification of farming programs and agroforestry systems (Ndoye and Tieguhong, 2004; Russell and Franzel, 2004).

Many of these species, of which some are very well known, produce edible products and traditional medicines. Common examples are *Gambeya lacourtiana* De Wild and *Picralima nitida* Durand, according to several authors (Nguenang *et al.*, 2010; Moupela *et al.*, 2011). Other fruit tree species remain unknown although they have been proven to be useful in local communities (Priso *et al.*, 2011). Actually seeds of woodland species take a longer time to germinate and therefore to mature (Gordon and Rowe, 1982; Cuisance, 1984; Bourobou, 1994; Tchoundjeu *et al.*, 1998; Jaenicke and Beniest, 2003), which makes farmers hesitate to grow them.

The vegetative propagation refers to the techniques of regeneration of plant materials using fragments or plant parts. According to Verheij (2005), various parts of a plant (such as leave, stem and root) can be used to regenerate a new individual. The propagation by cutting is carried out by planting a suitable part of the tree, shrub or creeper. For species which cannot be reproduce by cutting due to an inability to develop roots it is sometime necessary to circumvent this difficulty using layering. Indeed layering technique eases the growth of adventitious on the mother tree (Tchoundjeu *et al.*, 1997; Schroth *et al.*, 2004).

Currently, exotic fruit trees are grown along villages. In fact farmers tend to cultivate wild fruit trees due to a lack of knowledge about their floral biology and proper techniques of growing them (Bourobou, 1994). This situation explains the interest of Tchoundjeu *et al.* (1997; 2004) in this field, which ultimately aims at improving the living conditions of farmers without endangering the environment.

Pseudospondias microcarpa A. Rich. is poorly known of the public, thus the motivation of this study. One expected benefit of this research is the curbing of the duration of production of this species in order to integrate this practice into our cultural and agroforestry systems. It becomes therefore necessary to first identify the best way of propagating *P. microcarpa* among the techniques described earlier, that is, generative technique (seed sowing) and vegetative techniques (cutting and layering) using two different types of substrates. More specially, this study assesses the impact of substrates on the germination and growth in seedbed during the direct sowing, on the one hand, and performing the same assessment for the cutting and layering approaches, on the other hand.

Materials and methods

Study site

The study was carried out on the experimentation platform of *Institut National Supérieur d'Agronomie et de Biotechnologies* (INSAB), in Franceville, southeastern of Gabon. The geographic coordinates of this site are 13°34'23'' S and 13°34'23'' E, and the average altitude is 458 meters. The climate, of equatorial type, is characterized by four seasons. The average rainfall during the study was 32.3mm, the average temperatures was 23.8°C and the maximum relative moisture was 98%.

Substrates

Two types of substrates were used: substrate S1 was a mixture of soil collected onsite (66%) and a fraction of compost (34%) while substrate S2 contained only soil. Soil used as substrate was treated with fungicide (Préfongil) and the insecticide (Bofur 5GR) in the following densities: 2 l/ha for the fungicide and 12 kg/ha for the insecticide. Table 1 shows the physicochemical characteristics of the substrates used for the realization of this test.

Plant material

The plant material came from the species *P. microcarpa* A. Rich. Seeds to be planted were sorted prior to sowing while branches were used as basic plant materials for the vegetative propagation.

Seeds: The pulped fruits were dried for one day, seeds collected from these fruits (Figure 1) were immersed in water, and a final sorting of seeds was completed. This mixture was stirred up every 15 minutes under a laminar flow hood in order to monitor the digestion of the tegument (Roussel, 1984; Danthu *et al.*, 1992; 1996; 2003; Barnes, 2001) and to avoid contaminations. We sowed one seed per bag.

Table 1. Physicochemical characteristics of the two substrates.

Characteristics	Substrate n°1 (S1)	Substrate n°2 (S2)
pH_{water}	5,3	4,4
pH_{KCl}	5,0	3,6
Coarse silt (CS)	31,49	14,04
Fine silt (FS)	11,35	18,55
Clay (%)	16,90	22,70
Sand fine (SF)	15,32	15,34
Coarse sand (SC)	24,94	29,40

Table 2. Morphological characteristics of fruits and seeds.

Characteristics	Fruits	Seeds
	☐± σ	☐± σ
Length (cm)	3,1 (±0,28)	2,9 (±0,28)
Width (cm)	2,2 (±0,24)	1,0 (±0,11)
Mass (g)	10,3 (±1,26)	0,7 (±0,08)

Number: n=20 ; ☐± σ : Average ± standard deviation.

Cuttings: The cuttings originated from orthotropic branches with variable diameters ranging between 1.4 and 2.5 cm. They were 15 cm long with 3 to 4 nodes, and they were clothed in order to minimize their dryness. The collect of cuttings was done early in the morning in order to minimize sweating as much as possible. Usually, the upper part of the cuttings is cut in bevel while the lower cut is done at the base of a node. The cuttings, thereafter, are placed in the substrates within polyethylene bags. Transplanting is done in such a way that 2/3 of the cutting is buried underground while ensuring that more than a node remain above the substrate.

Marcotts: The layering was carried out on branches with ripened wood, from a vertical to a subvertical position, with diameter ranging between 1.7 and 3.6 cm. The successive stages of the layering include the circular cut in the bark of branch (using a knife on 10 cm in length), the treatment of cambium by a light scraping, the filling of transparent polyethylene bags with substrate, and the final coverage of the resulting marcott with another black-colored bag protecting the substrate from solar radiation.

Experimental device

The experimental device is completely randomized for each test. The experimental variables are the substrate (S1 and S2) and the plant materials (seeds, cuttings and marcotts) depending on the mode of propagation. For the propagation by seeds, the number of repetitions was 20 for each one of combinations "S1 X Seeds" and "S2 X Seeds". For the propagation by cutting, the number of repetitions for each combination was also 20. Finally, for the layering, the number of repetitions was of six (06) for each combination. A total of 120 experimental units were completed for this test.

Testing method and observations

The maintenance of seedlings in seedbed (watering and control of weeds) was regular and the daily observations continued over three months. In the case of layering, marcotts were wetted with 20 ml of water per week using a syringe. The observations took place before each watering in order to check for the occurrence of adventitious roots.

The parameters observed take into account the mode of propagation (sowing, cutting, and layering) and the type of substrate. They relate to the vegetative growth of the seedling. For the propagation by seed sowing, the sizes considered and measured were the time of germination, the duration of germination, the rate of germination and the rate of survival.

The germination time or waiting period (in days) is the time spent from the sowing to the first germination. The duration of germination or staggering (in days) expresses the gap between the first and the last germination. The rate of germination (T) can be obtained using the following formula:

$$T = \frac{G}{N} * 100$$

where G is the number of germinated seeds and N is the total number of seeds sown.

The parameters of growth, which are the size of the seedling (cm) and the length and the width of leaves (cm) were also taken into account.

For the propagation by cuttings, the response variables are the time of occurrence of the buds (days), the recovery rate of cuttings (%) and the lifetime after the recovery (days).

Finally, for the propagation by layering, the time of occurrence of the roots evaluated in weeks was the expected response. The rate of success of layering is the ratio of the number of rooted marcotts over the number of marcotts carried out.

Result analysis

The data collected at the time of the observations was analyzed using the software XL.STAT. The test of Student was performed to compare means and the Chi-Square test was used to analyze simple correlations. When the *p*-value and the correlations of Pearson between the treatments were found significant with a 5% threshold, we concluded that parameters were different.

Results and discussion

Generative propagation

Parameters of germination

Figures 1 and 2 illustrate the emergence of the species *P. microcarpa* in substrate S2, as well as the seedlings in substrate S1. The germination of *P. microcarpa* is aboveground with the first two dentale and opposite leaves simple. The (simple or compound) leaves, formed after the first ones, are opposite alternate. They are formed between the fourth and the fifth position.

The rates of germination and survival of seeds, which have not been treated with sulfuric acid were respectively 41.67% and 90% for S1, and 29.16% and

100% for S2. As Danthu *et al.* (1992 & 1996) observed on *Acacia sp.* seeds, the germination of seeds was irregular. It expanded over a period from 20 to 66 days whatever the substrate used. This irregularity is related to the fact that the pollination is open for *P. microcarpa*, which is a dioecious species. It is thus possible to have crossed pollinations between the tree-mother and other neighboring plant species. This situation would lead to obtaining hybrid individuals which could express sterility in the resulting generations. The seeds treated with the sulfuric acid did not germinate. This result is identical with the one that Jaouadi *et al.* (2000) obtained while they submitted *Acacia tortilis* on different abiotic constraints. Tables 2 and 3 respectively present the morphological characteristics of the fruits and seeds, and the parameters of germination of seeds of *P. microcarpa*. The weight of 1000 seeds was 700 grams.

Table 3. Parameters of seeds germination.

Parameters	S1	S2
Duration of germination (days)	20-66	20-66
Rate of germination (%)	41,67	29,16
Taux of survival (%)	90	100

Number : n=20.

Table 4 summarizes the trend of parameters observed (size of the stem, length and width of the leaves) and the comparison of means between the substrates for these various parameters of growth. During the trial period, substrate S1 produced a longitudinal growth higher than substrate S2 (Table 4). The statistical analysis helped compare and identify differences in the growth, on both types of substrates. Meanwhile the maximum length of leaves was reached at the end of the second week for substrate S1 whereas this time was three weeks for substrate S2.

The leaves had a better expression of growth in width in substrate S2. Just like for the growth in length of leaves, the maximum width of leaves was reached after two weeks in substrate S1, and after three weeks in the substrate S2.

Whatever the parameter of growth considered, the comparison of the means during the first five weeks of growth (Table 4) indicates that the effect of the substrate is statistically non-significant at a 5% threshold.

The absence of germination of seeds treated with the sulfuric acid can be explained by the duration of the treatment. Indeed, according to Verheij (2005) and Danthu *et al.* (2003), deepening seeds into concentrate acid solution causes a swelling of seeds, which lead to a tearing of teguments, a scrubbing of substances, and therefore a delay of germination. Indeed, acid is very abrasive, and for these reason it causes seeds to become much permeable to the chemical, which ultimately damages the embryo and its teguments. It's about a mechanical dormancy, which is characterized by permeability to water although the envelope of seed is thick and hard. The ending of dormancy is then inhibited, even when water manages to infiltrate. Our results agree with those of Gordons and Rowe (1982) who worked on *Vibumum* species, as well as Schroth *et al.* (2004) who worked on *Astrocaryum tucuma* seeds and Tchoundjeu *et al.* (2002a) who observed the vegetative propagation of *Prunus africana*.

The composition of the two substrates used has a known effect on the germination rates of untreated seeds. Indeed, the mixture soil-compost (S1) is richer in coarse silts compared to substrate S2, which was made up of soil only. In a general, the silts have the ability of preserving soil humidity as they also offer a better permeability of substrates (Danthu *et al.*, 1996; Jaenicke and Beniest, 2003). A poor substrate enhances the deformation of roots, contamination by pathogen and growth delay, according to Schiffers *et al.* (2007).

The lack of statistically significant difference between the means of the three parameters of growth considered in this study implies that the increase in dry matter is identical during the first five weeks in all seedlings, for both substrates S1 and S2. Therefore, the quantity of compost associated with the local soil did not have an impact on the increase in dry matter. The granulometric and physicochemical characteristics (pH) explain this indifference (Table 1).

Table 4. Evolution of morphometric parameters in the two substrates.

Parameters	Time of observation	Substrates	
		S1	**S2**
		☐± σ	**☐± σ**
Height of the seedlings	D7	7,88 (±1,52)	6,60 (±2,15)
	D14	8,83 (±1,70)	7,91 (±1,79)
	D21	10,01 (±2,11)	8,90 (±1,81)
	D28	11,51 (±2,41)	9,59 (±2,02)
	D35	12,23 (±2,59)	10,93 (±2,25)
Length of leaves	D7	4,64 (±1,01)	5,23 (±0,74)
	D14	5,56 (±1,25)	6,17 (±0,68)
	D21	5,61 (±1,28)	6,56 (±1,01)
	D28	5,61 (±1,28)	6,58 (±1,05)
	D35	5,61 (±1,28)	6,58 (±1,05)
Width of leaves	D7	2,82 (±0,87)	3,16 (±0,54)
	D14	3,50 (±0,85)	3,72 (±0,68)
	D21	3,55 (±0,85)	4,10 (±0,92)
	D28	3,60 (±0,86)	4,12 (±0,95)
	D35	3,60 (±0,86)	4,12 (±0,95)

Number : n=20 ; ☐± σ : Average ± standard deviation.

In addition, although it did not enhance a better growth on one of the substrates, the sexual reproduction has the advantage of producing healthy individuals by limiting the propagation of parasites at the seed level. This is the reason why Jaouadi *et al.* (2010) promote this method of plant regeneration.
Moreover, several genetic variations exist in dioecious plants, which is the case for *P. microcarpa*. Indeed, this dioecy implies a recombination of genes involving different genetic bases in seeds. It is thus possible to have plants which are phenotypically identical but with different genome.

Vegetative regeneration
Propagation by cutting: Table 5 and figures 3 and 4 represent on one hand the main parameters of propagation by cutting using the two substrates, and on the other hand, the recovery of cuttings in *P. microcarpa*.

The initiation of the buds precedes the recovery of cuttings characterized by the occurrence of compound leaves. In general, all aboveground nodes develop depending on the cutting's ability to regenerate new individuals.

No matter the substrate used, the recovery rate of the cuttings was 55%. The average survival times of buds were 33 days for substrate S1 and 41 days for substrate S2. The axillary growth was slow as the first days, followed by a drying up of the buds resulting in the death of seedlings.

The buds gave rise to compound leaves; however, the regenerated cutting lived only during a limited time.
The coefficient of correlation observed revealed the existence of a very weak bond between the various parameters considered, no matter the substrate. However, according to the table of distribution of

Student, the *t*-value computed from the coefficients of correlation compared to the table value of *t* led to a rejection of the dependence between variables. Indeed, according to the results of analysis, the variations of recovery times and diameters are neither dependent between themselves nor with the substrate.

Table 5. Parameters of resumption of the cuttings.

Parameters	Substrates	
	S1	S2
Time of appearance of the buds (days)	12	12
Duration of appearance of the buds (days)	9-13	9-15
Rate of resumption of the cuttings (%)	60	55
Rate of survival (%)	0	0
Duration of the life (days)	33	41

Number: n=20

More than 50% of the cuttings, planted in the substrates S1 and S2 resumed their growth but they desiccated thereafter. The absence of regeneration of certain cuttings is due to an early drying out of the cutting under the effect of the weather after the cutting's detachment from the mother tree, as Simons and Leakey (2004) recorded.

According to Kengué (2002), the formation of vegetative axes having persisted for a relatively long period of time before withering and desiccating is due to a small quantity of photosynthetic products in the stems. The growth happened thanks to the food reserves inside the cutting while the withering was caused by the depletion of these reserves due to the absence of a root system that could take over. Indeed, when a branch is separated from the mother plant, it becomes autonomous from a nutritive standpoint must first stimulate the formation of a scar callus, which induces the growth of the roots. Surely, the vegetative axes (leaves and buds) in growth have a stimulating effect on the root formation, but this phenomenon depends enormously first on the initiation of the callus.

The results of this study, with respect to the propagation by cutting of *P. microcarpa* using 15 cm long plant materials, agree with Bourobou (1994), who used macrocuttings of *Pseudospondias longifolia*. Both studies highlight the difficulty in inducing the root formation during the propagation by cutting in these two species.

Table 6. Parameters of resumption of the layers.

Parameters	Substrates	
	S1	S2
Time of appearance of roots (days)	11,2	12,4
Duration of appearance of roots (days)	9-13	9-15
Rate of resumption (%)	83	83
Rate of survival (%)	0	0
Duration of life (dyas)	33	41

Number: n=20

Layering: Table 6 and Fig. 5 and 6 present on one hand the characteristic parameters of the recovery of marcotts, and on the other hand, the rooting of a marcott and the aspect of two weaned marcotts.

The first roots were observed within nine weeks, while the last ones occurred in 15 weeks. The rate of success of the layering was 83% in the two substrates. Only two marcotts could not root up.

The two media of rooting appeared to be adequate for the root formation of *P. microcarpa* in spite of the differences observed in terms of occurrence of roots relative to the diameter of marcott and the nature of the substrates.

The matrices of correlation between the diameter of the marcott and the time of occurrence of the roots in the substrates S1 (- 0.262) and S2 (- 0.594) reveal that this time is independent of the diameter of the branch regardless the substrate used. Indeed, the coefficients of correlation are by far lower than the value read on the table of distribution of Student.

The layering thus seems to be a success overall. This fact corroborates with the assertions in connection with the insufficiency of reserves contained in the stems of the tree for a total recovery during cutting. Indeed, Tchoundjeu et al. (2002a), then Jaenicke and Beniest (2003) and Schippers *et al.* (2007) assert that there is, at the time of the layering, an accumulation of photosynthetic products (such as carbohydrates and auxins) in the upper part of the annealing. Therefore, the auxins activate the root formation in the presence of a wet substrate.

Fig. 1. Observation of the lifting of seed of *P. microcarpa* in the S2 substrate

Fig 2. Plantlets of *P.* two weeks old *microcarpa* led in the S1 substrate.

Fig. 3. Initiation of the buds at the base of a cutting of *P. microcarpa*

Fig. 4. Appearance of the leafy buds resulting from the cuttings of *P. microcarpa*.

Fig. 5. Rooting of the air layer of *P. microcarpa*

Fig. 6. Layers de *P. microcarpa*, separated at the end of fifteen weeks.

According to Tchoungdjeu *et al.* (1997) then Jaenicke and Beniest (2003), the clones resulting from the vegetative multiplication have an accelerated development and an early entry in production without young fragile stages. In addition, they present homogeneity by preserving the genetic constitution of the mother plant.

The above authors highlighted that the importance of choosing the vegetative technique in the domestication of woody species is linked to the evaluation of the potential of certain trees and to the reactions producing fruits of higher quality. It allows checking the existing reaction bond between the tree and its environment or management. In agreement with the above-cited authors, this study indicates that the vegetative technique is more adequate for propagating dioecious tree species and for producing fruits of great value.

Conclusion

The domestication program of *P. microcarpa* A. Rich. requires the knowledge of the floral biology of this species and the identification of the best method of its propagation, between the generative and the vegetative technique.

This study shows that the treatment of the seeds of *P. microcarpa* with sulfuric acid for four hours deteriorates the seeds and reduces their germination ability. Moreover, the evaluation of the increase in dry matter of seedlings, during the first five weeks of growth in the two substrates (soil only and soil-compost mixture) does not present a significant difference because of the similarities observed in their respective composition. While the cuttings of the species *P. microcarpa* present a potential for recovery, they wither and desiccate thereafter due to a deficiency of photosynthetic products and the lack of a callus formation. The layering, however, seems to be a better alternative for propagation for regenerating *P. microcarpa* because it allows an easy occurrence of adventitious roots on the marcotts. This technique remains the method most advisable for propagating the species *P. microcarpa*.

Following this study, which represents an initial step for domesticating *P. microcarpa*, further investigations need to be done notably by focusing more on the possibilities of its propagation. This dynamics could be articulated around the development of a better technique for treating seeds before sowing and the test of micropropagation of the species for the procurement in great quantity and in a short period of time very healthy plant materials. Finally, it is important to continue the observations after the weaning of the marcotts in order to evaluate and quantify the parameters of growth following the replanting of marcotts.

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