International Scholars Journals

International Journal of Agroforestry and Silviculture ISSN 2375-1096 Vol. 7 (10), pp. 001-010, October, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of cuttings diameter and indol acetic acid on rooting of *Pterocarpus erinaceus* Poir. stem cuttings

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Accepted 01 August, 2019

In order to develop a viable and large-scale *Pterocarpus erinaceus* seedling production strategy for reforestation, stem cuttings test was carried out using 15 cm length 1.5 to 3.5 cm diameter cuttings, harvested from healthy trees at approximately the same levels on young branches in the morning time. Various levels of Indol Acetic Acid (IAA) dose, cuttings diameters and soaking duration of cuttings into IAA were tested. Cuttings budding, shoots diameter and height as well as number of leaves were recorded. We performed a binomial logistic regression, analysis of variance and generalized linear regression to evaluate the effect of various factors on budding rates, bud bursting and rooting, to assess the effect of these factors on shoots diameter and height, and the effect on the number of leafy shoots. The results showed high rates of budding and bud bursting (>70), whatever the cuttings diameter. Cuttings treated with IAA showed the highest budding rate (>80%). Cuttings diameter significantly influence the number of leafy shoots per budded cuttings, shoots diameter and height ($p<0.05$). *P. erinaceus* can be successfully propagated by stem cuttings combining suitable cutting diameter (2 – 3 cm) and appropriate dose of IAA (1500 - 2000mg/l) for twenty minutes and one hour.

Key words: Cuttings diameter; Indol Acetic Acid; *Pterocarpus erinaceus*; vegetative propagation; seedlings growth.

INTRODUCTION

The priority challenge during the recent decades is to solve the crucial problem of dwindling forest resources, loss of biodiversity and vulnerability of ecosystem services to human communities. The continuous exploitation of wood, associated with the practice of extensive agriculture and urbanization are exposing more and more forest trees to strong degradation pressures. The scarcity of woody species is much greater for those that are valued by livestock, those that have a significant economic value, those that occupy an important place in daily socio-cultural activities and those that are indispensable in meeting the needs of the people's health. Among these species is *Pterocarpus erinaceus*, an endangered species on the IUCN Red List (Barstow 2018). It is a multipurpose species that faces strong anthropogenic pressure in its ecological habitat (Kokou et al. 2009, Fontodji 2015) due to the high technological performance of its wood (Segla et al., 2015). *P. erinaceus* has recently been screamed from its natural habitat for its timber which is traded internationally to Asian countries, mainly China. Several authors have pointed out the increased rate of illegal felling and over export of rosewood in African countries such as Benin, Gambia, Ghana, Guinea Bissau, Nigeria and Mozambique (Treanor 2015, Dumenu and Bandoh 2016, Jafaru 2017, Futukpor 2018).

Faced with this persistent threat, the domestication of the species is important to ensure its sustainability. Several actions have been carried out in favor of the species to reduce the pressure on it and make it an opportunity for the region. Dumenu (2019) assessing the impact of felling/export ban and CITES designation on the exploit-

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ation and trade of *Pterocarpus erinaceus* in Ghana, has concluded on the necessary *ex-situ* conservation measures such as seed banking, field gene banking, tissue culture and plantation development to conserve this endangered plant species in order to avoid the possibility of commercial extinction.

According to Monteuuis (2000), knowledge of the best conditions for seed germination and/or vegetative propagation by cuttings of species is a key to guarantee their domestication and conservation. However several trials initiated in recent decades on the cuttings of *Pterocarpus erinaceus* in Togo, Niger and Benin attempted to conclude on the impossibility or the difficulty of rooting despite the use of rooting hormones (Kokou et al. 2015; Bodjrènou et al. 2018). However Ky-Dembélé et al. (2015) indicated a potential for rooting of the species but in a relatively small proportion (37%).

This study aims to test the effect of various cuttings diameter, doses of indol acetic acid (IAA) and duration of cuttings soaking into IAA, on the rooting of stem cuttings of *Pterocarpus erinaceus*.

MATERIALS AND METHODS

Plant material

Pterocarpus erinaceus is a leguminous plant of the subfamily of Papilionoideae, used in cabinetmaking because of the high quality of its wood and for cattle feeding in the dry season as the nutrient content of its leaves is especially consistent. In the current study, only stem cuttings were used. Stems were harvested from healthy mature trees whose diameter at 1.30 m ranged from 15 to 35 cm. The cuttings were harvested in the morning before periods of high temperatures to limit their dehydration. Cuttings were collected at approximately the same levels on young branches. They were cut into 15 cm length segments, leafless with diameter comprising between 1.5 and 3.5 cm.

Study site location

The nursery was established on the experimental farm of Faculty of Agronomy, University of Parakou (Fig. 1). This site is located in North Benin, Department of Borgou at 407 km from Cotonou. The average altitude of experimental site is 350 m. It is located between 9 ° 15 and 9 °30 north latitude and between 2 ° 20 and 2 ° 45 east longitudes. Parakou city has a wet tropical climate of Sudanese types characterized by a rainy season that extends from May to October and a dry season from November to April. The average temperatures are fairly constant and close to 26 ° C and average annual rainfall is between 1150 and 1300 mm (Kora 2006). The biogeoclimatical conditions of the study site match those of the species natural habitat.

Experimental design and data collection

Two sets of experiments were carried out to assess the effect of IAA dose, diameter of cuttings and soaking duration on budding, degeneration and cuttings rooting of *Pterocarpus erinaceus.*

The first experimental test was conducted under the effect of three factors that are: diameter of the cuttings with two levels ([0-1.5 cm]; ([1.6 - 3cm]), the dose of IAA with 6 levels (0; 500; 750; 1000; 1500; and 2000 mg / l) and soaking duration in IAA with 4 levels (0; 10; 20 and 30 min). A total number of 48 (2 x 6 x 4) treatments, repeated 11 times were established. The data collected were related to the number of cuttings budged, and number of cuttings that degenerated after, diameter of shoots as well as number of leaves produced.

As all cuttings with a diameter comprising between 0 and 1.5 cm having budded (including the control) degenerated within 20 days after budding, while some of those of diameter greater than 2cm rooted, a second experimental test was settled in a split-plot design based on three factors which are: cuttings diameter with three levels ([1.5-2], [2.1 - 2.5], [2.6 - 3] and [3.1 - 3.5] centimeters), the dose of IAA with three levels (750; 1500; 2000 mg/l) and soaking duration in IAA with three levels (10seconds; 20minutes and 1 hour). A total number of 36 (4 x 3 x 3) treatments, repeated 3 times were established. The main factor was the cuttings diameter. The subsidiaries factors were randomly distributed in the sub-blocks. There was one control in each sub-block exempt of IAA treatment. The experimental test was conducted using leafless stem cuttings put in pots filled with forest soil substrate. The plots were manually watered 1 to 2 times per day. Data collected were related to the cutting budding, bud bursting, number of shoots, shoot diameter and height. The number of budded cuttings and leafy shoots were noted weekly for eight weeks. The number of rooted cuttings, diameter and height of leafy shoots were noted at the end of the tests (after 24 weeks).

Data statistical analysis

To assess the effect of IAA dose, cuttings diameter and soaking duration on budding, bud bursting and rooting of *Pterocarpus erinaceus* stem cuttings, binomial logistic regression was performed using the logitor function of the *mfx* package (Fernihough 2019). Histograms were plotted to show the rates of budding and bud bursting. In order to test the effect of these factors on the number of leafy shoots, the generalized linear model (glm) of poisson family was performed. Student Newman and Keuls (SNK) mean structuring tests were performed to categorize the treatments. Analyzes of variance (ANOVA) were performed to assess the effect of these factors on the diameter and height of leafy shoots. This was preceded by tests of normality and homogeneity of residual variances.

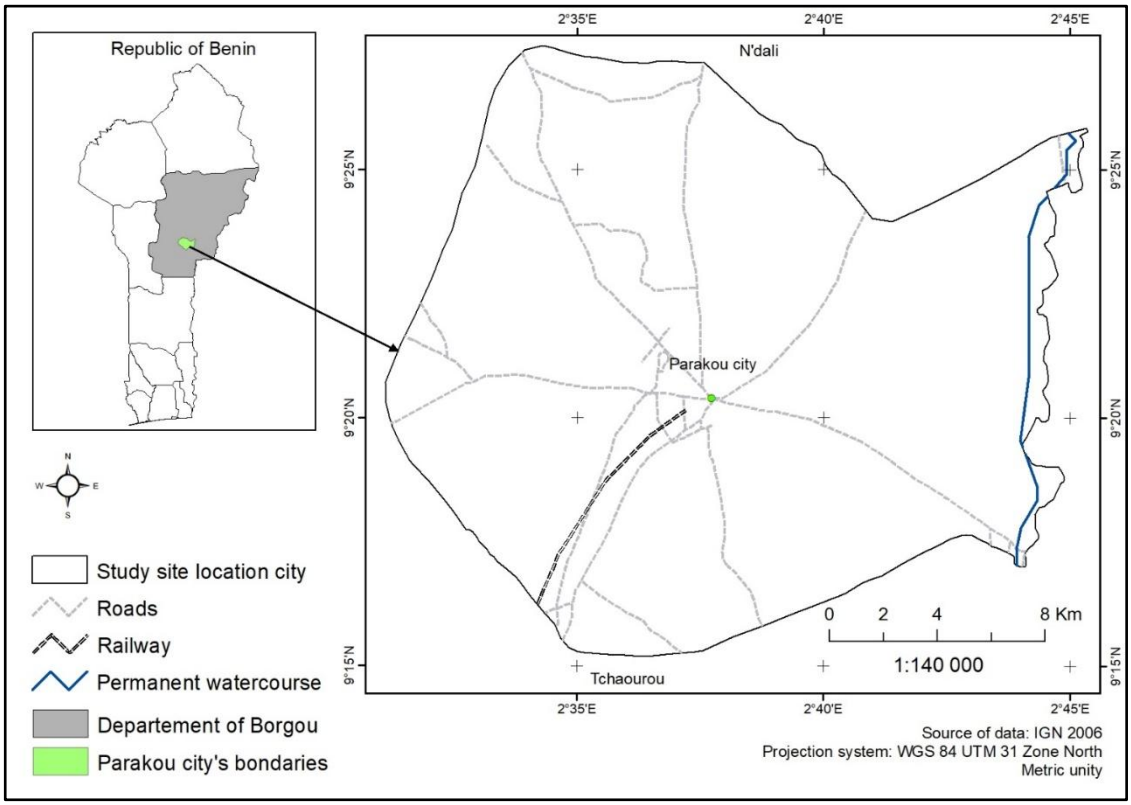


Figure 1. Location of the study site.

Boxplots were subsequently drawn to present the median values of number of leafy shoots, the diameter and height of leafy shoots according to the diameter class of cuttings, dose of IAA and soaking duration. All analyzes were performed using R version 3.5.1 software (R Core Team 2018).

RESULTS

Budding of the cuttings after eight weeks

The budding started the fourth day after establishment of the tests. For all treatments, the first buds appeared in less than a week similarly to the controls test. All cuttings with diameter comprising between 1.5 and 2cm that budded degenerated after three weeks. The budding rate of the cuttings is 79.8 % after eight weeks. The budding rates are statistically similar whatever the diameter class (78.89 %, 80.56 % and 80.56 % respectively for cuttings with diameter comprising [2 - 2.5], [2.6 - 3] and [3.1 - 3.5] centimeters). In contrast, levels of IAA dose and soaking duration of cuttings have statistically significant ($p < 0.05$) effects on the budding (Table 1). The chances of budding of the cuttings soaked in IAA at doses 750, 1500 and 2000 mg/l are respectively 5.70, 2.55 and 3.71 times higher, compared to the control tests (without application of IAA). All cuttings treated with IAA showed a budding rate very higher (>80 %) than the one of control tests (62.22 %; Fig. 2.a). Similarly budding rates after eight weeks range from 79.26 % for cuttings soaked for ten seconds to 89.63 % for cuttings soaked for one hour (Fig. 2.b).

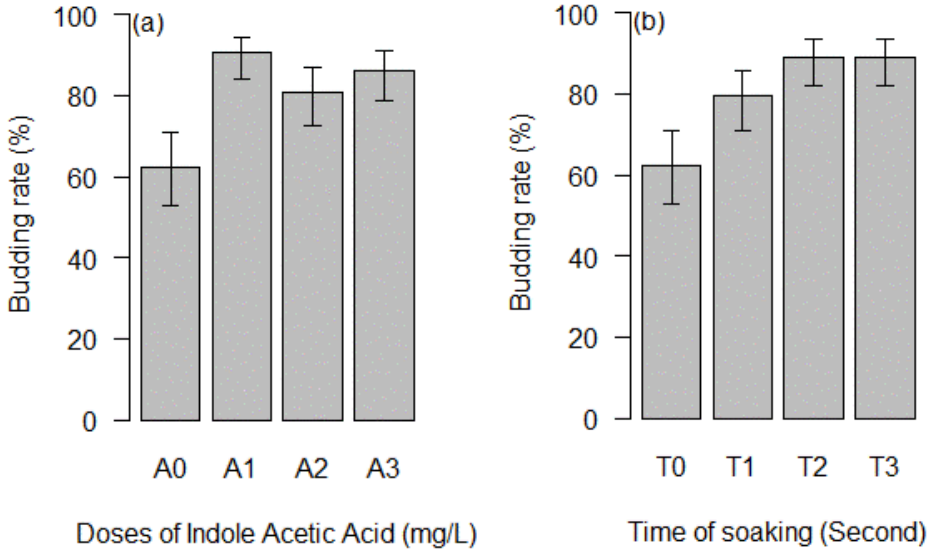
Pterocarpus erinaceus cuttings buds bursting after eight weeks

Cuttings Bud bursting rate after eight weeks was 72.2 %. But the bud bursting rates were statistically similar whatever the cuttings diameter (76.67 %, 70.56 % and 70 % respectively for diameter of [2 – 2.5], [2.6 – 3] and [3.1 – 3.5] centimeters). On the other hand, the dose of IAA and soaking duration of the cuttings have statistically significant effects ($p < 0.05$) on the bud bursting. Compared to control test, the probability of bud bursting is 5.21, 3.40 and 3.71 times higher respectively for cuttings soaked in IAA at 750, 1500 and 2000 mg/l (Table 2). The dose 750 mg/l showed the highest bud bursting rate (83.70 %). The duration of cuttings soaked into IAA proportionally increased the rate and chance of cuttings bud bursting. The bud bursting rates range from 71.85 % for cuttings soaked for ten seconds to 85.19 % for

Table 1. Effect of diameter class, IAA dose and soaking time on the budding of *P. erinaceus* after eight weeks.

Treatments	Odds Ratio	STD	P>|z|
IAA dose (mg/l)			
750	5.70	1.95	0.000
1500	2.55	0.72	0.001
2000	3.71	1.13	0.000
Soaking duration (seconds)			
10	2.32	0.64	0.002
1200	4.86	1.59	0.000
3600	4.86	1.59	0.000

STD: standard error, P: budding probability at 5 %.



Legend : A0 : control, A1 :750mg/l, A2 :1500mg/l, A3 :2000mg/l et T0 : control, T1 :10 seconds, T2 :1200 seconds, T3 :3600 seconds

Fig. 2. Budding rates of the cuttings according to doses of IAA (a) and time of soaking (b) after eight weeks.

cuttings soaked for one hour. Soaking of the cuttings in IAA significantly effects ($p < 0.05$) bud bursting (Table 2).

Effect of cuttings diameter, IAA dose and soaking duration on shoots growth

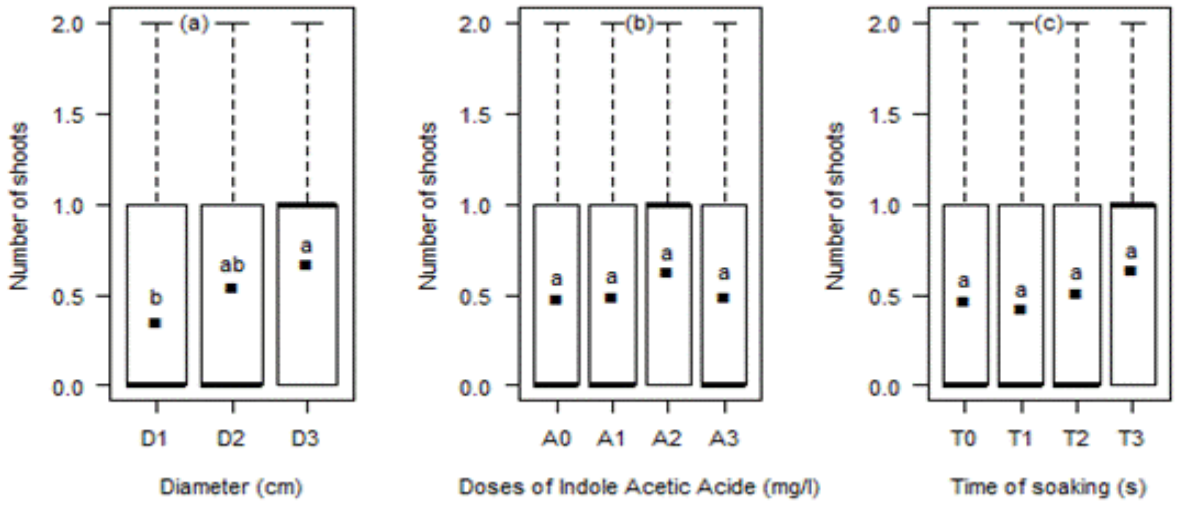
The diameter of the cuttings has a significant effect ($p<0.05$) on the number of leafy shoots per budded cuttings (Table 3). The average number of leafy shoots per budded cuttings slightly increased with cuttings diameter increasing (0.36; 0.55; and 0.68 respectively for cuttings belonging to [2 – 2.5], [2.6 – 3] and [3.1 – 3.5] centimeters diameter classes, Fig. 3). However, these values were statistically similar to those recorded from control tests (0.48, 0.49, 0.49 and 0.63) as well as those of soaking time of 10 seconds, 20 minutes and 1 hour (0.43, 0.52 and 0.64 respectively, Fig. 4).

The rooting rate at the end of the tests (24 weeks) is 29.0 %, 63.0 % and 07.9 % respectively in cuttings with diameter [2 – 2.5], [2.6 – 3] and [3.1 – 3.5] centimeters (Figure 5). According to the IAA dose applied to the cuttings, the rooting rates were 0 %, 17.7 %, 28.8 % and 74.8 % respectively in control tests and cuttings treated with 750; 1500 and 2000 mg/l of IAA. As far as soaking duration is concerned, the cuttings revealed rooting rates of 0%, 26.8% and 87.0% respectively for soaking duration of 10 second, 20 minutes and 1 hour.

Table 2. Effect of IAA dose and soaking duration on the bud bursting of *P. erinaceus* after eight weeks.

Treatments	Odds Ratio	STD	P>|z|
IAA dose (mg/l)			
750	5.21	1.51	0.000
1500	3.40	0.91	0.000
2000	3.71	1.01	0.000
Soaking duration (seconds)			
10	2.59	0.67	0.000
1200	4.94	1.42	0.000
3600	5.51	1.62	0.000

STD: standard error, P: budding probability at 5 %.



Legend : A0 : control, A1 :750mg/l, A2 :1500mg/l, A3 :2000mg/l et T0 : control, T1 :10 seconds, T2 :1200 seconds, T3 :3600 seconds.

Fig. 3. Boxplots of the average number of leafy shoots per budded cuttings after 24 weeks.

Table 3. Effect of diameter class, IAA dose and soaking duration on leafy shoots after 24 weeks.

Sources of variation	**Ddl**	**Residual deviance**	**Pr (>Chi)**
Diamater class	2	13,84	0,001
Dose of IAA	3	2,56	0,464
Duration of soaking	2	4,47	0,107
Diamaeter class x Dose of IAA	6	1,92	0,927
Diamater class x Time of Soaking	4	2,01	0,735
Dose of IAA x Time of Soaking	4	0,49	0,974

Ddl : Degree of freedom, Pr : Probability of significance at 5 %.

Diameter and height growth of leafy shoots after 24 weeks

The cuttings diameter has statistically significant effect on the diameter growth of leafy shoots ($p<0.05$), but not on their height growth (Table 4). Figure 6 showed the average diameter and average height of leafy shoots of rooted cuttings. The average diameter of leafy shoots are statistically similar for cuttings with diameter [2 – 2.5], [2.6 – 3] and [3.1 – 3.5] centimeters (3.11, 4.46 and 4.75 mm respectively). Similar trend is also observed as far as the applied IAA dose and the soaking duration are concerned.

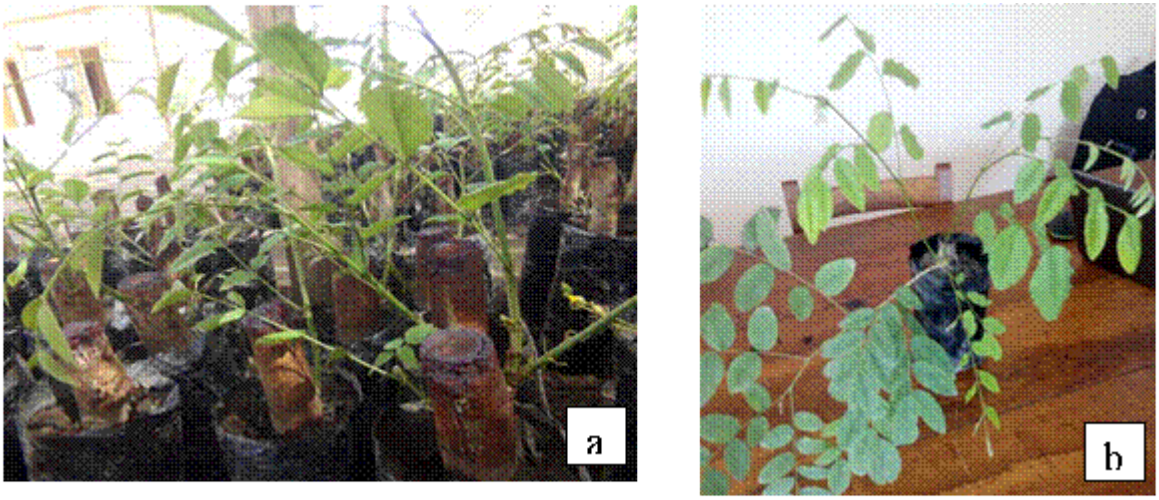


Fig. 4. Leafy shoots growth (a), cuttings with several leafy shoots (b).

Fig. 5. Rooted cuttings in forest soil substrate (a); roots (b) after 24 weeks.

Table 4. Effect of diameter class, IAA dose and soaking time on diameter and height of leafy shoots.

Sources of variation	Diameter of leafy shoots		Height of leafy shoots	
	F value	Pr(>F)	F value	Pr(>F)
Diameter class	25.40	0.000	10.12	0.052
Dose of IAA	3.05	0.081	1.17	0.654
Duration of Soaking	5.10	0.056	2.81	0.324
Dose of IAA x Time of Soaking	0.33	0.575	2.32	0.443

F: Fisher's statistic, Pr: Probability of significantly at 5%.

DISCUSSION

Budding ability of *Pterocarpus erinaceus* stem cuttings

The results showed a high rate of budding (more than 70%) of *Pterocarpus erinaceus* cuttings whatever the treatment applied (treated and untreated). All the batches of cuttings including control showed very high budding rates for diameter ranging from 1.5 cm to 2cm as well as the diameter ranging from 2.1cm to 3.5cm. The highest budding rates (from 79.26% to 89.63%) were obtained for the batches of cuttings soaked in IAA solutions whereas the lowest budding rate (62.2%) was produced by the

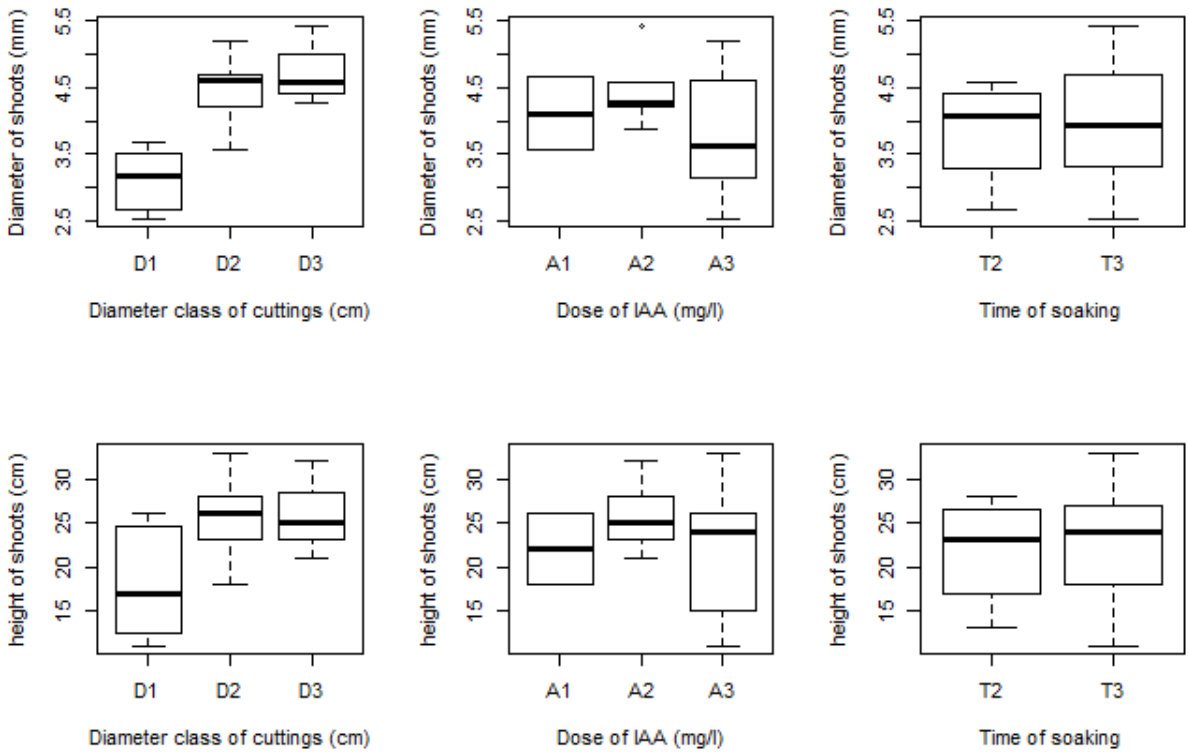


Fig. 6. Boxplots of the average diameter and height of leafy shoots.

Fig. 7. Rooted cuttings (a); cuttings with callus (b).

control. These values are higher than the budding rate recorded by Bodjrenou et al. (2018) on *Pterocarpus erinaceus* stem cuttings (35.5%) using naphthalene acetic acid (NAA) and Ky-Dembele et al. (2015) who used NAA and Indol Acetic Acid (37%). The results indicated that cuttings' budding is not related to any treatment. However, progressive drying out, sometimes accompanied by degeneration, was observed from around three weeks for cuttings with diameter comprising between 1.5 and 2 cm. The progressive decline of cuttings would certainly be due to the depletion of the reserves related to the growing maintenance needs of the shoots before rooting.

Other authors who tested stem cuttings of *Sclerocaria birrea* (Zida et al., 2018) and *Dispyros mespiliformis* (Noubissié-Tchiagam et al. 2011) revealed similar results.

These authors reported on the one hand that the cuttings without root system are kept alive on the basis of accumulated reserves, but degenerated progressively as these reserves are exhausted. The same authors justify the withering of the cuttings budded by the period of the year in which the tests were conducted in the sense that tests installed just before the return of the rains would probably have shown better survival rates.

As far as cuttings growth is concerned, the results obtained from diameter and height growth of leafy shoots revealed that the number and vigor of leafy shoots are related to the cuttings size, but not to the rooting hormone (IAA) applied. On the other hand, it appeared that the application of IAA at reasonable doses of 1500 and 2000 mg/l for twenty minutes up to one hour improves the rooting rate from 26.8% (1500 mg/l) to 87.0% (2000 mg/l). These results constitute a hope among the first studies on the vegetative propagation of *Pterocarpus erinaceus*. In terms of number, height and diameter of leafy shoots, the best performance recorded from the diameter of the cuttings could be explained by the use of IAA which has facilitated root induction because only the cuttings treated with hormone rooted.

Stem cuttings diameter and IAA as success factors for cutting of *P. erinaceus*

The efficiency of cutting success is largely related to rooting. Indeed the budding alone does not explain the chances of survival of the young shoots because it is based on the reserves stored by the cuttings to occur. So after the depletion of these reserves in the absence of a root system that can take over to feed the young shoot, it dries up and dies. This explains the favorable budding and bud bursting response observed in the three diameter classes. This result corroborates perfectly those of Kokou et al. (2015) and Bodjrènou et al. (2018) revealing that diameter has a marginal effect on bud development. However, contrary to previous trials that concluded that stem cuttings of *P. erinaceus* cannot be rooted and degenerated after a while (Bodjrènou et al., 2018; Kokou et al., 2015), our results show that cuttings with diameters between 2 and 3 cm can root perfectly when soaked in IAA dose of 1500 to 2000 mg/l for 20 minutes to 1 hour. In this experiment, cuttings with small diameter, non-soaked cuttings (control) as well as cuttings soaked in IAA for ten seconds have not rooted. The cuttings that survived with leafy shoots until the end of the tests (24 weeks) are those that rooted sufficiently (Fig. 7.a) or who made large amount of callus (Fig. 7.b). The callus formation is a necessary preliminary for cuttings rooting.

CONCLUSION

This study showed that the stem cuttings of *Pterocarpus erinaceus* can be used to propagate the species successfully. The cuttings with diameter comprising between 2 and 3 cm can root properly when they are soaked into indol acetic acid (IAA) at dose 1500 to 2000 mg/l for one hour. The cuttings with diameter higher than 3 cm have a rate of rooting lower than 1%. The cuttings diameter is an important factor for the leafy shoots development and hence rooting of *P. erinaceus* stem cuttings. We suggest that further investigations assess the influence of soaking duration on rooting ability and shoot growth vigor of stem cuttings of *P. erinaceus.*

ACKNOWLEDGMENT

We are grateful to Sud Expert Plantes Développement Durable (SEP2D) and its donors for having funded this work through research project N°AAP-1_45.

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