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Ecological Characterization and Mass propagation of *Mansonia altissima* A. Chev. in the Guinean Zone of Benin, West Africa

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ABSTRACT

The ecological characteristics of M. altissima in Benin were studied within the sacred groves of Adakplamè, its exclusive environment in Benin. The data collected in 40 rectangular plots of 1000 m² sized showed that M. altissima is more preferred species in this environment (R_i = 37.5%). Variables such as tree density, stand basal area, and mean diameter of tree were respectively 20 stems/ha, 0.88 m²/ha and 23.91 cm. Weibull 3-parameters distribution showed an « inverted J » shape with the predominance of medium class-sized individuals (DBH=15 cm). Germination test showed that M. altissima seeds were affected by a tegumentary dormancy making the germination rate decrease after a long conservation. Soaking of seeds in boiling water was an appropriate treatment to overcome this dormancy and to reduce the latency time and the average duration of germination. This also speed up seeds germination and allowed the highest germination rates. Mass propagation by cuttings showed a weak performance of regeneration. Naphthalene Acetic Acid (NAA) and cuttings diameter showed no significant effect on the regeneration of M. altissima cuttings.

Key words: *ecological characterization, vegetative propagation, M. altissima, Benin*

INTRODUCTION

Benin's population was estimated at about 10 million of inhabitants[1] and has an annual increase rate of 3.5%. The majority of the population depends on natural resources to meet their daily needs. Thus, agriculture, the exploitation of wood and Non-Timber Forest Products (fruits, medicinal and aromatic plants) are the important sources of income increasing for the rural populations and consequently improvement of their living conditions. However, some species undergo an increasing human pressure; others are endangered because of their overexploitation, or by the lack of natural regeneration by seedling, or just simply because of the disappearance of their ecological environments[2]. Among these species, *M. altissima* A. Chev. (Sterculiaceae) occupies a place of choice. This species is met in the African rainforest from Guinea and Ivory Coast till Central African Republic and the northern Congo.

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In Benin, this species is local endemic in the Guinean area and is found exclusively in the semi-deciduous rainforest of Adakplamè. The area size of this favorable environment is strongly reduced and continues to decrease because of encroachments from agriculture and the uncontrolled wood felling. *M. altissima* is Endangered (EN), and Critically Endangered (CR) in Benin[3]. It appears in the list of protected species mentioned in the article 36 of the Law 93-009 annexed at Decree n°96-271 of July 2nd 1996 under implement of forest regime in Benin. It is known for its economic, social, cultural, medicinal and pharmacological uses[4,5]. Taking into account the importance and different perceptible threats of extinction of this species in Benin, priority actions were recommended on the species[6]. Studies were recommended on the species ecology and process of regeneration[3]. The current survey concerns the ecological characterization and mass propagation trials of *M. altissima* in the ecosystem of sacred grove of Adakplamè using floristic inventory, structural characterization, and seeds germination and cuttings.

MATERIAL AND METHOD

Study area: The study was conducted in the district of Adakplamè and in the sacred groves of Kouvizoun. The district of Adakplamè is a rural area located in the Plateau department at about 130 km from Cotonou; the economic capital of Benin. The sacred groves Kouvizoun is a relic fragment of semi-deciduous rainforest of about 738 ha, of which a part is occupied by the whole agglomerations of Adakplamè. This region is characterized by a Guinean climate, with two seasons: a rainy season from March to November and a dry season from November to March. The average annual rainfall for the years 1995-2014 was 1678 mm. The average annual temperature was 26.5° C while average relative humidity was 75% per year.

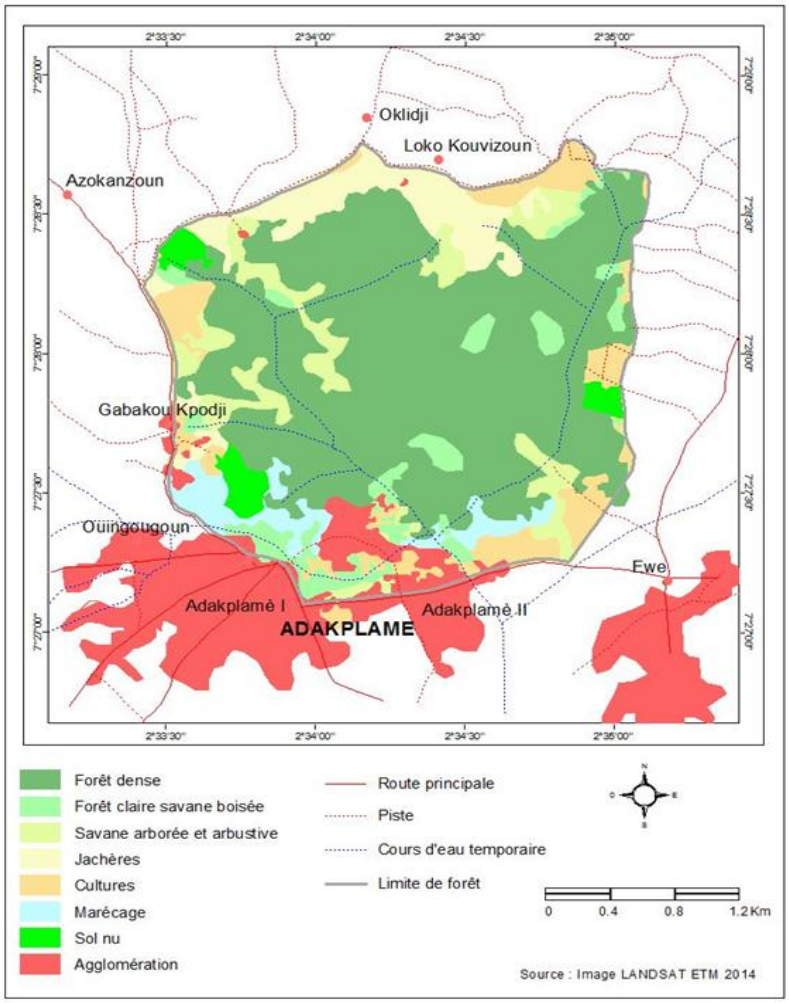


Fig. 1: Map showing the position of Adakplamè agglomerations surrounding the forest

Data collection: Dendrometric and structural characteristics of *M. altissima* were determined using data collected in 40 rectangular plots of 1000 m². Diameter at the breast height (DBH), bole height and total height were measured on each tree with DBH ≥ 10 cm. Regeneration (individuals with DBH<10 cm) was numbered in three sub-plots of 40 m² size per plot of 1000 m². Regeneration was systematically partitioned into two diameter size classes: seedlings (1 ≤ DBH <5) cm and saplings (5 ≤ DBH <10 cm).

Stem Cuttings of 1.5 cm and 3 cm diameter were collected from trees in forest. Afterward, they were cut in leafless cuttings of 15 cm of length. The Naphthalene Acetic Acid (NAA) at different concentrations (0; 500; 750; 1000; 1500 and 2000 mg/l) were applied to cuttings the same day during 10 sec. Cuttings have been introduced into the studied forest soil substratum previously watered. Hundred and sixty-eight (168) cuttings were planted in opened area and shady area. Seven (7) cuttings of the same diameter were used for each dose of NAA; making a total of 84 cuttings (2 x 6 x 7) each for opened and shady area. The experimental design was a total randomization. The seeds from fruits of *M. altissima* collected under mother trees in the forest in February 2015 were conserved after drying and sorting into a well closed bottle. This is such a traditional system of seeds conservation by farmers. After seven months of conservation, seeds were dispatched into 50 seeds sets and each set was submited to a pre-treatment before sowing. The control (T_0) is the one without seeds soaking ; T_1 refers to seeds soaking into plain water for 48 h ; T_2 refers to seeds soaking into plain water for 72 hours; T_3 and T_4 refer seeds soaking into boiled water for 60 seconds; T_5 refers to seeds soaking into boiled water until its coolness. As for T_0, T_1, T_2, T_3 and T_5, seeds were sown immediately after their abduction into black polyethylene pots filled with forest substratum. Each of these treatments was applied to 50 pots (one seed per pot) with 2 repetitions. Regarding T4, 50 seeds were randomly sown on traditional seedbed with 2 repetitions. Watering was done every day except when there was rain. The experimentation was conducted from September to November, 2015.

Data analysis

Characterization of *Mansonia altissima*: Different dendrometric parameters were calculated using the following equations:

Density (stems/ha) $N = \frac{n \times 10000}{S}$;

Average diameter (cm) $d_m = \frac{1}{n}\sum_{i=1}^{n} d_i$;

Average total height (m) $h_m = \frac{1}{n}\sum_{i=1}^{n} h_i$;

Basal surface area (m²/ha) $G = \frac{\pi}{4}\sum_{i=1}^{n} {d_i}^2 \frac{10000}{S}$

Mean diameter of tree (cm) $D_g = \sqrt{\frac{1}{n}\sum_{i=1}^{n} {d_i}^2}$.

Where n is the total number of trees within the plots ; S is the total area inventoried ; d_i denotes tree diameter i ; h_i is total height of tree i. Diameter and height structures of the species population were adjusted to Weibull 3 parameters distribution because of its "flexibility"[7]. The density function (f) of this distribution is given by the equation below:

$$f(x) = \frac{c}{b}\left(\frac{x-a}{b}\right)^{c-1} e^{-\left[\frac{x-a}{b}\right]^c}$$

Where a is the position parameter (a = 10 cm); b is the scale parameter; c is the shape parameter linked to the observed structure. Species richness index rarity weight[8] was calculated using the following equation:

$$R_i = \left(1 - \frac{n_i}{N}\right) \times 100$$

Where N denotes the total number of plots put in the forest and n_i the number of plots where *M. altissima* was found. R_i< 80% implies preferential species, very frequent; R_i> 80% for scarce species; $R_i = 100\%$ for extinct species.

Cuttings regeneration parameters were submitted to the analysis of variance in a linear model. Parameters measured on cuttings are the following:

- Rate of budding: the number of cuttings having budded, expressed as a percentage in relation to the total number of cuttings put in ground.

- Rate of leafy cuttings: the number of leafy cuttings, expressed as a percentage in relation with the total number of cuttings put in ground.
- Rate of survival: the number of cuttings having kept their leaves at the end of the test, expressed as a percentage in relation to the total number of cuttings put in ground.

Seeds germination parameters were also submitted to the analysis of variance.

For each treatment, parameters measured on seed germination are the following:

- Rate of seed germination: the number of seeds germinated divided by the total number of seeds sowed per treatment.
- Average duration of germination: the number of days between sowing and the last germination recorded.
- Latency time: the number of days before the first germination.
- Speed of germination: the time at which 50% of the seeds germinated (T_{50})[9].

$$T_{50} = t_1 + ((0{,}5 - n_1)/(n_2 - n_1)) \times (t_2 - t_1)$$

$t_{1:}$ the time at which n_1 of the seeds is germinated; $t_{2:}$ the time at which n_2 of the seeds is germinated; n_1 is accrued percentage of seeds germinated whose value is the closest of 50% per lower value; $n_{2:}$ accrued percentage of seeds germinated whose the value is the closest of 50% per higher value. The software Minitab 16 was used for Weibull 3-parameter distribution and the software R 3.2.4[10] for analysis of variance.

RESULTS

Dendrometric characteristics of *M. altissima* population: The average density of *M. altissima* trees in the study area was 20 stems/ha. The average diameter was 21.76 cm and its average total height was 12.47 m. The species showed a basal surface area of 0.88 (m²/ha) and the Diameter of tree with average basal surface area of 23.91 cm.

Structural characteristics of *M. altissima* population: Figure 2, a and b showed the diameter classes and height classes distribution of *M. altissima* trees (adult trees of DBH ≥10cm). The diameter structure was « inverted J » shape and left asymmetrical (1 <c <3.6) centered on 15 cm diameter class with a shape parameter equal to 1.397 (figure 2, a) while the height structure showed a «bell shape» structure also left asymmetrical (1 <c <3.6) centered on 10 to 16 m height class with a shape parameter equal to 2.680 (figure 2, b). The regeneration density was 462.12 stems/ha with 314 stems/ha of seedlings against 148 stems/ha of saplings.

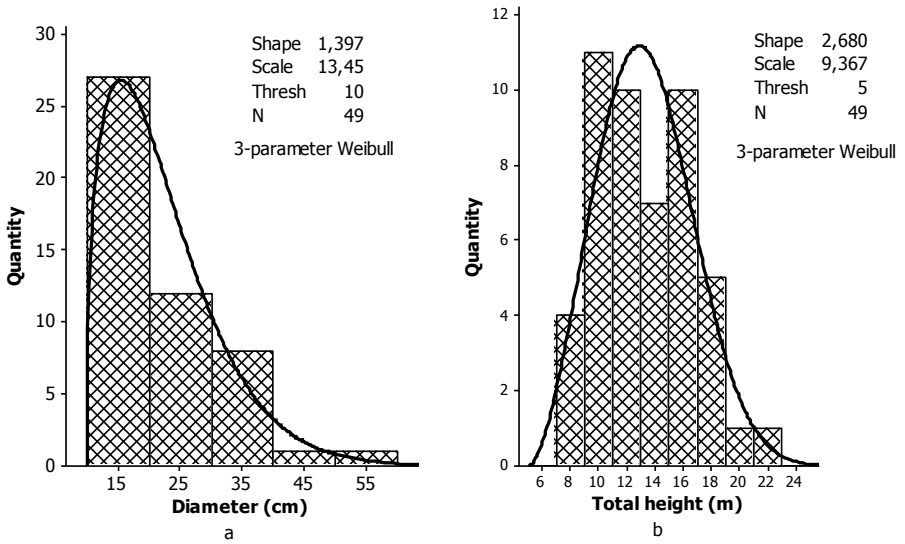


Fig. 2: Diameter structure (a); height structure (b) of *M. altissima* population

Vegetative propagation by cuttings of *M. altissima*: Tables 1, 2 and 3 show the effect of NAA, diameter and hadings, respectively on the rate of budding, rate of leafy cuttings and rate of survival.

Table 1: Effect of NAA, diameter of cutting and shading on the rate of budding

$R^2 = 0.63$ F = 1.88 ; p =0.15	Estimate	Std. Error	t	p	Mean (%)
NAA [500 mg/l]	-4e-16	2e+00	0e+00	1e+00	7.14
NAA [750 mg/l]	-1e+00	2e+00	-1e+00	3e-01	14.29
NAA [1000 mg/l]	3e-01	2e+00	2e-01	8e-01	25.00
NAA [1500 mg/l]	-1e+00	2e+00	-1e+00	3e-01	7.14
NAA [2000 mg/l]	3e+00	2e+00	2e+00	1e-01	28.57
Diameter [3 cm]	-1e+00	2e+00	-1e+00	3e-01	16.67
NAA500:Diam [3 cm]	1e+00	2e+00	7e-01	5e-01	7.14
NAA750:Diam [3 cm]	5e+00	2e+00	2e+00	0.037*	28.57
NAA1000:Diam [3 cm]	4e+00	2e+00	2e+00	1e-01	35.72
NAA1500:Diam [3 cm]	4e+00	2e+00	2e+00	0.058.	14.28
NAA2000:Diam [3 cm]	-7e-01	2e+00	-3e-01	7e-01	14.29

Table 2: Effect of NAA, diameter of cutting and shading on the rate of leafy cuttings

R^2 =0.76 ; F =3.38 ; p =0.023	Estimate	Std. Error	t	p	Mean (%)
NAA [500 mg/l]	-1.50	1.07	-1.40	0.19	3.75
NAA [750 mg/l]	-1.50	1.07	-1.40	0.19	3.57
NAA [1000 mg/l]	-1.50	1.07	-1.40	0.19	3.57
NAA [1500 mg/l]	-1.50	1.07	-1.40	0.19	17.86
NAA [2000 mg/l]	1.83	1.07	1.71	0.11	10.71
Diameter [3cm]	-1.50	1.07	-1.40	0.19	8 .33
NAA500:Diam [3 cm]	3.00	1.52	1.98	0.071.	7.14
NAA750:Diam [3 cm]	3.00	1.52	1.98	0.071.	7.14
NAA1000:Diam [3 cm]	5.36	1.52	3.54	0.00**	35.72
NAA1500:Diam [3 cm]	1.50	1.52	0.99	0.34	0.00
NAA2000:Diam [3 cm]	-1.83	1.52	-1.21	0.25	0.00

Table 3: Effect of NAA, diameter of cut and shading on the rate of survival

$R^2 = 0.81$; F = 4.72 ; p = 0.0063	Estimate	Std. Error	t	p	Mean (%)
NAA [500 mg/l]	3e-15	4e+00	0	1	0.00
NAA [750 mg/l]	-5e-15	4e+00	0	1	0.00
NAA [1000 mg/l]	-5e-15	4e+00	0	1	0.00
NAA [1500 mg/l]	-4e-15	4e+00	0	1	0.00
NAA [2000 mg/l]	7e+00	4e+00	1.73	0.11	7.14
Diameter [3cm]	3e-15	4e+00	0	1	3.70
NAA500:Diam [3 cm]	-7e-15	6e+00	0	1	0.00
NAA750:Diam [3 cm]	0e+00	6e+00	0	1	0.00
ANA1000:Diam [3 cm]	2e+01	6e+00	3.67	0.00**	21.43
ANA1500:Diam [3 cm]	-2e-15	6e+00	0	1	0.00
ANA2000:Diam [3 cm]	-7e+00	6e+00	-1.22	0.24	0.00

Analysis of Variance revealed that NAA, size of diameter and shading have no significant effect on the budding (Table 1). Only the interaction between NAA 750 mg/l and diameter of 3 cm showed a significant effect (t = 2.00; p = 0.037<5%) (Table 1). Significant effect of the factors was noticed on the leafy cuttings (F=3.38; p=0.023<5%) (Table 2). This effect is very significant between the 1000 mg/l NAA concentration and the diameter of 3cm (t=3.54; p= 0.004<1%) (Table 2). The effect of all factors on the survival rate was also very significant (F = 4.72; p=0.006<1%) (Table 3) with a significant interaction (t=3.67; p=0.003 <1%) between 1000 mg/l NAA concentration and the 3 cm diameter (Table 3).

Seeds germination kinetics: Figure 3 shows the seed germination kinetics.

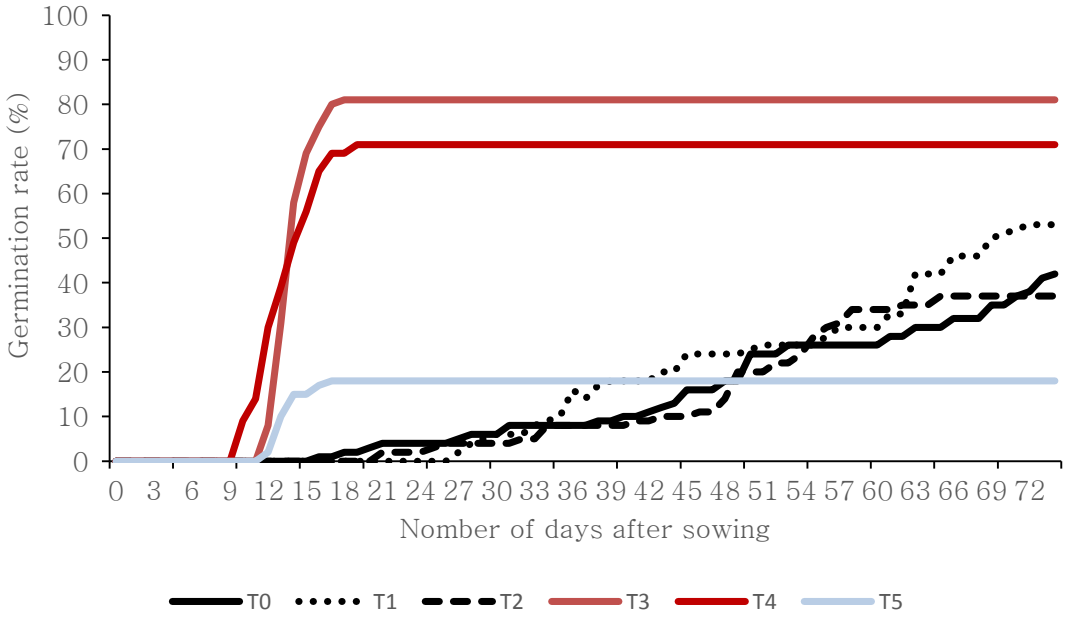


Fig. 3: Germination kinetics by seeds treatment of *M. altissima*

Germination curves of seeds soaked into boiling water (T_3 and T_4, figure 3) are above those of the control seeds and seeds soaked in water at air temperature (T_0, T_1 and T_2, figure 3). All curves showed three phases of germination:

- A first stage of latency corresponding to the number of days before the first germination;
- A second stage of the speed of germination, which spreads from 9th to the 16th day and from 11th to the 15th day respectively for T4 and T3 ;
- A third stage of germination speed corresponding to the germination rate after 75 days of observation for each treatment.

Latency time: The analysis of variance revealed that treatments have a highly significant effect (F = 66.55; p = 3.57e-05 <0.1%) on the latency time (table 4). Compared to the control, seed boiling (T_3, T_4 and T_5) reduced significantly the latency time from 16 days to respectively 12.5 days, 9 days and 11.5 days; While the treatments with plain water (T_1 and T_2) increased very significantly the latency time from 16 day to respectively 26.5 days and 21.5 days.

Table 4 : Effect of treatments on latency time

R^2 = 0.97 ; F = 66.55 ; p = 3.57e-05					
	Estimate	Std. Error	t	p	Mean (%)
T_1	10.5000	1.1547	9.093	9.93e-05***	26.5
T_2	5.5000	1.1547	4.763	0.003116**	21.5
T_3	-3.5000	1.1547	-3.031	0.023066*	12.5
T_4	-7.0000	1.1547	-6.062	0.000914***	09.0
T_5	-4.5000	1.1547	-3.897	0.008011**	11.5

Speed of germination: T_1 showed spread germination over all observation period (figure 2). This treatment enabled the germination rate to reach 50% in 69.33 days. T_3 and T_4 enabled to reach 50% of seeds germination in 13.66 and 14.15 days respectively, while T_0, T_2 and T_5 did not enabled to reach 50% of seeds germination during 75 days of observation. The 60 seconds boiled seeds (T_3, T_4 and T_5) showed grouped and accelerated germination of *M. altissima* seeds (figure 3).

Rate of germination: The analysis of variance revealed that treatments have a highly significant effect (F = 160; p = 2.68e-06 < 0.1%) on the seeds germination rate of *M. altissima* (table 5). The comparison of treatments to the control (T_0) revealed that treatments T_1, T_3 and T_4 were more effective. These treatments enabled to raise the germination rate of control seeds from 42% to 53%, 71% and 81% respectively. T_2 and T_5 showed negative effects, highly significant on the seeds germination.

Table 5 : Effect of treatments on germination rate

$R^2 = 0.99$; F = 160 ; p = 2.68e-06					
	Estimate	Std. Error	t	p	Mean (%)
T_1	11.00	2.58	4.26	0.005**	53
T_2	-5.00	2.58	-1.93	0.1	37
T_3	39.00	2.58	15.11	5.31e-06***	81
T_4	29.00	2.58	11.23	2.98e-05***	71
T_5	-24.00	2.58	-9.30	8.77e-05***	18

Average duration of germination: The Analysis of variance revealed that treatments have a highly significant effect (F = 395.3; p=1.81e-07<0.1%) on the average duration of seeds germination (table 6). Soakings into boiled water (T_3, T_4 and T_5) showed common time of seeds germination between 5 and 10.5 days. When seeds are sown directly in the pots filled with forest soil (T_3 and T_5), the average duration of germination is 5 days against 10.5 days when seeds are randomly sown on sprout-seed (T4).

Table 6 : Effect of treatments on average duration of germination

$R^2 = 0,99$; F = 395,3 ; p = 1,81e-07					
	Estimate	Std. Error	t	p	Mean (%)
T_1	-12,50	1,61	-7,78	0,0002***	45,0
T_2	-21,50	1,61	-13,38	1,08e-05***	36,0
T_3	-52,00	1,61	-32,35	5,80e-08***	5,5
T_4	-47,00	1,61	-29,24	1,06e-07***	10,5
T_5	-52,50	1,61	-32,66	5,48e-08***	05,0

DISCUSSION

Structural characteristics of *M. altissima* population: The weak value of the stand basal area is due to the small diameter of trees and to the weak expanse of environment. The weak value of density can be explained by the fact that *M. altissima* is a widely diluted species in its natural environment and very exploited by the local populations because of its resistance to the wood rot attacks. *M. altissima* occurs exclusively in dense forests and does not grow in others ecosystems such as woodlands and savannahs. Its stand density is weak compared to the one of *P. africana* (52.39 stems/ha) in savannah ecosystems[11] and of *B. aethiopum* (150 to 162 feet/ha) in the Sudano-Guinean and Guinean zones[12]. But such density is similar to the one found for *K. senegalensis* in dry dense forests[13]. A similar trend was noted by Agbangla *et al*[14] who explained the insufficiency of young trees, despite the abundance of regeneration and the excessive mortality of juveniles (1 to 10 cm) and young trees because of water deficit.

The diameter classes' distribution of the species is left-skewed with the predominance of young individuals of early class indicating a state of regeneration of a long time disturbed population. The overexploitation of woods by local populations entailed the disappearance of trees with large diameters. One finds more young trees of *M.*

altissima in ecosystems disturbed by logging and vegetation fires[15]. These results are similar to those found in the forest of Niaouli[14] in the Guinean and Sudanian zones for *P. africana*[11] and in the Guinean zone for *B. aethiopum*[12]. The diameter class-size distribution of *M. altissima* demonstrated an inadequacy of the species natural distribution to different human pressures suggesting the necessary protection of its environment.

The height class-size distribution was left-skewed with the predominance of the individuals of small heights. These results are similar to those found on *P. africana*[11] and *B. aethiopum*[12] in the three climatic zones. This structure can be explained by the density of the forest vegetation cover. After the stage of young plant, *M. altissima* requires clearly light for its growth[15]. This high value of the regeneration density (DBH <10 cm) is probably due to the abundant fruiting of the species[4]; but also to seeds dispersal mode. The seeds of *M. altissima*, very light and winged, are spread over wide area justifying the occurrence of seedlings in some gaps far from mother trees. However, as *M. altissima* is high light depending specie, its seedlings face some growing and survival problems justifying the weak saplings recruitment rate observed.

Mass propagation of *M. altissima*: The weak rate of budded cuttings shows that they haven't succeeded the stage of cicatrizing. However cicatrizing is a key stage of cutting, influenced by several factors such as the hormonal equilibrium between the auxin used and the other vegetal hormones[16]. The NAA might not induce a sufficient hormonal balance for cuttings cicatrizing. This could explain the weak success rate of the essay. Species specificity to respond to the auxin treatments is due to several factors of which the genotype and the environmental conditions; the species ability to root naturally and the stability of the different auxins in plants[17]. The requirements of cuttings as far as quantity and quality of applied auxin are concerned, vary according to the species and even with the variety[18]. Very weak survival rate observed revealed that the specie faces some difficulties of rooting or it doesn't respond to this propagation technics. The type of substratum could also explain this unsuccessful budding rate because there is a relationship between substratum and cuttings humidity[19]. The cuttings diameter and length have no effect on their regeneration. These results are contrary to the ones obtained for *Vitex doniana* when using cuttings of similar diameters (1 <diameter<4 cm) but of greater length (30; 50 and 70 cm)[20]. The smaller length of the cuttings (15 cm) could also explain the weak success rate of this essay.

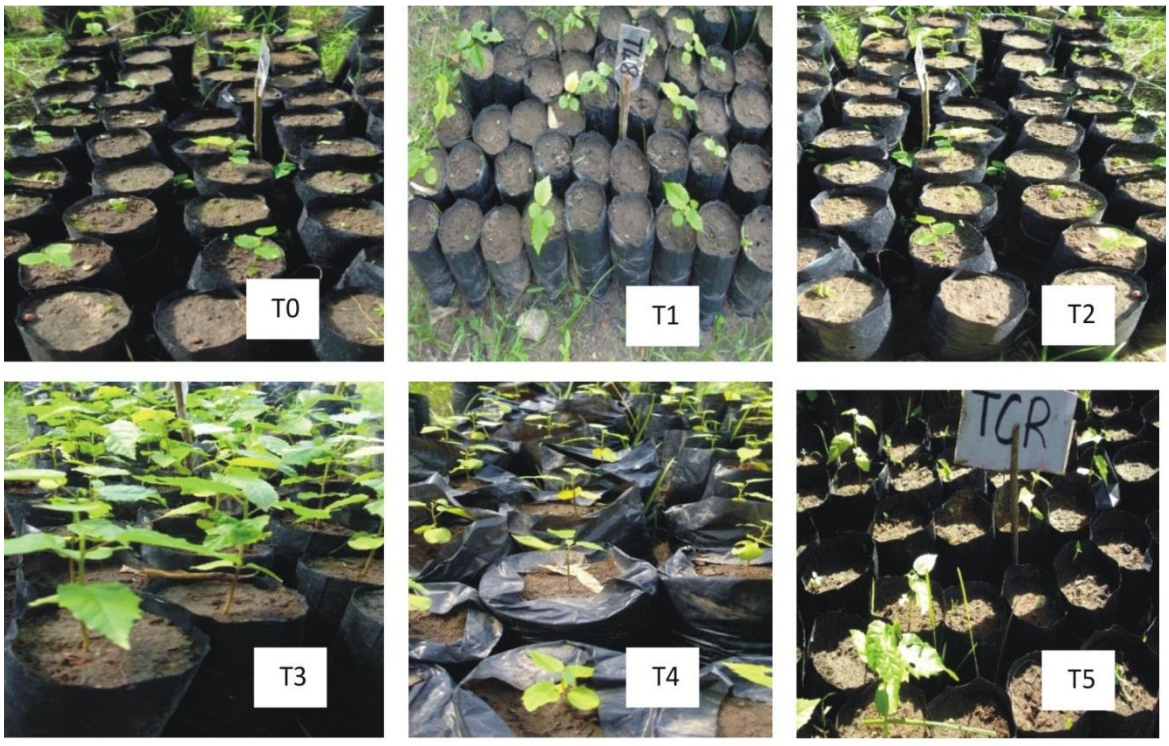
Seeds of *M. altissima* conserved during seven months showed signs of embryonic dormancy or tegumentary inhibition. A long conservation of seeds in natural conditions involves a slowing down of germination capacity after seven months[4]. This phenomenon may result from a tegumentary dormancy, due to impermeability of the integument or pericarp to water or the presence of inhibitory substance in the pericarp or integument[21]. The soaking of seeds into boiled water during a short period of time (60 seconds) involves the rapid imbibition of the integument and water entrance that enables metabolic reactions of seeds embryo and the rapid exit of the radicle[21,22]. The thermal shock created further to the soaked seeds water induced their fissuring thus their softening and then sufficient water and oxygen passing into the seeds, leading to quick germination[9]. When the duration of soaking long, there is excessive water penetration inside the seeds through cracks created by the thermal shock leading to the embryo asphyxia[9]. The duration of seeds soaking into boiled water depends therefore on the thickness and hardness of the seeds integuments[22,9]. Seeds soaked into plain water and the control seeds showed germination over 2 months. Soaking into boiled water accelerated seed germination speed and reduced significantly the average duration of germination[22,9].

CONCLUSION

Mansonia altissima population in Benin is strongly endangered and stretches toward extinction if nothing is done to protect its

habitat and to secure the forest resources. Although this habitat is favorable for the species, its structure is not adequate because of human pressures. Stem cuttings of *M. altissima* showed weak budding performances. The NAA didn't induce a sufficient hormonal balance for the cuttings cicatrizing. This hormone remains ineffective for *M. altissima* root induction. Otherwise, the seeds of *M. altissima* conserved for seven months may still show high germination rates when they undergo a pretreatment with boiled water during a short duration of 60 seconds. This pretreatment reveals more suitability for rapid, grouped and high germination of *M. altissima*. It may be advisable to the nurserymen particularly those of the Guinean zone in Benin for *M. altissima* plants to be used for enrichment of degraded ecosystems. Future research should access the modeling of actual and potential distribution of *M. altissima*, the improvement of knowledge on the biology and ecology in order to define a minimum of technical norms for the its sustainable conservation.

Picture 1: Some regenerate cuttings



Picture 2 : *M. altissima* nursery

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