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Determinants of the seeds germination of Mukulungu- *Autranella congolensis* (De Wild.) A.Chev. in nursery

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ABSTRACT

Subject description. *Autranella congolensis* is one of the most exploited species in the Congo Basin and generally suffers from a regeneration deficit. To guarantee its renewal and sustainability, silvicultural interventions based on planting or enrichment techniques from seedlings produced in nurseries should be encouraged.

Objective. This study aims to evaluate the germination determinants of *A. congolensis* seeds in nursery.

Methods. The seeds were collected from 87 samples of A. congolensis seed plants chosen in a range of dbh from 20 to 155 cm in North Congo, in the Celtis forest in a plot of 400 ha on argilo- soils to sandy clay. A completely randomized two-block device was set up in a greenhouse at the National Forest Research Institute (IRF) of the Scientific City of Brazzaville, at an average temperature of 24 ° C. A batch of seeds (200 seeds) was divided into eight treatments to test the modalities for breaking dormancy, at a rate of 25 seeds per treatment. The seeds were individually and immediately sown after pretreatment, on a substrate previously sterilized and composed of 25 % sawdust and 75 % humified earth in 0.5 phytocells.

Results. Results show that the trees density was 0.22 stem/ha, and population structure showed a Gaussian distribution according the diameter class, indicating that this species generally suffers from a regeneration deficit. Average germination observed under treatments combination was 25 % of total sown seeds. Indeed, germination rate was respectively 33 %, 31 %, 25 % and 23 %, for the treatments T1, T8, T2 and T6, with germination deadlines of 51 days for T1, 62 days for T2, 74 days for T6 and T8.

Conclusion. The pretreatments applied showed a relatively significant effect on the germination rate. Subsequent studies on the germination of *Autranella congolensis* seeds on other substrates under controlled conditions.

Keywords: *Autranella congolensis*, germination rate, nursery, forest in Celtis, Republic of Congo.

RESUME

Déterminants de la germination des graines de Mukulungu- Autranella congolensis (De Wild.) A.Chev. en pépinière

Description du sujet. *Autranella congolensis* fait partie des espèces les plus exploitées du Bassin du Congo et souffre généralement d'un déficit de régénération. Pour garantir son renouvellement et sa pérennité, des interventions sylvicoles basées sur les techniques de plantation ou d'enrichissement à partir des plantules produites en pépinière sont à encourager.

Objectif. Cette étude évalue les déterminants de la germination des graines de *A. congolensis* en pépinière.

Méthodes. Les graines ont été récoltées sur 87 échantillons de semenciers de *A. congolensis* choisis dans une gamme de dhp allant de 20 à 155 cm au Nord-Congo, dans la forêt à *Celtis* au sein d'une parcelle de 400 ha sur des sols argilo-sableux à sablo-argileux. Un dispositif complètement randomisé à deux blocs a été mis en place en serre à l'Institut national de Recherche Forestière (IRF) de la Cité Scientifique de Brazzaville, à la température moyenne de 24 °C. Un lot de graines (200 graines) a été réparti en huit traitements pour tester les modalités de levée de dormance, à raison de 25 graines par traitement. Les graines ont été individuellement et

immédiatement semées après prétraitements, sur un substrat préalablement stérilisé et composé de 25 % de sciure de bois et 75 % de terre humifiée dans des phytocells de 0,5.
Résultats. Les résultats révèlent que la densité des arbres était de 0,22 tige/ha, et la structure des populations des semenciers a affiché une distribution gaussienne des effectifs par classe de diamètre, démontrant que l'espèce souffre généralement d'un déficit de régénération. Le pourcentage moyen de germination observé a été de 25 %. Le taux de germination était de 33 %, 31 %, 25 % et 23 %, respectivement pour les traitements T1, T8, T2 et T6, avec des délais de germination de 51 jours pour T1, 62 jours pour T2, 74 jours pour T6 et T8.
Conclusion. Les prétraitements appliqués ont affiché un effet relativement significatif sur le taux de germination. Les études ultérieures sur la germination des graines d'*Autranella congolensis* sur d'autres substrats en conditions contrôlées.

Mots-clés : *Autranella congolensis*, taux de germination, pépinière, forêt à *Celtis*, République du Congo.

1. INTRODUCTION

Central Africa forests cover nearly 170 million hectares and constitute, after the Amazon massif, the second largest tropical forest massif on the planet (Hansen *et al*., 2013 ; De Wasseige *et al*., 2014). Over 60 million hectares of these forests are currently used for timber production in view of the permanent forest estate of the States, of whose 49 million hectares are currently allocated to forestry companies (Gourlet-Fleury *et al*., 2013). In the last two decades, Central African states have adopted management plans favorable to sustainable forest management (Nasi *et al*., 2012). The management plan is naturally based on the calculation of the reconstitution rates of the populations of commercial species (Durrieu de Madron and Forni, 1997). Species with a low reconstitution rate are those for which a potentially important reduction in the number of exploitable trees will be observed during the rotation, which could be to translate on the long term, by a rarefaction of seed trees and, consequently, a regeneration deficit (Gourlet-Fleury *et al*., 2013).

Regeneration, a critical phase in the life cycle of trees, is often seen as a set of processes eventually allowing forest reconstitution (Alexandre, 1982). After disturbance (logging, agriculture, windfall, etc.), forests can regenerate via the vegetative potential which corresponds to the seedling bank, the advective seminal potential, which corresponds to the seed rain and the edaphic seminal potential, which corresponds to the soil seed bank and/or seed bank (Lescure *et al*., 1989 ; Getachew Tesfaye *et al*., 2010).

According to Swaine and Whitmore (1998) and Baskin and Baskin (2003), seeds divid into two groups. Recalcitrant seeds which quickly lose viability when stored, owing to the high water content, and orthodox seeds, which can integrate the soil bank and survive for many years. Some seeds among the aforesaid two groups are generally dormant, which is defined as in a physiological or mechanical process preventing their immediate germination (Baskin and Baskin, 2003). This mechanism concerns a reduced number of timber-producing species in Central Africa. *Autranella congolensis* species (De Wild.) A. Chev, is one of them (Doucet, 2003 ; Gillet, 2013). *Autranella congolensis* is a commercial species of the moist forests of Central Africa.

This belonging to Sapotaceae family, known under the commercial name of "Mukulungu" (ATIBT nomenclature), is exploited for the quality of its wood used for the railway sleepers, bridges, quarter-sliced veneers, furniture and cabinetry (Debroux *et al*., 1998). According to the International Technical Association of Tropical Woods (ATIBT, 2019), *Autranella congolensis* is part of the most exploited species in forests of the Congo Basin.

The species presents a relatively distribution restrained in Africa, and therefore, would be considered potentially more vulnerable than the one with wide distribution (Hawthorne, 1995 ; Gillet *et al*., 2008 ; Gillet, 2013).
Primary seed dispersal is barochoric (Hawthorne, 1995 ; Doucet, 2003), and a secondary dispersal, ensured by animals is commonly evoked (Guion, 2011 ; Gillet, 2013). *A. congolensis* is a species of semi-heliophilic temperament (Doucet, 2003 ; Sepulchre *et al*., 2008), and generally suffers of a regeneration deficit, which translates, in moist semi-deciduous forest, by a Gaussian distribution numbers by diameter class (Durrieu de Madron and Forni, 1997, Gillet *et al*., 2008).

Due to its economic value and vulnerability, the species is enrolled on the IUCN Red List as a species, "critically endangered" (IUCN, 2012). Seedlings are often rare under the seed tree and at a distance from the attracted dispersing agent : the elephant (Letouzey, 1985 ; Debroux, 1996). In such a context, logging accelerates the regression of the species by the collection of seed trees. To ensure long-term management of populations of this species, it would be appropriate to well understand the mechanisms involved in the breaking dormancy. Seeds of the species *A. congolensis* are orthodox and the dormancy of seeds collected under seed trees can be broken by pulping and sowing in the earth or in the coarse sand (Debroux *et al*., 1998). Nevertheless, treatment of the seeds with sulfuric acid, in the boiling water and in the oven remains unexplored.

This study aims to evaluate the germination determinants of *A. congolensis* seeds in nursery.

2. MATERIAL AND METHODS

2.1. Study site

The seeds have been collected within the devices of DynAfFor project (Dynamics of Central African Forests), in the north of the Republic of Congo. Devices are installed in the forest concession allocated to CIB/OLAM, near the locality of Loundoungou in a forest to *Celtis* (Gond *et al*., 2013 ; Fayolle *et al*., 2014) (Table 1). *Celtis* forest is semi-deciduous, resting on sandy clay to clay sandy soils typical of alluvium (Fayolle *et al*., 2014 ; Freycon, 2014) (Table 1). In terms of chemical fertility, the soils of the Celtis forest are relatively rich. The *Celtis* forest has been regularly disturbed in the past (Morin-Rivat *et al*., 2014) by traditional human activities and can be qualified as old secondary forest (White, 1983).

Table 1. Synthetic description of the forest in Celtis, taking into account the rainfall recorded between 2000 and 2008 to Ouesso, Impfondo and Mbaïki (Verelst, 2009 ; Freycon, 2014).

	***Celtis* forest**
Geographical coordinates	02°18' - 02°22 N 17°31' - 17°34' E
Altitude (m)	410 - 460
Annual rainfall (mm/an)	1 729
Soil type	Acrisol - Arenosol - Gleysol
Geomorphology	Piedmont at the edge of the Congolese basin
Forest type	Semi - evergreen

2.2. Population structure of *Autranella congolensis*

All *A. congolensis* seed company of diameter at breast height (dbh) ≥ 20 cm have been inventoried and mapped in a plot of 400 ha within the *Celtis* forest. This inventory allowed to characterize the population structure of the species (distribution of numbers by diameter classes).

2.3. Experimental device and germination tests

June 24, 2019, in a plot of 400 ha, seed company of *A. congolensis* have been chosen in a range of dbh varying from 20 to 155 cm (divided in fourteen diameter classes). The diameter of 20 cm corresponds to minimum diameter in the management inventory. (Doucet, 2003 ; Gillet, 2013). The seeds of *A. congolensis* founded under each seed company have been collected, totaling a number of 200 seeds.

It was sent and received on June 29, 2019 at the National Forest Research Institute (IRF-Brazzaville) and kept cool for 17 days in a refrigerator at a temperature of around 5°C. A completely randomized device of two blocks has been set up in a greenhouse at the National Forest Research Institute (IRF). The batch of seeds (200 seeds) was divided in eight treatments to test the following conditions of breaking dormancy, at the rate of 25 seeds per treatment :

- T1 = seeds soaked in a sulfuric acid solution (H_2SO_4) diluted to 96% for 30 minutes ;

- T2 = seeds soacked in a sulfuric acid solution (H_2SO_4) diluted to 96% for 45 minutes ;
- T3 = seeds soaked in boiling water for 15 minutes ;
- T4 = seeds soaked in boiling water for 30 minutes ;
- T5 = seeds scarified (slightly remove part of the integument) ;
- T6 = seeds put in the oven at 110°C for 10 minutes ;
- T7 = seeds put in the oven at 110°C for 20 minutes ;
- T8 = seeds not submitted to any treatment (control).

The choice of these different modalities of breaking dormancy is based on previous studies bearing on integumentary dormancy of the seeds such as Mukulungu seeds (Footitt et Cohn, 1995 ; Delhaye, 2006 ; Kouadio, 2009 ; Balo Ilunga, 2015). After each immersion in sulfuric acid, seeds have been rinsed in distilled water for one hour, changing the rinsing water every 20 minutes three times (Footitt et Cohn, 1995 ; Delhaye, 2006 ; Kouadio, 2009).

Germination tests were realized in a greenhouse at the National Forest Research Institute, at an average temperature of 24°C and an average humidity of 60%. Seeds were individually and immediately sowed after treatments, put on a substrate previously sterilized and composed of 25% sawdust and 75% humified earth in 0.5 l phytocells, and covered with a layer of potting soil at 3 mm thickness. The humified earth has been incorporated to the substrate for ensuring the effect of the natural bacterial procession of the soil on the germination of the seeds at integumentary dormancy such as the seeds of Mukulungu (Diabate *et al*., 2005). Sowed seeds have been watered one day on two, and germination was monitored daily. The experiment ended October 17, 2019, after 12 weeks of follow-up. At the end of the experiment, the number of seeds having effectively germinated (NGS), the germination delay (DG, time interval which separates sowing and the first emergence) have been estimated for each treatment (De La Mensbruge, 1966 ; Mbolo, 1991 ; Danthu, 1993 ; Elazazi, 2016). Germination rate (GR %) has been then calculated (Maguire, 1962) using the following formula :

$$\mathrm{GR} = \frac{n}{N} \times 100$$

n = number of germinated seeds and, N = number of seeds sowned.

2.4. Data analysis

The population structure seed trees (distribution of numbers by diameter class) has been determined by a graphic description diameter of the trees.

The effect of seed pretreatment was determined by applying nonparametric tests of Scheirer-Ray-Hare and Dunn post hoc at the threshold of 5% using R Studio software (version 4.1.1, rcompanion and FSA packages). Generalized linear model (GLM) of analysis test Scheirer-Ray-Hare is the follows : $\mathbf{Y_{ijk} = \mu + B_i + T_j + B_i \times T_j + \square_{ijk}}$

Y : response variable ; **μ** : general mean value of the observations ; $\mathbf{B_i}$: effect of block ; $\mathbf{T_j}$ = effect of pretreatment and $\square_{\mathbf{ijk}}$: residual error.

3. RESULTS

3.1. Population structure of *Aurtranella congolensis* species

The observed density is 0.22 stems/ha (dbh ≥ 20 cm) in the Celtis forest. Population structure shows a Gaussian distribution of the effectives by diameter classes. Consequently, species generally suffers a regeneration deficit (figure 1).

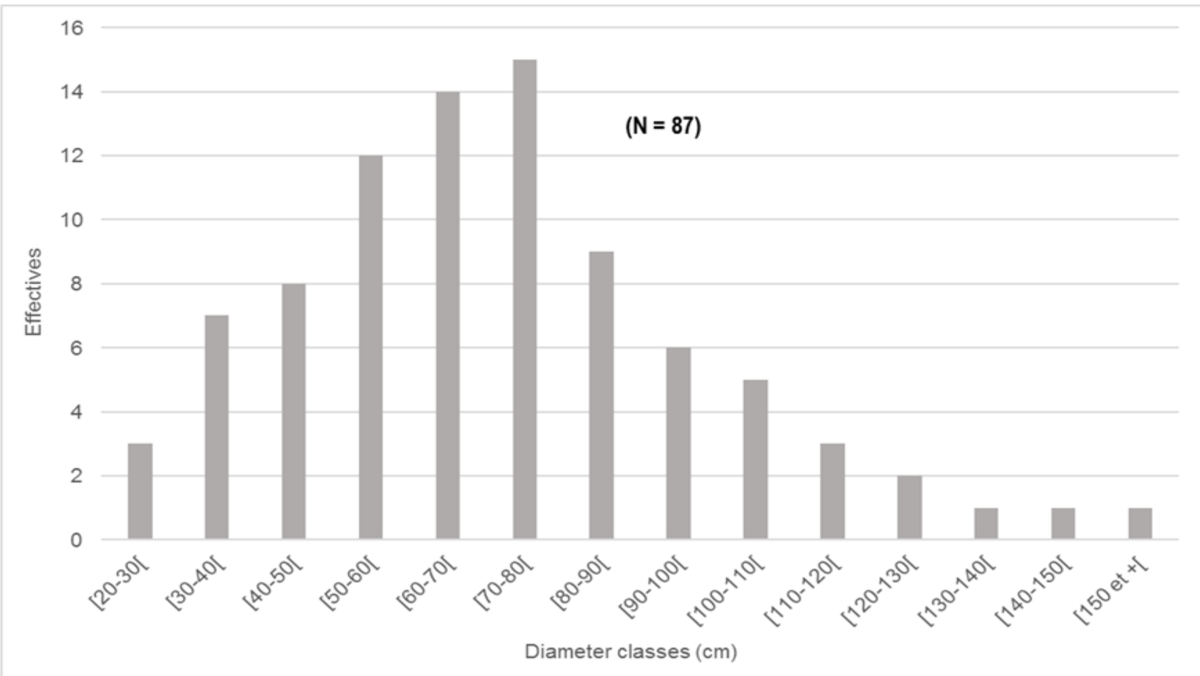

Figure 1. Population structure of *Autranella congolensis* within a 400 ha plot of the *Celtis* forest

3.2. Percentages and evolution of the average duration of seeds germination in terms of treatments.

At the end of the experiment and all treatments combined, the average germination percentage observed has been relatively low, since it only reached 25% of the seeds sowed. More precisely, germination rate was 33%, 31% 25% and 23%, respectively for treatments T1, T8, T2 and T6, with germination delays oscillating from 51 days for T1, 62 days for T2, 74 days for T6 and T8 (Table 2).

Nevertheless, the evolution of the average germination duration in terms of the treatments shows that the seeds that have undergone pretreatments showed relatively short germination average duration compared to those that were not treated (Figure 2). Table 2 presents the overall results according to the entire device.

Table 2. ***Overall result of the entire device***

Blocks	Treatments	Number of seeds sowed (NSS)	Number of germinated seeds (NGS)	Germination rate (GR%)	Germination delay (DG)
1	T1	13	4	31	51
1	T2	12	3	25	55
1	T3	12	0	0	-
1	T4	13	0	0	-
1	T5	13	0	0	-
1	T6	13	3	23	50
1	T7	12	0	0	-
1	T8	12	3	25	51
2	T1	12	4	33	41
2	T2	13	2	15	62
2	T3	13	0	0	-
2	T4	12	0	0	-
2	T5	12	0	0	-
2	T6	12	2	17	74
2	T7	13	0	0	-
2	T8	13	4	31	74

3.3. Effects of the treatments on seeds germination

The Scheirer-Ray-Hare analysis shows a non-significant effect of block (p-value = 0.24253), and pretreatment on the germination delay (p-value = 0.48753), as well as their interaction (p-value = 0.36163). For the germination rate alone, effect of the pretreatment has been significant (p-value = 0.04286) (Table 3). Nevertheless, structuring the means using Dunn's test reveals a similarity between T2 and T8, also between T2 and T6 (Figure 3).

Table 3. Descriptive statistics and effects of the different factors on the delay and rate of germination

Lineaments	Factors	Factor modalities	Mean	Std	Min	MAx	P-value
DG	Block	1	51.75	2.22	50	55	0.24253
		2	62.75	15.56	41	74	
	Treatment	T1	46	7.07	41	51	0.48753
		T2	58.5	4.95	55	62	
		T6	62	16.97	50	74	
		T8	62.5	16.26	51	74	
	Block×Treatment						0.36163
GR	Block	1	12.98	14.05	0	30.77	0.95520
		2	12.02	12.23	0	33.33	
	Treatment	T1	32.05	1.81	30.77	33.33	0.04286
		T2	20.19	6.80	15.38	25	
		T3	0	0	0	0	
		T4	0	0	0	0	
		T5	0	0	0	0	
		T6	19.87	4.53	16.67	23.08	

	T7	0	0	0	0	
	T8	27.88	4.08	25	30.77	
Block×Treatment						0.99948

4. DISCUSSION

4.1. Population structure of *Autranella congolensis* species shows a regeneration deficit

Population structure in the present study revealed the low number of the stems confirming the regeneration deficit of species. According to Sepulchre *et al*. (2008), in old secondary forests, mature or old, there is a link between the temperament of species and population structure. Semi-heliophiles species (case of species temperament of this study) frequently present a decreasing curve, followed by a bump and the noted hollow can corresponds when trees access at the canopy and accelerate their growth. Sciaphiles species are naturally illustrated by a regular decrease (exponential to more or less linear) in the number of plants with increasing diameter classes. Also, the heliophile species are represented by a decreasing exponential curve in a young forest from the colonization of a savannah or a abandoned field. According to Gillet (2013), population structures are often influenced by the origin of the forests that harbor the considered species.

Another parameter that could explain the regeneration deficit of species would be the regular fruiting diameter. According to Doucet (2003), regular fruiting diameter is the threshold from which efficient and regular fruiting of trees species can be observed in moist forests Central Africa. This diameter is determined based on a threshold of 70% of fertile individuals. Nevertheless, Gillet *et al*. (2008) studies demonstrate that the regular fruiting diameter of species *A. congolensis* would be 70 cm diameter. On the other side, a logging from 60 cm of diameter as he's currently planned in the Republic of Congo, does not seem compatible with the regular fruiting diameter estimated at 70 cm. Consequently, long-term maintenance of species is not guaranteed (Durrieu de Madron et Daumerie, 2004 ; Doucet *et al*., 2007).

According to Debroux (1998), species with stems to come up less present in most forest concessions in Republic of Congo are : acajou (*Khaya anthoteca*), ayous (*Triplochyton scleroxylon*), bilinga (*Nauclea diderrichii*), koto (*Pterygota bequaertii*), mukulungu (*Autranella congolensis*) and pao rosa (*Swartzia fistuloides*). Nonetheless, population structure of species *A. congolensis* can be locally very unfavorable, its geographical distribution is relatively limited to the Central Guinean Congolese region and the values of Minimum Exploitation Diameter (DME) are lower to its Regular Fruiting Diameter (DFR). Consequently, support to the regeneration imposes itself with all the more acutely for ensuring the sustainability of species.

4.2. Influence treatments on the germination rate of the seeds of species *Autranella congolensis*

It would suit emphasize beforehand that studies dedicated to the breaking dormancy and germination of the seeds of species *A. congolensis* are extremely rare. On the whole device put in place, seeds germination results of the species of *A. congolensis* showed a relatively low germination rate. On the 200 seeds sowed, with all treatments combined, 25 seeds germinated with an overall germination rate of 25 %.

In Democratic Republic of Congo, study of Pendge (1994) shows that the germination rate of the untreated seeds (control) was 29.9 % (on 258 seeds sowed) and the germination delay was 74 days. Also, for the pulped seeds, the germination percentages of the substrates earth and sandy were respectively 9% (on 100 seeds sown) and 5% (on 100 seeds sowned), procuring sharply lower germination rates, which could highlight the interest of breaking dormancy *via* sulfuric acid to increase the germination rate.

In the present study, the germination rate of untreated seeds (control) was 25-31 %, whereas germination delay was 74 days. Pretreatments applied to the seeds showed germination rates of 20% and 33 %, respectively for the T2 treatments (sulfuric acid for 45 minutes) ; T6 (Passage of the seeds in oven for 10 minutes) and T1 (sulfuric acid for 30 minutes). For all the treatments applied, germination delay was 55 days.

It could be noted nonetheless that the pretreatments reduced the germination delay by about 19 days, compared to the untreated seeds (control). These results could be justified by a relatively long dormancy of the seeds of *A. congolensis* species. The long germination delay (74 days for untreated seeds and 55 days for treated seeds) is presumably due to the thickness of lignified integument. According to Pendge (1994) and Debroux (1998), this integumentary dormancy sometimes prolongs up to 5 or even 12 months. It is the most frequent cause of delayed germination among tropical forest trees (Vazquez-Yanez, Orozco-Segovia, 1993). Also, Pangou (1986) and Kampé (2005) estimate that most of it's integumentary inhibition. For this purpose, it would not be excluded that the low germination rate could be attributed to the depth of sowing compared to the size of the seeds. Nonetheless, Heller (1990) rather consider two types of dormancy : integumentary dormancy due to the seminal envelopes and embryonic dormancy resulting from an unfitness of embryo to germinate.

These authors advise in particular to diversify other types of pretreatments in order to open some research perspectives. Nevertheless, the germination rate of the seeds species *A. congolensis* after passage through the intestines of elephants is still unknown and therefore constitutes a field of research to explore. The hypothesis according to which intestinal transit could facilitate cracking of seed integument and increase the speed and rate of germination therefore remains to be verified for this species as it was for *Balanites wilsoniania* (Chapman *et al*., 1992). One could only affim that germination is possible without this "elephant" effect.

4.3. Silvicultural implications

It seems relatively difficult to obtain a sustained and economically sufficient production of wood *Autranella congolensis*. Large trees are generally disseminated at low density. Natural regeneration is relatively poor, the low germination rate and seed dormancy hamper large-scale production of next stems. In addition, growth is slow, and it would probably take very long revolutions to allow sustainable exploitation. That makes *A. congolensis* a species with poor prospects for commercial timber production, and attention must be focused not only on its protection, but also on its assisted regeneration.

To alleviate the regeneration deficit generally observed of this species, silvicultural interventions based on planting or enrichment techniques are to be encouraged to contribute sustainably manage production forests, in order to ensure their renewal and sustainability (Favrichon, 1997 ; Doucet *et al*., 2016).

5. CONCLUSION

The present study shows that population structure of *A. congolensis* species presents a probable slowdown of the regeneration. Overall, the pretreatments applied produced a relatively significant effect on the germination rate. Consequently, assumptions of this study are confirmed. Nevertheless, considerable efforts of assisted regeneration must be deployed for this vulnerable species and on the IUCN Red List as a species, "critically endangered". If nothing is done for compensating the regeneration deficit, it is very likely that the species regresses strongly with successive rotations like other species such as the ayous (*Triplochiton scleroxylon*). Reforestation in post-exploitation plots with seedlings from nurseries would appear as the essential technique to ensure the sustainability of species.

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