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## Nursery germination trial of Tali seeds, *Erythrophleum suaveolens* (Guill. & Perr.) Brenan

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### ABSTRACT

The current low recruitment rate of the exploitable tree species arouses silvicultural interventions for long-term management of their populations. This study evaluated the germination of the *Erythrophleum suaveolens* seeds in nursery following different pretreatments. The seeds have been collected under seed trees of *E. suaveolens* chosen in a range from 30 to 155 cm North Congo in the *Celtis* forest within a plot of 400 ha on clay-sandy to sandy-clay soils. A complete design randomized of two blocks has been established in the greenhouse at respective average temperature and humidity of 24°C and 60%. The seed lot (350 seeds) has been divided into seven treatments for to test the modalities of breaking dormancy, at the rate of 50 seeds per treatment. The dormancy of the collected seeds was lifted by germination tests after treatment with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), in an oven at 140°C and in the boiling water. The seeds have been individually and immediately sowed after treatments. The germination rates were 68-76%, 36-56%, 16% and 12-28%, respectively for the T2, T1, T5 and T6 treatments, with germination times of 6 days for T1 and T2, 15 days for T5 and 15-20 days for T6. Treatments T3, T4 and T7 presented zero germination rates. The factors that can influence seeds variations of germination are discussed and recommendations inherent in the considerable efforts of assisted regeneration of this species which occupies a preponderant place in logging in Central Africa are formulated.

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**Keywords:** *Erythrophleum suaveolens*, seeds, germination, moist forest, *Celtis* forest, Republic of Congo.

### INTRODUCTION

Forestry legislation in Republic of Congo plans to promote sustainable timber exploitation; regularize the production of valuable species and guarantee the permanence of forestry activity by obliging forestry firm to

realize their activities in such a way as to ensure the regeneration of timber potential throughout the national territory. Foresters consider natural regeneration as all of the juvenile individuals in the understory of tree species (Sounou et al., 2009; Douh, 2018). For

ecologists, natural regeneration is perceived as a set of processes eventually allowing, reconstitution of the forest (Adjalla et al., 2022). The natural regeneration based on the activation of the different potentials described by Douh et al. (2014) and Adjalla et al. (2022). Among these potentials, the soil seed bank and/or seed bank seems the least well documented in Central Africa and even less in Republic of Congo (Douh et al., 2018). The seeds of some commercial species in Central Africa are generally dormant, which is defined as a physiological or mechanical process preventing their immediate germination (Baskin and Baskin, 2003). This is the case of the commercial species, *Erythrophleum suaveolens*. According to the International Tropical Timber Organization (OIBT) and the International Technical Association of Tropical Timber (ATIBT), *E. suaveolens* fait partie des espèces commerciales les plus exploitées des forêts du Bassin du Congo (OIBT, 2012; ATIBT, 2019). Cameroon and Congo are the main exporters (OIBT, 2012; de Wasseige et al., 2014). Belonging to the Leguminosae-Detarioideae family, known under the trade name of Tali (ATIBT nomenclature), the species is exploited for the quality of its wood, which is widely used and appreciated, notably in carpentry and for the heavy works (port works, quays and bridges, railway sleepers) (Okeyo, 2006). According to Doucet (2003), the species belongs to "non-pioneer light demander" temperament or moderate light. Juvenile individuals are briefly shade-tolerant.

But, they quickly need light for to pursue their development (Gorel et al., 2015). Nevertheless, analyzes of the population structures of *Erythrophleum suaveolens* show relatively high effectives in the 70 to 90 cm diameter classes (Gorel et al., 2015; Douh et al., 2018). A deficit of young stems naturally reflects the regeneration deficit of this species in Cameroon, Gabon and in Republic of Congo (Doucet, 2003; Gorel et al., 2015; Kouadio, 2009).

Thus, to compensate the regeneration deficit of the seedlings of *E. suaveolens*, artificial regeneration from seedlings produced in nursery would be desirable (Doucet et al., 2016). To guarantee long-term management of the populations of species, it would be appropriate to better understand the mechanisms involved in the breaking dormancy. The seeds of *E. suaveolens* species being dormant, they can be lifted per treatment to sulfuric acid, following the works of Kouadio (2009) and Douh et al. (2018). Nonetheless, the treatment of seeds to sulfuric acid, in the boiling water and to oven at 140 °C, would deserve to be continually explored in order to define the most appropriate treatment modalities. Also, it is apposite to know if the effect of a pretreatment also has an influence on the initial growth. The aim of this study is to determine the effect of the different seeds pretreatments on the germination performance and initial growth of *E. suaveolens* seedlings.

## MATERIALS AND METHODS

### Study site

The seeds have been collected within the devices of DynAffFor project (Dynamics of the Central African forests), in north of the Republic of Congo. The devices are installed in the forest concession attributed to CIB/OLAM, near the locality of Loundoungou in the *Celtis* forest (Fayolle et al., 2014).

The *Celtis* forest is semi-deciduous, resting on clay-sandy to sandy-clay soils typical of the 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 by traditional human activities and can be qualified as old secondary forest (White, 1983; Morin-Rivat et al., 2014).

### Experimental device and germination tests

In a plot of 400 ha, *Erythrophleum suaveolens* tree species have been chosen in a range of dbh varying from 30 to 155 cm. The

diameter of 30 cm corresponds to the regular fruiting diameter of the species (Doucet, 2003; Gillet, 2013; Douh et al., 2018). The *E. suaveolens* seeds founded under each seed tree have been collected, totaling 350 seeds. They have been conveyed then received at the National Forest Research Institute (IRF-Brazzaville) and kept cold for 17 days in a refrigerator at a temperature about 5°C. A complete randomized device of two blocks has been set up in greenhouse. The batch of seeds (350 seeds) has been divided in seven treatments to test the following modalities of breaking dormancy, at the rate of 50 seeds per treatment:

- T1 = seeds soaked in a solution of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) diluted to 96% for 30 minutes;
- T2 = seeds soaked in a solution of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) diluted to 96% for 45 minutes;
- T3 = seeds soaked in the boiling water for 15 minutes;
- T4 = seeds soaked in the boiling water for 30 minutes;
- T5 = seeds not having undergone any treatment (control);
- T6 = seeds put in the oven at 140 °C for 10 minutes;
- T7 = seeds put in the oven at 140 °C for 20 minutes.

The choice of these different modalities of breaking dormancy based on the previous studies bearing on the seeds with tegumentary dormancy like of *E. suaveolens* seeds (Kouadio, 2009; Fandohan et al., 2010; Balo Ilunga, 2015; Kanmegne et al., 2017; Douh et al., 2018).

After each immersion in sulfuric acid, seeds have been rinsed in the distilled water for one hour, changing rinse water every 20 minutes three times (Footitt and Cohn, 1995; Delhay, 2006; Kouadio, 2009; Douh et al., 2018). The germination tests have been realized in a greenhouse at the Scientific City of Brazzaville (National Forest Research

Institute), at an average temperature of 24°C and an average humidity of 60%.

The seeds have been individually and immediately sowed after treatment, placed on a previously sterilized substrate and composed of 25% sawdust and 75% humified earth in 0.5 l phytocel bags, and covered with a layer of soil 3mm thick. The humified earth has been incorporated to the substrate to ensure the effect of the natural bacterial procession of soil on the seeds germination with tegumentary dormancy like the seeds of *E. suaveolens* (Diabate et al., 2005).

Seeds sowed (NGS, number of seeds sowed) have been watered every other day, and germination has been monitored daily. The experiment lasted 12 weeks. At the end of experiment, the number of seeds having effectively germinated (NGG), the germination time (DG, time interval between sowing and the first emergence) have been evaluated for each treatment (De La Mensbruge, 1966; Mbolo, 1991; Danthu, 1993; Elazazi, 2016).

The germination percentage (TG%) has been calculated (Maguire, 1962). The height of the seedlings (in cm) has been measured at 5, 7 and 9 weeks after sowing on all the individuals of device having emerged.

### Data analysis

The effect of seed pretreatment on germination and initial seedling growth has been determined by applying nonparametric tests of Scheirer-Ray-Hare and Dunn's post hoc at the 5% threshold using RStudio software (version 4.1.2, rcompanion and FSA packages). The Generalized Linear Model (GLM) analysis is as follows:  $Y_{ijk} = \mu + B_i + T_j + B_i \times T_j + \epsilon_{ijk}$

$Y$  designates the response variable ;  $\mu$  designates the general value mean of the observations ;  $B_i$  designates the effect of the block ;  $T_j$  = designates the effect of pretreatment and  $\epsilon_{ijk}$  designates the residual error.

**Table 1:** Synthetic description of the *Celtis* forest, taking into account of the rainfall collected between 2000 and 2008 in Ouesso, Impfondo and Mbaïki (Verelst, 2009; Freycon, 2014; Douh et al., 2018).

<b><i>Celtis</i> Forest</b>	
	02°18' - 02°22' N
Geographical coordinates	17°31' - 17°34' E
Altitude (m)	410 – 460
Annual rainfall (mm/an)	1 729
Soil type	Acrisoil - Arenosoil – Gleysoil
Geomorphology	Piedmont on the edge of the Congolese bowl
Forest type	Semi-evergreen

## RESULTS

### Evolution of the germination depending on time

Globally, it is in the first and second week (W1 and W2) that we observed a relatively high trend of seeds germination (Figure 1). On the other hand, the last weeks (W4 and W5) have illustrated themselves by the relatively low germinations or even zero (Figure 1).

### Germination rates and times

At the end of the experiment and all treatments combined, the average germination percentage observed has been relatively low, since it only reached 22% of the seeds sowed. More precisely, the germination rates were 36-56%, 68-76%, 16% and 12-28%, respectively for treatments T1, T2, T5 and T6, with germination times of 6 days for T1 and T2, 15 days for T5, 15 to 20 days for T6 (Table 2). The treatments applied induce significantly different germination times (p-value = 0.00549). More specifically, sulfuric acid treatments would seem the most effective in reducing germination times to 6 days (Table 2).

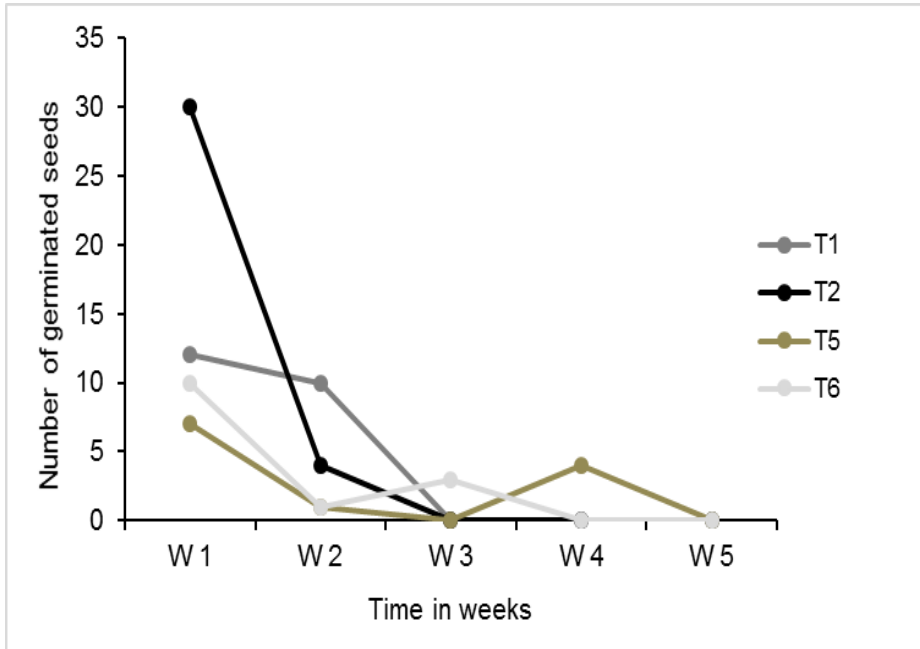
### Effects of the treatments on seeds germination

The results of Scheirer-Ray-Hare test show the existence of the significant differences between treatments (p-value = 0.04879).

The effect of treatments on seed germination therefore varies from one treatment to another (Figure 2). Treatment T5, which is the control treatment, had sensibly the same effects as T6 (Figure 2).

### Effect of the treatments on the initial growth of seedlings

The height of the seedlings varies according to the germination treatments used (p-values = 0.001669, 0.0007212 and 0.001063 respectively at 5, 7 and 9 weeks after sowing). The results show that sulfuric acid treatments promote better height growth of seedlings between 5 and 9 weeks after sowing (Figure 3). However, at 9 weeks, the control treatment promotes, just as much as the treatment with sulfuric acid diluted to 96% for 45 minutes, a better growth in height of the seedlings (Figure 3).



**Figure 1:** Evolution of the seeds germination in terms of time; W1 = First week; W2 = Second week...; T1= Treatment 1; T2= Treatment 2...

**Table 2:** Overall result of all device.

Blocks	Treatments	Number of seeds sowed (NGS)	Number of sprouted seeds (NGG)	Germination rate (TG%)	Germination time (DG)
<b>Block 1</b>	T1	25	14	56	6
	T2	25	19	76	6
	T3	25	0	0	-
	T4	25	0	0	-
	T5	25	4	16	15
	T6	25	3	12	20
	T7	25	0	0	-
<b>Block 2</b>	T1	25	9	36	6
	T2	25	17	68	6
	T3	25	0	0	-
	T4	25	0	0	-
	T5	25	4	16	15
	T6	25	7	28	15
	T7	25	0	0	-

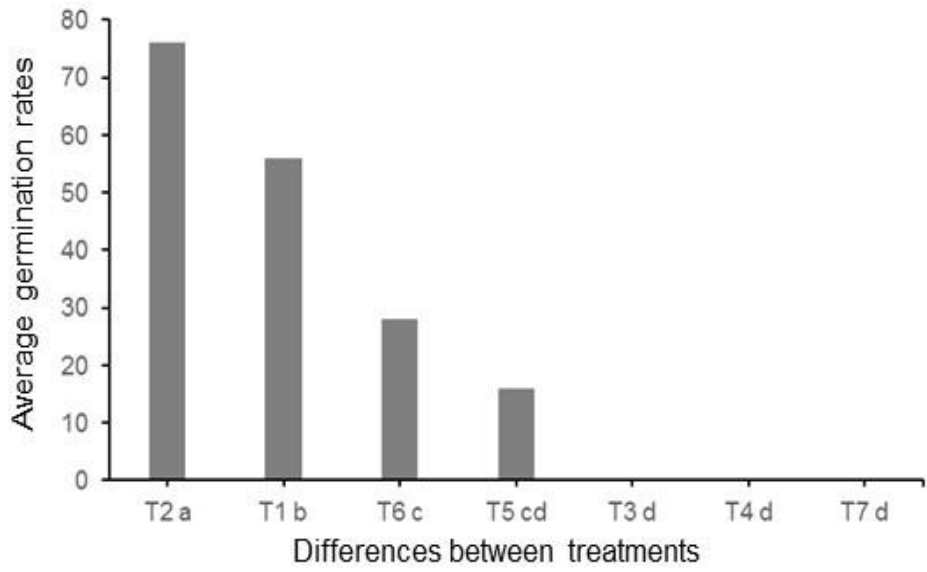


Figure 2: Average germination rate in terms of the treatments.

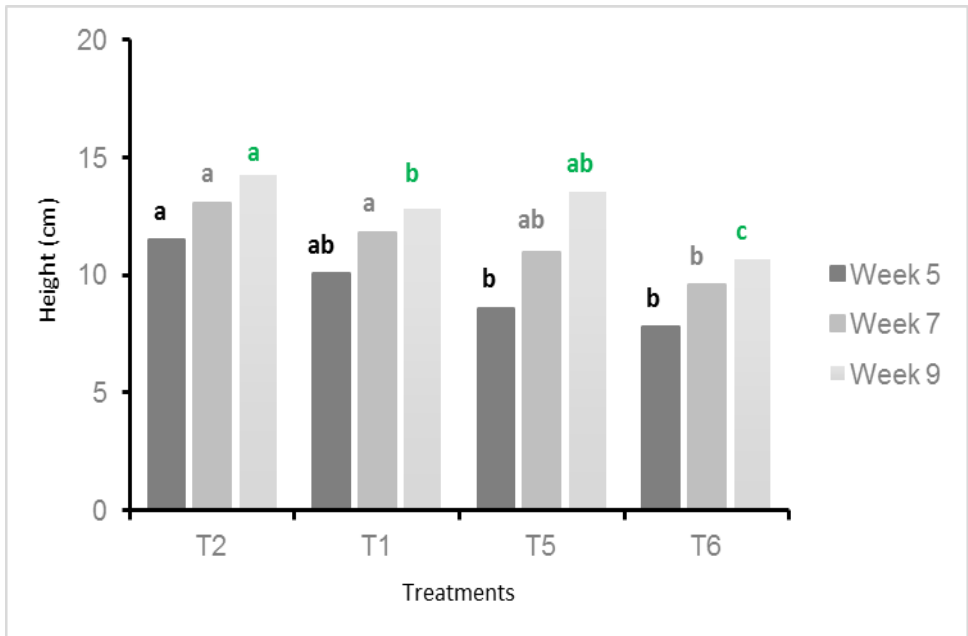


Figure 3: Average height of seedlings in terms of the treatments at different weeks.

## DISCUSSION

On the whole device put in place with seven treatments, the results of the germination seeds of *Erythrophleum suaveolens* species displayed a relatively low average germination rate. On 350 seeds sowed, and all treatments combined, 77 seeds germinated with an average germination rate of 22%.

Delhaye (2006) and Kouadio (2009) demonstrated that the seeds of *E. suaveolens* species, collected directly after dispersion and which are not treated (T5, control treatment in our case) presented relatively low germination percentages (0% and 32%, respectively) compared to those treated with sulfuric acid (70% and 98%, respectively). Nevertheless, the works of Azad et al. (2011) and Olatunji et al. (2012) displayed similar results for *Acacia auriculiformis* Cunn. ex Benth., a species whose seeds also present integumentary dormancy comparable to that of *E. suaveolens*.

In the present study, treatments 1 (96% diluted sulfuric acid for 30 min) and 2 (96% diluted sulfuric acid for 45 min) respectively presented the highest germination proportions (76-68% and 36-56%) than treatments 5 (seeds having undergone no treatment, control treatment) and 6 (seeds put in the oven at 140°C. for 10 min) (16% and 12-28%, respectively).

However, treatments 3 (seeds soaked in the boiling water for 15 min), 4 (seeds soaked in the boiling water for 30 min) and 7 (seeds put in the oven at 140°C for 20 min) did not displayed any germination proportion. This result can be attributed to probable destruction of the embryo by the heat. Similarly, the work of Kouadio (2009) displayed any germination for seeds soaked in the boiling water. Nevertheless, previous studies (Delhaye, 2006; Kouadio, 2009; Azad et al., 2011; Olatunji et al., 2012) demonstrated similar germination proportions with 95% dilute sulfuric acid. Consequently, sulfuric acid reveals as the best treatment for breaking dormancy integumentary dormant seeds like *E. suaveolens* seeds.

Contrary to results of the present study, the works of Douh et al. (2018) demonstrated

that at the end of the experiment, for all treatments combined, the average percentage of germination observed has been low, since it had only reached 12.9% of the seeds sowed, against 22% of the seeds sowed in the present study. More precisely, the germination rates were respectively 19.1% (control treatment), 4.3% (treatment 1) and 15.2% (treatment 2). The authors also underlined that the proportion of seeds germination was significantly lower for treatment 1 (seeds soaked in a solution of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) diluted to 95% for 20 minutes). We also point out that the higher proportion of germination of the control treatment in the work of Douh et al. (2018) could be explained by the stay of the seeds in the soil bank which would probably have initiated the lifting of dormancy, allowing rapid imbibition of the seeds. Contrary to the seeds of the present study which have been collected on the ground to feet of the seeds tree. Nevertheless, other studies are strongly encouraged in order to diversify other types of treatments.

Finally, as underlined by Douh et al. (2018) and Doucet et al. (2016), silvicultural interventions based on planting or enrichment techniques should be encouraged to contribute to the renewal and sustainability of species *E. suaveolens*, which is part of the four most exploited species in Central Africa.

This study reports a positive effect of sulfuric acid treatments on the height growth of the seedlings. The same effect is observed for the control treatment, 9 weeks after sowing. It would therefore be appropriate for to slice through on the existence of a proven positive effect of pretreatment on initial growth, a longer observation of the latter.

## Conclusion

Globally, the treatments applied showed a significant effect on the germination time and rate, as well as on the initial growth of the seedlings. Nonetheless, assisted regeneration considerable efforts must be made for this species which occupies a prominent place in logging in Central Africa. If nothing is done to compensate the regeneration deficit

observed, it is likely that the species regress strongly with successive rotations, as others species in the image of *ayous*, *Triplochiton scleroxylon*. The study suggests that the reforestation in the post-logging plots with seedlings from seeds collected from seeds tree and nursery regular monitoring is the essential technique to ensure the sustainability of species.

### COMPETING INTERESTS

The authors declare that they have no competing interest.

### AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all the authors. CD designed the study, wrote the protocol and the first draft of the manuscript. CGME analyzed the data and corrected the first draft of the manuscript. RGK collected the seeds in forest, and carried out the germination trial in the nursery. FK provided critical inputs towards the research, and corrected the manuscript. All the authors read and approved the final manuscript.

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