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Effect of three pretreatments on germination and nursery growth of *Erythrophleum ivorense* a. Chev, Aa little-known timber species in Cote d'Ivoire

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Abstract

Erythrophleum ivorense (A. chev), commonly known as Tali, is a tropical timber species found in savannah and forest zones. It is used for a wide range of purposes. The aim of this study is to contribute to the improvement of seed germination and seedling growth of *Erythrophleum ivorense* in nurseries by developing effective pre-treatment methods that will lead to a rapid lifting of seed dormancy. To achieve this, four pre-treatments were applied to *Erythrophleum ivorense* seeds. These were the control with no pretreatment (T0), soaking the seeds in ordinary water for 48 h (T1) and soaking the seeds in 95% dilute sulfuric acid for 20 min (T2). Germination and growth data were taken and statistically tested. The best germination rate was recorded when the seeds were soaked in 95% sulfuric acid for 20 min, with a value of 86%, while the lowest rate was obtained when the seeds were soaked in ordinary water for 48 h (10%). The analyses also showed that the different pre-treatments had a significant effect on growth parameters. Growth in height, diameter and number of leaves was greater in seedlings whose seeds had been treated with 95% dilute sulfuric acid for 20 min, with respective values of **(**19.19±4.13^{b}; 14.55±0.43^{b}; 6.51±1.75^{b}).

Pre-treatment with 95% dilute sulfuric acid for 20 min could be proposed for nursery propagation of *Erythrophleum ivorense* for long-term conservation. However, it should be used with precaution.

Keywords: Timber; Nursery propagation; *Erythrophleum ivorense*; Conservation; Côte d'Ivoire

1. Introduction

Tropical forests are home to the largest concentration of plant and animal species on the planet [1]. They are considered the world's largest reservoir of terrestrial biological diversity, both in terms of species and ecosystems. Tropical forests provide ecosystem goods and services to populations. They play a vital role in climate stability [2]. African forests are particularly important for regulating greenhouse gases, wood supply and non-timber forest products, and cultural services [3]. However, these forests are subject to numerous anthropogenic pressures, notably logging and agriculture. In Côte d'Ivoire, these various anthropic pressures have led to a drastic reduction in forest cover, resulting in the disappearance of many plant species, particularly local timber species. This was the case for *Erythrophleum ivorense* (A. Chev).

Erythrophleum ivorense (A. Chev), commonly known as Tali, is a tropical species found in savannah and forest areas. *E. ivorense* can reach 40 meters in height and 1.5 meters in diameter. *E. ivorense* is exploited for the quality and durability

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of its highly prized wood, particularly for joinery and heavy-duty work (railroad sleepers, harbour structures, quays and bridges) [4]. It is one of the lesser-known forest species in Côte d'Ivoire [5]. *E. ivorense* grows in tropical Africa, notably in Ghana, Côte d'Ivoire and Liberia [6]. It is found in primary and secondary evergreen forests and semi-deciduous rainforests [7]. *E. ivorense* is used in a number of areas. Its bark is used to treat smallpox [8]. Its bark is also used to treat convulsions, pain, heart problems and oedema caused by nematodes [3]. Its trunk is used as lumber in construction and furniture-making.

However, introducing the species into reforestation campaigns is made difficult by the fact that its propagation in nurseries is still poorly mastered. One of the reasons given for this is the thick, hard shell of the seed, which delays germination. In this context, this study aims to contribute to the improvement of seed germination and seedling growth of *Erythrophleum ivorense* by developing effective pre-treatment methods that will lead to a rapid lifting of seed dormancy. The results could be useful to the various state and private players involved in reforestation in Côte d'Ivoire.

2. Material and methods

The study material consisted of vegetal material and technical equipment.

The vegetal material consisted of *Erythrophleum ivorense* seeds harvested from stands in the Centre-Ouest region of Côte d'Ivoire. They were harvested six (06) months prior to the experiment. The seeds have an average thickness of 5.73 mm and an average length of 1.35 cm. The fruits are black, leathery, oblong pods, rounded at both ends. Seeds are protected by very tough lignified seed coats [3]. Like most Fabaceae, *Erythrophleum ivorense* has an intensity of dormancy that increases over time.

Figure 1 Seed tree (A) and seeds of *Erythrophleum ivorense* (B)

To carry out this experiment, the following technical equipment was used:

- a graduated ruler and digital calipers for measuring seedlings;
- 20 x 10 cm polyethylene bags for storing potting soil in the nursery;
- sulfuric acid (H2SO4)

2.1. Pre-treatment of seeds

Three types of pre-treatment are tested in order to lift seed dormancy. These are

- **Control (T0)**: No pre-treatment

The seeds underwent no pre-treatment whatsoever. They were simply rinsed with water to sort out the viable ones. Pre-treatment T0 therefore served as a control for all other pre-treatments.

- **Pre-treatment 1 (T1)**: Pre-treatment of seeds with ordinary water for 48 hours.

This pre-treatment involves placing the seeds in a container of water at room temperature (15°c) for 48 hours. This time corresponds to the maximum recommended seed soaking time [9]. The seeds were then removed and dried for 30 minutes.

- **Pre-treatment 2 (T2):** Pre-treatment with 95% dilute sulfuric acid for 20 min.

Seeds were soaked in a 95% solution of sulfuric acid (H_2SO_4) for 20 min. They are then removed, rinsed thoroughly with plain water and dried for 30 min.

2.1.1. Experimental set-up and sowing

For seeding, a traditional germinator containing humus-rich soil was set up at the Jean Lorougnon Guede University. This germinator was subdivided into three compartments, each of which received seeds according to the pre-treatments (Figure 2).

In each compartment, five rows containing 10 seeds each were arranged to facilitate counting and better monitor germination parameters. These seeds, arranged in rows, were covered with a thin layer of soil. A total of 150 seeds (50 per pre-treatment) were sown. During the 60-day experiment, the seeds were watered twice a day.

Figure 2 Experimental set- up to test germination of *Erythrophleum ivorense* seeds

2.1.2. Determination of germination parameters

From the second day after sowing, observations were made daily. Germination parameters (germination delay, germination duration, germination speed, germination rate) were observed and recorded.

Germination delay is the time in days between sowing and germination of each seed in a batch (number of days required for germination of each seed sown);

Germination duration or spread is the time in days between the first and last germination of a batch set;

Germination speed, defined as the time between the first and last germination. It is estimated by the percentage of germinated seeds as a function of time.

Germination rate (TG) is the proportion of germinated seeds in relation to the total number of seeds sown. It is expressed as a percentage. It is expressed according to the following mathematical formula:

$$TG = \left(\frac{ni}{N}\right) x\ 100$$

where ni: number of germinated seeds and N: total number of seeds sown.

2.1.3. Determining plant vigour

In addition to germination parameters, seedling vigor data were taken for two (02) months. For each pre-treatment, measurements were taken of seedling height (H), collar diameter (D) and number of leaves (Nf) for all seedlings that survived. Seedling height was measured with a ruler graduated in centimetres. Neck diameter was measured with an electronic caliper in millimetres. The number of leaves of each seedling were counted. These growth parameters were evaluated using a plant vigour parameter sheet.

2.1.4. Data analysis

The germination and growth parameters collected were subjected to statistical tests using R software. Averages and percentages of the parameters studied were calculated. An analysis of variance was performed to assess the significant difference between the different pre-treatments, and Student's t test was used for comparison of means.

3. Results

3.1. Effect of pre-treatments on germination parameters of *Erythrophleum ivorense* seeds

The analyses showed that the 95% dilute sulfuric acid for 20 min pre-treatment recorded the best germination rate than the other pre-treatments. In fact, the 95% dilute sulfuric acid for 20 min pre-treatment recorded a germination rate of 86% compared with the other two pre-treatments. The other two pre-treatments only recorded 12% and 10% respectively for the control (no pre-treatment) and soaking in cold water for 48h (Figure 3). The shortest germination time was obtained with seeds soaked in 95% dilute sulfuric acid for 20 min (T2). This ranged from 7 to 33 days, with an average of 19.71 days. On the other hand, seeds soaked in cold water (T1) had the longest germination time, ranging from 14 to 55 days, with an average of 30.53 days. Seeds treated in cold water for 48 h had a germination time ranging from 21 to 39 days, with an average of 29.55 days (Table 1).

In terms of germination time, seeds soaked in cold water for 48 hours (T1) had a shorter germination time than the other two pre-treatments. In fact, germination time for seeds soaked in cold water varied from 1 to 19 days, with an average of 9.56 days. The longest germination time, ranging from 1 to 52 days with an average of 18.71 days, was observed in seeds that had not undergone any pre-treatment (T0) (Table 1).

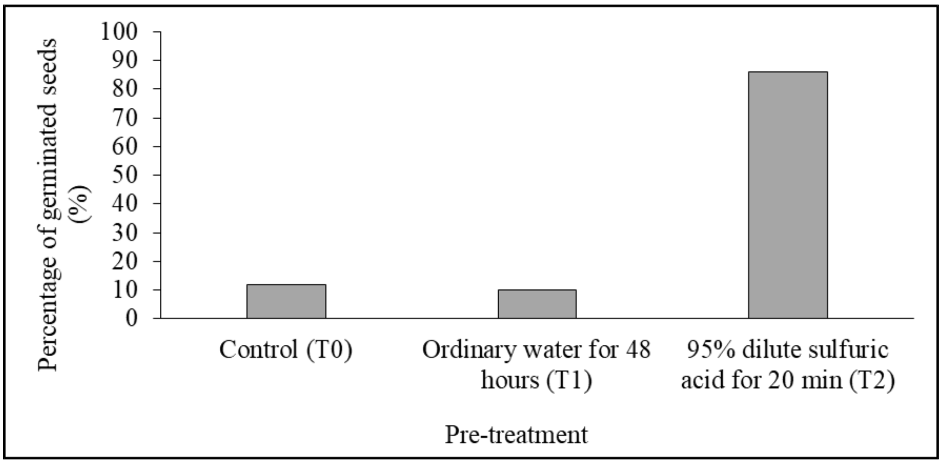


Figure 3 Germination rate of *Erythrophleum ivorense* seeds

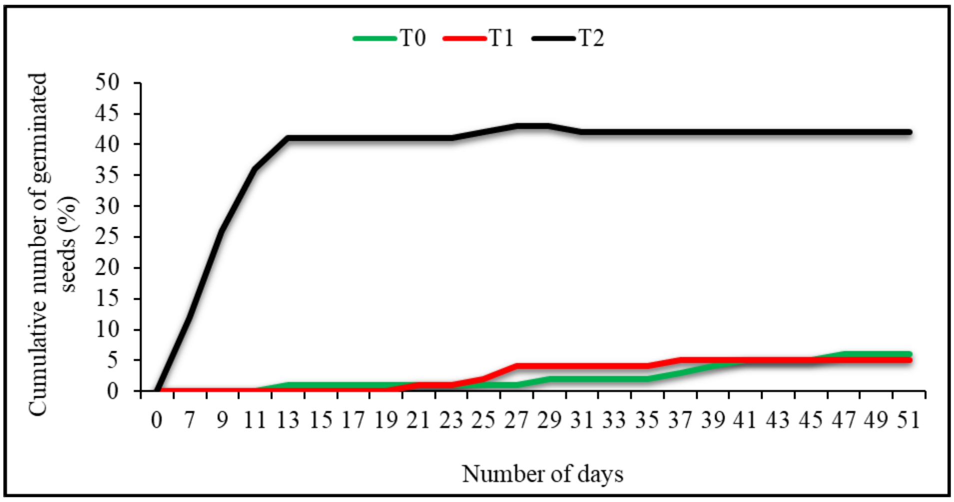
Table 1 Average germination time and duration for *Erythrophleum ivorense*

Germination parameters		Pre-treatments		
		Control (T0)	**Ordinary water for 48 hours (T1)**	**95% dilute Sulfuric acid for 20 min (T2)**
Germination delay (days)	Min	14	21	7
	Max	55	39	33
	Mean	30.53±11.53b	29.55±6.29ab	19.71±8.91a
Duration of germination (days)	Min	1	1	1
	Max	52	19	27
	Mean	18.87±14.17a	9.56±6.29a	13.71±8.91a

Values with identical letters on the same line are statistically identical at the 5% threshold. Max: maximum, Min: minimum

3.1.1. Germination dynamics of E. ivorense seeds

Figure 4 illustrates the daily dynamics of germination according to the pre-treatments applied to the seeds. Seeds treated with sulfuric acid (T2) recorded a higher number of germinated seeds per day than those soaked in cold water for 48h (T1) and the control (T0). In fact, the highest number of initial germinations was recorded for the sulfuric acid-soaked seeds, which began on day 7 after sowing, with one (01) seed in the process of germinating. Germination of seeds soaked in hot water also began on day 9 after sowing, with 7 seeds germinating. Germination of untreated seeds (control) began on day 11 with one seed. Seeds treated with sulfuric acid began to germinate on the 13th day after sowing with one seed. Seed germination stabilized at 13, 15, 17 and 19 days after sowing for sulfuric acid-treated seeds, the control, hot-water-treated seeds and cold-water-treated seeds respectively. After these dates, the seeds stopped germinating.



T0: control; T1: ordinary water for 48 hours; T2: 95% dilute sulfuric acid for 20 min.

Figure 4 Germination dynamics of *E. ivorense* seeds

3.1.2. Effects of different pre-treatments on the vigor of Erythrophleum ivorense seedlings in the nursery

Table 2 shows variations in the different growth parameters of *E. ivorense*. Analysis of variance showed a significant effect of the different pre-treatments on plant vigour parameters ($P<0.05$). Mean seedling collar diameter and mean seedling height for those not treated (T0) and those soaked in sulfuric acid were identical, with mean values of 14.18 mm and 14.18 cm; 14.55 mm and 19.19 cm respectively. On the other hand, seedlings whose seeds had been soaked in water for 48 h (T1) recorded an average of 10.70 mm and 10.55 cm respectively for seedling diameter and height. In terms of the number of leaves, seeds soaked in sulfuric acid (T2) recorded an average value of 6.5 or 7 leaves per seedling. By contrast, the other two pre-treatments recorded an average of 1.5 and 2 leaves per seedling.

Table 2 Average variation of plant vigour parameters of *Erythrophleum ivorense* seedlings according to pre-treatments.

	Pre-treatments			
Growth parameters	T0	T1	T2	Test statistics
Mean diameter	$14.18±0.26^b$	$10.70±5.23^a$	$14.55±0.43^b$	21.05***
Mean height	$9.88±1.44^a$	$1055±5.73^a$	$19.19±4.13^b$	15.27***
Number of leaves	$1.5± 1.73^a$	$2±00^a$	$6.51±1.75^b$	18.62***

Values with identical letters on the same line are statistically identical at the 5% threshold. T0: control; T1: plain water for 48 hours; T2: 95% dilute Sulfuric acid for 20 min.

4. Discussion

The best germination rate was obtained when *E. ivorense* seeds were soaked in sulfuric acid (86%). The high germination rate of seeds soaked in sulfuric acid could be explained by the fact that the sulfuric acid weakened the seed coats in order to lift their dormancy. This result corroborates those of Baskin & Baskin [10]. According to these authors, soaking seeds in sulfuric acid is a method commonly used to lift the dormancy of certain plant species. This pre-treatment disintegrates the cuticle and integument of the seeds, facilitating water uptake and gas exchange necessary for germination [11]. In addition, the longest germination times were recorded for untreated seeds (control) and seeds soaked in water for 48h. Our results corroborate those of Smith *et al.* [12]. Indeed, these authors showed in their work that the control and cold water soaking pre-treatments led to a prolonged germination time, as these pre-treatments failed to effectively break seed dormancy. The work of Ghassali *et al.* [13] on the germination of *A. farnesiana* and *A. karroo* showed that the delay in germination of certain seeds could reflect slow imbibition resulting from inherent seed properties, in particular the low permeability of the seed coat.

On the other hand, analysis of variance showed that pre-treatments carried out before sowing had an influence on the growth of *Erythrophleum ivorense* seedlings ($P< 0.05$) of the different pre-treatments on seedling growth parameters. The diameter and height of seedlings soaked in sulfuric acid were higher than those treated with hot and cold water, at 14.55 mm and 19.19 cm respectively. As for the number of leaves, seed soaking in sulfuric acid (T2) recorded an average value of 6.5, i.e. around 7 leaves per seedling. In contrast, the other two pre-treatments recorded an average of 1.5 and 2 leaves per seedling. Our results corroborate those of Baskin & Baskin [10]; Sanou [14]; Issa *et al*. [15]. Indeed, these authors have shown that the best pre-treatment in terms of growth in certain species is observed in seedlings from seeds soaked in sulfuric acid.

5. Conclusion

The study of *Erythrophleum ivorense* germination has enabled us to test three pre-treatments for lifting seed dormancy. The best germination rate, time and average duration were recorded when the seeds were soaked in sulfuric acid for 20 min. This pre-treatment could be proposed for nursery propagation of *Erythrophleum ivorense.* In addition, the study showed that the three pre-treatments applied to the seeds significantly influenced seedling growth (height, diameter and number of leaves).

Compliance with ethical standards

Disclosure of conflict of interest

There are no conflicts of interest.

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