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Impact of Pretreatment on the Germinative Characters of Seeds of *Stereospermum kunthianum* Cham. (Bignonaceae) for Its Domestication in Chad

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Abstract

The objective of this research is to lift the dormancy of seeds of *S. kunthianum* in with a view to domesticating them. In this experiment, we used the soaking of seeds in sulfuric acid for different durations (10 min, 30 min and 1 h). The treated seeds were sown in transparent germinators. The explants obtained were then transplanted into pots containing five types of substrate (black earth, fine sand, sawdust, mixtures 1/1 of sawdust/black earth and 1/1 of sawdust/fine sand). This second test made it possible to determine the rate of germination on the substrate. The results obtained compared to those of the control showed a clear reduction in the latency time or germination time (62 hours) instead of 6 days (control), in the germination time 3 days/7 days (control) and a very high germination rate (100%). At the 5% threshold, soaking for 10 min and 30 min (100%) is the best result on the germination of *S. kunthianum* seeds. Soaking for 1 hour gives (7%) very low. After transplantation of the explants, the percentages obtained independently of the treatments show that the 1/1 mixtures of sawdust/black earth (75.19%), black earth (73.33%) and sawdust/fine sand (66.30%) have a higher performance than the others. The type of germination is epigeal. This work makes it possible to domesticate *S. kunthianum* in Chad.

Keywords

Stereospermum kunthianum, Treatment, Substrate, Germination, Medicinal Plant and Chad

1. Introduction

The tropical forest represents one of the immense reserves of biological, food and medicinal resources that can be used by indigenous populations [1]. In 2003, the level of average annual destruction of forest resources due to human activities and climatic variations was 142,800 ha [2]. These authors add that the state of land occupation showed that wooded parks, gallery forests, shrub savannah and fallow lands experienced a regression from 110,274 ha to 43,950 ha between 1984 and 2016, *i.e.* an annual rate of regression of −2.58%. This phenomenon could be explained by a growing demand for non-timber forest products in general and particularly for medicinal plants. This demand is very strong in developing countries in general and in the forest region of Central and West Africa in particular, because modern medicine there is insufficient or even financially inaccessible for a large part of the population [3]. All these phenomena, although important for the immediate satisfaction of human needs, undoubtedly damage the state of conservation of biodiversity and harm populations of all generations.

According to [4], all parts of this plant are full of therapeutic virtues. This is what makes *S. kunthianum* an agro-forestry species, *i.e.* a multipurpose plant. Thus, the root is a powerful diuretic. It enters into the care of many other ailments such as: headache, gastritis, gonorrhea, dysentery, schistosomiasis, hematuria, jaundice. Root bark treats snakebite. Roots associated with leaves fight syphilis, gastritis, asthma and headache. The bark of the tree is haemostatic, healing, vermifuge and treats wounds, burns, leprosy, gonorrhea, bronchitis, pneumonia, cough, dysentery, gastritis, phagedenic ulcer and hypertension. The leaves combat asthenia, wounds and gonorrhea. The fruits are edible and treat coughs. This plant also has virtues that fight diarrhea, dysentery and flatulence in cattle. The wood is used in construction, making mortars and toothpicks. In West Africa the bark is used as a lip tint (lipstick). Its use as firewood is often prohibited in many localities because of its magico-religious power

In the Chadian pharmacopoeia, it is used for its therapeutic virtues in the treatment of hypertension, wounds, leprosy, general asthenia and poisoning.

Thus, the immense solicitation of *S. kunthianum* and the devastation of the area by the desert fire and the improbable regeneration of this species increase the risks of its disappearance. The survival of this species would now come from the implementation of appropriate management approaches associated with assisted regeneration techniques.

For many forest species, proper seed pretreatment is an important process to achieve satisfactory germination. Pre-treatments do not germinate the seeds, but allow the germination time to be minimized when all the required conditions are met. It is, by definition, the pre-treatment(s) carried out before, during or after conservation, which allows (attempts) the elimination of dormancy by their mechanical, chemical and physiological effects [5] [6]. It is according to the seed coat structure that the type of pretreatment is defined. The objective of this work is to determine the possibilities of improving the germination rate of *S. kun-*

thianum seeds for its domestication by using different pretreatments and substrates that are within the reach of local populations.

This research has made it possible to verify two hypotheses, one of which is "pretreatment with sulfuric acid breaks the dormancy of this species" and the second "certain substrates favor germination". To verify this hypothesis, the following materials and methods were used.

2. Materials and Methods

2.1. Place of Study

This study was conducted at the biology laboratory of the Higher Teachers' Training College in N'Djamena from January to July 2022. The coordinates of this school are: latitude 12˚1044882N and longitude 15˚057'3853"E (4325-R29, Ndjamena, Chad). The city of N'Djamena benefits from a Sahelo-desert climate of the Sudano-Guinean type, characterized by a rainy season (3 to 4 months) and a dry season (7 to 8 months) whose average annual rainfall rarely exceeds the 800 mm. The vegetation is savannah type with thorny dominance [7]. It is the shrubby savannahs with Acacia seyal that are widely distributed. The tree savannahs are either *Acacia seyal*, *Hyphaene thebaica* or *Sclerocarya birrea.* We observe here and there wooded savannahs or clear forests with Anogeissus leiocarpus. The population is mainly made up of baguirmians or barma, followed by Hausa and Sara; we note the presence of minor ethnic groups such as the Fulani, Bornous and Ouaddaiens [8]. The soils are tropical ferruginous, slightly leached with stains and rare concretions. They are hydromorphic and vertisols are found in depressions or on the edge of weakly flooded depressions. These are soils with a loamy-clayey to loamy clay texture, sometimes sandy-clayey [9].

2.2. Seed Collection Location

The seeds used during this experiment were collected in Mayo Kebbi-Ouest precisely in the villages of Berdé (09˚3491138N and 15˚57882E), Belé (09˚385621N and 15˚3925785E) and Belé Vansa (09˚3757808N and 15˚3123617E) all located on the Kelo-Pala section as shown in **Figure 1**. It corresponds to the southern fringe of the country and is located between isohyets 800 mm to 1200 mm (INSE, 1987). The rainy season generally lasts from May to November. It covers the regions of Mayo Kebbi, Tandjilé, the two logones and Moyen Chari. In this zone, the natural vegetation is of the wooded savannah type and the woody plants are tall, dense but composed of perennial and annual grasses which form a herbaceous carpet. The conventional Lake Chad Basin (1989) estimated between 1000 to 2000 plant species per 10,000 km^2 and that there are probably 2750 species in this area [10].

2.3. Technical Material

The technical equipment consists mainly of pots (a) of a sprayer (b), germinators (c) and substrates (d) (**Figure 2**).

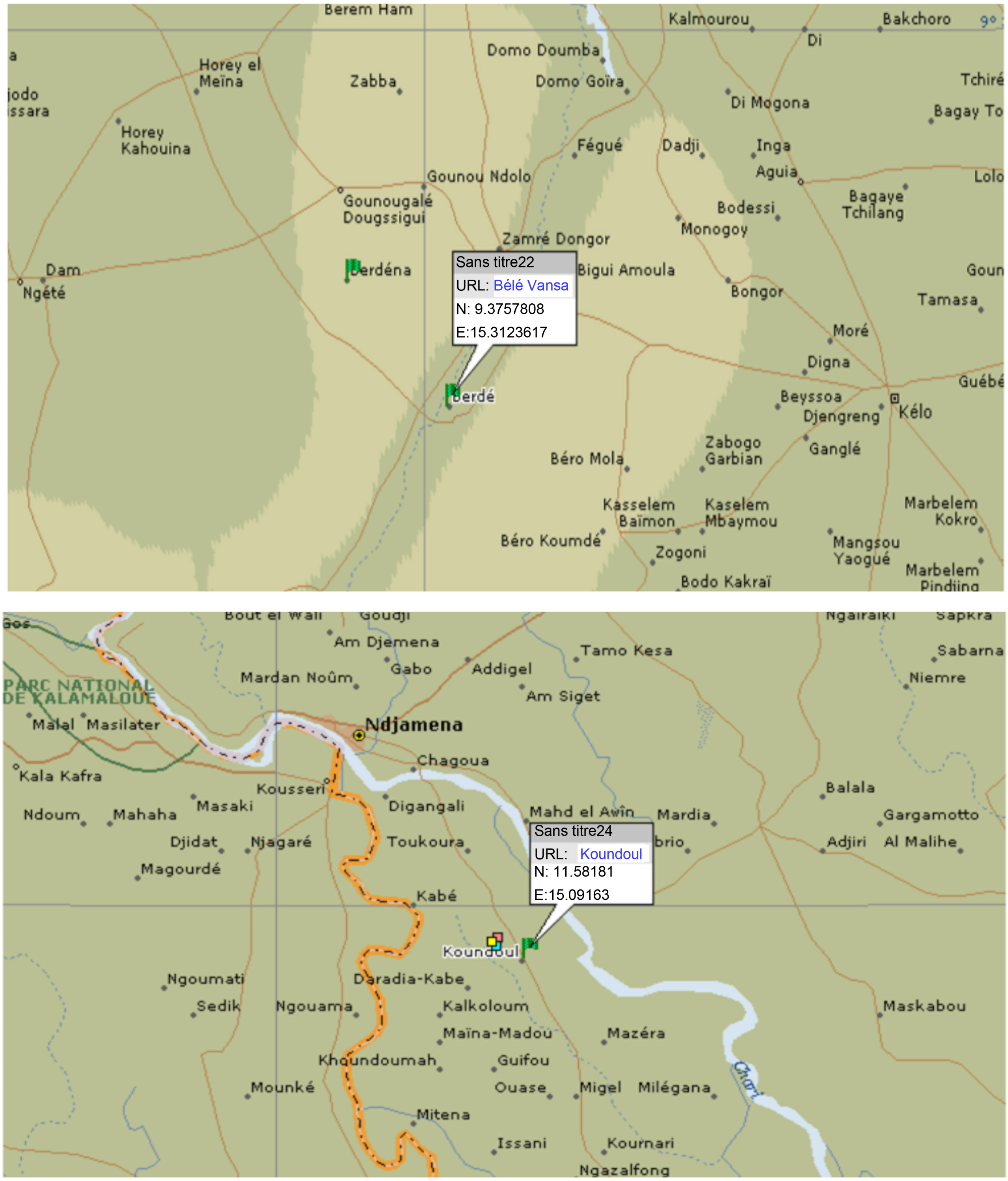

Figure 1. Geographical position of Koundoul and Bélé Vansa, seed.

2.4. Plant Material

According to [4] *S. kunthianum* is a large tree of the Bignonaceae family widespread in the wooded savannahs and dry leguminous forests of the Sudano-Guinean zone. Tree or shrub up to 10 - 12 m tall, with an open, rounded

Figure 2. Material used to conduct the germination experiment.

crown. Bark with thin and smooth scales, grey-purple to grey-green, with whitish edge and green rhytidome. Twigs pubescent become gray glabrous. Imparipinnate opposite leaves c. 32 cm long with 3 or 4 pairs of opposite or suboppsate leaflets. Blade with entire or crenulated, toothed, oval, elliptical or oblong edges, 5 to 10 cm long and 3.5 to 5 cm wide, with obtuse, pointed or apiculate apices, with asymmetrical rounded or wedge-shaped bases. Protruding, pinnate venation having 5 to 8 pairs of secondary veins. Terminal panicle inflorescence up to 25 cm long. Pink flowers streaked with purple, with corolla in tube about 5 cm long with 5 curly lobes on the edges. Fruits in cylindrical capsules, twisted when ripe, 40 to 60 cm long. Seeds flat with a papery wing at each end, 2.5 to 5 cm long. Flowering occurs in the dry season before the appearance of the first leaves in the Sahelo-Sudanian zone. But in Guinean areas, flowering occurs at the same time as the leaves. It is a species that adapts to all types of soil and is found in the Sahelo-Sudanian and Guinean zones. The parts used in this experiment consist of the seeds of *S. kunthianum* as shown in **Figure 3**.

2.5. Experimental Methods

2.5.1. Procedure

Soaking in concentrated sulfuric acid at 90˚ (**Figure 4**) for three different lengths of time (10 min (c), 30 min (b) and 1 h (a)) then rinsing with plain water for 24 h before sowing. For each duration, 30 × 3 seeds were used. That is a total of 90 × 3 seeds for the treatment. After treatment, the seeds were germinated in transparent germinators in which hydrophilic papers were placed beforehand. Arrangements were also made for sterilization of the germinators (**Figure 4**).

According to [11], this study makes it possible to determine the exact day of the appearance of the unobservable radical when the seed is directly sown in the substrates. The seeds soaked in sulfuric acid were germinated in transparent trays (**Figure 6(a)**) which served as germinators.

The device used to conduct this experiment is in complete randomized blocks with three pre-treatments. Three replicates per treatment were performed. Each experimental unit had 16 pots each with 5 seeds [11].

Untreated or control seeds were also sown to allow the effect of the treatments to be assessed by comparing results (**Figure 5(d)**).

2.5.2. Explants Transplantation Methods

The transplanting of the seedlings was carried out in 100 cm^3 polyethylene pots containing five types of the substrates. These are fine sand, black earth, sawdust,

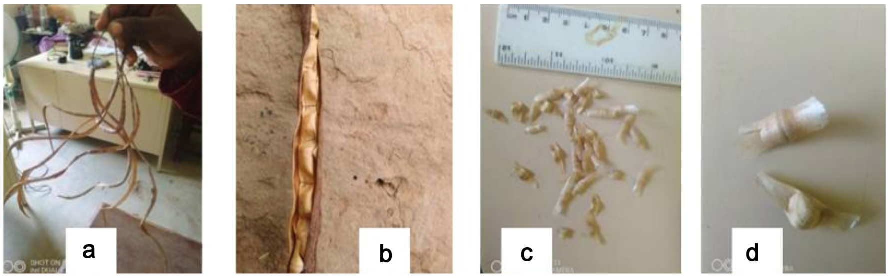

Figure 3. Fruits ((a) and (b)) and seeds ((c) and (d)) of *S. kunthianum.*

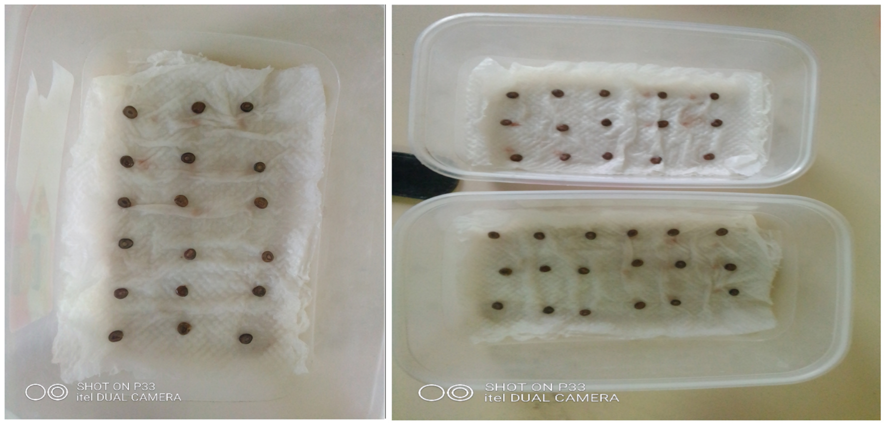

Figure 4. Arrangement of the contents of the germinators and sowing of the pre-treated seeds.

Figure 5. Seed acid soaking device and control (T0).

mixtures (1/1) of sawdust/black earth and sawdust/fine sand. After filling the pots and transplanting them, they were placed under a shed (the shade house). Watering was done every day for 1 month and two days for the rest of the time using a 16 liter Sprayer brand sprayer. The seedlings were followed during development until the third month (**Figure 9**). The device used to conduct this experiment is in randomized complete blocks. Six pots were used per substrate for a total of 60 seedlings. The experimental set-up consisted of 6 pots × 5 substrates, *i.e.* 30 pots and a total of 300 transplanted seedlings [11].

2.5.3. Monitoring and Recording of Data

The lifting of dormancy corresponds to the appearance of a radicle. This stage marks the end of the germination period (waiting period) but at the same time the beginning of the germination period. The following parameters were determined: type of germination, waiting time (germination delay) time elapsed between sowing and first germination; germination time (staggering) which is the time between the first and last germination; germination rate: T = G/N with G = number of seeds germinated and N = total seeds germinated according to the treatment. The percentages, mean values and standard errors of the parameters studied were calculated using the method of [12]. The comparison of the noted values was evaluated by the Chi-square analysis and by the Cramer test for the classification of the means.

3. Results

3.1. Dormancy Breaking Study

Thus, the transparency of the germinators made it possible to monitor the germination behavior of the seeds morning and evening (b). It happens that some seeds germinate (form the radicle) but do not grow above the substrate or take a long time to do so because the radicle did not follow the orientation of gravity very early (c). In some cases, the seedling cannot free itself from its seed coat attached to the substrate (d).

It very often happens that a germinated seed cannot grow above the substrate because the soil is very compact and retains the seed by its wings (**Figure 6(d)**).

The results obtained in the dry season (January, February and March) and rainy season (July and August) made it possible to establish the germination kinetics from the daily germination rates according to the scarification applied to the seeds as shown in **Figure 8** below:

3.2. Immersion for 10 min

The effect of soaking the seeds of *S. kunthianum* for 10 min in sulfuric acid on germination is presented by germination curves in **Figure 7**. It appears that the seasons do not significantly affect the germination parameters of this species. The latency time is 3 days on average after sowing. The germination time is 10.4 days on average. The average germination capacity is 72%.

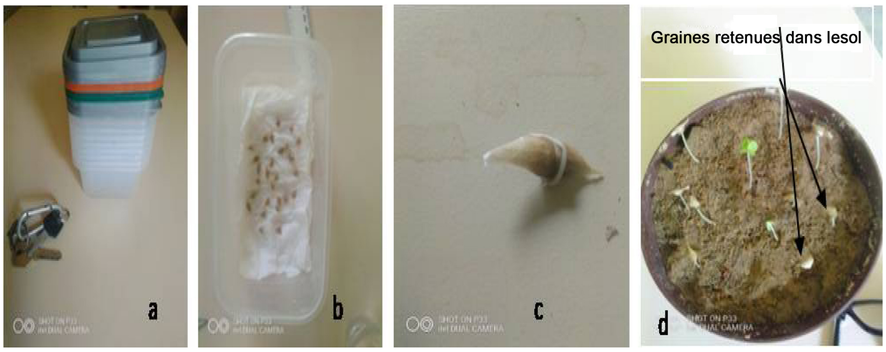

Figure 6. Well (a) and badly oriented (b) rootlets and seedlings retained by the nature of the substrate.

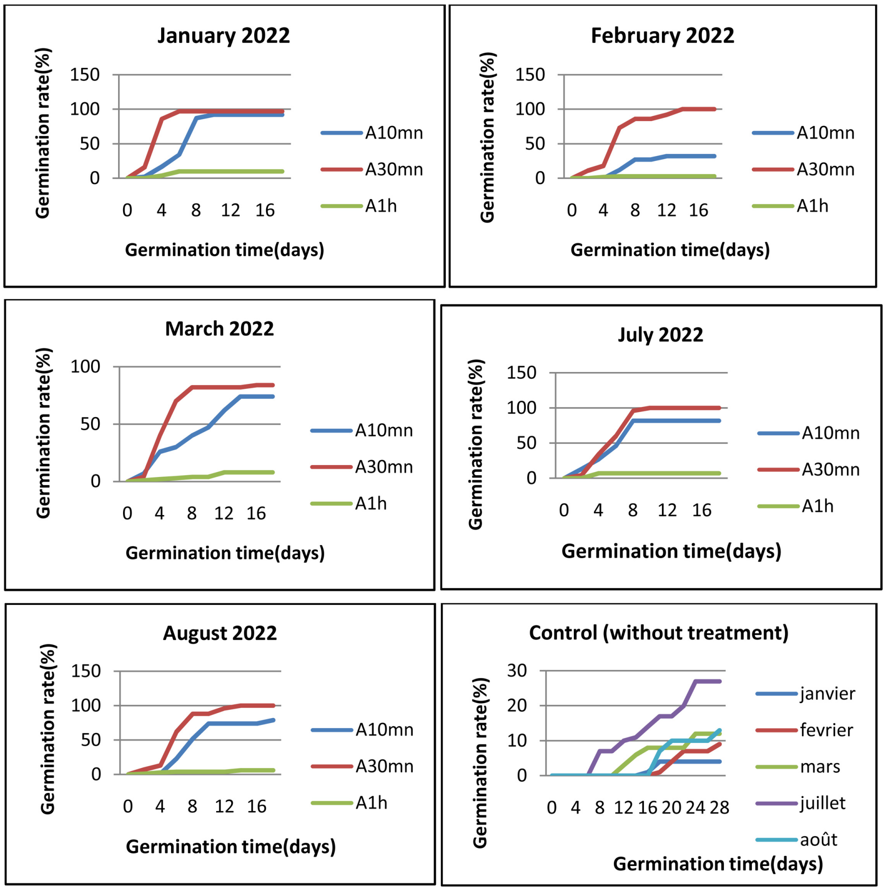

Figure 7. Cinétique de germination des graines selon les mois dans l'année.

3.3. 30 min Immersion

The effect of soaking *S. kunthianum* seeds for 30 min in sulfuric acid on germination is shown in the figure below. From the outset, it appears that the seasons do not significantly affect the germination parameters of this species. The average latency time is about 2 days after sowing. The average germination time is 11.2 days and the germination capacity is 96% and the germination rate is 9%/day.

3.4. 1 h Immersion

The effect of soaking *S. kunthianum* seeds for 10 minutes in sulfuric acid on germination is shown in the figure below. The average latency time is about 3 days after sowing. The average germination time is 8 days. The germination capacity is 7% and the germination rate is 0.9%/day.

3.5. Effects of Substrates on Transplanted Explants

Type of germination: The seedlings according to the scarification of the original seed, were transplanted on the 5 types of substrates. The exit of the seedlings from the support showed that germination is epigeal (**Figure 6(d)** since the cotyledonary leaves are raised above the substrate, this is explained by the faster growth of the hypo-cotyle axis of these seedlings. It also presents the image of a seedling whose cotyledonary leaves are lifted above the ground by the rapid growth of the stem characterizing so-called epigeal germination (a—the cotyledons still enclosed in the integumentary envelope and b—pre-leaves released of its envelope).

3.6. Explant Transplantation Survival Rate

Figure 9 below shows that on all the substrates, for the "Soaking in sulfuric acid" treatment, the plants from a soaking time of 30 minutes are those that best support the transplant mechanism (81.33%) followed by those with a soaking time of 10 minutes (63.11%) regardless of the substrate. At the 5% threshold, the P-value of the chi-square test between germination status and the sub-treatment of soaking in sulfuric acid is equal to 0 (Cramer's V equal to 0.38), *i.e.* that the germination of seeds of *S. kunthianum* depends moderately on the duration of the soaking of the seeds in sulfuric acid.

The germination of seeds soaked in sulfuric acid seems to be improved when the explants are transplanted on the substrate "Sawdust—black earth" (75.19%), "Black earth" (73.33%) or "Sawdust of wood-fine sand" (66.30%). At the 95% confidence level, the P-value of the chi-square test between the germination status of the seeds and the substrate is equal to 0 (Cramer's V equal to 0.30), *i.e.* the germination of seeds soaked in acid sulfuric depends moderately on the substrate.

It can be said from Table 1 that:

At the 5% threshold and independently of the substrate, seeds soaked in sulfuric acid for 30 minutes were 45.78% more likely to germinate than those soaked in sulfuric acid for one hour. Similarly, seeds soaked for 10 minutes are 27.56% more likely to germinate than those soaked in sulfuric acid for one hour;

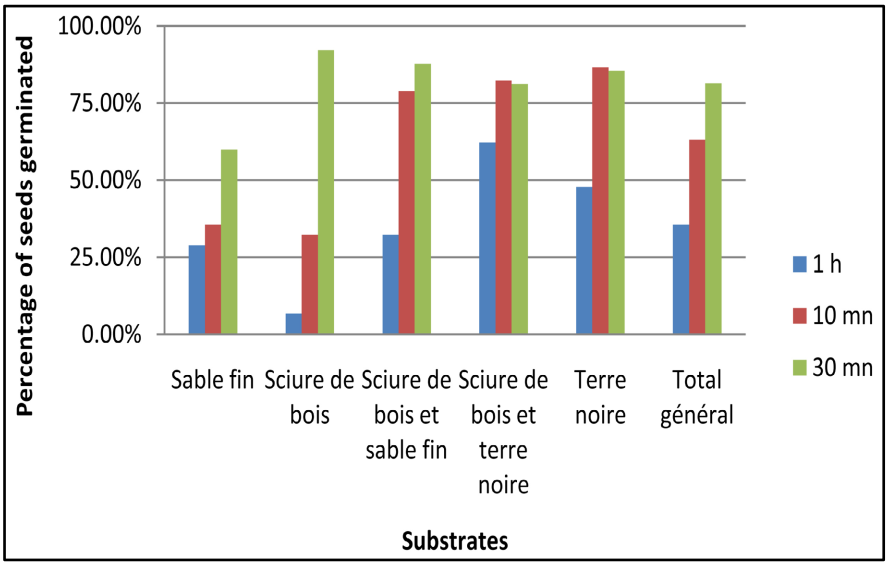

Figure 8. The percentage of germination following soaking in sulfuric acid.

Table 1. Analysis of average marginal effects.

Factor	Terms	Average marginal effect	P-value
Pre-treatment (reference = 1 h)	10 mn	0.2756	0.0000
	30 mn	0.4578	0.0000
Substrate (reference = fine sand)	Sawdust	0.0222	0.5326
	Sawdust and fine sand	0.2481	0.0000
	Sawdust and black earth	0.3370	0.0000
	Black earth	0.3185	0.0000

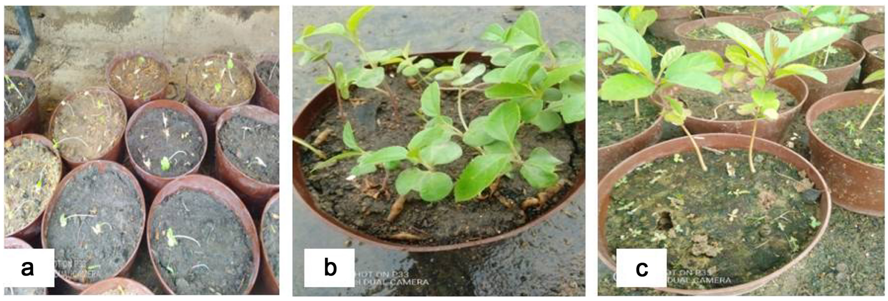

Figure 9. Ages of seedlings after transplantation (a) 7 days, (b) 1 month and (c) 3 months.

At the threshold of 5% and independently of the duration of soaking in sulfuric acid, the "Sawdust and black earth" substrate improves seed germination by 33.70% followed by the "Black earth" substrates (31.85%) and "Sawdust and fine sand" (24.81%) compared to the "Fine sand" substrate.

4. Discussion

The germination of *S. kunthianum* seeds studied highlighted the effectiveness of edaphic factors and pre-germination treatments. Immersion of seeds in concentrated sulfuric acid and substrate mixtures improve the germination qualities of seeds of this species. In fact, soaking the seeds of *S. kunthianum* for 10 and 30 minutes in sulfuric acid considerably reduces the lag time, the germination time and clearly improves the germination rate of these. **Figure 9** shows seedlings of different ages growing on black soil.

This germination is homogeneous, epigeal and rapid. These results corroborate those obtained by [13] during the germination of Acacia tortilis, those of [14] obtained during the germination of seeds of perennial Saharan spontaneous species and those [6] [15] in the germination of Antillis cystisoids. But immersion for more than 30 minutes in acid is not indicated for seeds because of the light seed coat like those of *S. kunthianum* seeds. Because it could cause damage to the embryo and reduce their germination rate [16]. In terms of the substrates, the black earth/sawdust mixture, the black earth and the fine sand/sawdust mixture showed better performance than the others.

5. Conclusion

According to this study, it appears that the germinative ability of *S. kunthianum* seeds can be improved by soaking its seeds in sulfuric acid for 10 to 30 minutes in any season. The use of a 1:1 substrate mixture of black earth/sawdust, black earth or fine sand/sawdust significantly increases this result. The method used for this germination is reproducible and less expensive. It can be reproduced easily by farmers and practitioners of local traditional medicine. But also, in the context of large-scale cultivation and regeneration for the fight against the desert (such as the Great Green Wall) this method can be used to obtain a homogeneous nursery in a short time. These results also make it clear that the domestication and sustainable conservation of the genetic specimen of *S. kunthianum* are therefore possible in botanical gardens and in home gardens by applying the methods used during this experiment.

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Authors' Contributions

The authors BT, AG, HA each have provided assistance in the preparation and reading of this article. BT and SIA participated in the initiation, collection, analysis and processing of field data. SIA contributed effectively to the scientific orientation of the manuscript.

Conflict Interests

The authors declare that they have no competing interests.

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