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Effects of Scarification and Substrates on Seed Germination Quality for Domestication of *Stereospermum kunthianum* (Bignoniaceae)

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Abstract: The demand for medicinal plants at the origin of phyto-drugs, like all ethnobotanical plants, threatens their existence today. The aim of this work was to determine suitable methods for optimizing the possibilities of regenerating *Stereospermum kunthianum*. To achieve this objective, 3 levels of mechanical scarification (light scarification, medium scarification and deep scarification) were applied to the seeds of this species using a rough instrument, and 5 days after germination, the seedlings were transplanted into 5 types of substrate to study their effects on seedling growth. The length of the seedlings was measured using a graduated ruler. The results obtained showed that the best latency time was 2 days for average seed scarifications. The highest germination half-time was 4.00 ± 0.06 days observed for average seed scarification, the highest germination speed was 22.50 ± 1.00 and the highest germination rate was 100.00% ± 0.00% for average scarification. The homogeneous substrate mixes black earth/woodsilk and Fine sand/woodsilk were more effective for seedling growth (1.9 cm/day in the juvenile state) and development. Medium seed scarification is the most effective method for regenerating *Stereospermum kunthianum*, and the black earth/wood shavings substrate mix is the best for its cultivation.

Key words: Scarification, substrate, germination, domestication, *Stereospermum kunthianum.*

1. Introduction

Among NTFPs (Non-Timber Forest Products), phyto-medicines are a valuable resource for the majority of people in Africa, where over 80% use them for health care [1]. Moreover, these medicinal plants are invaluable resources for the pharmaceutical industry [2]. Considerable economic benefits have been noted in the development of traditional medicine and the use of medicinal plants for the treatment of various diseases [3]. In 2003, the WHO (World Health Organization) estimated that 70% of the Chadian population relied on traditional plant-based medicine for primary care. This puts enormous pressure on medicinal plants [4]. These ever-increasing needs are currently a clear threat to the future existence of certain species, even if they can be seen when they are needed [5, 6]. In Chad, plant species traditionally exploited by local populations for food, health care and endangered species require special attention [7]. These include *Stereospermum kunthianum*.

Steresopermum kunthiana is a large tree in the Bignonaceae family found in the wooded savannahs and dry leguminous forests of the Sudano-Guinean zone. According to Arbonnier [8], all parts of this plant are rich in therapeutic virtues. This makes *Stereospermum kunthianum* a vulnerable species. The root is a powerful diuretic. It can also be used to treat headaches, gastritis, gonorrhea, dysentery, bilharzia, hematuria and jaundice. Root bark treats snakebite. Root and leaves combat syphilis, gastritis, asthma and headaches. Tree bark is hemostatic, healing, vermifuge and treats wounds, burns, leprosy, gonorrhea, bronchitis, pneumonia, coughs,

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dysentery, gastritis, phagedenic ulcer and hypertension. Leaves combat asthenia, sores and gonorrhea. The fruits are edible and cure coughs. This plant also has virtues that combat diarrhea, dysentery and cattle flatulence. The wood is used in construction, mortar making and toothpicks. In West Africa, the bark is used as a lip stain (lipstick). In the Chadian pharmacopoeia, this essence is sought after above all for its therapeutic virtues in the treatment of hypertension, wounds, leprosy, general asthenia and poisoning [9].

Thus, given the immense demand for *Stereospermum kunthianum* and the repeated devastation of the area by desert fire and the unlikely regeneration of this species, the risks of its disappearance increases. The survival of this species will now depend on the establishment of appropriate management approaches combined with assisted regeneration techniques. The aim of this work is to find ways of optimizing germination potential by scarifying *Stereospermum kunthianum* seeds.

2. Materials and Methods

2.1 Localization of Seeds Collection

The seeds used in this experiment were collected in the Mayo Kebbi-West region (Fig. 1), precisely in the villages of Berdé (09°3491138 N and 15°57882 E), Belé (09°385621 N and 15°3925785 E) and Belé Vansa (09°3757808 N and 15°3123617 E), all located on the Kelo-Pala stretch. It corresponds to the southern fringe of the country and lies between isohyets 800 mm and 1,200 mm [10]. The rainy season generally lasts from May to November.

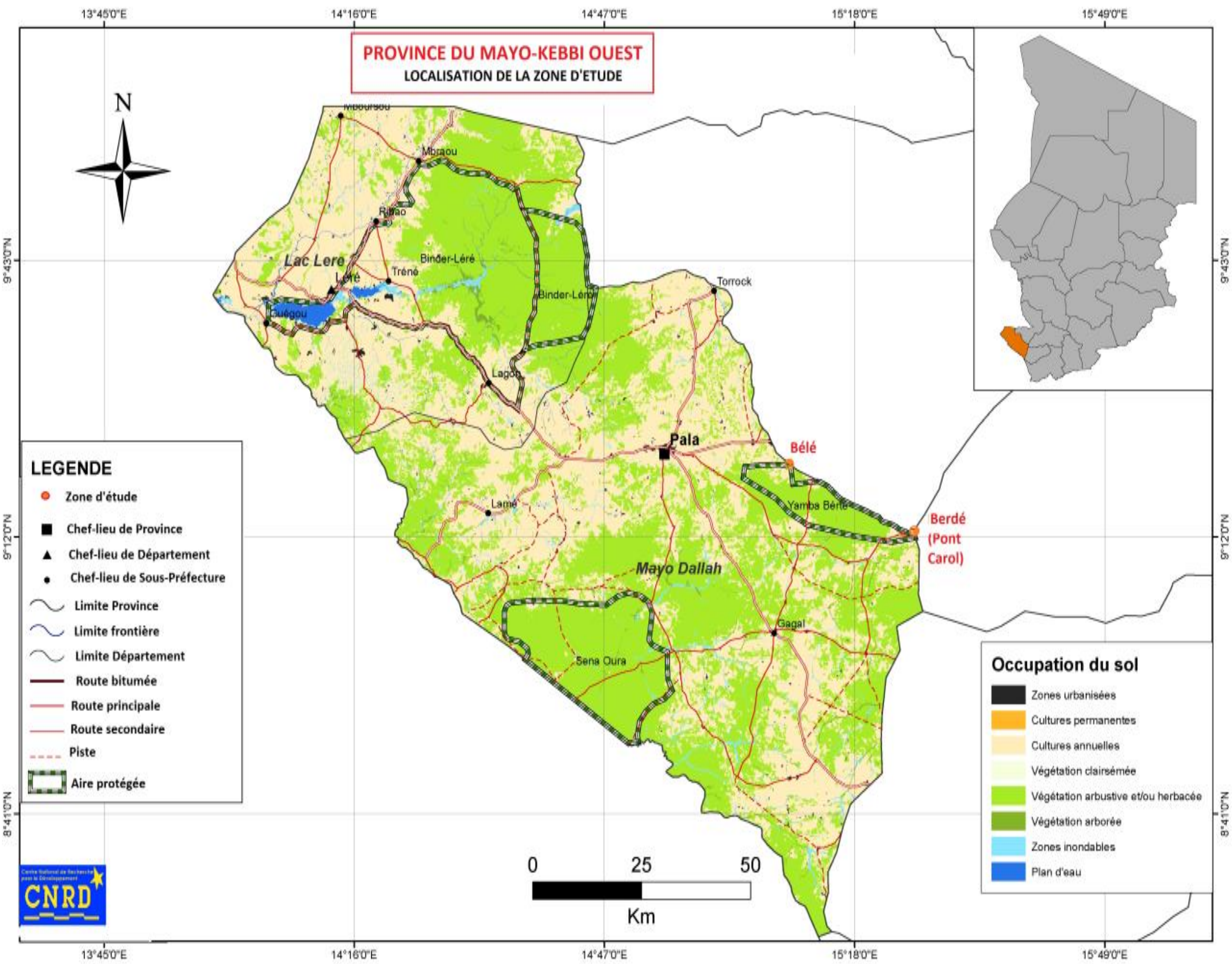

Fig. 1 Location of *Stereospermum kunthianum* seed collection site.

(a) normal (b) scarified

Fig. 2 Different states of *Stereospermum kunthianum* seeds.

2.2 Experimental Methods

2.2.1 Pretreatments

After collecting the seeds of this species (Fig. 2), three mechanical pre-treatments were applied to 270 seeds using a piece of abrasive concrete [9-11]. These were:

Light scarification, which consists in lightly wounding the first layer of the seed coat. This was carried out on 90 seeds;

Medium scarification, which consists in moderately wounding the seed coat, revealing an overlying black layer. This was carried out on 90 seeds;

Deep scarification, which involves cutting deep into the seed coat until the cotyledon appears. This was applied to 90 seeds.

2.2.2 Transplanting Methods for Seedlings

Tn (Black Earth), Sf (Fine Sand), Scb (Sawdust), Tn/scb (Black Earth/Wood Sawdust) and Scb (Fine Sand/Wood sawdust) were the five substrates used for this seedling growth test. To eliminate parasites from these substrates, they were sterilized in water boiled at 100 ℃ for 1h [12]. Seedlings were sown in 100 cm^3 polyethylene pots filled with the substrates. Each experimental unit comprised 16 pots, each containing 5 seeds and the seedling. Once the pots had been filled, they were placed under a shed to provide shade [13]. The set-up used to conduct this experiment was a randomized complete block design, with three replicates per secondary treatment.

2.2.3 Monitoring and Data Collection

The monitoring process consisted of a systematic daily count of seeds that had germinated. The data were used to calculate the following parameters.

(a) Latency time: Tl= jf-ji

with df: day of first observed germination; ji: day of sowing [14];

(b) Median germination time or half germination time: TMG = Σ ($n_i \times t_i$)/N

with n_i, the number of newly germinated seeds at time t_i,t_i, the number of days since the sowing date and N, the total number of germinated seeds[15];

(c) Germination speed

$$VG=(100(n_1+n_2+n_3+n_4))/(n_1t_1+n_2t_2+n_3t_3+n_4t_4)$$

n_x: being the number of germinated seeds for an observation x and t_x, the day corresponding to seed germination [15];

(d) Germination rate: $T= G/N$

where G= number of seeds germinated and N= total number of seeds germinated according to treatment.

(e) Growth rate: $V_c=L/d$

where L=seedling length and d=growth duration. This is the ratio of seedling length to seedling growth duration.

The percentages, mean values and standard errors of the parameters studied were calculated. The comparison of values was evaluated by ANOVA (Analysis of Variance) and by Duncan Ranks test for the classification of means.

3. Results

3.1 Sprouting Parameters

Table 1 shows the variation in seed germination parameters as a function of the different types of wounding applied to the seeds. It shows that:

(1) the lag time fluctuated from 2 days for medium scarified seeds to 3 days for light scarified seeds;

(2) germination half-time ranged from 4.00 ±0.06 days for medium-scarified seeds to 5.00 ±0.10 days for light-scarified seeds. Statistical analyses of variance showed no significant difference between seed treatment modalities at the 5% probability threshold. Mechanical scarification slightly improved the germination half-time of *Stereospermum kunthianum* seeds;

(3) the lowest germination speed was observed in lightly scarified seeds (16.00 ± 3.33) and the highest in medium scarified seeds (22.50 ± 1.00). Analysis of variance at the 5% level showed that there was a significant difference (p= 0.010) between seed treatment modalities for germination half-time. Scarification affected the germination rate of *Stereospermum kunthianum* seeds;

(4) germination rates ranged from 86.00% ± 0.10% for lightly scarified seeds to 100.00% ± 0.00% for moderately scarified seeds. At the 5% probability level, the difference between light and medium scarification was significant (p=0.001). Mechanical scarification was able to significantly improve the seed rate in *Stereospermum kunthianum*.

As *Stereospermum kunthianum*’s integument is thinner and less rigid, deep wounding was not possible. This method was therefore not used.

3.2 Evaluation of the Percentage of Seeds That Have Not Germinated and Rotten Seeds according to the Scarification of Stereospermum kunthianum Seeds

Fig. 3 shows the proportions of seeds that failed to germinate during the experiment. The percentages are significant and vary according to the sub-treatments applied to the seeds. The highest proportions of non-germinated seeds were recorded by the deeply scarified seeds (100%), as all the seeds rotted, and the control seeds (92.66%). The lowest proportion was observed in medium scarified seeds (0.00%). Statistical analysis of variance showed that there was a significant difference (p=0.002) between scarification methods at the 5% significance level for the proportion of ungerminated seeds in *S. kunthianum*.

3.3 Effects of Substrates on the Transplanting of Stereospermum kunthianum Seedlings

3.3.1 Germination Type

Removal of the seedlings from the substrate showed that seed germination in this species is epigeic (Fig. 4), since the cotyledonary leaves are raised above the substrate.

Table 1 Variation in seed germination parameters as a function of mechanical seed scarification methods.

Scarifications	Scarification seed germination parameters			
	Tl (day)	Tg (day)	Vg (day)	Taux(%)
Sc. light	3	5.00 ±0.10	16.00 ±3.33[b]	86.00 ±0.10[b]
Sc. medium	2	4.00 ±0.06	22.50 ±1.00[a]	100.00 ±0.00[a]
Sc. deep	0	0	0	0
Mean	2.5	4.50 ±0.08	19.25 ±2.16	93.00 ±2.21
P /F		0.420	0.010	0.001

[a,b,c] different numbers; Pr=p-value; F= Fischer; Tl=Latency time; Tg=Germination time and Vg=Germination speed.

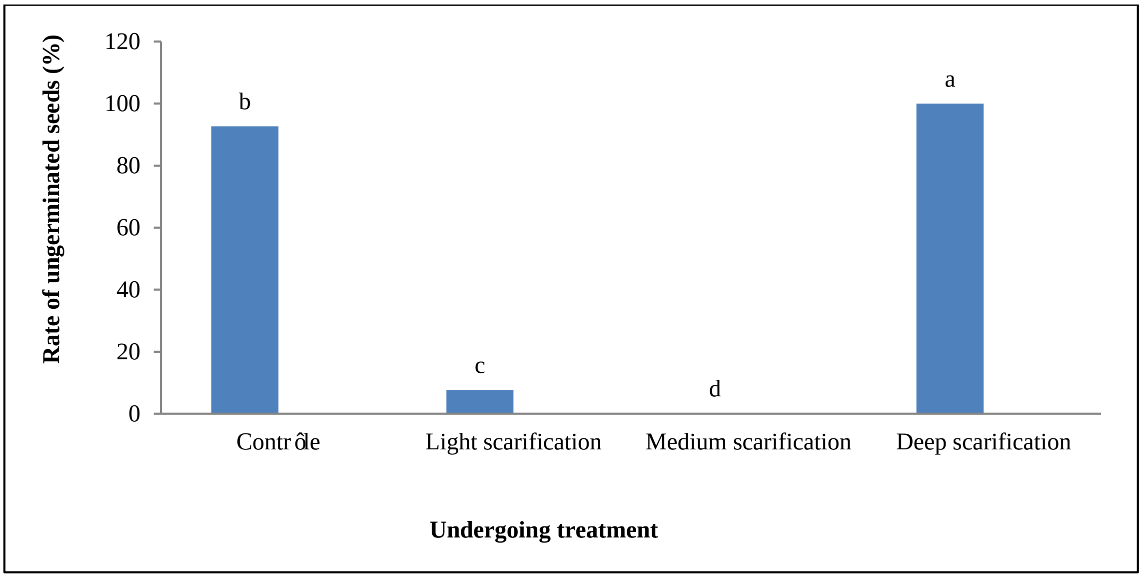

Fig. 3 Variation in non-germinated seed rates between treatments rate of ungerminated seeds.

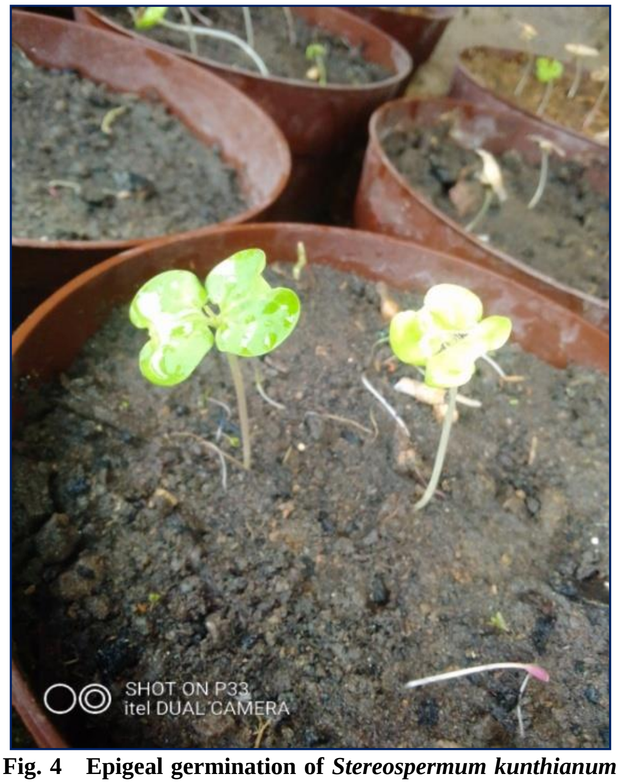

Fig. 4 Epigeal germination of *Stereospermum kunthianum* seeds.

3.3.2 Effects of Substrates on Development of Transplanted Seedlings

Seedlings were monitored during their development from the appearance of cotyledonary leaves above the substrate until the fourth month. Fig. 5 shows three stages in the growth of *Stereospermum kunthianum* seedlings.

3.3.3 Effect of Substrates on Seedling Growth

Fig. 6 shows that growth was weakest in sand (0.7 cm/day) and in the homogeneous (1/1) mixture of fine sand/wood shavings substrates (0.9 cm/day). Maximum growth was achieved in the equi-volumic (*v*/*v*) mixture of black earth/wood sawdust substrates (1.9 cm/day) and in the black earth substrate (1.4 cm/day). As a result, *Stereospermum kunthianum* grows faster on the homogeneous mix of black earth/wood shavings substrates. Statistical analysis of variance showed a significant difference between substrate effects (p=0.003) at the 5% threshold.

3.3.4 Substrate Effects on the Germination Rate of *Stereospermum kunthianum* Seeds

The variation in seed germination rates according to substrate is presented in Fig. 7, showing that germination rates fluctuated from 75.56% in sawdust to 97.78% in the homogeneous (1/1) black earth/sawdust mixture. Statistical analysis of variance at the 5% level shows a significant difference between substrate effects (p=0.000) for seed germination rates. Cramer's V value is equal to 0.24, so the substrates had an average intensity on seed germination. Homogeneous mixtures of black earth/wood sawdust and fine sand/wood sawdust remarkably improved germination rates.

(a) (b) (c) (d)

Fig. 5 Development of seedlings after transplantation. (a) 5-day-old seedlings, (b) 20-day-old seedlings, (c) 3-month-old seedlings, (d) 4-month-old seedlings.

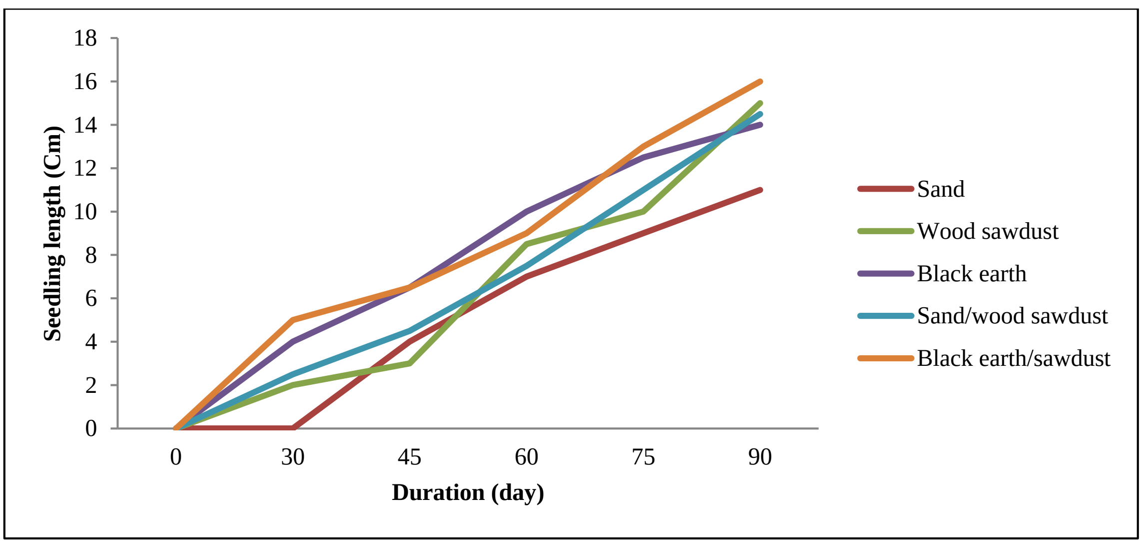

Fig. 6 Seedling growth curves for different substrates.

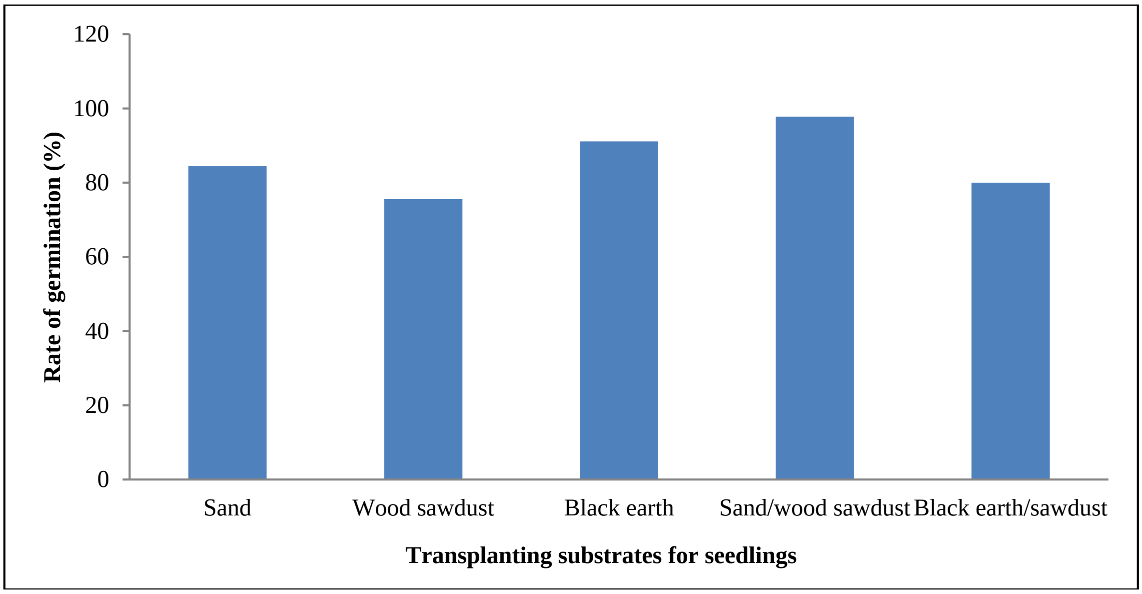

Fig. 7 Variation in seed germination rates with different substrates.

4. Discussion

The germination of the *Stereospermum kunthianum* seeds studied varied according to edaphic factors and the pre-germination treatments applied. The results obtained in the course of this work highlight the effectiveness of manual scarification and substrate mixtures in enhancing the germinative qualities of *Stereospermum kunthianum* seeds. In fact, light and medium scarification of *S. kunthianum* seeds considerably reduced latency and germination time, and significantly improved germination rates. Germination was homogeneous, epigeous and rapid. These results show that medium scarification is the best pre-treatment for improving germination in *S. kunthianum*. The performance of manual scarification has already been obtained for the germination of seeds of *Leuceana leucocephala* [16], *Alstonia boonei* [17], *Prosopis Africana* [12], *Ximenia Americanum* [18]. However, deep scarification is not indicated for seeds with light seed coats such as those of *S. kunthianum*. This could damage the embryo and reduce the germinative capacity of the seeds [19]. On the other hand, these results differ from those obtained by Sabre [20] when germinating *Antyllis cysthysoides*, which showed that deep scarification performed better than other methods. In terms of substrates, the black earth/sawdust mix and the fine sand/wooddust mix like black earth, showed better performance than the others on seedling growth and development. Sawdust and fine sand are not suitable for the development of *Stereospermum kunthianum* seedlings.

5. Conclusion

This work shows that the germination quality of *Stereospermum kunthianum* seeds can be improved by pre-treatment. Seed scarification considerably improves essential germination parameters such as latency, half-time, speed and germination rate. *Stereospermum kunthianum* germination is epigeic. Homogeneous mixtures (1/1) of black earth/wood shavings and fine sand/wood shavings result in better seedling development. For large-scale cultivation and regeneration, these methods can be used to obtain a homogeneous nursery in a short space of time. Scarification can easily be reproduced by farmers and practitioners of traditional local medicine, as this method is less costly. These results also suggest that sustainable conservation of the genetic specimen of *Stereospermum kunthianum* is therefore possible through domestication in botanical gardens and home gardens.

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