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Study of the Germination of Scarified Seeds of *Burkea africana* Hook. For Its Domestication in Chad

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Abstract

The objective of this work was to determine new approaches that could improve the germination quality of *Burkea africana* seeds under reproducible conditions and by means available to farmers with a view to its propagation. The tests concerned the influence of three pre-germinative treatments. It is about the light scarification (Scl), medium scarification (Scm) and deep scarification (Scd) of seeds in order to determine some parameters of germination that are the latency time, the time of germination, the rate of germination and the speed of germination. The treated seeds were sown in transparent germinators lined with hydrophilic paper and then after the appearance of radicles, the plants were transplanted on five types of substrates (black soil, fine sand, sawdust, mixtures 1/1 of sawdust-black soil and 1/1 of sawdust-fine sand). The results obtained by the deep (Scp) and medium (Scm) scarification compared to those of the light scarification and the control, showed a clear reduction of the lag time (3 days/15 days (control). At the 5% threshold, germination times 6.27×10^{-1} (Scd) and 6.01×10^{-1} (Scm) were significantly influenced. Germination rates 4.18×10^{-1} (Scd) and 3.92×10^{-1} (Scm) are also influenced by the pretreatment. Germination rates are significantly improved (80%). Regardless of the under treatment, the substrate "sawdust and fine sand" improves the germination of scarified seeds by 14.07% more compared to the substrate "fine sand" at the 5% threshold. The mechanical scarification and the type of substrates thus influence positively and homogeneously the germination of seeds of *Burkea africana*. The domestication of this species is thus possible and can be considered for useful purposes.

Keywords

Burkea africana, Scarification, Substrate, Germination and Chad

1. Introduction

In 2003, the average annual level of destruction of forest resources due to anthropogenic activities and climatic variations is 142,800 ha [1]. This phenomenon could be explained by an increasing demand for non-timber forest products in general and medicinal plants in particular [2]. 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 is insufficient or 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 the populations of all generations. For this reason, research should be carried out in the sense of preserving certain species that are most in demand for different practices in the Sahelo-Sudanese zones. Among these agroforestry plants, *Burkea africana* is one of the most widely used [4] and deserves special attention for its survival.

Burkea africana is a multi-purpose essence that is well known to traditional practitioners in the sub-Saharan region in general and in southern Chad in particular. In West Africa, according to [5], all the parts of this plant have therapeutic virtues capable of fighting several ailments. It is for example the case of the barks which treat effectively the rates, the migraine, the gonorrhea, the syphilis and they are also employed as antidote of the poisons of arrow. The roots treat abdominal pain. The leaves treat headache, epilepsy, edema and fever. The gum is sought after for its aphrodisiac properties. The flowers of this essence are also bitumized by bees.

Traditional Chadian medicine uses all organs of *Burkea africana* in the care of hypertension, wounds, leprosy, general asthenia and poisoning. The wood of this plant is much solicited for the construction of the frames of house, grain and mortars because of what it is very hard and resistant to the ravage of the termites. It is also valued for the quality of coal that it produces [4]. For [6], given the great demand for *Burkea africana*, the devastation of the area by bushfire, the advancement of the desert but also and the unlikely natural regeneration of this species that increase the regression of the population of this species, the risks of its disappearance become more and more evident. The survival of this species would be based on the implementation of appropriate management approaches associated with assisted regeneration techniques.

The results of this research allowed us to verify four hypotheses, one of which is "the pre-treatments by mechanical scarification of the seeds lifts the dormancy of this species", the second one "the seasons influence the germination of *Burkea*

africana" and the fourth one "the germination of *Burkea africana* is epigenetic" and the third one "some substrates favor the germination". To carry out this work, the following materials and methods were used.

2. Materials and Methods

2.1. Place of Study

This study was conducted in the biology laboratory of the Ecole Normale Supérieure of N'Djamena from January to July 2022. The coordinates of this school are: latitude 12˚1044882N and longitude 15˚0573853E (4325-R29, N'Djamena, Chad). The city of N'Djamena has a Sahelo-desert climate of the southern Guinean type, characterized by a rainy season (3 to 4 months) and a dry season (7 to 8 months) with an average annual rainfall that rarely exceeds 800 mm.

2.2. Seed Collection Location

The seeds used in this experiment were collected in Mayo Kebbi-West precisely in the villages Berdé (09˚3491138N and 15˚57882E), Belé (09˚385621N and 15˚3925785E) and Belé Vansa (09˚3757808N and 15˚3123617E), all of which are located on the Kelo-Pala section (**Figure 1**). It corresponds to the southern fringe of the country and is located between the 800 mm and 1200 mm isohyets [7]. The rainy season generally lasts from May to November. It covers the regions of Mayo Kebbi, Tandjilé, the two Logones and the Moyen Chari in southern Chad. In this zone, the natural vegetation is of the tree savanna type and the woody plants are tall and dense but composed of perennial and annual grasses that form a herbaceous carpet. The conventional basin of Lake Chad (1989) estimated that there are between 1000 and 2000 plant species per 10,000 km^2 and that there are probably 2750 species in this zone [8].

2.3. Technical Equipment

The technical equipment is presented in **Figure 2** and consists essentially of pots (a), a sprayer (b), germinators (c) and germination substrates (d).

2.4. Plant Material

Burkea africana is a tree of 10 to 12 m high, very branchy with a spreading top, with leaves arranged in a tuft at the end of the branches. The bark is very rough, cracked and scaly, grey or blackish, with a purplish-brown and fibrous edge having a brown rhytidome. Sticky sap. The branches are pubescent, reddish, gray or brown. Leaves alternate, bipinnate, 10 to 35 cm long, with 2 to 5 pairs of opposite or subopposite pinnules and 6 to 18 alternate leaflets per pinnule. Blade oblong, elliptic with obtuse or indented apices and rounded, asymmetrical bases. Leaves young pubescent, silvery on both sides becoming glabrous, dull green above and grey-green to yellowish below. Curved petiolules pubescent to about 2 mm long. Pinnate venation, not very prominent, with 6 - 12 pairs of secondary

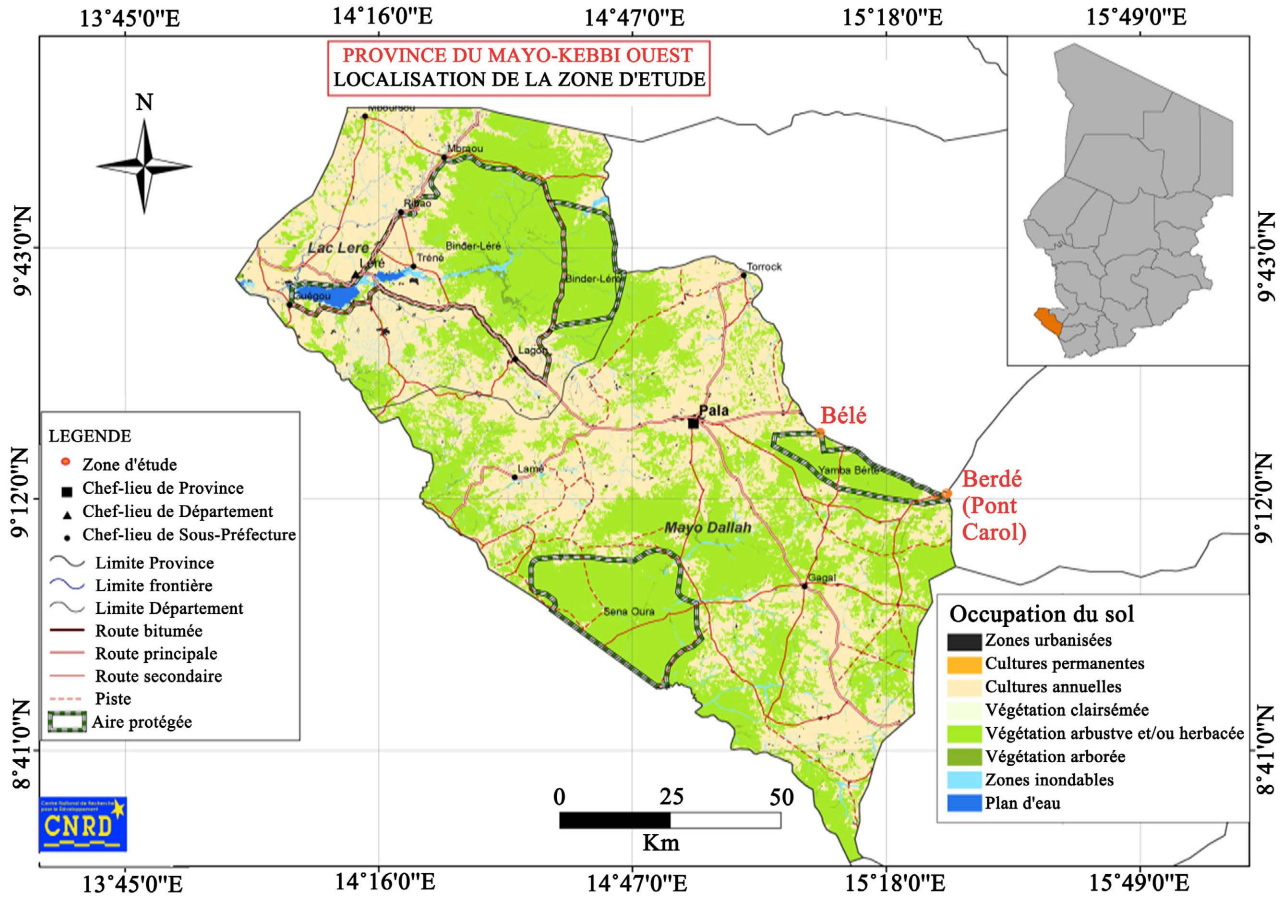


Figure 1. Geographical position of Berdé and Bélé Vansa, seed collection sites.

(a) (b)

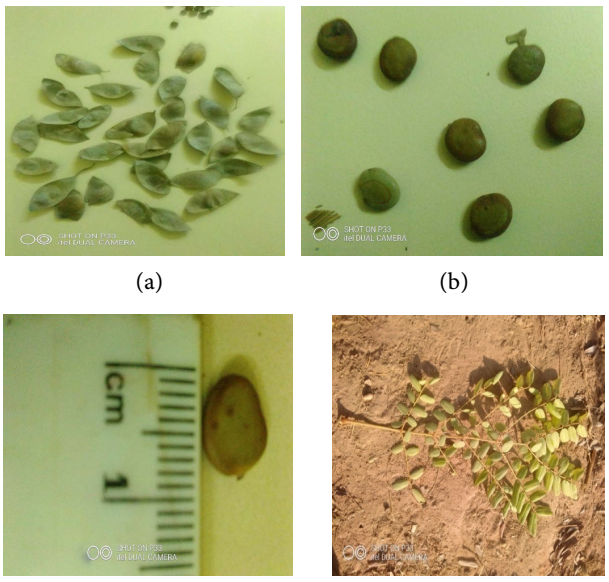
(c) (d)

Figure 2. Materials used to conduct the germination experiment.

veins (**Figure 3**). Inflorescence in terminal fascicles, simple or branched spikes 7 - 30 cm long. Flowers whitish sessile, 6 mm in diameter, with corollas of 5 oval, short, ciliated petals with 10 stamens. Fruit is an indehiscent, flat, elliptical, stipulate, more or less pubescent pod containing only one seed [5] [9]. This species generally flowers at the end of the dry season before leafing out. In Chad, this species is widespread in the savannahs of the Sudanian and Sudano-Guinean zones in the south. They are naturally spread by the wind, and sometimes by animals that readily consume the fruits (**Figure 3**).

2.5. Experimental Methods

In tropical regions, many species have seeds with a tough, hard, water-impermeable integument, thus inducing the phenomenon of dormancy called integumentary dormancy [2]. These seeds take a very long time to germinate [10] because they present a tegumentary dormancy and are subject to a real obstacle during germination in nature [11]. Indeed, this type of dormancy leads to a germination that lasts in time and this is what is incompatible with the production of seedlings on a medium or large scale, since for a reforester it is necessary to have a homogeneous and synchronous germination of seeds [2]. It is therefore essential to apply a treatment before sowing, to ensure a high final germination percentage but also a rapid and uniform germination after sowing [2] [11] [12]. However, the low resistance against anthropic factors, climatic effects and also the low regeneration rate of savannah species justifies and requires in-depth experiments on the conditions for successful germination of their seeds. Some plants are used for socio-economic interests that develop naturally, yet they are increasingly exploited by local populations. Most orthodox seeds are dormant until they are heavily soaked for germination to begin [13].



(a) (b)

(c) (d)

Figure 3. fruits (a), seeds ((b) and (c)) and leaf (d) of *B. africana*.

Physical pretreatment methods that facilitate the transition from dormancy include piercing or splitting the seed coat to make it more permeable, without damaging the embryo or endosperm of the grain [14].

2.5.1. Seed Pre-Treatment

Mechanical or manual scarification is a process by which, seeds are rubbed with an abrasive material to reduce the thickness of a seed's tough seed coat [15] and increase its level of permeability.

Using a piece of abrasive concrete, 270 seeds were scarified. Three kinds of sub-treatments (**Figure 4**) of mechanical scarification were applied to the seeds before sowing [11] [16]. Light scarification consists of lightly wounding the first layer of the seed coat; medium scarification consists of medium wounding of the seed coat revealing an overlying black layer. Deep scarification consists of completely removing the seed coat from one area of the seed (**Figure 4**). Each sub-treatment concerned 30 × 3 = 90 seeds, *i.e.* 270 seeds in total.

2.5.2. Modality of Sowing in the Transparent Germinators

The seeds (90) underwent a scarification before being distributed in three groups of 30 each in three different transparent germoirs. The hydrophilic papers moistened with tap water were placed beforehand at the bottom of each germoir and the seeds were put to germinate. The experimental unit is 30 seeds and repeated three times *i.e.*: 30 × 3 = 90 seeds. For all the experiment 90 seeds × 3 = 270 seeds were concerned. The set-up is randomized complete block (**Figure 5**). According to [17] [18], this method can accurately determine the day of radicle appearance of a seed that is not observable when the seed is directly inserted into the substrates. This experiment determined the latency time, germination time and germination rate of the seeds.

Seeds with no treatment or control were also sown to allow the effect of the treatments to be evaluated by comparison with the results (**Figure 5(c)**).

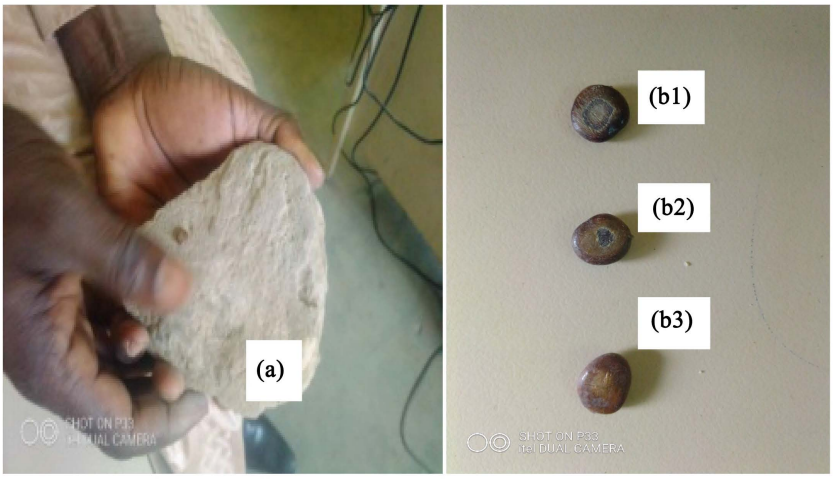


Figure 4. Scarification process (a), light scarification (b1), medium scarification (b2) and deep scarification (b3).

Figure 5. Sowing arrangement of scarified seeds: light (b1), medium (b2), deep (b3) in transparent pots (a) and the control (c).

2.5.3. Plants Transplantation Procedures

The transplantation of the plants was carried out in polyethylene pots of 100 cm^3 containing five types of substrates. These were fine sand, black soil, sawdust, mixtures (1/1) sawdust/black soil and sawdust/fine sand. After filling the pots and transplanting, they were placed under a shed (the shade house). Watering was done every day for one month and two days for the rest of the time using a 16 liter Sprayer according to [19].

2.5.4. Experimental Set-Up for Transplanting Sprouted Seeds

The setup used to conduct this experiment is randomized complete block design with three pretreatments (**Figure 6**). Three replicates per treatment were conducted. Each experimental unit had 16 pots with 5 seeds each [19].

2.5.5. Monitoring and Data Analysis

The monitoring process consisted of a systematic counting of the seeds whose dormancy was lifted every two days. The lifting of dormancy corresponds to the appearance of a radicle. This stage marks the end of the latency period (waiting time) but at the same time the beginning of the germination period. The following parameters were determined: type of germination, waiting time (germination time), time elapsed between sowing and the first germination; germination duration (staggering), which is the time between the first and the last germination; germination rate: T = G/N with G = number of germinated seeds and N = total number of germinated seeds according to the treatment. The collected data were subjected to analysis of variance (ANOVA) using XLSTAT 2017 software. When differences existed between the different treatments, the means were separated by the Waller Duncan test at the 5% significance level. The percentages, mean values and standard errors of the studied parameters were calculated following the method of [20]. The Chi-square analysis and the Cramer test were also used for the classification of the means.

3. Results

3.1. Dormancy Breaking Study

3.1.1. Influence of Pretreatments on Germination Parameters

From **Table 1**, it can be seen that the type of scarification significantly influenced

(a) (b)

Figure 6. Arrangement of transplant pots (a) and an experimental unit (b).

Table 1. Type effects of scarification on germination parameters.

	Tl	Tg	Vg
Scd	3	$6.27 \times 10^{-1} \pm 9.48 \times 10^{-2}$a	$4.18 \times 10^{-1} \pm 6.16 \times 10^{-3}$a
Scm	3	$6.01 \times 10^{-1} \pm 1.32 \times 10^{-1}$a	$3.92 \times 10^{-1} \pm 8.56 \times 10^{-3}$a
Scl	3	$2.27 \times 10^{-1} \pm 1.09 \times 10^{-1}$b	$1.29 \times 10^{-1} \pm 2.91 \times 10^{-3}$b
Pr > F		1.95×10^{-3}	2×10^{-4}
Significatif		Yes	Yes

Tl = Latency time; Tg = Germination time and Vg = Germination speed.

the latency time (Tl = 3 days), germination time (Tg = 2.27×10^{-1}) and germination velocity (Vg = 1.29×10^{-1}). The values of Tl, Tg and Vg obtained by deep scarification (Scd) and medium scarification (Scm) are significantly comparable. Light scarification (Scl) significantly influenced the same germination parameters but with values not comparable to those of Scp and Scm.

3.1.2. Influence of Seasons on Germination Rate

The germination rates obtained according to light scarification (Scl), medium scarification (Scm) and deep scarification (Scp) were used to establish the monthly germination curves presented in Figure 7. This germination was done in transparent trays and repeated during the months of January, February, March and then in July and August 2022.

Figure 7 shows that *Burkea africana* seeds can be artificially germinated in any season. Deep scarification is the most suitable for the treatment of seeds of this species (an average rate of 80% germination in any season) followed by medium scarification with a germination rate that varies between 60% and 75%. The light scarification has a low germination rate than the others. All this is compared to the results obtained by the controls.

3.2. Effect of the Transplantation of the Plants on the Substrates

The transplantation of the seedlings made it possible to determine the type of germination of the seeds and to study the correlative influence of pretreatment-substrates on the parameters of germination.

3.2.1. Seed Germination Type

After transplanting, the seedlings lifted the cotyledonary leaves above the substrate. This phenomenon is explained by the faster growth of the hypo-cotyled axis of the seedling compared to the epi-cotyled axis located above the pre-leaves: it is an epigeous germination (Figure 8).

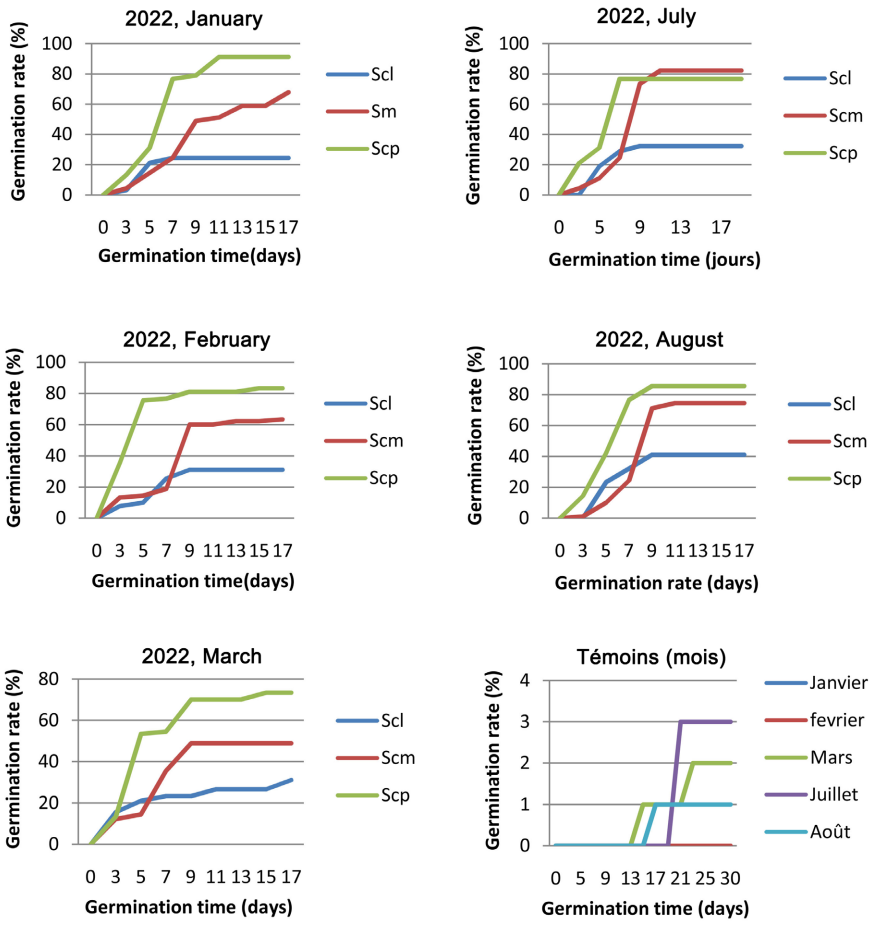


Figure 7. Germination kinetics of scarified seeds in transparent trays.

Figure 8. Epigeal germination of a *Burkea africana* seed.

3.2.2. Co Relational Influence of Pre-Treatment and Substrates

The seedlings, according to the pretreatment of origin, were transplanted and the recovery rates obtained on the types of substrates are presented in Figure 9.

According to the data, considering the treatment "mechanical scarification" and independently of the substrate, the sub-treatment "deep scarification" improves the most the percentage of germination of seeds (79.56%) followed by the sub-treatment "medium scarification" (60%) and "light scarification" (32.00%). The P-value of the chi-square test between germination status and mechanical scarification sub-treatment at the 5% threshold is equal to 0 (Cramer's V equal to 0.39), thus the mechanical scarification sub-treatment influences the germination of mechanically scarified seeds with a medium intensity.

According to the data, by applying the treatment "mechanical scarification", the percentage of seeds that germinated is 57.19%. Nevertheless, the substrate "sawdust and fine sand" improved the seed germination the most (65.19%) followed by the substrates "sawdust and black soil" (59.63%) and "sawdust" (59.26%). The P-value of the Chi-square test between germination status and substrate at the 5% threshold is equal to 0.002 (Cramer's V equal to 0.11), which means that germination of mechanically scarified seeds is weakly dependent on substrate.

3.2.3. Analysis of Marginal Effects at the Mean Point

The marginal effects at the mean point of the modalities of the variables under treatment and substrate of the sample of seeds treated by mechanical scarification were made from Table 2.

From the data in Table 2, it is evident that:

Deep and medium scarification improve the germination of seeds of this species respectively by 47.56% and 28% more compared to light scarification at the threshold of 5%; Regardless of the under treatment, the substrate "Sawdust and fine sand" improves the germination of scarified seeds by 14.07% more compared to the substrate "Fine sand" at the threshold of 5%. Considering this same threshold (5%), the substrates "Sawdust and black soil" and "Sawdust" increase

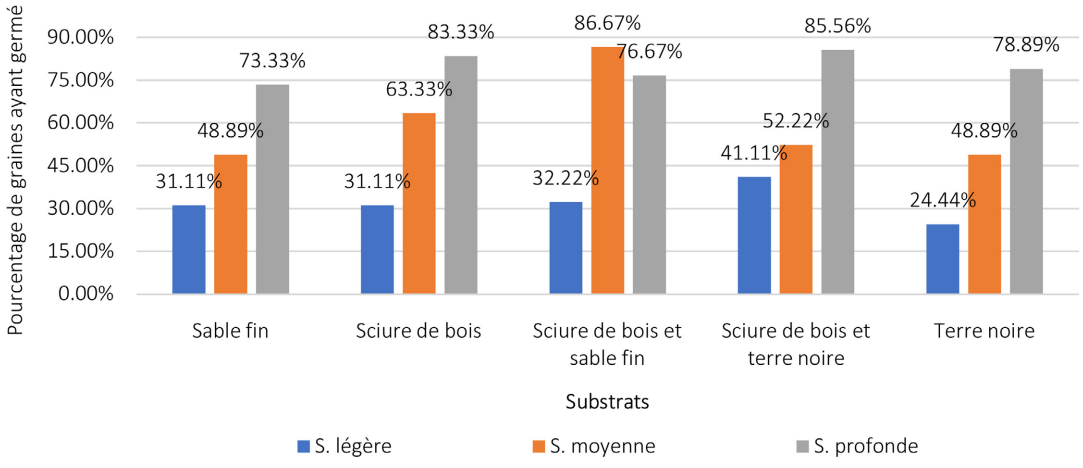


Figure 9. Percentage of seeds germinated according to the sub-treatment and the substrate.

the chances of germination of the seeds of the species 1 scarified respectively by 8.52% and 8.15% more compared to the substrate "Fine sand".

3.3. Influence of Substrates on Seedling Development

The images in Figure 10 show the development of young *Burkea africana* seedlings aged 5 days (a) and 17 days. The germination is normal whatever the substrates at first.

Table 2. Marginal effects at the mean point of the treatment and substrate variables.

Factors	Modalities	Marginal effect at mean point	P-value
Under treatment (reference modality = light scarification)	Medium scarification	0.2800	0.0000
	Deep scarification	0.4756	0.0000
Substrate (reference condition = fine sand)	Sawdust	0.0815	0.0372
	Sawdust and fine sand	0.1407	0.0003
	Sawdust and black soil	0.0852	0.0293
	Black earth	−0.0037	0.9251

(a)

(b)

(c)

Figure 10. Development of 5-day-old (a) and 17-day-old (b) et *B. africana* dead seedlings (c).

The size of the seedlings unfortunately remained the same for about 17 days. Since the cotyledonary leaves are normal, it is the root system that does not form early absorbing hairs (**Figure 10(c)**) to take up specific nutrients in the substrate. After this brief period of development, they started to perish (**Figure 10**) whatever the substrate. Bukea africana is a species of the Sudano-Guinean zone, this phenomenon can be explained by the desert climatic conditions of the experimental site, which are inappropriate for the growth of this species, and by the nature of the substrates used in these trials. It should be said that in the locality of N'Djamena where the experiment was carried out, there are no plants of this species. This would be due to the inappropriate climatic conditions.

4. Discussion

The germination behavior of *B. africana* seeds was studied by following both edaphic factors and pre-germination treatments applied. The results obtained during this work, highlight the effectiveness of manual scarification in the lifting of dormancy of the seeds of this species and the performance of substrate mixtures that significantly improve the germinatives qualities of its seeds. In fact, light, medium and deep scarification of *B. africana* seeds significantly reduced the dormancy time to 3 days. This result differs from that of [6], which states that the seeds of this species can germinate in 10 days as well as in 6 months, and the germination rate is often low. And that trials in Burkina Faso have shown that a mechanical scarification or a 15 - 20 minute sulfuric acid bath significantly increased the germination rate of the seeds of this plant. This difference with the result of [6], is due to the pre-treatment he applied to the seeds (chemical scarification and the duration of soaking in sulfuric acid). Deep scarification and medium scarification do not show a significant difference in their effects on seed germination of this plant. But both significantly influence the germination parameters of *B. africana*. At the threshold of 5%, the duration of germination and the speed of germination are significantly influenced compared to the light scarification and improve clearly the rate of germination of these. This germination is homogeneous, epigeneous and fast. The performance of manual scarification has already been obtained for the germination of seeds of *Leucaena leucocephala* [21], *Alstonia boonei* (Mapongmetsem *et al.*, 1999) of *Prosopis africana* [19], *Ximenia americanum*, [2] and not on *Burkea africana* seeds. However, these results corroborate those obtained by [10] [11], who showed in their work on the germination of *Anthyllis cytisoides*, the performance of deep scarification. Medium and deep scarification would degrade the seed coat in depth and facilitate the rapid entry of water to the embryo. Light scarification is not sufficient because the seed coat thickness remains poorly permeable to water. The seasons have no effect on the dormancy break of this species. In terms of substrates, the 1:1 black soil/sawdust mixture and the 1:1 fine sand/wood sawdust mixture as well as black soil and sawdust all showed better performance on improving the germination qualities of *Burkea africana* seeds. However, the development of the

seedlings presents difficulties as they die a few weeks after transplantation. This result corroborates that of [6] which confirms that seedlings can be kept for a long time in a humid environment, but they are subject to insect attacks and that seedlings frequently die in seedling trays or during transplanting.

5. Conclusion

In conclusion, mechanical scarification positively influences the dormancy break of *Burkea africana* seeds. Deep and medium seed scarification significantly improves the essential parameters of germination. The latency time and germination time are significantly reduced. Germination rate and germination speed are significantly improved. The germination of *B. africana* is epigenetic. The test substrates: black soil, sawdust, fine sand and the substrate mixtures 1/1 black soil/woodsawdust and fine sand/woodsawdust have a better germination rate. But the seedlings do not resist for a long time to the climatic conditions of the study area. They die two to three weeks after transplanting or transplanting in the substrates. This is what makes the cultivation of this plant difficult. Scarification can be easily replicated by local farmers and traditional medicine practitioners. These results also suggest that sustainable conservation of the genetic specimen of *Burkea africana* is therefore possible for any useful.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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