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EFFECT OF SEED PRETREATMENT ON GERMINATION OF *Bobgunnia madagascariensis* (Desv.) J.H. KIRKBR & WIERSEMA

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ABSTRACT

Knowledge of the method of seed germination is an important step of the domestication of crop plants. The objective of this study was to test the aptitude of germination of *Bobgunnia madagascariensis* (Desv.) using pretreatment condition. Eight batches of seeds were evaluated in two distinct substrates; namely sandy soil and ferralitic soil: (i) soaking of seed in cold water for 24 hours; (ii) soaking in hot water at 100 °C for 5 mm followed by in immersion into tap water for 24 hours, and (iii) scarification with razor blade. For each substrate, the treatments were compared to untreated controls. Results showed that seeds sown after scarification recorded the highest germination percentage (77.78%) with sandy soil ($P < 0.05$). Sandy soil also reduced the waiting time of germination (11.00 ± 0.00 days) and germination time (5.00 ± 0.5 days). Results of this study may serve as useful information for domestication of *Bobgunnia madagascariensis* (Desv.).

Key Words: *Bobgunnia madagascariensis*, domestication, seeds scarified

RESUME

La connaissance du mode de germination des graines est une étape importante de la domestication des plantes cultivées. L'objectif de cette étude était de tester l'aptitude à la germination de *Bobgunnia madagascariensis* (Desv.) en condition de prétraitement. Huit lots de semences ont été évalués dans deux substrats distincts ; à savoir sol sablonneux et sol ferralitique : (i) trempage des semences dans l'eau froide pendant 24 heures ; (ii) trempage dans de l'eau chaude à 100 °C pendant 5 mm suivi d'une immersion dans l'eau du robinet pendant 24 heures, et (iii) scarification avec une lame de rasoir. Pour chaque substrat, les traitements ont été comparés à des témoins non traités. Les résultats ont montré que les graines semées après scarification enregistraient le pourcentage de germination le plus élevé (77,78 %) avec un sol sableux ($P < 0,05$). Le sol sablonneux a également réduit le temps d'attente de la

germination (11,00 ± 0,00 jours) et le temps de germination (5,00 ± 0,5 jours). Les résultats de cette étude peuvent servir d'informations utiles pour la domestication de *Bobgunnia madagascariensis* (Desv.).

Mots Clés : *Bobgunnia madagascariensis*, domestication, graines scarifiées

INTRODUCTION

Bobgunnia madagascariensis (Desv.) is one of the most important leguminous trees possessing phytochemical compounds used for medicinal and other purposes (Amri, 2010). For medicinal purposes, leaves are used to cure scabies and cutaneous infections and root bark is mostly preferred as cure for toothaches (Amri, 2010). The roots are used to induce abortion, counteract venomous stings and bites, kill or expel intestinal worms and to treat leprosy. It has also been found useful in the treatment of fever (Coates, 2002). Other biological investigations have been reported on molluscidal activity of the pods of *B. madagascariensis* (Desv.) (Hostettmann *et al*., 2000).

A part from its medicinal properties, this species has also lactogenic properties. In Benin, several studies have led to the identification of several galactogenic species (Dassou *et al*., 2014; Imorou *et al*., 2021). Among these identified species, *B. madagascariensis* (Desv.) was reported as the most effective galactogenic species in terms of milk stimulation on cows (Imorou *et al*., 2021).

Multipurpose uses have resulted in over exploitation of the species to levels of extinction in some localities (Baatuuwie *et al*., 2019) mainly due to the high dependence on wild plant sources with little attention on species domestication. However, effective domestication will require knowledge on regeneration and other aspects of plant biology (Bohra *et al*., 2018). Propagating wild species for their restoration or reintroduction in native habitats can be an effective method of improving the size and viability of rare or threatened populations (Menges, 2008).

Despite its multiple uses, this species is still in the wild and is also under heavy pressure due to logging and extensive agriculture (Imorou *et al.*, 2021). In order to limit its disappearance, its domestication is proving to be necessary. Thus, understanding of seed germination of *B. madagascariensis* (Desv.) will allow the species to regenerate. According to Bellefontaine and Monteuuis (2000), knowledge of optimum germination conditions for the seeds of the species allows their domestication; contributes to their conservation as well as development; in addition to the conservation of biodiversity and the fight against desertification of the environments to which they were subservient.

Seed dormancy is one of the most important mechanisms of viability in plants. Generally, seed dormancy is little in plants that are domesticated from ancient times, compared to wild and native species (Munawar *et al*., 2015). Water absorption, enzymatic activity, embryo growth, seed coat rupture and plant growth are important steps of germination (Fenner and Thompson, 2005).

The objective of this study was to investigate the aptitude on germination of *B. madagascariensis* (Desv.) with a view to its good multiplication through the practical and reliable techniques of the seeds germination; thus to contribute to its conservation and its valorisation.

MATERIALS AND METHODS

Experimental site. The study was conducted at the Botanical Garden of the University of Abomey-Calavi (UAC), located in the municipality of Abomey-Calavi in the department of Atlantic in republic of Benin (Fig. 1). The geographical coordinates of this zone

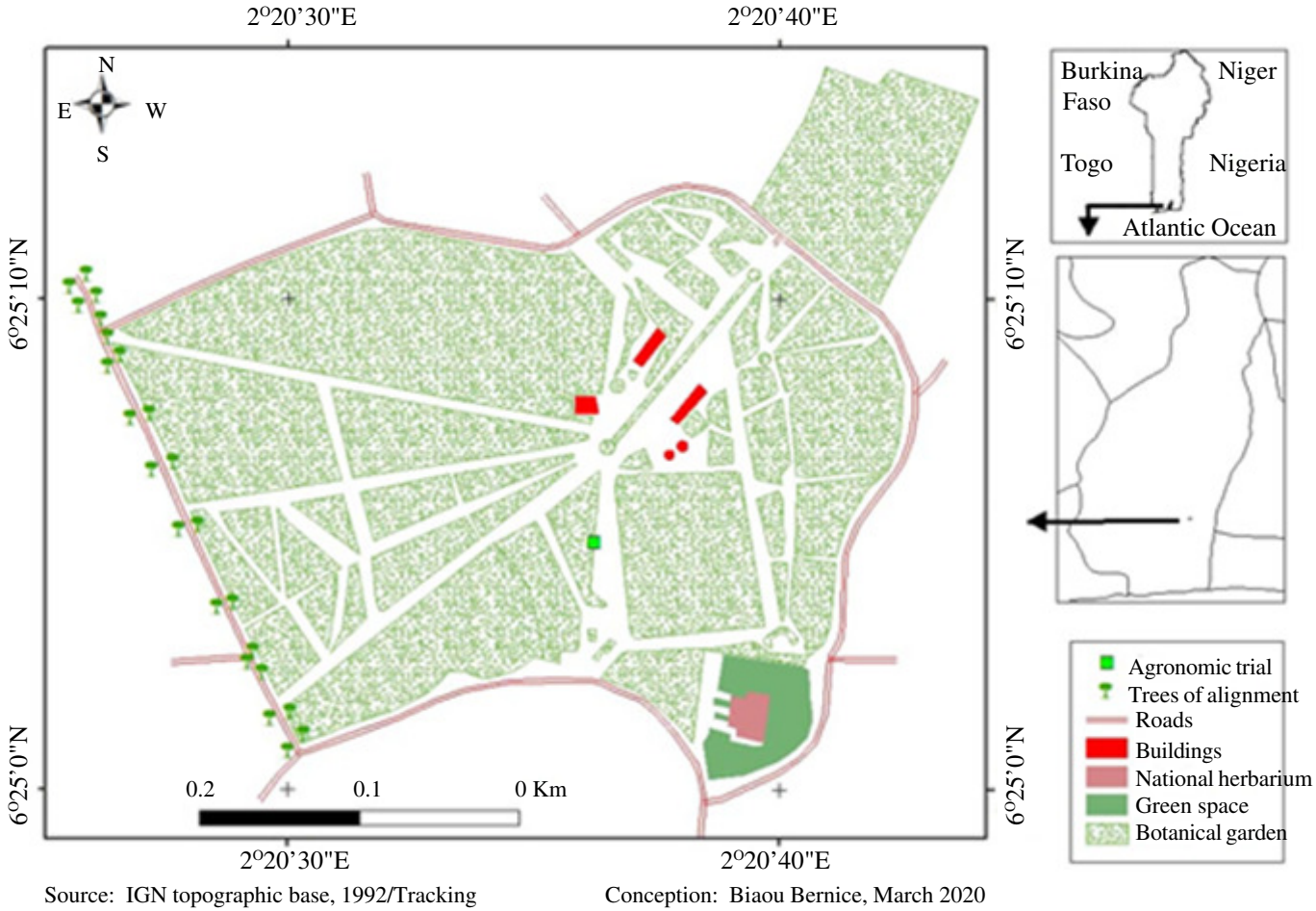

Figure 1. Map of study area.

are: altitude 24 m above sea level; 06°25.092'N and 002°20.607'E. The climate in the zone is sub-equatorial and is characterised by two rainy seasons (April to mid-July and September to November), and two dry seasons (December to March and mid-July to September) with fluctuations in recent years (Ahoton *et al*., 2011).

Plant materials and pre-germinated tests. *Bobgunnia madagascariensis* (Desv.) seeds (Fig. 2) were collected from Nikki (north of Benin) on January 15, 2020. Seed lots were subjected to three pre-germinated treatments before being sown in two separate substrates consisting of sandy soil and ferralitic soil, these included: (i) soaking the seeds in cold water for 24 hours; (ii) boiled in water at 100°C for five minutes, followed by rinsing twice in tap water and soaking for 24 hours in tap water; and (iii) scarification of the seeds coat with a razor blade. For each of the two substrates, these treatments were compared with untreated controls. For scarified seeds, the substrates used were sterilised for 1 hr in order to eliminate the spread unwanted disease organisms and pests to seedlings or potted plants; they were left to cool before being filled into the black polyethylene bags. Substrates were filled into the 20 x 10 x 8 cm^3 bags and placed under trees's shade at the Botanical Garden. The physico-chemical characteristics of the two substrates used are presented in Table 1.

Experimental design. The pots were arranged in randomised complete block design, with three replications and 8 treatments. Each

Figure 2. Seeds of *Bobgunnia madagascariensis* (Desv.).

TABLE 1. Physico-chemical characteristics of the substrates used (sandy soil and ferralitic soil)

Characteristics	Units	Sandy soil	Ferralitic soil
pH (H_2O)		6.47	6.9
pH (KCl)		5.92	5.98
Total N	(g kg^{-1})	0.4	0.3
Available P	(mg kg^{-1})	14.30	11.25
CEC	(cmol kg^{-1})	7.5	6.25
Exchange K^+	(cmol kg^{-1})	1.26	1.55
Clay	(%)	5.2	2.2
Silt	(%)	12	16
Sand	(%)	82.6	78.59

treatment consisted of 12 pots per block for a total of 96 pots per replication. In total, there were: 8 treatments x 12 pots x 3 replications equals to 288 pots. The treatments are described in (Table 2).

Data collection. The following data were collected: germination rates and germination dates (plant with two cotyledonous leaves on the surface of the substrate) from February 10, 2020 for the whole duration of the trial. These data allowed us to determine the waiting time (germination delay), time between sowing and first germination, the germination time (delay between first and last germination) per treatment and the germination percentage. The germination percentage (%) was determined using the Equation;

Germination % = G/N ×100

Where:

G = Total number of seeds that germinated per treatment.

N = The number of seeds sown per treatment (Ouédraogo *et al*., 2006; Ahoton *et al*., 2009);

Data analysis. The data collected were subjected a two-ways analysis of variance (substrate type and pretreatments). Significant mean values were then compared using the Least Significant Difference test at the 5% threshold (Dagnelie, 1998). To obtain normal distributions (analysis of variance hypothesis), the relativised values (germination rate) were transformed with (2arcsin"x/n) where n was the real value of germination percentage. The Statistical Analysis System version 9.4 (SAS v. 9.4) software was used for the statistical analyses.

RESULTS

Substrate and pretreatments. Table 3 presents the results of the two-ways analysis of variance considering substrate and pre-treatments. The results showed that substrate and pretreatments had a significant effect ($0.05 < P < 0.01$) on seed germination. Figure 3 shows effect of substrate and pretreatments on the seeds germination percentage. The analysis showed that seeds scarified recorded the highest germination percentage of 77.78±13.89% in relative to other pretreatments in sandy soil. The lowest percentage was obtained by seeds soaked in cold water for 24 hours (12.50 ± 4.17%). As regard substrate with ferralitic soil, seeds boiled at 100° C for five minutes and then soaked in tap water for 24 hours before sowing presented the highest germination percentage

TABLE 2. The different treatments of germination of *B. madagascariensis* (Desv.)

Treatments	Descriptions
T1	control seeds sown in the sandy soil
T2	seeds soaked in cold water for 24 hours before sowing in sandy soil
T3	seeds soaked in boiled water at 100°C during five minutes and then soaked in tap water for 24 hours before sowing in sandy soil
T4	seeds scarified and sown in the sterilized sandy soil.
T5	control seeds sown in the ferralitic soil
T6	seeds soaked in cold water for 24 hours before sowing in ferralitic soil
T7	seeds soaked in boiled water at 100°C during five minutes and then soaked in tap water for 24 hours before sowing in ferralitic soil
T8	seeds scarified and sown in the sterilized ferralitic soil.

TABLE 3. Results of analysis of variance (Value of Fisher) on germination percentage

Source of variation	Degree of freedom	Value of Fisher
		Seeds germination percentage (%)
Substrate	1	7.24 *
Pretreatments	3	10.96**
Replication	2	0.04 ns
Substrate* Pretreatments	3	3.65*

ns = not significant; * = significant at P < 0.05; **= significant at P < 0.01

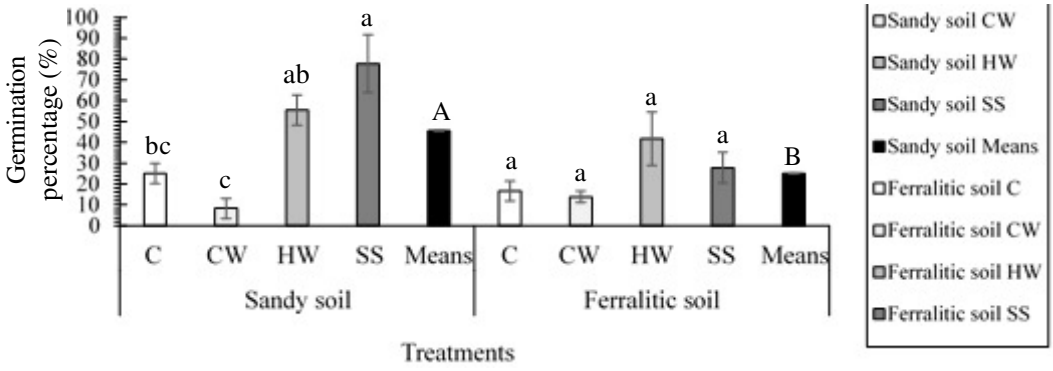

Figure 3. Effect of substrate and pretreatments on germination percentage. Vertical bars denote standard errors. Bars of the same types affected with the same letter are not significantly (P > 0.05) different according to the Least Significant Difference test.

of 41.66 ± 12.72%. On the other hand, the lowest rate was obtained by seeds soaked in cold water for 24 hours (13.89 ± 2.78%). In general, seeds scarified gave the best germination percentage with sandy soil.

Waiting time. Table 4 presents the results of the analysis of variance for waiting time of germination. The results indicated that substrate and pretreatments had a significant effect (P< 0.05) on the waiting time to seed germination. Figure 4 shows the effect of substrate and pretreatment on waiting time of germination. Waiting time of germination varied from 11.00 ± 0.00 to 30.00 ± 4.72 days for seeds sown in sandy soil; and from 11.33 ± 0.33 to 38.00 ± 2.00 days for those sown in ferralitic soil. The shortest days (11.00 ± 0.00 and 11.33 ± 0.33), were obtained with seeds scarified, with sandy soil and ferralitic soil, respectively. In general, seeds scarified and sown in sandy soil reduced the waiting time of germination.

Substrate and pretreatments. Table 5 presents the results of the analysis of variance realised on the germination time. The results indicated that the substrate together with pretreatment had a very highly significant (P< 0.05 to P< 0.001) effect on the time of germination. Interaction of substrate and pretreatment had also a significant (P< 0.05) effect.

Figure 5 shows the effect of substrate and pretreatment on the germination time. The seeds germination time varied on average from

TABLE 4. Analysis of variance table (F values) of the waiting time of germination

Source of variation	Degree of freedom	Value of Fisher
		Waiting time of germination (Days)
Substrate	1	4.99*
Pretreatments	3	4.27 *
Replication	2	1.21 ns
Substrate* pretreatments	3	1.51 ns

ns = not significant; *: significant at P<0.05

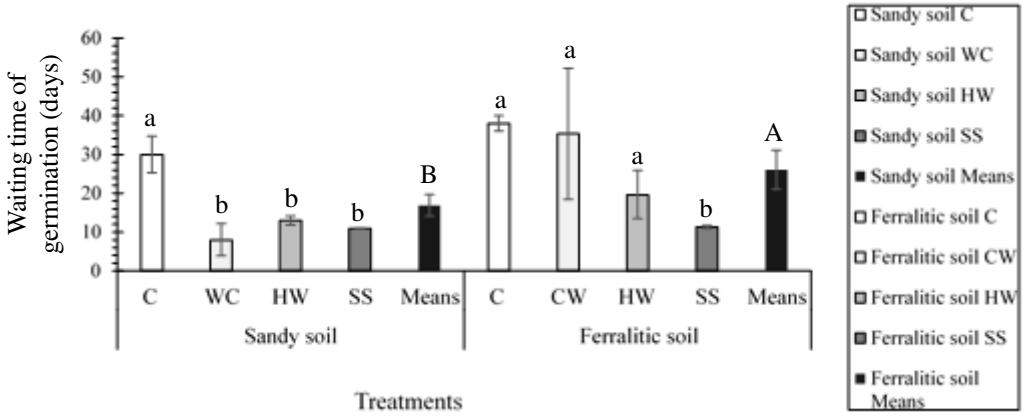

Figure 4. Effect of substrate and pretreatments on the waiting time of germination. Vertical bars denote standard errors. Bars of the same types affected with the same letter are not significantly (P > 0.05) different according to the Least Significant Difference test.

TABLE 5. Analysis of variance table (Value of Fisher) germination time

Source of variation	Degree of freedom	Value of Fisher
		Germination time (Days)
Substrate	1	6.39*
Pre-treatments	3	18.96***
Replication	2	1.40 ns
Substrate* Pretreatments	3	4.52*

ns = non significatif ; * = significatif at P<0.05 ; *** = significatif at P<0.001

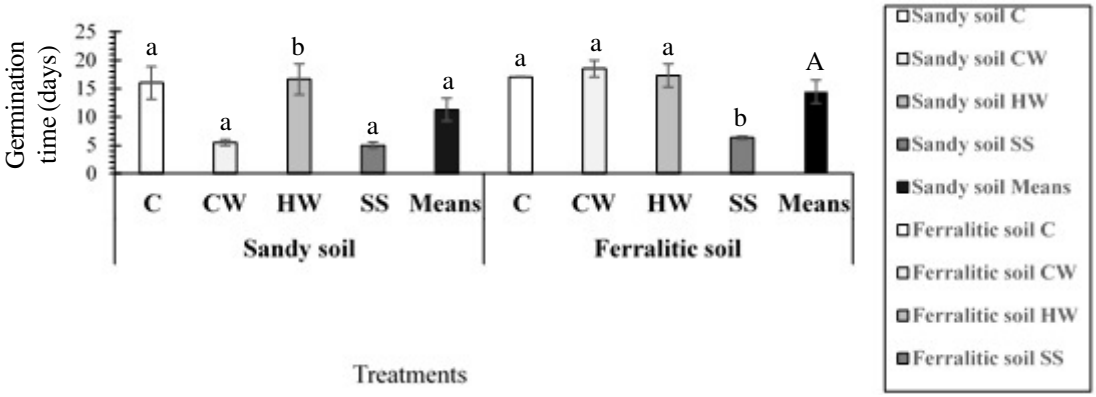

Figure 5. Effect of substrate and pretreatments on germination time. Vertical bars denote standard errors. Bars of the same types affected with the same letter are not significantly (P > 0.05) different according to the Least Significant Difference test.

5.00± 0.5 to 16.66± 2.72 days for seeds sown in sandy soil; and from 6.33±0.33 to 18.50±1.50 days for those sown in ferralitic soil. Sandy soil gave the shortest time (5.00± 0.5 days) of germination with seeds scarified. In general, seeds scarified and sown in sandy soil had reduced the germination time comparatively to ferralitic soil.

DISCUSSION

The results revealed that seeds scarified gave the best performance in terms of germination percentage, waiting time and germination time with sandy soil (Table 5). The lowest rate of germination was obtained with soaked seeds in cold water for 24 hours. Hossain *et al.* (2005) reported that seeds with hard, solid, impermeable seed coats germinated after pre-sowing treatments. However, breaking of seed dormancy varied from species to species. Therefore, it is important to determine the method and condition best suitable for each plant species.

The fact that physical scarification gave earlier germination in the present study, indicates that the more rapidly the seed coat is ruptured, the faster is the rate of germination. This is more so, since a major cause of seed dormancy is the presence of hard seed coats, which prevents the entrance of water, exchange of gases, and/or mechanically constrains the embryo (Amoakoh *et al*., 2017). The physical scarification allows water and air to enter into the seed reserves and stimulate germination, which ends up with the elongation of the embryonic axis (Holdsworth *et al*., 2008). Thus, seed becomes permeable to water only when the coat is disrupted, particularly at the lens (strophiole) region, which is usually the physically weakest part of the seed coat (Moïse *et al*., 2005; Jaganathan *et al*., 2017). Thus, in the absence of physiological dormancy, overcoming of physical dormancy may lead to immediate germination of the seeds upon imbibition. The rupturing of the seed coat is a mechanism which has triggered germination in many hard-seeded species with impermeable seed coats (Tigabu and Oden, 2001).

The use of seeds scarified to improve germination has been reported by Ahoton *et al*. (2009) on *Prosopis africana* with 85%; Barmukh and Nikam (2008) on *Pterocarpus marsupium* with 55. 3% within 15 days. Many authors have reported variables rates of germination on *Bobgunnia madagascariensis* (Desv.) following methods of pretreatments. Amri, (2010) in Tanzania, obtained 86% of germination percentage of seeds soaked in hot water for 10 minutes. Mojeremane (2012) in Côte d'Ivoire obtained 77% germination in 19

days of seeds soaked in hot water for 10 minutes. The low percentage obtained in our study is probably due to low seed viability as was reported by Mbuya *et al*. (1994) for germination of *Bobgunnia madagascariensis* (Desv.).

In the present study, scarified seeds have reduced the waiting time to 30±4.72 from 11± 0.00 days and time of germination from 16.00± 2.88 to 5.50±0.5 days. The rapid germination of physically scarified seeds was likely due to water and gases entering the embryo early, through the cracks that fired a sequence of biochemical reactions, resulting in the transformation of the embryo into a seedling early enough than other physical treatments. This could explain early days to emergence recorded among seeds that were pretreated. Physical scarification accelerated germination in *Bobgunnia madagascariensis* (Desv.). Scarified seeds of *Pterocarpus marsupium* reduced the number of days of germination (Barmukh and Nikam, 2008).

The relatively low germination percentage recorded with soaking of seeds in cold water for 24 hours implies that the method is ineffective for germination of *Bobgunnia madagascariensis* (Desv.) seeds. A similar observation was made by Danthu *et al*. (1995) and Amoakoh *et al*. (2017), who concluded that soaking in cold water was generally ineffective for germination of the seeds. The result of our study was, however, contrasted with the findings by Ibrahim and Otegbeye (2004) and Baatuuwie *et al*. (2019) who reported an increased percentage of seed germination with *Detarium microcarpum* and *Adansonia digitatata* with soaking water. This implies that different species have varying percentages at which their seed coats are permeable to water and gases (Owonubi *et al*., 2005).

With regard to substrates, sandy soil gave the greatest germination percentage and shortened the waiting time and the germination time. Sandy soil shortened the waiting time to seed germination from 30±4.72 to 11.00 ± 0.00 days as a result of seed scarification. This could be explained by the difference of physico-chemical properties of sandy soils. The soil had a light texture, high in sand content (82.6 %) which is favourable to rapid infiltration water and to germination. This finding agreed with the assertion of Dada *et al*. (2019) who reported that sandy soils are well aerated, light and easy to work, allow viable seeds to germinate easily and permits easy penetration of roots.

CONCLUSION

The results of the present study suggest that seeds of *Bobgunnia madagascariensis* (Desv.) possess physical dormancy. Thus, the most effective method of breaking dormancy is through seed scarification. On the other hand, sandy soil offers the best substrate for germination of *Bobgunnia madagascariensis* (Desv.) seeds. This study showed the aptitude through breaking dormancy of *Bobgunnia madagascariensis* (Desv.) seeds for germination.

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