Journal of Applied Biosciences 195: 20647 - 20655
ISSN 1997-5902

Seed germination and first growth performance of four *Sclerocarya birrea* (marula) provenances in Burkina Faso

Satassa Hien[1*], Lassina Traoré[1], K. Marie Laure Guissou[1], Anne Mette Lykke[2]
[1] Department of Training and Research in Science and Technology, University Norbert Zongo, Koudougou, Burkina Faso.
[2] Department of Ecoscience, Aarhus University, C. F. Møllers Allé 4, 8000 Aarhus.
Corresponding author: satsonhien@gmail.com

Submission 23rd January 2024. Published online at https://www.m.elewa.org/Journals/ on 31st March 2024.
https://doi.org/10.35759/JABs.195.2

ABSTRACT
Objective: In Sub-Saharan Africa, high pressure on savanna ecosystems has led to a decline in plant resources. This work aims to assess germination success by simple pretreatment of *Sclerocarya birrea* (marula) seeds from 4 sources in Burkina Faso.
Methodology and Results: Seeds were collected in farmer's fields, and from goat dung. A randomized complete block design with three replicates of 24 seeds was adopted. Variance analysis towards Student Newman and Keuls tests was used to compare growth parameters. There was no difference in germination success among the provenances of Wayen, Tiogo, Boromo, and Bondoukuy ($p>0.05$). Pretreatment influenced germination with higher values for seed that went through the digestive tract of goats irrespective of provenance. Significant differences among provenance were found for seedling height and collar diameter after four months' growth ($p<0.000$).
Conclusion and application of results: The study underlines the importance of using pretreatment. The Wayen provenance should preferably be used in a breeding program for reforestation and conservation of *Sclerocarya birrea*.
Keywords: *Sclerocarya birrea*, germination, plantation, pretreatment, restoration, West Africa.

INTRODUCTION
In Sub-Saharan Africa, the pressures on savanna ecosystems have led to the decline of plant resources (Kehlenbeck *et al.*, 2013). The control of regeneration patterns of local woody species is important for the sustainable management of tropical savanna ecosystems (Amani *et al.*, 2015). Seed germination success has a critical role in the conservation of indigenous tree species and reforestation efforts (Assogbadjo *et al.*, 2011). *Sclerocarya birrea* is one of the indigenous fruit species widely used in West Africa (Gouwakinnou *et al.*, 2011a; Moussa *et al.*, 2022), and offering economic and livelihood benefits to farmers (Dlamini, 2010). Despite its various uses, this species remains undomesticated in West Africa. Furthermore, its natural regeneration in fields and protected areas (Gouwakinnou *et al.*, 2009) is very scarce, due to damages of seed and seedling by domestic and wild animals (Daldoum *et al.*, 2012). Understanding of seed germination is essential for breeding programs

and conservation of *Sclerocarya birrea*, which is usually dioecious medium-sized tree. *Sclerocarya birrea* tolerates soils with very low fertility and exhibit a wide range tolerance for soil fertility (Msukwa *et al.*, 2016). It forms a natural association with arbuscular mycorrhizal fungi, which improves height and collar diameter growth (Haro *et al.*, 2021). The fruit of *Sclerocarya birrea* is a drupe with edible pulp and a hard endocarp encloses one to four seeds. Each seed has an operculum, which is ejected when germination starts (Muok *et al.,* 2011). Germination and propagation studies of *Sclerocarya birrea* have yielded various findings. The seeds do not germinate easily when they fall from the canopy, but can remain quiescent for more than six months' dormancy (Gaméné *et al.*, 2005). Thus, several authors have proposed pretreatment, to overcome dormancy and stimulate germination, e.g. passage through the acid digestive tract of mammals, or manual removal of the operculum (Setlalekgomo and Setlalekgomo, 2013), soaking seed in cold water for 24hours and treated with captain (a fungicide) (Dlamini, 2010), by sulphuric acid, mercuric chloride solution ($HgCl_2$), or cold stratification and dry storage (Moyo *et al.*, 2009). Also storage in brown paper bags at 22°C (Helm *et al.*, 2011), and burning (Pegg, 2014) were tested. Overall, the best germination rate was reported for soaking in cold water for 24 hours with 79.1% (Dlamini, 2010). However, expected water treatment, the other reported methods are risky for local people because they can cause injury if not used carefully. In contrast, some studies reported good germination rate by direct sowing without any pretreatment, of seeds at 68.33% (Hamidou *et al.*, 2014), and by soaking seeds in tap water for 24 hours at 63% (Abdelkheir and Warrag, 2014). It is therefore important to develop less dangerous methods that can be disseminated in rural areas to design efficient conservation management. The objective of this work was to assess germination success by simple pretreatment; of four different provenances of *Sclerocarya birrea* seeds and more specifically to assess the variation in germination success among provenances; assess the response to key pretreatment methods; and document the growth performance.

MATERIAL AND METHODS

Study area: The study was conducted at the Botanical Garden of University Norbert Zongo, Burkina Faso (altitude 295m, 12° 14' N and 02 ° 24' W) from June to November 2023. Located in North Sudanian phytogeographic sector, it is characterized by rainfall ranging between 700 and 900 mm and temperatures fluctuating between 28 and 37°C. The trial was run in real conditions during the wet season.

Seed collection: *Sclerocarya birrea* fruits were collected from native trees preserved in agricultural fields and village households in four sites across two phytogeographic zones (Wayen, Tiogo (north-sudanian sector), Boromo and Bondoukuy (south-sudanian sector)). Fruits were peeled, damaged seeds were removed and the remaining seeds were air-dried. Samples were kept in paper bags and stored at room temperature for one month until the experiment start. We tested two pretreatment methods: tap water soaking for one night (12 hours) and seeds passing through the digestive tract of goats. The results were then compared to the results of untreated seeds sowing.

Experimental design: A randomized complete block with three replicates of 24 seeds for each of four provenances (Wayen, Tiogo, Boromo and Bondoukuy) and three pretreatments [T0: untreated, T1: soaking in tap water for one night, and T2: passing through digestive tract of goats] (Figure 1). The T1 and T2 seeds served as treatments while T0 seeds served as a control.

Figure 1: Sampling design: randomized complete block with three replicates of 24 seeds for each of four provenances (Wayen, Tiogo, Boromo and Bondoukuy) and three pre-treatments.

Seeds of each provenance were sown on polyethylene plastic pots of 30 x 10 cm, initially filled with a mixture of soil (2/3), and compost (1/3). To maintain an ideal humidity condition, watering was carried out only in case of rain, twice a day: between 7 and 8 a.m., and between 5 and 6 p.m. Germinated seeds were recorded for five weeks after sowing. A seed was considered germinated when the two cotyledons had emerged. Watering was assured on no rainy days. Following germination tests, a nursery experiment was performed to examine seedling growth parameters. Seedling height and stem collar diameter (using a numeric caliper) were measured each month during four months.

Data analysis: For germination, following parameters were recorded: number of days to first emergence (E1), number of days to 50% emergence (E50), number of days between first and the last emergence called emergence spread (Es), and the proportion of seeds germinated during the period of observation called capacity (Ef). The mean emergence time (MGT) was calculated based on Fandohan *et al.* (2010):

$\mathrm{MGT} = \sum n_i d_i / n$; n = total number of seeds germinated during experimental period; ni = number of seed germinated on day di; di = day during germination period.

To determine significant differences in germination parameters among provenance and treatment, analysis of Variance (ANOVA) followed by the post hoc Student Newman and Keuls (SNK) test (p= 0.05). Analysis were run in R_studio (R.4.2.2).

RESULTS

Seed germination: Provenance did not influence seed germination (p > 0.05) (Table 1). There were significant effects of treatment on E1, E50, Es, and Ef, except on MGT.

Table 1: Results of the analysis of variance for the emergence of the first seedling (E1), time to 50% emergence (E50), emergence spread (Es), emergence efficiency (Ef) and mean germination time (MGT) of *Sclerocarya birrea* seeds of four provenances subject to three pretreatments

	Germination parameters					
	Degree of freedom	First emergence	50% emergence	Emergence spread	Emergence efficiency	Mean emergence time
Provenance	3	0.95ns	0.87ns	0.85ns	0.91ns	0.59ns
Pretreatment	2	0.001***	0.001***	0.001***	0.006**	0.11ns

Significant codes: ns= not significant at α = 0.05; ** significant at α = 0.01; *** significant at α = 0.001

Seed emergence varied according to the treatments (Figure 2). T2 showed shortest value of first emergence day (7 days in average). Time to 50% emergence was less than 14 days, and ermergence spread was 21 days. T2 was followed by T1 with 8 days to first emergence, 14 days to time to 50% emergence, and 24 days of emergence spread. The longest time of seed emergence was recorded for Treatment T0. First emergence took place 15 days after sowing, time to 50% emergence after 21 days and emergence spread was 29 days. Overall, irrespective of treatment, mean emergence time remains consistent throughout germination period.

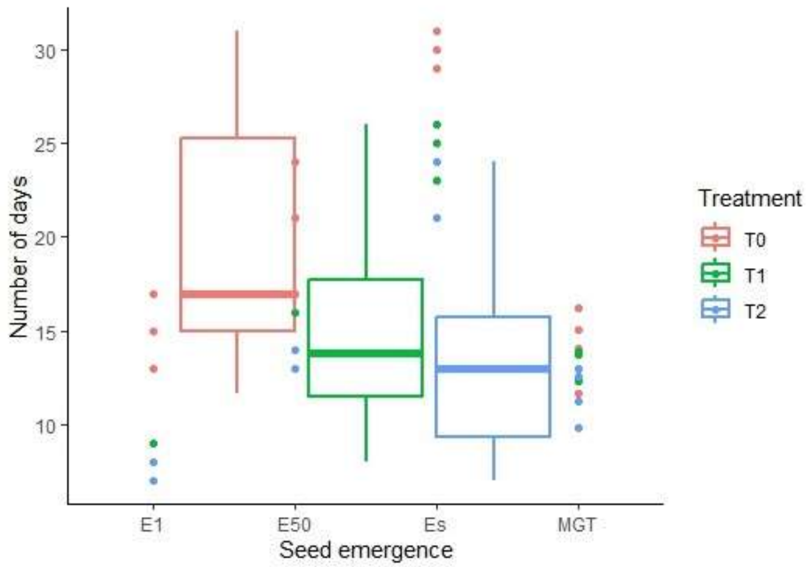


Figure 2: Emergence of first seedling (E1), time to 50% emergence (E50), emergence spread (Es), and mean germination time (MGT) of Sclerocarya birrea seeds by pre-treatments (untreated seed T0, seeds soaking in tap water for 12h T1, seeds passing through goats' digestive tracts T2).

During the five weeks of germination period, treatment showed different rates of germination (Figure 3). In the first week, T0 showed no germination. However, seeds from T0 start germinate in the second week of observation and gradually reached 70% of germination rate at the last week of germination period. On the other hand, T1 had low germination rate compared to T2 in the first week. The germination rate reached 50% for T1 and 70% for T2 during the second week of observation. The germination rate stays consistent after the fourth week respectively at 84.37% for T1 and 91.66% for T2.

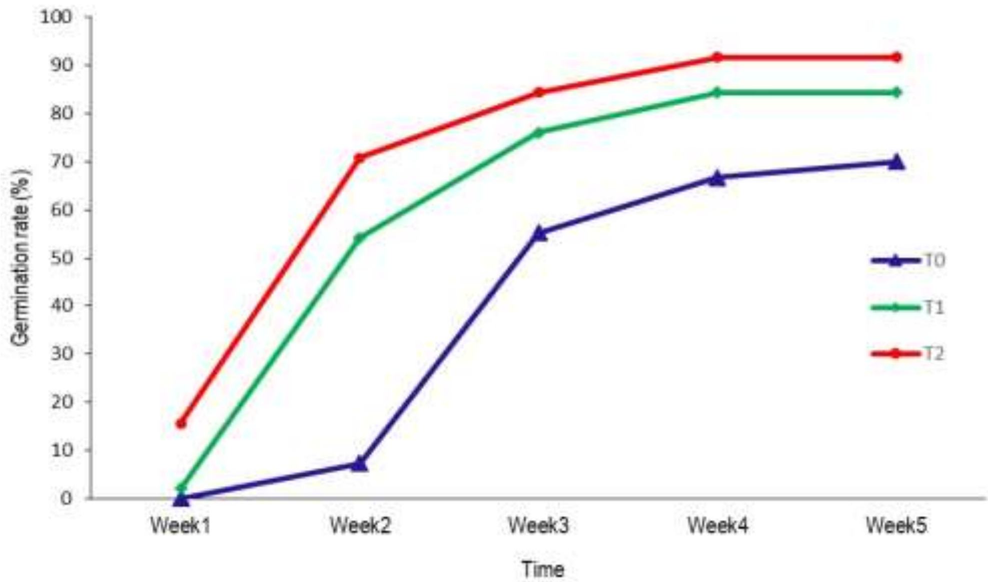
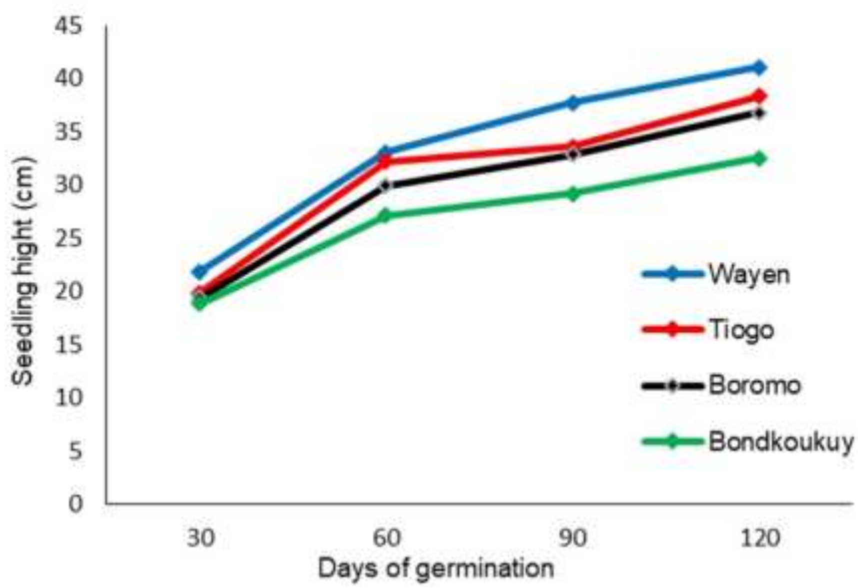


Figure 3: Germination success of Sclerocarya birrea seeds by pre-treatment (untreated seed T0, seeds soaking in tap water for 12h T1, seeds passing through goats' digestive tracts T2) over five weeks.

Seedling growth performance: The ANOVA revealed significant differences in seedling height among provenance after 30 days ($p<0.001$), 60 days ($p<0.000$), 90 days ($p<0.000$) and 120 days ($p<0.000$) (Figure 4). Seeds from Wayen exhibit the tallest seedlings, followed by Tiogo, Boromo and Bondoukuy. Globally, growth performance becomes more pronounced as the seedlings progress through various stages of development. Stem collar diameter showed the same scheme after two months of growth (Figure 4). Significant variations were also recorded among the provenances ($p < 0.001$) excepted in the first month of observation.



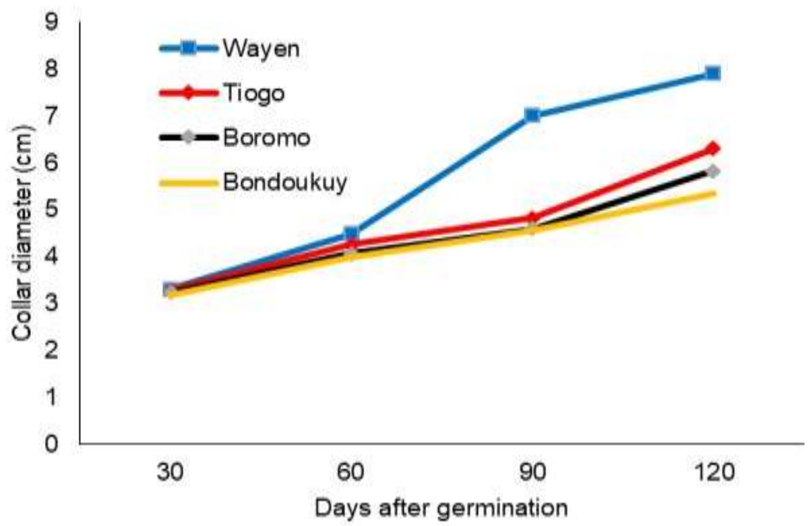


Figure 4: Stem height (A), and collar diameter (B) of Sclerocarya birrea seedlings from Wayen, Tiogo, Boromo and Bondoukuy.

DISCUSSION

Germination success of *Sclerocarya birrea:* This study highlights the germination success of recently harvested fruits of *Sclerocarya birrea* regarding provenance and non-risk pretreatment, techniques. The results indicate that provenance had no significant effect on seed germination, suggesting uniformity of sample in each locality. The same results were reported by Abdelkheir and Warrag (2014). Seeds collected from different provenances are submitted to the same environmental conditions such as temperature, soil humidity, soil quality, and other specific climatic conditions. This study showed that pretreatment breaks seed dormancy suggesting that passage through digestive tract of goats appears to be efficient, fast and exhibits higher germination capacity. Setlalekgomo and Setlalekgomo, (2013) also noted enhanced germination capacity of *Sclerocarya birrea* cores due to the passage through the tract of kudu (*Tragelaphus strepsiceros*). The effect of the ingestion of its fruits by herbivores increasing germination rate was also reported by Helm *et al.* (2011). Irrespective of seed size, Pegg, (2014) had reported that, antelope ingestion significantly enhanced germination rate of *Sclerocarya birrea*. Our finding suggests that endozoochory is an important mode of seed dispersal of *Sclerocarya birrea* as discuss in literature (Hall *et al.*, 2002). Seed soaking in tap water for one night; also increase germination of *Sclerocarya birrea*. Our results opposite the findings by Gaméné *et al.* (2005). Those authors state that *Sclerocarya birrea* seed require a postmaturation period (seeds stored for 18 months at 25 °C) to reach the maximum germination. Furthermore, previous studies have reported that, *Sclerocarya birrea* seed soaking in cold water for 48h can up grow 60-80% germination rate (Moyo *et al.*, 2009; Dlamini, 2010). Our trial experience 12h of soaking, suggesting this method should be quickly accepted by famers for short time, and because this technique did not present any risk of health. Untreated seed took long time more than 14 days of first emergence than the treated ones. This disagree with the findings by Hamidou *et al.* (2014) who had yet reported that, untreated seeds of *Sclerocarya birrea* had 8 days of latent period in Niger. The contrary with the above authors can due to seed origin and otherwise of climatic conditions of trial.

However, irrespective to pretreatment, technique and provenance, germination rate found in our study is higher than previous studies for *Sclerocarya birrea* (Dlamini, 2010; Halidou *et al.,* 2014, Abdelkheir and Warrag, 2014). Those authors have reported germination capacity varying up from 40% to 80%. Indeed, Muok *et al.* (2011) reported that a prolonged storage (one or more years) of *Sclerocarya birrea* seed has a greater effect on seed viability and may increase the capacity of germination.

Seedling growth performance: Next to germination, significant difference was observed in seedling growth among provenances. Similar results have been reported for *Adansonia digitata* L. (Assogbadjo *et al.,* 2011), *Tamarindus indica* L. (Fandohan *et al.,* 2010), and several species of Combretaceae (Amani *et al.,* 2015) in West Africa. Previously, Dlamini (2010) and Fandohan *et al.* (2010) reported a positive influence of seed morphometric traits on early growth of seedlings respectively for *Sclerocarya birrea* (subspecies *caffra*) and *Tamarindus indica*. These authors reported that larger seeds showed higher growth speeds than smaller ones. Our findings suggest that quick growth can be attributed to initial large food reserves in seeds among provenances. Emerging seedlings mainly depend on seed reserve for initial growth, which explains why seedling height and diameter in the first month were approximatively the same. However, results indicated that Wayen and Tiogo provenances (north sudanian phytogeographic sector) portrayed the highest values than Boromo and Bondoukuy (south sudanian phytogeographic sector) suggesting influence of climatic conditions on *Sclerocarya birrea* seedling growth. According to Gouwakinnou *et al.* (2011b) *Sclerocarya birrea* from the drier zone, generally have a higher weight of seeds and these results in a faster seedling growth as well as a higher collar diameter. Diallo *et al*. (2020) found that *Sclerocarya birrea* seedlings exhibit heritability in morpho-adaptive traits, including stem height, root length, secondary roots, and root biomass from nursery age. As the species population is facing a high risk of extinction because of the lack of its natural regeneration (Daldoum *et al.*, 2012), seed collection can be made in Wayen provenance. *Sclerocarya birrea* demonstrated a wide range tolerance for soil fertility (Msukwa *et al.,* 2016), and practical ex-situ conservation measures are urgently needed to preserve genetic diversity and maintain multiple specimens. This study highlights that seedlings growth parameters are indicators for selection based on the adaptation of plant material to ecological conditions.

CONCLUSION AND APPLICATION OF RESULTS

This study on the germination success and seedling growth of *Sclerocarya birrea* showed that pretreatment methods, such as soaking in tap water or passage through the digestive tract of goats, significantly improve germination rate. Provenance influence on seedling growth underscores that, seed from Wayen can be collected for any propagative technique and ex-situ conservation strategies, due to its better adaptation local habitat conditions. Furthermore, investigating both pretreatment methods and provenance provides help to understand of the ecological requirements and adaptive strategies of *Sclerocarya birrea*.

ACKNOWLEDGMENTS

This work was funded by CRES (Climate Change Resilience of Ecosystem Services) through a research project supported by Danida (Project No. 20-13-GHA). We are indebted to local farmers for their substantial contribution to this work, especially during seeds collection.

REFERENCES

Abdelkheir R, Warrag E. 2014. Variation in seed and germination traits of *Sclerocarya birrea* subsp. *birrea* populations.

Amani A, Inoussa MM, Guimbo ID, Mahamane A, Saadou M, Lykke AM., 2015. Germination et croissance de quatre espèces de Combretaceae en pépinière au Niger. *Tropicultura* 33 (2): 135-145.

Assogbadjo A E, Glèlè Kakaï R, Edon S, Kyndt T, Sinsin B., 2011. Natural variation in fruit characteristics, seed germination and seedling growth of *Adansonia digitata* L. in Benin. *New Savannas* 41 (1): 113-25.

Daldoum D M A, Massaud M M, Adam Y O., 2012. Distribution, fruit production and natural regeneration of *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *birrea* in the Nuba mountains, Sudan. *World Journal of Agricultural Sciences* 8 (3): 234-239.

Diallo F A, Sandwidi A, Dao M E C, Bationo-Kando P, Diallo B O., 2020. Caractérisation phénotypique des plantules de quatre populations de *Sclerocarya birrea* (A. Rich.) Hochst. au Burkina Faso. *Bois et Forêts des Tropiques* 344 : 33-46.

Dlamini C S., 2010. Provenance and family variation in germination and early seedling growth in *Sclerocarya birrea* sub-species *caffra*. *Journal of Horticulture and Savannary* 2 (9): 229-235.

Fandohan B, Assogbadjo A E, Glèlè Kakaï R, Sinsin B., 2010. Variation in seed morphometric traits, germination and early seedling growth performances of *Tamarindus Indica* L. *International Journal of Biological and Chemical Sciences* 4 (4): 1102–1109.

Gaméné C S, Erdey D, Baxter D, Motete N, Berjak P., 2005. Desiccation, germination and storage of *Sclerocarya birrea* seeds from Burkina Faso. Comparative storage biology of tropical tree seeds, pp. 40-56.

Gouwakinnou G N, Assogbadjo A E, Lykke A M, Sinsin B., 2011a. Local knowledge, pattern and diversity of use of *Sclerocarya birrea*. *Journal of Ethnobiology and Ethnomedicine* 7 (1): 1-9.

Gouwakinnou G N, Assogbadjo A E, Lykke A M, Sinsin B., 2011b. Phenotypic variations in fruits and selection potential in *Sclerocarya birrea* subsp. *birrea*. *Scientia Horticulturae* 129 (4): 777-783.

Gouwakinnou G N, Kindomihou V, Assogbadjo A E, Sinsin B., 2009. Population structure and abundance of *Sclerocarya birrea* (A. Rich) Hochst subsp. *birrea* in two contrasting land-use systems in Benin. *International Journal of Biodiversity and Conservation* 1 (6): 194-201.

Hall JB, O'Brien EM, Sinclair FL., 2002. *Sclerocarya birrea*: a monograph. School of Agricultural and Savanna Sciences. University of Wales, Bangor, p. 157.

Hamidou A, Iro D, Boubé M, Malick T, Ali M., 2014. Potential germination and initial growth of *Sclerocarya birrea* (A. Rich.) Hochst, in Niger. *Journal of Applied Biosciences* 76 (1): 6433-6443.

Haro H, Semdé K, Bahadio K, Sanon K B., 2021. Improved *Sclerocarya birrea* (A. Rich.) Hochst. growth by Mycorrhizal inoculation. *African Journal of Biological Sciences* 3 (1): 104-109.

Helm C V, Scott S L, Witkowski E T F., 2011. Reproductive potential and seed fate of *Sclerocarya birrea* subsp. *caffra* (marula) in the low altitude savannas of South Africa. *South African Journal of Botany* 77 (3): 650-664.

Kehlenbeck K, Asaah E, Jamnadass R., 2013. Diversity of indigenous fruit trees and their contribution to nutrition and livelihoods in Sub-Saharan Africa: examples from Kenya and Cameroon. Diversifying food and diets using agricultural biodiversity to improve nutrition and health, pp. 257-269.

Moussa A H, Mahamadou A G, Rayanatou I A., 2022. Caractérisation socio-économique et identification des usages de *Sclerocarya birrea* (A. Rich.) Hochst. au Niger. *Journal of Applied Biosciences* 174: 18113-18123.

Moyo M, Kulkarni M G, Finnie J F, Van Staden J., 2009. After-ripening, light conditions, and cold stratification influence germination of marula *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro seeds. *Horticultural Sciences* 44 (1): 119-124.

Msukwa V J, Munthali C R Y, Nyoka B I, Missanjo E., 2019. Phenology of *Sclerocarya birrea* (A. Rich.) Hochst. provenances. *Emerging Science Journal* 3 (1): 10-22.

Muok B O, Khumalo S G, Tadesse W, Alem S., 2011. *Sclerocarya birrea*, marula. Conservation and sustainable use of genetic resources of priority food tree species in sub-Saharan Africa. Biodiversity International, Rome: 12.

Pegg N., 2014. Antelope ingestion enhances germination of the Marula (*Sclerocarya birrea*), an Important African savannah tree. *African Journal of Ecology* 52 (4): 499-505.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Setlalekgomo M R, Setlalekgomo T., 2013. Effects of gut passage by Kudu (*Tragelaphus strepsiceros*) and pericarp on the germination percentage of Marula (*Sclerocarya birrea*) seeds. *Scientific Journal of Biological Sciences* 2 (6): 122-126.