Journal of Advances in Biology & Biotechnology

Volume 27, Issue 11, Page 827-835, 2024; Article no.JABB.126316
ISSN: 2394-1081

Improvement of Seed Germination and Seedling Growth of African Rosewood (*Pterocarpus erinaceus* Poir) by Seed Pretreatment, Seedling Substrate and Growing Medium

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Authors' contributions

This work was carried out in collaboration among all authors. Author TDEF wrote the manuscript, performed the statistical analysis and contributed to the interpretation of results. Author FIJ designed the study and contributed to the writing of the protocol and the interpretation of results. Author SMI executed field experiments and collected the data. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i111666

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/126316

Original Research Article

Received: 04/09/2024
Accepted: 08/11/2024
Published: 14/11/2024

ABSTRACT

Pterocarpus erinaceus is a highly valuable species endemic to West Africa. In Côte d'Ivoire, it is subject to abusive and illegal exploitation, compromising the survival of stands and, above all, its natural regeneration. The aim of this study was to determine the optimum conditions for the

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Cite as: *Fulgence, Thiemele Deless Edmond, Fofana Inza Jésus, and Soro Minfounga Issa. 2024. "Improvement of Seed Germination and Seedling Growth of African Rosewood (Pterocarpus Erinaceus Poir) by Seed Pretreatment, Seedling Substrate and Growing Medium". Journal of Advances in Biology & Biotechnology 27 (11):827-35. https://doi.org/10.9734/jabb/2024/v27i111666.*

germination and development of *Pterocarpus erinaceus* seedlings in the nursery. The experiment was conducted at the experimental site of the botanical garden of the Peleforo GON COULIBALY University (UPGC) following a randomized Fisher block design with three replications between May and August 2024. Four pretreatments were applied to *Pterocarpus erinaceus* seeds sown on two substrates in two environments. These were pretreatments (hulled seed, unhulled seed, unhulled seed soaked in ordinary water for 24 hours and hulled seed soaked in ordinary water for 24 hours), substrates (black earth and potting soil) and environments (tunnel and shade). The results showed that hulled seeds grown on sterilized black soil under tunnel conditions showed the best germination rate (81.67%) within a germination time of 3 days with a mean germination time of 7 days after sowing (DAS). These were followed by hulled seeds sown on black soil under shade conditions, with a 76.66% germination rate within a germination period of 3 days and an average germination time of 7.51 days after sowing. The seedlings obtained from these hulled seeds had a height of 20 cm, 7 leaves and a diameter of 1.45 mm in two months in the nursery under tunnel conditions, compared with 14.96 cm in height, 6 leaves and a diameter of 1.95 mm under shade conditions, which considerably reduced the time taken in the nursery compared with previous work. Thus, the technique of hulled seeds grown on black soil under tunnel conditions can be recommended for germinating *Pterocarpus erinaceus* seeds for planting purposes.

Keywords: Pterocarpus erinaceus; pretreatments; germination; seedling growth.

1. INTRODUCTION

Pterocarpus erinaceus is native to the Sahelo-Sudanian and Sudano-Guinean zones. Its range extends from southern Senegal to western Central African Republic (Ahoton et al., 2009). In the south, its range extends to the edge of the Côte d'Ivoire rainforest and the humid coastal savannahs of Guinea, Togo and Benin. *Pterocarpus erinaceus* is a multi-purpose plant. It provides excellent timber, highly prized for export. It is used in cabinet-making, construction and armament. African rosewood (*Pterocarpus erinaceus*) is therefore the world's best-selling tropical timber (Dumenu, 2019). It is also sought after by craftsmen in West Africa for the manufacture of various musical instruments (balafons, n'goni and djembes). In addition, Rosewood is one of the 4 woody species in northern Côte d'Ivoire most widely used in cattle feed and contributes to soil fertility (Silué et al., 2017 Peter et al.,2021), Finally, the bark, leaves and roots are used in pharmacopoeia to cure many ailments, including anemia, coughs, dysentery, malaria and infantile fever (Ouédraogo et al.,2017, Yusuf et al., 2020, Alagbe et al., 2024). Notwithstanding this importance, African rosewood is now under threat in Côte d'Ivoire. Indeed, Côte d'Ivoire ranks third among West African countries, where the exploitation of rosewood has increased over the last decade (Cites and Annexes, 2017). Moreover, scientific data on *Pterocarpus erinaceus* in Côte d'Ivoire are fragmentary. This lack of information prevents the implementation of sustainable management and conservation strategies for the species, which is exploited in natural formations in opposition to management principles (Ouédraogo et al.,2017, Cites, 2017). The species has not yet been domesticated, and its natural regeneration capacity is slow to reconstitute this vegetation cover (Bellefontaine and Monteuuis, 2002). Added to this, is the low natural germination rate of the seeds, which constitutes an obstacle to the valorization of this endangered species (Touré, 2001 Karsten et al., 2014). Pretreatments applied to the seed could improve their germination and remove the various obstacles encountered in domesticating the species. With this in mind, this study was carried out to determine the optimum conditions for germination and development of African rosewood seedlings in the nursery. Specifically, the aim is to identify the best seed pretreatments, seedling substrate and growing medium for seed germination and seedling growth and development in the nursery.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted at the experimental site of the botanical garden of the Peleforo GON COULIBALY University (UPGC). The experimental plot is located at longitude 5°38 West and latitude 9°26. The local climate is Sudanian, with a wet season from June to September and a dry season from October to May. It is characterized by slight temperature variations, around an average of 30°C. Average rainfall ranges from 900 to 1.500 mm/year.

Vegetation is characterized by scattered trees and shrubs, with a cover density of around 25-35%. The relief is monotonous, with altitudes varying on average between 300 and 400 m. The soils belong to the Ferrisol type (Dabin and Leneuf, 1960).

2.2 Plant Materials

The plant material consisted of mature fruits (Fig. 1a) and seeds (Fig. 1b) of *Pterocarpus erinaceus* collected from a stand of African rosewood at Peleforo Gon COULIBALY University.

2.3 Methods

2.3.1 Experimental design and trail conduct

The study was carried out in a randomized Fisher block design with three replications in two different growing environments (tunnel and shade) and on two substrates (black earth and potting soil). The tunnel consists of a frame covered with transparent polyethylene plastic film, which maintains a microclimate inside at a temperature of between 30°C and 35°C, with relative humidity varying between 80% and 90%. The 2 m-high shadehouse was covered with junta cloth to minimize the impact of incident sunlight on the plants. The potting soil was purchased commercially, and the black earth was taken from trees in the UPGC botanical garden and sterilized using heat from wood fires (100°C) to destroy any pathogen germs.

Four pretreatments were applied to the seeds. These were (1) unhulled seeds (SND : fruits containing wings were sown directly without prior treatment; (2) hulled seeds (SD: fruits were dehulled to recover seeds; (3) soaked hulled seeds (SDT: fruits were dehulled and soaked in water for 24 hours; and (4) soaked unhulled seeds (SNDT: fruits were soaked in ordinary water for 24 hours). In each medium, seeds were sown in nursery bags previously filled with soil and/or potting compost at a rate of one seed per bag. A total of eight treatments (4 pretreatments x 2 substrates) were applied in each block and each experimental plot consisted of 20 perforated polythene bags containing substrates.

2.3.2 Data collection

Germination data were collected every day after sowing for 1 month and were followed by growth and development data for seedlings in the nursery for up to two months. The germination parameters were (1) germination delay (DG), which is the time elapsed between sowing and first germination ; (2) germination rate (TG), which is an assessment of seed germination potential. This rate is obtained from the following formula: TG = (n /N) x 100. With n - number of germinated seeds and N - total number of seeds sown and (3) germination speed was expressed by the mean germination time (TMG), which is the time after which 50% of the seeds have germinated (Bamba et al., 2018). The speed is calculated according to the following formula :

$$TMG = \sum_{i=1}^{i=9} \frac{n_i t_i}{N}$$

with ni the number of germinated seeds at time ti (i ranging from 1 to 9) and N the number of germinated seeds at the end of the trial.

(a)

(b)

Fig. 1. (a) Fruits and (b) shelled seeds of *Pterocarpus erinaceus*

Growth and development parameters were collected from seedlings at two months after sowing. Parameters included seedling height (HP), collar diameter and number of leaves.

2.3.3 Data analysis

The data collected were analyzed using R software version 4.3.3. Normality tests, notably the Shapiro-Wilcoxon test, were performed to verify the distribution of the data. This helped to guide subsequent statistical tests, in particular the choice of ANOVA (Analysis of Variance) or Kruskal-Wallis test to assess the level of differentiation of each parameter measured between treatments. When these tests proved significant at the 5% level, post-hoc tests were performed, notably the Withney Newman test in the case of ANOVA and Dunn's test in the case of Kruskal-Wallis. A Student's t-test at the 5% threshold was performed to compare the means of the parameters measured between substrates.

3. RESULTS AND DISCUSSION

3.1 Germination Potential of Seeds on Substrates in Growing Media

3.1.1 Germination rate and germination time

According to Table 1, hulled seeds achieved better germination rates than the other pretreatments, regardless of the growing medium. The growing medium also had an impact on seed germination. Seeds grown in tunnels achieved better germination rates (TG) than those grown under shade. In fact, treatments SD_Ts (hulled seeds sown in black soil) and SD_Tx (hulled seeds sown in potting soil) had the best germination rates, respectively 81.67% and 73.33% under tunnel conditions, and 76.67% and 71.67% under shade. The highest rate was observed under tunnel conditions. This influence was much more marked for the germination rate of soaked hulled seeds (SDT), which was 60% under tunnel versus 23.33% under shade, thus demonstrating the beneficial effect of tunneling on *Pterocarpus erinaceus* seed germination. This difference in germination rate could be explained by the conditions inside the tunnel (high temperature and relative humidity), which boosted seed germination. These tunnel conditions have been used extensively in the production of plantain seedlings, using the PIF method to lift axillary bud dormancy and promote seedling proliferation (Kwa, 2003). Similar germination rates were obtained with other tree species such as *Acacia senegal* with 87% (Hamawa et al., 2020) and *Ricinodendron heudelotii* with 77% (Kouamé et al., 2012). These observations were obtained from seeds pretreated to boost germination after dormancy lifting. Several studies have shown that the seeds of species in the *Pterocarpus* genus are characterized by pericarpic dormancy, which must be lifted to ensure good germination (*Pterocarpus angolensis* (Laurie,1974, *Pterocarpus santalinus* (Naidu and Rajendrudu,2001), *Pterocarpus erinaceus* (Peteret al., 2014) In our study, seed hulling lifted this dormancy, which was materialized by a high seed germination rate, showing the involvement of the fruit shell in dormancy. Bamba et al.,2018 and Houphouet et al.,2022, also lifted seed dormancy using seed hulling, seed scarification was applied by Peter et al.,2021 and alternating seed soaking and drying was used by Yogeshwar et al.,2024.

Under shade, best results were also obtained with hulled seeds. The germination rates obtained in our study under shade conditions are higher than those of Bamba et al.,2018 and Adou et al.,2018 , who obtained rates below 69% under similar conditions. This high rate could be explained by the combination of several factors, among others, seed pretreatment, the use of healthy seed on healthy soil and the availability of vital natural resources such as oxygen, light and moisture and soil nutrients (Houphouet et al,2022).

The first day of germination was fairly short (3 days) with hulled seeds and longer (minimum 7 days) with unhulled seeds. It was constant between the two growing environments for most treatments. The last day of germination was fairly short (17 days) with hulled seeds and longer with unhulled seeds (22 days). The last day was longer in the tunnel than under shade for all treatments (Table 1). The germination peak varied from the 5th to the 7th day after sowing for hulled seeds, with an average of 5 days after sowing. It was longer for unhulled seeds, ranging from 14 to 19 days after sowing, with an average of 17 days after sowing. Peak germination was shorter under tunnel conditions than under shade conditions. The results in our study were better than those obtained by Bamba et al.,2018 and Houphouet et al.,2022, who respectively obtained germination times of 7 and 5 days. In addition, under *in vitro* culture conditions, the germination time were shorter (2 days) due to the optimal culture medium conditions (Johnson et al., 2020).

Table 1. Germinative characteristics of seeds according to treatments and Growing environments

Treatments	Growing environments	First day of germination	Last day of germination	Germination peak (day)	Germination rate (%)	Mean germination time
SD_Ts	Shade	3	15	7	76.67±2.88a	7.51±0.96c
SD_Ts	Tunnel	3	26	5	81.67±2.88a	7.11±2.07c
SD_Tx	Shade	3	19	6	71.66±11.54a	7.39±1.08c
SD_Tx	Tunnel	3	20	5	73.33±2.88a	7.16±1.08c
SDT_Ts	Shade	3	11	6	23.33±11.54cd	5.89±0.82c
SDT_Ts	Tunnel	3	18	5	60±17.32ab	7.95±1.33c
SDT_Tx	Shade	3	16	7	36.67±10.40bcd	6.51±0.78c
SDT_Tx	Tunnel	3	18	5	45±13.22bcd	7.81±1.90c
SND_Ts	Shade	9	26	14	40±8.66bcd	14.69±1.04ab
SND_Ts	Tunnel	13	26	19	55±13.22abc	18.39±1.09a
SND_Tx	Shade	8	21	15	36.67±18.92bcd	16.17±1.72a
SND_Tx	Tunnel	8	22	16	36.67±10.40bcd	18.97±3.50a
SNDT_Ts	Shade	7	19	17	20±13.22d	14.51±1.5ab
SNDT_Ts	Tunnel	7	19	18	17.5±10.60d	15.45±1.34ab
SNDT_Tx	Shade	10	20	17	20±15d	16.80±2.46a
SNDT_Tx	Tunnel	11	23	19	28.33±10.40bcd	15.87±1.13ab
P_value (treatments)					p=0.001 (**)	
P_value (media)					0.011 (**)	
P_value (treatments x media)					0.164 (ns)	

*SD_Ts: Soaked hulled seeds sown in black soil; SD_Tx: Soaked hulled seeds sown in potting soil; SDT_Ts: Soaked hulled seeds sown in black soil; SDT_Tx: Soaked hulled seeds sown in potting soil; SND_Ts: Unhulled seeds sown in black soil; SND_Tx: Unhulled seeds sown in potting soil; w SNDT_Ts: Soaked unhulled seeds sown in black soil; SNDT_Tx: Soaked unhulled seeds sown in potting soil; Means followed by the same letter in the same column are not significantly different at the 5% threshold. ** : Significant difference at 1%. ns : Difference not significant at 5% level*

Table 2. Effect of substrates on seed germination rate

Growing environments	Substrates	Germination rate (%)
Tunnel	Black earth	56.81±24.62a
	Potting soil	57.83± 19.63a
P-value (test T)		0.25 (ns)
Shade	Black earth	40.01±24.95a
	Potting soil	41.25± 23.17a
P-value (T-test)		0.9 (ns)

Means followed by the same letter in the same column are not significantly different at the 5% threshold

Table 3. Description of the growth parameters of seedlings in Growing environments at two months after sowing

Treatments	Growing environments	Plant height (cm)	Collar diameter (cm)	Number of leaves
SD_Ts	Shade	12.84±3.81b	1.95±0.66a	5.11±1.49ab
SD_Ts	Tunnel	20.38±5.21a	1.45±0.46b	6.4±2.28a
SD_Tx	Shade	8.42±2.19c	1.16±0.39c	4.48±1.36b
SD_Tx	Tunnel	10.25±3.89b	1.03±0.43b	4.96±2.11bab
SDT_Ts	Shade	12.03±4.89b	1.55±0.46b	5.25±1.54ab
SDT_Ts	Tunnel	17.69±5.13ab	1.44±0.44a	6.69±1.40a
SDT_Tx	Shade	7.43±2.09c	1.40±2.81a	4.5±1.04ab
SDT_Tx	Tunnel	11.56±3.03b	0.99±0.32	5.13±1.65ab
P-value		<0.001	<0.001	<0.001

Means followed by the same letter in the same column are not significantly different at the 5% threshold

The analysis of variance (Treatment: pretreatment_substrates and medium) revealed a significant difference ($p<0.001$) at the 5% threshold for the treatment and medium factors taken individually. However, their interaction was not significant ($p= 0.164$) (Table 1). Also, no significant difference ($P>0.05$) was found between substrates with regard to seed germination rates in the different growing media (Table 2).

3.1.2 Mean germination time

Seed germination speed was assessed by mean germination time (TMG). The results showed a highly significant difference ($p<0.001$) between treatments at the 5% threshold (Table 1). This difference was clearly observed between hulled and unhulled seeds. Hulled seeds recorded the best mean germination times, with times ranging from 5.89 to 7.95 days in the SDT-Ts (shade) and SDT-Ts (tunnel) treatments. Unhulled seeds had fairly long average germination times, ranging from 14.51 to 18.97 days in the SND-Ts (shade house) and SND-Tx (tunnel) treatments respectively. Substrate and growing medium also had little influence on germination speed.

3.2 Influence of the Growing Environments on Seedling Growth Parameters

The evaluation of the influence of the growing environments on the growth parameters of seedling from dehulled seeds showed a highly significant difference ($p<0.001$) between treatments in terms of the traits assessed. Table 3 shows that for most treatments, seedling heights and number of leaves are higher under the tunnel than under the shade, particularly for treatments SD_Ts and SDT_Ts. This suggests that the tunnel favours taller growth and the production of more leaves than the shadehouse. In addition, the results show that seedlings growing in the shade have larger diameters than those growing in the tunnel.

Seedlings under tunnel conditions grew to an average height of 20 cm in two months with the SD_Ts treatment. Height growth under tunnel conditions was higher than that obtained by Johnson et al.,2020 and Duvall,2008. These authors, working on the development of *Pterocarpus erinaceus* seedlings in nurseries in Togo and Mali respectively, obtained average heights of 15 cm after twelve months and 9 cm after sixteen weeks. This rapid growth in seedling height and number of leaves in our study could be explained by the high temperature and relative humidity of tunnel conditions. The development of our seedlings in the nursery was faster than those obtained by Adji et al., 2021. In their work, these authors observed an average seedling height of 15 cm, a diameter of 2.50 mm and 7 leaves after six months. Our seedlings, only two months old, already had a height of 20 cm, 7 leaves and a diameter of 1.45 mm under tunnel conditions. Tunnel conditions would thus reduce the time taken by seedlings in the nursery before planting as part of its silviculture. In our study, the seedlings were more vigorous on the sterilized black soil than on the potting soil under shade conditions, with higher collar diameters. This could be explained by the nutrient-rich nature of the soil collected from under the trees in UPGC's botanical garden. In fact, the garden is a natural ecosystem not exploited by man, whose soil has been enriched over the years by the decomposition of organic matter.

4. CONCLUSION

We conclude from the above findings that results obtained showed that applied treatments had a strong impact on the germination of Pterocarpus erinaceus seeds, with an effectiveness that depended on the growing environment. Dehulled seeds, stripped of their hulls, achieved rapid germination and a high germination rate, especially under tunnel conditions on sterilized black soil. Also, seedlings obtained from hulled seeds had good growth and development under tunnel conditions and also under shade, which considerably reduced the time spent in the nursery before planting as part of its silviculture and the reconstitution of disappearing natural resources. Thus, hulling seeds grown on good, healthy black soil in tunnel and under shade conditions can be recommended to growers and nurserymen for better seedling reproduction.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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