

Characterization of Germination and Growth of *Pterocarpus erinaceus* Poir. From Togo

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Abstract: *Pterocarpus erinaceus* Poir. is a woody species with high economic value, endemic to West Africa. Anthropogenic pressures, seminal production reduction, the rapid loss of seeds' germinal capacity, and especially the seedlings' difficulty to develop, endanger the regeneration and the survival rate of stands. This study aims to deepen scientific knowledge on the *Pterocarpus erinaceus* propagation through germination characterization, seeds' germinal capacity preservation, and its growth in Togo. Experimentations were carried out with seeds harvested in three phytogeographical zones in Togo. Results show that the germination percentages obtained are the same for the three phytogeographical zones i.e. 85%. Furthermore, the mean germination times (MTG) is short and equivalent to 5 days. Besides, storing the seeds in the refrigerator at 7°C or in a laboratory room at 25°C, allowed them to maintain their germinal capacity i.e. about 50% after 18 months. Finally, the seedlings growth is slow and characterized by rhythmicity, specifically observed by a slowdown between the 5th and 11th weeks in foliar development for all phytogeographical zones. After 16 weeks of development, an average seedlings have a size of 9.6 cm and carry 10 nodes and 4 leaves.

Keywords: *Pterocarpus erinaceus*, phytogeographical zone, germination, seed storage, growth, Togo

1. Introduction

The uncontrolled and unregulated exploitation of forest formations combined with the effects of climatic change lead to a rapid or even irreversible decline in some very useful species [1]. *Pterocarpus erinaceus* Poir, of the Fabaceae family, a tree in the Sahelian, Sudanian and Guinean zones [2, 3] is one of these overexploited species. Its inclusion in 2016 in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora [4] clearly indicates that its trade on world markets is jeopardizing its survival. Hence, in Togo, a moratorium has been imposed since June 2016. It prohibits any form of exploitation of the species for 10 years. This moratorium is intended not only to promote the natural recovery of stands but also to implement effective and efficient strategies for the production of plants and collect information regarding the biology and culture of the species [5].

In the process of regeneration, stand replenishment, or forest production, the most important and most vulnerable stage called the "establishment phase" is seed germination and seedling survival [6, 7]. It is essential to understand these physiological processes and to fully recognize the importance of good forest nursery cultivation practices and plant quality, to ensure the sustainability and profitability of agroforestry systems. The controlled production of quality plants becomes a primary concern, and at the same time constitutes a guarantee of success for domestication, its adoption in large-scale reforestation programs, and also its preservation from plausible extinction. Thus, high productivity, decisive in the establishment of vigorous plants of high quality requires a thorough study of the germinative characteristics of the seeds

harvested in the stands of *P. erinaceus* in Togo. According to Muok et al. [8], Thiombiano et al. [9], and Kokou et al. [10], in addition to the observation of reduced seminal production of trees, there is a rapid loss of germination capacity in the species. The production of high-performance plant material under controlled conditions is a fundamental step for forest planting and restoration programs. It is therefore essential to pay particular attention to the early years of plant development [5, 11]. According to studies by Bationo et al. [12] and Ouédraogo et al. [13], *P. erinaceus* plants have difficulty in passing the first stages of growth after germination.

The aim of this study is to further the knowledge of germination and understanding of the first weeks of growth stages of *P. erinaceus* seedlings. Specifically, it proposes to determine the germination characteristics of seeds from the three phytogeographical zones in Togo, evaluate the effect of shelf life and storage conditions on seed longevity, and study the initial growth of seedlings in the greenhouse after germination for 16 weeks. Reliable scientific data on these aspects is an essential phase in the implementation of effective strategies for the sustainable production and exploitation of *P. erinaceus*.

2. Materials et methods

2.1. Experimental site

The tests are carried out in parallel in the greenhouse and the Laboratory of Forestry Research on the university campus in Lomé, capital of Togo. The site belongs to ecological zone V of the country defined by Ern [14]. It enjoys a subequatorial climatic of Guinean type with an average rainfall varying

between 800 and 900 mm/year because the Togolese coastline is located on the drought diagonal that extends from Tema in Ghana to Grand-Popo in Benin. Average temperatures vary very little over the year (annual averages of $28 \pm 2^\circ\text{C}$) [15].

2.2. Provenance, disinfection of fruits and preservation of seeds

The seeds used come from five natural stands of *P. erinaceus* distributed over its entire geographical area in Togo along a south-north gradient (Figure 1). Fruit harvest took place in February 2018. From 25 to 31 individuals of good morphologic form [16] at were taken from each of the five sites. We selected trees separated by at least 20 meters to avoid seed selection from the same individual [17, 18].

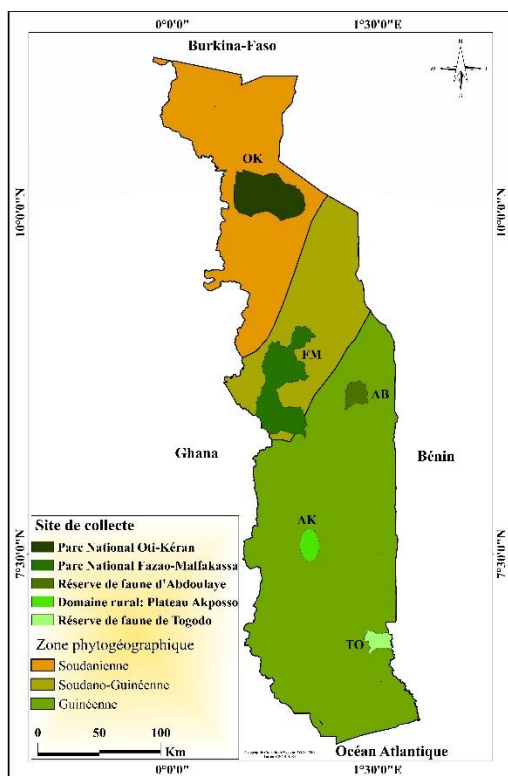


Figure 1: Location of plant material collection sites from north to south: Oti-Kéran National Park (OK), Fazao-Malfakassa National Park (FM), Abdoulaye Wildlife Reserve (AB), Akposso Plateau Rural Domain (AK) and Togodo Wildlife Reserve (TO)

Two types of sites have been surveyed, including four protected areas and one rural area (stands in unprotected areas). According to phytogeographical zones there are one site in the Sudanian zone (Oti-Kéran Park), one site in the Sudano-Guinean zone (Fazao-Malfakassa Park), and three sites in the Guinean zone (Abdoulaye Reserve, Akposso Plateau rural domain and Togodo Reserve). Table 1 describes the biophysical characteristics of the 3 phytogeographical zones in which the collections were done.

Three weeks after collection, the fruits were surface-sterilized by pre-soaking for 30 minutes in a solution of 1% sodium hypochlorite. Seeds were then extracted manually, sorted and measurements of length, thickness and individual weight were done.

The healthy sorted seeds are divided into two lots: one for the tests of characterization of the germination according to phytogeographical zones, and the other preserved in 49 mm high and 29 mm diameter plastic boxes for determining the conservation of germination capacity

Table 1: Biophysical characteristics of provenances

Phytogeographical zones	Biophysical characteristics		Collecting sites
	Climatic	Soils	
Sudanian zone	Sudanian tropical with one dry and one rainy season Annual rainfall: 800 to 1300mm/year Average temperature: 28°C	Tropical ferruginous soils with sandstone associated with pink quartzites and two-mica quartzites	Protected area: National Park Oti-Kéran
Sudano-guinean transition zone	Relatively nuanced climatic from subequatorial medium-altitude climatic to tropical semi-humid climatic with one dry and one rainy season Rainfall: 1200 mm to 1300mm/year Average temperatures: 24-26°C	Shallow, tropical ferruginous soils with a red silty-clay texture with ferruginous concretions	Protected area : National Park Fazao-Malfakassa
Guinean zone	Equatorial Guinean with two dry seasons and two wet seasons of unequal duration Rainfall: 1000 mm to 1400mm/year Average temperature: 27°C	Tropical ferralitic and ferruginous soils, deep hydromorphia, moderately leached and concretioned	Protected area: Abdoulaye Wildlife Reserve
			Protected area: Togodo Wildlife Reserve
			Rural Domain: Plateau Akposso

2.3. Germination test

The first germination test was carried out with disinfected seeds under a laminar flow hood. This disinfection consisted primarily of soaking in a 70° ethanol solution for 2 minutes. The seeds are then soaked in a 50% solution of Betadine (Povidone iodine: 10g per 100ml) for 3 minutes, then in a diluted solution (50%) of Domestos (sodium hypochlorite at 4.8 g per 100g; Unilever® France) for 2 minutes before finishing with 6 successive rinses with sterile distilled water. Thirty seeds per zone in Petriplates (90 x 90 mm²) with 10 seeds per box corresponding to 3 replicates of 10 seeds, are randomly used for germination tests. The germinations are performed in Petriplates with lids, and bottom lined with cotton soaked in distilled water to ensure permanent moisture to the seeds. The closed Petriplates are placed in a culture

room at $25 \pm 2^\circ\text{C}$, with a relative humidity of 60%, under a 16-hour photoperiodic light regime followed by 8 hours of darkness with a luminous intensity of $120 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Their position is changed every two days. Water is regularly supplied to keep the cotton moist. Seeds were considered as germinated upon radical emergence (it breaks through the seed coat). The cultures were observed daily and the germinated seeds were counted daily from the day of sowing.

2.4. Study of germinal capacity

Closed plastic boxes 49 mm high and 29 mm diameter, used for packaging and containing seeds obtained from the manual shelling of fruit, are kept under two conditions: (1) at room temperature in the laboratory at $25 \pm 2^\circ\text{C}$ with 60% relative humidity; (2) in the refrigerator at $7 \pm 2^\circ\text{C}$ with 11% relative humidity.

Regular and periodic germination tests are carried out to evaluate the conservation of germinal capacity and therefore viability of the seeds according to the storage conditions and duration after harvest. Since the objective is to keep the seeds for one year before assessing this capacity, germination tests are carried out 12 months, 14 months, 16 months and 18 months after harvest.

The parameters evaluated are: cumulative germination percentage (CGP), germination speed and mean germination time (MGT).

2.5. Effect of seed collection location on germination

For each of the three phytogeographical zones, germinations were carried out with seeds collected directly from tree crown and seeds collected on the ground under the trees. When considering each collection site, crown or soil, a total of 30 seeds per zones is used randomly in Petriplates (90 x 90 mm²) with 10 seeds per plate corresponding to 3 replicates of 10 seeds. The conditions for disinfection, germination and culture were the same as described above.

The parameters evaluated are: cumulative germination percentage (CGP), germination speed and mean germination time (MGT).

2.6. Seedling growth

After germination, growth is monitored in the greenhouse to assess the variability of the parameters chosen: the total height of the stem expressed in centimeters (cm), the number of nodes and the number of leaves formed. For this purpose, 20 seedlings per phytogeographical zone from the previous germination tests were selected based on their vigor. They are transplanted in polyethene bags (24 cm high and 7.5 cm in diameter) containing a mixture of sea sand and garden soil (composition in Appendix) in the proportion 50/50 (v/v). The seedlings are placed in a shady, secure compartment. The frequency of sprinkler irrigation is set at 4 waterings per day for 20 seconds per 90-minute interval between 10:00 and 15:00. This programming lasted 4 weeks and then changed to 2 manual waterings per day.

Growth measurements are made regularly weekly from day 15 after sowing for 16 consecutive weeks.

3. Statistical data analysis

The calculation of germination parameters is carried out as follows:

- The cumulative germination percentage (CGP):

$$CGP = \frac{\text{number of seeds germinated}}{\text{total number of seeds}} \times 100$$

- The germination speed expressed by the number of seeds germinated per day;
- And the mean germination time (MGT)

$$MGT = \frac{N1J1 + N2J2 + \dots + NnJn}{N1 + N2 + \dots + Nn}$$

with N1 representing the cumulative number of germinations during the first day (J1), N2 the cumulative number of germinations during the second day (J2),..., Nn the cumulative number of germinations during the last day (Jn).

The means of germination and seedling growth parameters (total stem height, number of nodes and leaves) of the populations in each zone are compared using an analysis of variance (ANOVA). The Tukey test, applied at the 5% threshold, is used to determine significant differences between the ANOVA group averages. All statistical analyses are performed with the R software [19].

4. Results

4.1 Study of germinal capacity

4.1.1. Characterization of germination

Daily germination kinetics indicated that germination began on day 2 and ended on day 15 (Figure 2). On day 5 after sowing 60% of the seeds germinated regardless of the origin of the seeds. On day 7 after sowing, the average percentage increases to at least 72% for the seeds of the Sudanian, Sudano-Guinean and Guinean zones. On day 11, germination averages 80%. At day 15 marking the end of the process, there was no significant difference (Tukey test at the threshold of 5%, $p 0.05 = 0.1311$) between the percentages obtained which were found to be high for the seeds collected into the Sudanian zone, 86.4%, Sudano-Guinean zone, 76.4% and in the Guinean zone, 86.6% (Figure 2).

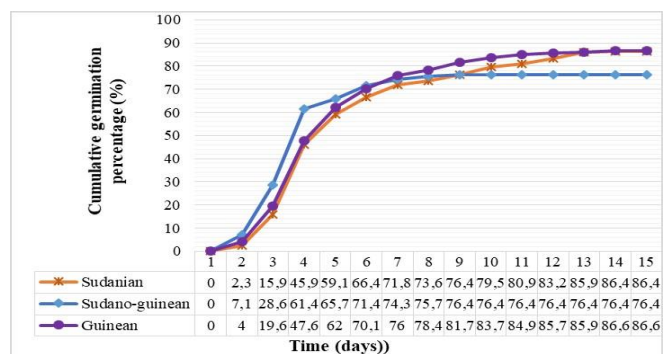


Figure 2: Evolution of *P. erinaceus* germination according to the phytogeographical zone of origin of the seeds

The process of seed germination was accomplished in 3 phases: the imbibition of the seeds, the *germination sensu stricto* and the elongation of the radicle. Imbibition begins from seed sowing (Figure 3A) until the first germinations are observed. It consists of rapid and passive water input (absorption) into the seeds whose volume visibly increases from the day of sowing to the next day, in 24 h (Figure 3A and 3B). Concomitantly with swelling of the seed and resumption of metabolic activities, root size increase is initiated and the root exerts pressure on the softened integuments through the entry of the water until their rupture contributing to its exit. It is from this moment that the first germinations are recorded and *germination sensu stricto* begins. On average on day 2, 5% of seeded seeds have roots that pierce the outer shell.

This cumulated percentage increases exponentially between day 3 and day 6. Within the time frame of 72 to 144 hours after sowing (72 hours), the same steps are repeated (swelling of the seeds, resumption of metabolic activity, increase root size and pressure on seed shells). Consequently, the majority of the seeds sown, finish the germination process. Thus, the average percentage of germination increases from 10 to 70% (Figure 3C).

From day 7 onwards, the cumulated germination percentages recorded, change less rapidly and even tend to stabilize. This is reflected by the plateau observed (Figure 2). In average, 7-20% of the seeds germinate at this same period. During this time, for 70% of the seeds that have germinated, the roots lengthen while the cotyledons still well soaked, grow and emerge from the seed coat (Figure 3D).

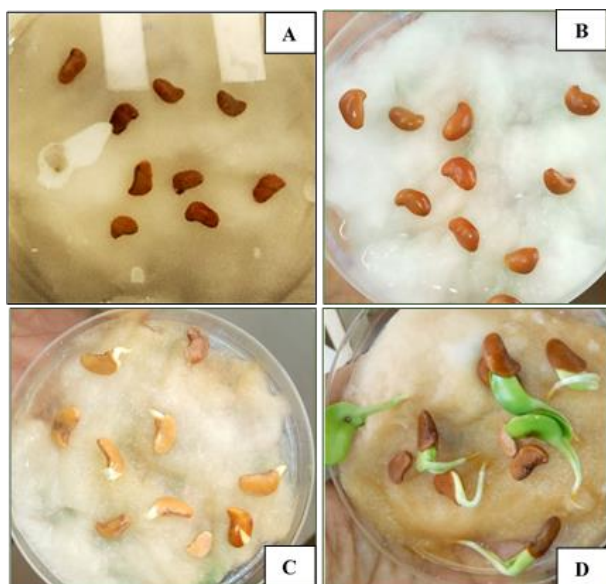


Figure 3: Sequence of *P. erinaceus* germination.

A = Sowing seeds on cotton; B = Seed imbibition; C = Germination *sensu stricto*; D = Lengthening of the radicle and greening of the cotyledons.

The germination speed reaches its highest point on day 4, regardless of the zone considered. In the Sudano-Guinean, it is highest with nearly 4 seeds sprouting simultaneously per day, which results in the highest peak in Figure 4 on the day considered. For the Sudanian and Guinean zones, 3 sprouted

seeds are assessed for the highest values. On days 5 and 6 in the Guinean zone, the germination speed changes on average to more than one germinated seed per day and then decreases again to less than one seed per day stopping on day 13. For seeds from the other two zones, more than one germinated seed on day 6. On the following days, less than one germinated seed per day is recorded. Sprouting stops on day 10 for seeds from the Sudano-Guinean zone and on day 14 for seeds from the Sudanian zone (Figure 4).

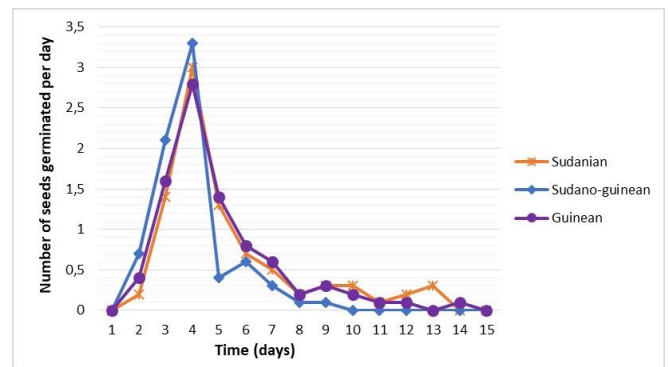


Figure 4: Germination speed of *P. erinaceus* according to phytogeographical zone

Mean germination times are equivalent ($p > 0.05$) regardless of the phytogeographical zone of the seeds. Whether in the Sudanian, Sudano-Guinean or Guinean zone, the mean germination times are around 5 days (Table 2).

Table 2: Average germination times of *P. erinaceus* according to phytogeographical zones. MGT = Mean germination time.

Phytogeographical zone	MGT (days)
Sudanian	5.1 a
Sudano-guinean	4.1 a
Guinean	4.9 a
p-value	0,065

Test of Tukey: the values followed by the same letter on one column are not significantly different at the threshold of alpha = 0.05.

4.1.2 Preservation of germinal capacity

Freshly harvested seeds showed that the cumulated germination percentages amount to 84% with a mean germination times of 5 days. The assessment of germination capacity after 12 months, does not reveal any decrease in germination percentages or change in mean germination time under any storage condition. Statistical differences begin to appear from the 14th month after the harvest (Figure 5).

With seeds from the Sudanian zone stored in the refrigerator at $7 \pm 2^\circ\text{C}$ and with 11% relative humidity (Figure 5A), the germination cumulated percentage is high and constant until month 16. The decrease noticed at month 18 is significant ($p = 1.8 \cdot 10^{-2}$) with a 53% decrease in germinative capacity. 18 months after storage, the seeds lost in average 40% of their initial germination capacity i.e. from 84% post-harvest germinative capacity to 47%.

For seeds from the Sudano-Guinean zone, the initial germination percentage of 84% remains constant until month 16. Same as in the Sudanian zone, the percentages of germination obtained do not vary during this period. At month

18, this percentage decreased significantly ($p= 4.0.10^{-4}$) to 37%. Henceforth, seeds from this zone lost more than 50% of their initial germinative ability i.e. from 84% to 37%.

Conservation has a highly significant effect ($p= 1.93.10^{-12}$) on germinative capacity at month 18 for seeds from the Guinean zone. Therefore, 49% of seeds germinate.

For seeds stored at $25 \pm 2^\circ\text{C}$ (Figure 5B) and specifically those from the Sudanian zone, the initial germination percentage remains constant until month 16. At month 18, the cumulated percentage recorded is 57 ($p= 2.8.10^{-3}$), which results in a serious decrease in germinative capacity of 30%.

Under the same storage condition, the decrease became significant ($p= 2.8 * 10^{-3}$) for the seeds in the Sudano-Guinean zone at month 14 (56%). The germinative capacity decreases radically and significantly in month 18 where seeds retain only 13%.

For seeds from the Guinean zone, the germination percentage decreases significantly ($p= 4.44.10^{-8}$) in month 18 i.e. 40%. The germinative capacity decrease by 50% after 18 months of seeds storage.

For the mean germination times assessed for the two storage conditions tested, there are significant differences (Table 3). At $7 \pm 2^\circ\text{C}$, after the first 12 months of storage, the observed MGT remains similar to the one observed initially at month 0.

Specifically, seeds from the Sudanian zone took an average of 6 days to germinate after 12 months (5.7 days), 14 months (5.9 days), 16 months (5.9 days) and then a statistically ($p= 4.83.10^{-2}$) longer time at 18 months, 8.6 days.

With the Sudano-Guinean zone seeds, the mean germination increases significantly ($p = 2.0.10^{-3}$) over the same period. From month 12 to month 16, the MGT is 7 days and extends by 2 days at the end of month 18 (9 days).

For seeds from the Guinean zone, the average germination becomes highly significant ($p = 1.75.10^{-13}$) from month 12 to month 18. During this period, the mean germination times are equivalent to each other (7 days on average), and therefore 2 days longer than recorded for month 0.

Observations made for the evaluation of this parameter at room temperature of laboratory i.e. $25 \pm 2^\circ\text{C}$, indicate that seeds from the Sudanian zone took 5.9 days in average to germinate after 12, 14 and 16 months; then 7.2 days to germinate after 18 months. Based on this observation, we deduce that the mean germination time becomes significantly longer with $p\text{-value}= 2.18.10^{-2}$.

For the seeds of the Sudano-Guinean zone, starting from month 14, we noticed that this time is lengthened in a highly significant way by 3 days, or 7 days to germinate, until month 18 ($p= 9.47.10^{-9}$).

For seeds from the Guinean zone, from month 12, the observed differences are significant ($3.94.10^{-7}$). There was an elongation of 3 days (6.9 days for month 12, 7 days for month 14, 7.6 days for month 16 and 7.9 days for month 18) to germinate, displaying the same behavior for both the MGT and the germination percentages recorded.

Regardless of seeds storage conditions, there was an overall slowdown in germination speed, but this slowdown occurred at month 18 when stored in the refrigerator and 4 months earlier (month 14) when stored at room temperature in the laboratory. However, it can be said that the seeds of *P. erinaceus*, whatever the phytogeographical zone, retains all their germinative capacity for one year and 50% beyond 14 months under accessible storage conditions.

The initial germination capacity assessment of *P. erinaceus* indicated a high cumulative percentage (84%) equivalent in the three phytogeographical zones. However, a certain and significant lengthening of the mean germination time of the seeds of all origins is observed, which shows an exceptional capacity of adaptation of the species in terms of survival by generative reproduction.

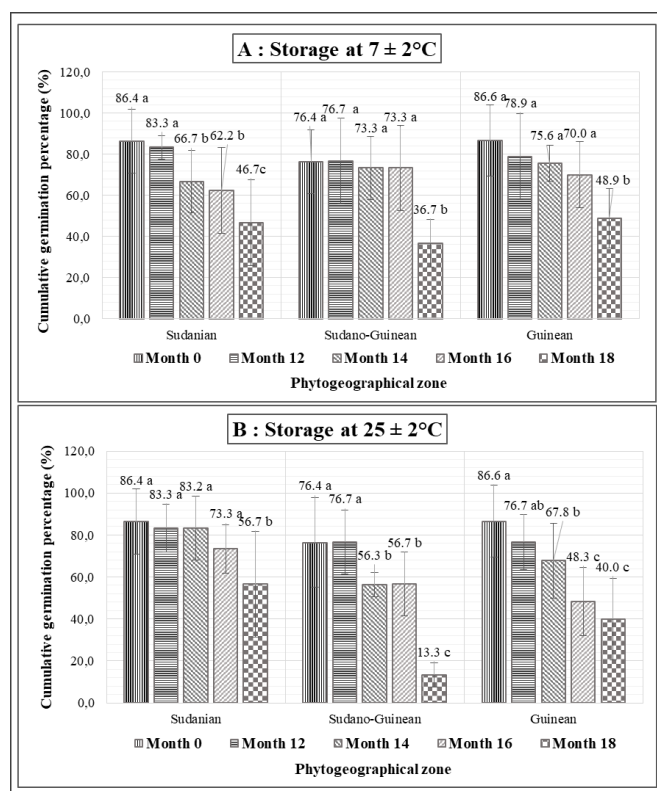


Figure 5: Cumulative germination percentage of *P. erinaceus* after seed storage.

A= Storage at $7 \pm 2^\circ\text{C}$ /11% relative humidity; B= Storage at $25 \pm 2^\circ\text{C}$ /60% relative humidity.

Test of Tukey: the values followed by the same letter on the phytogeographical zones histograms are not significantly different at the threshold of $\alpha = 0.05$.

Comparing the germination capacities of the seeds kept in the refrigerator ($7 \pm 2^\circ\text{C}$) and those kept at laboratory room ($25 \pm 2^\circ\text{C}$) for the timeline (12, 14, 16 and 18 months), does not reveal important differences ($p > 0.05$) regardless of the phytogeographical zone of origin. Consequently, the storage conditions ($7 \pm 2^\circ\text{C}$ or $25 \pm 2^\circ\text{C}$) did not influence the germination capacities recorded.

Table 3: Mean germination times of *P. erinaceus* after seed storage

Phytogeographical zones	7 ± 2°C / 11% relative humidity						25 ± 2°C / 60% relative humidity					
	Month 0	Month 12	Month 14	Month 16	Month 18	p-value	Month 0	Month 12	Month 14	Month 16	Month 18	p-value
Sudanian	5.4a	5.7a	5.9a	5.9a	8.6b	4.83.10 ⁻²	5.4a	5.9ab	6.7ab	6.9ab	7.2b	2.18.10 ⁻²
Sudano-Guinean	4.1a	6.5b	6.2b	6.9b	8.8c	2.0.10 ⁻³	4.1a	4.7a	6.6b	7b	7.3b	9.47.10 ⁻⁹
Guinean	4.9a	6.9b	6.4b	6.5b	6.6b	1.75.10 ⁻¹³	4.9a	6.9b	7b	7.6b	7.9b	3.94.10 ⁻⁷

Test of Tukey: the values followed by the same letter on one line are not significantly different at the threshold of alpha = 0.05.

4.1.3. Seed collection location and germination

All the seeds, whether they were collected directly from tree crown or on the ground under the trees in the different phytogeographical zones, germinate with high percentages i.e. more than 55% (Figure 6). In Sudanian and Guinean zones, there is no significant difference (p= 0.1193 and p= 0.1757) between the cumulative germination percentages recorded (87%) according to the crown and soil seeds collection location. It is only for the Sudano-guinean zone that the location seems to have an effect on the germination capacities of the seeds. The higher percentage is obtained with seeds collected directly on the crown compared to those collected on the ground with a highly significant difference of more than 20% (p = 1.07.10⁻³) for this zone (CGP crown = 77% > CGP soil= 55%, Figure 6).

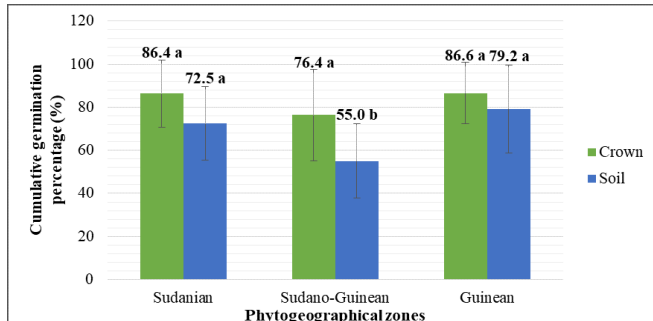


Figure 6: Cumulative germination percentage of *P. erinaceus* according to seed collection location

Mean germination time is around 4-6 days for seeds extracted from fruits collected on the ground in the three phytogeographical zones. The analysis of variance shows that in Sudanian and Guinean zones, regardless of collection location, the seeds germinate in 5 days (p > 0.05). It is only in the Sudano-guinean zone that the seeds collected on the ground recorded a mean germination time (p= 0.001) extended of two days compared to those collected directly on the crown (Table 4).

Table 4: Mean germination times (MGT) of *P. erinaceus* according to seed collection location

Phytogeographical zones	MGT (day)		p-value
	Crown	Soil	
Sudanian	5.1 a	6.5 a	0.3129
Sudano-Guinean	4.1 b	6.6 a	0.0010
Guinean	4.9 a	5.2 a	0.7447

Test of Tukey: the values followed by the same letter on one line are not significantly different at the threshold of alpha = 0.05

4.1.3. Study of the correlation between seeds morphological characteristics and germination

The analysis of correlations indicates that the germination capacities recorded have virtually no correlation with the morphological characteristics of seeds (Table 5). For the cumulative germination percentages, the correlations varied from 0.05 with thickness to 0.18 with seeds weight. For seeds mean germination times, the correlations remain low in the order of 0.18 between it and the length of seeds to 0.27 between it and its weight (Table 5). The only high, positive and statistically significant correlation (p = 0.001) is between the length of seed and its weight (r= 0.65).

Table 5: Correlation between seeds morphological characteristics and germination capacities of *P. erinaceus*

	MGT	CGP	LS	TS	WS
MGT	1	0.23 ***	0.18 ***	0.12	0.27 ***
CGP		1	0.06	0.05	0.18
LS			1	0.42 ***	0.65 ***
TS				1	0.36 ***
WS					1

Significance codes: *≤ 0.05; **≤ 0.01; *** = 0.001. MGT= Mean germination time; CGP= Cumulative germination percentage; LS= Length of seed; TS = Thickness of seed; WS= Weight of seed

4.2 Seedlings growth

4.2.1. Seedling height and number of nodes

Stem height growth curves showed a similar pattern for all three seedling lots, and this pattern is characterized by a slow and fairly regular pattern during the 16 weeks of culture

(Figure 6). Indeed, the first week after transplanting the seedlings, their mean height ranged from 2.7 ± 0.8 cm (Sudano-Guinean zone) to 2.4 ± 0.8 cm (Guinean zone). Between week 1 and week 4, the values of the increases recorded ranged from a minimum of 3 cm for the plantlets of the Sudanian (5.6 ± 1.2 cm) and Sudanese-Guinean (5.4 ± 21.9 cm) zones, to a maximum of 4 cm for those of the Guinean zone (6.2 ± 1.6 cm). From week 5, the speed of elongation of the stems decreases and until week 8. The total increase varies from 1.2 cm to 1.5 cm, or an average of 1.4 cm in this time interval (week 5 to week 8). Over the next eight weeks, the slowdown became more pronounced. This resulted in mean monthly gains in height of 0.5 cm from week 9 to week 12, and 0.6 cm from week 13 to week 16. At the end of the 16 weeks, the seedlings have equivalent sizes ($p=0.27$) of 9.1 ± 1.9 cm for those of the Sudanian zone, 9.3 ± 2.3 cm for those of the Sudano-Guinean zone and 9.9 ± 2.1 cm for those of the Guinean zone.

Analyzing the curves of the number of nodes on the seedlings over time, it is apparent that the behavior of the seedlings of the three phytogeographical zones is similar (Figure 6). Thus, in the first week, they have on average 2 nodes, 2.9 ± 0.7 nodes for those of the Sudanian zone, 2.4 ± 0.7 nodes for those of the Sudano-Guinean zone and 2.5 ± 0.8 nodes for those of the Guinean zone. In week 4, the initial number doubled to an average of 5.5 ± 0.8 nodes for the Sudanian zone, 4.5 ± 0.9 nodes for the Sudano-Guinean zone and 4.9 ± 1.1 nodes for the Guinean zone. The formation of nodes is similar from one zone to another. From this period, a mean monthly production of 2 nodes is evaluated until the end of the experiment. At the end, 11.1 ± 1.6 nodes, 9.7 ± 0.8 nodes, and 10.4 ± 1.9 nodes, respectively for Sudanian, Sudano-guinean and Guinean zones, were recorded. Comparison of these mean values indicates no influence ($p=0.88$) of phytogeographical zone of provenance on this parameter. Plantlets have an average of 10 nodes.

Comparison of these mean values indicates a significant influence ($p=0.0404$) of provenance for this parameter. Seedlings from the Sudano-Guinean zone produced fewer nodes (9 nodes) than those from other Sudanian (11 nodes) and Guinean zones (11 nodes).

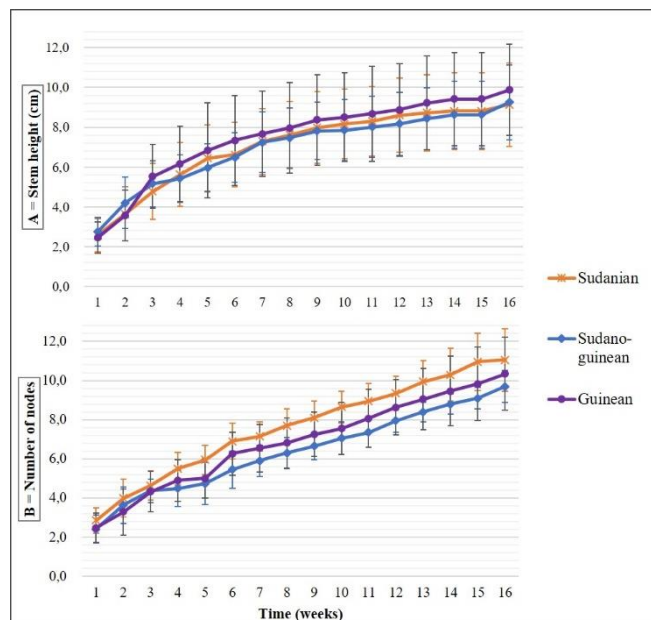


Figure 6: Evolution of height growth and number of nodes of *P. erinaceus* seedlings in the nursery over 16 weeks (error bars = standard deviations).

A= Height of the stem; B= Number of nodes.

4.2.2. Number of leaves

Leaf development over the weeks was marked by three phases, including a strong phase of production, followed by a slowdown and again production (Figure 7). During the first week of evaluation, seedlings had on average 2 leaves, 2.4 ± 0.7 for those in the Sudanian zone, 2.4 ± 0.7 for those in the Sudano-Guinean zone and 2.5 ± 0.8 for those in the Guinean zone. In week 4, an average production of 2 to 3 leaves was found for the three zones of the seedlings. From week 5, seedlings of Sudanian zone recorded the higher number of leaves (5.8 ± 0.89 leaves; $p=0.02$) compared to seedlings in the other two zones. It is only from this week that leaf fall begins and ends on average at week 8. Thus the seedlings lose on average one leaf until week 8. At week 10 the mean value for leaf number recorded is 3.0 ± 0.8 leaves. The resumption of foliar development occurs in weeks 11 and 12 with the emission of a new leaf and is recorded in week 16, 4.2 ± 1.2 leaves for the seedlings of the Sudanese zone, 3.9 ± 1.1 leaves for those of the Sudanese-Guinean zone and 4.1 ± 1.2 leaves for seedlings in the Guinean zone. In the end, the seedlings lose an average of 4 leaves and the results of the variance analysis according to the "phytogeographical zone" classification criterion applied to the three populations do not reveal any significant difference ($p=0.57$) in the total number of leaves emitted after 16 weeks of growth.

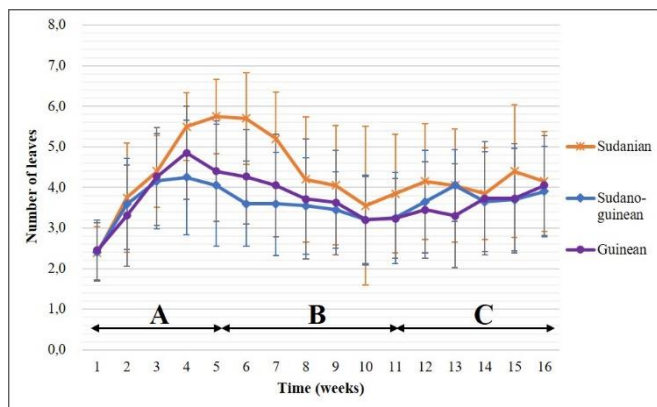


Figure 7: Evolution of leaf development of *P. erinaceus* seedlings in the nursery over 16 weeks (error bars = standard deviations).

A = Leaf formation; B = Loss of leaves; C = Formation of new leaves

The principal component analysis performed on data recorded in week 16 for the different growth parameters evaluated (height of the stem, number of nodes and leaves), indicates that all the variability (100% of the information) is represented by axes 1 and 2. Axis 1 (67%) contrasts phytoecological zones with aerial development (leaves and nodes) and axis 2 explains their distribution for the « height of the stem». The projection of the mean values of these parameters for the three populations corresponding to the three phytoecological zones (Figure 9), highlights the fact that the seedlings of the Sudanian zone discriminate by a higher foliar and nodal productions. The plants characterized by the higher stem heights are those of the Guinean zone, a characteristic correlated with axis 2 in Figure 9. Seedlings from Sudano-guinean zone appear to be the least productive compared to others.

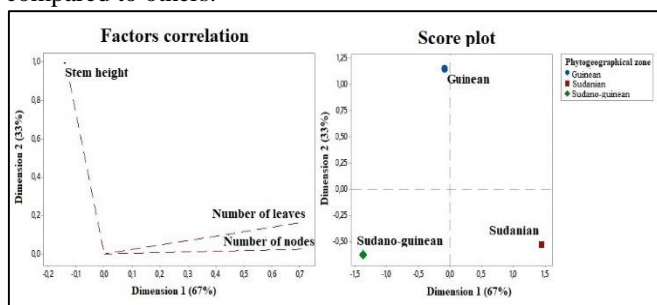


Figure 8: Factors and variables projection in the 1 x 2 factorial plane.

Monitoring of *P. erinaceus* seedlings over the 16 weeks highlights rhythmic growth. Although this growth is similar for the three phytoecological zones considering the three parameters chosen to evaluate it, the trends suggest that the most interesting seedlings would be those of the Guinean and Sudanian for which higher values of stem height, number of nodes and leaves are obtained after 16 weeks in the greenhouse.

5. Discussion

The present study investigated the behavior of *P. erinaceus* seeds of different origins located in three phytoecological zones and the growth of the seedlings obtained. *Pterocarpus*

erinaceus Poir. germinates in 5 days (mean germination time) and reaches on average about 90% of germination percentage. The values obtained attest to the good generative propagation capacity of the species, exceeding those obtained by Bamba et al. [20] in Ivory Coast, which estimated the germination capacity of this species' seeds at 68.5%. Statistical testing of final germination percentages indicated that there is no "provenance" effect on seed germination capacity. Indeed, if the phytoecological zones have an influence on the morphotype and thus the phenotype of the species [21], that have no influence on the germination percentages of the three provenance that are equivalent and high. The phenotype would be expected to influence seed germination capacity. However, it was shown that none of seed morphological characteristics (length, thickness and weight) showed a high correlation ($0.12 \leq r \leq 0.27$). The seeds of the North Sudanian zone, which are the longest and the heaviest recorded a cumulative germination percentage equivalent to those of South Guinean zone which are smaller and lighter. This nonproportional correlation between morphological characteristics and germination has also been reported for seeds of *d'Acacia fistula*, *Cassia hybrida*, *Acacia holosericea*, *Acacia carcinna* [22], de *Faidherbia albida* [23, 24] and *Cordia africana* [25].

In our experiment, germination lasted an average of 15 days and the seeds exhibited short mean germination times (MGT) of 5 days. The statistical tests did not reveal any significant difference for this parameter which is a genetic characteristic of the species which is not expected to change; the equivalent result obtained (MGT = 5 days) whatever the phytoecological zone of origin, and corresponds to what could be expected since it is the same species distributed over different climatic territories. Bewley and Black [26] explain that it is a stable germination energy derived from a species-specific genetic determinism.

During seed storage, the moisture content of a mature seed is in dynamic equilibrium with the hygrometry of the surrounding environment. Variation of this parameter from 60% to 11% in this study should result in a proportional decrease in the moisture content of *P. erinaceus* seeds. Seeds with a good ability to dehydrate, that are able without damage to be dried up to very low water levels of the order of 5-10% without losing their germinal capacity are called «orthodox» [27, 28]. The measurement of germinal capacity 18 months after the harvest of the seeds of *P. erinaceus*, resulted in the recording of germination percentages of nearly 50%. The germination capacity thus remains well beyond 18 months, which translates to an aptitude for survival for at least one unfavorable year (and even more) following the fructification of trees in natural environments.

The study of the germinal capacity of the seeds indicated that the decrease in germination percentages is progressive as the shelf life increases. These percentages are significantly lower from the 14th month on average. Similar results were obtained by Sanogo [11] by testing the germinal capacity of *Pterocarpus lucens* Lepr. seeds, packaged in glass bottles for 5 years. The germinal capacity of the seeds gradually decreased during storage in a freezer and a ventilated room.

The percentage of germination decreased by 56 to 30% on average after 5 years of storage under the test conditions. On the other hand, there was an improvement in the conservation of the germinal capacity of the seeds in the freezer with a significant decrease of 14% compared to 26% for the ventilated room. This medium minimized the increase in the water content of the seeds, which instead increased by more than 2% for those of the ventilated room. For *P. erinaceus* seeds, this increase would have caused them to deteriorate and would, therefore, explain a loss of viability. Besides, it was interesting to note that the amplitude of the decrease in germinal capacity during storage was equivalent under the conditions tested (25°C/60% relative humidity or 7°C/11% relative humidity).

Considering the mean germination times, the proportional increase in the time taken to complete the process, along with the duration of conservation, would be due to a slower mobilization of nutrient reserves, because of the state of seed degradation that would accelerate over time. This increase would occur more rapidly with seeds stored at 25°C than at 7°C. Barton [29] and Côme & Engelmann [30], talk about more significant maintenance of the viability of the seeds resulting from a slowdown in their ageing caused by this lowering of the temperature from 25°C to 7°C and water content of the seeds through relative humidity from 60% to 11%. This lengthening of the MGT can also be likened to an adaptation strategy of the species to cope with the variability of precipitation in its ecosystem [12, 31].

Several other authors working on the physiological and biochemical basis of the degradation of seed quality during storage have established that this slowdown would result from a decrease in metabolic activity of respiration, oxidation reactions, and pathogens on the surface of seed teguments or seeds [32–34]. The possible presence of pathogens can be controlled by a treatment to improve the health status or provide additional protection during germination. Therefore, the systematic use of seed disinfection by soaking in disinfectant solutions before germination is recommended [35]. It is essential to remember that the success of germination depends on the quality of seeds, thus on its physical, physiological and health characteristics [36, 37], but also on very external germination conditions provided them.

Seeds collection location, soil or directly on the crown, did not influence germination capacity. The only difference observed was for seeds from the Sudano-Guinean zone that appear to germinate better when taken from the tree (CGP crown 76% > PCG soil 55%). In the natural environment, the spread of seeds, fruit-eating insects or animals and/or granivores, herbivory can be major constraints not only for regeneration but also for the performance and survival of seedlings [38]. Thus, a decrease in the germination capacity of seeds taken from the soil can be explained by a mechanical degradation of the seeds left within the reach of any predator that can influence the probability of germination of the seeds [39]. The time taken to germinate is also lengthened by 2 days for seeds from the Sudano-Guinean zone unlike those from the other two zones. Insufficient information on the phenology of *P. erinaceus* makes it difficult to characterize

the age of the seeds collected in the soil. In addition, their behaviour during the germination process of this study is similar to the seeds germinated after at least 14 months of storage (refrigerator or laboratory ambient temperature) with a significant lengthening of the mean germination time. The seeds could therefore come from the previous fructification season, thus showing an ability to preserve the germinative faculties offset by a longer germination time.

Seedlings resulting from the germination of *P. erinaceus* seeds showed slow and discontinuous growth dynamic, similar for all three origins during the 16 weeks of cultivation. In the three zones of origin, the strongest growth period of the seedlings corresponds to the first 5 weeks after transfer to the greenhouse, where the height increases reach on average 0.7-1.6 cm/week whereas it is 0.1 to 0.5 cm/week the rest of the time. The production of nodes over the 16 weeks is regular with a rate of 2 nodes/month. It is by considering the production of leaves that the discontinuity of growth is more remarkable. The first five weeks correspond to a period of leaf formation when the elongation of the stems is more active. From week 6 to week 11 defoliation occurs and then from week 12 to week 16, again leaf production. The waves of active growth alternating with phases of low growth illustrated simultaneously by a maximum elongation, then a slowdown of stem growth and by an alternation of production and loss of leaves would, therefore, correspond to the kinetics of a “rhythmic” growth [40–42]. This growth is linked to the functioning of the caulinary apical meristem, coordinated by the growth regulators or phytohormones, mainly auxin whose localized accumulation is indispensable in the initiation of the primordia that will give birth to the leaves [43]. In our experiment, growth was observed by an elongation of the stem by its meristem, but also by the organogenesis processes (production of nodes and leaves). In the first week of growth, the seedlings had on average 2 leaves inserted on the stem at the nodes. Within 4 weeks of germination, there was an intense activity of the apical meristem. This resulted in an elongation of the stem and the establishment of important new territories (nodes and leaves). From week 5 to week 11, meristemic activity slowed down resulting in growth depression. Vogel [44] indicates that nutritious competition theory would explain rhythmic growth. In fact, the rapidly growing organs, the young leaves, would collect large quantities of water [45], sugars [46, 47] and various substances from the sap [48]; so there wouldn't be enough left for the adult leaves. This phenomenon would periodically increase the imbalance between the young leaves and the more mature (well developed) ones. It would be responsible for the rhythmic growth observed, by reallocating nutrients from the most developed organs and meristem to the youngest. The entry into diapause of the caulin apical zone caused by the extension of the young leaves which would divert to their benefit the essential water and mineral nutrient of the plant, simultaneously with the fall of the most mature ones would be justified [49, 50].

The observation of irreversible resting periods and necrosis of the apical bud, followed by drying of the aerial part, may be due to mycelial attacks favored by excess water in the soil

[44]. Corbineau et al. [51] highlight the hypothesis of root system activity in the determinism of the rhythmic functioning of the apical meristem. Indeed, since the development of the whole plant is the joint activity of the aerial and underground parts, the cessation of growth would result from the strategic activity of the apical meristem, that is to say, it would allow the establishment and more efficient recharge of underground reserves. This hypothesis was verified in *Isobertinia doka* Craib and Stapf., *Afzelia africana* Sm. and *Detarium microcarpum* Guill. And Perr. cultivated in rhizotron, where there has been in addition to an aerial rhythmic growth, a tuberization characteristic of the pivot. This accumulation of reserves would occur on average from the third week marking the phase of the slowing of the activity of the caulin apical bud [52]. Ouedraogo et al. [31] reported in the diagnosis of the degradation of stands in the natural environment, that the plantlets of four woody species, including *P. erinaceus*, have escaped climatic hazards and anthropogenic disturbances by their intrinsic ability to develop an underground woody tuber, a place of nutrient and energy reserves.

The study of the initial growth of *P. erinaceus* has thus shown that the species not only has a rhythmic growth but that this growth is slow. In 16 weeks, the seedlings of the three phytogeographical zones recorded an elongation of 10 cm (size) with the production of 10 nodes and 4 leaves. This growth dynamic was noted by Ouedraogo et al. [53] for *Boswellia dalzielii* Hutch. and Hamidou et al. [54] for *Sclerocarya birrea* (A. Rich.) Hochst. In their studies on sexual regeneration of *B. dalzielii*, Ouedraogo et al. [53] report an increase in height of 5 cm with the production of 6 leaves after 4 weeks of nursery cultivation and note, in addition, the development of a long hypertrophied root pivot containing important food and energy reserves. With the agroforestry species of interest *S. birrea*, Hamidou et al. [54] record a two-stage foliar production.

Based on the different facts, *P. erinaceus*, because of growth inhibition after a period of continuous growth, would belong to woody species with vegetative floristic potential where the biological form of expectation of the species is the seedling [55]. In conclusion, Hallé and Oldeman [56] would, therefore, be correct in stating that architecture and growth dynamics are genetically fixed and should be considered as constants of the individual and the species. It should be added, however, that natural variations in the environment would trigger the expression of these characters to some extent.

6. Conclusion and future scope

The present study on seed germination and primary growth of plants of *P. erinaceus* from three phytogeographical zones resulted in the first confirmation that the species has good potential for sexual regeneration. Germination is spread over 15 days with the first germinated seeds already recorded 24 hours after sowing and recording of short mean germination times (5 days on average). The comparison of seed germination capacity showed a higher performance of the three phytogeographical zones Sudanian-dry Guinean-wet zones and Sudano-Guinean semi-wet zone (87% on average).

The study of shelf life and conditions of viability of the seeds revealed possibilities of storing them with accessible ways for at least 18 months. About 50% of germinal capacity beyond 18 months was obtained, this germination capacity remaining similar under the conditions tested (storage in plastic bottles in the laboratory at 25°C/60% relative humidity or in the refrigerator at 7°C/11% relative humidity). In terms of seedling growth from greenhouse germination, it was relatively slow over the 16 weeks and marked by an alternation of active phase and resting phase accentuated at the level of foliar development. At the end of the follow-up, the plants measure on average 10 cm high and bear 10 nodes with 4 leaves. The similarity of seedling growth kinetics reflecting a homogeneity of progeny in the three phytogeographical zones, added to the growth renewal dynamics observed in foliar development, characterizes the high potential for adaptation of *P. erinaceus* to adverse conditions, but also to different types of climatic. There are therefore many favorable factors for its multiplication by seeds and thus the establishment of forest plantations. It would be interesting to continue this work by adding the study of the evolution of the root profile of plants during the first years of growth. To this could be added tests on the influence of other parameters such as exogenous nutrient intake, type of substrate, etc., for a better characterization of populations according to their yield. Besides, the vegetative multiplication of this species deserves further studies.

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