Effect of storage time of *Phaseolus lunatus* (L.) seeds on germinationand morphological traits

Abstract

Food security remains a major challenge, particularly in developing countries where post-harvest losses and inadequate storage practices limit the potential of many crops. *Phaseolus lunatus*, a nutrient-rich legume cultivated for its ability to enrich soils, is underutilized due to the low viability of its seeds after storage. This study aims to evaluate the effect of storage duration on the germination and morphological performance of two morphotypes (M1 and M2) of this species, to optimize seed management practices. The experiment was conducted using a randomized block design with two replications. Four storage durations were tested: less than two months (T1), one year (T2), two years (T3), and three years (T4). The measured parameters included germination rate, emergence time, number of leaves, plant height, and leaf biomass. Statistical analyses assessed the effects of morphotypes, storage duration, and their interactions. Results indicate that seed viability decreased significantly with storage duration, with germination rates dropping from 70% (M1) and 75% (M2) at T1 to 20% (M1) and 35% (M2) at T3, and reaching 0% after three years (T4). Emergence time also increased with storage duration, reaching 7.62 days at T3. Morphologically, seeds stored for less than two months (T1) produced more vigorous plants. M2 outperformed M1 in all conditions, demonstrating superior tolerance to seed aging. These findings underscore the importance of improved storage methods and highlight the potential of M2 for breeding programs. Effective management of *Phaseolus lunatus* seeds could significantly contribute to the sustainability of agricultural systems and global food security.

Keywords:Food security*, Phaseolus lunatus*, Seed conservation, Germination, Morphotypes, Breeding programs

1.INTRODUCTION

Food security remains a major challenge, particularly in a global context marked by the growing effects of climate change and the pressure of galloping demographics. Developing countries, particularly in Africa, face problems such as famine, malnutrition and food insecurity [1](FAO, 2019). Legumes play a crucial role in this struggle, thanks to their nutritional and agronomic qualities, offering a sustainable solution to improve food security while characterizing poverty. Among legumes, the Phaseolus genus stands out for its unique characteristics and key role in climate change resilience. Composed of some 80 species belonging to the Fabaceae family, certain species of this genus are of significant economic and agricultural interest [2, 3]. *Phaseolus lunatus* is particularly valued for its ability to fix atmospheric nitrogen, thereby boosting soil fertility and reducing production costs [4, 5]. Its nutritional richness in protein, dietary fiber, micronutrients and low fat content make this legume a food of choice, associated with a reduced risk of chronic diseases such as cardiovascular disease, diabetes and certain cancers [6, 7, 8].In Côte d'Ivoire, *P. lunatus* is mainly grown by women and small-scale farmers, often in combined cropping systems for self-consumption [9, 10]. However, this legume is still undervalued compared with other species of the Phaseolus genus, which limits its production potential. One of the main constraints is inadequate post-harvest seed management. Traditional conservation methods, often rudimentary, combined with long storage periods, lead to seed deterioration. This deterioration results in a reduction in germination capacity and agronomic potential, exacerbating post-harvest losses, estimated at between 30 and 40% in developing countries [11]. These limitations are holding back the expansion of cultivation of this species.Despite the importance of this issue, few studies have explored the effects of shelf life and storage practices on germination and morphological trait expression in *P.lunatus*. Yet effective seed management is an essential lever for improving agricultural productivity and enhancing food security [12]. This research therefore aims to fill this gap by identifying the optimal conditions for saving *P. lunatus*seeds . More specifically, the specific objectives are:

1. to evaluate the effect of storage time on *P. lunatus* seed germination,
2. to determine the impact of storage time on the expression of plant morphological characteristics, in order to propose practical recommendations for optimizing *P. lunatus* seed management.

The aim of this study is to provide a scientific basis for better seed management, reinforcing their role in sustainable agricultural systems and their contribution to global food security.

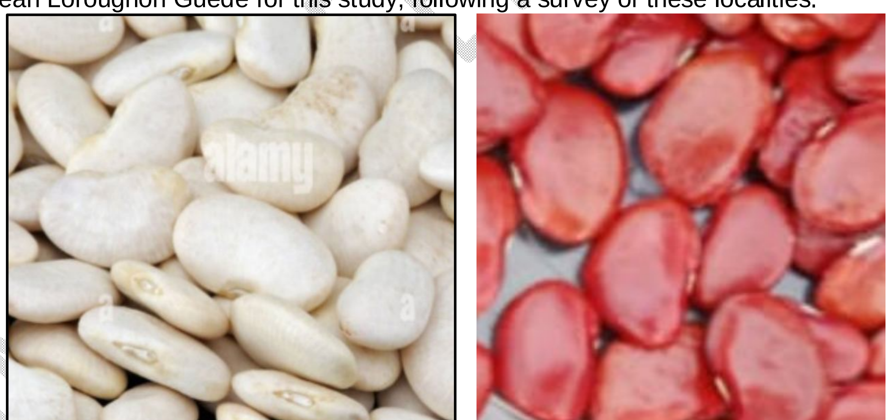
2. MATERIALS AND METHODS

2.1. Experimental site

The study took place on an experimental plot at the Université Jean Lorougnon Guédé. Located in the town of Daloa at 6°53 north latitude and 6°27 west longitude. With a population of 421,879 in 2021, Daloa is the fourth most populous city in Côte d'Ivoire, after Abidjan, the economic capital, and ahead of Yamoussoukro, the political capital. The town is located in the Haut Sassandra region in west-central Côte d'Ivoire. It is around 141 km from Yamoussokro, the political capital, and around 400 Km from Abidjan, the economic capital [13]. The soil is feralitic and the climate is tropical and humid, with one rainy season and two dry seasons [14].

2.2 Plant material

The plant material consisted of seeds of two morphotypes of *Phaseolus lunatus*. Morphotype1 (M1) has brown-colored seeds and morphotype 2 (M2) has white-colored seeds (Figure1). These morphotypes come from the southern and eastern regions of Côte d'Ivoire, from Adzopé and Bongouanou respectively. These are two production localities. These seeds were collected at the Université Jean Lorougnon Guédé for this study, following a survey of these localities.



A: Morphotype 1 (M1) B: Morphotype 2 (M2)

Figure 1:Seeds of two morphotypes of *Phaseolus lunatus*

2.3 Methods

2.3.1. Site preparation

The first stage in setting up the experiment began with the clearing of a 500 m² experimental plot. This was followed by ploughing to a depth of 20 cm at the sowing points, using a daba to loosen and aerate the soil.

2.3.2. Experimental design

The experimental set-up consisted of a randomized block with two replications. Two morphotypes were used in the experiment, the brown-seeded morphotype (M1) and the white-seeded morphotype (M2). In the block, each morphotype had four storage times. Seeds kept for less than two months (T1), kept for one year (T2), kept for two years (T3) and kept for three years (T4). Each morphotype was represented according to storage time on five lines. The lines were spaced 2 m apart, with 10 seeding points each. Seeding points were separated by 1 m, for a total of 200 seeding points for both morphotypes. Two seeds were sown per seeding point at a depth of 3 cm. Once the trial had been set up, the plot was maintained by regular weeding with the daba to facilitate good plant development and eliminate weeds. Daily watering was applied. Lastly, appropriate staking was required to encourage good growth and avoid stem overlapping.

2.3.3. Data collection

Data were collected on germination and morphological parameters. Germination parameters included emergence rate and seed emergence time. The emergence rate (%germination) was calculated as the ratio between the number of germinated seeds and the number of seeds sown. It is expressed as a percentage. Seed emergence time corresponds to the time between sowing and the appearance of cotyledonary leaves. It is expressed as the number of days after sowing (TE). For morphological parameters, the number of leaves, leaf width and length, collar diameter and stem height were measured.

2.3.4. Statistical analysis

Multivariate analysis and two-factor analysis of variance were performed. Where there was a significant difference, the smallest significant difference test was used to rank the variables concerned. All tests were performed using Statistica Version 7.1 [15].

3. Results and discussion

3.1 Results

3.1.1. Combined effect of morphotypes and shelf life

The results of the multivariate analysis of the traits studied are summarized in Table 1. These results indicated that the traits studied were significantly influenced by morphotypes, shelf life and their interaction.

Table 1: Multivariate analysis of parameters by morphotype and shelf life and their interaction according to Wilk's test

	F	*P*
Morphotype	23.648	<0.001
Storage Time	69.104	<0.001
Morphotype*Storage Time	14.394	<0.001

3.1.1 Influence of storage time on germination parameters (germination rate and emergence time)

Table 2 shows the results of the effect of storage time of Phaseolus lunatus seeds on germination parameters such as germination rate (%) and emergence time (TE, in days), for two morphotypes (M1 and M2).

These results showed that seeds stored for less than two months (T1) had the best germination rates (70 and 75%) in both morphotypes (M1 and M2), with germination rates decreasing progressively with storage time. Seeds stored for two years (T2) had germination rates of 20% and 35% for morphotypes M1 and M2 respectively, but no germination was observed for seeds stored for three years (T4).In terms of emergence time, seeds less than two months old (T1) showed the best results, with emergence times varying between 4.89 and 4.85 days after sowing for M1 and M2 morphotypes. Emergence time increased as the seeds aged.

Table 2: Influence of storage time on germination parameters (germination rate and emergence time)

Morphotype	Storage Time	Germination rate %	Emergence Time (jours)
M1	T1	70	4.89±0.74a
M1	T2	60	7±1.33b
M1	T3	20	7.62±1.30c
M1	T4	0	0
M2	T1	75	4,85±0,67a
M2	T2	65	6±1.41b
M2	T3	35	7.62±1.41c
M2	T4	0	0

T1: less than two months, T2: one year, T3: two years; T4: three years

3.1.2. Analysis of variance (ANOVA) on measured characteristics

Table 3 shows the results of the analysis of variance (ANOVA) performed on the various parameters measured. These results show that there was a significant difference in all germination and morphological parameters overall over the period of the work.

Table 3: Analysis of variance of observed parameters

	MC Effect	MC Error	*F*	*P*
ET (jours)	2.471	0.759	3.254	0.004
NL	25.172	7.899	3.186	0.005
PH (cm)	1232.472	292.834	4.208	0.000
CD (mm)	25.315	16.219	1.561	0.0159
Ll(cm)	4.545	1.132	4.014	0.000
LW (cm)	3.519	0.937	3.757	0.001

ET: Emergence time; NL: Number of leaves; PH: Plant height; CD: Colar diameter; LL: Leaf length, LW: Leaf width

3.1.3. Effect of storage time of *Phaseolus lunatus* seeds on morphological parameters

Table 4 shows the effect of *Phaseolus lunatus* seed storage time on morphological parameters of plants from two morphotypes (M1 and M2). The results showed that the best mean values observed for all morphological characters were recorded with seeds stored for less than two months (T1). Thus, storage time had a significant effect on all the growth parameters observed on the plants, i.e. number of leaves, plant stem, collar diameter, and growth in length and width of plant leaves (p=0.001)(table 4).At morphotype 1 level, the results showed that the average values observed in all these characters, the optimal duration of seed storage would be limited after one year of storage. In morphotype 2, on the other hand, the results show that the optimum storage time extends over two years. In this morphotype, the mean values of parameters observed after less than two months of storage were higher than those of morphotype 1, suggesting better overall performance. These results also show that, despite the drop in values observed after one and two years of storage, plants from morphotype 2 still performed better than those from morphotype 1.

Table 4: Influence of *Phaseolus lunatus* seed storage time on morphological parameters

Morphotype	Storage Time	Number of leaves	Plant Height(cm)	Colar Diameter (mm)	Leaflenght (cm)	LeafWidth(cm)
M1	T1	36.95±1.75b	221.96±10.53b	7.07±2.11a	12.86±0,55a	11.85±0.61a
M1	T2	30±2.14a	189.84±6.83a	5.47±0.26a	12±0.37b	10.95±0.32b
M1	T3	0	0	0	0	0
M1	T4	0	0	0	0	0
M2	T1	38±0.27a	238.83±4.79a	7.77±1.46a	12,9±0,2a	11.2±0.058a
M2	T2	33±0.74b	213.88±7.32b	5.45±2.66b	11,56b±0.29b	10.27±0.235b
M2	T3	27.9±0.71c	189.9±6.51c	5.37±2.45c	11,32c±1.65c	10.23±0.33c
M2	T4	0	0	0	0	0

3.2. Discussion

Firstly, assessing the impact of *Phaseolus lunatus* (L) seed storage methods on their germinative capacity and subsequent seedling development is essential to optimize production and preservation practices for this important food and economic legume. The results obtained in this study provide valuable information on the best choice of morphotypes and storage times to maximize emergence rates and ensure optimum plant growth.
The results of this study evaluated the effect of *P. lunatus* seed storage time and the differences between two morphotypes (M1 and M2) on germination and morphological parameters. They confirm the importance of proper seed management to preserve seed viability and optimize crop productivity.Multivariate analysis revealed significant differences between morphotypes, seed storage times and their interactions. These differences confirm the existing genetic variability within species of Phaseolus genus [12]. In the case of *P. lunatus*,results of the present study highlight existence of significant intra-specific variability, where morphotypes behave very differently in terms of storage and morphological expression, depending on storage times. This genotype-dependent specificity during long-term storage has also been reported by [16] and [17]for other plant species. In particular, M2 morphotype was distinguished by superior performance, which may be attributed to specific genetic or physiological characteristics. These results reinforce idea that certain lineages enable adaptive mechanisms favoring greater resilience to seed aging.Extending seed storage time led to a significant reduction in germination rate and an increase in emergence time. After three years of storage (T4), no germination was recorded, indicating a total loss of seed viability. This was due to degradation processes including lipid peroxidation, damage to cell membranes and alteration of enzymes essential for germination [18]. Seeds stored for less than two months (T1) showed superior vigour, with shorter emergence times and higher germination rates, underlining importance of optimal storage conditions for preserving seed quality [19]. These results corroborate studies carried out by[20] on *Phaseolus vulgaris*, which showed that morphotype and storage conditions had a major impact on seed germination. In particular, they observed that seeds stored under ambient conditions had lower germination rates than those stored at low temperatures. In another study,[21] reported that seed pre-treatment could significantly improve germination rates in Phaseolus sp. This would explain the better results obtained with T1 treatment in the present study. Seed germination capacity decreased with storage time, but this varied according to morphotype.M2 morphotype outperformed M1 in all parameters studied, both for germination and morphological traits. This superiority can be attributed to factors such as greater tolerance to oxidative damage, a more stable membrane structure or higher-quality energy reserves. These results indicate that M2 is a promising candidate for breeding programs aimed at improving seed tolerance to aging.

Seeds kept for a short period produced plants with better vegetative and reproductive growth. Plants derived from seeds saved for less than two months (T1) showed a higher number of leaves, larger plant size, and greater leaf biomass. These results confirm that seed degradation reduces not only germination capacity but also plant vigor, thus directly affecting crop yield potential [22](Bewley et al., 2013).The gradual decline in morphological performance with increasing storage time is concomitant with seed aging processes, notably the reduction in the quality of energy reserves and damage to essential cell structures. These effects underline the importance of regularly renewing seed stocks or improving storage conditions. The significant interaction between morphotypes and storage time indicates that morphotype performance differs according to storage time. M2 morphotype showed greater tolerance to ageing than M1, suggesting its potential for systems requiring prolonged seed storage. These results guide breeding efforts to identify and develop lines better adapted to prolonged storage conditions, thus helping to improve the sustainability of agricultural systems.

Conclusion and perspectives

This study on the effects of storage time on *Phaseolus lunatus* seeds revealed significant results on seed viability and morphological performance. These results showed that seed viability and germination rate decreased with increasing storage time, reaching 0% after three years. Seeds stored for less than two years (T1 and T2) showed optimal performance. In terms of plant development, seeds kept for less than two months produced more vigorous plants, characterized by superior morphological development. Finally, M2 morphotype showed greater tolerance to seed aging. These results highlight importance of optimal seed management to guarantee plant quality and crop yields. However, it would be even more interesting to:

- Test advanced preservation methods, such as controlled atmosphere storage.
- Study impact of aged seeds on agronomic performance under real conditions, to confirm the results obtained
- Develop training campaigns to inform farmer and seed bank managers about optimal seed storage and use practices.

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.

REFERENCES

1.FAO. The State of Food Security and Nutrition in the World. *FAO, Rome*.2019.

2.Baudoin, J. P., Mergeai, G., & Vanderborght, T. Genetic and genomic resources of *Phaseolus* beans. *Euphytica,*2002; *125*: 23-29.

3. Freytag, G. F., &Debouck, D. G.*Taxonomy, distribution, and ecology of the genus Phaseolus*. IBPGR.2002.

4. Ismet, T., &Firkret, D. Nitrogen fixation in legumes and soil fertility improvement. *Agricultural Science Journal,*2011,15: 89-97.

5. Romero AO, Damian HMA, Rivera TJA, Baez SA, Huerta LM, Cabrera HE.. The Nutritional value of Beans (Phaseolus vulgaris L.) and its importance for Feeding of Rural communities in Puebla-Mexico. *International Research Journal of Biological Sciences*;2013, 2(8): 59-65

6. Taylor C, Buckley J, Champ M, Patterson CA. The nutritional value and health benefits of pulses for obesity, diabetes, heart disease and cancer. *British Journal Nutrition,*2012;*108*: 1-165.

7. Zhang, C., Liu, G., Xue, S., & Wang, G. Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of

abandoned farmland in the Loess Plateau. *Soil Biology and Biochemistry*, 2016, 97: 40-4

8. Cheng, A., Raai, M. N. Nutritional and health benefits of legumes and their bioactive compounds. *Frontiers in Plant Science,* 2020;*11*, 1160.

9. Akoègninou A, van der Burg WJ, van der Maesen LJG. Flore Analytique du Bénin, Backhuys Publishers, Leiden, the Netherlands, 2006.

10. Nazir L, Feyissa T & Asfaw Z. Genetic diversity analysis of Lima bean (*Phaseolus lunatus* L.) Landrace from Ethiopia as reveled by ISSR marker. SINET: *Ethiopian Journal of Science;*2021, 44(1): 81–90.

11. Bonazzi, C., &Bimbinet, J. Post-harvest losses in developing countries: Prevention and recovery strategies. *Food Technology Review;*2003*,* 23: 45-52.

12. Singh, S. P., &Gepts, P. Genetic resources and domestication of *Phaseolus*. *Plant Breeding Reviews,* 2017.*41*, 1-26.

13. Diomandé M, Kouamé KB & Koko AC. Comparaison des propriétés chimiques de l'huile et tourteau d'arachide et de noix de cajou sur le marché de Daloa, Côte d'Ivoire. Engineering and Applied Sciences. 2017; 4(11) : 28-32.

14. N'guessan, Kouame., Jacob, K. N. D., Koutoua, Ayole., Blaise, Y. K., & Justin, Y. K. Influence de l'association culturale sur la capacité de nodulation de trois espèces de légumineuses : Arachide, Niébé et Soja vert. *Journal of Applied Biosciences*, 2020, 145: 14930- 14937.

15. StatSoft. STATISTICA (Data Analysis Software System), Version 7.1,Available at http://www.statsoft.com,2005.

16. Probert R.J., Daws MI. and Hay F.R. Ecological correlates of ex situ seed longevity: a comparative study on 195 species.*Annals of Botany*, ,2009, 104(1):57-69.

17. Walters, C., Hill, L. M., & Wheeler, L. J. Dying while dry: kinetics and mechanisms of deterioration in desiccated organisms. *Integrative and Comparative Biology*,2005, 45(5):751-758.

18. Rajjou, L., &Debeaujon, I. Seed longevity: Survival and maintenance of high germination ability of dry seeds. *Comptes Rendus Biologies,*2008, 331(10) :796-805.

19. Ellis, RH et Roberts, EH. The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, 1981, 9: 373-409.

20. Beninger, C. W., & Hosfield, G. L. Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *Journal of agricultural and food chemistry*,2003, 51(27):7879-7883.

21. Deshpande, S. S, & Damodaran, S. Food legumes: chemistry and technology. *In Advances in cereal science and technology*; 1990; Vol. 10 :147-241). AACC International Press.

22. Bewley, JD, Bradford, KJ, Hilhorst, HW, &Nonogaki, H.*Seeds: Physiology of Development, Germination, and Dormancy.* Springer Science & Business Media, 2013.