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Effect of Storage Method for *Phaseolus lunatus* Seeds on Germination and Expression of Morphological Traits

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Abstract | **Original Research Article**

Phaseolus lunatus plays an essential role in soil nutrition and fertility. However, poor seed saving practices often lead to significant post-harvest losses, reducing germination rates and crop yields. To solve these problems, it is necessary to understand how conservation methods affect seed quality and plant development. This study aimed to assess the effects of storage methods and morphotypes (M1 and M2) on germination performance and morphological traits of *Phaseolus lunatus*. The research was conducted at Jean Lorougnon GUEDE University. The experimental set-up was a randomised block design with two replications. Seeds of the two morphotypes were stored under different conditions (as pods and as shelled seeds) before being sown. Germination parameters, including emergence rate and time, as well as morphological characteristics such as plant height, number and size of leaves, were recorded and analysed using statistical methods. The results showed that storing hulled seeds significantly improved germination rates and reduced emergence time compared with storage in pods. Plants grown from hulled seeds also showed improved morphological characteristics. Among the morphotypes, M2 systematically outperformed M1, indicating greater genetic and physiological adaptability. This study highlights the crucial role of optimised seed preservation methods in improving the productivity of *Phaseolus lunatus*. Preservation of shelled seeds proved to be the most effective strategy. The superior performance of the M2 morphotype highlights its potential for breeding programs aimed at improving agricultural resilience and food security in resource-limited regions.
Keywords: *Phaseolus lunatus*, Seed saving, Morphotypes, Crop productivity, Food security.

INTRODUCTION

Food security is a major global challenge, particularly in a context marked by the growing effects of climate change and rapid population growth (GIEC, 2019; FAO, 2024). For many developing countries, meeting the challenges of food insecurity is a strategic priority. This challenge is exacerbated by recurrent food crises, post-harvest losses and the degradation of agricultural resources, which affect the most vulnerable populations (FAO, 2021). Legumes play an important role in this context, thanks to their nutritional value, their ability to enrich soils with nitrogen and their role in food diversification.

Among legumes, Phaseolus species stand out for their agronomic potential and economic importance. These plants, which belong to the Fabaceae family, comprise around eighty species (Baudoin *et al.*, 2022), some of which are widely cultivated around the world because of their ability to adapt to different environments (Zhang *et al.*, 2020). Beans in this family, such as *Phaseolus lunatus* (lima bean), are prized for their high protein, dietary fibre and micronutrient content, while being low in fat. What's more, their consumption helps to prevent chronic diseases such as diabetes, cardiovascular disease and certain cancers (Cheng *et al.*, 2020).

In Côte d'Ivoire, *Phaseolus lunatus* is mainly grown by small-scale farmers, often in association with other food crops. It has also been reported that eating legumes helps control cardiovascular disease (Marilyn *et al.*, 2011), diabetes (IRAD, 2013), obesity and colorectal cancer (*Taylor et al.*, 2012). Beans of Phaseolus genus are not well known to local populations, and their cultivation is essentially reserved for women or small farmers, who often grow them in association with other crops (Nazir *et al.*, 2021) for self-consumption (Akouègninou *et al.*, 2006). However, despite its potential, production of this species remains limited by significant post-harvest losses. These losses, estimated at around 30-40% of total production in developing countries, are attributed to inefficient conservation practices (Bonazzi & Bimbinet, 2003).

One of the main constraints identified is seed degradation due to inappropriate storage methods, which negatively influence germination and plant growth. Seeds are often stored in home-made conditions, such as perforated bags or untreated pods, without consideration for the conservation requirements specific to this species. This problem highlights the importance of studies aimed at identifying suitable conservation strategies to improve seed quality and, consequently, yields. The aim of this study is to optimise the conservation of *Phaseolus lunatus* seeds in order to improve their germination and morphological performance. Specifically, the study will aim to:

- Assess effect of storage methods on seed germination;
- Determine impact of different preservation methods on the morphological characteristics of the plants.

MATERIALS AND METHODS

Study site

The research was conducted at an experimental nursery belonging to the Jean Lorougnon Guédé University, situated in the town of Daloa. Geographically, Daloa lies between 6°53‘ north latitude and 6°27’ west longitude, within the Haut Sassandra region of west-central Côte d'Ivoire. The town is approximately 141 km away from Yamoussoukro, the country's political capital, and about 400 km from Abidjan, the economic hub (Diomande *et al.*, 2017). Daloa shares its borders with Vavoua to the north, Issia to the south, Duékoué to the west, and Bouaflé to the east. This region's vegetation falls within the mesophilic zone, previously dominated by dense forests that have been largely cleared to make room for cash crop cultivation. The soil type is predominantly ferrallitic, and the area experiences a humid tropical climate characterized by alternating dry and wet seasons (N'guessan *et al.*, 2012). The climatic conditions feature one rainy season and two distinct dry periods, with average temperatures ranging from 24.65°C to 27.75°C.

Plant material

The plant material consisted of seeds of two morphotypes of *Phaseolus lunatus*. Morphotype 1 (M1) is characterised by its brown colour and morphotype 2 (M2) by its white colour. These morphotypes come from Adzopé and Bongouanou. These are two high production localities where seed sampling was carried out and the seeds kept at Jean Lorougnon Guédé University (Daloa, Côte d'Ivoire) at room temperature for the purposes of this study.

Methods

Trial preparation

The first stage in setting up experiment began with clearing an area of 500 m² using a machete. After a 20 cm ploughing of the sowing points was carried out using a daba to loosen and aerate the soil and control certain pathogens and weeds that could hinder the proper development of plants. Two factors were studied. These were morphotype and seed storage method. The morphotype factor includes brown seeds (M1) and white seeds (M2). The storage method factor shows seeds stored in pods and shelled seeds stored in containers.

Experimental design

The experimental design was a completely randomised block with two replications. Each morphotype was represented according to storage mode on two lines. The lines were spaced 2 m apart and included 10 seedling points each. Each seeding point was 2 m apart, giving a total of 160 seeding points for the two morphotypes. Two seeds were sown per sowing point at a depth of 3 cm. After the trial was set up, the plot had to be regularly weeded with a daba to facilitate good plant development by eliminating weeds. The plants were watered daily. Finally, appropriate staking was necessary to encourage good growth and avoid mixing of stems.

Data collection

Data was collected on germination parameters and morphological parameters. Germination parameters concerned the emergence rate and duration of seed emergence. 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. The duration of seed emergence corresponds to the time between sowing and the appearance of the cotyledonary leaves. It is expressed as the number of days after sowing. For morphological parameters, the number of leaves, leaf area, collar diameter and stem height were determined.

Statistical Analysis

The data collected were subjected to multivariate and univariate analyses (MANOVA and ANOVA). Where the test was significant, the significant small difference test was used to rank the means of the individuals. All tests were performed using Statistica Version 7.1 (StatSoft, 2005).

RESULTS AND DISCUSSIN

Combined effects of morphotype and seed storage method

Table I presents a multivariate analysis of the parameters as a function of morphotype and storage method, as well as their interactions, using Wilk's test. The results show that all the factors studied have a significant effect ($p < 0.001$) on the parameters analysed for morphotype, storage method and their interaction.

Table 1: Multivariate analysis of parameters according to morphotype, storage type and their interaction using the Wilk's test

	ddl	*F*	*P*
Morphotype	6	23.648	<0.001
Storage method	6	28.954	<0.001
Morphotype*Stoage method	6	36.156	<0.001

Morphotype had a significant influence on the parameters studied. This highlights significant inter-morphotype variability, which could be linked to genetic and physiological differences between the M1 and M2 morphotypes. These results are consistent with those reported by Smýkal *et al.*, (2015), who highlight a high genetic diversity within legumes, influencing traits such as germination and growth. According to these results, the storage method significantly affects the parameters assessed. Seeds stored in pods and hulled seeds showed differences in germination and morphological performance. The storage method also had a significant effect on parameters analysed. Seeds stored in shelled form generally performed better in terms of germination and expression of morphological traits. This result could be explained by better preservation of energy reserves and reduced damage from moisture or insects (Abdoulaye *et al.*, 2011). These observations corroborate the work of Ellis and Roberts (1980), who demonstrated that storage conditions directly influence seed viability and longevity.

The highly significant interaction between morphotype and storage method indicates that morphotype responses vary according to storage method. Thus, the M2 morphotype seems to respond better to storage in pods compared with M1 morphotype. This significant interaction shows that specific characteristics of morphotypes influence their response to storage conditions. M2 morphotype seems to benefit more from storage in pods, while M1 shows a more marked improvement in shelled conditions. This variability may be attributed to structural differences in the seeds or to ecological and physiological adaptations specific to the morphotypes, as suggested by the work of Rao and Hodgkin (2002), who studied genetic and environmental interactions in the conservation of genetic resources.

These results show that in order to maximise germinative and morphological performance, conservation in dehulled form could be recommended, particularly in environments where there is a high risk of degradation by humidity or pests. However, for traditional farming practices or low-input systems, storage in pods could remain a viable option, particularly for morphotypes such as M2.

Effect of storage method on germination parameters

Table 2 shows effect of morphotype and storage method on two germination parameters: germination percentage and mean emergence time. The results showed significant variations between morphotypes and storage method.

Table 2: Effect of morphotype and storage method on germination parameters

Morphotype	Storage method	Germination rate (%)	Emergence time (Days)
M1	Pod	32.5	6.16±2.32
M1	Shelled	42.5	6.07±1.44
M2	Pod	45	5.78±1.51
M2	Shelled	48	5.62±1.59

The results showed that germination percentage varied according to morphotype and storage method. For M1 morphotype, germination was 32.5% for seeds stored in pods and 42.5% for those stored in shelled form. For M2 morphotype, germination reached 45% for seeds in pods and 48% for hulled seeds. These results show that the hulled storage method significantly improves the germination percentage, regardless of morphotype. The quality of this method could be explained by better protection of seeds against deterioration factors such as humidity and pathogens, which are often more problematic in pod storage conditions. These observations are consistent with the work of Ellis and Roberts (1980), who demonstrated that storing seeds in controlled environments prolongs their viability. The mean emergence time observed in these results was slightly shorter for seeds stored in the dehulled form in both morphotypes. These differences, although small, suggest that storage in the dehulled form enables the seeds to maintain a better physiological state, which would favour faster emergence. Well-preserved seeds are less exposed to environmental stresses that delay their metabolic activation during germination (Baskin & Baskin, 2014). The germination performance of M2 morphotype is generally better than that of M1, both in terms of germination percentage and average emergence time. This indicates a better physiological or genetic adaptation of M2, which could be exploited in varietal improvement programs. These results confirm observations of Smýkal *et al*., (2015), who highlighted the importance of intra-specific differences in legumes for adaptation to storage and growing conditions. The results in this table provide practical information for improving seed management. Storage in dehulled form is clearly advantageous for maximising germination percentage and reducing emergence time, especially for M2 morphotype. These practices could be recommended to growers, particularly in regions where storage conditions are often suboptimal.

Effect of morphotype and storage method on morphological characteristics

Table 3 presents the results concerning the effect of seed storage method on morphological characteristics as a function of morphotype.

Table 3: Effect of morphotype and storage method on morphological parameters

Morphot ypes	Storage method	Number of leaves	Plant high (cm)	Collar Diameter (mm)	Leave lenght (cm)	Leave widh (cm)
M1	Pod	32.05±8.08a	204.58±50.67a	4.37±9.45a	11.74±2.92a	10.7±2.65b
M1	Shelled	35±0.72b	207.90±16.03a	5.44±0.48a	12.19a±0.77a	11.06a±0.95a
M2	Pode	33.08±0.51a	219.94±27.08b	4.88±0.75a	11.87±0.47a	10.58±0.48b
M2	shelled	35±0.39b	226.24±24.97b	5.22a±0.35a	12.21±1.02a	11.15a±1.04a

The results recorded in Table 3 showed that plants grown from hulled seeds had a higher number of leaves. These results indicate that storage in hulled form promotes better leaf growth. This could be explained by better preservation of seed viability, which improves plant development (Ellis & Roberts, 1980). The results also showed that plants grown from hulled seeds were significantly taller. This confirms that the quality of seeds preserved in hulled form favours optimal vertical growth, probably due to their better physiological state at the time of germination. Studies such as Smýkal *et al.*, (2015) have shown that seed quality has a direct impact on plant performance.

Results obtained for stem neck diameter and leaf length showed no significant differences between storage modes for these parameters between the two morphotypes. Although the storage method influences other parameters, it would seem that stem diameter and leaf length are determined more by genetic factors specific to the morphotypes. Moreover, these characteristics are less sensitive to practical variations in storage method. However, in terms of leaf width, seeds stored in hulled form produced wider leaves for both morphotypes, compared with seeds in pods. Increased leaf width could be an indicator of potentially more efficient photosynthesis, favouring better productivity (Taîz *et al.*, 2015; Farqhar & Sharkey,1982).

In the overall results, M2 morphotype showed slightly better overall performance than M1 morphotype for most of the parameters studied. These differences can be attributed to inherent genetic variability, which gives M2 morphotype competitive advantages in terms of growth and development. These results corroborate the work of Rao and Hodgkin (2002), which highlights importance of genetic diversity in agronomic performance of legumes. In addition, the work of Nascimento *et al.*, (2012) showed importance of morphotype choice in optimising legume development, which is consistent with the results observed on seed storage between different morphotypes (M1 and M2).

These results have practical implications for the conservation and sustainable use of Phaseolus lunatus genetic resources. The diversity observed between morphotypes highlights the importance of appropriate genetic conservation strategies, including ex situ (gene banks) and in situ (preservation in the field) methods. Furthermore, the identification of the best-performing morphotypes, such as M2, can guide varietal improvement programmes to maximise yields in farming systems constrained by limited resources (Thormann & Parra-Quijano, 2016).

CONCLUSION

This study highlights the key role of storage method and genetic diversity in improving the germinative and morphological performance of Phaseolus lunatus. Storage in dehulled form and enhancement of M2 morphotype appear to be promising strategies for improving the productivity of this legume in traditional farming systems. The results obtained call for optimised conservation practices to be integrated into local farming systems. Farmers should be made aware of the importance of conservation methods and the specific features of morphotypes in order to maximise seed viability and crop performance. In addition, further studies could explore:

- Examining the genetic mechanisms underlying variability in morphotype performance.
- Effects of environmental conditions (temperature, humidity) on storage methods.
- Study the relationships between the morphological traits studied and yields.
- Determine impact of storage methods on production.

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