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Physiological Quality of *Cajanus cajan* (L.) Millsp. Seeds as Affected by Storage Duration

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

The legume *Cajanus cajan* (L.) Millsp. is a drought-tolerant, multifunctional crop whose productivity largely depends on maintaining seed physiological quality during storage. The objective of this study was to evaluate the effects of storage on the physiological quality of *Cajanus cajan* (L.) Millsp. (pigeon pea) seeds cultivated in Araçuaí-MG, Brazil. The experiment was conducted at the Federal Institute of Northern Minas Gerais -

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Araçuaí Campus, using a completely randomized design with four treatments (storage periods): T0 - 0 months of storage; T1 - 12 months of storage; T2 - 24 months of storage; and T3 - 36 months of storage, with four replications of 50 seeds each. The Analyse analysis performed included biometric evaluation, seed moisture content, germination, first germination count, germination speed index, shoot length, root length, and dry biomass of seedlings. The biometric data of the pigeon pea seeds from both treatments showed similar weight and size. The different storage periods did not statistically influence the germination speed index, hard seeds, normal and abnormal seedlings, and seedling dry biomass. The first count of germinated seeds and the number of dead seeds in treatment 3 (36 months) were significant. Therefore, storage in PET bottles proved to be efficient in maintaining seed quality. Seeds stored for 36 months showed higher germination speed and a lower number of dead seeds. The seeds exhibited similar biometric characteristics, with mean values of 5.50 ± 0.25 mm, 6.26 ± 0.51 mm, 4.57 ± 0.09 mm, 11.08 ± 0.924%, and 104.8 ± 9.24 g for width, length, thickness, seed moisture content, and thousand-seed weight, respectively.

Keywords: Biometry; germination; physiological potential, Cajanus cajan (L.) millsp.; storage duration.

1. Introduction

The species *Cajanus cajan* (L.) Millsp., commonly known as pigeon pea, belongs to the Fabaceae family and is recognized as a multifunctional legume widely adapted to tropical and subtropical environments. It is characterized as a shrubby plant with an annual or semi-perennial growth habit, capable of reaching up to four meters in height under favorable conditions. Although its center of origin remains under discussion, strong evidence points to South Asia, particularly India, as the primary domestication region, followed by its dissemination to Africa and the Americas (Varshney et al., 2010).

In Brazil, pigeon pea cultivation is mainly concentrated in semi-arid regions, especially in the Northeast, where it plays a significant role in smallholder farming systems. Despite its limited commercial scale, the species presents high agronomic potential due to its tolerance to water deficit, biological nitrogen fixation capacity, and versatility as forage, green manure, and grain crop (Odeny, 2007). In addition, its high protein content and biomass production make it a strategic component for sustainable agricultural systems, particularly under limiting environmental conditions.

Seed quality is a fundamental factor determining crop establishment and productivity. The use of high-quality seeds ensures the full expression of the genetic potential of cultivars, contributing to uniform emergence, vigorous seedling development, and increased yield (Marcos-Filho, 2015). Conversely, seeds with low physiological vigor may compromise plant establishment even under favorable environmental conditions, directly affecting productivity and economic returns (Finch-Savage and Bassel, 2016).

The physiological quality of seeds is influenced by several pre- and post-harvest factors, including crop management, harvesting time, drying processes, and storage conditions. Inadequate handling during these stages can accelerate seed deterioration, resulting in loss of viability and vigor (Bewley et al., 2013). Although germination tests conducted according to standardized protocols, such as the Rules for Seed Testing, are widely used to assess seed viability, complementary vigor tests are essential for a more accurate prediction of field performance.

High-quality seeds are defined by a combination of physical, physiological, genetic, and sanitary attributes. Physical quality refers to seed integrity and the absence of mechanical damage; physiological quality is associated with germination capacity and vigor; genetic quality ensures varietal purity and uniformity; and sanitary quality relates to the absence of pathogens that may compromise storage and field performance (Peske et al., 2012; Marcos-Filho, 2015; Maguire, 1962; Saxena et al., 2010).

Among post-harvest factors, storage plays a critical role in maintaining seed quality over time. Seed deterioration is an inevitable and irreversible process, mainly influenced by temperature, moisture content, and storage duration (Walters, 2015). Elevated temperature and moisture levels accelerate metabolic activity and oxidative processes, leading to membrane damage, enzyme degradation, and reduced germination potential (Sano et al., 2016). Therefore, the adoption of appropriate storage conditions is essential to preserve seed longevity and ensure satisfactory field performance.

Despite the recognized importance of seed storage, there is still limited information regarding the physiological responses of pigeon pea seeds subjected to prolonged storage, particularly under semi-arid conditions. This gap is especially relevant for smallholder systems, where seeds are often stored using low-cost and traditional methods.

In this context, the present study aimed to evaluate the effects of storage duration on the physiological quality of *Cajanus cajan* (L.) Millsp. seeds produced under Caatinga and Cerrado conditions in Brazil.

2. Materials and Methods

The experiment was conducted in the second semester of 2022 at the Biology Laboratory of the Federal Institute of Education, Science, and Technology of Northern Minas Gerais (IFNMG) in Araçuaí, MG. Pigeon pea seeds (*Cajanus cajan* (L.) Millsp.) were used, cultivated at Sitio Gravatá located in the Santo Antônio do Gravatá community, which belongs to the municipality of Araçuaí in the Vale do Jequitinhonha region of Minas Gerais. The seeds were from the 2019, 2020, 2021, and 2022 harvests.

According to Köppen, the municipality is classified as having a BSW climate type, characterized by a dry winter and a rainy season in the summer. It has an average annual precipitation of approximately 766 mm, with rainfall concentrated from November to March, and the monthly average temperature ranging from 26.4 to 21.6°C (Alvares et al., 2013). Araçuaí is located in a transition zone between the Caatinga, Cerrado, and Atlantic Forest biomes (Otoni, 2018).

After harvesting, the pods were selected to extract the seeds that showed any kind of damage, as well as impurities and foreign materials.

The seeds were manually harvested, and seed purity was ensured through standard cleaning and selection procedures. They were then stored in PET bottles, following the local practice of small-scale farmers, to preserve the seeds.The experimental design used was completely randomized, with four treatments (storage periods): T0 - 0 months of storage; T1 - 12 months of storage; T2 - 24 months of storage; and T3 - 36 months of storage, with four replications of 50 seeds each. The following evaluations were conducted for seed characterization and qualitative assessment: biometric evaluation, measuring width, length, and thickness of the seeds, as well as the weight of 100 seeds; seed moisture content (SMC); germination; first germination count (FGC); germination speed index (GSI); shoot length, root length; fresh and dry weight of seedlings (FW and DW).

In the biometric evaluation of the seeds, measurements were taken for size (width, length, and thickness) and the weight of 100 seeds. Using a digital caliper with a precision of 0.01 mm, the length, width, and thickness of the seeds were measured in millimeters (mm). The weight of 100 seeds was estimated by weighing four subsamples of 25 seeds per treatment using an analytical balance with a precision of 0.001 g (DOS SANTOS, 2020).

The seed moisture content was determined using the oven-drying method at 105 ± 3°C for 24 hours, according to the Rules for Seed Analysis (Brasil. Ministério da Agricultura, Pecuária e Abastecimento, 2009), with two repetitions of approximately 50g of seeds for each treatment.

For the germination test, 200 seeds were divided into four subsamples of 50 seeds each and alternately distributed on two sheets of special germination paper, covered with a third sheet, and rolled up. The paper sheets were moistened with distilled water in an amount equivalent to 2.5 times their dry mass. The rolls were then placed in transparent plastic bags and sealed with rubber bands to prevent water loss through evaporation. Evaluations were conducted on the fifth and ninth days following the Rules for Seed Analysis, considering seeds with primary root and shoot emergence as germinated seeds, and the results were presented as a percentage, according to the recommendations of Brasil (2009).

The germination speed index was determined by daily counting of germinated seeds from the third day to the seventh day after the test was set up. The germination speed index was calculated using the formula presented by McGuire (1962). The formula is ($GSI = G1/N1 + G2/N2 + ... + Gn/Nn$), where G1, G2, and Gn are the numbers of germinated seeds day by day, and N1, N2, and Nn are the number of days from the first, second, and last count.

Shoot and root lengths were measured at the end of the germination test, where primary roots and shoot lengths were measured for each replication using a digital caliper (Brasil. Ministério da Agricultura, Pecuária e Abastecimento, 2009).

The total fresh and dry biomass of the seedlings was determined by drying the seedlings in an oven, where they were placed in paper bags with forced air circulation at 60°C until reaching a constant weight, and the results were expressed in grams per seedling (Brasil. Ministério da Agricultura, Pecuária e Abastecimento, 2009).

The data from seed biometric characterization were analyzed through descriptive analysis and Pearson correlation. For the analysis of seed quality and physiological characteristics regarding storage, the results were subjected to analysis of variance at a 5% significance level and subsequent regression analysis. The statistical analysis was performed using the SISVAR software (Ferreira, 2019).

3. Results and Discussion

The biometric characteristics of *Cajanus cajan* seeds did not differ between treatments, indicating uniformity in seed size and weight. The average values for width, length, and thickness were 5.50 ± 0.25 mm, 26.00 ± 0.51 mm, and 4.57 ± 0.09 mm, respectively. Seed dimensions are relevant indicators of physical quality, as they influence germination performance and are essential for standardizing laboratory tests, ensuring greater reliability in the evaluation of seed physiological potential.

Seed size is directly associated with the amount of stored reserves, which plays a crucial role in seedling establishment, particularly under adverse environmental conditions. Larger seeds tend to exhibit greater vigor due to enhanced reserve mobilization capacity, supporting initial growth and increasing the likelihood of successful establishment (Leão-Araújo et al., 2020). The values observed in this study are consistent with those reported by Santos et al. (2020) for landrace pigeon pea varieties from Northern Minas Gerais and Bahia, indicating morphological stability within the species.

Regarding seed moisture content, an average value of 11.08% ± 0.92 was observed, which falls within the optimal range for safe storage. Maintaining moisture levels below 13% is essential to reduce metabolic activity and respiration rates, thereby limiting fungal development and delaying deterioration processes (Carvalho & Nakagawa, 2012). Under these conditions, seeds tend to maintain cellular integrity for longer periods, contributing to the preservation of physiological quality. Therefore, the moisture content observed in this study can be considered adequate for ensuring reliable seed analysis and maintaining viability during storage.

The use of PET bottles as a storage system, combined with low seed moisture content, played a fundamental role in maintaining seed quality during storage. Moisture levels below 13% are essential to reduce metabolic activity and respiration rates, thereby delaying deterioration processes. In addition, hermetic or semi-hermetic storage systems, such as PET containers, contribute to stabilizing internal conditions by limiting gas exchange and moisture fluctuations, which are critical factors affecting seed longevity (Corbineau et al., 2023; Hay et al., 2022).

Despite these advantages, storage duration remains a determining factor in seed viability. Even under adequate moisture and packaging conditions, seed deterioration is inevitable due to cumulative oxidative damage. Reactive oxygen species (ROS) progressively impair cellular membranes, proteins, and nucleic acids, leading to loss of vigor and reduced germination over time (Waterworth et al., 2016; Sano et al., 2020).

Seed moisture content is a key determinant of physiological quality from harvest to storage. Seeds reach maximum physiological potential at physiological maturity, when moisture content is optimal. Inadequate moisture levels during storage can accelerate deterioration processes, particularly through increased respiration and microbial activity. In this study, the observed moisture content (11.08%) is within the recommended range for safe storage, reinforcing the suitability of the adopted conditions.

The thousand seed weight (104.8 ± 9.24 g) reflects the reserve accumulation capacity, which is directly associated with seed vigor and seedling establishment. Larger seeds generally exhibit greater availability of stored assimilates, supporting early growth. However, during storage, the integrity of these reserves may be compromised by oxidative degradation, reducing the efficiency of reserve mobilization during germination (Bailly, 2004).

Storage duration significantly influenced the first germination count (FGC), which showed a quadratic response pattern. The highest germination performance observed at approximately 12 months may be associated with after-ripening processes, which can enhance germination by promoting metabolic adjustments in dry seeds. However, prolonged storage led to a marked reduction in germination, reaching 46% at 36 months.

The decline in germination is associated with seed ageing processes, including membrane disorganization, enzyme inactivation, and lipid peroxidation. These alterations impair cellular reorganization during imbibition, resulting in reduced germination and delayed seedling development. Furthermore, membrane damage increases solute leakage, which is commonly observed in deteriorated seeds and is directly linked to reduced physiological quality (Sano et al., 2020; Corbineau et al., 2023).

The first germination count (FGC) exhibited a quadratic response to storage duration (Fig. 1), with maximum germination (87.85%) observed at approximately 11.97 months. This initial increase in germination performance may be associated with after-ripening processes, which promote metabolic adjustments in dry seeds and enhance germination capacity. However, beyond this period, a progressive decline was observed, reaching 46.00% at 36 months, indicating the onset of deterioration processes associated with prolonged storage.

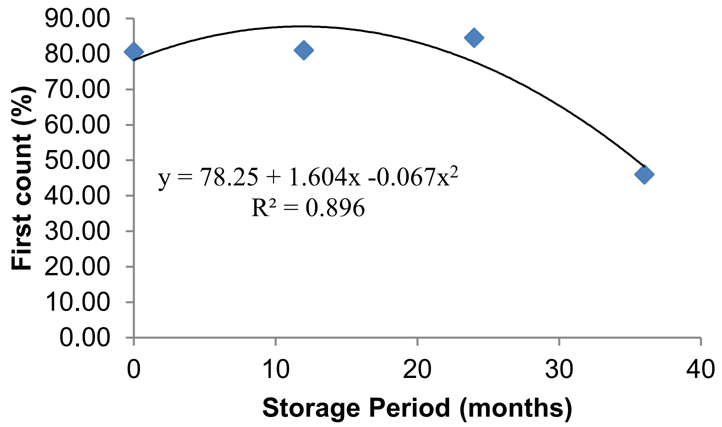

Fig. 1. First count of *Cajanus cajan* (L.) Millsp. seeds subjected to different storage conditions

In contrast, the percentage of dead seeds (DS) also showed a quadratic pattern (Fig. 2), with a peak at 9.81 months (12.81%), followed by a reduction to 0.5% at 36 months. At first glance, this reduction in dead seeds may appear contradictory to the observed decline in germination. However, this pattern suggests that deterioration initially affects seed vigor rather than viability.

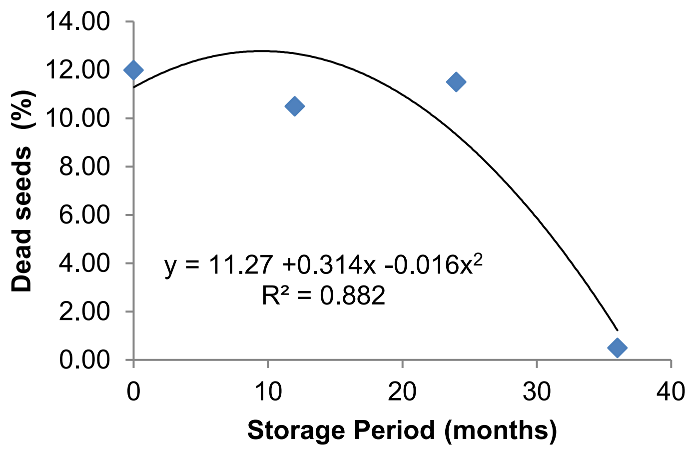

Fig. 2. Dead seeds of *Cajanus cajan* (L.) Millsp. subjected to different storage conditions

During early stages of seed ageing, metabolic and structural damage—such as membrane disorganization, reduced enzymatic activity, and oxidative stress—impair germination performance without immediately causing seed death. As a result, seeds may remain viable but exhibit delayed germination, reduced germination speed, or failure to develop into normal seedlings. This phenomenon is commonly associated with sublethal deterioration, in which seeds progressively lose vigor before losing viability (Bailly, 2004; Corbineau et al., 2023).

Therefore, the reduction in dead seeds observed at longer storage periods does not indicate an improvement in physiological quality, but rather reflects a shift in the deterioration process, where seeds transition from viable to low-vigor states before complete mortality occurs.

The coefficient of variation (CV) for FGC (20.20%) indicates moderate experimental variability, while the higher CV observed for dead seeds (40.30%) suggests greater dispersion and variability in this parameter, which may be associated with heterogeneous deterioration within the seed lot.

No significant effects of storage duration were observed for germination speed index (GSI), hard seeds (SD), normal seedlings (PN), abnormal seedlings (PA), and seedling dry mass (MSP). The absence of statistical differences in these variables suggests that the storage conditions were sufficient to maintain structural integrity and basic physiological performance for a certain period.

However, it is important to emphasize that the stability of these parameters does not necessarily indicate the absence of deterioration. Seed ageing is a gradual process that begins at the molecular level, often affecting biochemical and physiological processes before visible changes in germination or seedling development occur. Studies have shown that reductions in antioxidant activity and increases in oxidative damage can precede measurable declines in germination and vigor indices (Sano et al., 2020).

Regarding the germination speed index, although no statistical differences were observed among storage periods, it is widely recognized that higher GSI values are associated with faster and more uniform germination, reflecting greater seed vigor. The maintenance of GSI in this study is likely related to the adequate moisture content during storage, which helped preserve metabolic stability and delayed deterioration processes.

Nevertheless, the reduction observed in germination at longer storage periods indicates that vigor loss occurred, even in the absence of significant changes in GSI. This highlights the importance of using multiple parameters to assess seed quality, as single variables may not fully capture the complexity of deterioration processes.

No significant differences were observed for the percentage of hard seeds (SD) across storage periods. This behavior is consistent with the physiological characteristics of *Cajanus cajan*, in which the occurrence of hard seeds is generally low and more strongly influenced by genetic factors than by storage conditions. Variations in seed coat impermeability are typically associated with genotype-specific traits rather than storage duration, which explains the stability of this parameter under the conditions evaluated (Corbineau et al., 2023).

Similarly, no significant differences were detected for the percentage of normal (PN) and abnormal seedlings (PA). Although seed deterioration is expected to occur over time, the absence of statistical differences in these variables suggests that the storage conditions—particularly the adequate moisture content and packaging—were effective in preserving the structural integrity required for seedling formation. However, it is important to highlight that the maintenance of these parameters does not necessarily indicate the absence of deterioration, as early stages of seed ageing often affect metabolic and biochemical processes before visibly altering seedling morphology (Sano et al., 2020; Corbineau et al., 2023).

Seedling dry mass (MSP) also showed no significant variation among storage periods. This parameter is directly related to the efficiency of reserve mobilization during germination, reflecting the capacity of seeds to transfer stored assimilates to the embryonic axis. The stability observed suggests that, despite the effects of storage on germination percentage, the seeds retained a certain level of functional integrity for biomass accumulation during early seedling development (Bailly, 2004).

In contrast, seedling growth variables were more sensitive to storage duration. The highest root (43.97 mm) and shoot length (59.02 mm) were observed at 12 months of storage, while the lowest values were recorded at 36 months (17.31 mm and 31.99 mm, respectively). This reduction in seedling growth reflects the progressive decline in seed vigor associated with prolonged storage.

The decrease in seedling length is closely linked to reduced efficiency in reserve mobilization and energy metabolism during germination. Seed ageing leads to mitochondrial dysfunction, membrane degradation, and oxidative damage, which impair ATP production and limit the translocation of reserves to growing tissues (Bailly, 2004; Sano et al., 2020). As a result, even when seeds are able to germinate, their capacity to support vigorous seedling growth is significantly reduced.

The superior performance observed at approximately 12 months of storage reinforces the hypothesis that short-term storage may preserve—or even transiently enhance—physiological performance, possibly due to after-ripening effects. However, beyond this period, cumulative deterioration processes become predominant, negatively affecting seed vigor and seedling development (Corbineau et al., 2023).

Overall, these results demonstrate that seedling growth parameters are more sensitive indicators of vigor loss than germination percentage alone. Therefore, the combined evaluation of germination and seedling development is essential for accurately assessing the physiological quality of seeds subjected to storage.

4. Conclusions

Storage in PET bottles proved effective in maintaining seed quality over time. However, seeds stored for 36 months exhibited a reduction in germination and an increase in germination time—despite showing a lower percentage of dead seeds—indicating a loss of vigor rather than necessarily an immediate loss of viability.

The seeds displayed uniform biometric characteristics, with mean values of 5.50 ± 0.25 mm (width), 6.26 ± 0.51 mm (length), and 4.57 ± 0.09 mm (thickness). Furthermore, the moisture content was 11.08 ± 0.92%, and the thousand-seed weight was 104.8 ± 9.24 g, indicating adequate physical and physiological quality for storage.

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 writing or editing of this manuscript.

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Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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