Special Supplement: Pulses

How can the harvest method impact the seed quality of common bean with indeterminate growth habit?[1]

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ABSTRACT

Despite the economic and social significance of common bean (*Phaseolus vulgaris* L.), the national yield remains limited, partly due to crop establishment issues related to low seed quality. This study aimed to evaluate the seed quality of the TAA Dama cultivar (type III, with indeterminate growth habit) harvested using manual, semi-mechanized and mechanized methods. The seed quality was assessed using germination, accelerated aging, seedling emergence, electrical conductivity, computerized seedling image analysis, sodium hypochlorite (mechanical damage) and X-ray test. The semi-mechanized and mechanized harvest methods proved suitable for seeds from plants with indeterminate growth habit, provided that harvest parameters are properly adjusted. However, the presence of hard seeds, particularly in the manually-harvested lot, negatively affected the germination speed and seedling development. The radiographic imaging was effective in detecting internal seed damage, complementing the conventional quality assessments.

KEYWORDS: *Phaseolus vulgaris* L., seed mechanical damage, seed physical dormancy.

RESUMO

Como o método de colheita pode afetar a qualidade de sementes de feijão-comum com hábito de crescimento indeterminado?

Apesar da importância econômica e social do feijão-comum (*Phaseolus vulgaris* L.), a produtividade nacional permanece limitada, em parte devido a problemas de estabelecimento da cultura relacionados à baixa qualidade das sementes. Objetivou-se avaliar a qualidade de sementes de plantas de feijão-comum da cultivar TAA Dama (tipo III, com hábito de crescimento indeterminado) colhidas pelos métodos manual, semimecanizado e mecanizado. A qualidade das sementes foi avaliada por meio de germinação, envelhecimento acelerado, emergência de plântulas, condutividade elétrica, análise computadorizada de imagens de plântulas, hipoclorito de sódio (dano mecânico) e teste de raios X. Os métodos de colheita semimecanizada e mecanizada mostraram-se adequados para sementes de plantas com hábito de crescimento indeterminado, desde que os parâmetros de colheita sejam devidamente ajustados. No entanto, a presença de sementes duras, principalmente no lote colhido manualmente, afetou negativamente a velocidade de germinação e o desenvolvimento das plântulas. A imagem radiográfica foi eficaz na detecção de danos internos nas sementes, complementando as avaliações convencionais de qualidade.

PALAVRAS-CHAVE: *Phaseolus vulgaris* L., dano mecânico em sementes, dormência física de sementes.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption worldwide, serving as a crucial source of plant-based protein, minerals, antioxidants and bioactive compounds (Karavidas et al. 2022, Silva et al. 2024).

Seeds are the fundamental input for the production process, and harvest is one of the most critical stages in bean seed production. It must be carried out carefully, using a method suited to the producer's conditions and considering the developmental stage of the majority of the plant population. Ideally, seeds should be harvested at physiological maturity; however, this is often unfeasible, due to their high moisture content at this stage, particularly in genotypes with indeterminate growth habit. These genotypes are characterized

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by longer cycles and delayed maturation, resulting from a prolonged vegetative phase, which leads to excessive biomass production and asynchronous flowering and pod development (Kwak et al. 2012).

The growth habit in common bean is genetically determined and associated with the expression of traits such as the presence of vegetative (indeterminate) or reproductive (determinate) apical bud, internode length and climbing ability (Dawo et al. 2007). According to Li et al. (2018), the indeterminate growth habit is controlled by *PvTFL1y*, an ortholog of *Terminal Flower 1* (*TFL1*) in *Arabidopsis*, which encodes an apical meristem signaling protein derived from a common legume ancestor. The phenotypic expression of growth habit is classified into four main types: determinate bush (type I), indeterminate upright (type II), indeterminate prostrate or semi-prostrate (type III), and indeterminate climber (type IV).

In the 2023/2024 season, bean cultivation covered estimated 2.86 million hectares, reflecting a 5.8 % increase, and the total production reached 3.25 million tons, representing a 7 % increase, when compared to the previous season (Conab 2024). Consequently, breeding programs have focused on developing genotypes with this grain type, most of which exhibit indeterminate type II or III growth habit (Pereira et al. 2019, Vieira et al. 2022).

The harvest of common bean seeds can be performed manually, semi-mechanically or mechanically. According to Gupta et al. (2024), manual harvest involves performing all operations by hand, including uprooting, gathering and threshing, what requires a high labor input. Semi-mechanized harvest is a two-step process: plants are first uprooted and windrowed manually, followed by mechanical gathering, threshing, cleaning and seed conditioning using combine harvesters. Fully mechanized harvest employs a self-propelled harvester to perform all steps simultaneously. This method is suitable for larger farms with modern technology and gentle slopes. However, improper harvest practices, such as incorrect machine settings or poor seed handling, can compromise seed quality.

During mechanized harvest, seeds act as static bodies that collide with components such as threshing cylinders, conveyors and sieves inside the machinery, leading to impacts that may cause mechanical damage. This damage can have immediate effects, reducing seed germination and vigor. It includes fractures in the embryonic axis - potentially resulting in abnormal seedlings - or damage to cotyledons, which delays nutrient translocation to the embryo (Oliveira et al. 2021). Additionally, cracked seed coats or superficial abrasions can create entry points for pathogens, increasing the risk of infestations (Lemes & Catão 2024). Even with optimal machine adjustments, mechanical damage still occurs, influencing seed lot quality to varying degrees - a challenge exacerbated by the growing demand for agricultural mechanization.

Seed quality is fundamental to achieving the crop's productive potential. According to Marcos-Filho (2020), it encompasses a set of attributes that determine seed value for planting, including genetic, physical, physiological and sanitary characteristics. The X-ray technique enables non-destructive visualization of internal seed structures, offering a promising tool for detecting mechanical damage in legume seeds such as common bean (Mondo et al. 2009), mung bean (Machado et al. 2020), cowpea (Rego et al. 2023), soybean (França-Silva et al. 2023) and green manure species (Zacharias et al. 2024). Therefore, continued research into factors associated with mechanical damage during harvest is essential to refine practices and ensure high-quality seeds. Accordingly, this study aimed to evaluate the effects of manual, semi-mechanized and mechanized harvest methods on the seed quality of common bean genotypes with indeterminate growth habit.

MATERIAL AND METHODS

The experiment was conducted during the 2023 growing season (May-August) in a commercial farm dedicated to common bean seed production in Avaré, São Paulo state, Brazil (23º05'56"S, 48º55'33"W and altitude of 766 m). The used cultivar was TAA Dama (growth habit type III).

According to the Köppen climate classification, the region has a subtropical climate (Cwa), characterized by hot summers and dry winters. The soil is classified as a Dystrophic Red Oxisol (*Latossolo Vermelho Distrófico*) (Santos et al. 2018), corresponding to Ferralsol (FAO 2015). During the seed production period, the average air temperature was 18 ºC, the mean relative humidity was 70 %, and the total rainfall was 140 mm (Brasil 2023). Irrigation was managed according to the crop development stages; however, system failures occurred during the seed maturation.

The treatments consisted of three harvest methods performed during the same period: manual, semi-mechanized and mechanized. In the manual method, plants were cut at the ground level using pruning shears, placed in polyethylene bags and transported to a ventilated shed. The seeds were manually threshed immediately after collection. For the semi-mechanized harvest, the plants were uprooted and arranged in rows in the field for natural drying under direct sunlight. After drying, the material was processed with a New Holland CR 5.85 combine harvester operating at 800 rpm. The mechanized harvest was performed using the same harvester model set to a cylinder rotation speed of 800 rpm and a forward speed of approximately 2.5-3.0 km h^{-1}. At the harvest time, the seed moisture content ranged 12.2-15.9 %.

Post-harvest analyses were conducted at the laboratory. Initial procedures included purity analysis and seed size standardization using the uniformity test (Brasil 2009). The study was conducted on seed fractions retained between 14/64" (5.5 mm) × ¾" (19 mm) and 11/64" (4.5 mm) × ¾" (19 mm) sieves. The seeds were stored in Kraft paper bags inside a dry chamber at 20 ºC and 40 % of relative humidity for two months. The seed quality was evaluated using the following tests:

Moisture content: determined using the oven method at 105 ± 3 ºC, for 24 hours (Brasil 2009), with two subsamples of approximately 5 g per treatment. The moisture content was assessed at harvest and after the accelerated aging test, and the results were expressed as percentage on a wet basis;

Germination: conducted with 8 replicates of 25 seeds per treatment. The seeds were placed in rolls of germination paper moistened with water at 2.5 times the paper's mass and kept in a germination chamber at 25 ºC. Evaluations followed the criteria established by Brasil (2009) and were performed on the fifth (first count) and the ninth day after sowing. The results were expressed as percentage of normal seedlings;

Accelerated aging: seeds from each treatment were arranged in a single layer on a metal screen suspended inside transparent plastic boxes (11 × 11 × 3.5 cm) containing 40 mL of distilled water, to create an environment of approximately 100 % of relative humidity. The boxes were sealed and placed in a water-jacketed chamber at 41 ºC, for 48 hours (Bertolin et al. 2011). After aging, the seeds underwent germination testing and were evaluated after five days. The results were expressed as percentage of normal seedlings;

Electrical conductivity: conducted with 8 replicates of 50 seeds per treatment. The seeds were previously weighed using a precision scale (0.001 g) and soaked in 75 mL of deionized water in 180-mL plastic cups. The containers were maintained in a germination chamber at 25 ºC, for 24 hours (Silva et al. 2013). At the end of this period, the electrical conductivity was measured using a Digimed® conductivity meter (model DM-32). The results were expressed in µS m^{-1} g^{-1} of seeds;

Sodium hypochlorite (mechanical damage): performed with 8 replicates of 50 seeds per treatment. Visibly, the damaged seeds were excluded prior to testing. The samples were placed in plastic cups containing 50 mL of a 5.25 % sodium hypochlorite solution and kept at room temperature under laboratory benches for 10 min (Krzyzanowski et al. 2023). Afterwards, the seeds were rinsed with running water and placed on paper towels for evaluation. Swollen seeds were separated and counted in each replicate. The results were expressed as percentage of seeds exhibiting mechanical damage;

Seedling emergence: conducted with 8 replicates of 50 seeds per treatment. The seeds were sown on a 6-cm layer of medium-textured sand in plastic boxes (32 × 28 × 10 cm), then covered with a 3-cm sand layer. Water was added to 60 % of the substrate's maximum retention capacity, and the boxes were maintained under standard laboratory conditions. Daily counts were recorded to determine the emergence speed index (Maguire 1962). The final seedling emergence was recorded on the fourteenth day and expressed as percentage of seedlings with fully expanded cotyledon leaves;

Computerized seedling image analysis: conducted with 5 replicates of 20 seeds per treatment. The seeds were arranged in two parallel rows on two sheets of germination paper, covered with a third sheet, and rolled. The substrate was moistened with water equivalent to 2.5 times its mass, and the rolls were placed in a germination chamber at 25 ºC, in the dark. After three days, seedlings (normal and abnormal) and non-germinated seeds were transferred to a blue ethylene vinyl acetate (EVA) sheet for contrast. Images were captured using an HP Scanjet 200 scanner, mounted in an inverted position inside an aluminum box (60 ×

50 × 12 cm), set to 300 dpi resolution and connected to a computer (3.50 GHz Intel Core i7, 16 GB RAM, 1 TB HDD). The images were saved in the jpeg format and the image processing performed using the Vigor-S software, which calculated the hypocotyl length (cm), primary root length (cm), total seedling length (cm) and indices for growth, uniformity and vigor (scale: 0-1,000, directly proportional to vigor). Growth and uniformity contributed with 70 and 30 %, respectively, to the vigor index, whereas the hypocotyl and primary root contributed with 10 and 90 %, respectively, to the growth index (Castan et al. 2018). The number of hard seeds was also recorded as described by Brasil (2009);

X-ray: 200 seeds per treatment were affixed with double-sided transparent adhesive tape onto acetate sheets (210 × 297 mm) and numbered. Radiographic images were obtained using a Faxitron digital X-ray system (model MX-20 DC 12) connected to a computer (Intel Core 2 Duo, 3.16 GHz, 2 GB RAM, 160 GB HDD). The seeds were positioned at 28.6 cm from the X-ray source, and exposure was set at 32 kV, for 10 seconds. The images were saved in the tif format for analysis. After imaging, the seeds were subjected to germination testing by placing them in groups of 10 on the upper third of the germination substrate, to allow for individual seedling development. After five days, seedlings (normal and abnormal) and non-germinated seeds were photographed using a digital camera (Sony Cyber-shot, model DSC-W530). Seedling performance was compared with the corresponding X-ray images;

Statistical analysis: the experiment followed a completely randomized design. Original data were tested for normality and homogeneity using the Shapiro-Wilk and Bartlett tests, respectively, at 5 % of significance ($p \leq 0.05$). The data were then subjected to analysis of variance (Anova), and means compared using the Tukey test ($p \leq 0.05$). The X-ray test results were analyzed by comparing the embryo integrity (as observed in radiographs) with the corresponding seedling performance. All statistical analyses were performed using the R software, version 4.4.2.

RESULTS AND DISCUSSION

The semi-mechanized harvest method resulted in the highest seed moisture content at harvest (15.9 %), followed by the mechanized (13.9 %) and manual (12.2 %) methods (Figure 1). After two months of storage under cold and dry conditions, the moisture content stabilized between 8 and 9 % for all treatments. These differences remained within the recommended two-percentage-point range, ensuring reliability in the assessment of the physiological potential (Rodrigues et al. 2020).

At physiological maturity, a high water content in seeds typically makes mechanized harvest unfeasible, due to the risk of mechanical damage. To address this, desiccants are often applied to reduce moisture before harvest (Rosado et al. 2019, Silva et al. 2021). The high moisture content observed in the semi-mechanized method may be attributed to uprooted plants undergoing natural drying below the soil surface. As hygroscopic structures, seeds gain or lose water in response to ambient temperature and humidity. Prolonged field exposure increases their susceptibility to environmental fluctuations (Abati et al. 2022).

Although the harvest method did not significantly influence the final germination, it affected the germination speed (Figure 2). No significant differences were observed among the treatments in the production of normal seedlings, as measured by the germination, accelerated aging and seedling emergence tests (Figures 2A, 2C and 2D), with all treatments exceeding the 80 % minimum required for seed commercialization in Brazil (Brasil 2013). However, the first germination count was

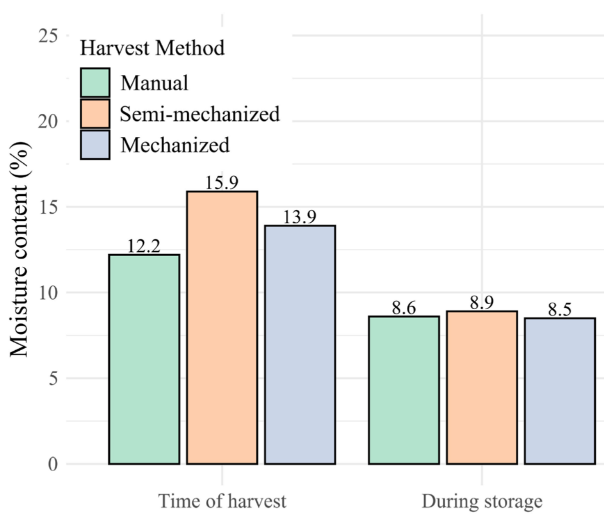

Figure 1. Average moisture content of common bean seeds harvested by different methods, determined at harvest and after storage.

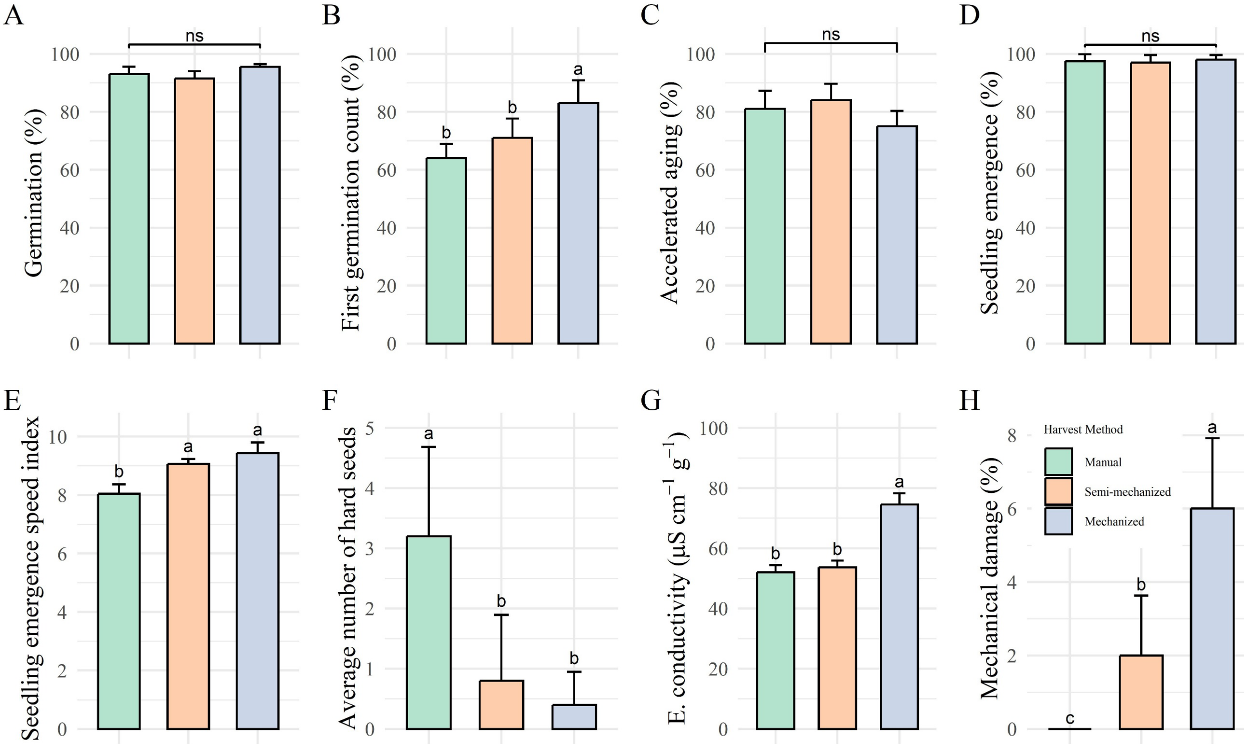

Figure 2. Quality characterization of common bean seeds harvested using different methods. Bars followed by the same letter do not differ significantly by the Tukey test at 5 % of probability.

significantly higher in the mechanized (83 %) and semi-mechanized (71 %) methods than in the manual method (64 %) (Figure 2B). Likewise, the seedling emergence speed index was greater in the mechanized (9.44) and semi-mechanized (9.07) treatments, when compared to the manual (8.05) one (Figure 2E).

The reduced first germination count (Figure 2B) and seedling emergence speed index (Figure 2E) in the manually harvested seeds are associated with a higher incidence of hard seeds (Figure 2F), which delay germination and seedling emergence due to slower water uptake. "Hard seeds" refer to seeds that fail to absorb water within a normal time frame (Figure 3). This condition is caused by seed coat impermeability, a common dormancy mechanism in Fabaceae species, including common bean, and is associated with the species' evolutionary and domestication processes (Brasil 2009, Soltani et al. 2021, Wen et al. 2024).

Conversely, the mechanically harvested seeds exhibited a significantly higher electrical conductivity (74.52 µS cm^{-1} g^{-1}) than seeds harvested by the other methods (Figure 2G), indicating a greater electrolyte leakage and reduced membrane integrity. This outcome is attributed to increased mechanical damage during mechanized harvest (Figure 2H). Although this method may reduce the hard seed occurrence, it can cause latent damage to the seed coat and embryo, compromising the physiological potential. As expected, the sodium hypochlorite test detected no

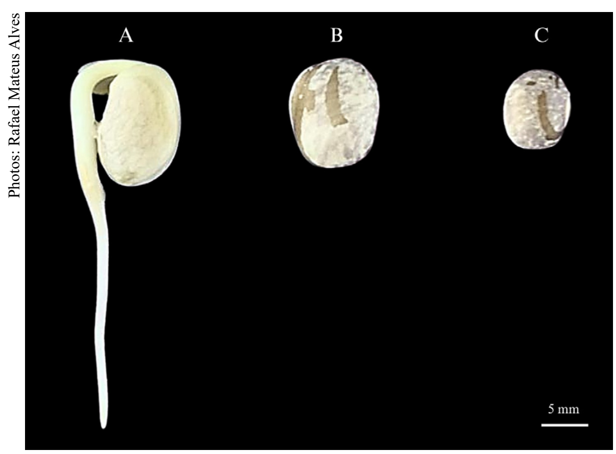

Figure 3. Representative image of a common bean: seedling (A), imbibed seed (B) and hard seed (C), at three days after the test initiation.

mechanical damage in seeds from the manual method, as these seeds were extracted and cleaned without machinery, minimizing physical impact.

Vigor tests offer complementary information about seed lot quality (Marcos-Filho 2020). Electrical conductivity reflects the integrity of cell membranes - higher values indicate reduced vigor (Ribeiro et al. 2025). The higher mechanical damage observed in the mechanized method may be further explained by the characteristics of the TAA Dama cultivar, which has indeterminate growth habit. This trait may have led to harvest seeds with higher moisture content, making them more susceptible to mechanical injury. Although mechanical harvest caused greater damage, confirmed by both the electrical conductivity and sodium hypochlorite tests, no significant deterioration in germination or emergence was observed.

Mechanical damage arises from abrasions and impacts that seeds endure during harvest, processing and bagging (Benaseer et al. 2018). These injuries, both internal and external, may compromise seed quality (Altizani-Júnior et al. 2023). Therefore, a strict control of post-harvest operations is essential to reduce damage during seed production (Sukhanova et al. 2023). However, the degree of damage varies depending on the crop and conditions. For instance, Astanakulov et al. (2021) found higher mechanical damage in semi-mechanized harvest of mung bean (*Vigna radiata* L. Wilzek), whereas Eckert et al. (2011) reported no significant difference between mechanized and semi-mechanized methods for common bean seeds, being consistent with the findings of the present study.

The seedling image analysis using the Vigor-S software confirmed the higher frequency of hard seeds in the manually harvested lot (Figure 2F), which delayed germination and seedling development (Figure 4). This aligns with the results of the first germination count and seedling emergence speed index (Figures 2B and 2E). As a result, seedlings from manual harvest exhibited reduced development, with shorter hypocotyls and primary roots, leading to decreased total seedling length and increased developmental variability (Figures 4A-4C). These deficiencies reflected in lower seedling growth, uniformity and vigor indices, relatively to the semi-mechanized and mechanized methods (Figures 4D, 4E and 4F).

The computerized seedling image analysis, a vigor test based on seedling development, showed

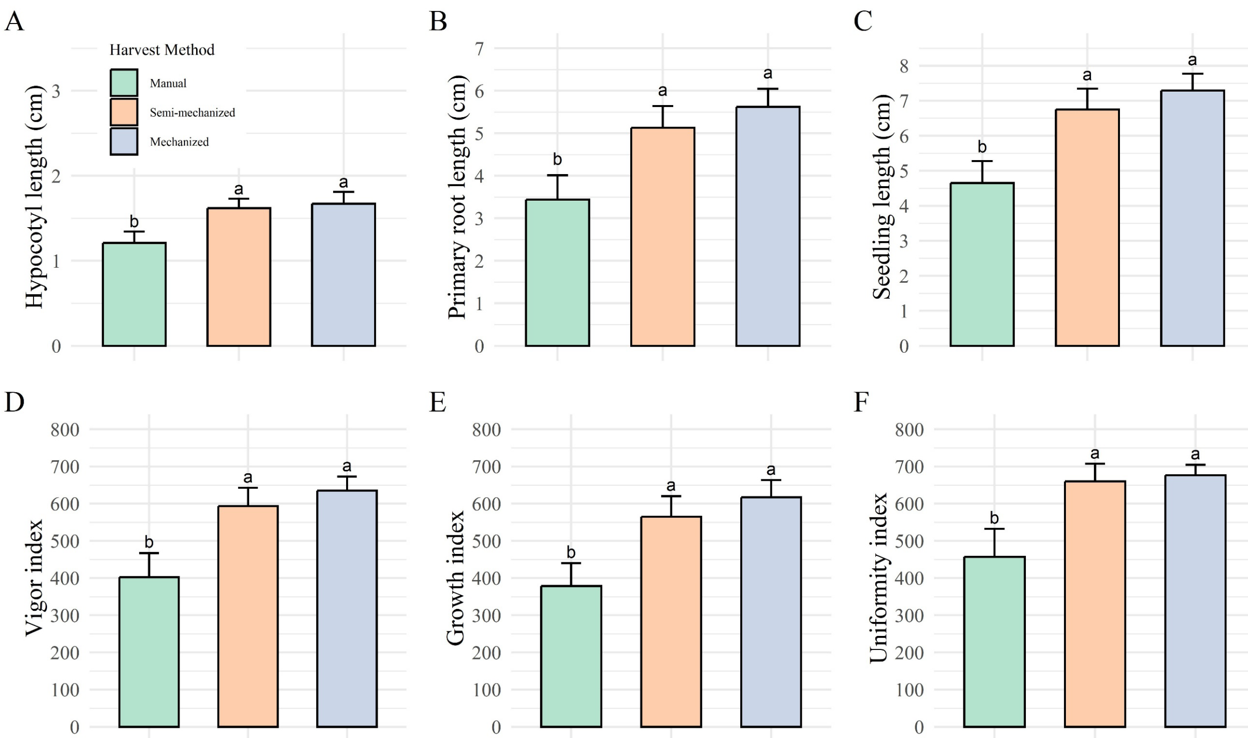

Figure 4. Computerized image analysis of common bean seedlings from seeds harvested using different methods. The same letters indicate no significant differences among the treatments, as determined by the Tukey test at 5 % of probability.

reductions in several parameters due to the presence of hard seeds, particularly in the manually harvested lot (Figure 5A). The selection of pods and seeds at the physiological maturity stage - typically an advantage for ensuring a higher germination potential - was not reflected in the manual harvest results, due to the occurrence of hard seeds. This characteristic is not visually detectable at harvest, but became evident during the computerized image analysis, in which some seeds failed to imbibe water after three days (Figures 5A and 5B). The occurrence of physical dormancy in leguminous seeds may vary significantly across cultivars, populations, growing seasons, individual plants, and even among seeds within the same pod (Smýkal et al. 2014).

In the present study, failures in the irrigation system during seed development likely induced water stress, contributing to the higher incidence of hard seeds. According to França et al. (2020), the formation of hard seeds in *Phaseolus vulgaris* is strongly influenced by both genotype and water availability during seed development, with drought conditions increasing the frequency of hard seeds. In this context, the semi-mechanized and mechanized harvest caused small fissures in the seed coat, what facilitated the water uptake - reflected in the lower hard seed incidence but higher mechanical damage in these treatments (Figure 2H). It is important to note that the sodium hypochlorite test does not differentiate between superficial and internal damage, or detect latent injuries that may affect seed quality. Therefore, any lesion, regardless of severity, is classified as mechanical damage in this test. For this reason, the X-ray analysis was employed as a complementary tool to provide a more comprehensive evaluation of internal tissue integrity and its relationship to seed performance.

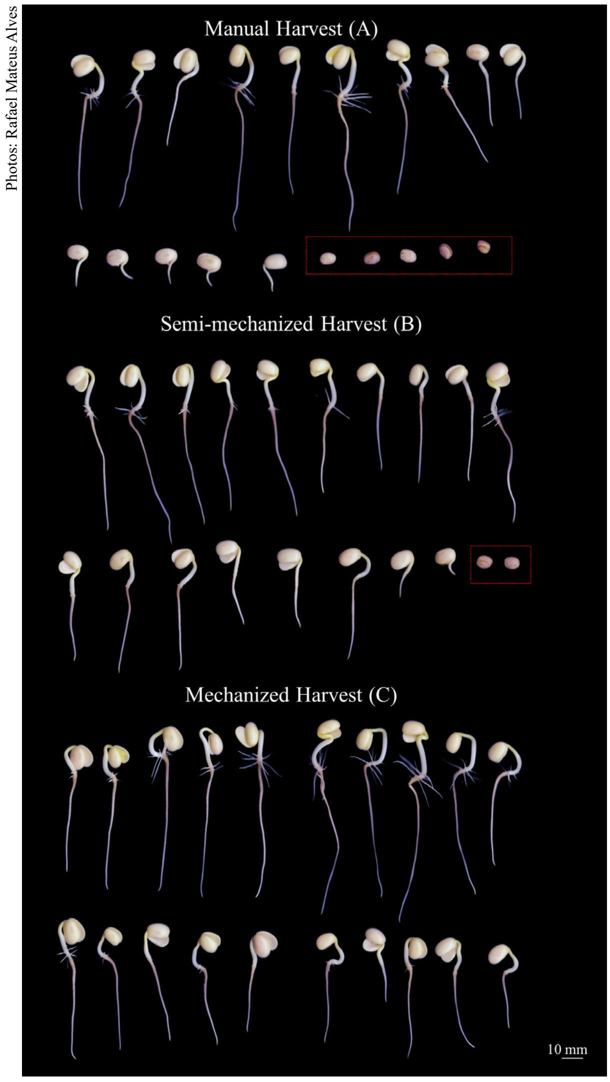

Figure 5. Visual appearance of three-day-old common bean seedlings obtained from seeds harvested using manual (A), semi-mechanized (B) and mechanized (C) methods.

The radiographic analysis revealed no significant differences in the frequency of internal damage among the seeds harvested using manual, semi-mechanized or mechanized methods. In the X-ray images, the tissue density is associated with grayscale values - denser, healthy tissues appear light gray due to greater resistance to radiation, whereas deteriorated tissues with lower density appear darker (França-Silva et al. 2023). Figure 6A illustrates an intact seed, whose normal seedling is shown in Figure 6B. In contrast, the superficial mechanical damage, such as seed coat fractures (Figure 6C), did not prevent the development of normal seedlings (Figure 6D).

The X-ray images demonstrated that high-pressure impacts may severely damage the physiological potential of common bean seeds, especially when the embryonic axis is affected (Mondo et al. 2009). However, the absence of severe internal damage in this study indicates that the harvester was properly calibrated, despite the mechanized method exhibiting a higher proportion of seeds with superficial mechanical damage (Figure 2H). This damage did not compromise the overall physiological quality of the seed lot, if compared to other harvest methods.

The analysis of seeds in Figures 6E and 6G, along with their respective seedlings (Figures 6F and 6H), shows embryonic malformations, including cotyledon fissures. While the seed in Figure 6F produced a normal seedling, the seedling in Figure 6H exhibited an asymmetry that could compromise its survival. Figures 6I and 6K display tissue deterioration characterized by dark regions on the X-ray images, indicating a lower tissue density. The seed in Figure 6I produced a normal seedling (Figure 6J), but that in Figure 6K was non-viable (Figure 6L). These outcomes emphasize that the damage impact depends not only on severity, but also on its location, extent and depth within the seed.

Photos: Rafael Mateus Alves

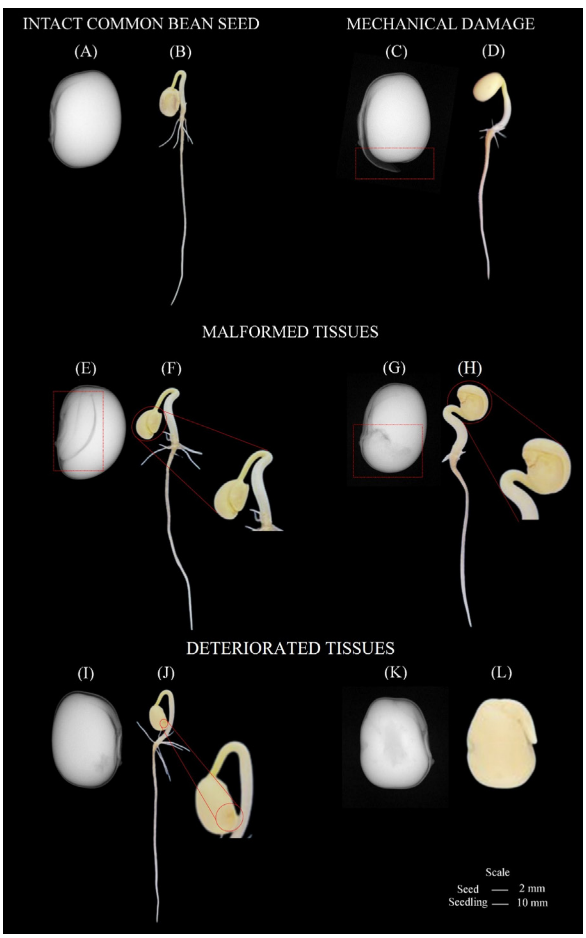

Figure 6. Radiographic image showing the internal structures of an intact common bean seed (6A) and its resulting normal seedling (6B). For comparison, seeds with mechanical damage (6C), tissue malformations (6E and 6G) and tissue deterioration (6I and 6K) are shown alongside their respective normal seedlings (6D, 6F, 6H and 6J) and one non-germinated seed (6L).

Understanding the causes and consequences of structural malformations and tissue deterioration is critical for improving seed quality and achieving uniform seedling establishment. Damage to the cotyledons, which play a vital role in early photosynthesis, may limit the energy supply necessary for initial seedling growth.

CONCLUSIONS

1. The semi-mechanized and mechanized harvest methods did not compromise the overall physiological quality of common bean seeds and are suitable for the commercial seed production of genotypes with indeterminate growth habit (type III), provided that the technical parameters for harvest and threshing are properly calibrated;
2. The presence of hard seeds, especially in manually harvested lots, negatively affects the germination speed and seedling development, compromising the stand establishment and uniformity;
3. The X-ray image analysis proved effective in identifying internal structural alterations and damage in common bean seeds.

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