

Original Article

## Physiological quality of seeds of arabic coffee cultivars stored for a period of two years

Qualidade fisiológica de sementes de cultivares de café arábica armazenadas por um período de dois anos

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### Abstract

The rapid loss of viability, coupled with the difficulties and limitations in conserving coffee seeds, are some of the challenges that nurseries are currently facing. Thus, the objective of this work was to analyze the physiological quality of stored Arabica coffee seeds from cultivars recommended for planting in Brazilian mountainous regions. Seeds from 10 Arabica coffee cultivars were used: Catucaí-785/15, Catucaí-2SL, Catucaí-24/137, Japan, Arara, Acauã, Catuaí IAC-81, Mundo Novo IAC-379/19, Catuaí IAC-62, and Caturra IAC-479. The seeds were collected in the Arabica coffee seed production field, in the municipality of Marechal Floriano-ES. They were subsequently processed and dried in the shade, at room temperature, until reaching a humidity of  $35 \pm 1\%$ , analyzed and stored in a natural laboratory environment ( $25 \pm 2^\circ\text{C}$ ), for 24 months. The following were analyzed: seed water content, germination, germination speed index, electrical conductivity and potassium leaching. The experimental design used was completely randomized, with ten cultivars, two storage times, with four replications of 25 seeds. Storing arabica coffee seeds for 24 months results in a drop in the germination percentage. Stored seeds of the Catucaí 24/137 and Arara cultivars showed germination percentages similar to those of newly harvested seeds. Seeds of the Catuaí IAC-62 cultivar maintain vigor during storage. The stored seeds of the Caturra IAC-476 and Japi cultivars showed a reduction in physiological quality. Electrical conductivity and potassium leaching tests are efficient in identifying seeds in an advanced state of deterioration. Seeds of Arabica coffee cultivars stored for 24 months, under the conditions of the present study, produce abnormal seedlings.

**Keywords:** *coffee arabica* L., germination, longevity, seedling production.

### Resumo

A rápida perda de viabilidade atrelada às dificuldades e limitações enfrentadas na conservação das sementes de café, são alguns dos desafios que os viveiristas têm enfrentado atualmente. Assim, objetivou-se com o presente trabalho avaliar a qualidade fisiológica de sementes de cultivares de café arábica armazenadas, recomendadas para plantio nas regiões serranas brasileiras. Foram utilizadas sementes de 10 cultivares de café arábica: Catucaí-785/15; Catucaí-2SL; Catucaí-24/137; Japi; Arara; Acauã; Catuaí IAC-81; Mundo Novo IAC-379/19; Catuaí IAC-62 e Caturra IAC-479. As sementes foram colhidas no campo de produção de sementes de café arábica, no município de Marechal Floriano-ES. Em seguida, foram processadas e secas até atingir umidade de  $35\% \pm 1\%$ . Posteriormente, foram submetidas aos testes de germinação e vigor, e o restante das amostras armazenadas em ambiente natural de laboratório ( $25 \pm 2^\circ\text{C}$ ) por 24 meses. Após esse período, foram submetidas aos testes de condutividade elétrica, lixiviação de potássio, germinação e vigor. O delineamento experimental utilizado foi o inteiramente casualizado, com dez cultivares, dois tempos de armazenamento, com quatro repetições de 25 sementes. O armazenamento de sementes de café arábica por 24 meses, resulta em queda na porcentagem de germinação. Sementes dos cultivares Catucaí 24/137 e Arara, apresentam porcentagem de germinação semelhantes aos das sementes recém-colhidas. Sementes do cultivar Catuaí IAC-62 apresentam resistência ao impacto negativo do armazenamento sobre o índice de velocidade de germinação. As sementes dos cultivares Caturra IAC-476 e Japi após 24 meses de armazenamento apresentam os menores índices de germinação e vigor. Os testes de condutividade elétrica e lixiviação de potássio são eficientes no auxílio da identificação de sementes que estão em estado avançado de deterioração. Sementes de cultivares de café arábica armazenadas por 24 meses, nas condições do presente estudo, proporcionam plântulas anormais.

**Palavras-chave:** *Coffea arabica* L., germinação, longevidade, produção de mudas.

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## 1. Introduction

Brazil is considered the largest producer and exporter of coffee beans in the world, in addition to occupying second place in the ranking of consumption of the drink, factors that demonstrate the great economic importance that coffee farming has in the country. In the year 2022, Brazilian production was 50.92 million processed bags (60 kg), with a production area of approximately 1,841.5 hectares. The prospects are good, given that in the last decade it has been observed that the global demand for coffee has increased by around 2.0% per year, providing great challenges and opportunities for the coffee production chain (Brasil, 2022; ICO, 2023).

To meet the growing global demand for coffee, the production of high quality seedlings related to physical, physiological, genetic and sanitary characteristics are extremely important in the production process, with the use of good quality seeds being essential for the formation of seedlings (Trujillo et al., 2019). Therefore, in search of greater sustainability, the search for seeds of coffee cultivars that, in addition to having good productive capacity, are resistant to attacks by diseases such as rust (*Hemileia*) has increased. *Vastatrix*) and cercosporiosis (*Cercospora coffeicola*) (Carvalho et al., 2017).

One of the biggest challenges for coffee nurseries is the rapid loss of seed viability, which ends up concentrating the production of seedlings in periods close to the grain harvest, mainly from June onwards, a period in which temperatures are lower and unfavorable for coffee planting. Therefore, the formation and maintenance of seed stocks for safe propagation in the medium and long term are negatively affected (Penido et al., 2021; Vilela et al., 2021).

The conservation of seeds of the genus *Coffea* presents limitations due to recalcitrant behavior, a factor related to its intermediate and recalcitrant characteristics regarding desiccation tolerance and storage behavior. Therefore, the quality of coffee seeds is affected mainly by the water content, drying conditions, packaging and storage, with seeds of these species being characterized by low longevity, which under inadequate storage conditions can suffer irreversible damage to their viability (Ellis et al., 1991; Abreu et al., 2014; Coelho et al., 2015; Fantazzini et al., 2018), resulting in the beginning of degradation of cell membranes and a drop in seed vigor (Delouche and Baskin, 1973). The conductivity test electrical as a seed vigor test he has been considered as one of the most promising by the International Seed Analysis Association (ISTA, 2017), due to the disorganization of cell membranes and the loss of cellular constituents as exudates in the imbibition water (Abdul-Baki and Baker, 1973; Delouche and Baskin, 1973).

Electrical conductivity and potassium leaching tests have been widely used in studies to analyze the integrity of membranes, making it possible to establish an association between these tests and the deterioration of coffee beans (Santos et al., 2009; Angélico et al., 2011; Isquierdo et al., 2011; Alves et al., 2017).

The germination test, in addition to determining the productive potential of a specific batch, can also be used to analyze the quality of stored seeds and the formation of seedlings (Malau et al., 2018; Wibowo et al., 2020).

However, there is a need to complement the information obtained in germination tests under ideal conditions, in relation to field conditions, whose conditions are adverse, in which complementation is used with seed vigor tests, highlighting the seed test. potassium leaching (Marcos Filho, 2015).

Based on the factors mentioned, carrying out research that demonstrates the effects of storage on the physiological quality of different seeds of coffee cultivars is essential to evaluate the quality standard of the seeds. With this, the objective was to study the physiological quality of seeds of stored Arabica coffee cultivars, seeking to improve and adapt the seedling production system.

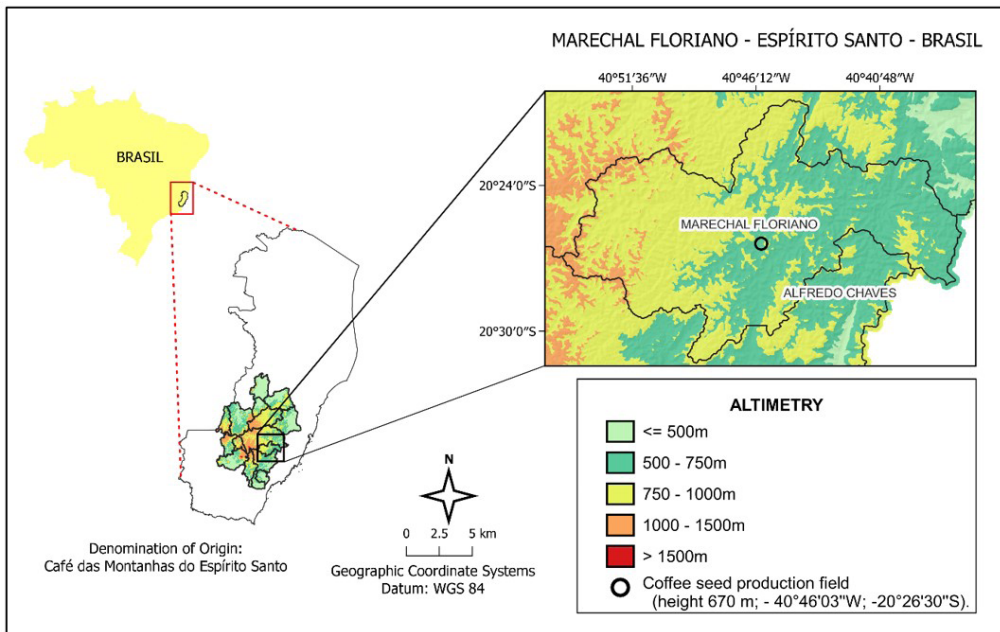
## 2. Material and Methods

The experiment was conducted at the Seed Analysis Laboratory, at the Center for Agricultural Sciences and Engineering at the Federal University of Espírito Santo, in Alegre, ES. Using Arabica coffee seeds from cultivars: Group 1. Catucaí: CV Catucaí-785/15, Catucaí-2SL (sel. CAK) and Catucaí-24/137 and Japi (all selected from the cross between the groups Icatu × Catucaí) - tolerant to coffee rust; Group 2. Sarchimor: Arara (yellow cross Icatu 2944 × Sarchimor 1669/20) and Acauã (cross Mundo Novo IAC-388/17 × Sarchimor IAC-1668) - tolerant to coffee rust; Group 3. Others: Catucaí IAC-81 (cross between Mundo Novo × Caturra), Mundo Novo IAC-379/19 (cross between Sumatra and Bourbon Vermelho) and Catucaí IAC-62 (cross between Mundo Novo × Caturra) and Caturra IAC- 479 (Red Bourbon mutation) - cultivars susceptible to coffee rust, harvested in the coffee seed production field accredited by MAPA, located in the municipality of Marechal Floriano (altitude 670 m; - 40°46'03"W; - 20°26'30"S), mountainous region of the state of Espírito Santo, Brazil (Figure 1).

The harvest was carried out manually, in the middle third of the plants. The fruits were processed in a washer-separating machine, eliminating poorly formed (floating) fruits and separating the ripe ones. Subsequently, manual pulping was carried out, with natural fermentation for 24 hours and elimination of mucilage, using 30% water in relation to the volume of coffee. Drying was carried out on a suspended patio 1 m above the ground, made of black polyolefin mesh, until reaching a moisture content of  $35 \pm 1\%$  (bu) and taken to the Seed Analysis Laboratory, where they were packaged in 0.10 mm plastic packaging and stored in a natural environment under laboratory conditions ( $25 \pm 2$  °C) for 24 months, to simulate field conditions. In most cases, storage is done in an environment with room temperature. Subsequently, the following were analyzed:

Seed water content - was determined by the greenhouse method, at  $105 \pm 3$  °C for 24 hours (Brasil, 2009), using three replications of 10 seeds of each cultivar.

Germination - the endocarp (parchment) of the seed was removed by immersion in 70% (v/v) alcohol for two minutes, followed by washing in distilled water and immersion in a 2% (v/v) sodium hypochlorite solution) for three minutes and the germination test was set up using four replications of 25 seeds for each treatment. Sowing was done on rolls of germitest type paper moistened with distilled water in



**Figure 1.** Location of the coffee seed production field accredited by the Ministry of Agriculture and Livestock (MAPA).

a proportion equivalent to 2.5 times the mass of dry paper, which were kept in a BOD type germination chamber, regulated at an alternating temperature of 20-30 °C, in the absence of light. The evaluations were carried out 15 and 30 days after sowing, calculating the percentage of normal seedlings (Brasil, 2009), and the results expressed as a percentage of germination.

Germination speed index - was determined concomitantly with the germination test, and the number of seeds with primary root protrusion equal to or greater than 2 mm was calculated daily, until the 30th day (Maguire, 1962). Electrical conductivity test - was carried out with four replications of 50 seeds for each cultivar, with the seeds weighed on a 0.0001 g precision scale, and immersed in beakers containing 75 mL of distilled water and kept in BOD at 25 °C. Analyzes were carried out on the samples after five hours, measuring the electrical conductivity present in the solution, using a DIGIMED DM-32 conductivity meter. Electrical conductivity was obtained by dividing the value obtained by the mass of the 50 seeds, and the results were expressed in  $\mu\text{S cm}^{-1} \text{g}^{-1}$ .

Potassium leaching test - was carried out with four replications of 50 seeds for each cultivar, with the seeds weighed on a 0.0001 g precision scale, and immersed in beakers containing 75 mL of distilled water and kept in BOD at 25 °C. Potassium analyzes were carried out after five hours, using a DIGIMED DM-62 flame photometer, and the results were in potassium leachate in ppm/g of sample (Prete, 1992).

Aerial part length - was determined 30 days after sowing, with the aid of a millimeter ruler, by measuring the length between the collar and the apex of the leaf of ten seedlings, taken at random, and the result expressed in  $\text{cm plant}^{-1}$ .

Root length - was determined 30 days after sowing, with the aid of a millimeter ruler, measuring ten seedlings from the neck to the tip of the largest root and the results expressed in  $\text{cm plant}^{-1}$ .

Fresh and dry weights of seedlings - were determined 30 days after sowing, using an analytical balance (0.0001 g). After obtaining the fresh mass, the seedlings were placed in *Kraft paper bags*, kept in a convection oven at 65 °C for 72 hours (constant mass) and the results were expressed in  $\text{mg plantule}^{-1}$ .

The experimental design used was completely randomized, with ten cultivars (Catucaí-785/15; Catucaí-2SL; Catucaí-24/137; Japi; Arara; Acauã; Catuaí IAC-81; Mundo Novo IAC-379/19; Catuaí IAC -62 and Caturra IAC-479) and two storage times (freshly harvested and stored for 24 months), with four replications of 25 seeds. The means were compared using the F test at a 5% probability level and the Scott Knott mean grouping test was performed. Principal component analysis was performed to group the coffee cultivars according to the characteristics analyzed through visual examinations in graphical dispersions. All statistical analyzes were performed using the R software (R Core Team, 2023).

### 3. Results and Discussion

For newly harvested seeds (zero month of storage), no significant differences were observed between the cultivars analyzed in relation to the germination variable, whose averages varied from 87 to 99%. However, among the seeds stored for 24 months, the cultivar Catuaí IAC-62 stood out, which presented a higher germination average (99%), while the cultivars Japi and Caturra IAC-476 presented the lowest

averages (2 and 9%, respectively) (Table 1). The reduction in germination observed in stored seeds may be associated with a decrease in enzymatic activity, reflected in a lower respiratory potential in the seed, consequently with a reduction in energy production (ATP) and in the supply of nutrients for the seed to germinate, as observed in soybean seeds after premature aging (Xin et al., 2014).

When comparing newly harvested seeds with seeds stored for 24 months, only the seeds of the Catucaí 24/137 and Arara cultivars did not show a significant difference between times (0 and 24 months), with averages for the Catucaí 24/137 cultivar of 95 and 87%, respectively and Arara 88 and 87%, respectively. In the other cultivars, the germination averages of newly harvested seeds were higher when not stored (Table 1), corroborating the results obtained by Wibowo et al. (2020).

Among the recently harvested seeds, the cultivars Catucaí 785/15 and Catucaí 2 SL/CAK presented the highest average germination speed index (1.70 and 1.75, respectively), while in seeds stored for 24 months, the cultivar Catucaí IAC-62 presented the highest GSI (1.59), and cultivars Caturra IAC-476 and Japi presented the lowest averages (0.15 and 0.03, respectively) (Table 2). This behavior shows that during the storage period the seeds present a reduction in physiological potential, culminating in a reduction in germination and vigor, as observed by several authors (Popinigis, 1985; Carvalho and Nakagawa, 2012; Marcos Filho, 2015; Wibowo et al., 2020; Lima et al., 2019, 2023). Comparing the cultivars in terms of storage times (0 and 24 months), it can be seen that the Catucaí IAC-62 cultivar showed a smaller reduction in vigor analyzed by IVG, whose results were similar between times (1.50 and 1.59, respectively). However, in the other cultivars, the GSI averages of newly harvested seeds were higher when compared to the averages of seeds stored

for 24 months, suggesting an increased degree of seed deterioration during storage (Delouche, 2002). According to Ferreira et al. (2022), the deterioration of coffee seeds throughout storage is intrinsically linked to the decrease in their total antioxidant capacity.

This antioxidant capacity plays a crucial role in repairing or minimizing potential damage caused during storage. This factor can be decisive in the percentages of germination and the germination speed index (GSI). As the total antioxidant capacity decreases, the vulnerability of seeds to environmental adversities increases, with negative impacts on physiological quality. Coffee seeds stored at 81% relative humidity at a temperature of 20 °C showed a rapid loss of viability, associated with extensive loss and oxidation of antioxidants, accumulation of fatty acids and selective loss of phospholipids, especially phosphatidylethanolamine (Dussert et al., 2006).

In the analysis of the water content of the seeds after 24 months of storage (Table 3) associated with physiological quality (Tables 1 and 2), there were variations in the responses in relation to the water content, which initially was 35±1%. The water content in recalcitrant seeds varies with the species, ranging between 12-31% (Roberts, 1973). However, the seeds of the Japi and Caturra IAC-476 cultivars, which showed less fluctuation in water content during storage (32.13 and 29.33%, respectively), showed a greater reduction in germination (9 and 2%, respectively) and vigor (IVG) (0.15 and 0.03, respectively), a behavior that may be associated with a reduction in their antioxidant capacity (Ferreira et al., 2022), increasing the deterioration of seeds throughout storage and a greater occurrence of microorganisms during germination (Delouche, 2002).

Mainly, considering that coffee seeds have restrictions on desiccation (Ellis et al., 1991), and in the laboratory environment there are large fluctuations in moisture

**Table 1.** Germination (%) of seeds of arabica coffee cultivars recently harvested (time 0) and stored for 24 months.

Cultivars	Times (months)	
	0	24
Catucaí 785/15	95 a <sup>*(1)</sup>	76 c
Caturra IAC-476	95 a*	9 e
Catuaí IAC-81	93 a*	82 b
Mundo Novo	90 a*	73 c
Catucaí 2 SL/CAK	99 a*	65 d
Catucaí 24/137	95 a <sup>ns</sup>	87 b
Catuaí IAC-62	87 a*	99 a
Acauã	92 a*	64 d
Japi	94 a*	2 e
Arara	88 a <sup>ns</sup>	87 b
CV (%)	9.31	

<sup>(1)</sup>The means followed by the same lowercase letter, in the columns between the cultivars, do not differ from each other, using the Scott-Knott mean grouping test, at a 5% probability level. \*Significant and <sup>ns</sup>Not significant in the lines, between times, by the F test (p<0.05). CV: Coefficient of variation.

**Table 2.** Germination speed index (GSI) of arabica coffee cultivar seeds recently harvested (time 0) and stored for 24 months.

Cultivars	Times (months)	
	0	24
Catucaí 785/15	1.70 a <sup>*(1)</sup>	1.13 b
Caturra IAC-476	1.53 b*	0.15 d
Catuaí IAC-81	1.53 b*	1.22 b
Mundo Novo	1.48 b*	1.00 c
Catucaí 2 SL/CAK	1.75 a*	0.89 c
Catucaí 24/137	1.57 b*	1.24 b
Catuaí IAC-62	1.50 b <sup>ns</sup>	1.59 a
Acauã	1.51 b*	0.90 c
Japi	1.55 b*	0.03 d
Arara	1.55 b*	1.20 b
CV (%)	9.48	

<sup>(1)</sup>The means followed by the same lowercase letter, in the columns between the cultivars, do not differ from each other, using the Scott-Knott mean grouping test at a 5% probability level. \*Significant and <sup>ns</sup> Not significant in the lines, between times, by the F test (p<0.05). CV: Coefficient of variation.



content, in which the seeds, being hygroscopic, tend to establish an equilibrium with humidity of the environment. Thus, the different responses of genotypes, factors linked to environmental conditions, such as temperature and relative humidity, can exert a significant influence on the preservation of the physiological quality of stored seeds, making the process even more complex (Roberts, 1973; Popinigis, 1985; Delouche, 2002; Meneghello, 2014; Marcos Filho, 2015; Lima et al., 2023). Seeds of *Eugenia involucrata* lost viability when kept in a natural environment for 30 days or when their water content was reduced to 39.84% (Hossel et al., 2016), while seeds of *Chrysophyllum cainite* L. with a reduction in water content to 34% showed 85% germination and at lower values lost viability (Nascimento et al., 2022).

Due to the complexity of coffee plant physiology, the causes of the rapid loss of forecast and low tolerance to desiccation observed in coffee seeds have not yet been fully elucidated (Ferreira et al., 2022).

The viability study of coffee seeds is an important step, given the risks and fluctuations that the storage of these seeds may suffer. Their physiological quality and viability can be increased with the use of storage techniques and extend their storage period (Corrêa et al., 2022)

When evaluating the physiological quality of seeds, electrical conductivity and potassium leaching tests are commonly used. The values obtained in the electrical conductivity (EC) and potassium leaching (LK) analyzes (Table 4) in the seeds of the cultivars stored for 24 months were lower than those observed in seeds of other species in the process of deterioration, suggesting the loss of integrity of the cell membrane system and ion leaching (Abdul-Baki and Baker, 1973; Delouche and Baskin, 1973). Behavior assigned to the compromise of membranes, which with the deterioration process have a reduced repair capacity and in contact with water culminate in the release of compounds such as cations, anions, sugars, organic acids, amino acids, which can stimulate the development of pathogens (Bewley et al., 2013; Marcos Filho, 2015).

While electrical conductivity measures the total amount of ions in solution, the potassium leaching test focuses exclusively on measuring potassium. It is worth highlighting that the values obtained in this study were lower than those frequently cited in the literature for seeds in the process of deterioration (Caixeta et al., 2013; Malta et al., 2005). However, the seeds used in this study have a moisture content between 29 and 43% (Table 3), Vazquez (1995) points out that the higher the initial water content of the seeds, the lower the conductivity values, reducing the release of electrolytes.

The Caturra IAC-476 and Japi cultivars, which presented lower moisture values, presented lower germination percentages and germination speed index (GSI) (Tables 1, 2 and 3) and presented higher electrical conductivity and potassium leaching values (Table 4), suggesting greater structural disorganization of the membranes with greater exudation of organic solutes from the interior of the seeds during imbibition, due to the increase in porosity and permeability to solutes (Simon, 1974).

In the analyzes of the physiological quality of the seeds (Figure 2), the results obtained were effective

in explaining 99.87% of the variations present in the original characteristics, highlighting CP1 with 96.34% and CP2 with 3.53%. This statistical approach provides a graphical representation of the relationships previously discussed, establishing visual connections between the results in Tables 1 and 2 with Table 4, in which the variables germination and GSI are mainly associated with the cultivar Catucaí IAC-62, while the variables EC and LK are associated with the cultivars Caturra IAC-476 and Japi. The increase in the amount of electrolytes in the imbibition water is directly associated with the degradation of membranes and the subsequent loss of permeability control (Simon, 1974), showing that higher levels of EC and LK are indicative of damage to cell membranes in the seeds of these cultivars.

The exudation of cellular constituents is inversely related to seed vigor (Woodstock, 1988). This exudation reflects not only the loss of membrane integrity, but also the consequent loss of compartmentalization of cellular constituents,

**Table 3.** Seed water content (% bu) of arabica coffee cultivars after 24 months of storage.

Cultivars	Water content (% bu) <sup>(1)</sup>
Catucaí 785/15	39.63
Caturra IAC-476	29.33
Catuai IAC-81	38.99
Mundo Novo	37.13
Catucaí 2 SL/CAK	38.59
Catucaí 24/137	38.80
Catuai IAC-62	43.09
Acauã	38.60
Japi	32.13
Arara	40.26

<sup>(1)</sup>Average values of four replications, expressed as a percentage, wet basis.

**Table 4.** Electrical conductivity (EC) and potassium leaching (LK) of arabica coffee cultivars stored for 24 months.

Cultivars	EC ( $\mu\text{S cm}^{-1} \text{g}^{-1}$ )	LK (ppm/g)
Catucaí 785/15	4.43 b <sup>(1)</sup>	1.43 b
Caturra IAC-476	49.58 a	31.66 a
Catuai IAC-81	3.19 b	1.33 b
Mundo Novo	3.30 b	0.73 b
Catucaí 2 SL/CAK	3.92 b	0.49 b
Catucaí 24/137	4.35 b	1.75 b
Catuai IAC-62	2.97 b	0.79 b
Acauã	4.31 b	1.46 b
Japi	47.87 a	31.92 a
Arara	3.89 b	0.62 b

<sup>(1)</sup>The means followed by the same lowercase letter, in the columns between cultivars, do not differ from each other at a 5% level, using the Scott- Knott test.

creating a substrate suitable for the development of microorganisms and, therefore, accelerating the seed deterioration process. The degradation of membranes observed in this study highlights the beginning of a decline in seed quality (Oliveira et al., 2015). The increase in the amount of reactive oxygen species (ROS) emerges as a central element that triggers oxidative damage to lipids, proteins and DNA (Bailly, 2004; Waszczak et al., 2018; Zhang et al., 2021). As seeds age, greater concentrations of ROS attack polyunsaturated fatty acids in membrane phospholipids, causing the long-chain fatty acids to break down into smaller compounds, modifying membrane permeability and leading to membrane destruction (Oenel et al., 2017; Ebone et al., 2019; Ratajczak et al., 2019).

In the germination of seeds stored for 24 months, abnormal seedlings were formed in relation to shoot length (SL), root length (RL), fresh mass (FM) and dry

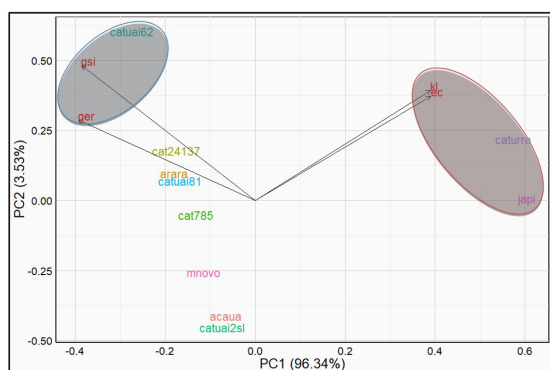
mass (DM) (Brasil, 2009). Similar results were observed in coffee seeds stored at 25 °C (Araújo et al., 2008). The emergence of abnormal seedlings, characterized by the death of tissues in different regions of the seed, can be attributed to a variety of processes that occur during seed deterioration. This includes membrane degradation, genetic damage, changes in respiratory activity, transformations in enzymes and proteins, adjustments in carbohydrate and lipid metabolism, hormonal imbalances, presence of toxic metabolites, action of microorganisms and changes in synthesis rates (Coolbear, 1995).

However, in recently harvested seeds (Table 5), the cultivars Arara, Catucaí 24/137, Catuai IAC-62 and Catucaí 785/15 presented the highest average shoot length (3.7; 3.7; 3.5 and 3.4 cm, respectively), while the cultivar Caturra IAC-476 presented the lowest average (1.9 cm). Considering the root length, the highest averages (3.8 and 3.7 cm, respectively) were observed in the cultivars Catucaí 785/15 and Catucaí 24/137 and smaller CR (1.5 cm) in seedlings of the cultivar Caturra IAC-476.

The cultivars Acauã, Catucaí 785/15, Catucaí 24/137 and Catucaí IAC-62 presented the highest average fresh weight (5.10; 5.00; 5.00 and 5.00 grams, respectively). However, the Caturra IAC-476 and Mundo Novo cultivars presented the lowest MF averages (4.00 and 4.25 grams, respectively). The reduction in dry mass of seedlings from stored seeds was similarly observed in passion fruit seedlings after 120 days of storage (Lima et al., 2023).

The cultivars Catucaí 785/15, Catuai IAC-81, Catucaí 24/137, Acauã, Japi, Mundo Novo and Arara presented the highest average dry mass (1.00; 1.00; 1.00; 1.00; 0.99, 0.98 and 0.98 grams, respectively).

This work highlights the importance of continuous research to optimize storage conditions for coffee seeds. Given the complexity of this process and the specific difficulty associated with the intermediate and recalcitrant characteristics of coffee seeds in relation to desiccation tolerance (Ellis et al., 1991), the need for specific and personalized approaches in this context is emphasized. This



**Figure 2.** Scatter diagram in relation to the first two main components, obtained from germination data (ger), germination speed index (gsi), potassium leaching (kl) and electrical conductivity (ec), of seeds of ten arabica coffee cultivars after 24 months of storage (cat785 = Catucaí 785/15; caturra = Caturra IAC-476; catuai81 = Catuai IAC-81; mnovo = Mundo Novo; catuai2sl = Catucaí 2 SL/CAK; cat24137 = Catucaí 24/137; catuai62 = Catuai IAC-62; acaua = Acauã; japi = Japi; arara = Arara).

**Table 5.** Shoot length (SL), root length (RL), fresh mass (FM) and dry mass (DM) of newly harvested Arabica coffee cultivar seeds.

Cultivars	SL (cm)	RL (cm)	FM (g)	DM (g)
Catucaí 785/15	3.4 a <sup>(1)</sup>	3.8 a	5.00 a	1.00 a
Caturra IAC-476	1.9 d	1.5 d	4.00 c	0.94 b
Catuai IAC-81	2.8 c	2.9 b	4.50 b	1.00 a
Mundo Novo	2.7 c	2.1 c	4.25 c	0.98 a
Catucaí 2 SL/CAK	2.8 c	3.2 b	4.75 a	0.93 b
Catucaí 24/137	3.7 a	3.7 a	5.00 a	1.00 a
Catuai IAC-62	3.5 a	3.3 b	5.00 a	0.93 b
Acauã	3.1 b	2.2 c	5.10 a	1.00 a
Japi	3.0 c	2.3 c	4.60 b	0.99 a
Arara	3.7 a	2.5 c	4.50 b	0.98 a
CV (%)	9.30	14.19	11.5	4.81

<sup>(1)</sup>The means followed by the same lowercase letter, in the columns between the cultivars, do not differ from each other, using the Scott- Knott mean grouping test at a 5% probability level. CV: Coefficient of variation.

approach is crucial to overcoming the inherent challenges and ensuring the effective preservation of seed quality, thus contributing to continued success in agricultural production.

#### 4. Conclusions

Storing arabica coffee seeds for 24 months results in a drop in the germination percentage.

Stored seeds of the Catucaí 24/137 and Arara cultivars showed germination percentages similar to those of newly harvested seeds.

Seeds of the Catuai IAC-62 cultivar maintain vigor during storage.

The stored seeds of the Caturra IAC-476 and Japi cultivars showed a reduction in physiological quality.

Electrical conductivity and potassium leaching tests are efficient in identifying seeds in an advanced state of deterioration.

Seeds of arabica coffee cultivars stored for 24 months, under the conditions of the present study, produce abnormal seedlings.

In view of this, it is suggested that new storage techniques and environmental conditions be developed so that coffee seeds can be stored for a longer period without losing their vigor.

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#### References

- ABDUL-BAKI, A.A. and BAKER, J.E., 1973. Are changes in cellular organelles or membranes related to vigor loss in seeds? *Seed Science and Technology*, vol. 1, no. 1, pp. 89-125.
- ABREU, L.A.S., VEIGA, A.D., PINHO, E.V.R.V., MONTEIRO, F.F. and ROSA, S.D.V.F., 2014. Behavior of coffee seeds regarding tolerance to desiccation and storage. *Journal of Seed Science*, vol. 36, no. 4, pp. 399-406. <http://doi.org/10.1590/2317-1545v36n41008>.
- ALVES, G.E., BORÉM, F.M., ISQUIERDO, E.P., SIQUEIRA, V.C., CIRILLO, M.A. and PINTO, A.C.F., 2017. Physiological and sensorial quality of Arabica coffee submitted in different temperatures and drying airflows. *Acta Scientiarum. Agronomy*, vol. 39, no. 2, pp. 225-233. <http://doi.org/10.4025/actasciagron.v39i2.31065>.
- ANGÉLICO, C.L., PIMENTA, C.J., CHAGAS, S.J.R., CHALFOUN, S.M., PEREIRA, M.C. and CHALFOUN, Y., 2011. Diferentes estádios de maturação e tempos de ensacamento sobre a qualidade do café. *Coffee Science*, vol. 6, no. 1, pp. 8-19.
- ARAÚJO, R.F., ARAÚJO, E.E.N., CECON, P.R. and SOFIATTI, V., 2008. Conservação de sementes de café (*Coffea arabica* L.) despoldado e não despoldado. *Revista Brasileira de Sementes*, vol. 30, no. 3, pp. 71-78. <http://doi.org/10.1590/S0101-31222008000300010>.
- BAILLY, C., 2004. Active oxygen species and antioxidants in seed biology. *Seed Science Research*, vol. 14, no. 2, pp. 93-107. <http://doi.org/10.1079/SSR2004159>.
- BEWLEY, J.D., BRADFORD, K.J., HILHORST, H.W.M. and NONOGAKI, H., 2013. *Seeds: physiology of development, germination and dormancy*. 3rd ed. New York: Springer, 392 p.
- BRASIL, 2009. *Regras para análise de sementes*. Brasília: MAPA-ACS, 395 p.
- BRASIL. Companhia Nacional de Abastecimento – CONAB, 2022 [viewed 28 December 2023]. *Acompanhamento da safra brasileira de café: quarto levantamento, safra 2022* [online]. Brasília: CONAB, vol. 9, no. 4. Available from: <http://www.conab.gov.br/info-agro/safras/cafe/boletim-da-safra-de-cafe>
- CAIXETA, I.F., GUIMARÃES, R.M. and MALTA, M.R., 2013. Qualidade da semente de café pelo retardamento do processamento pós-colheita. *Coffee Science*, vol. 8, no. 3, pp. 249-255.
- CARVALHO, N.M. and NAKAGAWA, J., 2012. *Sementes: ciência, tecnologia e produção*. Jaboticabal: FUNEP, 590 p.
- CARVALHO, A.M., CARDOSO, D.A., CARVALHO, G.R., CARVALHO, V.L., PEREIRA, A.A., FERREIRA, A.D. and CARNEIRO, L.F., 2017. Comportamento de cultivares de cafeeiro sob a incidência das doenças da ferrugem e cercosporiose em dois ambientes de cultivo. *Coffee Science*, vol. 12, no. 1, pp. 100-107. <http://doi.org/10.25186/cs.v12i1.1248>.
- COELHO, S.V.B., FIGUEIREDO, M.A., CLEMENTE, A.C.S., COELHO, L.F.S. and ROSA, S.D.V.F., 2015. Physiological and biochemical changes in coffee seed dried in silica gel and saturated saline solutions. *Pesquisa Agropecuária Brasileira*, vol. 5, no. 6, pp. 483-491. <http://doi.org/10.1590/S0100-204X2015000600007>.
- COOLBEAR, P., 1995. Mechanisms of seed deterioration. In: R.E. GOUGH, ed. *Seed quality: basic mechanisms and agricultural implications*. New York: CRC Press, pp. 233-277.
- CORRÊA, J.P., LANDGRAF, P.R.C. and REZENDE, T.T., 2022. Coffee seed storage. *Research, Society and Development*, vol. 11, no. 14, e28111435945.
- DELOUCHE, J.C. and BASKIN, C.C., 1973. Accelerated aging techniques for predicting the relative storability of seeds lots. *Seed Science and Technology*, vol. 1, no. 2, pp. 427-452.
- DELOUCHE, J.C., 2002. Germinação, deterioração e vigor da semente. *Seed News*, vol. 6, no. 6, pp. 45-48.
- DUSSERT, S., DAVEY, M.W., LAFFARGUE, A., DOULBEAU, S., SWENNEN, R. and ETIENNE, H., 2006. Oxidative stress, phospholipid loss and lipid hydrolysis during drying and storage of intermediate seeds. *Physiologia Plantarum*, vol. 127, no. 2, pp. 192-204. <http://doi.org/10.1111/j.1399-3054.2006.00666.x>.
- EBONE, L.A., CAVERZAN, A. and CHAVARRIA, G., 2019. Physiologic alterations in orthodox seeds due to deterioration processes. *Plant Physiology and Biochemistry*, vol. 145, pp. 34-42. <http://doi.org/10.1016/j.plaphy.2019.10.028>. PMID:31665665.
- ELLIS, R.H., HONG, T.D. and ROBERTS, E.H., 1991. An intermediate category of seed storage behavior? *Journal of Experimental Botany*, vol. 42, no. 238, pp. 653-657. <http://doi.org/10.1093/jxb/42.5.653>.
- FANTAZZINI, T.B., ROSA, S.D.V.F., PEREIRA, C.C., PEREIRA, D.S., CIRILLO, M.A. and OSSANI, P.C., 2018. Association between the artificial aging test and the natural storage of coffee seeds. *Journal of Seed Science*, vol. 40, no. 2, pp. 164-172. <http://doi.org/10.1590/2317-1545v40n2188120>.
- FERREIRA, I.A., FÁVARIS, N.A.B., ROSA, S.D.V.F., COELHO, S.V.B., RICARDONI, M.A. and COSTA, M.C., 2022. Trolox equivalent antioxidant capacity of *Coffea arabica* L. seeds. *Ciência e*

- Agrotecnologia*, vol. 46, e000522. <http://doi.org/10.1590/1413-7054202246000522>.
- HOSSEL, C., HOSSEL, J.S.A.O., ALEGRETTI, A.L., WAGNER JUNIOR, A. and DALLAGO, A., 2016. Hidrocondicionamento e atmosfera modificada ou controlada para a conservação da viabilidade de sementes de cerejeira-da-mata. *Pesquisa Aplicada & Agrotecnologia*, vol. 9, no. 2, pp. 99-106. <http://doi.org/10.5935/PAet.V9.N02.11>.
- INTERNATIONAL COFFEE ORGANIZATION – ICO, 2023 [viewed 29 December 2023]. *Public market information* [online]. Available from: <https://icocoffee.org/resources/public-market-information/>
- INTERNATIONAL SEED TESTING ASSOCIATION – ISTA, 2017. *International rules for seed testing*. Bassersdorf: ISTA, 296 p.
- ISQUIERDO, E.P., BORÉM, F.M., CIRILLO, M., OLIVEIRA, P.D., CARDOSO, R.A. and FORTUNATO, V.A., 2011. Qualidade do café cerejea desmucilado submetido ao parcelamento da secagem. *Coffee Science*, vol. 6, no. 1, pp. 83-90.
- LIMA, P.A.M., GUILHEN, J.H.S., MACIEL, K.S., ALEXANDRE, R.S., FERREIRA, A. and LOPES, J.C., 2023. Physiological quality of 'BRS' passion fruit seeds grown at different altitudes and storage environments. *Comunicata Scientiae*, vol. 14, e3993. <http://doi.org/10.14295/cs.v14.3993>.
- LIMA, P.A.M., MACIEL, K.S., ALEXANDRE, R.S. and LOPES, J.C., 2019. The physiological quality of yellow passion fruit ('*Passiflora edulis*' sims. f. '*flavicarpa*' deg.) seeds with different water content placed in a cold chamber room and environmental conditions. *Australian Journal of Crop Science*, vol. 13, no. 3, pp. 452-457. <http://doi.org/10.21475/ajcs.19.13.03.p1462>.
- MAGUIRE, J.D., 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, vol. 2, no. 2, pp. 176-177. <http://doi.org/10.2135/cropsci1962.001183X0002000200033x>.
- MALAU, S., SIAGIAN, A., SIRAIT, B. and AMBARITA, H., 2018. Germination performance of *Coffea arabica* L. genotypes from different altitude, precipitation and temperature of seeds producing farms in Sumatera Utara of Indonesia. *IOP Conference Series. Earth and Environmental Science*, vol. 205, no. 1, pp. 1-6. <http://doi.org/10.1088/1755-1315/205/1/012013>.
- MALTA, M.R., PEREIRA, R.G.F.A. and CHAGAS, S.J.R., 2005. Condutividade elétrica e lixiviação de potássio do exsudato de grãos de café: alguns fatores que podem influenciar essas avaliações. *Ciência e Agrotecnologia*, vol. 29, no. 5, pp. 1015-1020. <http://doi.org/10.1590/S1413-70542005000500015>.
- MARCOS-FILHO, J., 2015. *Fisiologia de sementes de plantas cultivadas*. Abrates, 659 p.
- MENEGHELLO, G.E., 2014. Qualidade de sementes: umidade e temperatura. *SEED News*, vol. 18, no. 6, pp. 28-33.
- NASCIMENTO, G.R., LOPES, J.C., LIMA, P.A.M. and ALEXANDRE, R.S., 2022. Características físicas, morfológicas e fisiológica de sementes da fruta caimito (*Chrysophyllum Cainito* L.). *International Journal of Developmental Research*, vol. 12, no. 3, pp. 54370-54375. <http://doi.org/10.37118/ijdr.24015.03.2022>.
- OENEL, A., FEKETE, A., KRISCHKE, M., FAUL, S.C., GRESSER, G., HAVAUX, M., MUELLER, M.J. and BERGER, S., 2017. Enzymatic and non-enzymatic mechanisms contribute to lipid oxidation during seed aging. *Plant & Cell Physiology*, vol. 58, no. 5, pp. 925-933. <http://doi.org/10.1093/pcp/pcx036>. PMID:28371855.
- OLIVEIRA, A.P.L.R., CORRÊA, P.C., REIS, E.L. and OLIVEIRA, G.H.H., 2015. Comparative study of the physical and chemical characteristics of coffee and sensorial analysis by principal components. *Food Analytical Methods*, vol. 8, no. 5, pp. 1303-1314. <http://doi.org/10.1007/s12161-014-0007-4>.
- PENIDO, A.C., RODRIGUES, V.O., CARVALHO, M.V., KREPISCHI, L.S., PEREIRA, C.P. and OLIVEIRA, J.A., 2021. Effect of chemical treatment on the physiological and sanitary quality of stored coffee seeds. *Journal of Seed Science*, vol. 43, no. 3, e202143009. <http://doi.org/10.1590/2317-1545v43237239>.
- POPINIGIS, F., 1985. *Fisiologia da semente*. Brasília: Agiplan, 289 p.
- PRETE, C.E.C., 1992. *Condutividade elétrica do exsudato de grãos de café (Coffea arabica L.) e sua relação com a qualidade da bebida*. Piracicaba: Universidade de São Paulo, 125 p. Tese de Doutorado em Agronomia.
- R CORE TEAM, 2023 [viewed 28 December 2023]. *R: a language and environment for statistical computing* [online]. Vienna: R Foundation for Statistical Computing. Available from: <https://www.r-project.org/>
- RATAJCZAK, E., MAŁECKA, A., CIERESZKO, I. and STASZAK, A.M., 2019. Mitochondria are important determinants of the aging of seeds. *International Journal of Molecular Sciences*, vol. 20, no. 7, pp. 1568. <http://doi.org/10.3390/ijms20071568>. PMID:30925807.
- ROBERTS, E.H., 1973. Predicting the storage life of seeds. *Seed Science and Technology*, vol. 12, no. 4, pp. 499-514.
- SANTOS, M.A., CHALFOUN, S.M. and PIMENTA, C.J., 2009. Influência do processamento por via úmida e tipos de secagem sobre a composição, físico química e química do café (*Coffea arabica* L.). *Ciência e Agrotecnologia*, vol. 33, no. 1, pp. 213-218. <http://doi.org/10.1590/S1413-70542009000100030>.
- SIMON, E.W., 1974. Phospholipids and plant membrane permeability. *The New Phytologist*, vol. 73, no. 3, pp. 377-420. <http://doi.org/10.1111/j.1469-8137.1974.tb02118.x>.
- TRUJILLO, H.A., GOMES-JUNIOR, F.G. and CICERO, S.M., 2019. Digital images of seedling for evaluating coffee seed vigor. *Journal of Seed Science*, vol. 41, no. 1, pp. 60-68. <http://doi.org/10.1590/2317-1545v41n1204651>.
- VAZQUEZ, G.H., 1995. *Condicionamento fisiológico de sementes de soja: efeitos sobre a germinação, vigor e potencial de armazenamento*. Piracicaba: Universidade de São Paulo, 135 p. Tese de Doutorado em Agronomia.
- VILELA, A.L.O., ROSA, S.D.V.F., COELHO, S.V.B., FÁVARIS, N., FANTAZZINI, T.B. and BAUTE, J.L., 2021. Antioxidant and antimicrobial activity of cathode and anode water in *Coffea arabica* L. seeds. *Journal of Seed Science*, vol. 43, no. 3, e202143011. <http://doi.org/10.1590/2317-1545v43244602>.
- WASZCZAK, C., CARMODY, M. and KANGASJÄRVI, J., 2018. Reactive oxygen species in plant signaling. *Annual Review of Plant Biology*, vol. 69, no. 1, pp. 209-236. <http://doi.org/10.1146/annurev-arplant-042817-040322>. PMID:29489394.
- WIBOWO, A., NUGROHO, D. and SUMIRAT, U., 2020. Seed germination performance of nine arabica coffee (*Coffea arabica* L.) varieties under the laboratory condition after six months of storage period. *Pelita Perkebunan*, vol. 36, no. 3, pp. 203-211. <http://doi.org/10.22302/jicri.jur.pelitaperkebunan.v36i3.450>.
- WOODSTOCK, L.W., 1988. Seed imbibition: a critical period for successful germination. *Journal of Seed Technology*, vol. 12, no. 1, pp. 1-15.
- XIN, X., TIAN, Q., YIN, G., CHEN, X., ZHANG, J., NG, S. and LU, X., 2014. Reduced mitochondrial and ascorbate-glutathione activity after artificial aging in soybean seed. *Journal of Plant Physiology*, vol. 171, no. 2, pp. 140-147. <http://doi.org/10.1016/j.jplph.2013.09.016>. PMID:24331429.
- ZHANG, K., ZHANG, Y., SUN, J., MENG, J. and TAO, J., 2021. Deterioration of orthodox seeds during ageing: influencing factors, physiological alterations and the role of reactive oxygen species. *Plant Physiology and Biochemistry*, vol. 158, pp. 475-485. <http://doi.org/10.1016/j.plaphy.2020.11.031>. PMID:33250322.