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Comparison between coffee seedlings produced by traditional methods and from cryopreserved seeds

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ABSTRACT: Coffee seeds are classified as intermediate, tolerating partial desiccation but being sensitive to low temperatures. They can be stored under cold conditions, allowing only short-term conservation. Cryopreservation overcomes these limitations, providing a viable method for long-term seed conservation and greater flexibility in seedling production. This study aimed to evaluate the development of coffee seedlings from cryopreserved seeds, comparing them with seedlings from seeds stored under cold (10°C) and dry (50% relative humidity) conditions. Seeds of the 'Catuaí Amarelo' IAC 62 cultivar were harvested at 42% moisture content (wet basis, wb) and subjected to four treatments: drying in a stationary dryer to 12% (S1) or 32% (S2) wb and stored in cold conditions; drying to 17% wb in saturated NaCl solution (S3) or silica gel (S4) and stored in liquid nitrogen. After six months, germination and tetrazolium tests were performed to assess physiological quality. Seeds were then used for seedling production in a nursery and greenhouse, where height, shoot diameter, number of leaves, leaf area, and shoot and root dry weight were evaluated. S1, S2, and S4 seeds showed similar results across most physiological variables, while S3 consistently exhibited lower performance. Overall, seedlings grown in the greenhouse exhibited greater vegetative development than those produced in the nursery.

Index terms: *Coffea arabica* L., cold storage, cryogenics, liquid nitrogen, plant propagation.

RESUMO: As sementes de café são classificadas como intermediárias, tolerando a dessecação parcial, mas sendo sensíveis a baixas temperaturas. Elas podem ser armazenadas sob condições frias, o que permite apenas a conservação por curto prazo. A criopreservação supera essas limitações, oferecendo um método viável para a conservação de sementes por prazo e maior flexibilidade na produção de mudas. Este estudo teve como objetivo avaliar o desenvolvimento de mudas de café provenientes de sementes criopreservadas, comparando-as com mudas oriundas de sementes armazenadas sob condições frias (10 °C) e secas (50% de umidade relativa). Sementes da cultivar 'Catuaí Amarelo' IAC 62 foram colhidas com 42% de teor de água (base úmida, bu) e submetidas a quatro tratamentos: secagem em secador estacionário até 12% (S1) ou 32% (S2) bu e armazenamento sob refrigeração; secagem até 17% bu em solução saturada de NaCl (S3) ou em sílica gel (S4) e armazenamento em nitrogênio líquido. Após seis meses, foram realizados testes de germinação e de tetrazólio para avaliar a qualidade fisiológica. As sementes foram então utilizadas para a produção das mudas em viveiro e em casa de vegetação, onde foram avaliados altura, diâmetro do caule, número de folhas, área foliar e massa seca da parte aérea e das raízes. As sementes S1, S2 e S4 apresentaram resultados semelhantes na maioria das variáveis fisiológicas, enquanto S3 apresentou desempenho consistentemente inferior. De modo geral, as mudas cultivadas na estufa exibiram maior desenvolvimento vegetativo do que aquelas produzidas no viveiro.

Termos para indexação: *Coffea arabica* L., armazenamento a frio, criogenia, nitrogênio líquido, propagação de plantas.

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INTRODUCTION

Coffee is one of the most important and widely grown agricultural commodities in the world, playing a crucial role in the global economy. Brazil is expected to reach a record coffee harvest in 2026, with total production estimated at 71 million 60-kg bags, representing an increase of 21.8% compared to the previous crop year. Of this total, arabica coffee production is projected to reach 46.70 million bags (Forbes, 2025). *Coffea arabica* L., a predominantly autogamous species, maintains genetic uniformity when propagated by seeds. However, commercial crops are established through seedling transplantation, a method that ensures greater phenotypic consistency, higher field survival rates, and improved initial plant establishment compared to direct seeding. However, the seeds of this species are classified as intermediate, positioned between orthodox and recalcitrant seeds, with low tolerance to desiccation and to exposure to low temperatures (Ellis et al., 1990). This feature limits conventional cold storage, which leads to loss of seed viability after around six months of storage (Araujo et al., 2008; Coelho et al., 2015). Thus, propagation of the species is restricted to the harvest period, hindering planning for seedling production and increasing vulnerability to climate conditions at the time of planting (Figueiredo et al., 2021).

Different drying methods can be used in seed production and play a critical role in determining seed tolerance to ultra-low temperatures, directly influencing the success of cryopreservation. These methods include the use of silica gel (José et al., 2011) and saline solutions such as NaCl (Bazin et al., 2011; Choudhury et al., 2011; Coelho et al., 2015). Silica gel acts as a desiccant, promoting controlled water removal from seeds and reducing the risk of cellular damage associated with ice crystal formation during the cryopreservation process. NaCl saline solutions, in turn, create an environment with controlled relative humidity, allowing slower drying rates compared to silica gel, which may be advantageous for species sensitive to rapid dehydration (Coelho et al., 2015).

Despite these approaches, there is no consensus regarding the optimal moisture content and drying method required to maintain coffee seed viability under cold and dry storage conditions (Ellis et al., 1990). This uncertainty is particularly relevant in the context of cryopreservation, as seed water content and its physical state are key determinants of cellular stability at liquid nitrogen temperatures (Souza et al., 2024). In this context, cryopreservation emerges as a viable alternative for the long-term conservation of coffee seeds (Coelho et al., 2017; Coelho et al., 2019; Coelho et al., 2024), enabling seedling production at different times of the year. However, the success of this technique is directly dependent on the initial quality of the seed lots, the drying method and rate applied, and the moisture content established prior to storage. Inadequate drying rates or moisture levels may compromise seed physiological quality and, consequently, seedling development (Souza et al., 2024).

C. arabica seeds can be stored at relatively high moisture levels (31.5% wet basis - wb) without compromising quality for up to nine months at 7 °C, provided they are placed in permeable packaging (Araujo et al., 2008). Additionally, studies have shown that cryopreserved seeds dried to 17% moisture (wb) maintain high quality after storage in liquid nitrogen (Coelho et al., 2017). However, the relationship between these treatments and the ability of seeds, dried using different methods, to produce seedlings under various environmental conditions has not yet been investigated.

Although traditionally, coffee seedlings are produced in conventional nurseries, protected environments, such as greenhouses, may provide better environmental conditions for seedling growth, enhancing physiological quality and vigor (Rebouças et al., 2014), though this makes production more costly. However, the response of seedlings produced from cryopreserved seeds under these different systems has not yet been studied and is little understood.

Given this knowledge gap, this study aimed to evaluate the effect of different drying methods and moisture contents, as well as of cold and dry storage and of storage in liquid nitrogen (cryopreservation), of *C. arabica* L. seeds on the physiological performance of seedlings produced in a nursery and in a greenhouse.

MATERIAL AND METHODS

This study was carried out in the seed research laboratory (*Laboratório Central de Pesquisa em Sementes - LCPS*) of the Seed Sector and in the Coffee Production Sector of the Department of Agriculture (*Departamento de Agricultura - DAG*) of the *Universidade Federal de Lavras* (UFLA), Lavras, MG, Brazil.

Fruit harvest and seed processing: fruits from *C. arabica*, cultivar *Catuaí Amarelo IAC 62*, at the physiological maturity stage was selectively harvested from the middle part of the branches of the middle third of the plants in a crop field of the Procafé Experimental Farm in Varginha, Minas Gerais, with an altitude of 980 m and a highland tropical climate (Cwb), according to the Köppen classification.

After harvest, the fruit was further selected for uniform ripeness, and it was mechanically pulped. Mucilage was then removed from the seeds through fermentation in water for 24 hours in an environment (25 °C) and pre-dried in the shade to remove surface moisture. After pre-drying, the seeds had a 42% moisture content (wb) and 94% germination rate.

Treatment preparation: the treatments were set up by combining different seed drying and storage methods. The seed lot obtained was separated into portions. A portion of the seed lot was dried in a stationary dryer until reaching 12% or 32% moisture content (warm basis - wb) and then kept in cold and dry storage for six months. These treatments were used because they represent practices adopted in coffee seedling production. The other portion of the seed lot was dried to 17% moisture content in silica gel or in saturated NaCl saline solution and then cryopreserved in liquid nitrogen (Table 1). These seeds were cryopreserved, following protocols developed for the *C. arabica* species, for the same six-month period. In all the treatments, drying and storage were conducted under controlled conditions.

Seed drying procedures: in drying in the small-scale stationary dryer, the coffee seeds with parchment were dried until reaching 32% moisture (wb), considered as the high-moisture treatment, or until reaching 12% moisture (wb), considered as the low-moisture treatment. The seeds from these two treatments were subsequently kept in cold and dry storage at 10 °C and 45% relative humidity for a period of six months (Table 1).

To apply the other two treatments (Table 1), the seeds, likewise with parchment, were dried to 17% moisture (wb) following the methodology of Coelho et al. (2017) and Figueiredo et al. (2017) either in saturated NaCl solution or in silica gel and subsequently stored in liquid nitrogen at -196 °C, where they remained under cryopreservation for six months.

For drying to 17% moisture (wb), both in silica gel and in saturated saline solution, the seeds were arranged in a single layer on metal screens in a transparent gerbox (germination box) and kept at 25 °C until reaching the desired moisture level. Within the boxes and below the metal screens, 60 grams of silica gel or saturated NaCl solution (prepared with 40 grams of NaCl and 10 mL of water) was added. Drying was monitored by weighing on a precision balance (0.001 g) until the seeds reached the desired moisture level, which was determined by the laboratory oven method at 105 °C for 24 hours (Brasil, 2025), with two replications of 10 seeds. The results were expressed as a percentage based on the wet weight of the seeds.

Seed cryopreservation procedures: the seeds with 17% moisture (wb) dried in silica gel or in saturated saline solution were placed in tri-laminate aluminum foil envelopes (Coelho et al., 2018, 2019) and then directly immersed in liquid nitrogen (-196 °C) for ultra-rapid cooling of approximately -200 °C/minute (Dussert et al., 2001), where they remained

Table 1. Description of the drying and storage treatments of the *Coffea arabica* L. seeds used for seedling production.

Treatment	Moisture (% wb)	Drying methods	Storage
S1	12	Stationary dryer	Cold storage*
S2	32	Stationary dryer	Cold storage*
S3	17	Saturated NaCl solution	Liquid N **
S4	17	Silica gel	Liquid N**

*Temperature of 10 °C and relative humidity of 50%; **Under a temperature of -196 °C.

for six months (Table 1). After that period, they were removed and immediately rewarmed in a water bath at 40 °C for two minutes, as described by Dussert et al. (2001). After rewarming, the seeds were surface dried with paper towels, and their parchment was manually removed for seed physiological evaluations.

Evaluation of seed physiological quality: the assessment of seed physiological quality was conducted following a drying procedure and subsequent storage for six months. The evaluation was conducted through a germination test, with four replications of 25 seeds per treatment. Germination testing was conducted using germination paper moistened with water in the amount of 2.5 times the dry weight of the paper in a seed germinator at 30 °C, according to the methodology established by the Rules for Seed Testing (Brasil, 2025). The evaluation of normal seedlings was performed at 30 days, with normal seedlings defined as those with a well-developed primary root and at least two healthy and well-formed lateral roots. In contrast, abnormal seedlings were those that did not meet these requirements.

In the germination test, the following evaluations were also conducted: seedlings with radicle protrusion at 15 days, strong normal seedlings at 30 days (characterized by a hypocotyl hook of at least 3-cm length, and seedlings with expanded cotyledonary leaves at 45 days.

After 45 days, the shoots of normal seedlings were separated from the roots using a scalpel, and the plant material was placed in paper bags and dried in a forced-air circulation laboratory oven at 60 °C until constant weight. Dry weight was determined using a precision balance, and the results were expressed in mg.seedling^{-1}.

Procedures for seedling production: the seeds from the four different treatments (Table 1) were used before and after six months in cold storage or cryostorage. The seeds removed from cold storage were sown immediately. The cryopreserved seeds were warmed in a water bath at 40 °C for 2 minutes before being sown.

The seeds were sown in plastic bags suitable for seedling production (11 × 22 cm) in Tropstrato® commercial substrate. Two seeds were sown per bag, and after emergence, one was eliminated. The seedlings were produced in two environments, in a nursery in the Coffee Growing Sector, and in a greenhouse in the Seed Sector, both at the Department of Agriculture (DAG) of UFLA. Crop treatments were carried out according to the recommendations of (Bergo et al., 2002).

Evaluation of the seedlings produced: Evaluations were carried out when at least 90% of the seedlings of the treatment that exhibited the best development had three to four pairs of true leaves (Barros et al., 2025). The seedlings used for data collection in each experimental plot were evaluated using the following measurements:

a) shoot diameter: measured at the point just below the level of the cotyledonary leaves using a digital caliper, expressed in mm;
b) mean seedling height: measured as the distance from the root collar to the terminal bud of the orthotropic shoot, expressed in cm;
c) leaf area: calculated as the product of the width × length × 0.667 (leaf area coefficient) × 2 (pair of leaves) (Barros et al., 2025), with results expressed in cm^{-2};
d) number of pairs of true leaves: counted from the number of pairs of fully expanded leaves from the seedlings used for data collection from each plot, with the results expressed as mean number of true leaves per seedling;
e) dry weight of the shoots and roots: obtained after drying the roots and shoots separately in a forced-air circulation laboratory oven at 60 °C until reaching constant weight. They were then weighed, and mean results were expressed as gram.seedling^{-1}.

Statistical analyses and experimental design: A randomized block experimental design was used with three replications in a triple factorial arrangement: four seed treatments (S1, S2, S3, and S4) (Table 1), two points in time (before and after six months of storage), and two seedling production locations (greenhouse and nursery), for a total of 16 experimental plots. The four central seedlings in each experimental plot were considered for data collection.

Analysis of variance was used on the data from seedling evaluation, as well as from evaluation of seed physiological quality, and the mean values were compared using the Scott-Knott test ($p > 0,05$) through the Sisvar® statistical analysis program (Ferreira, 2014).

RESULTS

The physiological evaluation of seeds showed a significant effect ($p > 0.05$) of the interaction between the storage and drying treatment factors for the variable's percentage of radicle protrusion, normal seedlings, strong normal seedlings, seedlings with expanded cotyledonary leaves at 45 days, and shoot dry weight. In contrast, root dry weight was not significantly affected by the factors individually or by their interaction.

The seed physiological quality the S4 treatment (cryopreserved after drying in silica gel to 17% moisture (wb)), were statistically similar to those of the seeds from S1 (cold storage with moisture contents of 12% moisture (wb)) and S2 (32% moisture (wb)) treatments (100% and 91% of root protrusion, respectively) (Figure 1A) (Table 1). In contrast, the results of the seeds dried to 17% moisture (wb) in saturated saline solution (S3) were significantly lower than those of the other treatments for all the variables analyzed (Figures 1, 2, and 3).

Regarding radicle protrusion (Figure 1A), the 6-month storage period did not show a negative impact. The seeds dried to 17% moisture (wb) in saturated NaCl solution (S3) or in silica gel (S4) showed a higher percentage (58% and 87%, respectively) of radicle protrusion after storage in liquid nitrogen.

The best results for the percentage of normal seedlings before storage were from the treatments with moisture contents of 12% (wb) (S1) and 32% (wb) (S2), with averages of 96% and 91%, respectively. However, after storage, the best results were from the wetter seeds (S2) (78%) and the seeds dried to 17% moisture (wb) in silica gel and cryopreserved (S4). They showed a higher percentage of normal seedlings (79%) compared than those from cold storage. The seeds that were dried more slowly in saturated NaCl solution and cryopreserved (S3) showed lower results for stored (8%) and non-stored (5%) treatments (Figure 1B).

For the normal seedling variable, seeds from the S4 treatment after storage for six months in liquid nitrogen showed the same percentage or higher percentages compared to the non-cryopreserved treatments (S1 and S2) (Figure 2B). In contrast, cryopreservation had a negative effect on the results of strong normal seedlings (Figure 2). Moreover, the percentages of this variable in the S1 and S2 treatments declined after six months in cold storage (Figure 2A). In the S4 treatment, the percentages did not differ from each other before and after cryopreservation.

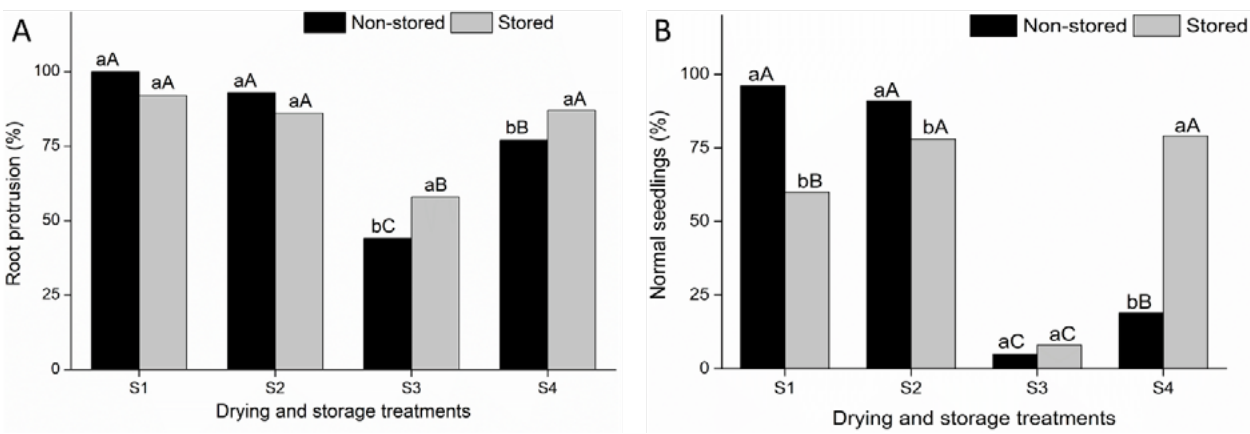

Figure 1. Percentage of root protrusion (A) and percentage of normal seedlings (B) of *Coffea arabica* L. seeds before and after six months of storage. Treatments: seeds dried in a stationary dryer until reaching 12% moisture (wb) (S1) or 32% moisture (wb) (S2) and kept in cold and dry storage for six months; seeds dried to 17% moisture (wb) in saturated saline solution (S3) or in silica gel (S4) and stored in liquid nitrogen for six months. Lowercase letters compare the effect of storage within the same treatment, and uppercase letters compare the treatments at each storage time. Same letters do not differ from each other according to the Scott-Knott test at the 5% probability level.

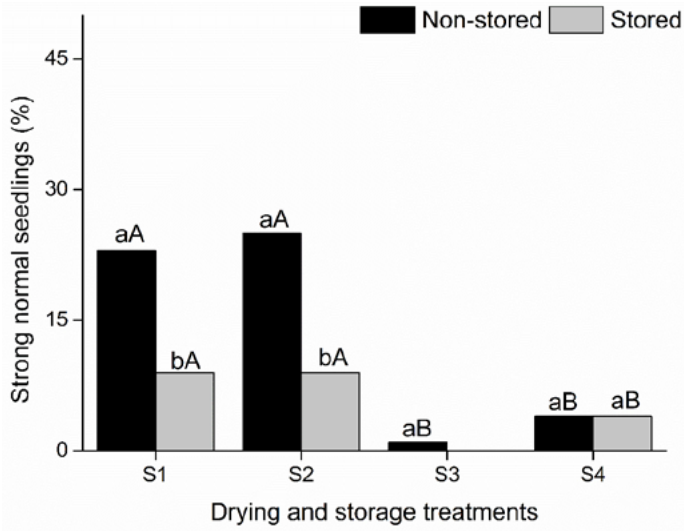

Figure 2. Results of *Coffea arabica* L. seeds before and after six months of storage. Treatments: seeds dried in a stationary dryer until reaching 12% moisture (wb) (S1) or 32% moisture (wb) (S2) and kept in cold and dry storage for six months; seeds dried to 17% moisture (wb) in saturated saline solution (S3) or in silica gel (S4) and stored in liquid nitrogen for six months. *Lowercase letters compare the effect of storage within the same treatment, and uppercase letters compare the treatments at each storage time. Same letters do not differ from each other according to the Scott-Knott test at the 5% probability level.

There was a significant reduction in the percentages of seedlings with expanded cotyledonary leaves and in shoot dry weight for the seeds from the S1 treatment after six months of storage (Figure 3). In contrast, the seeds dried with silica gel and cryopreserved (S4) showed significantly higher results after cryostorage, and this was statistically similar to the seeds from the S2 treatment after storage. For the root dry weight variable, no significant differences were observed.

For the results of the number of pairs of true leaves, mean seedling height, and leaf area, a three-way interaction was observed among the factors under study, that is, seed treatments, evaluation times, and production location. A trend of better results was observed for most evaluation variables of the seedlings produced in a greenhouse across all the treatments studied at both storage times. For some treatments, this difference was statistically significant (Table 2).

In accordance with the results of seed physiological analysis, seedling evaluation was also lower for the S3 treatment, corresponding to the seeds that were dried in saturated saline solution and cryopreserved, in both environments and at the storage times evaluated (Table 2).

For the three variables analyzed (Table 2), the S1 and S2 treatments at the first evaluation time had better results than the other treatments in both growing environments. However, after six months of storage, the S4 seedlings grown in a greenhouse had values statistically similar to those of the S1 and S2 treatments.

Regardless of the drying and storage treatments, the performance of the seedlings grown from freshly harvested seeds, without storage, was statistically superior than that of the seedlings grown from stored seeds regarding mean shoot diameter, shoot dry weight, and root dry weight (Table 3).

The seedlings grown from seeds dried in saturated saline solution and then cryopreserved (S3) showed significantly lower results compared to the other treatments (Table 4), just as shown from other variables evaluated in this study. It is noteworthy that the seedlings derived from cryopreserved seeds after drying in silica gel were not statistically different from the seedlings from non-cryopreserved seeds in evaluation of shoot diameter and root dry weight, but they were lower regarding shoot dry weight (Table 4).

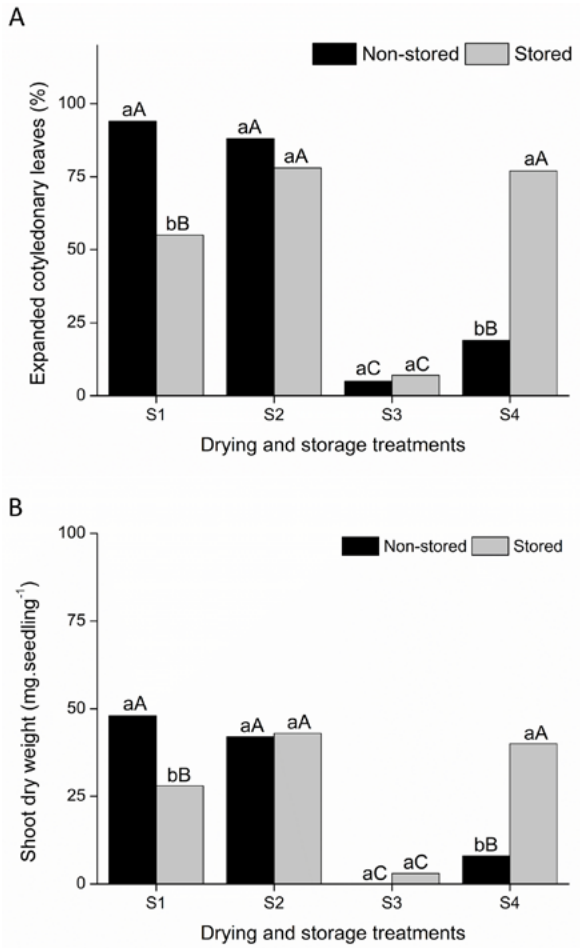

Figure 3. Results of *Coffea arabica* L. seeds before and after six months of storage. Treatments: seeds dried in a stationary dryer until reaching 12% moisture (wb) (S1) or 32% moisture (wb) (S2) and kept in cold and dry storage for six months; seeds dried to 17% moisture (wb) in saturated saline solution (S3) or in silica gel (S4) and stored in liquid nitrogen for six months. A) expanded cotyledonary leaves, B) shoot dry weight in gram.seedling^{-1}. *Mean values followed by the same letters do not differ from each other according to the Scott-Knott test at the 5% probability level. Lowercase letters compare the effect of storage within the same treatment, and uppercase letters compare the treatments at each storage time.

Table 2. Means of number of pairs of true leaves, seedling height, and leaf area of seedlings from *Coffea arabica* L. seeds evaluated before and after storage. Treatments: seeds dried in a stationary dryer until reaching 12% moisture (wb) (S1) or 32% moisture (wb) (S2) and kept in cold and dry storage for six months; seeds dried to 17% moisture (wb) in saturated saline solution (S3) or in silica gel (S4) and stored in liquid nitrogen for six months.

		Before storage				After storage			
		Treatment							
Variable	Location	S1	S2	S3	S4	S1	S2	S3	S4
No. of pairs of leaves	Greenhouse	4 aA (a)	4 aA (a)	0 aC (a)	2 aB (a)	2 bA (b)	3 aA (b)	0 aB (a)	2 aA (a)
	Nursery	3 aA (a)	3 aA (a)	1 aB (a)	1 aB (a)	3 aA (a)	2 aB (b)	0 aC (a)	0 bC (a)
CV (%)		31.16							

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Table 2. Continuation.

		Before storage				After storage			
		Treatment							
Variable	Location	S1	S2	S3	S4	S1	S2	S3	S4
Seedling height (cm)	Greenhouse	16.46 aA (a)	16.42 aA (a)	1.29 aC (a)	4.96 aB (a)	4.34 bA (b)	6.82 aA (b)	0.58 aB (a)	5.30 aA (a)
	Nursery	11.67 bA (a)	10.50 bA (a)	1.92 aB (a)	2.63 aB (a)	8.96 aA (a)	6.31 aA (b)	0 aB (a)	1.46 bB (a)
CV (%)		32.56							
Leaf area (cm^{-2})	Greenhouse	200.79 aA (a)	183.65 aA (a)	23.98 aC (a)	82.23 aB (a)	38.73 aA (b)	56.84 aA (b)	2.27 aA (a)	42.11 aA (a)
	Nursery	90.56 bA (a)	94.37 bA (a)	19.26 aB (a)	17.16 bB (a)	75.32 aA (a)	47.29 aA (a)	0 aB (a)	11.80 aB (a)
CV (%)		49.23							

*Means followed by the same lowercase letters in the columns and uppercase letters in the rows within treatments, and lowercase letters in parentheses in the rows among storage types, do not differ from each other according to the Scott–Knott test at the 5% probability level. CV: coefficient of variation.

Table 3. Means of shoot diameter, shoot dry weight, and root dry weight of seedlings grown from *Coffea arabica* L. seeds evaluated before and after storage.

Evaluation time	Variables		
	Shoot diameter (mm)	Shoot dry weight ($gram.seedling^{-1}$)	Root dry weight ($gram.seedling^{-1}$)
Before storage	1.82 a	0.63 a	0.19 a
After storage	0.97 b	0.28 b	0.09 b
CV (%)	84.47	76.51	75.63

*Mean values followed by the same lowercase letters in the columns do not differ from each other according to the Scott-Knott test at the 5% probability level.

Table 4. Means of shoot diameter, shoot dry weight, and root dry weight of seedlings derived from *Coffea arabica* L. seeds under the following treatments: seeds dried in a stationary dryer until reaching 12% moisture (wb) (S1) or 32% moisture (wb) (S2) and kept in cold and dry storage for six months; seeds dried to 17% moisture (wb) in saturated saline solution (S3) or in silica gel (S4) and stored in liquid nitrogen for six months.

Treatment	Variables		
	Mean shoot diameter (mm)	Shoot dry weight ($gram.seedling^{-1}$)	Root dry weight ($gram.seedling^{-1}$)
S1	2.08 a	0.72 a	0.20 a
S2	2.05 a	0.66 a	0.18 a
S3	0.15 b	0.16 b	0.04 b
S4	1.32 a	0.28 b	0.14 a
CV (%)	84.47	76.51	75.63

*Mean values followed by the same lowercase letters in the columns do not differ from each other according to the Scott-Knott test at the 5% probability level. CV: coefficient of variation.

DISCUSSION

For cryostorage to be successful, the coffee seed sample must be sufficiently dehydrated before exposure to liquid nitrogen to prevent the formation of intracellular ice crystals during cooling (Figueiredo et al., 2021). Adequate

dehydration minimizes the risk of structural and biochemical damage caused by water expansion during intracellular ice crystal formation. Therefore, the choice of the drying method is a factor that determines the effectiveness of cryostorage, ensuring recovery of highly viable and functional samples. In this study, cryopreservation of coffee seeds after slow drying in saturated saline solution led to worse results at both evaluation times (Figures 1, 2, and 3).

In cryopreservation studies with *Coffea arabica* L. seeds, Dussert et al. (1998) reported that a high percentage of radicle protrusion does not always lead to the development of normal seedlings. This distinction is particularly relevant in cryopreservation studies, where seeds may initiate germination but fail to develop into vigorous, viable seedlings due to physiological damage or suboptimal moisture content during drying and storage (Coelho et al., 2024). The same result was observed in the present study (Figures 1.A and 1.B), which may be explained by the greater sensitivity of the endosperm to stress factors, such as stress brought about by exposure to ultra-low temperatures (Table 1) (Coelho et al., 2024). In these cases, the endosperm is sufficiently intact to allow the emergence of viable embryos; however, the structural or biochemical damage caused by cryopreservation may limit the availability of seed reserves essential for continued seedling development. As a result, seeds exhibit radicle protrusion but fail to develop into normal seedlings, showing that radicle protrusion alone is not a sufficient parameter to evaluate the success of cryopreservation (Coelho et al., 2017; Vilela et al., 2022).

The seeds from the S3 treatment dried in saturated NaCl solution showed a lower percentage of normal seedlings compared to those dried in a stationary dryer until reaching 12% moisture (wb) and to those dried in silica gel until reaching 17% moisture (wb) (S1 and S4), both before and after storage. Coelho et al. (2018), studying on the cryopreservation protocol for *Coffea canephora* Pierre seeds, also observed that slow drying in saturated NaCl solution was detrimental to normal seedlings formation; they also found better results for seeds dried more rapidly in silica gel. That may be due to an internal osmotic imbalance caused by the saturated saline solution, which can compromise the ability of cells to recover after rehydration, resulting in lower physiological performance of the samples (Coelho et al., 2015).

Similarly, Figueiredo et al. (2017) observed good physiological results in coffee seeds cryopreserved after rapid drying in silica gel until reaching 17% moisture (wb). Rapid drying is advantageous because it effectively reduces the amount of free water in the cells, minimizing the risks of intracellular ice crystal formation during the cryopreservation process (Pammenter and Berjak, 2014). Furthermore, rapid drying in silica gel is provided for controlled drying, which helps maintain cell structure, assisting seed recovery after rewarming (Coelho et al., 2019).

The *C. arabica* seedlings produced from seeds cryostored for six months after drying to 17% moisture (wb) in silica gel (S4) showed the same physiological performance as the seedlings produced from freshly harvested seeds (Table 2). The seeds dried in saturated saline solution (S3), just as observed in evaluation of seed physiological quality, showed a lower result in seedling production. That indicates that the quality of the seeds, as reflected in the physiological evaluations, has a direct impact on seedling development. The results of this study are consistent with those of Pammenter and Berjak (2014) and Coelho et al. (2015), who report that rapid drying in silica gel leads to results superior to those from slow drying in saturated saline solution for cryopreservation of intermediate seeds such as coffee.

The moisture content of stored seeds had an impact on seed germination and seedling growth. Seeds stored at 12% moisture (S1) had better germination rates and seedling development compared to those stored at 32% moisture (S2). Lower moisture contents reduce metabolic activity, slow respiratory rates, and limit enzymatic reactions involved in seed deterioration, thereby contributing to the preservation of membrane integrity and cellular organization during storage (Corbineau, 2024). In contrast, high seed moisture content favors the onset of deteriorative processes, such as lipid peroxidation, protein denaturation, and loss of membrane selectivity, which directly impair seed viability and vigor (Coelho et al., 2019). Elevated moisture levels also enhance seed respiration and create favorable conditions for the proliferation of storage fungi, further accelerating deterioration and decreasing germination potential (Ranganathan and Groot, 2023). Thus, maintaining seeds at optimal moisture contents during storage is critical for preserving physiological quality, resulting in higher germination capacity and more uniform seedling establishment (Kameswara et al., 2017).

For the variables number of pairs of true leaves, seedling height, and leaf area, the S1 and S2 treatments in the first evaluation time had better results than the other treatments at both growing locations (Table 2). These data show the importance of conservation methods that promote initial seedling development, especially in nursery and greenhouse, essential environments for production of healthy seedlings. It is noteworthy that after the storage period, the seedlings from the S4 treatment developed in a greenhouse showed values statistically equal to those of the S1 and S2 treatments. This is an important result because it indicates the real possibility of using cryopreserved seeds for producing *Coffea arabica* L. seedlings at any time of the year, leading to a more flexible supply of seedlings for growers.

The growth of *Coffea arabica* L. seedlings in a greenhouse was superior to the growth in the conventional nursery system. This result may be since the greenhouse provides a more controlled environment than the nursery, with irrigation, temperature, and sunlight favorable to seedling development. Silva et al. (2013) compared the growth parameters of coffee seedlings grown in a greenhouse and in a nursery and also observed better results in the greenhouse in all the evaluations carried out.

Cryopreservation of seeds ensures safe conservation provided that care is taken in the different steps of the preparatory process for immersing the seeds in liquid nitrogen, as well as in rewarming (Coelho et al., 2017; Figueiredo et al., 2017). Therefore, it is noteworthy that the seeds dried in silica gel until reaching 17% moisture and immersed in liquid nitrogen had mean values similar to the non-cryopreserved seeds in some evaluations. This indicates that they have potential for use in seedling production since they developed normally, producing healthy plants suitable for planting in the field. However, cryopreservation of coffee seeds after drying them to 17% moisture in saturated saline solutions is not recommended, considering their poorer performance in all the evaluations in this study.

CONCLUSIONS

Coffee seedlings can be produced from cryopreserved seeds. However, the physiological quality of the seeds is affected by the drying process that precedes cryopreservation. Drying the seeds to 17% silica gel followed by storage in liquid nitrogen preserves their physiological quality, making it comparable to that of conventional storage methods. Conversely, drying in a saturated NaCl solution significantly reduces seed germination. Furthermore, seedlings grown in a greenhouse exhibited superior vegetative performance compared to those grown in a nursery.

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AUTHORS' CONTRIBUTION

Marcela Andreotti Ricaldoni: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - original Draft, Visualization; Gabriela Ribeiro Gontijo: Formal analysis, Data curation, Visualization and Writing - Review & Editing; Ana Luiza de Oliveira Vilela: Visualization and Writing, Review & Editing; Cristiane Carvalho Pereira and Ana Cristina de Souza contributed with: Formal analysis and methodology; Sttela Dellyzete Veiga Franco da Rosa: Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding Acquisition.

DATA AVAILABILITY

Additional data will be made available by the authors upon reasonable request.

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