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Tolerance of *Coffea arabica* L. seeds to sub zero temperatures

Tolerância de sementes de *Coffea arabica* L. à temperaturas sub zero

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ABSTRACT

Preservation of the quality of coffee seeds is hindered by their intermediate behavior in storage. However, long-term storage at sub zero temperatures may be achieved by adjusting the water content of the seeds. The aim of this study was to evaluate the tolerance of coffee seeds to freezing, in relation to physiological and enzymatic modifications. Coffee seeds were dried in two manners, rapid and slow, to water contents of interest, 0.67, 0.43, 0.25, 0.18, 0.11, and 0.05 g H_2O g^{-1} dw (dry basis). After drying, the seeds were stored at a temperature of -20 ºC and of 86 ºC for 24 hours and for 12 months, and then compared to seeds in cold storage at 10 ºC. The seeds were evaluated through calculation of percentage of normal seedlings, percentage of seedlings with expanded cotyledonary leaves, dry matter of roots and of hypocotyls, and viability of embryos in the tetrazolium test. Expression of the enzymes superoxide dismutase, catalase, and peroxidase were evaluated by means of electrophoretic analysis. Only seeds dried more slowly to 0.18 g H_2O g^{-1} dw present relative tolerance to storing at -20 °C for 12 months. Coffee seeds do not tolerate storage at a temperature of -86 ºC for 12 months. Water contents below 0.11g H_2O g^{-1} dw and above 0.43 g H_2O g^{-1} dw hurt the physiological quality of coffee seeds, regardless of the type of drying, temperature, and storage period. Coffee seed embryos are more tolerant to desiccation and to freezing compared to whole seeds, especially when the seeds are dried to 0.05 g H_2O g^{-1} dw. The catalase enzyme can be used as a biochemical marker to study tolerance to freezing in coffee seeds.

Index terms: Drying; silica gel; saturated saline solutions; freezing; antioxidant enzymes.

RESUMO

A preservação da qualidade de sementes de café é dificultada pelo comportamento intermediário no armazenamento. Porém, a conservação a longo prazo em temperaturas subzero pode ser conseguido com o ajuste do teor de água das sementes. Objetivou-se neste trabalho avaliar a tolerância de sementes de café ao congelamento, com relação às modificações fisiológicas e enzimáticas. As sementes foram submetidas a dois tipos de secagem, rápida e lenta, até os teores de água de interesse, de 0,67, 0,43, 0,25, 0,18, 0,11, 0,05 g H_2O g^{-1} dw (base seca). Após secagem, as sementes foram armazenadas em temperatura de -20 e de -86 ºC, por 24 horas e por 12 meses, sendo comparadas às sementes armazenadas em câmara fria a 10 ºC. As sementes foram avaliadas pela porcentagem de plântulas normais, plântulas com folhas cotiledonares expandidas, matéria seca de raízes e de hipocótilos e viabilidade dos embriões no teste de tetrazólio. A expressão das enzimas superóxido dismutase, catalase e peroxidase foi avaliada por meio de análise eletroforética. Apenas as sementes secadas lentamente até 0,18 g H_2O g^{-1} dw apresentam relativa tolerância ao armazenamento a -20 °C por 12 meses. Sementes de café não toleram o armazenamento à temperatura de -86 ºC por 12 meses. Umidades abaixo de 0,11g H_2O g^{-1} dw e acima de 0,43 g H_2O g^{-1} dw são prejudiciais à qualidade fisiológica das sementes de café, independentemente do tipo de secagem, temperatura e período de armazenamento. Embriões de sementes de café são mais tolerantes à dessecação e ao congelamento quando comparado às sementes inteiras, principalmente quando as sementes são secadas até 0,05 g H_2O g^{-1} dw. A enzima catalase pode ser usada como um marcador bioquímico para estudar a tolerância de sementes de café ao congelamento.

Termos para indexação: Secagem; sílica gel; soluções salinas saturadas; congelamento; enzimas antioxidantes.

INTRODUCTION

The degree of dehydration and the storage temperature tolerated by live plant cells divide seeds into three categories. Orthodox seeds bear water loss to water content near 5% and can be stored at low temperatures for long periods of time. Recalcitrant seeds do not exhibit such characteristics and quickly lose quality under these conditions (Roberts, 1973). *Coffea arabica* L. seeds partially tolerate water loss (around 10% to 13% moisture) and are sensitive to storage at temperatures below zero (°C); they are thus considered to be intermediate seeds (Ellis; Hong; Roberts, 1990).

Seed storage at sub-zero temperatures is alternative used in germplasm banks to conserve seed viability of various species grown throughout the world for long periods of time.

However, this technique is not yet totally safe for intermediate seeds like coffee seeds, and this species is thus still conserved in plant collections in the field (Dussert et al., 2012).

The challenge of exposing seeds to below-zero temperatures is freezing the seed without the formation of ice crystals in the intracellular medium, which causes rupture of the cell membrane system, resulting in loss of semi-permeability and of compartmentalization of cell structures (Pammenter; Berjak, 2014). The formation of ice crystals in the cells occurs if the water content in the plant tissues is high. Therefore, water needs to be removed before freezing occurs to avoid collapse of cells. The effects of water content and temperature are interdependent, and critical water content always increases as temperature decreases (Dussert et al., 2003; Dussert; Engelmann, 2006). Pammenter and Berjak (2014) report that removal of water from the seed can cause physical damage to tissues, disordering metabolism and consequently affecting seed germination capacity.

Seed capacity to tolerate storage at negative temperatures also depends on tolerance of seed tissue to desiccation (Pukacki; Juszczyk, 2015). Species considered intermediate and recalcitrant, such as those of the *Coffea* genus, have limitations in this tolerance, making it necessary to know the degree of sensitivity to freezing, associated with tissue response to drying.

The environmental conditions to which seeds are subjected during storage directly affect physiological quality, causing degenerative physiological modifications, such as delay in germination, leaching of solutes, changes in enzyme activity, and loss of cell compartmentalization (Marcos Filho, 2015). The effects of processes related to seed deterioration are not always detected through physiological analyses such as the germination test and vigor analyses. Analysis of the enzymatic profile has been used for better understanding of the mechanisms involved in the seed deterioration process, related to the oxidative processes caused by exposure to cold.

When seeds are exposed to environmental stresses, such as storage at below-zero temperatures, there is an increase in reactive oxygen species (ROS), such as the superoxide radical (O_2^-), hydroxyl (OH^-), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2); excessive production of these radicals hurts seed quality. However, plant cells have enzymatic antioxidant systems, which prevent and/or remove the radicals produced (Sharma et al., 2012). Balance between excessive production of ROS and the ability of activating the antioxidant defense system in seeds will be reflected in greater or lesser tolerance of seeds to stresses of dehydration and exposure to below-zero temperatures.

Ellis, Hong and Roberts (1990), in a study on the effects of drying in seeds of different cultivars of *Coffea arabica* L. to water contents below 10%, as well as the effects of storage at temperatures of 15, 0, and -20 °C, observed that most cultivars do not withstand exposure to sub-zero temperatures. According to studies on the critical level of water for maintenance of the physiological quality of seeds of the *Coffea* genus, Eira et al. (1999) observed that for the species *Coffea arabica* L., the critical level of water for storage at the temperature of -20 °C is 0.12 Kg/Kg.

Considering the difficulty of storage of coffee seeds and, moreover, the fact that the lower limits of temperature withstood by these seeds have not been established, the aim of this study was to evaluate the tolerance of coffee seeds to freezing, in relation to physiological and enzymatic modifications.

MATERIAL AND METHODS

Obtaining seeds

This study was carried out in the Seed Analysis Laboratory of the Agriculture Department of the Universidade Federal de Lavras (UFLA), Lavras, MG, Brazil. Seeds from the 2012/2013 crop season of *Coffea arabica* L., cultivar Catuaí Amarelo IAC 62, were used. Coffee fruits were harvested on the Procafé Experimental Farm in Varginha, Minas Gerais, altitude 980 m, with a highland tropical climate (Cwb), according to the Köppen classification.

Fruits in the cherry maturation stage were selectively harvested from the middle branches of the plants in the middle part of the branches. After harvest, the fruits were selected for uniformity of the stage of maturation and mechanically pulped. The seeds were then "fully washed" through fermentation in water for 24 hours and then kept in the shade for pre-drying to remove surface moisture. For uniformity of size, seeds removed from the circular sieve no. 20/64 were used.

Drying seeds

The seeds in this study were used to evaluate tolerance to exposure to temperatures above and below zero (°C) after desiccation. Seeds were dried to different water contents in environments with controlled temperatures and relative humidity. Two types of drying were used, fast drying and slow drying. For both types of drying, a hermetic environment was used in gerbox

acrylic boxes, supplied with screens and sealed with plastic wrap to avoid changes in relative humidity within the containers.

For fast drying, the seeds were placed in a single layer over the gerbox screen, with activated silica gel in the box below the screens. During drying, the silica was exchanged before a change in its color, which indicates an increase in relative humidity. For slow drying, the seeds were also placed in a single layer over the screens of the gerbox, but containing saturated saline solutions that were able to maintain a stable internal relative humidity. Each saturated saline solution was prepared by dissolving the specific salt in water. To obtain seeds with 0.67 g H_2O g^{-1} dw, lithium chloride salt (LiCl) was used. For moisture of 0.43 g H_2O g^{-1} dw, sodium chloride (NaCl) was used; for the other moisture levels of 0.25, 0.18, 0.11, and 0.05 g H_2O g^{-1} dw, magnesium chloride hexahydrate ($MgCl_2$. $6H_20$) was used. The salts, the concentrations, and the relative humidity provided by the saline saturated solutions are summarized in Table 1.

The containers with the saline solutions, silica gel, and seeds were placed in B.O.D. chambers at a constant temperature of 25 ºC. Water loss during drying was monitored by continuous weighings on a balance with precision of 0.001 g until the seeds reached the water contents of interest.

Seed storage

The seeds with different water contents obtained at the two drying speeds were placed in hermetic packages and stored at temperatures of -20 ºC and -86 ºC. These seeds were compared to others that were in cold and dry storage (10 ºC and 45% RH) for the same periods of time, 24 hours and 12 months. After these periods, the seeds underwent physiological and enzyme evaluations.

Seed thawing

The seeds stored at temperatures of -20 ºC and -86 °C were thawed in a water bath for 2 minutes at 40 ºC, according to the methodology of Dussert et al. (1998). For thawing, the seeds were rapidly removed from their respective packages and directly immersed in the water bath. Afterwards, they were dried on paper toweling and their parchments were manually removed before physiological and biochemical evaluations.

The seeds that remained at the temperature of 10 °C after the period of 24 hours and 12 months were removed from their packages and left on the laboratory counter for 6 hours to establish equilibrium with room temperature. After that, their parchments were removed manually and they underwent physiological and biochemical evaluations.

Determination of water content

The water content of seeds was determined using a laboratory oven at 105 ºC for 24 hours (Brasil, 2009), and the results were expressed in percentages based on seed dry weight.

Physiological evaluation

Physiological evaluation of the seeds was performed by percentage of normal seedlings, of seedlings with expanded cotyledonary leaves and dry matter in the hypocotyl and radicle in the tests of germination, and embryo viability in the tetrazolium test. The germination test was performed with four replications of 25 seeds between sheets of germitest paper, moistened with water at 2.5 times the weight of the dry paper. The seeds were kept in a germinator regulated at a constant temperature of 30 ºC, and the percentage of normal seedlings was evaluated after 30 days following the guidelines of the RAS (Brasil, 2009). At 45 days after the beginning of the germination test, the seedlings with expanded cotyledonary leaves were counted, and the results were expressed in percentage.

Seedling dry matter was determined at 45 days after the germination test. The hypocotyl-radicle axes of the normal seedlings were isolated, placed in paper bags and dried in an air circulation laboratory oven at 60 ºC for 5 days or until constant weight. After this period, the dry matter of roots and shoots of the seedlings was determined, and the results were expressed in milligrams per seedling.

Table 1: Salts used for slow drying of *Coffea arabica* L. seeds.

Salt	Concentration	Equilibrium relative humidity at 25 ºC (%)
Lithium chloride (LiCl)	50 g/1000 mL H_2O	95
Sodium chloride (NaCl)	Saturated solution	75
Magnesium chloride hexahydrate ($MgCl_2$. $6H_20$)	Saturated solution	35

Four replications of 10 seeds were used for the tetrazolium test; the seeds were imbibed in distilled water for a period of 48 hours at 30 ºC (Clemente et al., 2011). After this period, the embryos were removed with the aid of a scalpel so as to avoid damaging them. The embryos were immersed in 0.5% tetrazolium solution in the absence of light for a period of 3 hours at 30 ºC for coloring, at which time they were evaluated and the results were expressed in percentage of viable embryos.

Isoenzyme electrophoresis

For biochemical analyses, the seeds were macerated in liquid nitrogen in the presence of polyvinylpyrrolidone, and the samples were stored at a temperature of -86 ºC (deep freezer) until the time of analyses. The method proposed by Alfenas (2006) was used for extraction, electrophoretic run, and revelation of the isoenzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (PO).

Statistical design

The experimental design was completely randomized in a 2×6×3 factorial arrangement, with two drying speeds, six seed water contents, and three storage temperatures, with four replications. Analyses were performed separately for each storage time. The results of the physiological tests were subjected to analysis of variance through the SISVAR statistical program (Ferreira, 2011).

RESULTS AND DISCUSSION

Table 2 shows the water contents of the coffee seeds before and after storage for twelve months at 10, -20, and -86 ºC. There was an increase in water content of the seeds as initial water contents and storage temperatures decreased. This increase in water content is greater in seeds dried in silica and below 0.18 g H_2O g^{-1} dw and may be due to the thawing of seeds in a water bath for two minutes. Seeds with lower water contents and dried more rapidly absorbed water more rapidly than seeds dried more slowly. Seeds stored at 10 ºC also showed an increase in water content after 12 months, though they had not passed through thawing in a water bath; however, this increase was less compared to those seeds exposed to sub-zero temperatures.

There was significant interaction of the factors investigated, drying speed, water content, and storage temperature; and the effects of seed moisture and sub-zero temperatures depend on the drying method. The results of seed physiological evaluation are shown in Figures 1 and 2.

Germination of the control treatment, that is, before drying and storage, was 96%, with water content in the seeds at 0,72 g H_2O g^{-1} dw under these conditions. The germination test data show (Figure 1) that seeds can be dried to 0.18 g H_2O g^{-1} dw without loss in the germination potential of the seeds.

Table 2: Water content (g H_2O g^{-1}dw), before and after storage for twelve months at different temperatures, of *Coffea arabica* L. seeds dried in saturated saline solutions and in silica gel.

Drying Speed	Drying Agent	Water Content (before storage)	Water Content (after storage)		
			10 ºC	-20 ºC	-86 ºC
Slow	Diluted solution of LiCl	0.672	0.658	0.587	0.715
	Saturated saline solution of NaCl	0.414	0.391	0.418	0.447
	Saturated saline solution of $MgCl_2$. $6H_20$	0.263	0.214	0.263	0.302
	Saturated saline solution of $MgCl_2$. $6H_20$	0.175	0.189	0.218	0.279
	Saturated saline solution of $MgCl_2$. $6H_20$	0.122	0.127	0.188	0.233
	Saturated saline solution of $MgCl_2$. $6H_20$	0.055	0.087	0.217	0.214
Fast	Silica gel	0.647	0.689	0.639	0.664
	Silica gel	0.466	0.435	0.443	0.451
	Silica gel	0.255	0.212	0.287	0.279
	Silica gel	0.176	0.167	0.235	0.321
	Silica gel	0.103	0.121	0.274	0.259
	Silica gel	0.044	0.074	0.224	0.227

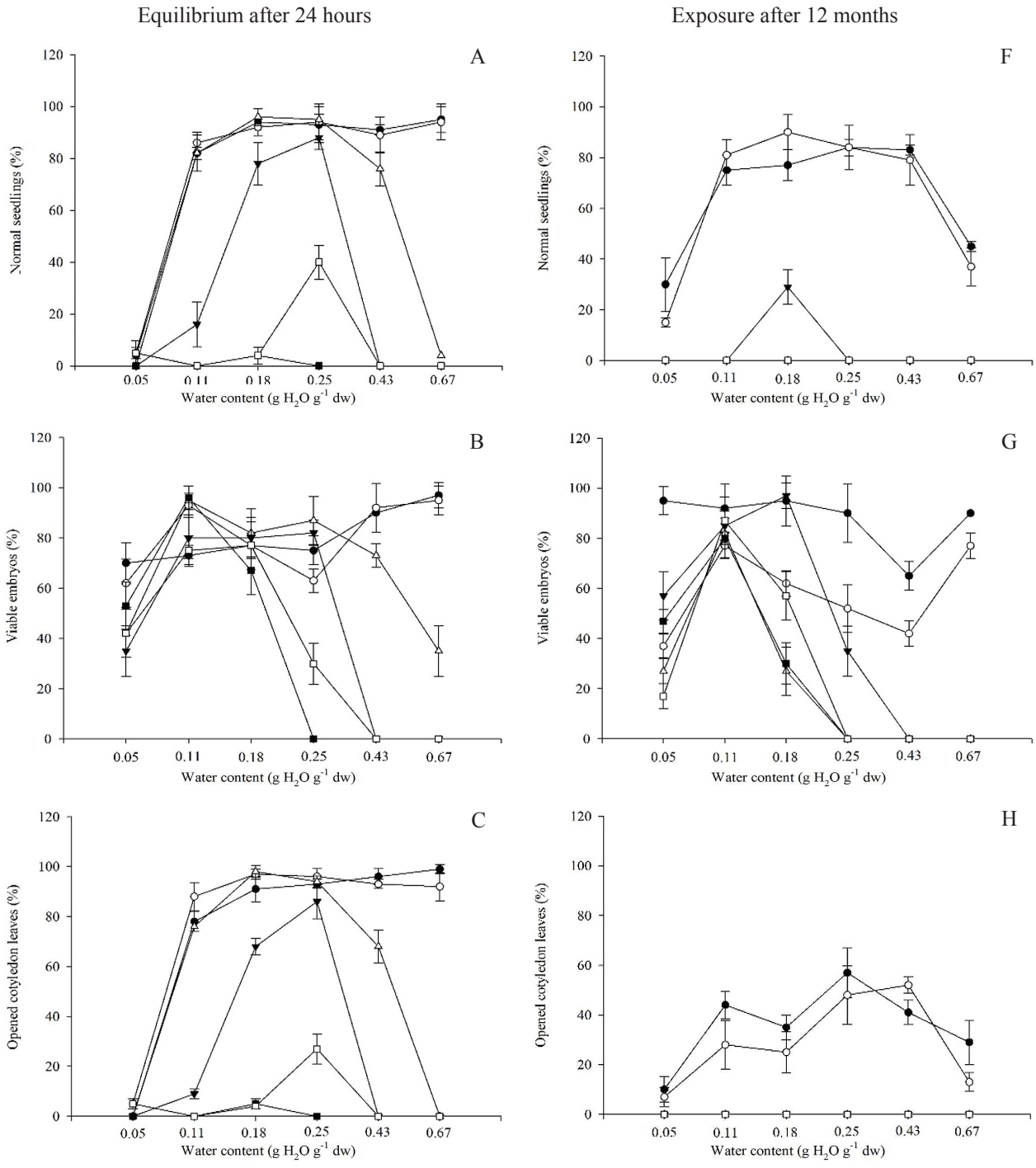

Figure 1: Mean percentage of normal seedlings, of seedlings with expanded cotyledonary leaves, and of embryo viability in the tetrazolium test, and root and hypocotyl dry matter of *Coffea arabica* L. seeds that underwent fast or slow drying and were stored at temperatures of 10, -20, and 86 ºC for 24 hours and 12 months.

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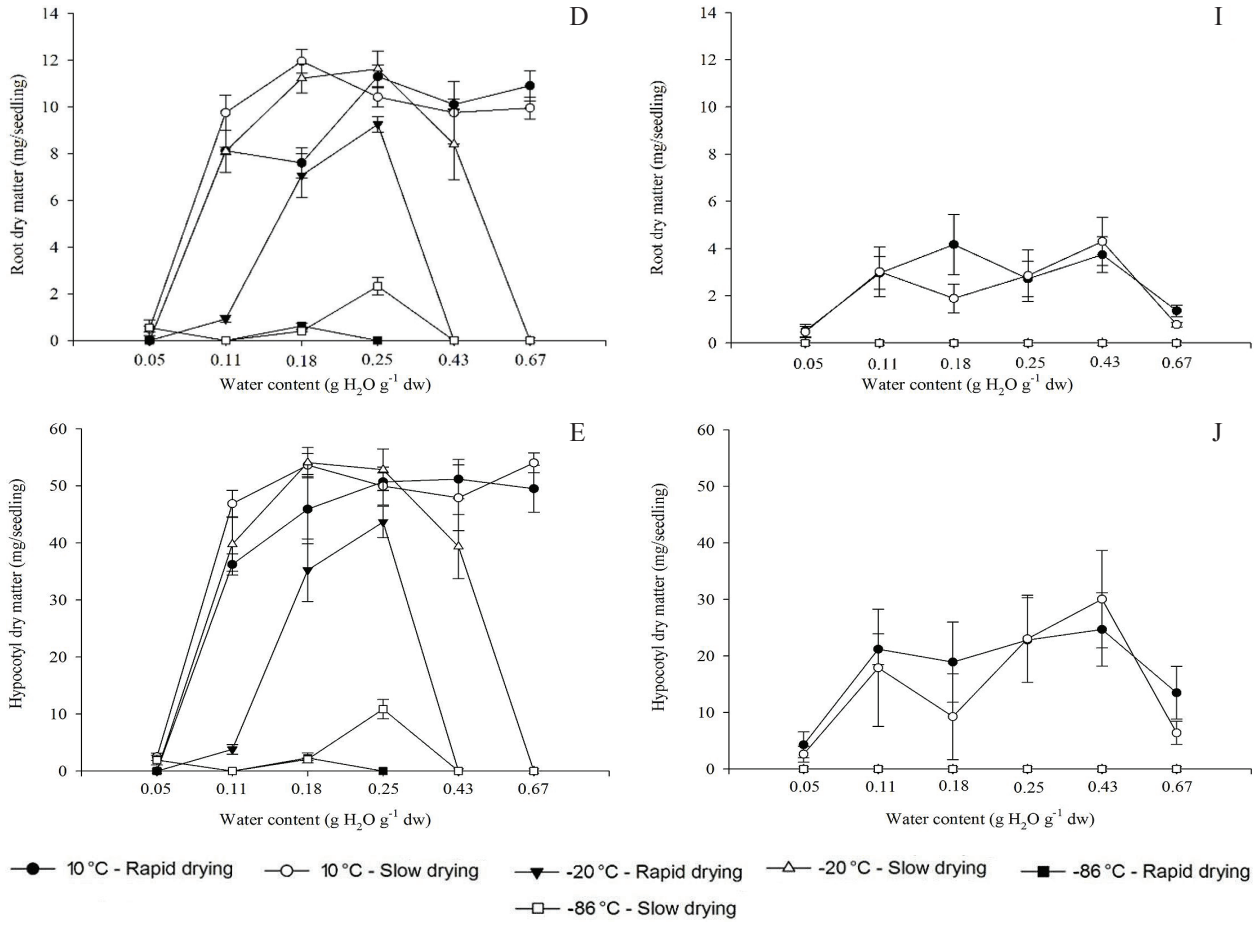

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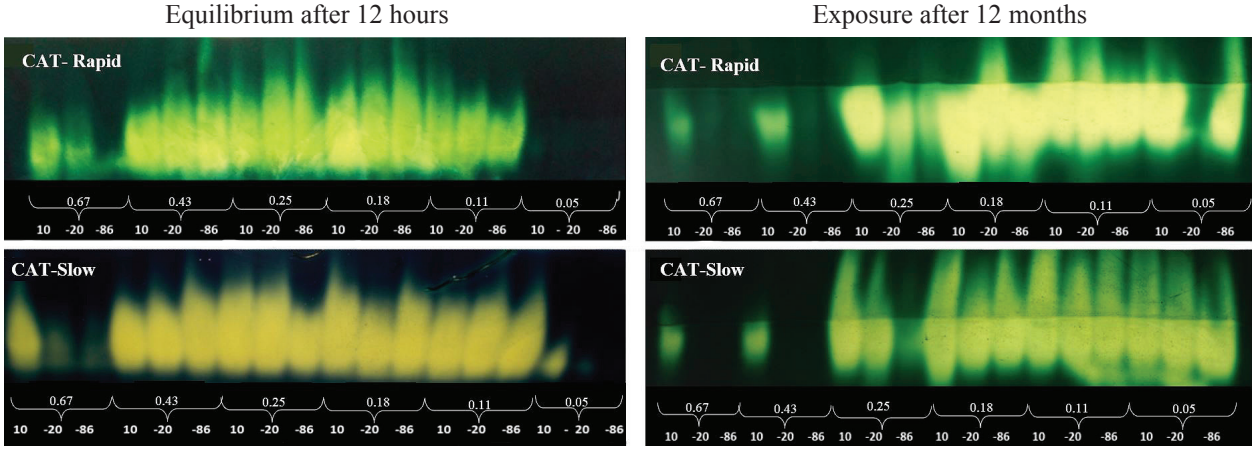

Figure 2: Isoenzymatic pattern revealed for the enzymes catalase (CAT), superoxide dismutase (SOD), and peroxidase (PO) in *Coffea arabica* L. seeds under fast and slow drying to the water contents of 0.67, 0.43, 0.25, 0.18, 0.11,and 0.05 g $H_2O.g^{-1}$ dw, and stored at the temperatures of 10, -20, and -86 ºC for 24 hours and 12 months.

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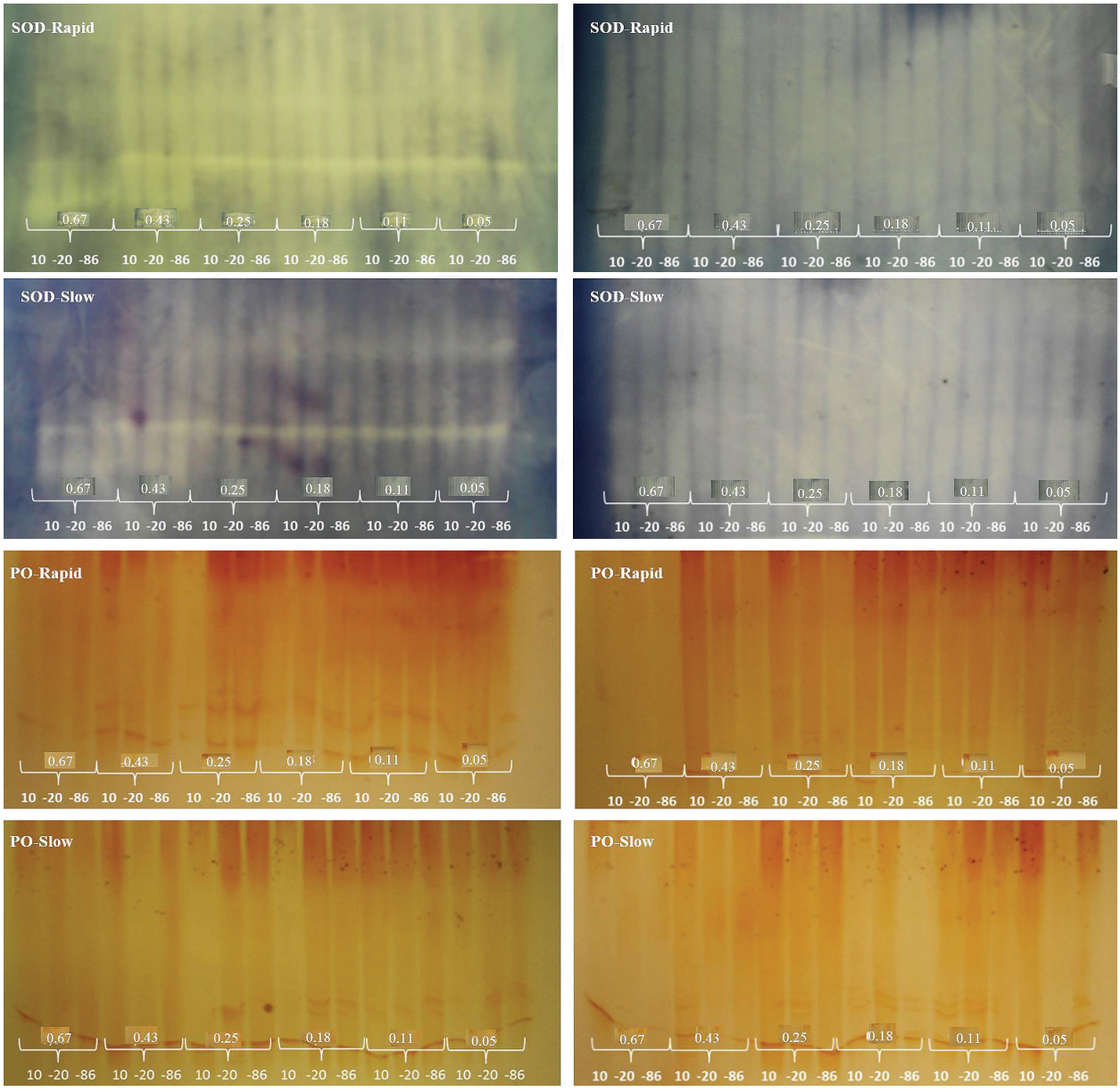

Figure 2: Continuation...

These results show that seeds in cold and dry storage (10 ºC, 50% RH) had better results than seeds stored at sub-zero temperatures investigated. Seeds with water content from 0.11 to 0.43 g H_2O g^{-1} dw still had a normal seedling percentage of around 80% after twelve months of storage. For this temperature of 10 °C, vigor tests showed (Figure 1) that slow drying to water contents of 0.11 and 0.18 g H_2O g^{-1} dw is more recommended for storage of coffee seeds than fast drying. Water loss to contents near 0.05 g H_2O g^{-1} dw, regardless of the manner of drying, is highly damaging to coffee seed quality, reducing germination potential to values below 30%.

After 12 months of cold and dry storage (10 ºC, 50% RH), embryos of the coffee seeds dried slowly to 0.05 g H_2O g^{-1} dw had viability greater than 90% in the tetrazolium test, though seed germination was very low, indicating greater tolerance of embryos to drying and storage stresses in relation to the whole seeds (Figure 1).

For storage at the temperature of -20 °C, coffee seeds dried more rapidly to water contents from 0.11 to 0.25 g H_2O g^{-1} dw tolerate exposure to this condition for 24 hours, with germination above 80% (Figure 1). As seed water content increases or decreases, the percentage of normal seedlings declines drastically, arriving nearly at zero when these seeds have extreme water contents, whether very low, at 0.05, or very high, at 0.67 g H_2O g^{-1} dw. After slow drying, only the seeds dried from 0.18 to 0.25 g H_2O g^{-1} dw tolerate exposure to the temperature of -20 ºC for 24 hours, with a survival rate above 70% for normal seedlings (Figure 1). After 12 months of storage, only those dried more slowly to 0.18 g H_2O g^{-1} dw present some percentage of normal seedling, indicating relative desiccation tolerance to the temperature of -20 ºC (Figure 1).

Seeds undergoing fast drying to 0.25 g H_2O g^{-1} dw partially tolerate exposure to the temperature of -86 ºC for 24 hours (Figure 1, A). Under these conditions, the percentage of survival of these seeds is near 40%. Coffee seeds dried in saturated saline solutions, that is, dried more slowly, do not exhibit tolerance to temperature. Coffee seeds did not tolerate exposure to -86 °C for 12 months, with null germination and vigor values.

Ellis, Hong and Roberts (1990), studying storage of different cultivars of *Coffea arabica* L. at a temperature of -20 ºC, observed that seeds survive only when they have adjusted water content from 0.10 to 0.12 g H_2O g^{-1} dw. Subsequently, Eira et al. (1999), in studies on tolerance to desiccation and to freezing in six different species of the *Coffea* genus, observed that the C. *arabica* species is one of the most tolerant to storage at -20 ºC, and the ideal water content for this storage is 0.20 g H_2O g^{-1} dw. However, the authors observed that after 12 months of exposure to this temperature, seeds lose viability.

Results obtained in vigor testing (Figure 1) show that seeds with water contents of 0.18 and 0.25 g H_2O g^{-1} dw have better physiological quality after remaining 24 hours at temperatures below zero, indicating this to be the moisture range at which coffee seeds best tolerate exposure to cold. It was also observed that coffee seeds dried rapidly in a moisture range of 0.11 to 0.43 g H_2O g^{-1} dw and stored at -20 ºC remain viable and without significant loss of vigor compared to the temperature of -86 ºC for 24 hours. Seeds with high water content do not tolerate exposure to sub-zero temperatures, drastically losing physiological quality.

In relation to seed vigor after exposure to sub-zero temperatures for 24 hours, it was observed that seeds better tolerate the temperature of -20 ºC compared to the temperature of -86 °C (Figure 1). Seeds surviving under these conditions, that is exposure to -20 ºC for 24 hours, were those dried slowly to water contents of 0.25 g H_2O g^{-1} dw. Coffee seed vigor is compromised after 12 months of storage when exposed to temperatures of -20 and -86 °C, in all treatments. Only those seeds with water contents of 0.11 and 0.18 g H_2O g^{-1} dw and stored at 10 ºC exhibited higher vigor. Water content of 0.05 g H_2O g^{-1} dw is highly damaging to coffee seed vigor, regardless of the type of drying and storage temperature.

An interesting result was found in the tetrazolium test, for even though the seeds had not survived reduction in water content to 0.05 g H_2O g^{-1} dw, the embryos extracted from these seeds proved to be viable at this same water content, regardless of the storage temperature and drying rate (Figure 1). A similar situation occurred for coffee seeds dried to 0.11 g H_2O g^{-1} dw and exposed to sub-zero temperatures. In general, it can be observed that the embryos had viability in the tetrazolium test, indicating more tolerance to exposure to sub-zero temperatures when the seeds contained water contents from 0.11 to 0.25 g H_2O g^{-1} dw. However, embryos extracted from seeds with moisture above 0.43 g H_2O g^{-1} dw did not tolerate exposure to these temperatures.

Ellis, Hong and Roberts (1990, 1991); Gentil (2001), and Hong and Ellis (1992) observed that the critical moisture level, considered lethal for coffee seeds, lies between 0.04 and 0.05 g H_2O g^{1} dw. In the present study, however, the embryos of coffee seeds with 5% moisture exhibited high viability in the tetrazolium test, which was not observed in evaluation of normal seedlings in the germination test (Figure 1). From these results, greater sensitivity of the endosperms in relation to embryos is evident when seeds are dried to low water contents and exposed to sub-zero temperatures.

These results corroborate those obtained by Coelho et al. (2015), who, upon studying the effect of different drying rates on the quality of coffee seeds stored at 10 ºC, observed that the damage from drying is more related to the endosperm than to the embryo. In a similar manner, Dussert and Engelmann (2006) demonstrated that endosperms of coffee seeds are more sensitive to damage from drying and from immersion in liquid nitrogen compared to zygotic embryos.

In relation to the enzymatic systems studied, it was observed that the bands of all the enzymes vary both in quantity and in intensity. Thus, the type of drying, final water content, and the storage temperature had an influence on the degree of biochemical deterioration of the seeds.

In regard to expression of the catalase enzyme (Figure 2), low activity was observed when the seeds had water content of approximately 0.67 g H_2O g^{-1} dw and were

stored at temperatures below zero (-20 and -86 ºC) for 24 hours, or no activity when they remained 12 months stored in these temperatures (Figure 2). Under these conditions also, germination potential of the seeds is not observed. When cells with high water contents are exposed to negative temperatures for a period of time, cell rupture and death occur due to formation of ice crystals in the intracellular medium (Dussert et al., 2012). This rupture of the cells may have compromised the physiological quality and the operation of the enzyme system of the seeds, and thus enzymatic expression was not observed in these treatments.

Seeds dried to 0.05 g H_2O g^{-1} dw did not exhibit catalase enzyme activity when exposed for 24 hours to the temperatures studied (Figure 2). However, after remaining 12 months in these same conditions, there was an increase in expression of this enzyme, even though there was not germination in these treatments. It was observed that seeds dried to water contents below 0.18 g H_2O g^{-1} dw showed an increase in water content after storage for 12 months at sub-zero temperatures. This may be connected with an increase in activity of the catalase enzyme in seeds dried to 0.05 g H_2O g^{-1} dw after 12 months of storage.

According to Sofo et al. (2015), reduction in catalase enzyme activity promotes accumulation of H_2O_2, increasing lipid peroxidation and damage to the plant membranes under environmental stresses. Thus, increase in the expression of this enzyme is required for eliminating the excess of H_2O_2 and reducing lipid peroxidation.

For the enzyme superoxide dismutase (Figure 2), it was observed that for 24 hours of exposure, both in rapid drying and in slow drying, there was the presence of two bands in the gels, except for the moisture of 0.67, in which only one band appears for the temperatures studied in this water content. When the seeds are exposed to the storage temperatures over a period of 12 months (Figure 2), lower expression of the enzyme was observed, and the presence of bands was not observed for any of the water contents and temperatures studied.

Superoxide dismutase is a group of isoenzymes found in the chloroplast, mitochondria, and peroxisomes, and constitute the first line of defense against the formation of free radicals under stress conditions (Wattanakulpakin et al., 2012). Thus, this enzyme is among the most important defense systems when connected with the series of events necessary for complete detoxification of the ROS (Wattanakulpakin et al., 2012).

From the expression profile of the peroxidase enzyme (PO), it can be observed that the seeds dried in silica gel, that is, in a fast manner, generally had greater expression than the seeds dried in a slow manner, especially to the lowest water contents (Figure 2). After 12 months of storage at all the temperatures studied, a decrease in expression of peroxidase in moist seeds (0,067 g H_2O g^{-1} dw) is observed, in both types of drying.

Activity of the peroxidase enzyme is reduced in the seed deterioration process. Consequently, membrane function is changed, which provokes lipid peroxidation, degradation of nucleic acids, and inactivation of enzymes (Garg; Manchanda, 2009). Reduction in the activity of scavenger enzymes increases the sensitivity of seeds to oxidative stress (Gomes; Garcia, 2013). Superoxide dismutase, catalase, and peroxidase are enzymes that eliminate free radicals and peroxides, as well as malate dehydrogenase, acid phosphatase, and glutamate dehydrogenase, which are indicative of deterioration, since they are involved in cell metabolism (Taveira et al., 2012). Thus, information on the activity of certain enzymes can be used in studies on seed deterioration (Gomes; Garcia, 2013).

Taveira et al. (2012), studying the protein profile of coffee seeds dried under different methods, observed greater activity of the catalase enzyme in seeds of worse physiological performance. Saath et al. (2014) and Brandão Júnior, Vieira and Hilhost (2002) found reduction in the activity of the catalase enzyme in coffee seeds with lower physiological performance after processing and drying.

In general, the results obtained in this study indicate that coffee seeds do not survive storage for long periods of time at the sub-zero temperatures investigated. However, recent studies have shown that adjustment between the final water content of seeds, the drying method, and their freezing and thawing rate can determine survival of coffee seeds in liquid nitrogen and need to be better studied.

CONCLUSIONS

Only seeds dried more slowly to 0.18 g H_2O g^{-1} dw present relative tolerance to storing at -20 °C for 12 months. Coffee seeds do not tolerate storage at a temperature of -86 ºC for 12 months. Water contents below 0.11g H_2O g^{-1} dw and above 0.43 g H_2O g^{-1} dw hurt the physiological quality of coffee seeds, regardless of the type of drying, temperature, and period of storage. Coffee seed embryos are more tolerant to desiccation and thawing compared to whole seeds, especially when the seeds are dried to 0.05 g H_2O g^{-1} dw. The catalase enzyme can be used as a biochemical marker to study tolerance to freezing in coffee seeds.

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