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Drying of arabica coffee and its effect on the gene expression and activity of enzymes linked to seed physiological quality

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ABSTRACT. The reduced longevity of coffee seeds has been attributed to their sensitivity to desiccation. Studies related to gene expression and enzyme activity in coffee seeds under drying are important for understanding the effects of drying on their physiological quality. The aim of this study was to investigate the molecular aspects of seeds under different drying methods and associate them with physiological quality. Coffee seeds with different water contents were dried both slowly and rapidly. Enzymatic activity was analysed, as well as the expression of genes that encode the enzymes superoxide dismutase, catalase, peroxiredoxins, isocitrate lyase, and endo-ß-mannanase. There was a significant effect of drying speed and final water content on enzyme activity and on the expression of the different genes analysed. In seeds under rapid drying, there was greater expression of the genes that encode the enzymes catalase and endo-ß-mannanase. Greater expression of the *1 CYS PRX* and *SOD* genes and greater activity of the ICL isoenzymes were found in seeds with superior physiological quality, but greater activity of the endo-β-mannanase and CAT enzymes occurred in seeds with lower physiological quality.

Keywords: desiccation tolerance; enzyme profile; gene transcription; oxidative stress; endo-ß-mannanase.

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Introduction

Coffee is one of the most important agricultural products traded worldwide and in Brazil, and it is the second most important item in international commodity trade. Of the 103 species of coffee plants described to date (Davis, Govaerts, Bridson, & Stoffelen, 2006), the species most commonly grown throughout the world, *Coffea arabica*, is also the species with the most studies regarding the level of sensitivity to desiccation of its seeds. According to the results of most of these studies, *C. arabica* seeds can be partially dehydrated without the loss of viability (Ellis, Ho, & Roberts, 1991; Dussert et al., 2006; Dussert, Couturon, Engelmann, & Joët, 2012).

C. arabica seeds are classified as intermediate seeds (Ellis et al., 1991); that is, they are able to tolerate desiccation levels that are considerably lower than those in recalcitrant seeds. However, they do not tolerate extreme losses of water as do orthodox seeds, which tolerate desiccation to a water content of less than 7%, with little effect on viability (Robert, 1973). Nevertheless, the drying method used has an effect on the physiological quality of these seeds. This sensitivity to desiccation can compromise the conservation of coffee seeds in storage since safe storage requires low water content and low temperatures (Dussert et al., 2012).

An efficient drying process is one that, in addition to reducing the water content of the product, increases its post-harvest conservation potential and preserves its physical characteristics and technological properties, attributing commercial value (Coelho et al., 2015). The use of seeds of high physiological quality is one of the main factors responsible for obtaining coffee seedlings with greater vigor under field conditions, resulting in higher yield.

Given this situation, enhancing the conservation of coffee seeds has been the aim of various studies, and in the results obtained from these studies, considerable sensitivity of the seeds to dehydration has been

observed, which in most cases has caused a rapid loss of viability (Rosa, Brandão Júnior, Von Pinho, Veiga, & Castro e Silva, 2005; Coelho et al., 2015; Coelho, Rosa, & Fernandes, 2017). Understanding the effects of variable dehydration sensitivity has been made difficult by an excessive number of factors analysed in the same experiment. Thus, to understand the effects of drying on the physiological quality of coffee seeds, it is fundamental to analyse gene expression and enzyme activity and to identify proteins related to antioxidant defence mechanisms, such as the enzymes superoxide dismutase (SOD), catalase (CAT), and peroxiredoxins (PRX), and enzymes related to the β-oxidation of lipids, such as isocitrate lyase (ICL) and seed deterioration (endo-β-mannanase).

Thus, the aim of the present study was to investigate the molecular aspects of seeds under different drying methods and associate these aspects with physiological quality.

Material and methods

Acquisition and hulling of coffee seeds

C. arabica seeds of the cultivar Catuaí Amarelo were used. Fruits in the cherry maturity stage were selectively harvested from the middle branches of the plants and in the middle parts of the branches in a coffee field of the Fundação Procafé in Varginha, Minas Gerais State, Brazil. After harvest, the fruit was mechanically pulped, and mucilage was removed from the seeds by fermentation in water for 24 hours at 25°C. The seeds were then placed on screens in a single layer for the removal of surface water.

Drying of seeds

The coffee seeds were dried in environments with controlled temperature and relative humidity until reaching different water content levels. Seeds were dried in two manners, rapid drying, using silica gel, and slow drying, using saturated saline solutions, in gerbox-type acrylic germination boxes that were appropriately sealed, providing a hermetic environment.

For rapid drying, the seeds were placed on screens in gerbox-type boxes containing 80 grams of activated silica gel below the screens. Slow drying was also conducted in gerbox-type boxes, the same as rapid drying, but saturated saline solutions were used below the screens containing the seeds. Saline solutions are able to maintain stable internal relative humidity in an environment under constant temperature. Lithium chloride at 5% was used, and after 105 hours and consequent stabilization of the water content, the lithium chloride was replaced with magnesium chloride. The containers were kept in BOD-type chambers under a constant temperature of 25°C (Table 1).

Table 1. Saline solutions used in slow drying of *Coffea arabica* L. seeds.

Salt for saline solution	Concentration	Equilibrium relative humidity at 25°C
LiCl	50 g 1,000 mL^{-1} H_2O	95%
$MgCl_2$. $6H_20$	Saturated solution	35%

Water loss during drying was monitored by continual weighing on a precision balance with 0.001 g resolution, and water content was determined by roasting the seeds in a laboratory oven at 105°C (Brasil, 2009) until the seeds reached the water content values of interest (30, 20, 10, and 5%). Water content was controlled by the loss of weight and calculated by the equation described by Cromarty, Ellis, and Roberts (1985): Wf = Wi (100-Mi)/(100-Mf), where Wf = the weight of the sample (g) after drying; Wi = the weight of the sample (g) before drying; Mi = the degree of moisture (%) before drying; and Mf = the degree of moisture (%) desired after drying. The initial water content of the seeds was 38%.

The following treatments were used for molecular and physiological analyses: seeds that were not dried, with a 38% water content (control); seeds dried to 30, 20, 10, and 5% water contents by rapid drying; and seeds dried to 30, 20, 10, and 5% water contents by slow drying.

Determination of water content

Water content was determined by the laboratory oven method at 105°C for 24 hours, using two samples of 10 seeds per replication. The results were expressed as percentages based on the wet weight of the seeds, according to the Rules for Seed Analysis (*Regras para Análise de Sementes* - RAS) (Brasil, 2009).

Physiological analyses

The parchment was removed from all the seeds with the aid of tweezers before carrying out the physiological tests. The germination test was performed with four replications of 25 seeds. The seeds were sown on sheets of germination paper moistened with water at an amount 2.5 times the weight of the dry paper. The seeds were kept in a seed germinator and regulated to a temperature of 30°C, and the percentage of normal seedlings was evaluated at 30 days, according to the recommendations of the RAS (Brasil, 2009).

At 45 days after the beginning of the germination test, the seedlings that had fully expanded cotyledonary leaves were counted, and the results were expressed as percentages.

To conduct the tetrazolium test, four replications of 25 seeds were placed in a container with distilled water for 48 hours at 30°C (Clemente, Carvalho, Guimarães, & Zeviani, 2011). After that period, the embryos were removed with the aid of a scalpel and immersed in 0.5% tetrazolium solution in the absence of light for a period of 3 hours at 30°C for staining. The results of the evaluation of viability were expressed as the percentage of viable embryos (Brasil, 2009).

Experimental design and statistical analyses

For analysis of the results of physiological quality evaluations, a completely randomized design was used in a factorial arrangement [(2 × 4) + control] composed of two drying methods (slow and rapid), four water content values (30, 20, 10, and 5%) and a control (additional treatment) with a 38% water content. The Sisvar 7.7 Beta software was used (Ferreira, 2014), and the mean values were compared by the Tukey test at 1% probability. Through the same software, Dunnett's test was used for comparison of the interaction of the mean values between each factor and the mean of the controls at 5% probability.

Molecular analyses

Analyses of the enzymes superoxide dismutase, isocitrate lyase, and catalase

The electrophoresis technique was used for these analyses. Samples of 50 coffee seeds from each treatment were macerated in liquid nitrogen and stored in a deep freezer at -85°C. The protein fraction was extracted, and 200 µl of extraction buffer pH 8.0 (0.2 M Tris, 0.1% β-mercaptoethanol, 0.4% PVP, 0.4% PEG, and 1 mM EDTA) was added to 100 mg of the macerated seeds. The homogenized material was incubated on ice for 1 hour and then centrifuged at 16,000 × g at 4°C for 60 minutes. Supernatant from each treatment (40 µl) was applied to polyacrylamide gels at 4.5% (stacking gel) and 7.5% (separating gel). The buffer gel/electrode system used was tris-glycine pH 8.9. Electrophoresis was performed at 150 V for 6 hours, and the gels were analysed for the following enzymatic systems, superoxide dismutase, isocitrate lyase, and catalase, according to the method described by Alfenas (2006).

Analysis of the endo-ß-mannanase enzyme

A quantity of 300 µL of the extraction buffer (0.1 M Hepes, 0.5 M NaCl pH 8.0, ascorbic acid at a rate of 5 mg of the acid for each mL of buffer) was used in each microtube with 100 mg of the macerated material from each sample. Then, the microtubes containing the samples were shaken in a vortex-type shaker for 1 minute and centrifuged at 14,000 rpm for 30 minutes at 4°C. The supernatant was applied to a gel containing 6 mL of locust bean gum (LBG) (Sigma), 0.24 g of agarose (Qbiogene), and 24 mL of buffer pH 5.0 (11 mL of 1 M citric acid, 50 mL of Na_2HPO_4, and 149 mL of distilled water). Aliquots were applied in 2-mm holes made in the gel with the aid of a hole punch. The gel was incubated for 21 hours and analysed according to the method proposed by Silva, Toorop, Van Aelst, and Hilhorst (2004). The activity of the endo-ß-mannanase enzyme was calculated according to Downie, Hilhorst, and Bewley (1994).

Gene expression

The RNAs of the seeds were extracted according to the manual of the Concert™ Plant RNA Purification Reagent (Invitrogen). After extraction, all the samples were treated with DNA-free DNase (Ambion), and cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) with 1 µg of RNA, as recommended by the manufacturer. Detailed methods can be found in the Supplementary Material.

For analysis of the expression of the selected genes (Table 2), the ABI PRISM 7500 Real-Time PCR (Applied Biosystems) apparatus was used, with SYBR Green detection of cDNA samples obtained from

the seeds under slow and rapid drying with different water content values (30, 20, 10, and 5%) in biological triplicates. The efficiency of the designed primers was determined through the dilution curve by absolute quantification.

Table 2. Specific primers used in the real time PCRs.

Gene	Primers	
	Forward	Reverse
ICL	attgggacctgcctagaacc	Cacctggacctcactccttc
ENDOBETA	cacaatcatggcatgggaact	Attgactgtccttccggagtaatc
CAT 3	tgactgccacacgatcatcttc	Cgggaggcagtgggaaa
SOD	gcatggctcttcgaactctagtc	Gccgcgcagctgttg
1 CYS PRX	ttccgcagggataccagact	Ccggttacacatcgtgaatcgcag
24S	gaccaatcgtcttctttccagaaa	Tcaactcagccttggaaacattag
GPDH	ttgaagggcggtgcaaa	Aacatgggtgcatccttgct

In the expression trial, 1 µL of cDNA (1:5 dilution), 0.4 µL of forward/reverse primer (10 µM), and 5 µL of SYBR Green Master Mix (Applied Biosystems), were used in each 10 µL reaction. The samples were pipetted in technical triplicates, and one control without cDNA (NTC) was included for each pair of primers. The levels of the transcripts are presented as a ratio (relative expression) of the absolute value of expression of the target gene to the absolute value of expression of the *24S* and *GPDH* normalization genes. The gene expression values for the dried seeds were plotted, taking the gene expression values of the seeds that were not dried as a reference. The data correspond to the mean values of three biological samples. The relative expression was analysed by the method of Pfaffl, Tichopad, Prgomet, and Neuvians (2004). The thermal conditions of the reaction were 2 minutes at 50°C and 10 minutes at 95°C for initiation, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C, ending with 15 minutes at 95°C. At the end of cycling, a denaturation curve at 60 - 95°C showed the specificity of the PCR. The data were collected, exported by the 7500 Fast Software (Version 2.1) program, and analysed in an Excel (Microsoft) spreadsheet.

Results

Physiological analyses

The results confirmed that the drying rate decreased as the seeds lost water. In seeds with an initial water content of 38% (wet basis), under rapid drying, a drying time of 100 hours was needed to reach a 5% water content, with a mean drying speed of 0.099% h^{-1}. Under slow drying, a drying time of 190 hours was needed to reach the same water content, with a mean drying speed of 0.065% h^{-1}. The mean water content values attained for seeds dried at the two drying speeds are shown in Table 3.

Table 3. Mean values of the water content of seeds subjected to rapid drying in silica gel and slow drying in saturated saline solution.

Water content of interest (%)	Water content obtained (%)	
	Slow drying	Rapid drying
30	32.05	32.40
20	20.80	23.23
10	9.92	11.02
5	5.41	3.56

The interaction between the drying speed and seed water content factors was not significant for the response variables percentage of normal seedlings at 30 days (germination), percentage of viability in the tetrazolium test, and percentage of seedlings with expanded cotyledonary leaves. For these variables, the seed response varied only with the degree of moisture.

The results for the percentage of normal seedlings at 30 days (germination) are presented in Table 4, which shows a significant reduction in germination in seeds dried to a 10% or lower water content. The germination of seeds with a 5% water content was drastically reduced, confirming that drying to a water content below 10% is harmful to the physiological quality of coffee seeds, causing a reduction in germination.

Table 4. Mean percentages of normal seedlings at 30 days (germination), viable embryos in the tetrazolium test, and expanded cotyledonary leaves (ECL) from coffee seeds with different water content values.

Water content (%)	Germination (%)	Viable embryos (%)	ECL (%)
30	90 ab	93 a	86 ab
20	92 a	89 a	89 a
10	82 b ɤ	91 a	78 b ɤ
5	4 c ɤ	77 b ɤ	2 c ɤ
Control	92	98	91
CV (%)	9.82	5.64	11.17

Mean values followed by the same lowercase letter in the column do not differ from each other by the Tukey test at 1% probability. ɤ denotes mean values that differ from the control at the level of 5% probability by Dunnett's test.

A significant reduction in the percent of seedlings with expanded cotyledonary leaves at 45 days (Table 4) was observed for seeds with a 5% water content. Furthermore, according to these results, coffee seeds dried to a 20% water content did not differ from those of the control treatment, that is, seeds that were not dried.

By the tetrazolium test, there were no differences in the viabilities of embryos from the seeds that were dried or not dried, except for the values observed in seeds dried to a 5% water content (Table 4). At a 5% water content, the seed germination percentage was nearly zero; nevertheless, the germination values of viable embryos were greater than 70%.

In most of the physiological analyses performed, drying to a 20% water content did not reduce seed quality.

Molecular analyses

Dried seeds had greater catalase enzyme activity than undried seeds, regardless of the speed at which the seeds were dried (Figure 1A). Seeds dried slowly had more stable enzyme activity; that is, there was less variation in the size of the bands as drying advanced. There was greater enzyme activity in seeds under rapid drying to a 10% water content than in seeds under slow drying to a 10% water content. Seeds that did not pass through the drying process and seeds with a 30% water content had low enzyme activity. In general, the activity of catalase increased as the seeds were dried, that is, as the water content declined.

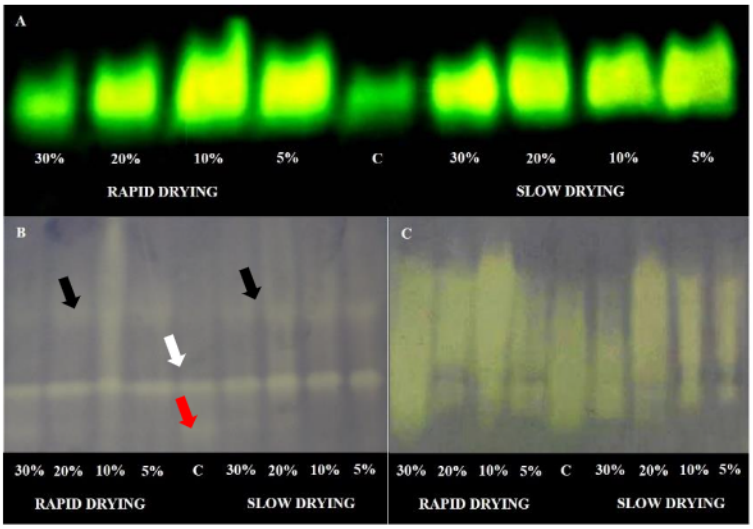

Figure 1. Enzyme activity of A) catalase, B) superoxide dismutase, and C) isocitrate lyase in *C. arabica* seeds under rapid or slow drying with different water content values and in seeds that were not dried (control).

Just as greater activity of the catalase enzyme was observed in the more rapidly dried seeds, expression of the *CAT3* gene was also greater in seeds under rapid drying than in those under slow drying (Figure 2A). Under rapid drying, the expression of this gene was greater in seeds with a high moisture content and diminished as those seeds dried. Under slow drying, the expression of *CAT3* varied little.

In relation to the superoxide dismutase enzyme, three isoforms were observed (arrows) in the electrophoretic profile (Figure 1B): one of them was present in all the treatments analysed (white arrows); another was present only in the undried seeds (red arrows); and the third was present in seeds under rapid or slow drying with a 20% or lower water content (black arrows).

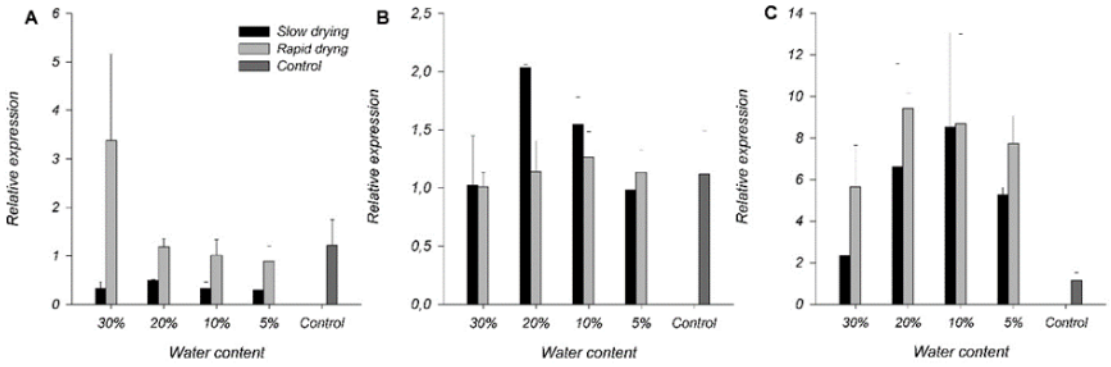

Figure 2. Profile of the relative expression of the *CAT3* (A), *SOD* (B), and *ICL* (C) genes in *C. arabica* seeds under rapid drying or slow drying with different water content values and in seeds that were not dried (control).

The relative expression of the gene that encodes the superoxide dismutase enzyme is shown in Figure 2B. Greater expression was observed in seeds under slow drying to 20 and 10% water contents than in seeds under rapid drying. However, there was no difference in the gene expression in seeds under slow drying or rapid drying when the seeds were dried to a 30% water content. In general, the expression of this gene in undried seeds (control) had greater similarity to that observed in seeds under rapid drying.

Seeds under different drying treatments showed variation in the activity of the enzyme isocitrate lyase (Figure 1C). Greater activity was observed in seeds under rapid drying than in those under slow drying. Under rapid drying, greater activity was found in seeds with 30 and 10% water contents, while under slow drying, greater expression was observed in seeds dried to a 20% water content.

In regard to transcripts (Figure 2C), greater expression was observed in seeds under rapid drying than in those under slow drying to 30 and 5% water contents. When dried to 20 and 10% water contents, there was no difference between the seeds under slow drying and those under rapid drying, although the highest expression values for this gene in coffee seeds were observed in these treatments. It should be noted that the lowest expression was observed in undried seeds (control).

Data regarding the activity of the endo-ß-mannanase enzyme are shown in Figure 3A. The results obtained in this study show that the drying method did not affect the activity of this enzyme when the seeds were dried to 30 and 20% water contents. In addition, there was greater activity of this enzyme in seeds under slow drying to water content values of 10 and 5% than in seeds under rapid drying. Furthermore, the lowest physiological performances were observed for these treatments, as evaluated by germination and vigor tests.

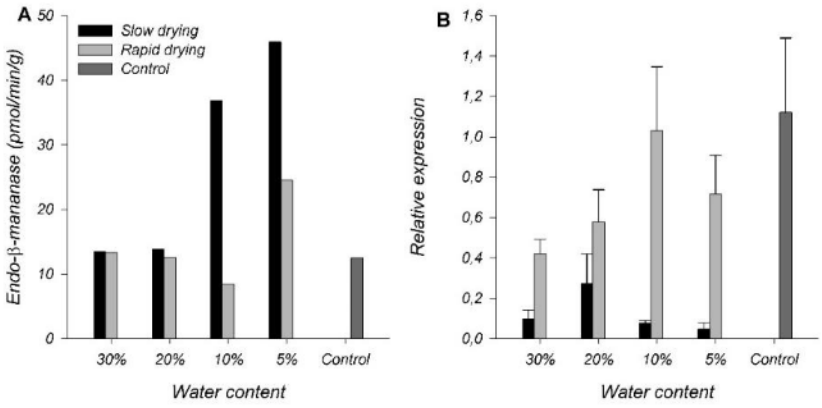

Figure 3. Endo-ß-mannanase enzyme activity (A) and relative expression profile of *MAN A* (B) in *C. arabica* seeds under rapid drying or slow drying with different water content values and in seeds that were not dried (control).

The results of the gene expression analysis obtained by the real-time PCR technique did not follow the same pattern as that found in the analysis of endo-β-mannanase activity, as observed for the expression of the *CAT*, *SOD*, and *ICL* genes.

Greater *MAN A* gene expression was found in seeds under rapid drying, whereas greater activity of the MAN A enzyme was found in seeds under slow drying (Figure 3B). Initially, this observation may appear

inconsistent, but when enzyme activity is evaluated, the response generated represents the activity of all the functional endo-ß-mannanase isoforms determined at that time. Nevertheless, in the evaluation of transcript expression, only one isoform was evaluated: *MAN A*. As is known, the presence of the transcript does not mean that the respective polypeptide product is being translated in the same proportion, and even if it is being translated proportionally, the enzymes may or may not be active and functional. Furthermore, as reported in the theoretical literature, there are various isoforms of endo-β-mannanase in coffee that have distinct functions. From the results found, it can be inferred that these isoforms respond differently to desiccation at different levels.

The relative expression levels of the *1 CYS PRX* gene are shown in Figure 4. Greater expression of this gene was observed in seeds under slow drying to a 20% water content than in seeds under rapid drying. For seeds dried to 30, 10, and 5% water contents, greater expression of this gene was observed for rapid drying than for slow drying. Lower expression of the gene was found in undried seeds (control) than in seeds that were dried slowly to a 20% water content.

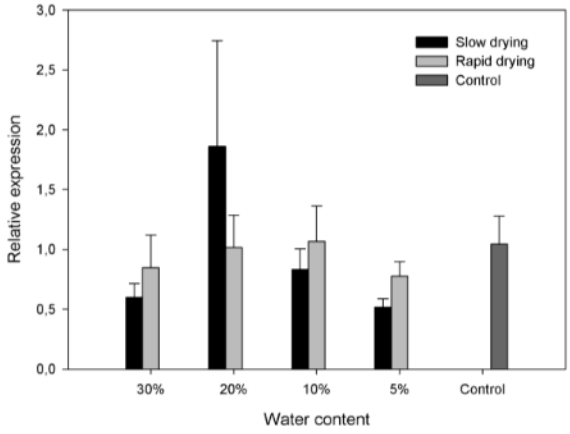

Figure 4. Profile of the relative expression of *1 CYS PRX* in *C. arabica* seeds under rapid drying and slow drying with different water content values and in seeds that were not dried (control).

Discussion

In the present study, drying speed did not affect coffee seed germination. The most important factor among those studied was the final water content attained after drying. Veiga et al. (2007) likewise did not observe significant differences in the germination of *Coffea arabica* seeds under slow drying versus rapid drying. In contrast, José, Silva, Davide, Melo, and Toorop (2011) studied the best manner of drying seeds of *Magnolia ovata* (a species with intermediate seeds, similar to coffee seeds) and observed that germination was more negatively affected by rapid drying than by slow drying for the same final water content.

In the present study, drying seeds to a water content below 10%, which is considered low for species with recalcitrant characteristics such as coffee, is extremely harmful to the physiological quality of the seeds. The germination percent of the seeds with 5% water content was nearly zero. The critical moisture level that is considered lethal for coffee seeds is between 4 and 5% (Ellis et al., 1991; Hong & Ellis, 1992). According to Coelho et al. (2015; 2017); drying to this level is generally harmful to coffee seeds, regardless of the speed at which they are dried, and vigor is reduced with the loss of water.

Tolerance to desiccation in coffee seeds varies among the seed tissues, and the embryonic axis is generally more tolerant than the whole seed. This is probably due to the more rapid drying of the axis during development and to the lower proportion of water bound up in its structure (Marcos Filho, 2015). In light of this result, it can be inferred that the main location of damage as a consequence of drying is the endosperm. Similar results were found by Coelho et al. (2015; 2017) upon comparing the results of the tetrazolium test in coffee embryos with the results of the formation of normal seedlings. The better physiological performance of the embryos compared to the whole seeds also corroborates the results of Dussert et al. (2006), who showed that the endosperms are more sensitive to drying and to immersion in liquid nitrogen than are zygotic embryos.

An efficient drying process not only reduces the water content of the seed but also increases its post-harvest conservation and preserves its physical characteristics and technological properties, resulting in

commercial value (Rosa et al., 2005). To achieve satisfactory results, it is necessary to invest in studies that aim at the maximum quality control of seeds and are monitored by germination and vigor analyses to ensure their high performance.

The results show that drying increases catalase enzyme activity. Catalase expression is lower in seeds with higher moisture levels. These results corroborate those obtained by Santos, Von Pinho, and Rosa (2013), in which seeds that were not dried (control) exhibited lower activity of this enzyme. Similar results were observed by Veiga et al. (2010) and Coelho et al. (2015).

Brandão Junior, Vieira, and Hilhost (2002) found catalase enzyme activity only in seeds that were dried, and greater activity was observed in seeds in the more advanced stage of development and with better physiological potential. To evaluate the protein profiles and physiological performance of coffee seeds under different methods of processing and drying, Taveira, Rosa, Borém, Giomo, and Saath (2012) evaluated the activity of various enzymes related to oxidative stress. Greater activity of the catalase enzyme was found in the seeds that had worse physiological performance, such as those observed in this study. This greater activity can be explained by the need for greater activation of these enzymes as seed deterioration evolved, especially at water contents below 10%.

There are enzymes in which a decline in activity leads to greater vulnerability of the seeds or of their specific parts to the attack of free radicals and peroxidation. Lower enzyme activity may also mean greater difficulty in mobilizing reserves (amylases, proteases, lipases). For other enzymes, such as "scavengers", greater activity indicates greater intensity of the removal of toxic products formed as deterioration progresses; that is, these enzymes act more intensely in more deteriorated seeds (Len, Koh, & Tan, 2019). It is important to highlight that the efficiency of repair mechanisms depends on the efficient action of enzymes (Marcos Filho, 2015).

Under drying stress, seeds react by increasing the expression of some genes and silencing or attenuating the expression of others. In the present study, greater expression of the *CAT3* gene was observed in seeds under rapid drying than in seeds under slow drying, and the greatest expression was in seeds dried rapidly to a 30% water content (Figure 2A). The *CAT3* gene is present in seeds and young plants; its expression is related to removal of the H_2O_2 produced during the degradation of fatty acids in the glyoxysome (Len et al., 2019).

Apparently, different drying rates determine different metabolic processes, such that some events generally occur more intensely in seeds with a relatively higher water content (Marcos Filho, 2015). From this result, it can be inferred that seeds with higher moisture provide stronger signals of the stress imposed on them, in this case drying. As drying advances and seed water content decreases, the stress imposed seems to be reduced, which results in lower expression of the *CAT3* gene. A comparison of catalase enzyme activity and expression of the *CAT3* gene showed that the activity of this isoenzyme is higher in seeds with a lower water content, that is, in seeds that remained for a longer time in the drying process and in which there was the need for translation of this antioxidant enzyme, probably for the elimination of reactive oxygen species (ROSs).

Santos et al. (2013) analysed the expression of the *CAT3* gene in coffee seeds with different water content values that were dried in rapid and slow manners and observed this same pattern of gene expression, with greater expression in seeds with a higher water content and a significant difference between the two drying speeds tested. The authors also observed greater gene expression in seeds under rapid drying than in those under slow drying.

The superoxide dismutase enzyme is among the most important enzymes in the defence system, which is a connected series of events necessary for the complete elimination of free radicals or reactive oxygen species (Len et al., 2019). Greater expression of this enzyme in early or late phases of the seed maturation process is indicative of its defence activity in the reduction of superoxide and subsequent reduction in free radical formation, whether alongside the deterioration process or through seed immaturity (Len et al., 2019). According to Wang, Zhang, Deng, Yuan, and Shen (2017), the greatest difference among the SOD isoforms is in the regulation sequences in the genes that encode these proteins, and each protein responds in a different way to oxidative stress at different levels.

Coelho et al. (2015) studied two drying methods, rapid and slow, and storage of *C. arabica* seeds at subzero temperatures. They observed greater expression of the superoxide dismutase enzyme in seeds dried slowly than in those dried rapidly. Baliza et al. (2012) observed lower expression of this enzyme in coffee seeds in the sugarcane green stage, and this lower expression was associated with lower physiological quality. These contrasting results can be explained by the fact that high or low expression of this enzyme is linked to the deterioration process, and a combined biochemical and physiological analysis is necessary.

The considerable increase in SOD transcripts under stress conditions generally corresponds to a much more moderate increase in enzyme activity (Wang et al., 2017). Based on the results obtained in the present study, a direct relationship between gene expression and enzyme activity was not found. These observations may indicate that the action of probable post-transcriptional, translational, and post-translational mechanisms may be promoted during the drying process (Nelson & Cox, 2013).

In the present study, a greater isocitrate lyase enzyme activity was observed (Figure 2) in rapidly dried seeds. This enzyme is related to the germination process and acts in the glyoxylate cycle (Taiz & Zeiger, 2004). In that cycle, the insoluble lipids of the seeds are transformed into soluble sugars (sucrose), which are easily moved to the radicle and apical meristems (Gonçalves, Mielniczki-Pereira, Borges, & Valduga, 2016). The activity of this enzyme increases during germination, reaching maximum values when the highest rate of lipid degradation and sucrose synthesis occurs (Bewley, Bradford, Hilhorst, & Nonogaki, 2013).

According to Selmar, Bytof, Knopp, and Breitenstein (2006), the gene that encodes the isocitrate lyase enzyme has higher levels of expression in dehydrated coffee seeds than in moist seeds. Gaspari-Pezzopane, Bonturi, Filho, Favarin, and Maluf (2012) evaluated the expression of different genes associated with coffee fruit development and maturation processes. Four of the 28 genes evaluated had a stable expression profile in all cultivars and crop seasons and, therefore, were validated as candidate genes for phenological markers of coffee fruit. Among them, the *ICL* gene had substantially greater expression in cherry fruit than in other stages, therefore indicating that the high expression of this gene may be linked to the high quality of the seeds. In addition, these analyses suggest that this gene has the potential to become a molecular marker in the final phases of coffee fruit maturation. However, its expression does not show a clear correlation with tolerance/sensitivity of coffee seeds to desiccation.

In addition to participating in the germination process, some studies have related endo-ß-mannanase activity to the coffee seed deterioration process, since there is an increase in the activity of this enzyme throughout storage, when seeds lose physiological quality (Veiga et al., 2007; Ferreira et al., 2018). Thus, the loss of coffee seed quality, among other factors, may be related to the increase in the activity of this enzyme. The endo-β-mannanase enzyme has the ability to hydrolyse internal bonds of mannan polymers, causing degradation of the cell walls in the region surrounding the embryo and facilitating radicle emergence, but this enzyme may also be related to the deterioration process.

There are two steps that characterize the process of weakening the structures of the endosperm that surround the coffee embryo: the first consists of an increase in the activity of the cellulase enzyme, and the second consists of an increase in endo-ß-mannanase activity (Schroder, Atkinso, & Redgwell, 2009). The activity of this enzyme is regulated by the presence of phytohormones, such as abscisic acid, which acts to inhibit the action of enzymes, and gibberellin, which induces the degradation of the endosperm and, consequently, promotes germination (Bewley et al., 2013; Silva et al., 2004).

In this study, the seeds were not soaked for the determination of endo-β-mannanase activity; thus, it can be inferred that the activity observed is related to the deterioration process, that is, to disordered degradation of the cell walls. For the activity of this enzyme to be associated with the germination of seeds with better physiological performance, the extraction of the enzyme and detection of its activity should be performed after the emergence of the radicle begins, that is, in germinated seeds. The activity of endo-β-mannanase in coffee seeds increases only 10 days after soaking, and it is associated with softening of the endosperm cap and with the provision of carbohydrates for embryo growth during the germination process (Silva et al., 2004).

Peroxiredoxins are antioxidant enzymes that are part of the complex protection mechanism involved in the repair of damage caused by free radicals during desiccation and the beginning of imbibition, and they are also involved in maintenance and protection during dormancy (Mowla, Thomson, Farrant, & Mundree, 2002). The *1 CYS PRX* gene is solely expressed in seeds and only in the parts tolerant to desiccation (Aalen, 1999). Developing seeds show an increase in the level of *1 CYS PRX* expression, and this level is maintained in mature seeds during storage (Mowla et al., 2002). To date, there have been no records of studies on the expression of the gene that encodes the PRX enzyme in coffee and its relation to seeds with different water content values.

In the present study, greater expression of the *1 CYS PRX* gene was observed in seeds under slow drying to a 20% water content. For seeds dried to 30, 10, and 5% water contents, greater expression of this gene was observed during rapid drying. Compared to seeds dried in a slow manner to a 20% water content, seeds that were not dried (control) showed lower expression of this gene. Soeda et al. (2005) observed lower levels of RNA for this gene in seeds of *Brassica oleracea* after osmoconditioning when the seeds were dried rapidly and higher levels when the seeds were dried slowly, which can also be observed in the current study.

Conclusion

The physiological quality of coffee seeds is more related to water content than to the drying speed of the seeds. Greater activity of the catalase and endo-β-mannanase enzymes is associated with lower physiological quality in coffee seeds. Greater expression of the isoenzyme ICL is related to greater physiological quality in coffee seeds. There is higher expression of the *CAT3* and *MAN A* genes in rapidly dried seeds. The *ICL* gene is more highly expressed in dried seeds than in undried seeds. Greater expression of the *1 CYS PRX* and *SOD* genes is associated with high physiological quality in coffee seeds.

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References

Aalen, R. B. (1999) Peroxiredoxin antioxidants in seed physiology. *Seed Science Research*, *9*(4), 285-295. DOI: https://doi.org/10.1017/S096025859900029X

Alfenas, A. C. (2006) *Eletroforese e marcadores bioquímicos em plantas e microorganismos*. Viçosa, MG: UFV.

Baliza, D. P., Caixeta, F., Pinho, E. V. R. V., Cunha, R. L., Martins, D. C., & Rosa, S. D. V. F. (2012). Physiological quality of coffee seeds produced under different levels of solar radiation and maturation stages. *Journal of Seed Science*, *34*(3), 416-423. DOI: http://dx.doi.org/10.1590/S0101-31222012000300008

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). *Seeds: Physiology of development, germination and dormancy* (3rd ed.). New York, NY: Springer.

Brandão Júnior, D. S., Vieira, M. G. G. C., & Hilhost, H. W. M. (2002). Aquisição da tolerância à dessecação nos diferentes estádios de desenvolvimento de sementes de cafeeiro (*Coffea arabica* L.). *Ciência e Agrotecnologia*, *26*(4), 673-681. DOI: https://doi.org/10.1590/S0101-31222002000100004

Brasil (2009). *Regras para análise de sementes*. Brasília, DF: MAPA/ACS.

Clemente, A. C. S., Carvalho, M. L. M., Guimarães, R. M., & Zeviani, W. M. (2011). Preparo das sementes de café para avaliação da viabilidade pelo teste de tetrazólio. *Journal of Seed Science*, *33*(1), 38-44. DOI: https://doi.org/10.1590/S0101-31222011000100004

Coelho, S. V. B., Rosa, S. D. V. F., & Fernandes, J. S. (2017). Cryopreservation of coffee seeds: A simplified method. *Seed Science and Technology*, *45*(3), 1-12. DOI: https://doi.org/10.15258/sst.2017.45.3.15

Coelho, S. V. B., Figueiredo, M. A., Clemente, A. C. S., Coelho, L. F. S., & Rosa, S. D. V. F. (2015). Alterações fisiológicas e bioquímicas em sementes de café secas em sílica gel e soluções salinas saturadas. *Pesquisa Agropecuária Brasileira*, *50*(6), 483-491. DOI: https://doi.org/10.1590/S0100-204X2015000600007

Cromarty, A. S., Ellis, R. H., & Roberts, E. H. (1985). *The design of seed storage facilities for genetic conservation*. Rome, IT: International Board of Plant Genetic Resources.

Davis, A. P., Govaerts, R., Bridson, D. M., & Stoffelen, P. (2006). An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae). *Botanical Journal of the Linnean Society*, *152*(4), 465-512. DOI: https://doi.org/10.1111/j.1095-8339.2006.00584.x

Downie, B., Hilhorst, H. W. M., & Bewley, J. D. (1994). A new assay for quantifying endo-β-mannanase activity using Congo Red dye. *Phytochemistry*, *36*(4), 829-835. DOI: https://doi.org/10.1016/S0031-9422(00)90446-1

Dussert, S., Couturon, E., Engelmann, F., & Joët, T. (2012). Biologie de la conservation des semences de caféiers : aspects fondamentaux et conséquences pratiques. *Cahiers Agricultures*, *21*(2), 106-114. DOI: https://doi.org/10.1684/agr.2012.0552

Dussert, S., Davey, M. W., Laffargue, A., Doulbeau, S., Swennen, R., & Etienne, H. (2006). Oxidative stress, phospholipid loss and lipid hydrolysis during drying and storage of intermediate seeds. *Physiologia Plantarum*, *127*(2), 192-204. DOI: https://doi.org/10.1111/j.1399-3054.2006.00666.x

Ellis, R. H., Ho, G. T. D., & Roberts, E. H. (1991). An intermediate category of seed storage behaviour? II. Effects of provenance, immaturity, and imbibition on desiccation-tolerance in coffee. *Journal of Experimental Botany*, *42*(5), 1167-1174. DOI: https://doi.org/10.1093/jxb/42.5.653

Ferreira, V. F., Ricaldoni, M. A., Rosa, S. D. V. F., Figueiredo, M. A., Coelho, S. V. B., & Fantazzini, T. B. (2018). Endo-β-mannanase enzyme activity in the structures of *Coffea arabica* L. seeds under different types of processing and drying. *Ciência Rural*, *48*(12), 1-7. DOI: http://dx.doi.org/10.1590/0103-8478cr20170839

Ferreira, D. F. (2014). Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. *Ciência e Agrotecnologia*, *38*(2), 109-112. DOI: http://dx.doi.org/10.1590/S1413-70542014000200001

Gaspari-Pezzopane, C., Bonturi, N., Filho, O. G., Favarin, J. L., & Maluf, M. P. (2012). Gene expression profile during coffee fruit development and identification of candidate markers for phenological stages. *Pesquisa Agropecuária Brasileira*, *47*(7), 972-982. DOI: https://doi.org/10.1590/S0100-204X2012000700014

Gonçalves, I. L., Mielniczki-Pereira, A. A., Borges, A. C. P., & Valduga, A. T. (2016). Metabolic modeling and comparative biochemistry in glyoxylate cycle. *Acta Scientiarum. Biological Sciences*, *38*(1), 1-6. DOI: https://doi.org/10.4025/actascibiolsci.v38i1.24597

Hong, T. D. & Ellis, R. H. (1992). Optimum air dry seed storage environments for arabica coffee. *Seed Science and Technology*, *20*, 547-560.

José, A. C., Silva, E. A., Davide, A. C., Melo, A. J. S., & Toorop, P. E. (2011.) Effects of drying rate and storage time on *Magnolia ovate* Spreng. seed viability. *Seed Science and Technology, 39*(2), 425-434. DOI: https://doi.org/10.15258/sst.2011.39.2.14

Len, J. S., Koh, W. S. D., & Tan, S. X. (2019). The roles of reactive oxygen species and antioxidants in cryopreservation. *Bioscience Reports*, *39*(8), 1-25. DOI: https://doi.org/10.1042/BSR20191601

Marcos Filho, J. (2015). Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola*, *72*(4), 363-374. DOI: https://doi.org/10.1590/0103-9016-2015-0007

Mowla, S. B., Thomson, J. A., Farrant, J. M., & Mundree, S. G. (2002). A novel stress-inducible antioxidant enzyme identified from the resurrection plant *Xerophyta viscosa* Baker. *Planta*, *215*(5), 716-726. DOI: https://doi.org/10.1007/s00425-002-0819-0

Nelson, D. L. & Cox, M. M. (2013). *Lehninger principles of biochemistry* (6th ed.). New York: W.H. Freeman Publishers.

Pfaffl, M. W., Tichopad, A., Prgomet, C., & Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: Bestkeeper – Excel-based tool using pair-wise correlations. *Biotechnology Letters, 26*(6), 509-515. DOI: https://doi.org/10.1023/B:BILE.0000019559.84305.47

Roberts, E. H. (1973). Predicting the storage life of seeds. *Seed Science & Technology*, *1*, 499.

Rosa, S. D. V. F., Brandão Júnior, D. S., Von Pinho, E. V. R., Veiga, A. D., & Castro e Silva, L. H. (2005). Effects of different drying rates on the physiological quality of *Coffea canephora* Pierre seeds. *Brazilian Journal of Plant Physiology*, *17*(2), 199-205. DOI: http://dx.doi.org/10.1590/S1677-04202005000200002

Santos, G. S., Von Pinho, E. V. R., & Rosa, S. D. V. F. (2013). Gene expression of coffee seed oxidation and germination processes during drying. *Genetics and Molecular Research, 12*(4), 6968-6982. DOI: https://doi.org/10.4238/2013.December.19.16

Schroder, R., Atkinson, R. G., & Redgwell, R. J. (2009). Re-interpreting the role of endo-β-mannanases as mannanendotransglycosylase/hydrolases in the plant cell wall. *Annals of Botany, 104*(2), 197-204. DOI: https://doi.org/10.1093/aob/mcp120

Selmar, D., Bytof, G., Knopp, S. E., & Breitenstein, B. (2006). Germination of coffee seeds and its significance for coffee quality. *Plant Biology*, *8*(2), 260-264. DOI: https://doi.org/10.1055/s-2006-923845

Silva, E. A. A., Toorop, P. E., Van Aelst, A. C., & Hilhorst, H. W. (2004). Abscisic acid controls embryo growth potential and endosperm cap weakening during coffee (*Coffea arabica* L., cv. Rubi) seed germination. *Planta*, *220*(2), 251-261. DOI: https://doi.org/10.1007/s00425-004-1344-0

Soeda, Y., Konings, M. C. J. M., Vorst, O., van Houwelingen, A. M. M. L., Stoopen, G. M., Maliepaard, C. A., ... van der Geest, A. H. M. (2005). Gene expression programs during *Brassica oleracea* seed maturation, osmopriming, and germination are indicators of progression of the germination process and the stress tolerance level 1. *Plant Physiology, 137*(1), 354-368. DOI: https://doi.org/10.1104/pp.104.051664

Taiz, L., & Zeiger, E. (2004). *Fisiologia vegetal*. Porto Alegre, RS: Artmed.

Taveira, J. H. S., Rosa, S. D. V. F., Borém, F. M., Giomo, G. S., & Saath, R. (2012). Perfis proteicos e desempenho fisiológico de sementes de café submetidas a diferentes métodos de processamento e secagem. *Pesquisa Agropecuária Brasileira, 47*(10), 1511-1517. DOI: http://dx.doi.org/10.1590/S0100-204X2012001000014

Veiga, A. D., Guimarães, R. M., Rosa, S. D. V. F., Von Pinho, E. V. R., Castro e Silva, L. H., & Veiga, A. D. (2007). Armazenabilidade de sementes de cafeeiro em diferentes estádios de maturação e submetidas a diferentes métodos de secagem. *Revista Brasileira de Sementes*, *29*(1), 83-91. DOI: http://dx.doi.org/10.1590/S0101-31222007000100012

Veiga, A. D., Von Pinho, É. V. R., Veiga, A. D., Pereira, P. H. A.R., Oliveira, K. C., & Von Pinho, R. G. (2010). Influência do potássio e da calagem na composição química, qualidade fisiológica e na atividade enzimática de sementes de soja. *Ciência e Agrotecnologia*, *34*(4), 953-960. DOI: 10.1590/S1413-70542010000400022.

Wang, W., Zhang, X., Deng, F., Yuan, R., & Shen, F. (2017). Genome-wide characterization and expression analyses of superoxide dismutase (*SOD*) genes in *Gossypium hirsutum*. *BMC Genomics*, *18*(376), 1-25. DOI: https://doi.org/10.1186/s12864-017-3768-5