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Accelerated aging test for coffee seeds requires higher temperature

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Abstract

The vigor tests are important tools used in the internal quality control by the seed companies. The accelerated aging test is a sensitive method for assessing the vigor of seed lots, as well as for estimating their storage potential. However, for coffee seeds there is little information about its use and efficiency.The objective of this study was to investigate methods of the accelerated aging test suitable for *Coffea arabica* L. seeds. Two studies were carried out. In the first, different temperatures (42°C, 44°C, and 46°C) and exposure times (0, 24, 48, 72, 96, 120, 144, 168, and 192 h) were investigated for the accelerated aging test. In the second, the best combinations of temperatures and exposure times were tested on coffee seeds from five different cultivars at temperatures of 44 and 46 ºC at times of 24, 48, 72, and 96 h of incubation. After each aging period, the water content of the seeds was determined, and physiological quality was evaluated by the germination test. The temperature of 42°C leads to slow deterioration of the seeds and is not recommended for evaluation of *Coffea arabica* L. seed vigor at the exposure times from 24 to 192 h tested here. The accelerated aging test, carried out at a temperature of 44ºC for 72 hours or at 46ºC for 48 hours, allows the *Coffea arabica* seed lots to be separated into different vigor levels.

Keywords: *Coffea arabica* L.; deterioration; quality control; physiological quality; vigor test.
Abbreviations: RAS - Rules for Seed Analysis, BOD - Biochemical Oxygen Demand, CV- Coeficient of variation.

Introduction

Brazil is the country with the largest coffee production and export volume in the world, and commodity coffee is very important in the Brazilian trade balance. Coffee not only has economic importance but also enormous social, dietary, and cultural relevance (Conab, 2020). Given the importance of coffee for Brazil, it is necessary to develop new technologies that can assist researchers and technicians in resolving the bottlenecks involved in coffee growing and increase its sustainability.

The *Coffea arabica* L. species is propagated by seeds that are intermediate to recalcitrant regarding desiccation tolerance and storage behavior (Ellis et al., 1990). This seed trait and the slow and uneven germination of the seeds hinder the production of seedlings with the quality desired at the ideal time of planting.

The intermediate to recalcitrant *Coffea arabica* L. seeds have limited longevity; they are not able to maintain satisfactory germination capacity for more than six months after harvest (Figueiredo et al., 2017; Pertel et al., 2003). Thus, seed storage can lead to irreversible damage to seed viability and this is one of the biggest obstacles for coffee producers and seed technicians (Coelho et al., 2018).

Seed testing must provide reliable information on seed physiological potential so that appropriate decisions may be made during production and subsequent trade (Bittencourt et al., 2012). The standard germination test, however, does not provide sufficient results to evaluate the physiological potential of seeds arriving from the field, since the test is performed under ideal water availability, aeration, and temperature conditions (Brasil, 2009). The standard test can be complemented by vigor tests to assist decision making and develop more effective quality control (Bittencourt et al., 2012).

Accelerated aging is a vigor test that provides reliable information regarding seed physiological quality. In this test, seeds are placed under high temperature and relative humidity conditions to estimate the relative storage potential of different seed lots (Kavan et al., 2019). According to Matera and Suzukawa (2019), this test can predict the level of deterioration and storage potential of the seed, and may correlate these features with field emergence. The test may also reveal physiological differences in seeds from different seed lots with similar germination.

The accelerated aging test may be used to evaluate the vigor of diverse plant species, and many seed production companies have included the test in their quality control programs (Rocha et al., 2018).

Few references appear in the scientific literature on using the accelerated aging test to evaluate the physiological conditions of *Coffea arabica* L. seed lots. Pertel et al. (2003) and Fantazzini et al. (2018) worked with the accelerated aging test at the temperature of 42°C and different exposure times for coffee seeds. They observed that seed deterioration occurred over long periods of exposure, indicating that other ambient conditions within the aging chamber should be investigated. For the coffee seed germination test, the Brazilian seed analysis guidelines, RAS (Brasil, 2009), prescribe a temperature of 30°C, a higher temperature than for most other crops, which may indicate that a higher temperature can be used in the accelerated aging test for this species.

Thus, the aim of this study was to investigate methods of the accelerated aging test suitable for *Coffea arabica* L. seeds.

Results and discussion

Study 1. Methodologies of the accelerated aging test

The two coffee seed lots tested in this study exhibited the same initial germination, without any statistical difference ($p < 0.05$), and different vigor levels as assessed by the different tests (Table 1).

The initial water content of the coffee seeds of Lot 1 was 13.29% and of Lot 2 was 12.70% (Table 2), a difference of less than two percentage points between the lots. This is an important requirement for performing vigor tests, because to obtain consistent results, the test must be set up with samples whose water content does not vary by more than 2.0 percentage points. Water absorption by seeds is a factor that can affect the results of the accelerated aging test (Silva et al., 2010). According to Marcos Filho (2015b), wetter seeds are more sensitive to test conditions and are therefore subject to more intense deterioration.

There was a gradual increase in seed water content as time increased in the accelerated aging test, regardless of the incubation temperature. According to Marcos Filho (2015b), increases in water content favor an increase in seed temperature as a result of respiratory processes and greater microorganism activity. An increase in time of exposure to the adverse conditions provided by the accelerated aging test leads to a greater increase in the water content of the exposed seeds.

There was interaction between temperature and exposure time for both lots in the accelerated aging test (Table 3 and Figure 1), with reduced germination results. In addition, the deterioration rate increased with the increase in exposure time to test conditions within each temperature tested. These results were also observed by Pertel et al. (2003) and other authors (Fantazzini et al., 2018).

At the temperature of 42°C, significant reductions in germination percentages from the accelerated aging test occurred only beginning at 144 h incubation for Seed Lot 1, and at 120 h for Seed Lot 2 (Table 3). For the temperature of 44°C, reduction occurred beginning at 72 h for both lots. At the temperature of 46°C, a reduction in percentage from accelerated aging occurred beginning at 48 h for both lots. The increase in temperature and in exposure time led to a drastic reduction in percentage of accelerated aging. In addition, beginning at 96 h at 44ºC and at 48 h at 46ºC, there was significant difference between accelerated aging and seed germination, both for Lot 1 and for Lot 2 (Table 3, letters x and y).

According to Marcos Filho (2015b), temperature, seed exposure time, seed water content, or the genotype affect physiological deterioration of the seeds during the accelerated aging test. The test is considered a method that simulates stress conditions, and these conditions generate high respiration and consumption of reserves, which leads to degenerative changes in seed metabolism (Moraes et al., 2016) leading to their deterioration.

For Lot 2 at the different temperatures tested, there was an increase in the accelerated aging germination percentage in the seeds subjected to the 24-h exposure period compared to the control treatment (0 h) (Table 3). This may be related to the pre-conditioning provided by seed exposure to a high moisture condition in a 24-h period, much like the effects of priming on vegetable seeds (Ferreira et al., 2020).

Pertel (2003) observed that the deterioration in *Coffea arabica* L. seeds by natural aging (storage) at 6 months in ambient temperature and 12 months in cold storage (10°C) was more drastic than that by artificial aging using the temperature of 42°C and 120 h of exposure. These results corroborate the present study, in which seeds under accelerated aging at 42°C and 120 h of exposure exhibited a high percentage of germination, very similar to the control treatment, in both seed lots tested (Table 3).

Fantazzini et al. (2018) studied the response of *Coffea arabica* seeds under natural aging and concluded that the artificial aging time from 6 to 10 days under a temperature of 42°C and 100% relative humidity seems to be too long. They recommended that other ambient conditions within the accelerated aging environment should be investigated, with the aim of reducing the time of the test for simulating deterioration during natural storage of coffee seeds. A vigor test should be fast, economical, simple, and useful (Marcos Filho, 2015a). Therefore, the accelerated aging test using a temperature of 42°C did not meet these basic requirements, because deterioration occurred in a slow manner, showing low capacity for placing seeds under stress conditions.

Temperatures of 44°C and 46°C exposed seeds to stress in a more accentuated and rapid manner. For the temperature of 44°C, reduction in accelerated aging germination percentage occurred in a more gradual manner, exhibiting four levels of deterioration for Lot 1 and 2 (Table 3; Figure 1). For Lot 1, the group with more deteriorated seeds occurred at 144 h of exposure on, whereas for Lot 2, the group with greater deterioration occurred only at 192 h of exposure (Figure 1).

In relation to the temperature of 46°C, reduction in accelerated aging germination percentage occurred in a more drastic manner than at the other temperatures evaluated (Figure 1). There were 4 levels of deterioration for Lot 1, and 7 levels for Lot 2. For Lot 1, the group of more deteriorated seeds was formed beginning at 120 h of exposure, and for Lot 2, the group with greater deterioration arose beginning at 144 h of exposure (Table 3). The increase in time of exposure to the test conditions resulted in an increase in water content in the seeds. This condition plus the higher temperature of 46°C imposed by the aging test resulted in a more accentuated accelerated deterioration

process in these seeds than in the seeds placed under lower temperatures.

After the period of 144 h of exposure to the temperature of 46°C, the germination percentage of seeds under accelerated aging was drastically reduced for both lots, with rates lower than 10%, showing that increased exposure time from this period on no longer differentiated levels of deterioration (Table 3; Figure 1). Silva et al. (2010) found that increase in temperature had more drastic effects on seed deterioration than increase in exposure time. This was confirmed in the present study at the temperature of 46°C. Prolonging exposure in the accelerated aging test tends to favor the activity of pathogens in the seed deterioration process, which interferes in the results (Mahjabin and Abidi, 2015). Pathogen activity is likely one of the reasons for drastic reduction in germination after long times of exposure to adverse conditions.

Lots 1 and 2 responded in different ways in their ability to bear the stress imposed by the conditions of the accelerated aging test at the different temperatures tested, but the temperatures of 44°C and 46°C proved to be more effective for evaluating deterioration. At those temperatures, the seed lots showed significant differences in relation to degree of deterioration, allowing 4 to 7 levels of vigor to be distinguished throughout the exposure periods in BOD.

Study 2. Accelerated aging test in different seed lots of Coffea arabica L.

According to the initial physiological quality, assessed by the germination test, the seed lots of the cultivars tested showed statistically equal germination percentages, corresponding to 87% (‘Catuaí Amarelo 2 SL’), 82% (‘Catucaiam’), 88% (‘Asa Branca’), 83% (‘Acauã’), and 86% (‘Guará’).

Differences for initial seed water content were less than two percentage points among the cultivars (Table 4), which is desirable for performing vigor tests.

By the seed water content in the periods of exposure to the accelerated aging test at 44°C and 46°C (Table 4), differences were not greater than 2%, which is desirable according to Marcos Filho (2015b), confirming the uniformity of conditions in carrying out the test within each exposure time tested.

The results of the accelerated aging test showed significant interaction between temperature and exposure time (Table 5). Within each temperature tested, the germination percentage in seeds from accelerated aging tended to decrease with an increase in the period of exposure to the test. This was also observed by Kavan et al. (2019) where advance in the deterioration process is mainly determined by interaction among genetic inheritance, seed water content, and temperature.

The results of the accelerated aging test of the cultivars at a temperature of 44°C and exposure for 24 and 48 h showed significant reductions in germination in relation to their statistically equal germination percentages before the test (Table 5). However, differences were not significant among the cultivars, as verified by Student's *t*-test. At 72 h of exposure at this same temperature, the germination percentages of the artificially aged seeds showed significant differences in reduction and were grouped into two vigor levels. There was an amplitude of 32 percentage points between the cultivar with the greatest reduction, Catucaí, with 34%, and the cultivar with the least deterioration, Asa Branca, with 66% germination (Table 5).

At 96 h, the effects of the temperature of 44°C were more drastic, clustering the seeds of cultivars into three vigor levels, with the seeds of Guará, Catucaí, and Catucaiam showing less vigor and Asa Branca showing the most vigor. In this combination of 44°C and a period of 96 h there was amplitude of 50% between the highest (Asa Branca), and the lowest level of deterioration (Guará) (Table 5).

Regarding the temperature of 46°C, the 24-h period also had a slight effect on deterioration; however, at 48 h, it is already possible to separate the seed lots into two groups, with an amplitude of 23 percentage points between the cultivar with the highest level of deterioration (57%, Acauã) and the one with the lowest level (80%, Guará) (Table 5).

At 72 and 96 h, deterioration was drastic and the seeds clustered into three distinct levels of physiological performance, with a range of 31 percentage points at 72 h and 26 percentage points at 96 h between the highest and the lowest percentages (Table 5). However, in the combination of 96 h and 46°C, four of the five cultivars had accelerated aging percentages below 20%, implying that the test had a drastic effect in this arrangement and is thus unsuitable for vigor evaluation. The combination of 46°C and a 96-h exposure period led to a result similar to that of 72 h, with most of the seed lots near total inviability (Table 5).

When the temperature of 46°C was used in the test, after 48 h, the seeds of all cultivars tested showed a significant reduction in accelerated aging, whereas at 44°C, this reduction only occurred after 72 h of incubation (Table 5).

Comparing the results of the accelerated aging test at both 44°C and 46°C with that of seed germination before the tests, some cultivars showed statistically equal performance by Student's *t*-test, even at the highest temperature, especially the seeds of Catucaiam and Guara, with germination percentages of 75% and 80% after 48 h at 46°C (Table 5), indicating greater resistance of these cultivars to the deterioration process.

Up to 48 h, there are lower speeds of quality reduction, with this drop being more pronounced at 46°C for the cultivars tested (Figure 2). After 48 h, there is a tendency towards faster aging, mainly at a temperature of 46°C. At a temperature of 44°C, all cultivars showing a pronounced drop already at 72 h. At a temperature of 44°C and 72 h, the cultivars separate two vigor levels, with Catucaí showing the lowest percentage of germination after accelerated aging; at 96 h, the cultivars separated into three levels. At a temperature of 46°C there is a separation of cultivars into three distinct vigor levels; at 96 h of exposure at this temperature, there is a sharp drop in seed quality and the same cultivar separation, according to physiological performance.

The accelerated aging test can indicate the storage potential of *Coffea arabica* seed lots, as described by Marcos Filho (2015a), and it can be used as a complement to official tests for the purpose of assisting decision making for more effective quality control, as reported by Bittencourt et al. (2012).

A vigor test should be fast, economical, simple, and useful (Marcos Filho, 2015a). Thus, according to this study for vigor estimation, the combination of parameters recommended for the accelerated aging test would be the temperature of

Table 1. Characterization of the initial quality of seed lots of *Coffea arabica* L., Catuaí Amarelo IAC62 by the following tests: tetrazolium (TZ), germination (G), root protrusion (RP), strong normal seedlings (SNS), seedlings with expanded cotyledonary leaves (ECL), shoot dry matter (SDM), root dry matter (RDM), seedling emergence (E), emergence speed index (ESI), and electrical conductivity (EC).

Lot	TZ (%)	G (%)	RP (%)	SNS (%)	ECL (%)	SDM (g)	RDM (g)	E (%)	ESI	EC
1	98 a	94 a	98 a	26 a	94 a	2.63 a	0.59 a	90 a	25.6 b	10.8 a
2	98 a	88 a	99 a	27 a	89 b	2.52 b	0.55 a	80 b	30.8 a	9.8 a
CV (%)	1.16	4.99	1.95	7.46	5.42	4.99	4.85	11.10	18.64	16.61

*Means followed by the same lowercase letter in the column do not differ from each other by Student's *t*-test at 5%.

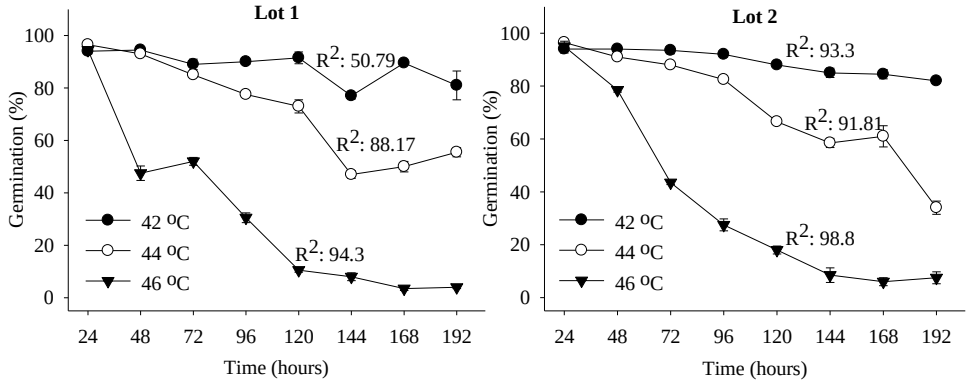

Figure 1. Accelerated aging of seeds of *Coffea arabica* L., Catuaí Amarelo IAC6, from Lot 1 and Lot 2 using different temperatures and time periods of incubation.

Table 2. Water content (%) of seeds from seed lots of *Coffea arabica* L., Catuaí Amarelo IAC62, after accelerated aging using different temperatures and time periods of BOD incubation.

Time (h)	Temperature					
	42°C		44°C		46°C	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
0	13.29	12.70	13.29	12.70	13.29	12.70
24	25.06	26.15	25.23	24.04	26.01	25.27
48	28.14	25.99	24.83	30.19	32.10	29.82
72	34.07	33.88	34.65	34.43	33.86	34.18
96	34.63	27.47	35.98	35.79	35.86	36.39
120	34.67	37.89	37.98	36.39	34.93	41.70
144	33.71	36.42	40.44	39.58	33.08	36.44
168	37.26	38.46	38.38	40.11	38.77	38.51
192	39.43	38.29	38.70	40.28	37.62	38.61

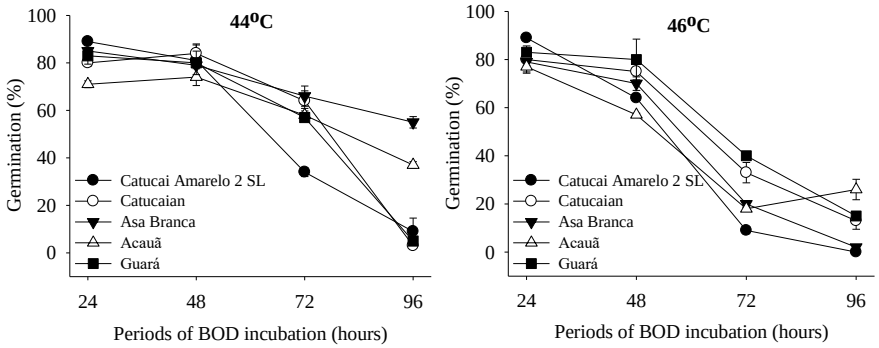

Figure 2. Germination percentages of seeds subjected to accelerated aging from five *Coffea arabica* L. cultivars using the temperatures of 44°C and 46°C and different periods of incubation in BOD.

Table 3. Percentages of accelerated aging of seeds of *Coffea arabica* L., Catuaí Amarelo IAC62, from Lot 1 and Lot 2 using different temperatures and periods of time in the BOD.

Seed Lot 1			
Time (h)	Temperature		
	42°C	44°C	46°C
24	x 94 a A	x 97 a A	x 95 a A
48	x 95 a A	x 93 a A	y 48 b B
72	x 89 a A	x 85 b A	y 52 b B
96	x 90 a A	y 78 c B	y 31 c C
120	x 92 a A	y 73 c B	y 11 d C
144	y 77 b A	y 47 e B	y 8 d C
168	x 90 a A	y 50 e B	y 4 d C
192	x 81 b A	y 56 d B	y 4 d C
Germination of lot 1 (without accelerated aging)	94 x		
Seed Lot 2			
24	x 94 a A	x 97 a A	x 95 a A
48	x 94 a A	x 91 a A	y 79 b B
72	x 94 a A	x 88 b A	y 44 c B
96	x 92 a A	y 83 b B	y 28 d C
120	x 88 b A	y 67 c B	y 18 e C
144	y 85 b A	y 59 d B	y 9 f C
168	y 85 b A	y 61 d B	y 6 f C
192	y 82 b A	y 34 e B	y 8 f C
Germination of lot 2 (without accelerated aging)	88 x		

*Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by the Scott-Knott test at 5%. Means following the letter x do not differ from the Lot 1 or Lot 2 by Student's *t*-test at 5%.

Table 4. Initial water content and water content after accelerated aging in seed lots of five cultivars of *Coffea arabica* L. at the temperatures of 44°C and 46°C and periods of 24, 48, 72, and 96 h of incubation

Lot	Cultivar		44°C				46°C			
		0 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	Catucaí Amarelo	13.88	25.06	33.89	36.89	41.06	24.96	35.63	37.51	41.76
2	Catucaiam	13.91	25.70	32.89	36.60	38.99	25.17	35.14	38.29	40.97
3	Asa Branca	13.97	25.23	34.88	36.10	39.79	24.98	35.52	37.86	42.37
4	Acauã	14.15	26.15	33.91	35.60	38.71	24.60	35.21	37.96	40.16
5	Guará	13.69	25.27	33.80	35.47	39.26	26.07	32.46	37.17	39.27
Differences (%)		0.73	2.11	1.99	1.42	2.07	1.81	3.17	1.12	2.47

Table 5. Accelerated aging of seed lots of five cultivars of *Coffea arabica* L. using the temperatures of 44°C and 46°C and exposure times of 24, 48, 72, and 96 h.

Lot	Cultivar	0 h	44°C				46°C			
			24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	Catucaí Am.2 SL	x 87	x 89 Aa	y 81 Aa	y 34 Bb	y 9 Cc	x 89 Aa	y 64 Bb	y 9 Cc	y 0 Dc
2	Catucaiam	x 82	x 80 Aa	x 84 Aa	y 64 Ba	y 3 Cc	x 80 Ab	x 75 Aa	y 33 Ba	y 13 Cb
3	Asa Branca	x 88	x 85 Aa	y 79 Aa	y 66 Ba	y 55 Ca	y 79 Ab	y 70 Ba	y 20 Cb	y 2 Dc
4	Acauã	x 83	y 71 Ab	y 74 Aa	y 58 Ba	y 37 Cb	y 77 Ab	y 57 Bb	y 18 Cb	y 26 Ca
5	Guará	x 86	x 83 Aa	x 80 Aa	y 57 Ba	y 5 Cc	x 93 Aa	x 80 Ba	y 40 Ca	y 15 Db
CV%			20.8							

*Mean values followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by the Scott-Knott test at 5%. **The letters x and y compare seeds without accelerated aging (0 h) and each percentage at each temperature and time.

46°C and a 48-h or 72-h exposure period because they are fastest, most sensitive, and most suitable for evaluation of *Coffea arabica* L. seed vigor. The results imply that the incubation temperature has a more harmful effect on germination than exposure time. Results similar to those found in the present study were obtained in other studies using the accelerated aging test:

Rocha et al. (2018) for popcorn seeds, and Aquino et al. (2018) for *Piptadenia moniliformis* (Bent) seeds.

The *Coffea arabica* cultivars Catucaí Amarelo 2SL, Catucaiam, Asa Branca, Acauã, and Guará used in this study

showed different responses in the incubation time and temperature combinations of the accelerated aging test. Even though these cultivars were produced in the same place and under the same pre- and post-harvest management practices, the intention of this study was not to evaluate the tolerance of different genetic materials, but rather to evaluate the response of coffee seeds, with the same initial germination percentage, in the accelerated aging test.

From this study, it can be inferred that the combinations of incubation temperatures and times of 44°C for 96 h and 46°C for both 48 and 72 h are sufficient to stratify seed lots with the same initial germination percentages into different vigor levels. These combinations can be used in the accelerated aging test to classify the seed lots used in this study into different vigor levels. However, additional studies should be carried out with a larger number of lots and cultivars to determine the best combinations of temperature and time for coffee seeds.

It is important to emphasize that the accelerated aging test methodology used for orthodox seeds, such as corn and soybean, establishes a temperature of 42°C and 48 h, This temperature is indicated as the upper limit for preserving proteins, which theoretically denature at temperatures higher than this. However, for coffee, the temperature of 42°C does not effectively age the seeds, as seen in the present study and also by Fantazzini et al. (2018). Moreover, the temperatures of 44°C and 46°C do not cause drastic effects, at least up to 48 h of seed exposure. It is noteworthy that the temperature recommended for germination of *Coffea arabica* L. seeds is 30°C, much higher than the temperature of 25°C recommended for most crops (Brasil, 2009). This suggests that higher temperatures can be used in the accelerated aging test for seeds of this species.

Materials and methods

Location and plant material

Fruit from the *Coffea arabica* L. species was collected from crop fields of an experimental farm of the Fundação Procafé (Coffee Technology Support Foundation) in the municipality of Varginha, MG, Brazil, in the 2018/2019 crop year.

Seed collection, selection, and processing

Fruit in the cherry maturity stage was manually harvested from the middle parts of the middle branches of the plants. The harvested fruit was selected for uniform maturity and mechanically pulped. Mucilage was then removed from the seeds by fermentation in water for 24 h at 25°C. The seeds were placed on screens in a single layer in the shade to remove surface water and then dried in a fixed-bed dryer at 26°C until reaching 12% moisture. After drying, the parchment was carefully removed from the seeds with the aid of a tweezers.

The experiment was divided into two studies. In the first, three different temperatures and nine different exposure times were used in the accelerated aging test on seeds from two seed lots of *Coffea arabica* L., Catuaí Amarelo IAC/62. The two seed lots were studied separately. Only two seed lots were used in this first study, in view of the large number of treatments resulting from the combination of multiple times and temperatures. In the second study, the best temperature and time combinations found in the first study were tested on five commercial coffee cultivars.

Study 1. Methodologies of the accelerated aging test

Two seed lots of *Coffea arabica* L., Catuaí Amarelo IAC/62, were used in the experiment, which were initially characterized physiologically. Three incubation temperatures – 42°C, 44°C, and 46°C – and nine exposure times – 0, 24, 48, 72, 96, 120, 144, 168, and 192 h – were investigated, with four replications. Transparent plastic germination boxes (gerboxes) with dimensions of 11 × 11 × 3 cm were used, containing 40 mL of distilled water at the bottom of the containers to ensure relative humidity near 100%. The seeds were distributed in a single layer on metallic screens in the plastic boxes for uniform exposure to the humidity within the boxes. The boxes were sealed and kept in a BOD incubating laboratory oven at the temperatures and times defined above.

***Study 2. Accelerated aging test in different lots of Coffea arabica L. seeds*.**

In the second study, tests were conducted on five coffee cultivars: Catucaí Amarelo 2 SL, Catucaiam, Asa Branca, Acauã, and Guará. The two best temperatures found in study 1 – 44°C and 46°C – and the four best exposure times – 24, 48, 72, and 96 h – were used in the accelerated aging test, with 4 replications. The gerbox method with 40 mL of distilled water at the bottom of the container was used once more. The seeds were distributed on metallic screens in a single layer in the plastic boxes. The boxes containing the seeds were sealed and kept in a BOD incubating laboratory oven at the temperatures and times defined above.

In both studies, the seeds were initially assessed to characterize the seed lots through determination of water content, dry matter, and the emergence speed index and through performance of the germination test, electrical conductivity test, tetrazolium test, and emergence test. After each incubation period and temperature tested, the seed water content was determined and the germination test was carried out.

Physiological evaluations

Water content was determined by the laboratory oven method at 105°C for 24 h, with four replications of 25 seeds. The results were expressed in percentage based on seed fresh weight or wet basis, according to the instructions of the Rules for Seed Analysis (Regras para Análise de Sementes - RAS) (Brasil, 2009).

The **germination test** was performed with four replications of 50 seeds without parchment for each treatment, sown in rolls of germination paper moistened with distilled water in the amount of 2.5 times the weight of the dry paper. The seeds were placed in a seed germinator regulated to 30°C and exposed to light. The percentage of root emergence at 15 days after sowing and the percentage of normal seedlings at 30 days after sowing were determined according to the criteria established by the RAS (Brasil, 2009). The percentage of strong normal seedlings at 30 days after sowing, counting those that had a hypocotyl hook of three centimeters or more, and the percentage of seedlings with expanded cotyledonary leaves at 45 days after sowing were also determined.

Root dry matter and shoot dry matter were determined in the normal seedlings obtained in the germination test at 45 days after sowing. Roots and shoots were separated and then dried in a forced air circulation laboratory oven at 60°C for 5 days. After that period, the amount of dry matter was determined on a precision balance of 0.001-g resolution.

The **tetrazolium test** was performed with four replications of 25 seeds from each treatment. The seeds, without parchment, were soaked for 36 h at 30°C. The embryos were extracted with the aid of a scalpel and kept in a solution of distilled water and polyvinylpyrrolidone (PVP) antioxidant from the time of their extraction from the endosperms until being placed in tetrazolium solution. After extraction, the embryos were soaked in 0.5% tetrazolium solution in dark bottles and kept at a temperature of 30°C for 3 h, as described in the RAS (Brasil, 2009), with modifications (Clemente et al, 2011). Embryo viability was analyzed with the aid of a stereoscopic magnifying glass with 10 X magnification.

The **electrical conductivity test** was performed with four replications of 50 seeds, which were weighed, placed in cups with 75 mL of deionized water, and kept at a temperature of 25°C, protected from light, in a BOD type chamber. After 24 h of soaking, electrical conductivity was read using a Digimed CD-21 conductivity meter, according to the method described by Vieira et al. (1994). The results were expressed in $\mu S\ cm^{-1}\ g^{-1}$.

For **emergence under controlled conditions,** plastic trays were used containing soil and sand in a 2:1 proportion as substrate. Four replications of 50 seeds were sown at random over a layer of 2/3 of the substrate. After sowing, the seeds were covered with the remaining 1/3 of the substrate. After these materials were set up, the substrate was irrigated until reaching 60% of field capacity. The trays were kept in a plant growth chamber at a temperature of 30°C in alternating 12-h periods of light and dark. The percentage of normal seedlings was determined at 80 days after sowing, when stabilization of seedling emergence occurred, and the emergence speed index (ESI) was determined according to the formula proposed by Maguire (1962).

Experimental design and statistical analyses

A completely randomized experimental design was used in both studies. In the first, each seed lot was analyzed separately. A 3 × 9 factorial arrangement was used, consisting of three temperatures (42, 44 and 46ºC) and nine periods of incubation in BOD (0, 24, 48, 72, 96, 120, 144, 168, 192 h), with four replications. In the second, a 2 × 4 × 5 factorial arrangement was used, consisting of two temperatures (44 and 46ºC), four periods of incubation in BOD (24, 48, 72, and 96 h), and seed lots from five cultivars of *Coffea arabica* L. (Catucaí Amarelo 2 SL, Catucaiam, Asa Branca, Acauã, and Guará), with four replications. Analysis of variance was performed on the results of the physiological tests using the SISVAR statistical program (Ferreira, 2014), and the means were compared by the Scott-Knott test at the level of 5% probability in the first study, and by a regression test in the second study.

Conclusions

The temperature of 42°C leads to slow deterioration of the seeds and is not recommended for evaluation of *Coffea arabica* L. seed vigor at the exposure times from 24 to 192 h tested here.

The accelerated aging test, carried out at a temperature of 44ºC for 72 hours or at 46ºC for 48 hours, allows the *Coffea arabica* seed lots to be separated into different vigor levels.

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