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Applicability of the tetrazolium test to coffee seeds with different types and levels of stresses

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ABSTRACT

For sale or use of coffee seeds after harvest, the coffee grower must have information regarding their initial quality, which must meet the standards established in the Sistema Nacional de Sementes e Mudas (SNSM). However, slow germination of coffee seeds makes it difficult to obtain results from evaluation of the seed physiological quality. An alternative for quick evaluation of coffee seed viability is the tetrazolium test. However, there are still questions regarding the true potential of the test for estimating the quality of seed lots with different levels of quality. The aim of this study was to evaluate the applicability of the tetrazolium test in estimating the viability of coffee seeds with different types of stress and levels of quality. Seeds of *Coffea arabica* L. were used, which were placed under the following treatments to obtain different types and levels of stresses: natural processing or fully-washed processing, different times of artificial aging, drying at high temperature, exposure to below-zero (°C) temperature, and storage under conditions without climate control. After obtaining the seed lots, the seeds were evaluated physiologically by the tetrazolium test and the germination test. The tetrazolium test overestimates the potential for production of normal seedlings of seed lots of *Coffea arabica* L. that have the lowest level of quality. The discrepancies between the results of viability in the tetrazolium test and the germination test vary according to the level of physiological quality and the type of stress the coffee seeds were under. We emphasize that the tetrazolium test in the SNSM as an alternative to the germination test in ascertaining the quality of coffee seeds must be reviewed since the results may significantly differ from the ability to produce normal seedlings.

Key words: Germination; Viability; Physiological quality; *Coffea arabica* L.; Quality control.

1 INTRODUCTION

Among the activities addressed by the National System of Seeds and Seedlings (Sistema Nacional de Sementes e Mudas - SNSM) are production, certification, analysis, and sale of units for plant propagation, seeds, and seedlings, governed by Law 10.711 of 5 August 2003 and regulated by Decree 10.586 of 18 December 2020, with the essential purpose of making quality seeds and seedlings available and assurance of genetic identity and control of generations (Brasil, 2020).

The activity of seed analysis, performed in official laboratories accredited by the Brazilian Ministry of Agriculture (Ministério da Agricultura, Pecuária e Abastecimento - MAPA), has the purpose of determining the identity and quality of a seed sample by means of methods, standards, and procedures that have been made official and established for each species. As an integral part of activities of the SNSM, seed analysis is mandatory for trade, transport, and use and inspection of seeds for purposes of production, and it is essential for making seeds with genetic identity and quality available (Brasil, 2020).

In the case of coffee seeds, the Rules for Seed Analysis (Regras para Análise de Sementes - RAS) establishes a period of 30 days for conducting the germination test, obtaining the result, and issuing the report of analysis, and this is necessary for sale of seeds to seedling producers (Brasil, 2009). Nevertheless, this is regarded as a long period, considering that coffee seeds have slow germination and seedling production is also slow, requiring a minimum of six months to obtain seedlings ready for planting (Rosa et al., 2010).

In this context, the use of the tetrazolium test is legally permitted, which is characterized by its speed in ascertaining the germination potential of coffee seeds by means of evaluation of embryos, according to Instrução Normativa (Administrative Act) IN – n° 35 of 29 November 2012 of the MAPA (Brasil, 2012). However, there are still questions regarding the true potential of the test in estimating seed quality, especially in the case of different levels of quality. The biggest differences between the results of the tetrazolium test and the germination test are more frequent in lower quality seed lots. These differences have been attributed to a probable greater sensitivity of the endosperms of the seeds to stress conditions compared to embryos (Coelho; Rosa; Fernandes, 2015; Dussert et al., 2012; Figueiredo et al., 2017).

According to the directives of the Rules for Seed Analysis (Brasil, 2009), staining can be performed after extraction of the embryos or simply through exposing them without removing them from the endosperm. In this regard, Zonta et al. (2009) compared the different methods of the tetrazolium test used for coffee seeds. The authors evaluated exposure of the portion of the endosperm that contains the embryo and exposure of the embryo alone to a 0.1% tetrazolium solution for 16 hours at 35 °C. According to the results obtained, the method performed with total removal of the embryo from the endosperm was more promising for evaluation of the viability and vigor of coffee seeds, with values equivalent to the germination test. Staining the portion of the endosperm that contains the embryos exhibited problems regarding staining, raising questions during evaluation of seed viability.

Performed in a shorter time, the tetrazolium test minimizes the activity of possible adverse factors that may come to interfere in evaluation of the germination potential of the seeds. Attack of microorganisms that develop during the germination test can impede correct evaluation of seedlings, resulting in discrepancies found between the results of the two tests (Clemente et al., 2011), especially in seed lots with worse physiological quality.

Thus, the aim of this study was to evaluate the applicability of the tetrazolium test to estimate the viability of coffee seeds under different types of stress and levels of quality.

2 MATERIAL AND METHODS

2.1 Plant material

Fruit of the species *Coffea arabica* L., cultivar Catuaí Amarelo IAC 62, were harvested at physiological maturity (cherry stage) from the experimental farm of the Fundação Procafé in Varginha, MG, Brazil, and taken to the Universidade Federal de Lavras (UFLA), MG, where they were mechanically pulped. The mucilage was then removed from the seeds by fermentation in water for a period of 24 hours at ambient temperature, and then the seeds were placed in a single layer on a suspended screen and kept in the shade for pre-drying and removal of surface moisture.

After processing, the initial moisture content of the seeds was determined and initial physiological quality was evaluated through the germination test and viability test in tetrazolium solution in the Central Seed Laboratory of UFLA.

2.2 Obtaining seeds with different levels of quality

After initial evaluation, the newly-harvested seeds were placed under different stress conditions to obtain seeds with different levels of quality. Different methods were used for that purpose, aiming to obtain three levels of quality of the coffee seeds. For the highest level of quality, under all types of stress, seeds dried in the shade until reaching 12% moisture content were used.

2.3 Stress by different types of processing

Two methods of coffee seed processing were used. Part of the seeds was demucilaged and dried in a dryer under a temperature of 35 °C until reaching 12% moisture content. The other part was dried in the fruit itself (natural coffee) in a dryer at 35 °C until reaching 12% moisture content, with later pulping, in accordance with Isquierdo et al. (2013).

2.4 Stress through artificial aging

The seeds were placed under different periods of artificial aging, defined according to results of preliminary experiments performed on the coffee seeds. Coffee seed lots with differences in physiological quality were obtained when the coffee seeds remained for four and eight days in a Biochemical Oxygen Demand (B.O.D.) growth chamber at 42 °C and 100% relative humidity (RH).

The seeds were placed on an aluminum screen fixed in Gerbox acrylic boxes containing 40 mL of distilled water, which were closed with a lid to obtain 100% relative humidity within and kept in a B.O.D. growth chamber at 42 °C for the pre-established times.

2.5 Stress by drying at high temperature

The seeds were placed under two different drying treatments. Part of the seeds was dried in fixed-bed dryers with air heated to 50 °C, until reaching 30% moisture content. After reaching 30% moisture content, the seeds were dried with air heated to 40 °C until the seeds reached 12% moisture content. Thus, stress was applied through intermittent temperature. The other part was dried at 50 °C until reaching 12% moisture content, thus applying stress through continuous high temperature, a procedure adapted from Taveira et al. (2012).

2.6 Stress through exposure to below-zero temperature

The seeds were exposed to below-zero (°C) temperature, in accordance with the method used by Coelho et al. (2015). Part of the seeds with 17% moisture content, obtained after drying in a dryer at 35 °C, was placed in impermeable packaging and kept in a freezer (-20 °C) for 24 hours. The other part of the seeds, with 15% moisture content, obtained after natural drying in the shade, was placed in impermeable packaging and kept in a deep-freezer (-86 °C) for 24 hours.

After the period of 24 hours, the seeds were removed from the packaging and rapidly thawed by immersion in a water bath at a temperature of 40 °C for 2 minutes, in accordance with the method proposed by Dussert et al. (1999). After thawing and a resting period for stabilization of equilibrium temperature, the seed parchment was removed.

2.7 Stress by storage under conditions without climate control

The seeds were placed for different periods of storage under conditions without climate control; they were placed in tri-laminate paper bags and stored without climate control for periods of four and eight months.

After obtaining all the seed lots with different type of stress and levels of quality, moisture content was determined and physiological quality was evaluated through the germination test and the tetrazolium test. In addition to the percentage of normal seedlings, the percentage of root emergence, percentage of seedlings with expanded cotyledonary leaves, and the dry matter of the seedlings were evaluated in the germination test.

2.8 Determination of seed moisture content

The moisture content of the seeds was determined by the laboratory oven method at 105 °C for 24 hours (Brasil, 2009), using two 10-seed subsamples. Results were expressed in mean percentage dry basis.

2.9 Physiological evaluations

- **Germination test:** This was carried out with four replicates of 50 seeds for each treatment, sown in germination paper moistened with distilled water in the amount of two and a half times the weight of the dry paper. The rolls of paper containing the seeds were placed in a seed germinator regulated to 30 ºC in the presence of light (Brasil, 2009). The percentages of root emergence at 15 days and of normal seedlings at 30 days after sowing were determined. Normal seedlings were considered as those with a primary root and at least two lateral roots. At the end of the germination test, the percentage of seedlings with expanded cotyledonary leaves at 45 days after sowing was also determined.
- **Dry matter of seedlings:** The dry matter of seedlings was determined at 45 days after sowing when the shoots were separated from the roots using a scalpel, and the plant material was placed in paper bags and then to dry in a forced air circulation oven at 60 ºC for 4 to 5 days or until constant weight. Dry matter was determined on a precision balance.
- **Tetrazolium test:** Coffee seeds coming from artificial aging treatments remained in water for 24 hours and for the other treatments for 36 hours for extraction of embryos. The extracted embryos were kept in Polyvinylpyrrolidone (PVP) antioxidant solution, washed in running water, and immersed in 0.5% tetrazolium solution using dark containers, where they were kept at a temperature of 30 ºC for 3 hours (Brasil, 2009; Clemente et al., 2011). Embryo viability was analyzed with the aid of a stereoscopic magnifying glass with 10X magnification for visualization of the external and internal appearance. After a longitudinal cut, they were classified as viable or inviable according to the location and extent of damage (Brasil, 2009).

2.10 Experimental design

The seeds placed under the different stress conditions did not exhibit similar physiological performance within the levels of quality as was planned to compose the experimental design in a single factorial arrangement. To achieve the aims of this study of analyzing the interaction among the effects of the types of stress and levels of quality, a completely randomized design was used in a hierarchical factorial arrangement. The first source of variation was considered as the five types of stress on the seeds (different types of processing, artificial aging, exposure to below-zero temperature, high drying temperature, and storage under conditions without climate control). The second source of variation was the three levels of quality (level 1 - highest, level 2 – medium, and level 3 - lowest) corresponding to the levels of stress within each type. Analysis of variance was conducted on the data and the means were compared by the Scott-Knott test at 5% probability (Ferreira, 2014).

3 RESULTS

3.1 Profile of the newly-harvested seeds

The results of evaluations of newly-harvested seeds before they were placed under different types of stress are shown in Table 1. The seeds had 38.9% moisture content and initial quality of 90% normal seedlings and 100% viable embryos. These seeds were used as the treatment of the highest level of quality and were also placed under the different types of stress to obtain the different levels of quality.

The seeds placed under the different types of stresses, i.e., by types of processing, artificial aging, drying at high temperature, exposure to below-zero temperature, and storage under conditions without climate control, did not exhibit similar physiological performance within the medium and low levels of quality as was planned for them to obtain in order to compose a single factorial arrangement design. Thus, to achieve the aims of the study of analyzing the interaction between the effects of the types of stresses and levels of quality, a hierarchical factorial arrangement was used in analyses of variance, i.e., the levels of quality were hierarchized within each type of stress. The single factorial arrangement was not possible, because it requires balanced levels.

From results of the analyses, there was a significant effect from the type of stress alone, as well as from the levels of quality hierarchized within each type of stress, for all the variables analyzed (data not shown). Table 2 shows the results of the test of comparison of the means of the variables of physiological evaluation of the seeds coming from each type of stress.

According to the data of Table 2, there was a reduction in physiological quality of the seeds under different types of stress, i.e., from different types of processing, artificial aging, exposure to below-zero temperature, drying at high temperature, and storage under conditions without climate control, in all the variables studied. The negative effect was more intense in seeds subjected to below-zero temperatures and to high temperature and in those stored under conditions without climate control.

In the germination test (Table 2), the seeds under different types of processing, as well as those aged artificially, had higher percentages of root emergence, of normal seedlings, and of expanded cotyledonary leaves and higher shoot dry matter and root dry matter. The most vigorous seeds, which resulted in higher percentages of seedlings with expanded cotyledonary leaves, were also those subjected to different types of processing and artificial aging.

In the tetrazolium test (Table 2), the highest viability of the embryos was obtained in seeds under different types of processing, followed by the artificially aged seeds.

Just as for the results of the germination test, the percentage of viable embryos in the tetrazolium test was also lower in the seeds exposed to below-zero temperature, in those dried at high temperature, and in those stored under conditions without climate control. Nevertheless, the results of viability of these embryos were less discrepant than the percentage of normal seedlings observed in the germination test.

There was a significant effect on the physiological quality of the coffee seeds from the level of quality hierarchized within each type of stress, as shown by data in Tables 3 to 7 from the results of the quality variables, mainly analyzing the relation between the results of germination (normal seedlings) and of viability in the tetrazolium test.

In general, the different types of processing used to obtain seeds with different levels of quality provided seeds with levels of quality near each other. There was no significant difference between the high and medium levels, except for the variables of root dry matter and viable embryos (Table 3). Only the seeds classified as having a lower level of quality showed a significant difference compared to the others.

In evaluation of viable embryos (Table 3), the values were comparable to the percentages of normal seedlings obtained in the germination test, indicating that for seeds under the different types of processing, the tetrazolium test was adequate for estimating seed quality within the levels of quality studied.

For the root emergence variable (Table 3), significant differences were not observed among the levels of quality and, according to viable embryos, a significant effect was observed among the high, medium, and low levels.

The results of comparison of the means of the quality variables for the seeds under the artificial aging treatments are shown in Table 4.

Table 1: Initial moisture content (IM) and root emergence (RE), normal seedlings (NS), seedlings with expanded cotyledonary leaves (CL), root dry matter (RDM), shoot dry matter (SDM), viable embryos in the tetrazolium test (VE) in newly-harvested or pre-dried *Coffea arabica* L. seeds.

Profile	IM (%)	RE (%)	NS (%)	CL (%)	RDM (mg/seedlings)	SDM (mg/seedlings)	VE (%)
Newly-harvested seeds	38.9	94	90	88	0.76	3.48	100

Table 2: Normal seedlings (NS), viable embryos in tetrazolium solution (VE), root emergence (RE), and seedlings with expanded cotyledonary leaves (CL), shoot dry matter (SDM), and root dry matter (RDM) of *Coffea arabica* L seeds.

Type of stress	Variable of physiological quality					
	NS (%)	VE (%)	RE (%)	CL (%)	SDM (mg/seedlings)	RDM (mg/seedlings)
Different processing	82 a	85 a	93 a	77 a	3.13 a	0.55 a
Artificial aging	80 a	82 b	92 a	73 a	3.00 a	0.57 a
Exposure to below-zero temperature	47 b	78 c	60 b	42 b	1.76 b	0.34 b
Drying at high temperature	38 c	65 d	50 c	35 c	1.46 c	0.27 c
Storage under conditions without climate control	35 c	54 e	49 c	32 c	1.36 c	0.28 c
CV (%)	8.74	5.17	6.09	9.87	10.23	11.37

Mean values followed by the same lowercase letter in the columns do not differ statistically from each other by the Scott-Knott test at the level of 5% probability.

Table 3: Effect of the level of quality factor hierarchized within the stress-by-different-types- of-processing factor on the physiological quality of *Coffea arabica* L. seeds.

Quality variable	Stress by different types of processing			
	Level of quality			
	Level 1 (High)	Level 2 (Medium)	Level 3 (Low)	Mean
Normal seedlings (%)	87 a	85 a	74 b	82
Viable embryos (%)	93 a	87 b	76 c	85
Root emergence (%)	95 a	94 a	91 a	93
Expanded cotyledonary leaves (%)	81 a	81 a	68 b	77
Shoot dry matter (mg)	3.39 a	3.47 a	2.52 b	3.13
Root dry matter (mg)	0.66 a	0.51 b	0.49 b	0.55

Mean values followed by the same lowercase letter in the row do not differ statistically from each other by the Scott-Knott test at the level of 5% probability.

Table 4: Effect of the level of quality factor hierarchized within the stress-by-artificial-aging factor on the physiological quality of *Coffea arabica* L. seeds.

Quality variable	Stress by artificial aging			
	Level of quality			
	Level 1 High	Level 2 Medium	Level 3 Low	Mean
Normal seedlings (%)	87 a	78 b	74 b	80
Viable embryos (%)	93 a	75 b	79 b	82
Root emergence (%)	95 a	96 a	85 b	92
Expanded cotyledonary leaves (%)	81 a	71 b	66 b	73
Shoot dry matter (mg)	3.39 a	2.77 b	2.83 b	3.00
Root dry matter (mg)	0.66 a	0.55 b	0.49 b	0.57

Mean values followed by the same lowercase letter in the row do not differ statistically from each other by the Scott-Knott test at the level of 5% probability.

The artificial aging treatments led to two different levels of quality, evaluated by means of all the quality variables, except for percentage of root emergence.

The values of normal seedlings and of viable embryos show the same classification of the seeds in levels of quality. The seeds had values near each other in the germination test and tetrazolium test, with the same classification in levels of quality.

Nevertheless, a tendency is observed toward overestimation of quality in the tetrazolium test in the highest and lowest level of quality, and underestimation in the medium level. But, in general, these values are comparable. In the tetrazolium test, the percentage of viable embryos was underestimated in the seeds classified as having a medium level of quality. The same result can be observed for percentage of normal seedlings, indicating that artificial aging causes damage both to endosperms and to embryos of coffee seeds.

The results for the seeds under drying at high temperature are shown in Table 5.

Seeds under the drying at high temperature treatments had severe damage, as observed in the results of the germination test from the percentages of normal seedlings of the seeds at the medium and low levels of quality. Three different levels of quality were obtained, which was confirmed by all the variables, except for root dry matter, which classified the seeds in only two levels of quality. For the other variables, the high drying temperature led to seeds with three levels of quality (high, medium, and low), possibly through damage to the membrane system caused by the high drying temperature (Siqueira et al., 2017).

Comparison of the germination test (% normal seedlings) with the tetrazolium test (% of viable embryos) showed that seeds with percentages considerably below the 70% limit considered for sale of coffee seeds had high values of viability in the tetrazolium test. Seeds classified as medium level of quality, with 20% normal seedlings, exhibited 66% viability. The quality of seeds classified in the low level, with 8% normal seedlings, also had quality overestimated by the tetrazolium test, exhibiting 36% viability.

According to the results of Table 6, the seeds exposed to below-zero temperature could be separated into three decreasing levels of quality (high, medium, and low) according to the results of all the evaluations made in the germination test, as well as by the tetrazolium test, in coffee embryos.

Table 5: Effect of the level of quality factor hierarchized within the stress-by-drying-at-high-temperature factor on the physiological quality of *Coffea arabica* L. seeds.

Quality variable	Stress by drying at high temperature			
	Level of quality			
	Level 1 High	Level 2 Medium	Level 3 Low	Mean
Normal seedlings (%)	87 a	20 b	8 c	38
Viable embryos (%)	93 a	66 b	36 c	65
Root emergence (%)	95 a	36 b	20 c	50
Expanded cotyledonary leaves (%)	81 a	16 b	7 c	35
Shoot dry matter (mg)	3.39 a	0.70 b	0.30 c	1.46
Root dry matter (mg)	0.66 a	0.09 b	0.04 b	0.09

Mean values followed by the same lowercase letter in the row do not differ statistically from each other by the Scott-Knott test at the level of 5% probability.

Table 6: Effect of the level of quality factor hierarchized within the stress-by-exposure-to-below-zero-temperature factor on the physiological quality of *Coffea arabica* L. seeds.

Quality variable	Stress by exposure to below-zero temperature			
	Level of quality			
	Level 1 High	Level 2 Medium	Level 3 Low	Mean
Normal seedlings (%)	87 a	53 b	0 c	47
Viable embryos (%)	93 a	78 b	65 c	78
Root emergence (%)	95 a	85 b	0 c	60
Expanded cotyledonary leaves (%)	81 a	46 b	0 c	42
Shoot dry matter (mg)	3.39 a	1.90 b	0 c	1.76
Root dry matter (mg)	0.66 a	0.35 b	0 c	0.34

Mean values followed by the same lowercase letter in the row do not differ statistically from each other by the Scott-Knott test at the level of 5% probability.

The seeds classified at the highest level of quality exhibited higher values in all the variables studied. As the quality decreased to medium and low levels, a reduction was observed both in the percentage of normal seedlings and in the variables that estimate seed vigor. The below-zero temperature provoked drastic stresses in seeds classified as low level of quality, impeding their germination, and they exhibited zero percentage of normal seedlings. Although these seeds had null physiological performance in the germination test, they exhibited 65% viable embryos in the tetrazolium test, indicating an overestimation of the quality of the low level seeds through the tetrazolium test.

The results of the analyses of the effects of levels of quality hierarchized within the type of stress brought about by storage of seeds under conditions without climate control are shown in Table 7.

The results shown in Table 7 allowed classification of the seeds in three levels of quality (high, medium, and low) in all the variables. Thus, as in the seeds under stress by exposure to below-zero temperature, there was also drastic damage in seeds stored under conditions without climate control, and these seeds had zero germination at the lowest level of quality and 19% at the medium level.

Comparison of the results of the germination test with those of the tetrazolium test showed that the latter overestimated the quality of the seeds of the highest level and of the medium level of quality (Table 7). The seeds at the lowest level of quality, which had zero percent normal seedlings, indicating seed death in the germination test, also had a practically zero percent (4% viability) in the tetrazolium test, indicating embryo death. This result differs from what was observed for seeds under stress by drying at high temperature (Table 5), which also had very low percentages of normal seedlings, but high viability in the tetrazolium test.

4 DISCUSSION

In this study, different responses of the seeds were observed, as shown by results of viability by the tetrazolium test and of the germination test when the seeds were under different types of stress. According to these results, the potential of the tetrazolium test for estimating the physiological quality of coffee seeds varies according to the type of stress the seeds were exposed to and according to their levels of quality from these stresses. In seeds with germination above 70%, such as those under the different types of processing (Table 3) and artificial aging (Table 4), the tetrazolium test has comparable results, although with a tendency toward overestimation. In seeds under stresses from drying at high temperature (Table 5), from exposure to below-zero temperature (Table 6), and from storage in an environment without climate control (Table 7), in which damage to seeds was drastic, the tetrazolium test exhibits highly discrepant and overestimated results in relation to the results obtained in the germination test. Although the low quality seeds were subject to these drastic stresses, with zero physiological performance in the germination test, 65% of the embryos were designated as viable in the tetrazolium test after freezing damage from below-zero temperature and 4% as viable after storage in an environment without climate control. This indicates different sensitivities to the types of stress from the different seed structures, i.e., from the endosperms and the embryos.

The low percentage of normal seedlings in the germination test may be a reflection of the higher sensitivity of the endosperms, in relation to the embryos, to the adverse conditions to which the seeds were subjected, as was observed by Coelho et al. (2015), suggesting that the endosperms are more susceptible to the stresses caused by high drying temperatures. From the results observed in the present study, the endosperms were also more susceptible to the damage brought about by below-zero temperature.

This sensitivity of the coffee seed structures was also found in the germination test by Coelho et al. (2015) and Coelho, Rosa and Fernandes (2017), upon comparing the data from the tetrazolium test in coffee embryos to the data on formation of normal seedlings. The better physiological performance of the embryos compared to the whole seeds also corroborates the results of Dussert et al. (2012) and Sershen et al. (2012), which showed greater sensitivity of the endosperms than of the zygotic embryos to drying and to low temperature.

Studies conducted to investigate the formation of coffee seedlings from seeds with low levels of quality showed that the embryos extracted from these seeds are able to form normal seedlings when grown apart from the endosperms and that there are strong indications that the endosperm structures seem to be more sensitive than the embryos to the stresses that lead to seed deterioration (Santos; von Pinho; Rosa, 2013). This fact, corroborated by other authors (Coelho et al., 2015; Dussert et al., 2012) confirms that the embryos may be less sensitive to deterioration than the endosperms.

This greater sensitivity of the endosperms of coffee seeds in relation to the embryos was also found in studies on the effects of drying, in which embryos that were cultured had better development compared to normal seedlings in the germination test for seeds under stress from drying (Dussert; Engelmann, 2006; Coelho et al., 2015; Figueiredo et al., 2017). According to Dussert and Engelmann (2006), the drying process causes oxidative stress, generating reactive oxygen species, which may affect the viability and vigor of coffee seeds.

The tetrazolium test is performed under more environmentally friendly than those provided for the germination test, in addition to being performed in less period of time, which mitigates the action of possible factors adverse effects, such as microorganisms, not allowing that these factors interfere with embryo evaluation. According to França Neto et al. (2018) and Krzyzanowski et al. (2020) differences of up to 5% between the tests of viability and germination are acceptable.

In general, the different types of processing used to obtain seeds with different levels of quality provided seeds with levels of quality near each other. According to Dussert and Engelmann (2006) and Abreu et al. (2014), the drying process causes oxidative stress, generating reactive oxygen species, which may affect the viability and vigor of coffee seeds.

Table 7: Effect of the level of quality factor hierarchized within the stress-by-storage-under-conditions-without-climate-control factor on the physiological quality of *Coffea arabica* L. seeds.

Quality variable	Stress by storage under conditions without climate control			
	Level of quality			
	Level 1 High	Level 2 Medium	Level 3 Low	Mean
Normal seedlings (%)	87 a	19 b	0 c	35
Viable embryos (%)	93 a	68 b	4 c	54
Root emergence (%)	95 a	51 b	0 c	49
Expanded cotyledonary leaves (%)	81 a	17 b	0 c	32
Shoot dry matter (mg)	3.39 a	0.70 b	0 c	1.36
Root dry matter (mg)	0.66 a	0.16 b	0 c	0.28

Mean values followed by the same lowercase letter in the row do not differ statistically from each other by the Scott-Knott test at the level of 5% probability.

In addition to the damage caused by aging of seeds, the high moisture content, together with higher air temperatures, contributes to increase the incidence of seed pathogens (Carvalho; Guimarães; Silva, 2011), which may cause seed deterioration and death, impeding evaluation in the germination test, as well as considerably compromising germination and seedling development (Clemente et al., 2011).

Finally, we emphasize that deliberation for use of the tetrazolium test in the SNSM as an alternative to the germination test to ascertain the quality of coffee seeds and to issue the report of analysis needs to be reviewed, considering that the results may differ significantly from the true ability of the seeds to produce a normal seedling.

5 CONCLUSIONS

The tetrazolium test overestimates the potential of seed lots of *Coffea arabica* L., cultivar Catuaí Amarelo IAC 62, that have low level of quality for producing normal seedlings.

The discrepancies between the results of viability in the tetrazolium test and of normal seedlings in the germination test vary according to the level of physiological quality and the type of stress the seeds of *Coffea arabica* L., cultivar Catuaí Amarelo IAC 62, were exposed to.

The use of the tetrazolium test in the SNSM as an alternative to the germination test to ascertain the quality of *Coffea arabica* L., cultivar Catuaí Amarelo IAC 62, seeds needs to be reviewed, considering that the results may differ significantly from the ability to produce normal seedlings.

Seeds of *Coffea arabica* L., cultivar Catuaí Amarelo IAC 62, confirmed as dead in the germination test may exhibit viability in the tetrazolium test of up to 65%.

6 AUTHORS' CONTRIBUTION

TBF performed the experiments, analysis and wrote the manuscript. SDV oriented, supervised and guided the whole experiments, analysis and manuscript. DSP assisted in the statistical analyses. SVBC and SDVFR reviewed and approved the final version of the manuscript. PHAS, ALOV and FASR assisted in the experiment and in the final review of the manuscript.

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