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Physiological quality of seeds from traditional and new recommended groups of Arabic coffee cultivars to highland regions

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***Abstract*— *The quality of a seed lot is an essential factor in the planning and implementation of any agricultural production program. Quality seeds provide strong and uniform plant growth. This study aims to analyze the physiological quality of seeds from different groups of Arabica coffee cultivars recommended for planting in the Brazilian highland regions. The seeds were picked from an Arabica coffee seed production field, accredited by the Brazilian Ministry of Agriculture, Livestock, and Supply, located in the municipality of Marechal Floriano, state of Espírito Santo (ES), Brazil. The experimental design was completely randomized, with four replications of 50 seeds each. The treatments were composed by traditional cultivars and new Arabica coffee recommendations from Catuaí (Catuaí IAC-62, Catuaí IAC-81), Caturra (Caturra IAC-479), Catucaí (Catucaí-785/15, Catucaí-2SL/CAK, Catucaí-24/137, Japi), Mundo Novo (Mundo Novo IAC-379/19), and Sarchimor (Acauã, Arara) groups. The seeds were manually harvested in sieves, at the point of physiological maturation, processed, and dried until reaching a humidity of 35% ± 1%. Immediately following this, the seeds were taken to the Seed Analysis Laboratory, Department of Agronomy, Center for Agricultural Sciences and Engineering, Federal University of Espírito Santo (CCAE-UFES), Alegre-ES, Brazil. The parameters analyzed were germination (%), germination speed index (GSI), length of shoot and root, and the fresh and dry mass weight of the seedlings. The rust-resistant cultivars of the Catucaí group, particularly Catucaí-785/15 and Catucaí-24/137, showed higher values of germination and seed vigor, which suggested a higher physiological quality of the seeds. These cultivars should have lower seedling disposal in the nursery, and a higher yield in the final seedling production. Cultivar Caturra IAC-479 showed the lowest performance.*

I. INTRODUCTION

Coffee has an important socioeconomic function in Brazil, and Arabica coffee (*Coffea arabica* L.) is grown in approximately 13% of the Brazilian area. This requires intensive labor throughout the year, which generates employment and distribution of income (Barbosa et al., 2012). The state of Espírito Santo is the second major Brazilian coffee producer, and this crop has been the main agricultural economic activity in 80% of the municipalities. Arabica coffee has been cultivated predominantly in the sloping areas and at high altitudes, ranging from 600 m to 1,200 m above sea level (asl). The average productivity in this Brazilian state is around 1,727 kg ha-1, and the total production of Arabica coffee in 2020 has been circa 270.42 thousand tons, involving 26,313 small rural properties and 53 thousand families in the production system (Schimidt et al., 2004; Conab, 2020).

In the last ten years there has been a significant increase in the demand for seeds from the tolerant/resistant cultivars compared to the coffee-rust, with good adaptation, productivity, and quality of the drink being observed. Cultivating Arabica coffee with these characteristics needs less pesticides, and reduces the risk of poisoning to the applicator and to the environment, and also reduces production costs. An improvement in the productivity, resistance to disease, quality of the coffee, and diversity in the sensory and organoleptic profiles of the beverage has been observed (Carvalho et al., 2012; Matiello et al., 2020). Traditionally, the cultivars used belonged to the Mundo Novo, Catuaí, and Caturra groups. However, several Brazilian research institutions have developed and indicated new Arabica coffee cultivars for use in different Brazilian regions. Particularly, those cultivars that have rust-tolerance/resistance, high productivity, and high quality of drink, mainly from the Catucaí and Sarchimor groups (Carvalho, 2012; Krohling et al., 2018; Matiello et al., 2020).

The quality of a seed lot is an essential factor in the planning and implementation of any agricultural production program; good quality seeds provide strong and uniform plants. The physiological quality of the seeds has been characterized by their strength and energy for germination, these being the sum of attributes that give them the potential to quickly transform into normal seedlings (Carvalho; Nakagawa, 2000). Use of high-quality seeds is essential for rapid and uniform establishment of plants in the field, particularly for coffee growing. The germination capacity and the real physiological potential of the seeds are essential qualities for making decisions involving the commercialization and use or disposal of a seedling lot (Reis et al., 2010). Coffee seeds have slow and variable germination, making it difficult for the process of seedling production. A germination test is used to determine the seed potential and evaluate its quality during the storage period, and also to estimate the success of seedling production in a nursery (Wibowo et al., 2019).

Coffee seedling producers have to purchase coffee seeds from seed producers accredited by the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA). The quality of seeds must be in accordance with the standards of normative instruction N. 35 of November 2012 (Brasil, 2012). This physiological quality is influenced by the genotype and reaches the maximum value during its maturity, and is considered to be the maximum potential of seed quality, such as germination and seedling vigor, which are genetically controlled (Espindula et al., 2018).

Researches to determine the physiological quality of coffee seeds of different cultivars, available in the market, have a lot of significance, as they provide accurate information to seed producers and coffee seedling producers on the uniqueness of the germination and vigor of each cultivar. The inadequate management of seeds in the coffee nursery affects the formation of seedlings, causing irregular development of the coffee plantation, delay in the start of the productive phase, and reduction in the crop yield (Macêdo et al., 2011; Matiello et al., 2020). This study aims to analyze and compare the physiological quality of seeds of different groups of Arabica coffee cultivars recommended for planting in the Mountain region, in the state of Espírito Santo (ES), Brazil.

II. MATERIAL AND METHODS

The Arabica coffee seeds were harvested in a coffee seed production field accredited by the MAPA, located in the municipality of Marechal Floriano (altitude 670 m; -40°46'03"W; -20°26'30"S), Mountain region of Espírito Santo State, Brazil. Ten liters of fruits at stage 88-BBCH coffee scale (complete physiological maturity) (Arcila-Pulgarin et al., 2002) of each cultivar were manually picked in July 2020, from the middle-third of the plants. The fruits were processed in a separator-washing machine, with the aim of eliminating the coffee fruits that floated (malformed) from the ripe ones. The fruits were processed in a manual pulp remover, and soon after, were submitted to a natural fermentation process for 24 hours, using clean water to remove the mucilage; 30% of water, in relation to the coffee volume, was used. Subsequently, the fruits were dried on a black polyethylene mesh suspended 1 m from the soil (Alixandre et al., 2019) until it had a moisture content of 35% ± 1% (bu). It was later sent to the Laboratory of Analysis of Seeds, Department of

Agronomy, Center for Agricultural Sciences and Engineering, Federal University of Espírito Santo (CCAE-UFES), Alegre-ES, Brazil.

The experimental design used was completely randomized with four replications, using 50 seeds per plot. Cultivars (cv) that constituted the treatments were grouped into: Group 1. Catucaí: Cv Catucaí-785/15, Catucaí-2SL/CAK), Catucaí-24/137, and Japi (all selected from the cross between the groups Icatu x Catuaí) — coffee-rust tolerant; Group 2. Sarchimor: Arara (cross between yellow Icatu 2944 x Sarchimor 1669/20), and Acauã (cross between Mundo Novo IAC-388/17 x Sarchimor IAC-1668) — coffee-rust tolerant; Group 3. Others: Catuaí IAC-81 (cross between Mundo Novo x Caturra), Catuaí IAC-62 (cross between Mundo Novo x Caturra), Mundo Novo IAC-379/19 (cross between Sumatra x Bourbon Vermelho), and Caturra IAC-479 (mutation of Red Bourbon) — coffee-rust susceptible cultivars. Under laboratory conditions, the seed endocarp (parchment) was initially removed, and immediately seed asepsis was done by immersion in 70% alcohol (v/v) for 2 minutes, in 2% sodium hypochlorite solution (v/v) for 3 minutes, and then in a captan solution (2.5% w/v). After each phase of this procedure, the seeds were washed in distilled water, with the aim to remove any residue of chemicals used (BRASIL, 2009). Seed quality analysis were performed using the following procedures:

Seed water content: Was determined in an oven at 105 ± 3°C, using two sub-samples of 50 seeds (Brasil, 2009).

Germination test: Was performed with four replications of 50 seeds for each treatment; rolls of three Germitest® papers, previously dampened with distilled water in a proportion of 2.5 times the dry weight of the papers, were used as the substrate. The seeds were kept in a germination chamber [Biochemical Oxygen Demand (B.O.D.)] at a constant temperature of 30 ± 1°C, in a 24-hour dark phase. Seed germination was monitored daily and evaluations consisted of two counts of normal plants: Fifteen days after the start of the test, evaluation was for radicular protrusion (≥ 2 mm), and 30 days after the tests began, evaluation was for the number of developed healthy seedlings (Brasil, 2009). The results were expressed as the germination percentage (%TG).

Germination speed index (GSI): Was determined together with the germination test, by adding the number of seeds that showed protrusion of the primary root (≥ 2 mm), daily (Maguire, 1962). The count was done up to the thirtieth day after sowing. The GSI was calculated daily, by adding the number of seeds germinated and dividing it by the number of days between sowing and germination: $GSI = G1/N1 + G2/N2 + ... + Gn/Nn$, where GSI = germination speed index; G1, G2, and Gn = number of seedlings obtained in the first, second, third, and last counts; N1, N2, ... Nn = number of days after sowing, in the first, second, and last counts.

Root length and aerial part of the seedlings: The measurement was performed after 30 days of sowing, wherein the normal seedlings of each repetition were measured with the aid of a graduated ruler (cm), from the tip of the root to the apex of the aerial part, and the results were expressed in cm $seedling^{-1}$.

Fresh and dry mass of seedlings: The seedlings, after the previous analysis were weighed in an analytical balance (0.0001 g) to obtain the fresh weight. Subsequently, they were packed in Kraft paper bags, kept in an oven, with the temperature set at 65 + 2°C for 48 hours (until reaching constant weight). The samples were then weighed and the results of the dry mass weight was expressed in g $seedling^{-1}$.

The data were submitted to Lilliefors tests, to verify the normality of the errors, before performing the Analysis of Variance (ANOVA) of the variables. The Cochran and Bartlett tests were used to verify the homogeneity of the variances. The data of the variables were submitted to ANOVA, and the means compared by the Scott Knott test ($p < 5\%$). The principal component analysis was used to group the genotypes, with regard to the characteristics studied, by means of a visual examination of their graphic dispersions. For statistical analysis, the R program (R Core Team, 2020) was used.

III. RESULTS AND DISCUSSION

There was no difference in the seed germination of the Arabica cultivars in the final evaluation, and total germination values ranged from 88% to 99%. All cultivars showed germination values above 70%, the minimum required by normative instruction N. 35, of November 2012, from MAPA, for commercialization of coffee seeds in Brazil (BRASIL, 2012). However, cv Catucaí-785/18 (95%), and Catucaí-2SL/CAK) (95%) showed the highest germination values in the first germination count. Cultivar Catuaí IAC-81, Catucaí-24/137, Catuaí IAC-62, Acauã, Japi, and Arara, showed germination values from 86% to 90%; cv Mundo Novo IAC-379/19 (75%), and Caturra IAC-479 (80%) showed the lowest germination percentage values obtained (Table 1).

Seeds from cv Catucaí-785/15 and Catucaí-2SL/CAK showed higher GSI values than the others, even as, cv Mundo Novo IAC-379/19 showed lower GSI values, with less vigor. The difference in vigor among the cultivars was pointed out in the first germination count and in the GSI values; although, all cultivars had good germination potential in the final evaluation. It was evident that cv Catucaí 785/15 and Catucaí-2SL/CAK showed greater vigor in relation to the others. Cultivar Catuaí IAC-81, Catucaí-24/137, Catuaí IAC-62, Acauã, Japi, and Arara, presented TG values ranging from 87% to 95%; and cv Caturra IAC-479 and Mundo Novo IAC-379/19 showed a different behavior, with a lower vigor rate (Table 1).

Table 1. First count germination (FCG), total germination (TG), and germination speed index (GSI) of seeds of different groups of Arabica coffee cultivars

Groups	Cultivars	FCG(%)*		TG(%)*		GSI*	
Catucaí	Catucaí-785/15	95	a	95	a	1.70	a
	Catucaí-2SL/CAK	95	a	99	a	1.75	a
	Catucaí-24/137	90	b	95	a	1.57	b
	Japi	86	b	94	a	1.55	b
Sarchimor	Acauã	86	b	92	a	1.51	b
	Arara	88	b	88	a	1.55	b
Other	Caturra IAC-476	75	c	95	a	1.54	b
	Catuaí IAC-81	88	b	93	a	1.54	b
	Mundo Novo	80	c	90	a	1.49	b
	Catuaí IAC-62	87	b	87	a	1.50	b
Mean		**87**		**92.8**		**1.57**	
CV (%)		**6.17**		**5.37**		**5.31**	

*Means followed by the same letter in the column do not differ statistically from each other by the Scott Knott test (p <5%).

The cultivars Catucaí-24/137 (3.68 cm), Arara (3.67 cm), Catuaí IAC-62 (3.47 cm), Catucaí-785/15 (3.42 cm), and Acauã (3.15 cm) (Table 2) presented the highest growth of the aerial part of the seedlings, even as lower growth values were observed in cv Caturra IAC-479 (1.91 cm). Cultivars Catucaí-785/15 (3.81 cm), and Catucaí-24/137 (3.72 cm) presented higher values of root length in relation to cv Caturra IAC-479 (1.52 cm), which presented less growth. The growth of the aerial part of the seedlings was directly proportional to the length of the roots. This factor could be associated with the production of cytosine, a hormone, which despite being produced in other parts of the plant, was produced in greater quantities in the roots. Limitation in root metabolism could reduce the production of hormones and limit the growth of the aerial part of the seedling (SANTOS, 2020). Cultivar Acauã (5.18 g) had the highest fresh weight, differing from the other cultivars, followed by cv Catuaí IAC-62 (4.91 g), Catucaí-24/137 (4.87 g), and Catucaí-785/15 (4.71 g). The lowest transfer of fresh mass was observed in cv Caturra IAC-479 (4.08 g). Cultivars Catuaí IAC-81 (1.15 g), Catucaí-785/15 (1.12 g), Acauã (1.12 g), and Catucaí-24/137 (1.09 g) showed the highest dry mass accumulation values, and differed from the other cultivars.

The first two main components (dimensions) were used for the composition of equations 1 and 2.

CP1(DIM 1) = 0.74 LAP + 0.91RL+ 0.63SFW + 0.38SFW + 0.96FCG + 0.25TG + 0.60GSI (1);

CP2(DIM 2) = - 0.58 LAP + 0.01RL - 0.54SFW - 0.17FSW + 0.16FCG + 0.87TG + 0.76GSI (2)

First germination count (FCG), and seedling root length (RL) (Equation 1, Figure 1A) are highlighted in the first main component, and total germination (TG), and germination speed index (GSV) (equation 2, Figure 1). Strong correlations are also observed between the variables fresh seedling weight (SFW) and length of seedling aerial part (LAP), according to the acute angles formed between them.

Table 2. Length of aerial part (LAP), root length (RL), seedling fresh weight (SFW), and seedling dry weight (SDW) of different groups (group) of Arabica coffee cultivars (cv)

Group	Cultivar (cv)	LAP (cm)		RL (cm)		SFW (g)		SDW (g)	
Catucai	785/15	3.42	a	3.81	a	4.71	b	1.12	a
	2SL/CAK	2.76	b	3.21	b	4.56	c	0.95	b
	24/137	3.68	a	3.72	a	4.87	b	1.09	a
	Japi	3.01	b	2.35	c	4.61	c	1.04	b
Sarchimor	Acauã	3.15	a	2.16	c	5.18	a	1.12	a
	Arara	3.67	a	2.51	c	4.45	c	1.01	b
Other	Caturra	1.91	c	1.52	d	4.08	d	0.98	b
	Catuaí 81	2.80	b	2.88	b	4.48	c	1.15	a
	Mundo	2.69	b	2.06	c	4.44	c	0.99	b
	Catuaí 62	3.47	a	3.35	b	4.91	b	0.94	b
Mean		3.05		2.76		4.63		1.04	
CV (%)		9.29		14.19		4.34		7.48	

*Means followed by the same letter in the column do not differ statistically from each other by the Scott Knott test (p <5%).

The dispersion of the 10 cultivars, with regard to the germination characteristics, based on the coordinates related to the first two main components (CP1 and CP2 — Dimensions) showed that four distinct groups were formed. These two components absorbed 75.36% of the variation in the original characteristics, with CP1 (Dim1) responsible for 46.47%, and CP2 (Dim2) for 28.89%. The first group (A) was comprised of cv Caturra IAC-476 (black); the second group (B) was comprised of cv Catucaí-2SL/CAK (red); the third group (C) of cv Catucaí-785/15 and Catucaí-24/137 (green); and the other groups of cultivars formed group D (blue) (Figure 1B).

Cultivars Caturra IAC-476 and Catucaí-2SL/CAK did not show similarity between themselves or with the other groups; cv Catucaí-785/15 and Catucaí-24/137 (group C) showed similarity between them, and dissimilarity with the other groups; and cv Catuaí IAC-81, Mundo Novo IAC-379/19, Catucaí IAC-62, Acauã, Japi, and Arara showed similarities with regard to the studied germination characteristics (Figure 1B). The results obtained by the main components (Figure 1) confirmed those obtained by the Scott Knott test (Tables 1 and 2), which highlighted, in general, that cv Catucaí-785/15, and Catucaí-24/137 (group C) showed superior characteristics of physiological quality than the others, followed by cultivars of group D and group B. The cv Caturra IAC-476, in general, presented germination characteristics inferior to the others.

The difference in the physiological quality of coffee seeds may be associated with the ability to adapt to environmental, biotic, and abiotic stresses, which can have a disadvantageous influence on the physiology of the plant. It may directly influence the chemical composition, accumulation of reserves, and hormonal balance of the seeds. In this context, it is worth mentioning that the methodology adopted in this study, carefully standardized factors such as, climate, crop management, and management during the harvest and post-harvest, which enabled conditions for maximum expression of the physiological quality of the seeds in the analyzed conditions (Carvalho, 2012; Taiz et al., 2017).

Cultivars Catucaí-785/15 and Catucaí-24/137 have shown high vigor and resistance to rust (*Hemileia vastatrix* Berk & Br) under field conditions, in Brazil. This contributes to the maintenance of a higher index of leaf area, generating a higher rate of liquid photosynthesis throughout the period of fruit development. These conditions enable the production of seeds with greater accumulation of reserve, density, size, hardness of the integuments, and a more balanced chemical and hormonal composition. Thus, it is possible to suggest that these factors may have contributed positively to a higher physiological quality of the seeds of these cultivars. The less vigor of the plant and the high susceptibility to coffee-rust, a characteristic of cv Caturra IAC-479, may have contributed negatively to the lower physiological quality of its seeds (Giomo et al., 2008; Krohling et al., 2018).

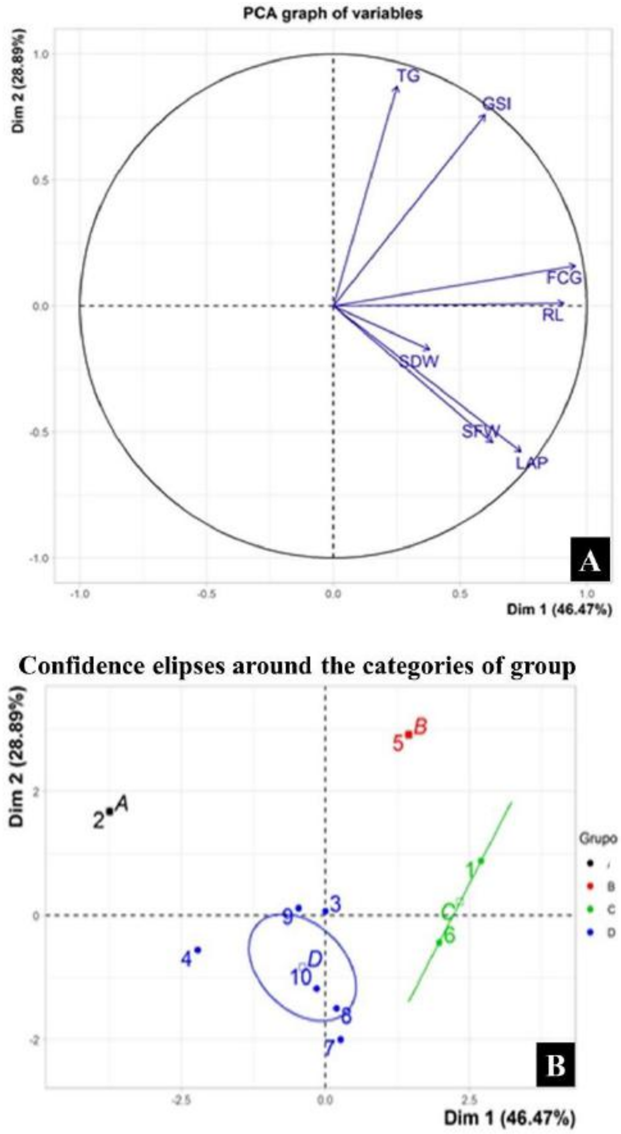

Fig.1 – Dispersion diagram in relation to the first two main components of the genotypes: 1. – Catucaí-785/15; 2. – Caturra IAC-479; 3. – Catuaí IAC-81; 4. – Mundo Novo IAC 379-16; 5. – Catucaí-2SL/CAK); 6. – Catucaí-24/137; 7. – Catuaí IAC-62; 8. – Acauã; 9. – Japi; and 10. – Arara. Variables: first count germination (FCG), total germination (TG), germination speed index (GSI), length of aerial part (LAP), seedling root length (RL), fresh seedling weight (FSW), and seedling dry weight (DSW).

Another factor that can explain the difference obtained in the physiological quality among the cultivars is related to the speed of reorganization of the cell membrane of the seeds during the imbibition process; considering that membranes reach maximum organization at the stage of physiological maturity. However, from that moment, as a result of water loss by natural or artificial means, a process of structural disorganization begins. Regardless of the degree of deterioration of the seeds, the membrane system reorganizes itself during the imbibition process, regaining control over its permeability. Therefore, the faster the reorganization of the membrane occurs, the greater is the tendency to increase the vigor of the seeds (Araújo, 2011; Bewley et al., 2013; Marcos Filho, 2015). A difference in vigor has been observed between cultivars of the Sarchimor group (IPR-98, IPR-100, IPR-105, and IPR-106) in which cv IPR-98 has the lowest percentage of germination, radicle protrusion, germination after premature aging, and greater electrical conductivity. It suggests that a difference in vigor among seeds of cultivars within the Sarchimor group may be related to the integrity of the seeds verified by electrical conductivity. Thus, it is possible that the better vigor performance of cv Catucaí-785/15 and Catucaí-24/137 may be related to the higher speed of reorganization of seed membranes during the imbibition process, inducing greater vigor (Dalvi et al., 2013; Francisco et al., 2019).

Germination and vigor of the Arabica coffee seeds can be impaired when infected with disease. The microflora associated with coffee seeds varies depending on the area of cultivation and the climatic conditions of the production region. Fungi of the genera *Aspergillus* and *Penicillium* are usually present on the seed surface shortly after being processed; these can colonize in seeds under field conditions with abundant humidity (90–95%) and under storage. However, other fungi such as *Fusarium* spp., *Colletotrichum* spp., and *Phoma* spp., can also infect seeds (Squarezi et al., 2002). These fungi are very common in the cultivation in mountain regions, where it is humid when seeds are collected. No fungi had been observed during the entire experiment period, showing that appropriate conditions of harvest and processing of the fruits carried out in the methodology adopted in this study are limiting to the contamination process.

The physiological quality of the *Coffea canephora* 'Apoatã' seed varies depending on the genotype. The genetic variability between cultivars can generate seeds with different sizes, densities, tegument hardness, and concentration of substances that inhibit germination, such as, caffeine and phenolic compounds. Thus, it is possible to confirm that the study of germination and vigor is something complex, as it involves numerous metabolic processes in which several factors can act simultaneously, and the whole process is controlled by the interaction between genetic and environmental factors (Teixeira, 2012; Rubim et al., 2014; Espindula, 2018; Posse et al., 2019).

The information obtained in this study can significantly contribute to improving the efficiency of the production process of coffee seedlings. Cultivars that present seeds with higher physiological quality tend to have a higher rate of initial development of seedlings in the nurseries, and also a lower rate of discarded seedlings. This can provide production of higher quality seedlings and also greater commercial gain for coffee seedling producers. Further studies are suggested with seed evaluation of cultivars from different regions and altitudes, in order to evaluate the effect of the interaction between the cultivars and environmental conditions on the physiological quality of coffee seeds.

IV. CONCLUSIONS

- All seeds of the Arabica coffee cultivars tested fall within the MAPA requirements for commercialization.
- The new rust-resistant cultivars of the coffee group Catucaí, particularly cv Catucaí-785/15 and Catucaí-24/137, present greater germination and vigor in seeds.
- Cultivar Caturra IAC-479, shows inferior performance in relation to the physiological quality of seeds.
- Arabica coffee seeds sold in the sampled region have an excellent physiological quality.
- Crops with adequate nutrition, fruit harvest in the physiological stage of the ripe cherry, correct handling in the post-harvest period, and manual removal of the endocarp, allow for maximum expression of the physiological quality potential of Arabica coffee seeds.

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