

## Digital images of seedling for evaluating coffee seed vigor<sup>1</sup>

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**ABSTRACT** – The digital image analysis of seedlings has become largely employed in seed quality-control programs due to its feasibility, objectivity and fast results. Despite these advantages, no studies have yet demonstrated the efficiency of this technique for analyzing coffee seed vigor. The present study aimed at evaluating the efficiency of the Seed Vigor Imaging System (SVIS<sup>®</sup>) in determining the vigor of coffee seeds (*Coffea arabica* L.) from digital images of seedlings. Also, the results of these analyses were contrasted with conventional vigor tests. Six seed lots from each cultivar, *Bourbon* and *Catucaí* 20/15, were used. The research was conducted in two experimental times, and the seed vigor was determined by the tests of first germination count, accelerated aging, electrical conductivity, primary root protrusion speed, as well as by the analysis of scanned images of seedlings, made possible by the SVIS<sup>®</sup> software. For the Bourbon and Catucaí 20/15 cultivars, the SVIS<sup>®</sup> analysis was able to discriminate lots of both high and low vigor (vigor indexes of 317 to 752, and 181 to 703, respectively). A similar outcome was obtained from the conventional tests appraised in this research. Eventually, it was possible to conclude that the digital analysis of 20-day-old seedlings by SVIS<sup>®</sup> is an efficient method to evaluate coffee seed vigor.

Index terms: *Coffea arabica* L., seed physiological potential, computer vision, image processing of seedlings.

## Imagens digitalizadas de plântulas na avaliação do vigor de sementes de café

**RESUMO** – A análise de imagens digitalizadas de plântulas é cada vez mais utilizada em programas de controle de qualidade de sementes devido à sua praticidade, objetividade e rapidez na obtenção dos resultados. Apesar dessas vantagens, ainda não existem pesquisas comprovando a eficiência dessa técnica para a análise do vigor de sementes de café. Assim, esta pesquisa teve como objetivo avaliar a eficiência da análise de imagens digitalizadas de plântulas pelo *Seed Vigor Imaging System* (SVIS<sup>®</sup>) na determinação do vigor de sementes de café (*Coffea arabica* L.), e compará-la com os testes convencionalmente empregados. Foram utilizadas sementes das cultivares Bourbon e Catucaí 20/15, cada cultivar representada por seis lotes. A pesquisa foi conduzida em duas épocas de avaliação e o vigor determinado por meio das avaliações de primeira contagem de germinação, envelhecimento acelerado, condutividade elétrica, velocidade de protrusão da raiz primária e análise de imagens digitalizadas de plântulas com o SVIS<sup>®</sup>. Tanto para a cultivar Bourbon como para a cultivar Catucaí 20/15, a análise SVIS<sup>®</sup> permitiu discriminar lotes com vigor mais baixo e mais alto (índices de vigor de 317 a 752, e 181 a 703, respectivamente), de maneira semelhante aos testes convencionais utilizados nesta pesquisa. Concluiu-se que a análise de imagens digitalizadas de plântulas com 20 dias de idade após a semeadura pelo SVIS<sup>®</sup> é eficiente na avaliação do vigor de lotes de sementes de café.

Termos para indexação: *Coffea arabica* L., potencial fisiológico de sementes, visão computacional, processamento de imagens de plântulas.

### Introduction

The commercial coffee planting is usually performed through seedlings obtained from seeds. Thus, the production of high-quality seeds, which comprises physical, physiological, genetic,

and sanitary features, is essential for the success of a culture.

In order to assess the physiological potential of seeds, germination and vigor tests are employed to study their behavior, when they are subjected to both optimal conditions and adverse ones (Marcos-Filho, 1999). Seed vigor evaluation

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is fundamental to select lots that germinate alike and to distinguish those with high or low performance during storage and in the field (Marcos-Filho, 2015). In coffee seeds, such characterization is particularly important, as slow germination and fast deterioration of the physiological potential of seeds are regarded as critical problems for the species.

Currently, the evaluation of coffee seed vigor is primarily conducted through tests of accelerated aging (Araujo et al., 2008; Giomo et al., 2008; Hilst et al., 2012), electrical conductivity (Hilst et al., 2012; Carvalho et al., 2012), seedling emergence (Favarin et al., 2003; Giomo et al., 2008), exudate leaching (Hilst et al., 2012), and primary root length (Araujo et al., 2008). Nonetheless, some of these tests, such as the accelerated aging and seedling emergence ones, demand a considerable amount of time to be concluded (more than 25 days); whereas others, as the electrical conductivity and exudate leaching, are faster but prone to present variations in outcome due to physical injuries that may go undetected to the unaided eye.

New procedures designed specifically for analyzing seed vigor are always welcome, especially when they have low cost and provide fast highly accurate results. On that account, the analysis of seedling growth through computer-aided methods has become more frequent in appraising seed vigor. Among the recent technologies used for such purpose, the Seed Vigor Imaging System (SVIS<sup>®</sup>), proposed by Sako et al. (2001), and the Automated Analysis of Seed Vigor System (Vigor-S<sup>®</sup>), used by Castan et al. (2018), are prominent alternatives. Both processes are based on the imaging of seedlings by a scanning device, followed by the post-evaluation through some specialized software. In SVIS<sup>®</sup>, the vigor index is determined according to the speed and uniformity of seedling development, in contrast with the maximum value estimated for seedlings at ages pre-established in the software programming. After processing the images, the software automatically generates numbers for the indices of vigor and growth uniformity (from 0 to 1000), and also establishes the length of the seedlings.

The SVIS<sup>®</sup> was already used successfully for determining the vigor of lettuce seeds (Sako et al., 2001). It was also adapted for several other species, such as soybean (Hoffmaster et al., 2003; Marcos-Filho et al., 2009), corn (Hoffmaster et al., 2005), cucumber (Chiquito et al., 2012), beans (Gomes-Junior et al., 2014), eggplant (Silva and Cicero, 2014), and other plants, as reported by Marcos-Filho (2015). In recent research featuring the rootstock citrumelo Swingle [*Citrus paradisi* Macfad. Duncan grapefruit. X *Poncirus trifoliata* (L.) Raf.], Gomes-Junior et al. (2017) proved the effectiveness of SVIS<sup>®</sup> in characterizing the vigor of seeds extracted from fruits at different maturation stages. The outcomes evidenced the potential of the computer analysis

of seedling images to assess the vigor of seeds that germinate slowly and possess intermediate tolerance to desiccation. The success of this method in obtaining results similar to those of the tests usually applied to coffee seeds supports its inclusion in seed selection programs.

The objective of this study was to check the efficacy of the method of digital analysis of images of seedlings, performed by the software Seed Vigor Imaging System (SVIS<sup>®</sup>), in determining the vigor of coffee seeds (*Coffea arabica* L.), and ultimately compare it with conventional tests.

## Material and Methods

The research was conducted in the laboratories of Seed Analysis and Image Analysis. Both belong to the Department of Crop Science at the *Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo* (USP/Esalq), Piracicaba, in the Brazilian state of São Paulo (SP).

Six coffee seeds (*Coffea arabica* L.) lots of each cultivar, Bourbon and Catucaí 20/15, with different physiological potential, were used. The experiment was carried out in two parts, one in June, and the other in October 2017. In the meantime, the seeds were packed in 0.01 mm-thick vapor-exchange-resistant polyethylene bags, which were stowed in an ambient with a temperature of 20 °C and relative air humidity of 40-45% (Van der Vossen, 1979). The following evaluations were conducted:

**Moisture content:** it was determined by the oven method at 105 °C ( $\pm 3$  °C) for 24 hours. Two 5-gram samples of seeds from each lot were tested, and the results were expressed in mean percentage (wet basis), as proposed by Brasil (2009).

**Germination:** it was assessed in four replications of 50 seeds for each lot. Seeds had their parchment manually removed and then were spread on paper towel rolls, previously moistened in a proportion of 2.5 times the dry paper weight. The rolls were then placed inside a germinator set at 30 °C for 30 days. The counts were performed 15 (first germination) and 30 days after the test setup (Brasil, 2009). The results were expressed in mean percentage of normal seedlings per lot.

**Accelerated aging:** the seeds were placed inside plastic boxes (11 cm x 11 cm x 3 cm) containing 40 mL of water. They were distributed in single layers, over a suspended metal screen. The containers were covered and kept in a BOD aging chamber set at 41 °C, with relative humidity above 90%, for 72 hours (Marcos-Filho, 1999). After that period, the moisture content of the seeds was determined, and the germination test was carried out 15 days after the original setup. The results were expressed in mean percentage of normal seedling per each lot.

**Electrical conductivity:** four replications with 25 physically

pure seeds from each lot were weighed. Then, they were subject to imbibition in plastic cups containing 75 mL of deionized water, for 24 hours at 25 °C. The electrical conductivity of the solution was measured with a Digimed conductivity meter, model DM-32. These results were expressed in  $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$  of seeds, as proposed by Carvalho et al. (2012).

**Primary root protrusion speed:** it was determined during the second trials by using four replications of 50 seeds from each lot. The seeds had the germination induced, according to the method previously described for the germination test. The evaluations comprised the count of seedlings with a primary root of at least 2 mm long, as suggested by Machado et al. (2012). Accounted seedlings were removed from the rest of the group, and the procedure was repeated daily with the remaining ones, always at the same time, until the protrusion of the primary roots ceased. At the end of the counts, the primary root protrusion speed index was calculated through the equation proposed by Maguire (1962).

**Evaluation of vigor, uniformity, and seedling length with the software Seed Vigor Imaging System (SVIS®):** some preliminary testing was carried out to check the most suitable seedling age to perform the analysis. So seedlings could be obtained, five replications of 20 seeds from each lot were used. They were distributed in two rows on the upper-thirds

of two paper towel sheets, and then covered with another one. The paper was moistened in a proportion of 2.5 times the dry paper weight, and the rolls were kept in a germinator at 30 °C, for 20 days. After that, seedlings, as well as non-germinated seeds, were transferred to a blue EVA sheet (Ethylene Vinyl Acetate), dimensioned 30 cm x 22 cm. They were digitalized with an HP Scanjet 200 device, which had been installed in an inverted position inside the aluminum box (60 cm × 50 cm × 12 cm). The scanner was set at a 100-dpi resolution and connected to a computer. The images were processed by the SVIS® software, which highlighted in red the structures of the root axis/ hypocotyl of the seedlings, and in green the non-germinated seeds (Figure 1). Some manual corrections (performed with the aid of a mouse) were necessary in cases which the software had not completely done the marking. Last, the system generated indices of seedling growth uniformity and vigor (ranging from 0 to 1000). Also, it determined the total seedling length (in centimeters), by converting 1 pixel into 0.0254 cm (Sako et al., 2001; Hoffmaster et al., 2003; Marcos-Filho et al., 2006). In the SVIS® settings, a maximum size of 1.8 inches (4.57 cm) was input for 20-day-old seedlings, as determined by the previous testing. The contribution of the values of growth and uniformity for the calculation of the vigor index was of 70% and 30%, respectively. These are

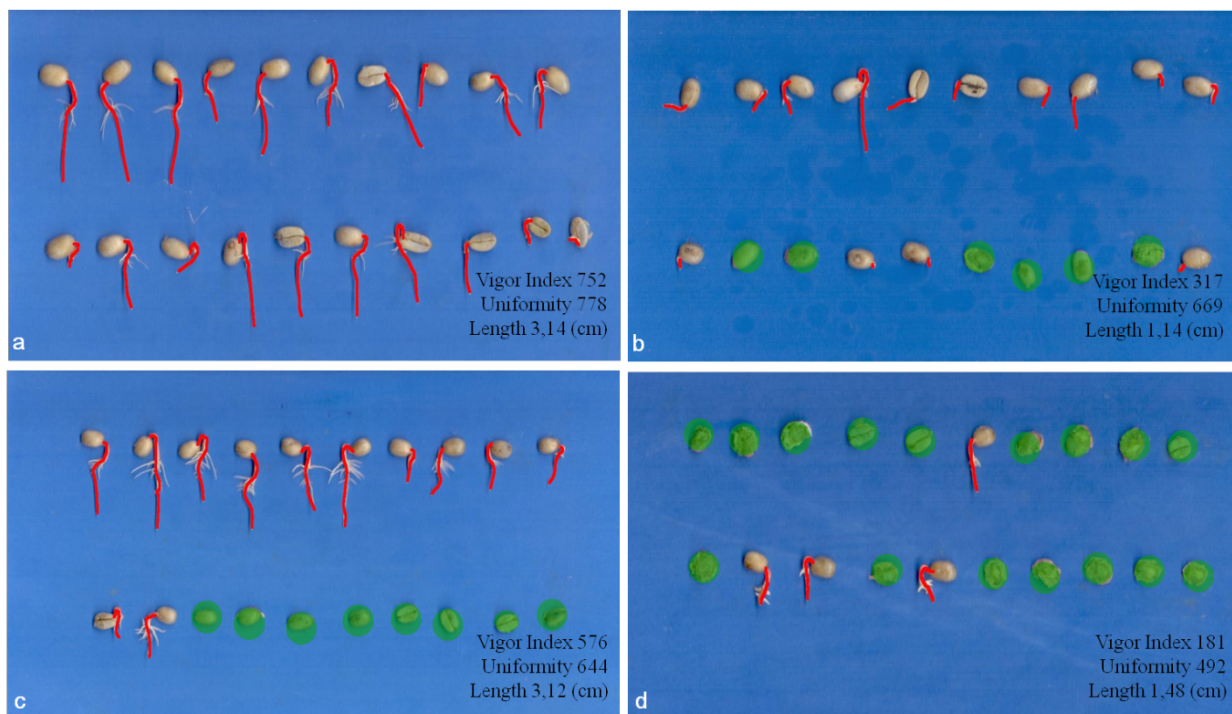


Figure 1. Vigor differences among coffee seed lots (*Coffea arabica* L.) of the cultivars Bourbon (a, b) and Catucaí 20/15 (c, d) aged 20 days, after being assessed by the software Seed Vigor Imaging System (SVIS®) – within the same experimental time. The evaluated seedlings are highlighted in red, and the non-germinated ones are colored green.

further based on the proportionality observed in the root axis/hypocotyl of each seedling.

*Statistical analysis:* the experiments were performed according to a completely randomized design, with a six-level (lots) unifactorial arrangement corresponding to the treatments. The data were subjected to the Shapiro-Wilk normality test, and also to the Bartlett test, at a 5% significance level, focused on assessing the homogeneity of the variances. The uniformity index of the Bourbon cultivar and the vigor index of the Catucaí 20/15 (both variables obtained in the second set of trials) were submitted to a logarithmic transformation. The means were compared through the Tukey's test at a 5% probability level, by using the statistic software InfoSat (Di Rienzo et al., 2008). In this case, the statistical analysis was performed for each lot individually.

## Results and Discussion

The uniformity in seed moisture content is essential for obtaining consistent results when investigating the physiological potential (Marcos-Filho et al., 1987). That being the case, in general, no alteration was perceived in the physiological behavior of the seed lots studied, even though their moisture content varied considerably between the two set of experiments. In the first one, the seeds of Bourbon cultivar showed a moisture content ranging from 32.7 to 40.7%; whereas, in the second trials, the values of this feature proved to have been reduced at different intensities in all lots (Table 1). The moisture content of coffee seed lots is highly unpredictable, once it is related to the production, processing, and storage conditions.

Regarding the germination in the first set of experiments, lots 1, 4, and 6 presented the highest percentages (above 90%), thus statistically differing from lots 3 and 5, which displayed the lowest values (82% and 71%, respectively). As for the vigor assessed by the accelerated aging and electrical conductivity tests, lot 5 had the weakest performance of all lots (Table 1).

Similar results were obtained in the second group of trials for the cultivar Bourbon. In this case, lot 5 performed worse than lots 1, 4, and 6, both in the first germination count and accelerated aging tests. Moreover, it was inferior to all the other lots in the electrical conductivity test. In addition, the primary root protrusion speed index also statistically identified lot 5 as the least vigorous one (Table 1).

One of the consequences of the seed deterioration process is a decrease in the germination speed and seedling growth rate, which tends to occur in a fast pace, prior to the reduction in the number of normal seedlings (Marcos-Filho, 2015). The guidelines of Rules for Seed Testing (Brasil, 2009) does

not take into account the evaluation of normal seedlings for determining the primary root protrusion index. In spite of that, the outcome of this work demonstrated the ability of the latter parameter in identifying differences in vigor among coffee seed lots. It is particularly advantageous as it is easier to execute and provides results faster than the first germination count. In other species, such as corn (Toledo et al., 1999; Matthews and Khajeh-Hosseini, 2006), broccoli (Martins et al., 2002), *Brassica napus* (Matthews et al., 2012), pearl millet (Machado et al., 2012), tomato (Ermis et al., 2015), and leek (Ozden et al., 2017), the primary root protrusion test was also a consistent reference to estimate seed vigor.

The vigor index and seedling length determined with the SVIS<sup>®</sup> in the first trials identified statistical differences between lot 5 (worst performance) and the others, which performed alike (Table 2). The uniformity index also classified lot 5 as the least vigorous one, but without differing it from lot 3. The results of the SVIS<sup>®</sup> analysis in the second trials were similar to those of the previous one, with lot 5 showing a vigor index inferior to that of the other lots, and a uniformity index lower than that of lots 1, 2, 3, and 4 (Table 2). All lots lacking seedling growth uniformity were perceived as less vigorous by the SVIS<sup>®</sup> (Figure 1). The uniformity index is an important indicator of seed vigor because it evaluates the seedling capacity of developing homogeneously (Marcos-Filho et al., 2009). Thus, it is a key aspect for the standardization of coffee seedling production.

Regarding the second set of trials, lot 6 presented the longest seedlings (2.88 cm), according to the SVIS<sup>®</sup>. This value is statistically bigger than that of lots 1, 2, and 5 (Table 2). Comparing the results of seedling length, as provided by the image analysis, with those obtained from the first germination count and accelerated aging tests, it could be noticed an equivalency in the classification of the seed lots. This fact evidences the sensitiveness of the SVIS<sup>®</sup> technique, which identified more subtle vigor variations among the lots during the trials. As affirmed by Guedes et al. (2009), seedling length is a more sensitive parameter for sorting lots with similar vigor, especially compared to traditional measurement methods, which are based on the number of normal seedlings in the first germination count.

The utmost goal of the vigor analysis is to identify physiological-potential differences in lots of commercially important seeds consistently. In this sense, this evaluation is a more sensitive parameter than the germination test (Marcos-Filho, 2015). By comparing the results from the first germination count, accelerated aging, electrical conductivity, and primary root protrusion speed tests (Table 1) with those obtained via computer image analysis of seedlings (Table 2), it became evident the equivalency between the methods for

sorting seed lots of Bourbon coffee in different physiological potential levels (Figures 1a, 1b), in both evaluation periods.

The computer image analysis of seedlings performed by SVIS® require the adjustments of some parameter, such as the number of seedlings per sample, contribution of the growth and uniformity values to the vigor index calculation,

seedling age, and maximum estimated length for seedlings of that age. Studies with sweet corn seeds, taking into account contributions of 30/70, 50/50, and 70/30 for the growth and uniformity values, respectively, showed that the highest weight of growth (composition 70/30) produced more consistent results (Gomes-Junior et al., 2009). On the other

Table 1. Mean values of the parameters initial moisture content (MC), germination percentage (G), first germination count (FGC), accelerated aging (AA), moisture content after accelerated aging (MC AA), electrical conductivity (EC), and primary root protrusion speed (PRPS) of coffee seeds lots of the cultivars Bourbon and Catucaí 20/15. All assessed at two different experimental times.

Cultivar	Lot	MC	G	FGC	AA	MC AA	EC	PRPS	
				%			$\mu\text{s.cm}^{-1}.\text{g}^{-1}$	index	
First experiments									
Bourbon	1	32.7	91 ab*	46 ab	80 a	40.8	8.90 a	-	
	2	36.4	85 bc	42 abc	85 a	39.9	9.15 ab	-	
	3	36.4	82 c	37 bc	80 a	40.5	8.47 a	-	
	4	40.7	93 a	37 bc	91 a	44.9	7.91 a	-	
	5	35.9	71 c	32 c	58 b	41.3	14.67 c	-	
	6	37.2	95 a	51 a	84 a	40.1	11.48 b	-	
	CV (%)	-	4.04	10.48	6.45	-	10.34	-	
	Second experiments								
		1	19.9	91 a	45 a	89 a	42	8.83 a	1.01 a
		2	18.5	84 ab	38 ab	84 ab	40.8	8.66 a	0.97 ab
		3	23.1	81 ab	35 ab	81 ab	40.8	9.15 a	0.84 c
		4	34	91 a	45 a	91 a	44.1	8.12 a	1.02 a
		5	15.3	72 b	22 b	68 b	40.4	12 b	0.63 d
	6	14.3	88 a	40 a	86 a	39.3	8.53 a	0.87 bc	
	CV (%)	-	7.02	18.79	8.85	-	8.73	5.97	
First experiments									
Catucaí 20/15	7	30.7	58 b	14 b	35 b	48.9	15.21 a	-	
	8	29.8	54 b	8 b	39 ab	50.2	16.65 a	-	
	9	31.8	89 a	66 a	40 ab	49.3	14.08 a	-	
	10	30.8	84 a	64 a	52 a	51.4	12.81 a	-	
	11	33.4	80 a	60 a	29 b	48.9	15.73 a	-	
	12	30.2	81 a	59 a	36 ab	48.9	16.15 a	-	
	CV (%)	-	7.29	13.9	18.04	-	21.92	-	
	Second experiments								
		7	21.8	37 b	8 b	23 b	38.7	23.34 b	0.45 b
		8	22.7	34 b	8 b	25 b	39.3	17.34 a	0.56 ab
		9	23.1	32 b	4 b	24 b	40.8	15.55 a	0.66 a
		10	26.7	37 b	16 a	31 b	34.3	29.32 cd	0.57 ab
		11	26.7	32 b	18 a	26 b	41.8	25.56 bc	0.63 ab
	12	21.9	51 a	18 a	58 a	38	33.49 d	0.53 ab	
	CV (%)	-	9.59	25.74	21.11	-	8.01	16.13	

\*Means followed by the same letter in the column do not differ from each other, according to the Tukey's test at a 5% probability level.

hand, in bean seeds, the compositions 30/70, 40/60, 50/50, 60/40, and 70/30 did not affect the precision of the results (Gomes-Junior et al., 2014).

Table 2. Mean values of the parameters vigor index (Vigor), uniformity index (Unif.), and seedling length (SL), determined through computer analysis of images of coffee seedlings (SVIS®) of lots Bourbon and Catucaí 20/15 cultivars. All assessed at two different experimental times.

Cultivar	Lot	Vigor	Unif.	SL
		index		cm
Bourbon	First trials			
	1	752 a*	778 a	3.14 a
	2	697 a	801 a	2.72 a
	3	633 a	756 ab	2.5 a
	4	740 a	825 a	2.75 a
	5	317 b	669 b	1.14 b
	6	696 a	792 a	2.76 a
	CV (%)	12.77	6.44	17.26
	Second trials			
	1	629 a	826 a	2.11 bcd
	2	599 a	825 a	1.99 cd
	3	649 a	803 a	2.35 abc
	4	686 a	788 a	2.68 ab
	5	382 b	676 b	1.46 d
6	691 a	754 ab	2.88 a	
CV (%)	11.2	16.16	15.14	
Catucaí 20/15	First trials			
	7	703 ab	718 a	3.32 a
	8	468 d	604 b	2.74 abc
	9	670 abc	719 a	3.09 ab
	10	772 a	742 a	3.48 a
	11	557 bcd	712 a	2.37 bc
	12	527 cd	717 a	2.18 c
	CV (%)	13.94	6.93	13.66
	Second trials			
	7	214 c	504 b	1.64 b
	8	230 bc	524 b	2.07 ab
	9	258 bc	524 b	2.45 ab
	10	334 b	536 b	1.89 b
	11	181 c	492 b	1.48 b
12	576 a	644 a	3.2 a	
CV (%)	3.7	5.86	26.9	

\*Means followed by the same letter in the column do not differ from each other, according to the Tukey's test at a 5% probability level.

To determine the age of seedlings, the usual reference is the time established for the germination count, as proposed in Brasil (2009), anticipated in one or two days. Examples that illustrate this methodology include 3-day-old soybeans (Hoffmaster et al., 2003; Marcos-Filho et al., 2009), 3-day-old sweet corn (Gomes-Junior et al., 2009; Alvarenga et al., 2012), 4-day-old cucumber (Chiquito et al., 2012), 4- or 3-day-old beans (Gomes-Junior et al., 2014), and 21-day-old Swingle citrumele (Gomes-Junior et al., 2017). However, in the present work, preliminary tests performed in seedlings aged 15 days (first germination count) revealed inconsistencies in the software analysis, mainly due to insufficient seedling growth. More consistent results were obtained with seedlings aged 21 days. In this case, the highest vigor lots showed an average seedling length of 3.14 and 3.12 cm (Figures 1a and 1c). Such outcome emphasizes the importance of adjusting the seed vigor parameters of each particular species before the automated analysis. Also, the necessity to compare the results with those produced by more conventional tests should be considered.

The moisture content of the cultivar *Catucaí* 20/15 assessed in the first experimental time varied from 29.8 to 33.4%. In the second trials, the values decreased in all cases, more or less sharply depending on the lot (Table 1). As for the germination, in the first evaluations, lots 9, 10, 11, and 12 showed the highest percentages, which statistically differed from those of lots 7 and 8 (58 and 54%, respectively). In the following set of experiments, these values were considerably reduced in all seed lots (Table 1). Such a significant decline in germination can be attributed to the sanitary conditions of the seeds, since during the test a high fungi incidence was noticed in the non-germinated seeds and abnormal seedlings. They were identified as belonging to the genera *Penicillium* ssp., *Fusarium* ssp., *Aspergillus* ssp., *Cladosporium* ssp., and *Alternaria* ssp.

According to the results from the first germination count of the first experimental time, the lots received a classification similar to that produced by the germination test. That is, the physiological potentials of lots 7 and 8 were inferior to that of lots 9, 10, 11, and 12. A similar outcome was observed in the second trials, when lots 7, 8, and 9 were assessed as the least vigorous ones. Considering the accelerated aging test, in the first analysis, lots 7 and 11 showed less vigor than lot 10, but they did not differ statistically from lots 8, 9, and 12. In the second evaluation time, lot 12 was considered as the most vigorous one. Also, the decrease in vigor appraised by the accelerated aging test was less pronounced than that detected by the first germination count (Table 1).

On the other hand, the conductivity test performed in the second set of trials classified lot 12 as less vigorous than lots 7, 8, 9, and 11. Such difference was not perceived during the

previous experiments (Table 1). The guidelines for this test suggest removing seeds with scratched tegument or injured by insects or pathogens (Vieira and Krzyzanowski, 1999). Therefore, the contradiction between the results from the second trials and those from the first germination count and accelerated aging can be attributed to the presence of seeds with damages (either caused physically or by the coffee berry borer), which somehow went undetected during sampling. These seeds are responsible for increasing the concentration of leached ions in the solution.

Concerning the primary root protrusion speed, the presence of fungi in the seeds affected the accuracy of the results. Nonetheless, they were still consistent with those obtained by other vigor tests, chiefly because the primary root protrusion speed also identified lot 7 as less vigorous than lot 9 (Table 1). The use of this test in several species has increased due to its fast-performing low-cost procedures, and the efficiency it bears in classifying the vigor of the lots. When applying different vigor tests to different species, Marcos-Filho (2015) observed that the most deteriorated seeds germinated more slowly. This behavior was remarked by the longer time taken for the primary root to project since the beginning of water absorption. The results of the present research confirmed the promising use of this parameter in coffee seed quality control programs.

The results from the SVIS® analysis were equivalent to those provided by the tests conventionally used for assessing the vigor of Catucaí 20/15 coffee seeds. In the first experimental time, lot 10 was placed among those with superior vigor by the first germination count test. It was also considered the most vigorous one, according to the accelerated aging and vigor index tests, as well as by the seedling length gauged via SVIS® (Table 2). Even though the physiological potential drastically decreased by the second trials, the results from the SVIS® analysis were consistent, evidencing the noticeable differences among the values of vigor index, uniformity, and seedling length of the lots (Figures 1c, 1d), in both occasions.

The SVIS® analysis identified lot 12 as the most vigorous one (Table 2), and so did the first germination count and accelerated aging tests (Table 1). Similarly, lots 7, 8, and 9 were included among the least vigorous, according to both the computer analysis and the conventional tests mentioned above.

Some relation between the methods was also observed in other species. Studies with citrumelo Swingle seeds also showed the equivalency between the vigor indices produced by the SVIS®, and those obtained from the first germination count (Gomes-Junior et al., 2017). Likewise, in seeds of soybean (Marcos-Filho et al., 2009), peanuts (Marchi et al., 2011), and sweet corn (Alvarenga et al., 2012), the results from the SVIS®

analysis were consistent with those provided by the accelerated aging test. In many other plants, such as corn (Hoffmaster et al., 2005; Castan et al., 2018), cucumber (Chiquito et al., 2012), bean (Gomes-Junior et al., 2014), and pearl millet (Javorski et al., 2018), the computer imaging of seedlings was also efficient in evaluating the vigor of seed lots.

The present work showed that, in comparison with conventional tests, the analysis of 20-day-old seedlings through the SVIS® software also provided results consistent enough to discriminate levels of vigor in seed lots of Bourbon and Catucaí 20/15 coffee. Therefore, this is a promising addition for seed quality control programs, once it can help to produce seedlings more efficiently. The advantages of this method include the standardization of the analyses, the increase in data reliability, and the possibility of storing data as images, instead of sheer numbers.

## Conclusions

The analysis of digitalized images of 20-day-old seedlings by the software SVIS® allows to identify vigor differences among coffee seed lots efficiently. The sensitiveness of this method is equivalent to that of the conventional tests.

## References

- ALVARENGA, R.O.; MARCOS-FILHO, J.; GOMES-JUNIOR, F.G. Avaliação do vigor de sementes de milho superdoce por meio da análise computadorizada de imagens de plântulas. *Revista Brasileira de Sementes*, v.34, n.3, p.488-494, 2012. <http://dx.doi.org/10.1590/S0101-31222012000300017>
- ARAUJO, R.F.; ARAUJO, F.E.; CECON, P.R.; VALDINEI, S. Conservação de sementes de café (*Coffea arabica* L.) despulpado e não despulpado. *Revista Brasileira de Sementes*, v.30, n.3, p.071-078, 2008. <http://dx.doi.org/10.1590/S0101-31222008000300010>
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2009. 395p.
- CARVALHO, C.A.M.; GUIMARÃES, R.M.; SILVA, T.T.A. Condicionamento fisiológico em matriz sólida de sementes de café (*Coffea arabica* L.) com e sem pergaminho. *Revista Brasileira de Sementes*, v.34, n.1, p.94-98, 2012. <http://dx.doi.org/10.1590/S0101-31222012000100012>
- CASTAN, D.O.C.; GOMES-JUNIOR, F.G.; MARCOS-FILHO, J. Vigor-S, a new system for evaluating the physiological potential of maize seeds. *Scientia Agricola*, v.75, n.2, p.167-172, 2018. <http://dx.doi.org/10.1590/1678-992x-2016-0401>



- CHIQUITO, A.A.; GOMES-JUNIOR, F.G.; MARCOS-FILHO, J. Avaliação do potencial fisiológico de sementes de pepino utilizando sistema computadorizado de análise de imagens de plântulas (SVIS®). *Revista Brasileira de Sementes*, v.34, n.2, p.255-263, 2012. <http://dx.doi.org/10.1590/S0101-31222012000200010>
- DI RIENZO, J.A.; CASANOVES, F.; BALZARINI, M.G.; GONZALEZ, L.; TABLADA, M.; ROBLEDO, C.W. InfoStat versión 2008. Argentina: Grupo InfoStat FCA Universidad Nacional de Córdoba, 2008. 331p.
- ERMIS, S.; OZDEN, E.; DEMIR, I. Early radicle emergence count after controlled deterioration (CD) predicts emergence potential better than final standard germination after CD in tomato varieties. *Seed Science and Technology*, v.43, n.2, p.278-283, 2015. <https://doi.org/10.15258/sst.2015.43.2.06>
- FAVARIN, J.L.; COSTA, J.D.; NOVENBRE, A.D.C.; FAZUOLI, L.C.; FAVARIN, M.G.G.V. Características da semente em relação ao seu potencial fisiológico e a qualidade de mudas de café (*Coffea arabica* L.). *Revista Brasileira de Sementes*, v.25, n.2, p.13-19, 2003. <http://dx.doi.org/10.1590/S0101-31222003000400003>
- GIOMO, G.S.; NAKAGAWA, J.; GALLO, P.B. Beneficiamento de sementes de café e efeitos na qualidade física. *Bragantia*, v.67, n.4, p.997-1010, 2008. <http://dx.doi.org/10.1590/S0006-87052008000400024>
- GOMES-JUNIOR, F.G.; MONDO, V.H.V.; CICERO, S.M.; McDONALD, M.B.; BENNETT, M.A. Evaluation of priming effects on sweet corn seeds by SVIS®. *Seed Technology*, v.31, n.1, p.95-100, 2009. <http://www.jstor.org/stable/23433510>
- GOMES-JUNIOR, F.G.; CHAMMA, H.M.C.P.; CICERO, S.M. Automated image analysis of seedlings for vigor evaluation of common bean seeds. *Acta Scientiarum Agronomy*, v.36, n.2, p.195-200, 2014. <http://dx.doi.org/10.4025/actasciagron.v36i2.21957>
- GOMES-JUNIOR, F.G.; ARRUDA, N.; MARCOS-FILHO, J. Swingle citrumelo seed vigor and storability associated with fruit maturity classes based on RGB parameters. *Scientia Agricola*, v.74, n.5, p.357-363, 2017. <http://dx.doi.org/10.1590/1678-992x-2016-0173>
- GUEDES, S.R.; ALVES, E.U.; GONÇALVES, E.P.; SANTOS, S.R.N.; LIMA, CR. Testes de vigor na avaliação da qualidade fisiológica de sementes *Erythrina velutina* Willd. (Fabaceae-Papilionoideae). *Ciência e Agrotecnologia*, v.33, n.5, p.1360-1365, 2009. <http://dx.doi.org/10.1590/S1413-70542009000500023>
- HILST, P.C.; DIAS, D.C.F.S.; ALVARENGA, E.M.; SOUZA, B.L. Test of exudates color hues for evaluating the physiological potential of coffee (*Coffea arabica* L.). *Revista Brasileira de Sementes*, v.34, n.2, p.212-217, 2012. <http://dx.doi.org/10.1590/S0101-31222012000200004>
- HOFFMASTER, A.L.; FUJIMURA, K.; MCDONALD, M.B.; BENNETT, M.A. An automated system for vigor testing three-day-old soybean seedlings. *Seed Science and Technology*, v.31, n.3, p.701-713, 2003. <http://cat.inist.fr/?aModele=afficheN&cpsid=15163127>
- HOFFMASTER, A.F.; XU, L.; FUJIMURA, K.; MCDONALD, M.B.; BENNETT, M.A.; EVANS, A.F. The Ohio State University seed vigor imaging system (SVIS®) for soybean and corn seedlings. *Seed Technology*, v.27, n.1, p.7-24, 2005. <http://www.jstor.org/stable/23433211>
- JAVORSKI, M.; CASTAN, D.O.C.; SILVA, S.S.; GOMES-JUNIOR, F.G.; CICERO, S.M. Image analysis to evaluate the physiological potential and morphology of pearl millet seeds. *Journal of Seed Science*, v.40, n.2, p.127-134, 2018. [http://www.scielo.br/scielo.php?script=sci\\_abstract&pid=S2317-15372018000200127&lng=pt&nrm=iso&tlng=en](http://www.scielo.br/scielo.php?script=sci_abstract&pid=S2317-15372018000200127&lng=pt&nrm=iso&tlng=en)
- MACHADO, C.G.; MARQUES, R.P.; MARTINS, C.C.; CRUZ, S.C. Precocidade na emissão da raiz primária para avaliação do vigor de sementes de milho. *Semina Ciências Agrárias*, v.33, n.2, p.499-506, 2012. <http://www.redalyc.org/articulo.oa?id=445744112007>
- MAGUIRE, J.D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, v.2, n.1, p.176-177, 1962. <https://dl.sciencesocieties.org/publications/cs/abstracts/2/2/CS0020020176>
- MARCHI, J.L.D.; CICERO, S.M.; GOMES-JUNIOR, F.G. Utilização da análise computadorizada de plântulas na avaliação do potencial fisiológico de sementes de amendoim tratadas com fungicida e inseticida. *Revista Brasileira de Sementes*, v.33, n.4, p.652-662, 2011. <http://dx.doi.org/10.1590/S0101-31222011000400007>
- MARCOS-FILHO, J.; CICERO, S.M.; SILVA, W.R. *Avaliação da qualidade das sementes*. Piracicaba: Fealq, 1987. 230p.
- MARCOS-FILHO, J. Testes de vigor: importância e utilização. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Ed.). *Vigor de sementes: conceitos e testes*. Londrina: ABRATES, 1999. cap.1, p.1.1.1-26.
- MARCOS-FILHO, J.; BENNETT, M.A.; McDONALD, M.B.; EVANS, A.F.; GRASSBAUGH, E.M. Assessment of melon seed vigour by an automated computer imaging system compared to traditional procedures. *Seed Science and Technology*, v.34, n.2, p.485-497, 2006. <https://doi.org/10.15258/sst.2006.34.2.23>
- MARCOS-FILHO, J.; KIKUTI, A.L.P.; LIMA, L.B. Métodos para avaliação do vigor de sementes de soja, incluindo a análise computadorizada de imagens. *Revista Brasileira de Sementes*, v.31, n.1, p.102-112, 2009. <http://dx.doi.org/10.1590/S0101-31222009000100012>
- MARCOS-FILHO, J. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola*, v.72, n.4, p.363-374, 2015. <http://dx.doi.org/10.1590/0103-9016-2015-0007>
- MARTINS, C.C.; SENEME, A.M.; CASTRO, M.M.; NAKAGAWA, J.; CAVARIANI, C. Comparação entre métodos para a avaliação do vigor de lotes de sementes de couve-brócolos (*Brassica olerae* L. var. italica PLENK). *Revista Brasileira de Sementes*, v.24, p.96-101, 2002. <http://dx.doi.org/10.1590/S0101-31222002000100016>
- MATTHEWS, S.; KHAJEH-HOSSEINI, M. Mean germination time indicator of emergence performance in soil of seed lots of maize (*Zea mays* L.). *Seed Science and Technology*, v.34, n.2, p.339-347, 2006. <https://doi.org/10.15258/sst.2006.34.2.09>
- MATTHEWS, S.; WAGNER, M.H.; KERR, L.; MCLAREN, G.; POWELL, A.A. Automated determination of germination time courses by image capture and early counts of radicle emergence lead to a new vigour test for winter oilseed rape (*Brassica napus*). *Seed Science and Technology*, v.40, n.3, p.413-424, 2012. <https://doi.org/10.15258/sst.2012.40.3.12>



- OZDEN, E.; MAVI, K.; SARI, E.; DEMIR, I. Radicle emergence test predicts longevity (half viability period) of leek seed lots. *Seed Science and Technology*, v.45, n.1, p.243-247, 2017. <https://doi.org/10.15258/sst.2017.45.1.06>
- SAKO, Y.; McDONALD, M.B.; FUJIMURA, K.; EVANS, A.F.; BENNETT, M.A. A system for automated vigour assessment. *Seed Science and Technology*, v.29, n.3, p.625-636, 2001. <https://www.eurofinsus.com/media/162083/seed-vigor-imaging-system.pdf>
- SILVA, V.N.; CICERO, S.M. Análise de imagens de plântulas para avaliação do potencial fisiológico de sementes de berinjela. *Horticultura Brasileira*, v.32, n.2, p.145-151, 2014. <http://dx.doi.org/10.1590/S0102-05362014000200004>
- TOLEDO, F.F.; NOVENBRE, A.D.L.C.; CHAMMA, H.M.C.P.; MASCHIETTO, R.W. Vigor de sementes de milho (*Zea mays* L.) avaliado pela precocidade de emissão da raiz primária. *Scientia Agricola*, v.56, n.1, p.191-196, 1999. <http://dx.doi.org/10.1590/S0103-90161999000100026>
- VAN DER VOSSEN, H.A.M. Methods of preserving the viability of coffee seed in storage. *Seed Science and Technology*, v.7, n.1, p.65-74, 1979. <http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=CAFE.xis&method=post&formato=2&cantidad=1&expresion=mfn=005765>
- VIEIRA, R.D.; KRZYZANOWSKI, F.C. Teste de condutividade elétrica. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇANETO, J.B. (Ed.). *Vigor de sementes: conceitos e testes*. Londrina: ABRATES, 1999. p.4.1-4.26.



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