

Test of exudates color hues for evaluating the physiological potential of coffee (*Coffea arabica* L.) seeds¹

Paulo Cesar Hilst^{2*}, Denise Cunha Fernandes dos Santos Dias²,
Eveline Mantovani Alvarenga², Bruna Luiza de Souza²

ABSTRACT - Coffee seeds have slow and irregular germination, losing fast their viability during storage, and the standard germination test of these seeds requires at least 30 days. Besides, the results may not reflect the actual physiological quality of these seeds. The objective of this work was to develop a fast and practical test for evaluating the viability of coffee seeds, which is based on the interpretation of different color hues of exudates from seeds. Coffee seeds of the cultivar Catuai 44 from six lots were submitted to germination, accelerated aging, and electrical conductivity tests. In the exudates color hue test, coffee seeds without the parchment and the silvery pellicle (four replications of 10 seeds each) were distributed on top of paper towels moistened and then maintained into a germinator, at 25 °C for 24, 48, 72, 96, and 120 h. Three classes of color hues were established: colorless, light color hue, and dark color hue, assigning the values of 0, 1, and 3, for each class, respectively. The proposed exudates color hue test can be recommended for the fast assessment of viability for coffee seeds. The most promising results were obtained for seeds with 12% moisture content, after imbibition periods of 72, 96, and 120 h; and with 30% moisture content, after imbibition periods of 72 and 120 h.

Index terms: leaching, seed quality, seed analysis, germination, vigor.

Teste de coloração de exsudatos para avaliação do potencial fisiológico de sementes de café (*Coffea arabica* L.)

RESUMO - Sementes de café têm germinação lenta e desuniforme, perdendo rapidamente a viabilidade durante o armazenamento e a condução do teste de germinação exige, pelo menos, 30 dias. Além disso, os resultados obtidos poderão não mais refletir a real condição fisiológica das sementes. Objetivou-se desenvolver um teste rápido e prático para a avaliação da viabilidade de sementes de café, baseado na interpretação das diferentes intensidades dos tons da coloração dos exsudatos das sementes. Foram utilizadas sementes de seis lotes da cultivar Catuai 44, que foram avaliadas pelos testes de germinação, envelhecimento acelerado e condutividade elétrica. No teste de coloração dos exsudatos, sementes sem o pergaminho e a película prateada (quatro repetições de 10 sementes cada) foram distribuídas sobre papel toalha umedecido e mantidas em germinador a 25 °C por 24, 48, 72, 96 e 120 h. Foram estabelecidas três classes de intensidade de tons da coloração: ausência de coloração; intensidade de coloração leve; e coloração forte, atribuindo valores de 0, 1 e 3, para cada classe, respectivamente. O teste de coloração do exsudato proposto pode ser recomendado para avaliação rápida da viabilidade das sementes de café. Os resultados mais promissores foram obtidos em sementes com 12% de umidade, após períodos de embebição de 72, 96 e 120 h; e com 30% de umidade, após períodos de embebição de 72 e 120 h.

Termos para indexação: lixiviação, qualidade, análise de sementes, germinação, vigor.

¹Submitted on 03/23/2011. Accepted for publication on 10/18/2011.

²Departamento de Fitotecnia, Universidade Federal de Viçosa, 36570-000 - Viçosa, MG, Brasil.

*Corresponding author <pchilst@yahoo.com.br>

Introduction

The propagation of the arabica coffee plant is done by means of seedlings, which are developed from seeds that commonly present slow and irregular germination, besides rapidly losing their viability during storage.

These facts may bring problems to seed producers, once the official method for evaluating the quality of commercial coffee seed lots is the germination test, which demands at least 30 days (Brasil, 2009) to provide results that may not reflect the actual physiological conditions of those seeds.

Considering these facts, the need for tests allowing a fast evaluation of the physiological condition of such seeds has increased, thus guaranteeing a more precise decision making about commercialization or disposal of seed lots.

The tests for fast evaluation of seed viability or vigor are fundamental techniques to substantiate the prompt decision making during the different steps of seed production, especially between the maturation phase and the future sowing in the field. Furthermore, they represent important components in seed quality control programs, allowing for the optimization of information achievement upon the disposal of low quality seed lots during the reception procedure at the processing unit and the streamlining of the management with a more efficient use of the infrastructure available (Marcos-Filho, 2005).

Abdul-Baki and Anderson (1973) reported that the physiological quality evaluation of seeds, by means of tests requiring short periods of time, is correlated with the seed enzymatic and respiratory activities or with the integrity of the cellular membranes. According to Delouche (1975), the seed deterioration process begins with the loss of selective permeability of the cellular membranes and ends with the loss of the germination capacity.

Some fast tests can be used for evaluating the physiological quality of coffee seeds as an alternative to the germination test, being highlighted the LERCAFÉ test (Reis et al. 2010; Zonta et al., 2008), the tetrazolium test (Lopez, 1988; Dias and Silva, 1998), and the electrical conductivity test (Costa and Carvalho, 2006).

Based on the sodium hypochlorite antioxidant and bleaching properties Reis et al. (2010) developed a fast test for evaluating the quality of coffee seeds denominated LERCAFÉ, which is performed by immersing the seeds in a sodium hypochlorite solution followed by visual evaluation of the color displayed on the region close to the seed embryos. Low quality seed displayed embryos with a greenish color, which did not occur in the high quality seeds.

The tetrazolium test is stressed as the most used for the fast evaluation of coffee seeds quality, being based on the activity of dehydrogenase enzymes. Although successfully used, this method presents as an inconvenience the difficulty for the embryo extraction, which requires accurate technique and a well trained seed analyst (Vieira et al., 1998).

Based on the cellular membranes reorganization capacity during imbibition (Matthews and Powell, 1981), the electrical conductivity test although very much used for evaluating seed vigor of several different species, has not presented consistent results for coffee seeds. Costa and Carvalho (2006) were able to classify seed lots into different levels of physiological quality by using this method for coffee seeds. For Soto et al. (1995), the electrical conductivity test was more efficient in evaluating the viability of coffee seeds than the tetrazolium test. According to Malta et al., (2005), however, the electrical conductivity values are significantly influenced by the presence of defective seeds or seeds mechanically damaged, thus favoring an inadequate interpretations for the quality of the seed lots analyzed.

On carrying out the electrical conductivity test on coffee seeds, differences have been observed on the hue of colors of the lixiviation from seeds of different lots. Some of them presented a light brown hue while others had a dark brown hue. This finding emphasized the possibility of trying to associate this characteristic with the physiological quality of the seeds. In a study with exudates of coffee seeds Sera and Miglioranza (2000) observed that after a 12 h imbibition period, the low quality seed lots generally generated exudates of darker hue on moistened paper towels, what can be an indicative of the physiological quality level of the seed lots. The brown exudate on the paper towel may be associated to the loss of substances lixiviated during the seed deterioration process (Sera e Miglioranza, 2006). These same authors observed a negative correlation between the hues of the seed exudates and the seed germination, after different imbibition periods.

Considering that the evaluation of the exudates hues has as principle the permeability of the membranes system, such characteristic (hues of the color of exudates) can constitute a promising alternative for the fast quality evaluation of coffee seeds. Therefore, information concerning the methodology and the applicability of the test of color hues of exudates for coffee seeds as well as its validation for application on commercial seed lots constitute important aids to the sector of seed production of this plant species.

The objective of the present research was to develop a

fast and practical test for evaluating the physiological quality of coffee seeds based on the evaluation of the intensity of the color hues of seed exudates, with improvements on the already available methodology.

Material and Methods

The research was carried out at the Departamento de Fitotecnia, at Universidade Federal de Viçosa. Six different seed lots of coffee (*Coffea arabica* L.) seeds, cv. “Catuaí 44”, were used for the experiment. The seeds of each lot were maintained under laboratory environmental conditions during all the experimental period. For characterization of the physiological quality of the seed lots, the following tests were performed:

Moisture content: after removal of the parchment from the seeds, the oven method at $105\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ was used in two subsamples of 30 seeds each, per treatment, according to the Rules for Seed Analysis (Brasil, 2009). Results were expressed in percent wet basis.

Germination: four subsamples of 50 seeds each (without the parchment) were arranged on top of four sheets of paper towels moistened with sterile distilled H_2O , in the proportion of 2.5 times the mass of the dry substrate. The paper towel sheets were then turned into rolls, which were maintained into a seed germinator at $30\text{ }^{\circ}\text{C}$ (Brasil, 2009). The evaluations were performed at 21 and 30 days after starting the test, and the results were expressed as the mean percent of normal seedlings for each seed lot.

Accelerated aging: approximately 250 coffee seeds without the parchment were arranged on top of aluminum mesh trays, which were then attached to the top of germination boxes (gerbox) containing 40 mL of sterile distilled H_2O at the bottom. Immediately after, the gerbox were lidded and maintained into BOD type incubators set at $45\text{ }^{\circ}\text{C}$ for 72 h. After that period, four subsamples of 50 seeds each were placed to germinate as already described for the germination test (Brasil, 2009) evaluating the percent normal seedlings obtained at the 21st day after sowing.

Electrical conductivity: the methodology reported by Vieira e Krzyzanowski (1999) was adopted for this test, in which eight subsamples of 50 seeds each were used. Each subsample was weighed on a 0.001 g precision scale and then placed into plastic cups containing 75 mL of distilled water which were afterwards maintained into a BOD incubator at $25\text{ }^{\circ}\text{C}$ for 24 h. The electrical conductivity was determined by a conductivimeter. Results were expressed in $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ of seeds.

For the adequacy of the methodology of the test of color hues, four coffee seed samples, with 10 seeds each, were used for each seed lot. Those seeds were immersed in distilled H_2O , for approximately 10 min., for the careful removal of the silvery pellicle with the aid of tweezers for not damaging the seed. Immediately after that procedure, the seeds were arranged (with the flat side in contact with two sheets of paper towels moistened with sterile distilled H_2O , in the proportion of 2.5 times the mass of the dry substrate) and placed on top of aluminum mesh screens coupled to a gerbox containing 40 mL of sterile distilled H_2O at the bottom. The gerbox were then lidded and placed into a germinator at $25\text{ }^{\circ}\text{C}$, for 24, 48, 72, 96, and 120 h. After each period of time, the seeds were moved from their original position within the gerbox for the visualization of the color hue of the exudate formed under each seed. Three intensities of color hues were established: colorless; light color hue; and dark color hue; assigning the values of 0, 1, and 3 to each class, respectively (Figure 1). Starting from the sum of the values obtained in each replication, the mean values of each seed lot were obtained. After each evaluation, the seeds were returned to their original position and the gerbox was maintained inside the germinator until the next evaluation moment.

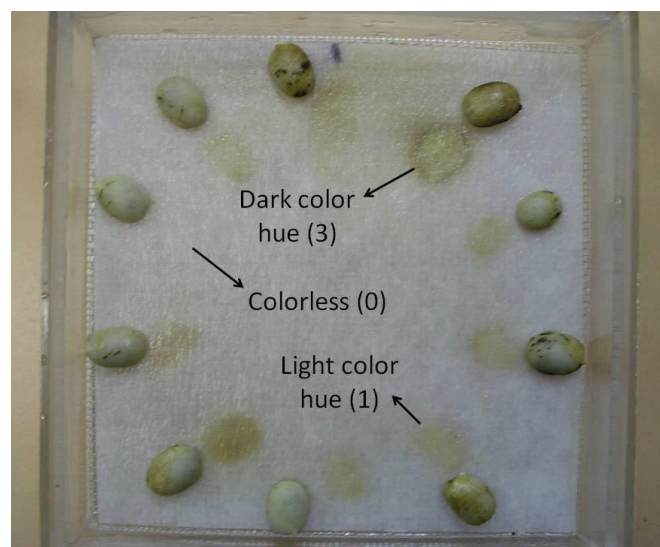


Figure 1. Illustration of the intensity of the color hues of exudates from coffee (*Coffea arabica* L.) seeds after a 24 h imbibition period attributing the values of: 0 (colorless); 1 (light color hue); and 3 (dark color hue) for high, median and low physiological quality of those seeds, respectively.

A completely randomized experimental design with four replications was used in the experiment. The treatment means were compared by the Tukey test at 5% probability.

Results and Discussion

The moisture content of seeds from all the six lots of seeds varied from 11.8% to 12.3% (Table 1). The percent germination of seeds from lot 1 was significantly higher than those obtained for the lot 3, and the remaining seed lots presented intermediary physiological quality. In the accelerated aging test, the seed lots 4 and 6 were classified as more vigorous in relation to the lot 3, which was identified as the one that presented the

worst physiological quality; and the remaining seed lots presented an intermediary position. Therefore, both the germination test and the accelerated aging test were coherent on the indication of the seed lot 3 as the one with the worst physiological quality. Higher values of electric conductivity were obtained for the lots 1 and 6 followed by the lot 3, thus indicating lower seed vigor. These seed lots had lower performance than the lots 2 and 4, indicated as having the best physiological quality and that presented the lowest ions lixiviation. It can also be verified that the seed lot 3 (indicated by the tests of germination and accelerated aging as the one with the worst quality) was also classified as of lower quality by the electric conductivity test results.

Table 1. Mean values obtained in the determination of the moisture content (MC) and in the tests of germination (TG), electrical conductivity (EC), and accelerated aging (AA) of coffee seeds from six lots.

Seed lot	MC (%)	TG (%)	EC ($\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$)	AA (%)
1	11.8 B*	84.5 A*	23.53 A*	54.5 AB*
2	12.0 AB	78.5 AB	12.67 C	55.0 AB
3	12.2 A	66.0 B	20.76 AB	41.5 B
4	12.2 A	83.3 AB	11.78 C	69.5 A
5	11.8 B	80.5 AB	17.19 B	53.5 AB
6	11.9 B	74.5 AB	21.48 A	64.0 A
CV (%)	0.85	10.36	9.38	13.36

*Means followed by the same letter within the column are not statistically different by the Tukey test at 5% probability.

It has to be emphasized, however, that the information obtained with the electric conductivity test for coffee seeds have been conflicting. Costa and Carvalho (2006) were able to sort coffee seed lots into different levels of physiological quality by using the individual method in the evaluation of the electric conductivity. Differently, Malta et al. (2005) did not obtain consistent information when assessing the electric conductivity of seeds of that species; what had also been found by Lima et al. (2003). The disorganization of the cellular membranes of defective coffee seeds allows for the easier penetration and diffusion of water within the seed (Illy et al., 1982), factors that are also related to the electric conductivity of seeds.

Therefore, it can be verified that there has been coherence among the tests results shown on Table 1, in relation to the indication of the seed lot 3 as the one with the worst physiological quality; although the seed lots 1 and 6 were also pointed as having low quality by the electric conductivity test. Nevertheless, concerning the

seed lot of best quality, it is possible to affirm that none of the seed lots significantly differed among each other by analyzing the results of the tests of seed germination and accelerated aging (Table 1). By the electrical conductivity test, however, the seeds with better physiological quality were those from the seed lots 2 and 4, followed by the ones from seed lot 5 (Table 1).

By analyzing the data obtained in the test of color hues of exudates it is noticeable that for all imbibition periods evaluated it is possible to sort the seed lots into different levels of physiological quality (Table 2). It can yet be observed that for all the imbibition periods the seed lot number 6 did not statistically differed from the seed lots 1, 2, 3, and 5, but presented figures significantly higher than the seed lot 4, which presented the best performance in relation to physiological quality. The seeds of this lot 4 presented lower proportion of exudates with dark brown hue. Within the periods of 48 and 72 h, the seed lots 1, 3, and 6 were classified as the ones with the worst physiological quality

and were significantly inferior to seed lot 4 (with the best performance) (Table 2). These results are similar to those obtained with the electric conductivity test (Table 1). It can also be observed in that table that the seed lot 4 was classified among the lots of better physiological quality considering

all the quality evaluation tests used in the experiment. The coffee seeds of this lot presented germination of 83.3%, high vigor by the accelerated aging test, and the lowest figure for electric conductivity, which indicate that this lot has a high physiological quality (Table 1).

Table 2. Mean values obtained starting from the sum of the values attributed to each seed evaluated by the test of color hues of exudates from coffee seeds of six lots, after five different imbibition periods.

Seed lot	Imbibition periods				
	24h	48h	72h	96h	120h
1	7.3 AB*	8.5 A*	10.5 A*	10.8 AB*	11.0 AB*
2	4.5 AB	4.8 AB	6.8 AB	7.8 AB	7.8 AB
3	7.8 AB	8.5 A	10.3 A	11.0 AB	11.8 A
4	0.0 B	0.0 B	0.0 B	1.5 B	2.0 B
5	4.5 AB	5.8 AB	5.8 AB	6.3 AB	6.8 AB
6	9.3 A	10.8 A	10.8 A	11.3 A	11.8 A
CV (%)	36.82	42.43	27.32	18.17	16.53

*Means followed by the same letter in the column are not statistically different by the Tukey test at 5% probability.

In reference to the worst performance, it is possible to affirm that through the tests of germination and accelerated aging the lowest values were obtained for seeds from the lot 3. This seed lot was also included among those of worse physiological quality in the test of electric conductivity and in the test of exudates color hues; and in the same tests, the seeds lots 1 and 6 (48 and 72 h imbibition) and the seed lot 6 (120 h imbibition) were also identified as of inferior quality. Low quality seeds exhibit exudates of darker hues or brown. According to Sera e Miglioranza (2006), the brown exudate may be linked to the lixiviation of soluble substances during the seed deterioration process. The authors observed negative correlation of the color hues of the exudates from seeds after different imbibition periods with the germination, i.e. darker hues exudates were associated to lower seed germination capacity. In general, the higher the seed vigor the larger will be the capacity of reestablishing the cellular membranes integrity during imbibition and lower will be the amount of lixiviates liberated for the external medium (Matthews e Powell, 1981). In the specific case of coffee seeds the larger lixiviation is also related to the alterations of the exudates hues, i.e. exudates more concentrated tend to produce hues of more intense brown.

By the results obtained and according to the observations of Sera e Miglioranza (2006) it is possible to state that there is an actual association between the exudates color hues and physiological quality of coffee

seeds. Nevertheless, to classify coffee seeds into lots of different levels of physiological quality, there is the need of improving the studies concerning the test of exudates color hues, in order to achieve more consistent results allowing for a reliable differentiation among seed lots of high, median and low physiological quality. Based on the periods of seed imbibition studied it is also possible to obtain information on the physiological quality of coffee seeds already with an imbibition period of 24 h. This would allow for that the decisions referring to post-harvest management and to commercialization of the seed lots could be taken before the results of the germination test were issued, which takes at least 30 days (Brasil, 2009).

The need for adjusting the methodology, however, especially on results evaluation criteria, still persists since that may not be possible to differentiate seed lots that could have distinct quality levels and that could present different proportions of seed qualities within each class but that, at the end, would present the same result.

Conclusions

There is actually a negative correlation between the color hues of exudates from coffee seeds and their physiological quality; and the test of exudates color hues is promising for a fast evaluation of the viability of these seeds;

It is possible to classify coffee seed lots into high and

low physiological quality using the test of exudates color hues after a 24 h imbibition period and the subsequent distribution of these seeds on H₂O moistened paper towel sheets. For a more efficient separation of the seed lots into levels of quality, however, there is still the need of further adjustments on the evaluation criteria.

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