

Suitability of the electrical conductivity test and antioxidant enzyme activity to assess the vigor of lentil (*Lens culinaris* Medik.) seeds

Adequação do teste de condutividade elétrica e atividade de enzimas antioxidantes para avaliar o vigor de sementes de lentilha (*Lens culinaris* Medik.)

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Highlights

There is a demand for fast and efficient vigor tests for lentil seeds.

These tests are relevant to speed up lot quality control.

The electrical conductivity test is efficient for classifying the vigor of lots.

The activity of antioxidant enzymes is related to physiological potential.

Abstract

Adapting methodologies that enable the assessment of lentil seed vigor is of utmost importance to ensure the marketing of lots with outstanding field performance. This study aimed to define the appropriate conditions for conducting the electrical conductivity test for lot classification according to vigor and assess whether the activity of antioxidant enzymes is related to the physiological potential of lentil seeds. For this, seeds from seven lots of the cultivar Silvina were subjected to different tests to characterize their initial quality, and the results were compared with those obtained in the electrical conductivity test and enzyme activity. Four soaking periods (4, 8, 12, and 24 h) associated with three water volumes (75, 100, and 150 mL) at 25 °C were used to assess electrical conductivity. The SOD, CAT, and APX enzymes were also assessed. The electrical conductivity test and enzyme activity were efficient in assessing the physiological potential with information related to other vigor tests. The electrical

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conductivity test conducted with 50 seeds immersed in 75 mL of water at 25 °C for 24 h allowed the separation of lots into vigor levels. The activity of CAT, SOD, and APX enzymes can be related to the physiological potential of lentil seeds.

Key words: Physiological potential. Methodology. Soaking period. Antioxidant system. Enzyme activity.

Resumo

Adequar metodologias que possibilitem avaliar o vigor das sementes de lentilha é de suma importância para garantir a comercialização de lotes com ótimo desempenho em campo. Objetivou-se definir as condições adequadas para a realização do teste de condutividade elétrica para classificação de lotes quanto ao vigor, como também avaliar se a atividade de enzimas antioxidantes possui relação com o potencial fisiológico das sementes de lentilha. Para tanto, sementes de sete lotes cv. Silvina foram submetidos a diferentes testes para a caracterização da sua qualidade inicial e os resultados comparados com os obtidos no teste de condutividade elétrica e atividade enzimática. Foram utilizados para avaliar a condutividade elétrica quatro períodos de embebição (4, 8, 12 e 24 h), combinados com três volumes de água (75, 100 e 150 mL) sob temperatura de 25 °C. Foram realizadas também a avaliação das enzimas SOD, CAT e APX. O teste de condutividade elétrica e o de atividade enzimática foram eficientes para a avaliação do potencial fisiológico com informações relacionadas com outros testes de vigor. O teste de condutividade elétrica conduzido com 50 sementes imersas em 75 mL de água, a 25 °C, por 24 h permite a separação dos lotes em níveis de vigor. A atividade das enzimas CAT, SOD e APX pode ser relacionada ao potencial fisiológico das sementes de lentilha.

Palavras-chave: Potencial fisiológico. Metodologia. Período de embebição. Sistema antioxidante. Atividade enzimática.

Introduction

Lentil (*Lens culinaris* Medik.) has been gaining worldwide prominence, concerted efforts to promote its consumption owing to its high nutritional value, being rich in proteins, carbohydrates, fiber, and vitamins (Merga & Haji, 2019; Nascimento & Silva, 2019).

In Brazil, the crop has shown great potential in the Midwest, South, and Southeast regions as a great option for winter or second-crop cultivation (Nascimento & Silva, 2019). Thus, the demand for seeds has been increasing. In this context, the definition of appropriate methodologies to evaluate the physiological potential of lentil seeds is important to ensure high-quality standards.

The electrical conductivity test stands out as a quick, practical, and efficient method for assessing seed vigor. It is a biochemical test based on the ability to reorganize the cell membrane system, which is one of the first manifestations of the seed deterioration process (Vieira & Marcos, 2020). Thus, determining the electrical conductivity value of the seed soaking solution indirectly allows information to be obtained about the intensity of damage caused to cell membranes resulting from the deterioration process (Powell, 1986). Less vigorous seeds release a higher amount of solutes, as the speed of membrane restructuring during the soaking process is slower (Torres et al., 2015).

Although the electrical conductivity test is simple and easy to perform, some factors such as water volume for soaking seeds, soaking time, number of seeds per replication, and temperature influence the results (Figueiredo et al., 2021; Vieira & Marcos, 2020). For many species, the test is performed with samples of 50 seeds immersed in 75 mL of distilled or deionized water at 25 °C for 24 hours (Vieira & Marcos, 2020). This procedure is mainly recommended for efficiently assessing the vigor of large-seed legumes such as peas, soybeans, and beans (Baalbaki et al., 2009; Marcos, 2015). However, Dias and Marcos (1996) observed that shorter soaking periods for soybeans (8 and 12 hours) allowed the identification of more pronounced differences in vigor between lots, while evaluations conducted after 16 hours were more sensitive, allowing more detailed classification of lots into vigor levels. Machado et al. (2011) assessed temperatures of 20 and 25 °C and a volume of 75 mL of water in pea seeds and concluded that the test conducted at 25 °C for 24 hours was adequate for differentiating lots.

J. O. Araújo et al. (2022) tested the suitability of the electrical conductivity test for chickpea seeds with 75, 100, and 150 mL for periods of 4, 8, 12, and 24 hours at 25 °C and recommended the use of 50 seeds immersed in 150 mL of water for 24 hours. Moreover, Figueiredo et al. (2021) indicated that the test for linseed should be conducted with 25 seeds in 75 mL of water at 30 °C.

Silva et al. (2020) evaluated electrical conductivity in lentil seeds after soaking for 3, 6, 9, 12, and 15 hours in 75 mL at 25 °C and concluded that the 12-hour soaking period was the most appropriate. However, the authors did not test longer soaking periods

to facilitate analyses during business hours in routine laboratories and did not test other water volumes for test adequacy. Therefore, testing different combinations of soaking periods and water volumes is important.

Considering that the disruption of the membrane system is due to the formation of reactive oxygen species (ROS), which cause lipid peroxidation, protein oxidation, and enzymatic changes (Kumar et al., 2015), the determination of enzyme activity of the antioxidant system can also provide interesting information about the physiological potential of seeds and may be related to the results of electrical conductivity. The enzymes superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), and ascorbate peroxidase (APX) stand out, playing a fundamental role in neutralizing the harmful effects of ROS (Ebone et al., 2019).

J. O. Araújo et al. (2021) found that the activity of the CAT and APX enzymes in chickpeas is related to the physiological seed potential. Also, Bandeira et al. (2014) reported that the evaluation of the activity of the SOD, CAT, and APX enzymes allowed the separation of lots of soybean seeds according to vigor.

Thus, this study aimed to adapt the electrical conductivity test methodology for lentil seeds and assess whether the activity of antioxidant enzymes is related to the physiological potential of the seeds.

Material and Methods

The research was conducted at the Laboratory of Seed Research of the Department of Agronomy of the Federal University of Viçosa, in Viçosa, Minas Gerais,

Brazil. Seven lots of lentil seeds of the Silvana cultivar supplied by Embrapa Vegetables were used. The seeds from each lot were first characterized regarding their physiological potential by the following tests:

Degree of moisture (DM): determined by the oven method at 105 °C for 24 hours, with two replications of 50 seeds (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009).

Germination (G) and first germination count (FGC): four replications of 50 seeds were distributed on paper towels moistened with water in an amount 2.5 times the weight of the dry paper. Rolls were formed and kept in a seed germinator at 20 °C. The percentage of normal seedlings obtained on the fifth day after sowing was calculated for the first germination count, while germination was determined by the percentage of normal seedlings 10 days after sowing (MAPA, 2009).

Emergence (E) and seedling emergence rate index (ERI): conducted in a growth chamber with four replications of 50 seeds sown 1.0 cm deep in plastic trays containing a mixture of soil and sand in a proportion of 1:2 moistened until reaching 60% of retention capacity (MAPA, 2009). Daily counts of the number of seedlings that emerged were performed until the stand was completely stabilized to calculate the percentage of emergence and ERI, according to Maguire (1962).

Seedling dry mass (SDM): performed with four replications of 20 seedlings. The cotyledons were removed using a scalpel and the seedlings from each replication were placed in paper bags and kept in an air circulation oven at 65 °C for 72 hours. After drying, the samples were taken from

the oven and placed in a desiccator for 30 minutes until weighed to avoid moisture gain or loss. They were then accurately weighed on a scale (0.0001 g) and the obtained weight was divided by the number of seedlings in each replication, with the result expressed in mg seedling⁻¹ (Krzyzanowski et al., 2020).

Cold test (CT): conducted similarly to the germination test by adding 60 mL of soil over the seeds after sowing. The rolls were placed in plastic bags and kept in a BOD at 10 °C for seven days (Cicero & Vieira, 2020). After this period, they were taken from the plastic bags and kept in a seed germinator at 20 °C for five days to evaluate the percentage of normal seedlings (Marcos, 2015).

Assay I - suitability of the electrical conductivity test to evaluate the vigor of lentil seeds

Electrical conductivity (EC): four replicates of 50 seeds were weighed (0.001 g precision) and placed in plastic cups containing the respective volumes of distilled water: 75, 100, and 150 mL. The cups were kept in a BOD incubator at 25 °C for 4, 8, 12, and 24 hours. Electrical conductivity was evaluated after each period using a conductivity meter (Digimed CD 21) and the results were expressed in seeds $\mu\text{S cm}^{-1} \text{g}^{-1}$ (Vieira & Marcos, 2020).

Assay II - Quantification of the activity of antioxidant enzymes

Four replications with 50 seeds from each lot were placed to soak in a moistened paper towel roll and kept in a germinator at 20 °C for 16 hours. The seed coat was

removed using a scalpel after this period. Subsequently, the plant material was frozen in liquid nitrogen and lyophilized. Then, the lyophilized material was macerated in a ball mill, stored in Eppendorf tubes, and maintained inside a desiccator to prevent moisture loss until the enzyme activity was quantified. The activity of the superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymes was quantified by absorbance readings on a Multiskan GO microplate spectrophotometer.

Crude enzyme extracts, used to determine the activity of antioxidant enzymes, were obtained employing 0.1 g of plant material to which 2 mL of extraction medium, potassium phosphate buffer (0.1 M, pH 6.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulphonyl fluoride (PMSF), and 1% polyvinylpyrrolidone (PVPP) were added (p/v) (Chaffai et al., 2005). Then, centrifugation was performed at 15,000 xg for 15 minutes at 4 °C to remove the oil layer from the supernatant. The protein contents of enzyme extracts were determined by the method of Bradford (1976), using BSA as standard. An aliquot of 2.5 µL of the enzyme extract was added to 1 mL of the Bradford reagent, followed by stirring. The absorbance of the sample was read after 20 minutes using a spectrophotometer at 595 nm.

SOD was determined according to the protocol proposed by Del Longo et al. (1993) by adding the crude enzyme extract to a reaction medium consisting of 50 mM sodium phosphate buffer, pH 7.8, containing 13 mM methionine, 75 µM p-nitro blue tetrazolium (NBT), 0.1 mM EDTA, 2 µM riboflavin, and 50 µL of enzyme extract. The reaction was carried out and SOD activity was defined as

the amount of enzyme necessary to inhibit NBT photoreduction by 50% (Beauchamp & Fridovich, 1971). The results were expressed as $\text{U min}^{-1} \mu\text{g}^{-1}$ protein.

CAT was determined according to the protocol proposed by Havir and Mchale (1987) by adding the crude enzyme extract to the reaction medium, consisting of 50 mM potassium phosphate buffer, pH 7.0, 12.5 mM H_2O_2 , and 100 µL of enzyme extract. Enzyme activity was calculated using the molar extinction coefficient of $36 \text{ M}^{-1} \text{ cm}^{-1}$ (Anderson et al., 1995), and the results were expressed as $\mu\text{mol min}^{-1} \text{ mg}^{-1}$ protein.

APX was determined by adding 150 µL of crude enzyme extract to 3 mL of reaction medium consisting of 50 mM potassium phosphate buffer, pH 7.8, 0.25 mM ascorbic acid, 0.1 mM EDTA, and 0.3 mM H_2O_2 . Enzyme activity was calculated using the molar extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ (Nakano & Asada, 1981), and the results were expressed as $\text{nmol min}^{-1} \text{ mg}^{-1}$.

Experimental design and statistical analysis

A completely randomized experimental design was used. For each lot, physiological characterization data and data from Assay II (enzyme activity) were subjected to analysis of variance, and the means were compared using the Tukey test at the 5% probability. For Assay I, electrical conductivity test data were analyzed in a triple factorial scheme (lots x water volumes x soaking periods). The means of the qualitative factors for each treatment were compared using the Tukey test at 5% probability. Polynomial regression analysis was performed for quantitative factors. Pearson's

linear relationship coefficient (r) was applied to the variables of the initial physiological characterization and all combinations of the electrical conductivity test. PCA was also performed for Assay II using data from the SOD, CAT, and APX enzymes and data from the characterization of the initial physiological quality. The statistical software R 4.1.1 (R Core Team [R], 2022) was used for all analyses.

Results and Discussions

Characterization of the initial quality of lentil lots

Table 1 shows the data relating to the characterization of the initial physiological quality. The degree of moisture of the different lentil lots was uniform, varying from 13.1 to 13.4%. This trait is essential, as it is essential to standardize moisture so that assessments are standardized and results are consistent (Marcos, 2015; Worma et al., 2019).

Table 1

Characterization of the initial quality of seven lots of lentils of the cultivar *Silvina* analyzed by the degree of moisture (DM), germination (G), first germination count (FGC), emergence (E), emergence rate index (ERI), seedling dry mass (SDM), and cold test (CT)

Lot	DM (%)	G (%)	FGC (%)	E (%)	ERI (index)	SDM (mg seedling ⁻¹)	CT (%)
1	13.16	95 b	53 c	91 a	10.43 a	0.12 ab	91 ab
2	13.09	92 b	63 bc	88 b	9.66 b	0.11 ab	89 ab
3	13.19	83 c	58 c	72 c	8.08 c	0.08 c	74 c
4	13.39	94 b	70 b	91 a	10.84 a	0.10 b	92 ab
5	13.15	99 a	86 a	92 a	10.13 a	0.13 a	99 a
6	13.41	94 b	89 a	85 b	9.29 b	0.10 b	94 a
7	13.20	91 b	71 b	92 a	10.29 a	0.11 ab	87 b
F	-	34.29*	22.29*	8.9*	17.73*	88.39*	21.73*
CV (%)	-	1.82	8.09	5.44	4.45	8.5	3.67

* = significant by the F-test at a 5% probability; F = calculated F value; CV = coefficient of variation. Means followed by the same letter in the column do not differ from each other by the Tukey test at a 5% probability.

The germination of seeds from lot 5 was higher than that of the other lots, followed by lots 1, 2, 4, 6, and 7, while lot 3 stood out with the lowest percentage of germination. The evaluation of the first germination count showed that lots 6 and 5 presented the

highest percentages (above 80%), followed by lots 4 and 7, with lower values for lots 1 and 3 although not differing from lot 2. The first count is considered a complementary test to the germination test, consisting of an indication for classifying lots regarding

vigor, expressing differences in germination rate between lots (Krzyzanowski et al., 2020). Thus, differences in the performance of lots 1, 2, 4, 6, and 7 not observed by the germination test could be detected when the germination rate was assessed (Table 1).

The results of seedling emergence for the physiological potential classification of lots were similar to those of the emergence rate index (ERI), with lots 1, 4, 5, and 7 being followed by lots 2 and 6, considered intermediate, and lot 3 presenting the worst performance. The seedling dry mass data from different lots had better performance for lot 5 and lower value for lot 3, with lots 4 and 6 showing an intermediate position. In general, lots whose seedlings have higher dry mass content have higher vigor, indicating a higher transfer of dry mass from the seed reserve tissue to the embryonic axis. On the other hand, lots that do not show this efficiency are characterized as lower-vigor lots (Krzyzanowski et al., 2020). The cold test allowed the separation of lots 5 and 6 with the best performances under stress caused by low temperatures, which were superior to lots 7 and 3 (Table 1).

In general, based on the tests used for the initial characterization of different lots in terms of physiological potential, lot 5 was among those with the best performance, while lot 3 was among the worst performing, with some variations regarding medium vigor lots depending on the test (Table 1). The use of lots with different levels of physiological potential is fundamental in studies on evaluating seed vigor, especially when it is intended to define or test new methodologies

for this purpose. New methodologies should be tested to allow classifying lots in terms of vigor similar to that obtained in tests already established for the species under study.

Assay 1 - Suitability of the electrical conductivity test to assess the vigor of lentil seeds

A comparison of the performance of different lots of lentil seeds in different soaking periods and water volumes (Table 2) showed the separation of lots relative to their physiological potential with 75 mL within the 4 hours of soaking. Furthermore, lot 3 showed the lowest vigor, as observed by the initial physiological potential characterization tests (Table 1). Regarding the best-performing lots, lot classification was not so clear, with variations depending on the time up to 12 hours of soaking. However, the best performance was observed after 24 hours for lot 5, followed by lots 1, 7, and 4. Similar results were also observed for the 100-mL volume. Lot 3 showed the worst performance at all times tested in the 75 mL and 100 mL volumes, with electrical conductivity values significantly higher than those of the other lots. According to the principle of this test described by Vieira and Marcos (2020), it indicates higher disorganization of the cell membrane system. In this context, water volumes of 75 and 100 mL were efficient in separating the different vigor levels. Furthermore, lots of low-vigor lentil seeds can be identified using the electrical conductivity test with 4 hours of soaking.

Table 2
Electrical conductivity ($\mu\text{S cm}^{-1} \text{g}^{-1}$) of lots of lentil seeds under different soaking periods and water volumes

Soaking period	Lot	75	100	150
4 hours	1	49.77 Ac	36.55 Bc	22.97 Cc
	2	58.17 Ab	41.35 Bb	28.09 Cab
	3	75.05 Aa	50.60 Ba	31.92 Ca
	4	51.80 Ac	36.28 Bc	24.38 Cb
	5	48.28 Ac	35.07 Bc	21.47 Cc
	6	58.85 Ab	40.69 Bb	28.19 Cab
	7	51.21 Ac	36.47 Bc	22.84 Cc
8 hours	1	63.86 Ad	48.02 Bc	31.84 Cc
	2	75.83 Ab	54.28 Bb	38.74 Cab
	3	90.76 Aa	63.40 Ba	43.44 Ca
	4	69.09 Ac	49.04 Bc	34.30 Cb
	5	62.41 Ad	47.11 Bc	31.42 Cc
	6	72.03 Ab	44.32 Bc	38.66 Cab
	7	63.81 Ad	47.47 Bc	31.85 Cc
12 hours	1	75.23 Ad	56.56 Bcd	39.05 Cc
	2	87.40 Ab	63.52 Bb	43.80 Cb
	3	104.24 Aa	72.08 Ba	51.02 Ca
	4	79.34 Ac	58.78 Bc	41.97 Cbc
	5	71.93 Ae	55.10 Bd	39.16 Cc
	6	87.68 Ab	64.76 Bb	44.92 Cb
	7	75.05 Ad	56.22 Bcd	39.85 Cc
24 hours	1	96.84 Ac	76.56 Bc	52.30 Cc
	2	111.04 Ab	85.64 Bb	57.55 Cb
	3	128.97 Aa	96.30 Ba	64.76 Ca
	4	100.16 Abc	77.95 Bc	57.55 Cb
	5	89.04 Ad	68.73 Bd	51.32 Cd
	6	112.28 Ab	85.23 Bb	57.63 Cb
	7	98.16 Ac	76.29 Bc	53.01 Cc
CV (%)		3.23		

* = significant by the F-test at a 5% probability; F = calculated F value; CV = coefficient of variation. Means followed by the same letter in the column do not differ from each other by the Tukey test at a 5% probability.

Lot 3 was identified as having the worst performance only at 12 and 24 hours when 150 mL of water was used (Table 2). The combinations of 150 mL for 12 hours showed no detailed separation for higher-vigor lots, as lots 1, 4, 5, and 7 did not differ from each other. On the other hand, the differentiation was clearer in the combinations of 150 mL for 24 hours, with higher vigor for seeds from lot 5.

As already mentioned, the electrical conductivity test is based on the loss of integrity of cell membranes, which is one of the initial events in the deterioration process and, consequently, the reduction of physiological potential in seeds. Therefore, ions, sugars, salts, and other compounds are exuded when placed to soak in water, increasing the electrical conductivity of the soaking solution. Membrane reorganization can be higher and faster depending on the seed deterioration level (Bewley & Black, 1994). Therefore, the membrane reorganization mechanism may be inefficient or absent in deteriorated seeds with low vigor, causing higher leaching of electrolytes (Fessel et al., 2010). As it is based on a typical

event at the beginning of deterioration, the test can be used to detect seed deterioration in its initial phase, in addition to allowing results to be obtained shortly, speeding up decision-making regarding lot management.

A comparison of the different water volumes within each soaking period (Table 2) showed a significant reduction in electrical conductivity values with an increase in water volume for all lots. According to Dalanhol et al. (2014), higher soaking volumes allow for higher dilution of exudates and, consequently, a reduction in electrical conductivity values, as observed in studies with chickpea seeds (J. O. Araújo et al., 2022).

Therefore, there is a higher concentration of exudates and leachates from lentil seeds in the volume of 75 mL and, consequently, the highest electrical conductivity values and lower concentrations of exudates and leachates in the volume of 150 mL, resulting in lower conductivity values. This trend can be confirmed by Figure 1, which shows the difference in the color concentration of the solution in the different volumes.

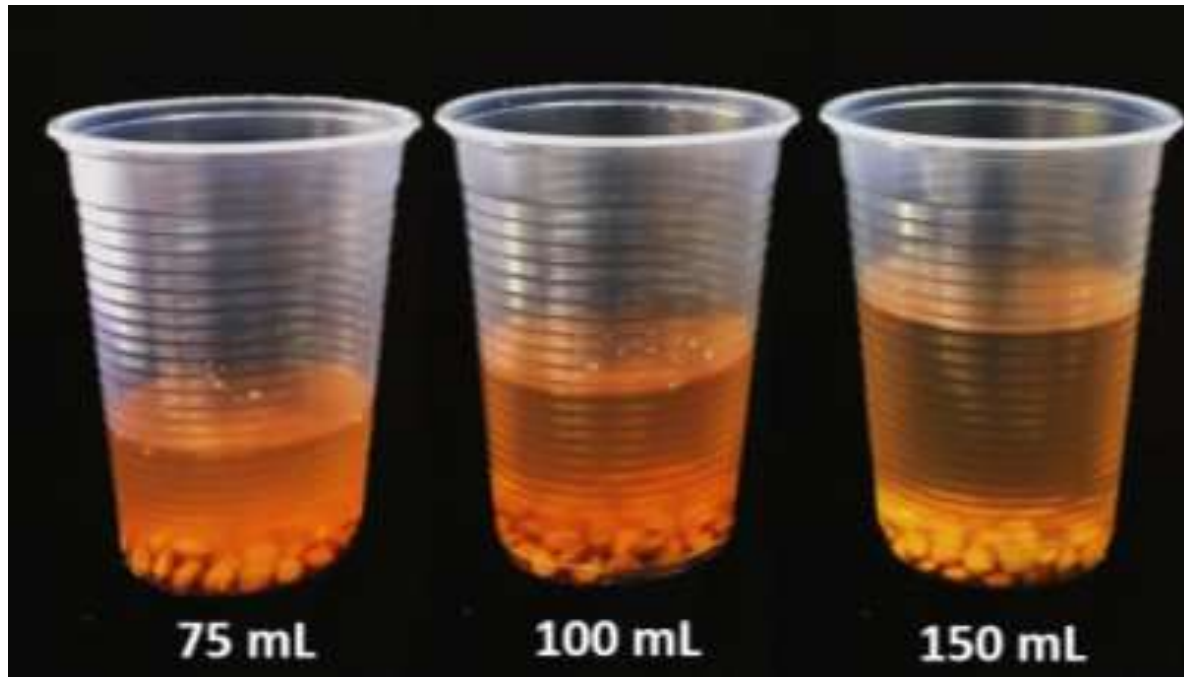


Figure 1. Solution volumes for lentil seeds after 24 hours of soaking. Plastic cups with a capacity of 300 mL were used for all volumes.

The increase in the soaking period led to an increase in electrical conductivity for all volumes (Figure 2). The highest electrical conductivity values (which indicate lower vigor due to higher release of leachate) among all analyzed volumes were obtained for seeds from lot 3, whereas the lowest values

were observed for seeds from lot 5, leaving the other lots in an intermediate position. The increase in electrical conductivity with increasing soaking period was also found in lentil (Silva et al., 2020), chickpea (J. O. Araújo et al., 2022), and white oat seeds (Sponchiado et al., 2014).

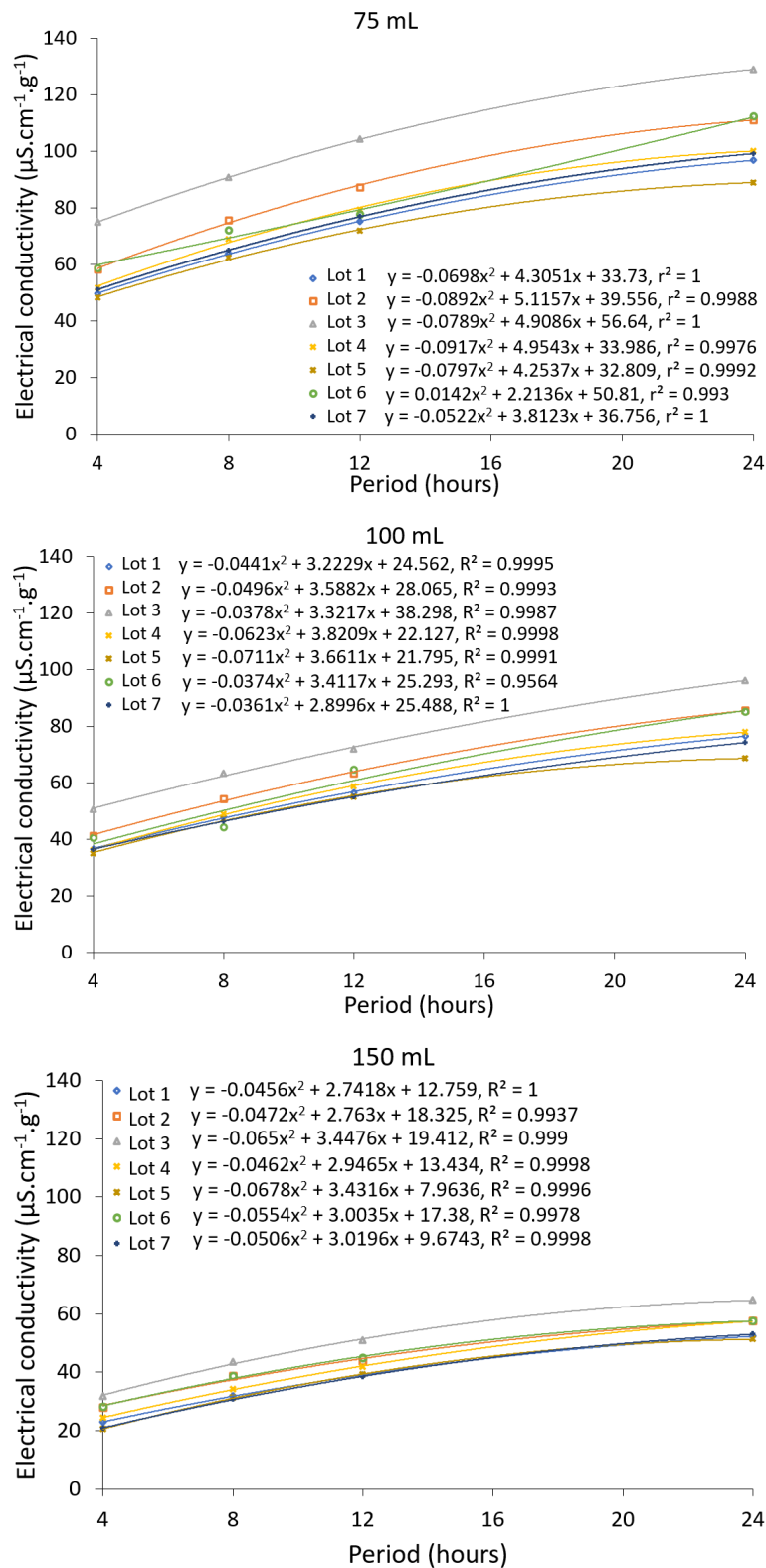


Figure 2. Electrical conductivity of seven lots of lentil seeds of the cultivar Silvina after soaking for 4, 8, 12, and 24 hours in 75, 100, and 150 mL of water.

Differences between the conductivity values of different lots of chickpea seeds could already be observed in the initial soaking periods (4 and 8 hours) and maintained up to 24 hours, allowing the separation of lots at different levels of physiological potential, regardless of the water volume (J. O. Araújo et al., 2022). However, the authors emphasize the importance of considering that the 8-hour and 12-hour periods are not as practical for conducting the test as the 24-hour period, considering the most appropriate times for carrying out the readings. This period has been recommended for most Fabaceae seeds such as pea (Machado et al., 2011), cowpea (Santos et al., 2022), *Vigna unguiculata* (Moura et al., 2017), soybean (Vieira & Marcos, 2020), and chickpea (J. O. Araújo et al., 2022). Shorter soaking periods (4 and 8 hours) for soybean seeds allowed the identification of more pronounced differences in vigor between lots, while evaluations performed after 16 hours were more sensitive to variations in seed vigor (Dias & Marcos, 1996). The electrical conductivity test in mung bean (*Vigna radiata*) seeds allowed the separation of lots in terms of vigor after 3 hours of soaking (R. F. Araújo et al., 2011), proving to be suitable for evaluating their physiological potential using 50 seeds and 75 mL of water.

Importantly, there was a distinction between lots for the volume of 75 mL relative to different vigor levels, with lot 3 standing out with the highest values in all evaluation periods, a difference that was also visible with 100 mL (Figure 2). However, this separation in the 150-mL is not so clear, which reinforces the discussion for the data in Table 2 referring to these periods. The 75-mL volume for pea seeds was also the most suitable for assessing seed vigor from the first soaking hours (8 and 16 hours) until 24 hours (Machado et al., 2011).

Negative correlations can be observed between the results of the initial quality tests and those of electrical conductivity in the different procedures tested. Also, these negative correlations indicate that the higher the amount of leachate released by the seeds, the lower the vigor.

In general, the correlation analysis confirms the results obtained with the mean comparison tests (Table 2), which showed a distinction between the physiological potential of lots in most electrical conductivity treatments, with some variations between them in terms of sensitivity. Table 2 shows an efficient separation of the lots in terms of vigor for the 75-mL volume from the first hours of soaking (4, 8, and 12 hours), extending up to 24 hours, which can be confirmed by the high correlations (Figure 3).

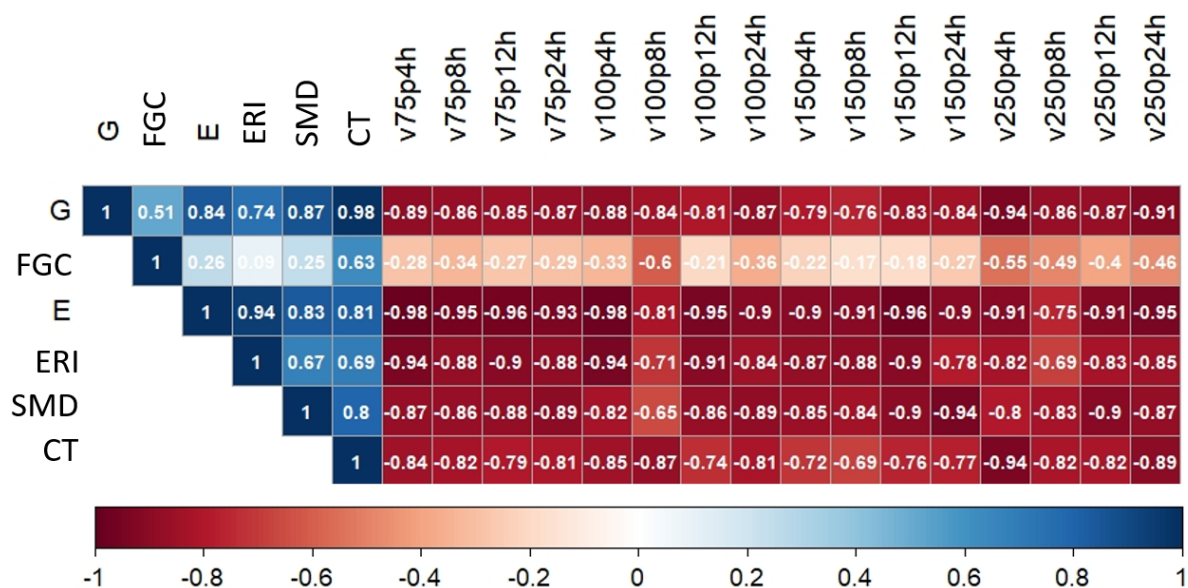


Figure 3. Pearson's linear correlation coefficients estimated between the variables of germination (G), first germination count (FGC), emergence (E), emergence rate index (ERI), seedling dry mass (SMD), cold test (CT), and the combinations of different water volumes (v75, v100, and v150) and soaking periods (4, 8, 12, and 24 hours) of the electrical conductivity test in seeds from seven lots of lentil of the cultivar Silvina.

Our results confirm the efficiency of the electrical conductivity test in separating different lots of lentil seeds in terms of physiological potential. This test is recommended for seeds from the family Fabaceae, such as peas (Ferreira et al., 2017), chickpeas (J. O. Araújo et al., 2022), soybeans (Prado et al., 2019; Fessel et al., 2010; Dias & Marcos, 1996), cowpea (Santos et al., 2022), and different varieties of *Vigna unguiculata* (Moura et al., 2017).

Assay II - Quantification of the activity of antioxidant enzymes

The activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were similar, allowing us to observe higher activity in the seeds of lot 5, which did not differ significantly from those of lot 1, and lower activity in the seeds of lot 3 compared to those of lots 5 and 1. Lot 3 was statistically similar to lots 2, 4, and 6, which did not differ from lot 1 (Figure 4).

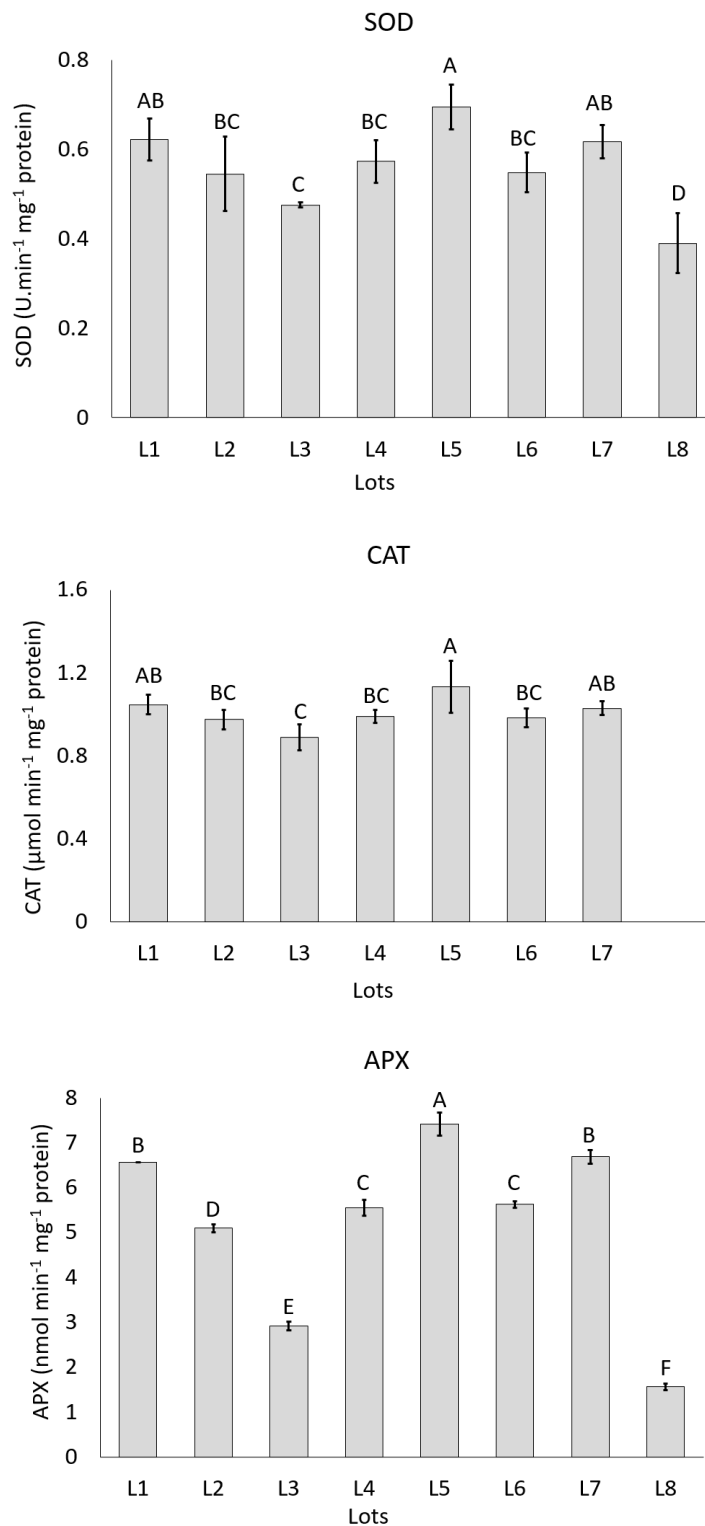


Figure 4. Activity of the superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymes in seeds from seven lots of lentil seeds of the cultivar Silvina. The same letters do not differ by the Tukey test ($P < 0.05$). Bars represent the standard deviation.

The differences between the ascorbate peroxidase (APX) activity in the seeds of different lots were even clearer, with higher activity for lot 5, followed by lots 1 and 7, which were higher than lots 4 and 6, followed by lot 2. Lot 3 stood out with the lowest activity (Figure 4).

These results regarding enzyme activity are consistent with those obtained in most of the tests used to characterize the initial physiological quality of the lots (Table 1). In this sense, lots 5 and 1 were generally superior to lot 3. Furthermore, the highest stratification of lots in terms of enzyme activity was obtained with the APX determination.

The activity of antioxidant enzymes can be an interesting tool to be correlated with the results of vigor tests, being an indication of the deterioration level of lots. J. O. Araújo et al. (2021) found that the activity of CAT and APX was related to the vigor results obtained in tests assessing the physiological potential of chickpea seeds. The determination of the activity of SOD, CAT, and APX enzymes was efficient in differentiating lots with small differences in vigor for *Vigna unguiculata* (Deuner et al., 2011), soybean (Bandeira et al., 2014), and corn (Marini et al., 2013). Bandeira et al. (2014) found that the activity of SOD, CAT, and APX in soybean seeds was more sensitive than the germination, first count, and seedling growth tests for differentiating the physiological potential of lots. According to R. F. Araújo et al. (2018), the reduction in CAT activity was related to the loss of quality of pepper (*Capsicum chinense* and *Capsicum frutescens* L) seeds.

Some authors have stated that the activity of antioxidant enzymes is efficient in differentiating lots in terms of vigor but the results regarding these enzymes must be interpreted with caution when referring to their isolated use for assessing vigor. Relating the activity of these enzymes to the results of specific tests to assess vigor is considered more appropriate as a complementary information and not as a method of assessing physiological potential. The higher activity of antioxidant enzymes in the seeds of a given lot is an indication of less deterioration and, consequently, less vigor (Morais et al., 2021).

Therefore, the balance between the production and intracellular removal of ROS, especially hydrogen peroxide, is assumed to be directly related to the ability of the cells to maintain high SOD, CAT, and APX activity. The efficient performance of the antioxidant defense system is important to maintaining cellular homeostasis, thus avoiding oxidative damage (Kumar et al., 2015), and may be directly related to the physiological potential of the seeds (Gomes & Garcia, 2013).

The negative scores of component 1 (PC1) in the principal component analysis (PCA) show that lot 5 is directly related to the most vigorous variables in the initial characterization of the lots and enzyme activity, followed by lots 1, 7, and 4. However, lot 3 is in the opposite direction to the variables related to seed vigor, located in the positive scores of component 1, standing out as the lot with the lowest vigor. Lots 2 and 6 showed intermediate performance between positive and negative PCA scores (Figure 5).

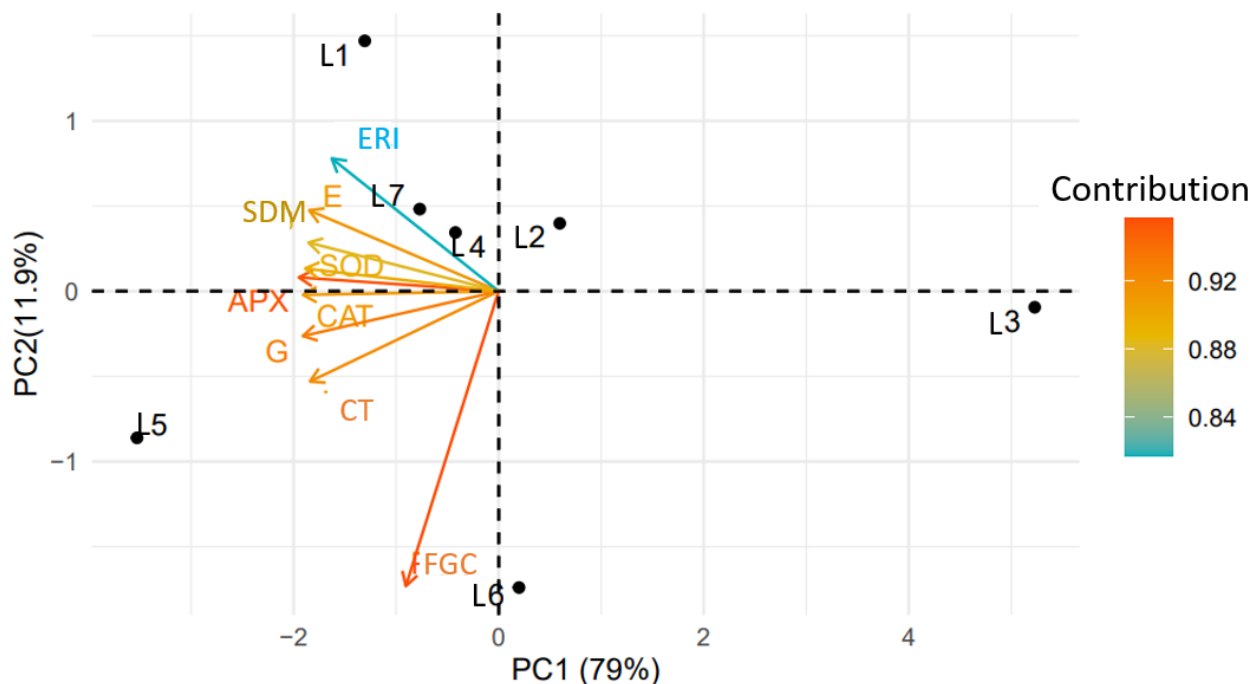


Figure 5. Principal component analysis (PCA) of the variables germination (G), first germination count (FGC), emergence (E), emergence rate index (ERI), seedling dry mass (SDM), cold test (CT), and the activity of antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) in seven lots of lentil seeds of the cultivar *Silvina*.

Seed vigor reflects a set of characteristics that determine their performance potential in the field and under storage (Marcos, 2020). In this context, tests to measure vigor are fundamental for the development of modern agriculture, especially when they allow obtaining quick and accurate results for classifying lots in terms of physiological potential, enabling faster internal decision-making regarding seed quality control. The results found in this study point to different efficient methodologies for assessing the vigor of lentil seeds that can be used in laboratories of seed analysis, scientific research organizations, and quality control programs in companies.

Conclusions

The electrical conductivity test conducted with 50 seeds immersed in 75 mL of water at 25 °C for 24 hours allows the separation of lots into vigor levels.

The activity of CAT, SOD, and APX enzymes can be related to the physiological potential of lentil seeds.

Acknowledgments

To the Federal University of Viçosa (UFV), the National Council for Scientific and Technological Development (CNPq), the Coordenação de Aperfeiçoamento de

Pessoal de Nível Superior - Brasil (CAPES - Finance Code 001), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

References

- Anderson, M. D., Prasad, T. K., & Stewart, C. R. (1995). Changes in isozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotylus of maize seedlings. *Plant Physiology*, *109*(4), 1247-1257. doi: 10.1104/pp.109.4.1247
- Araújo, J. O., Dias, D. C. F. S., Miranda, R. M., & Nascimento, W. M. (2022). Adjustment of the electrical conductivity test to evaluate the seed vigor of chickpea (*Cicer arietinum* L.). *Journal of Seed Science*, *44*(2), e202244003. doi: 10.1590/2317-1545v44258666
- Araújo, J. O., Dias, D. C. F. S., Nascimento, W. M., Martins, A. O., & Limão, M. A. R. (2021). Accelerated aging test and antioxidant enzyme activity to assess chickpea seed vigor. *Journal of Seed Science*, *43*(1), e202143038. doi: 10.1590/2317-1545v43253934
- Araújo, R. F., Abud, H. F., Silva, L. J., Araújo, E. F., Pinto, C. M. F., & Silva, F. W. S. (2018). Physiological changes and antioxidant enzymes activity in Biquinho and Malagueta pepper seeds during the maturation process. *Revista Ceres*, *65*(6), 534-545. doi: 10.1590/0034-737X201865060009
- Araújo, R. F., Zonta, J. B., & Fontes, E. (2011). Teste de condutividade elétrica para sementes de feijão-mungo-verde. *Revista Brasileira de Sementes*, *33*(1), 123-130. doi: 10.1590/S0101-31222011000100014
- Baalbaki, R., Elias, S., Marcos, J., F^o., & Mcdonald, M. B. (2009). *Seed vigor testing handbook*. AOSA.
- Bandeira, J. M., Marini, P., Borba, I. C. G., Martins, A. B. N., Amarante, L., & Moraes, D. M. (2014). Antioxidative metabolism: a tool to detect small differences in the vigor of soybean seeds. *Iheringia*, *69*(2), 285-292. <http://isb.emnuvens.com.br/iheringia/article/view/92/99>
- Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, *44*(1), 276-287. doi: 10.1016/0003-2697(71)90370-8
- Bewley, J. D., & Black, M. (1994). *Seeds: physiology of development and germination*. Plenum Press.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(2), 248-254. doi: 10.1006/abio.1976.9999
- Chaffai, R. R., Marzouk, B., & Ferjani, E. E. (2005). Aluminum mediates compositional alterations of polar lipid classes in maize seedlings. *Phytochemistry*, *66*(16), 903-912. doi: 10.1016/j.phytochem.2005.06.028
- Cicero, S. N., & Vieira, R. D. (2020). Teste de frio. In F. C. Krzyzanowski, R. D. Vieira, J. B. França-Neto, & J. Marcos F^o. (Eds.), *Vigor de sementes: conceitos e testes* (pp. 277-307, Cap. 6). Londrina.

- Dalanhol, S. J., Rezende, E. H., Abreu, D. C. A., & Nogueira, A. C. (2014). Teste de condutividade elétrica em sementes de *Bowdichia virgilioides* Kunth. *Floresta e Ambiente*, 21(1), 69-77. doi: 10.4322/floram.2014.013
- Del Longo, O. T., González, A., Pastori, G. M., & Trippi, V. (1993). Antioxidant defenses under hyperoxygenic and hyperosmotic conditions in leaves of two lines of maize with differential sensitivity to drought. *Plant Cell Physiology*, 34(7), 1023-1028. doi: 10.1093/oxfordjournals.pcp.a078515
- Deuner, C., Maia, M. S., Deuner, S., Almeida, A., & Meneghello, G. E. (2011). Viabilidade e atividade antioxidante de sementes de genótipos de feijão-miúdo submetidos ao estresse salino. *Revista Brasileira de Sementes*, 33(4), 711-720. doi: 10.1590/S0101-31222011000400013
- Dias, D. C. F. S., & Marcos, J., Fº. (1996). Testes de condutividade elétrica para avaliação do vigor de sementes de soja (*Glycine max* L. Merrill). *Scientia Agricola*, 53(1), 1-9. doi: 10.1590/S0103-90161996000100005
- Ebone, L. A., Caverzan, A., & Chavarria, G. (2019). Physiologic alterations in orthodox seeds due to deterioration processes. *Plant Physiology and Biochemistry*, 145(19), 34-42. doi: 10.1016/j.plaphy.2019.10.028
- Ferreira, L. B. S., Fernandes, N. A., Aquino, L. C., Silva, A. R., Nascimento, W. M., & Leão-Araújo, E. F. (2017). Temperature and seed moisture content affect electrical conductivity test in pea seeds. *Journal of Seed Science*, 39(4), 410-416. doi: 10.1590/2317-1545v39n4181021
- Fessel, S. A., Panobianco, M., Souza, C. R., & Vieira, R. D. (2010). Teste de condutividade elétrica em sementes de soja armazenadas sob diferentes temperaturas. *Bragantia*, 69(1), 207-214. doi: 10.1590/S0006-87052010000100026
- Figueiredo, J. C., David, A. M. S. S., Silva, C. D., Soares, L. M., Pereira, K. K. G., Silva, R. A., & Alves, R. A. (2021). Teste de condutividade elétrica em sementes de linho. *Investigacion Agraria*, 23(2), 81-85. doi: 10.18004/investig.agrar.2021.diciembre.2302686
- Gomes, M. P., & Garcia, Q. S. (2013). Reactive oxygen species and seed germination. *Biologia*, 68(3), 351-357. doi: 10.2478/s11756-013-0161-y
- Havir, E. A., & Mchale, N. A. (1987). Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiology*, 84(2), 450-455. doi: 10.1104/pp.84.2.450
- Krzyzanowski, F. C., França, J. B., Neto, Gomes, F. G., Jr., & Nakagawa, J. (2020). Teste de vigor baseados em desempenhos de plântulas. In F. C. Krzyzanowski, R. D. Vieira, & França, J. B. Neto. (Eds.), *Vigor de sementes: conceitos e testes* (pp. 79-127, cap. 2). Londrina.
- Kumar, S. P. J., Prasad, S. R., Banerjee, R., & Thammineni, C. (2015). Seed birth to death: dual functions of reactive oxygen species in seed physiology. *Annals of Botany*, 116(4), 663-668. doi: 10.1093/aob/mcv098
- Machado, C. G., Martins, C. C., Santana, D. G., Cruz, S. C. S., & Oliveira, S. S. C. (2011). Adequação do teste de condutividade

- elétrica para sementes de *Pisum sativum* subsp. *Arvense*. *Ciência Rural*, 41(6), 988-995. doi: 10.1590/S0103-84782011005000062
- Maguire, J. D. (1962). Speed of germination and in selection and evaluation for seeding emergence and vigor. *Crop Science*, 2(2), 176-177. doi: 10.2135/cropsci1962.0011183X000200020033x
- Marcos, J., F^o. (2015). *Fisiologia de sementes de plantas cultivadas*. ABRATES.
- Marcos, J., F^o. (2020). Testes de vigor: Importância e utilização. In F. C. Krzyzanowski, R. D. Vieira, J. B. França-Neto, & J. Marcos F^o. (Eds.), *Vigor de sementes: conceitos e testes* (pp. 17-72, Cap.1). Londrina.
- Marini, P., Bandeira, J. M., Borba, I. C. G., Martins, A. B. N., Moraes, D. M., Amarante, L., & Villela, F. A. (2013). Antioxidant activity of corn seeds after thermal stress. *Ciência Rural*, 43(6), 951-956. doi: 10.1590/S0103-84782013005000064
- Merga, B., & Haji, J. (2019). Economic importance of chickpea: Production, value, and world trade. *Cogent Food and Agriculture*, 5(1), 1-12. doi: 10.1080/23311932.2019.1615718
- Ministério da Agricultura, Pecuária e Abastecimento (2009). *Regras para análise de sementes*. Secretaria de Defesa Agropecuária - MAPA/ACS. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise_sementes.pdf
- Morais, T. C., Dias, D. C. F. S., Pinheiro, D. T., Gama, G. F. V., & Silva, L. J. (2021). Physiological quality and antioxidant enzymatic action in sunflower seeds exposed to deterioration. *Revista Caatinga*, 34(3), 570-579. doi: 10.1590/1983-21252021v34n308rc
- Moura, M. C. F., Lima, L. K. S., Santos, C. C., & Dutra, A. S. (2017). Teste da condutividade elétrica na avaliação fisiológica em sementes de *Vigna unguiculata*. *Revista de Ciências Agrárias*, 40(4), 714-721. doi: 10.19084/RCA17034
- Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, 22(5), 867-880. doi: 10.1093/oxfordjournals.pcp.a076232
- Nascimento, W. M., & Silva, P. P. (2019). *Grão-de-bico: nova aposta do agronegócio brasileiro*. Seed News. <https://seednews.com.br/artigos/2969-grao-de-bico-nova-aposta-do-agronegocio-brasileiro-edicao-maio-2019>
- Powell, A. A. (1986). Cell membranes and seed leachate conductivity in relation to the quality of seed for sowing. *Journal of Seed Technology*, 10(2), 81-100. doi: jstor.org/stable/23432796
- Prado, J. P., Krzyzanowski, F. C., Martins, C. C., & Vieira, R. D. (2019). Physiological potential of soybean seeds and its relationship to electrical conductivity. *Journal of Seed Science*, 41(4), 407-415. doi: 10.1590/2317-1545v41n4214988
- R Core Team (2022). *A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org>

- Santos, B. G., Vale, L. S. R., Oliveira, C. G. B., Moura, M. M., Alves, S. M. A., Pereira, W. J. F^o., Marques, M. L. S., & Carvalho, R. C. M. (2022). Electrical conductivity test in evaluating the physiological potential of Cowpea Seeds. *Brazilian Journal of Development*, 8(2), 11220-11231. doi: 10.34117/bjdv8 n2-183
- Silva, E. C., Menechini, W., Galvão, C. S., Oliveira, L. A. B., Ferreira, N. C. F., & Silva, L. S. (2020). Adequacy of electrical conductivity test for lentil seeds. *Scientia Agraria Paranaensis*, 19(3), 307-311, 2020. doi: 10.18188/sap.v19i3.22566
- Sponchiado, J. C., Souza, C. A., & Coelho, C. M. M. (2014). Teste de condutividade elétrica para determinação do potencial fisiológico de sementes de aveia branca. *Semina: Ciências Agrárias*, 35(4), 2405-2414. doi: 10.5433/1679-0359.2014v35n4 Suplp240
- Torres, S. B., Paiva, E. P., Almeida, J. P. N., Benedito, C. P., & Carvalho, S. M. C. (2015). Teste de condutividade elétrica na avaliação da qualidade fisiológica de sementes de coentro. *Revista Ciência Agronômica*, 46(3), 622-629. <http://www.ccarevista.ufc.br/seer/index.php/ccarevista/article/view/3881>
- Vieira, R. D., & Marcos, J., F^o. (2020). Teste de condutividade elétrica. In F. C. Krzyzanowski, R. D. Vieira, J. B. França-Neto, & J. Marcos F^o. (Eds.), *Vigor de sementes: conceitos e testes* (pp. 333-375, Cap. 8). Londrina.
- Worma, M., Segatto, C., Stefen, C., Buba, G. P., & Leolato, L. S. (2019). Qualidade fisiológica de sementes de milho produzidas com adubação biológica e bioestimulante em diferentes preparos de solo. *Revista Engenharia na Agricultura - Reveng*, 27(3), 187-194. doi: 10.13083/reveng.v27i3.893