SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2025v46n2p587

Vigor of canola seeds as assessed by the electrical conductivity test

Vigor de sementes de canola avaliado pelo do teste de condutividade elétrica

Soryana Gonçalves Ferreira de Melo[1]; Ítallo Jesus Silva[2]; Lucas da Costa Oliveira[3]; Guilherme Henrique Fernandes Carneiro[3]; Guilherme Vieira Pimentel[4]; Raquel Maria de Oliveira Pires[4]; Marcela Carlota Nery[5]*

Highlights

Canola's economic importance makes assessing seed physiological potential essential.
Seed lot characterization tests correlate with the electrical conductivity test.
The electrical conductivity test demonstrates efficiency in determining seed vigor.

Abstract

Canola (*Brassica napus* L. var. oleifera) is one of the main oilseeds for both edible and industrial purposes. Methodologies for assessing the physiological potential of canola seeds are essential to ensure the commercialization of high-quality lots. This study aimed to adapt the methodology for evaluating the physiological quality of canola seeds using the electrical conductivity test. Seeds from three lots of three canola cultivars Hyola 575 CL, Nuola 300, and Diamond were tested for initial quality, and the results were compared with those from the electrical conductivity test. To adapt the electrical conductivity test methodology, seeds were subjected to two temperatures (25 °C and 30 °C), three volumes of deionized water (25, 50, and 75 mL), and five imbibition periods (2, 4, 6, 8, and 10 h). At 25 °C, a volume of 25 mL allowed lot stratification by vigor at 4, 8, and 10 h. At 30 °C, 25 mL was sufficient for the 10 h period. The highest values were observed at 10 h, indicating the time required for solute leaching and electrical conductivity measurement. Therefore, for the electrical conductivity test on canola seeds, it is recommended to immerse 50 seeds in 25 mL of deionized water for 10 h at either 25 °C or 30 °C.

Key words: *Brassica napus* L. var. oleifera. Physiological potential. Immersion period. Water volume.

[1] Drª in Plant Production, Universidade Federal dos Vales Jequitinhonha e Mucuri, UFVJM, Diamantina, MG, Brazil. E-mail: soryana.melo@ufvjm.edu.br
[2] M.e in Plant Production, UFVJM, Diamantina, MG, Brazil. E-mail: itallo.jesus@ufvjm.edu.br
[3] Students Graduate in Agronomy, UFVJM, Diamantina, MG, Brazil. Brazil. E-mail: costa.lucas@ufvjm.edu.br; henrique.guilherme@ufvjm.edu.br
[4] Profs. Drs., Department of Agriculture, Universidade Federal de Lavras, UFLA, Lavras, MG, Brazil. guilherme.pimentel@ufla.br; raquelmopires@ufla.br
[5] Profª Drª, Department of Agronomy, UFVJM, Diamantina, MG, Brazil. E-mail: nery.marcela@ufvjm.edu.br
* Author for correspondence

Received: May 17, 2024 Approved: Mar. 12, 2025

Resumo

A canola (Brassica napus L. var. oleifera) é uma das principais oleaginosas para fins comestíveis e industriais. Metodologias para avaliar o potencial fisiológico de sementes de canola, assegurando a comercialização de lotes de alta qualidade são necessárias. Nesse sentido, o objetivo deste estudo foi adequar a metodologia para avaliação da qualidade fisiológica de sementes de canola por meio do teste de condutividade elétrica. Foram utilizadas sementes provenientes de três lotes de três cultivares de canola, Hyola 575 CL, Nuola 300 e Diamond que foram submetidas a testes para caracterização de sua qualidade inicial e os resultados comparados com aqueles obtidos no teste de condutividade elétrica. Para adequação da metodologia do teste de condutividade elétrica, as cultivares foram submetidos a duas temperaturas (25 °C e 30 °C), três volumes de água deionizada (25, 50 e 75 ml), em cinco períodos de embebição (2, 4, 6, 8 e 10 horas). Na temperatura de 25 °C foi possível estratificar os lotes em níveis mais altos de vigor utilizando um volume de 25 mL durante 4, 8 e 10 horas. Já a 30 °C, o volume de 25 mL mostrou-se satisfatório para o período de 10 horas. O período de 10 horas foi o que apresentou os valores mais altos, sendo, portanto, o tempo necessário para a lixiviação dos solutos e a medição da condutividade elétrica. Portanto, para a condução do teste de condutividade elétrica em sementes de canola, recomenda-se imergir 50 sementes em 25 mL de água deionizada por 10 horas a 25 °C ou 30 °C.
Palavras-chave: *Brassica napus* L. var. oleífera. Potencial fisiológico. Período de imersão. Volume de água.

Introduction

Canola (*Brassica napus* L. var. *oleifera*) is a globally important oilseed crop and the third most cultivated, surpassed only by oil palm and soybean (Boersch, 2024). Its seeds contain 42% to 48% oil, and 21% to 33% protein (Raboanatahiry et al., 2021). Additionally, canola is widely used for forage, edible oil, and biodiesel production (Sadras & Calderini, 2021).

In Brazil, canola has been gaining prominence, with significant efforts directed toward its tropicalization and expansion into various regions of the country (Araújo et al., 2021; Guiducci et al., 2020; Guimarães et al., 2020). Public-private partnerships play a key role in accelerating technological advancements and supporting the development of the crop in the Brazilian Cerrado, particularly in the MATOPIBA region, which includes the states of Maranhão, Tocantins, Piauí, and Bahia (R. Lima et al., 2023). Investments are also being made to explore new biofuel markets, recognizing canola as a low-carbon feedstock when cultivated sustainably (US Canola Association, nd).

Making canola production viable in Brazil entails an increasing demand for high-quality seeds (Gularte et al., 2020). In this context, ensuring the production of high-quality seeds and establishing standardized methods for assessing their physiological quality are essential (Ávila et al., 2005).

The electrical conductivity test is considered one of the most promising methods for evaluating seed vigor due to its strong theoretical foundation, objectivity, speed, ease of execution, and reproducibility, making it suitable for standardization as a routine test (Internacional Seed Testing Association [ISTA], 2011).

The test measures the release of solutes, such as amino acids and inorganic ions, through cell membranes. Seeds with lower physiological potential typically have disorganized cell membranes, resulting in reduced selective permeability. During imbibition, these damaged membranes allow the uncontrolled release of ions and organic solutes into the external environment, which can be detected by measuring electrolyte levels in the solution. The loss of membrane integrity—an early stage of seed deterioration caused by biochemical degradation or physical ruptures—is a key factor influencing seed vigor. This difference in vigor can be assessed indirectly through the intensity of electrolyte release detected by the electrical conductivity test (Vieira & Marcos, 2020).

Although the electrical conductivity test is relatively simple and easy to perform, several factors, such as water volume, imbibition time, number of seeds per replicate, and temperature, can influence the results (Figueiredo et al., 2021; Vieira & Marcos, 2020). For many species, the test uses 50 seeds immersed in 75 mL of distilled or deionized water at 25 °C for 24 h (Vieira & Marcos, 2020). This procedure is particularly effective for assessing the vigor of seeds such as lentils (Limão et al., 2024) and soybeans (Vieira & Krzyzanowski, 1999).

Different imbibition periods have been determined for physiological quality assessment for small-seeded species in the Brassicaceae family, such as broccoli, radish, crambe, and canola. In broccoli, 1 to 2 h were identified as critical (Magro et al., 2011). For radish, a 17 h imbibition period was effective (Mavi et al., 2014), while for crambe, significant results were achieved after 16 h (J. J. P. Lima et al., 2015). In canola, imbibition periods of 8 h (Milani et al., 2012) and 6 to 9 h (Bezerra et al., 2024) were recommended for better lot stratification using deionized water.

Given this, obtaining accurate data through an effective methodology that distinguishes seed lots with different physiological qualities is crucial for canola seed storage and commercialization. Therefore, this study aimed to adapt the methodology for evaluating the physiological quality of canola seeds using the electrical conductivity test.

Material and Methods

Twelve lots of canola seeds from three cultivars (Diamond, Nuola 300, and Hyola 575), collected from different locations and harvests, were used in the study. These were distributed as follows: four lots of the Diamond cultivar [Lot 1 (Diamantina, 2021), Lot 2 (UFLA, 2021), Lot 3 (UFLA, 2019), and Lot 4 (Diamantina, 2019)]; four lots of the Nuola 300 cultivar [Lot 5 (Diamantina, 2021), Lot 6 (UFLA, 2021), Lot 7 (Diamantina, 2021), and Lot 8 (Diamantina, 2019)]; and four lots of the Hyola 575 cultivar [Lot 9 (Diamantina, 2021), Lot 10 (UFLA, 2019), Lot 11 (UFLA, 2019), and Lot 12 (Embrapa, 2019)].

The experiments were conducted at the Seed Laboratory of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM), located on the JK Campus, where the following tests were performed:

Moisture content: Determined using the oven method at 105 °C for 24 h (Ministério da Agricultura, Pecuária e Abastecimento, [MAPA], 2013). Two replicates were used for each lot, with 1 g of seeds per replicate.

Germination test: performed according to the criteria established by the Rules for Seed Testing (Ministério da Agricultura, Pecuária e Abastecimento, [MAPA], 2013), with four replicates of 50 seeds per lot. Seeds were placed to germinate in plastic (Gerbox®; 11 × 11 × 3.5 cm) on three sheets of Germitest® paper moistened with distilled water, using 2.5 times the weight of dry paper. The seeds were then placed in a Biochemical Oxygen Demand (BOD) germinator at a constant temperature of 20 °C with constant light. Evaluations were made on the fifth day (first germination count) and finished on the seventh day (final count), recording the number of normal seedlings.

Emergence test: conducted with four replicates of 50 seeds per lot. The seeds were sown in plastic boxes containing a mixture of sand and soil in a 2:1 ratio, moistened with distilled water to 60% of field capacity (Krzyzanowski et al., 2020). The boxes were kept in a growth room at 20 °C with a constant photoperiod. After emergence began, evaluations were made daily, with the initial stand recorded on the fifth day. The test was terminated once the emergence percentage stabilized for three consecutive days, and the number of normal seedlings that emerged was recorded.

Cold test: performed following the recommendations of Cicero and Vieira (2020). Four replicates of 50 seeds per lot were placed on Germitest® paper moistened with water equivalent to 2.5 times the weight of the dry paper. The rolls were grouped in sets of four (replicates), secured with rubber bands, sealed in plastic bags, and kept in a BOD incubator at 10 °C for seven days. After this period, the rolls were removed from the bags and placed in a germinator at 25 °C. The percentage of normal seedlings was assessed on the fifth day.

Accelerated aging: conducted according to the methodology described by Marcos (2020), using plastic Gerbox® containers with individual compartments and an aluminum screen tray inside. Seeds were arranged in a single layer on the screen. Forty milliliters of distilled water were added to each Gerbox®. The boxes were covered and placed in a BOD germination chamber set at 42 °C for 24 h. After the aging period, the seeds underwent the germination test as previously described, with evaluations performed on the fifth day after sowing. The water content of the seeds was also determined.

Electrical conductivity test adaptation: the methodology described by Vieira and Marcos (2020) (Figure 1) was used. Four replicates of 50 seeds per lot were weighed on a 0.001 g precision scale and then soaked in plastic containers containing 50, 75, and 100 mL of deionized water. The containers were kept in a BOD incubator at 25 °C and 30 °C for imbibition periods of 2, 4, 6, 8, and 10 h. After each imbibition period, the containers were removed, shaken, and the electrical conductivity was measured using a conductivity meter (model mCA 150, TECNOPON). The results were expressed in $\mu S \cdot cm^{-1} \cdot g^{-1}$.

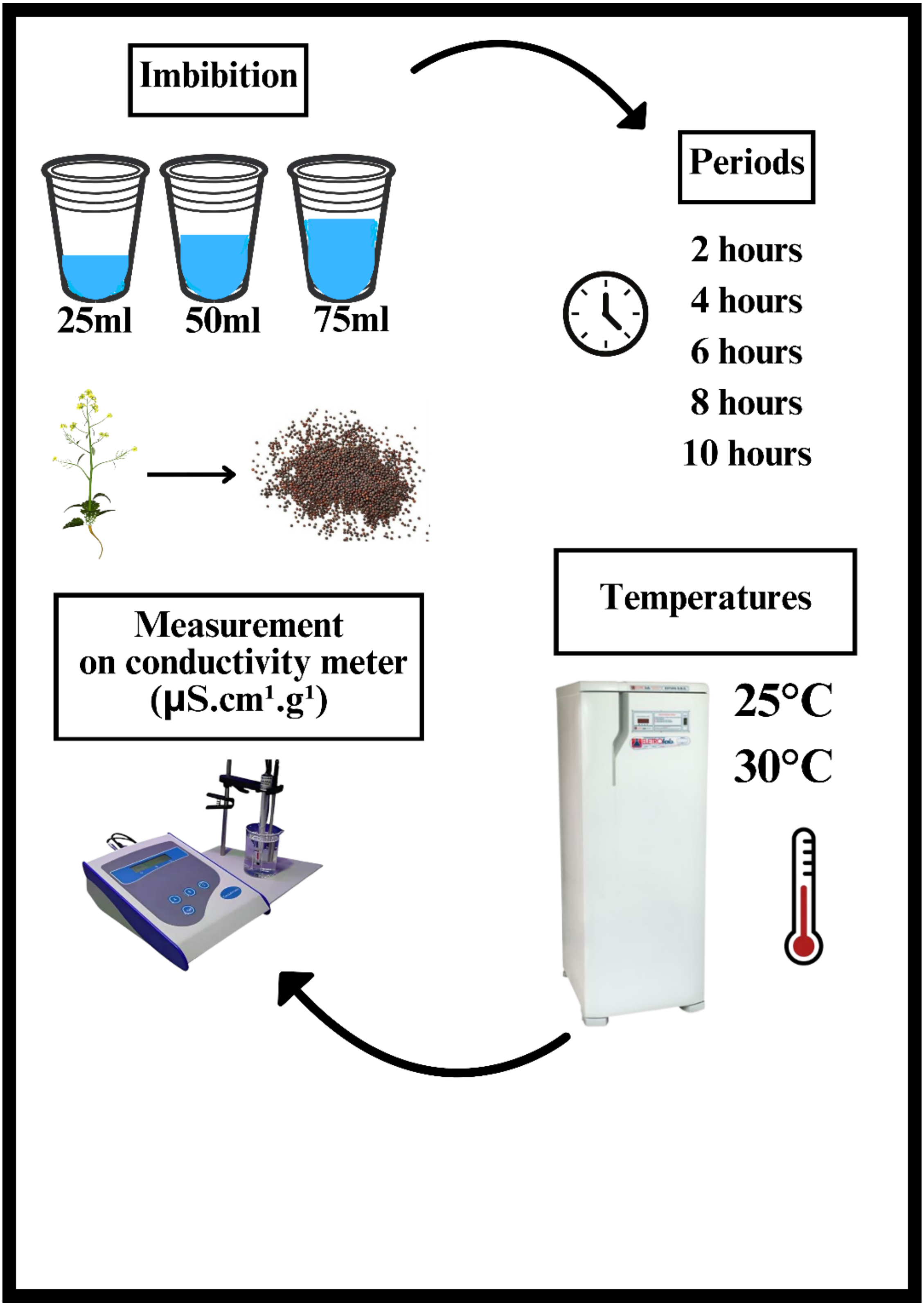

Figure 1. Illustrative diagram of the electrical conductivity test on canola seeds.

The tests for the initial characterization of the lots were conducted in a completely randomized design experiment, with four replicates per lot. The electrical conductivity test was arranged in a triple factorial design: 12 lots, three volumes of water (25, 50, and 75 mL), and five imbibition periods (2, 4, 6, 8, and 10 h). The test was carried out at two temperatures: 25 °C and 30 °C. The means of the qualitative factors were compared using the Scott-Knott test ($p < 0.05$), while for the quantitative factor, a response surface analysis was performed. Cluster analysis, dendrogram, cophenetic correlation, and the "Ward" model were applied, with a cutoff point in the Euclidean distance at K = 4, using data from the initial characterization and the conductivity test. The statistical analyses were performed using the statistical software R 4.1.2 (R Core Team [R], 2022).

Results and Discussion

Figure 2 illustrates the results related to the initial characterization of the physiological quality of the lots. The canola seeds exhibited similar moisture content across all lots, ranging from 8.87% to 10.71%. According to Marcos (2015), it is crucial to establish a standardized evaluation to ensure uniformity in seed moisture content, allowing for safe comparisons of the physiological potential between the lots analyzed.

There was maximum germination (100%) in lots L1, L4, L5, L8, and L10, while lot L11 showed the lowest mean, with 90%. The other lots reached intermediate values (Figure 2), all with germination above 90%. Therefore, all lots meet the minimum standards for the production and sale of canola seeds, as established by Normative Instruction No. 45 of MAPA, which requires a germination percentage above 80% (Ministério da Agricultura, Pecuária e Abastecimento, [MAPA], 2013).

For the first germination test count, there were significant differences between the lots, with lot L11 showing the lowest value (14%) compared to the others (Figure 2). The vigor, emergence, initial stand, cold, and accelerated aging tests revealed differences in lot performance that were not detected by the germination test (Figure 2). Therefore, these tests provided greater discrimination between the lots than the germination test, as they captured deterioration processes that compromised germination capacity. According to Milani et al. (2012), vigor tests, such as the first germination count and emergence test, are sensitive tools for evaluating seed quality across different lots, identifying variations that the germination test alone does not detect.

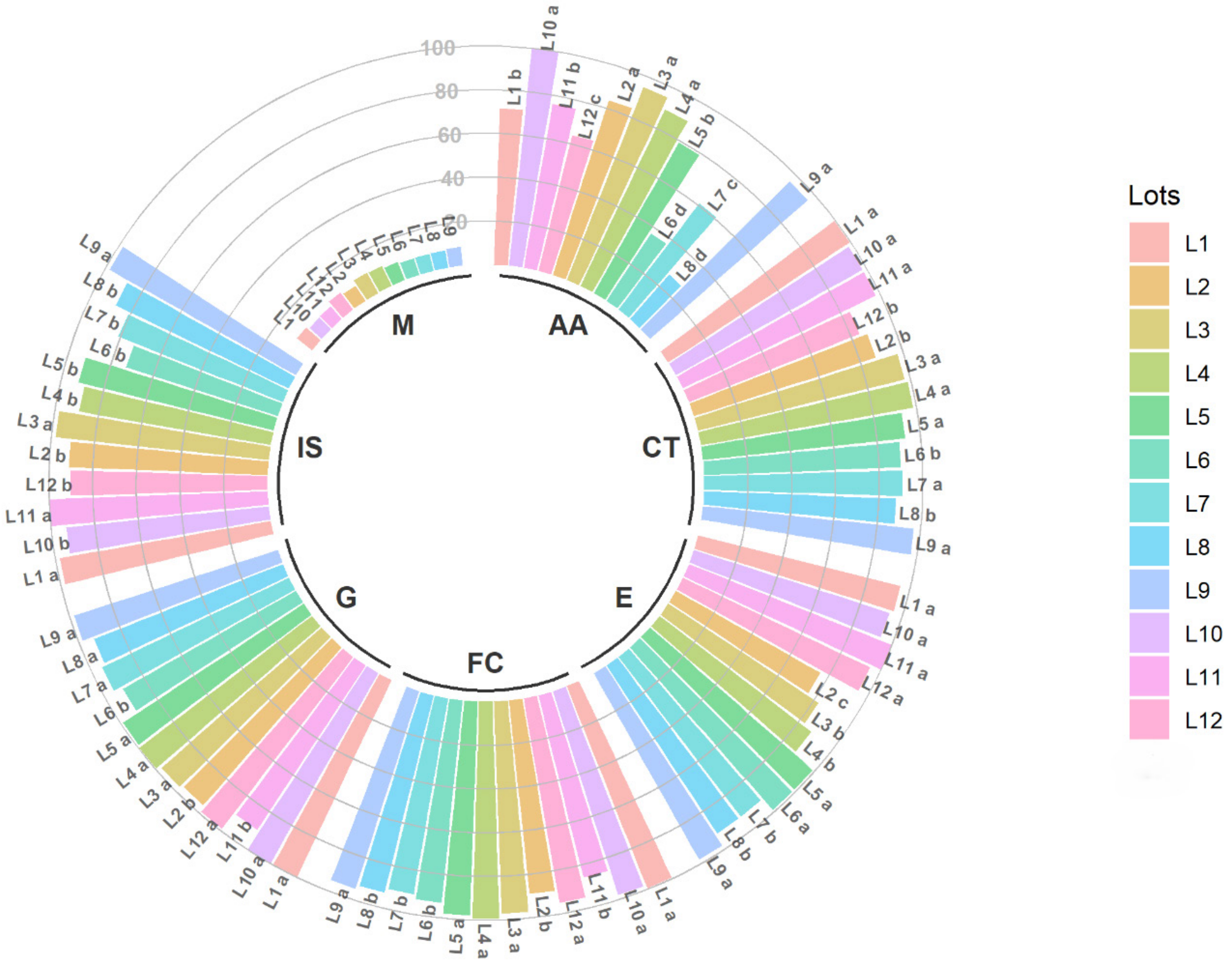

Figure 2. Radar chart of the characterization of the initial quality of 12 lots of canola cultivars Diamond, Nuola 300, and Hyola 575.
Evaluated variables: degree of moisture (M), germination (G), first germination count (FG), emergence (E), initial stand (IS), cold test (CT), and accelerated aging (AA). Means followed by the same lowercase letter in the chart for each analysis do not differ from each other, according to the Scott-Knott test (p>0.05).

Vigor tests are extremely important for distinguishing seed lots, as they assess essential characteristics such as the capacity for rapid and uniform germination, tolerance to water and heat stress, longevity, and physiological integrity. These attributes determine the seeds' performance under different environmental conditions (Marcos, 2020). Bezerra et al. (2024) also emphasize the importance of vigor assessment for canola seed stratification, noting that vigor testing reflects tolerance to heat stress, longevity, physical integrity, and the ability to develop vigorously under specific environmental conditions. These characteristics are fundamental for identifying lots with higher resistance to adverse conditions, optimizing the performance of canola crops.

In general, based on the tests used for the initial characterization of the different lots in terms of physiological potential, lots L1, L4, L9, and L10 exhibited superior performance in key criteria, such as germination speed and uniformity, tolerance to thermal stress, longevity, and physiological integrity. In contrast, other lots showed lower performance, with varying results among those classified as having medium vigor, depending on the test performed. Lots L6, L7, L8, and L12 had low germination percentages after aging, indicating reduced physiological potential under high relative humidity and temperature conditions. In the cold test, lots L2, L6, and L12 did not withstand thermal stress as well as the others, while only lot L2 exhibited low emergence due to the intensity of the test conditions (Figure 2). The use of lots with varying physiological potential is essential in studies evaluating seed vigor, particularly when the objective is to define or assess new methodologies (Limão et al., 2024).

According to the results of the electrical conductivity test, there was a significant interaction between the factors studied (Tables 1 and 2). The higher conductivity values recorded by the conductivity meter indicate a greater release of solutes into the substrate, which is associated with lower seed vigor. Conversely, more vigorous seeds exhibited lower conductivity values. Vieira and Marcos (2020) explain that the electrical conductivity test measures the extent of solute release, including amino acids and inorganic ions, through seed cell membranes. Seeds with lower physiological potential have less organized membranes with reduced selective permeability, depending on the degree of deterioration. This structural weakness leads to uncontrolled diffusion of ions and organic solutes into the surrounding solution during imbibition, which is reflected in the electrolyte concentration in the imbibition medium.

For seeds subjected to a temperature of 25 °C, significant differences were observed across imbibition periods of 2, 4, 6, 8, and 10 h and in water volumes of 25, 50, and 75 mL, allowing the classification of the lots into up to four vigor levels (Table 1). Within each combination of imbibition period and volume, different stratifications were observed: two vigor levels in 75 mL for 2 and 4 h and in 50 mL for 2 h; three vigor levels in 50 mL for 4 h and in 75 mL for 6 and 8 h; five vigor levels in 50 mL for 10 h; and six vigor levels in 25 mL for 10 h. Additionally, the 25 mL volume at 8 h allowed the classification of the lots into seven vigor levels (Table 1).

At a temperature of 30 °C (Table 2), significant differences were also observed among the factors, leading to the classification of up to seven vigor levels among the lots. The 25 mL volume at 10 h stratified the lots into seven vigor levels, while the 25 and 50 mL volumes at 6 and 10 h allowed classification into six levels. The 4- and 8-h periods at 25 and 50 mL resulted in five vigor levels. On the other hand, the 25 mL volume for 2, 6, and 10 h, as well as the 50 and 75 mL volumes for 2, 6, and 8 h, categorized the lots into four and three vigor levels, respectively. The lowest level of discrimination was observed in the 75 and 50 mL volumes for 2 and 4 h, which distinguished only two vigor levels. It is noteworthy that the 25 mL volume for 10 h provided the highest level of stratification, distinguishing seven vigor levels. When comparing the different imbibition volumes, the 25 mL volume demonstrated superior differentiation capability, particularly in the 10-h period, where it achieved vigor stratification (seven levels) comparable to the initial seed quality classification.

For seeds of small species, particularly those in the Brassicaceae family, the optimal imbibition period varies by species. Magro et al. (2011) observed differences in the vigor of broccoli seeds after 1 and 2 h of imbibition. Similarly, Mavi et al. (2014) found that a 17-h imbibition period for radish seeds provided efficient measurements for evaluating seed quality.

According to J. J. P. Lima et al. (2015), imbibition crambe seeds for 16 h in 50 mL of water at 25 °C, using 0.45 g of seeds, was effective for classifying the physiological quality of seed lots. For canola seeds, Milani et al. (2012) used 50 seeds immersed in 25 mL of deionized water for 8 h, which allowed for better classification of seed lots based on physiological quality. Bezerra et al. (2024) also demonstrated the effectiveness of this test for canola seeds, with optimal stratification achieved using 50 or 75 seeds immersed in 75 mL of deionized water for 6 or 9 h.

The electrical conductivity test, performed with distilled water, is an essential tool in laboratory routines, as it provides fast and efficient results for differentiating seed lots. This test enables the identification of seeds with cell membrane damage, ensuring both its reliability and efficiency of seed quality assessments.

This study supports the use of electrical conductivity testing for canola seeds based on consistent findings from previous research. Higher electrical conductivity values indicate lower vigor due to reduced cell membrane integrity and greater solute leaching during hydration (Vieira & Marcos, 2020). This methodology provides an effective assessment of seed lot physiological quality, facilitating a more accurate and efficient classification.

When analyzing the response surface plot for the 'volume' and 'period' factors in the electrical conductivity test at 25 °C and 30 °C (Figure 3), a clear trend is observed: an increase in imbibition period and a reduction in volume result in higher electrical conductivity values. In contrast, larger imbibition volumes are associated with lower conductivity values.

Table 1
Electrical conductivity (μS.cm⁻¹. g⁻¹) of 12 canola seed lots under different imbibition periods and water volumes at 25 °C

	2 h			4 h			6 h			8 h			10 h		
Lot	25 mL	50 mL	75 mL	25 mL	50 mL	75 mL	25 mL	50 mL	75 mL	25 mL	50 mL	75 mL	25 mL	50 mL	75 mL
1	44.06 dA	25.11 bA	21.55 bA	53.26 dA	33.06 cB	26.06 bB	48.33 dA	41.64 dA	29.46 cA	76.44 fA	41.29 dB	31.59 cB	63.98 fA	53.82 eA	31.94 dB
2	63.74 cA	14.09 bB	21.96 bB	53.18 dA	59.14 bA	45.94 aA	47.82 dA	35.01 dA	37.12 cA	144.21 dA	72.08 cB	53.67 cB	178.31 dA	89.47 cB	62.26 cC
3	46.82 dA	22.76 bA	21.71 bA	57.90 dA	34.49 cB	29.53 bB	49.35 dA	38.60 dA	25.63 cA	54.55 fA	43.09 dA	31.85 cA	150.81 dA	71.69 dB	54.19 cB
4	106.28 bA	73.59 aB	52.63 aB	130.39 bA	79.17 aB	51.40 aC	181.63 bA	138.41 bB	64.91 bC	215.31 bA	104.79 bB	71.38 bC	207.93 cA	108.53 cB	81.49 bC
5	32.29 dA	24.69 bA	20.25 bA	60.75 dA	28.95 cB	23.13 bB	49.69 dA	39.05 dA	24.65 cA	33.22 gA	47.23 dB	95.69 aB	85.42 eA	45.93 eB	38.37 eB
6	50.04 dA	25.03 bA	28.66 bA	153.57 aA	61.54 bB	49.41 aB	124.17 cA	63.83 cB	53.94 bB	188.36 cA	97.22 bB	68.06 bC	187.01 cA	112.82 cB	85.24 dC
7	79.45 cA	36.59 bB	37.48 aB	112.08 bA	64.64 bB	39.08 bC	129.31 cA	52.42 cB	41.25 cB	113.50 eA	57.51 dB	43.92 cB	252.81 bA	127.01 bB	97.75 bC
8	151.49 aA	71.73 aB	51.09 aB	157.22 aA	82.81 aB	66.57 aB	298.50 aA	166.12 aB	115.86 aC	270.64 aA	154.25 aB	90.75 cC	308.16 aA	157.50 aB	120.33 aC
9	37.79 dA	36.66 bA	19.15 bA	45.53 dA	30.55 cA	21.04 bA	53.01 dA	35.17 dB	26.17 cB	63.75 fA	40.72 dB	31.67 cB	84.79 eA	47.84 eB	40.36 dB
10	66.70 cA	32.32 bB	26.56 bB	84.66 cA	45.37 cB	31.90 bB	114.94 cA	55.08 cB	41.24 cB	122.94 eA	70.47 cB	46.92 cC	172.64 dA	100.56 cB	54.45 cC
11	37.49 dA	26.26 bA	18.74 bA	51.86 dA	30.66 cB	23.83 bB	59.73 dA	35.09 dB	25.54 cB	73.48 fA	42.05 dB	31.10 cB	81.78 eA	45.28 eB	33.24 dB
12	67.02 cA	36.06 bB	27.01 bB	93.54 cA	47.19 cB	33.90 bB	49.11 dA	35.72 dA	31.05 cA	108.76 eA	58.95 cB	44.38 cB	165.74 dA	67.49 dC	110.88 aB
CV (%)								21.47							

Means followed by the same uppercase letter in the row in each period and lowercase letter in the column for each volume do not differ from each other according to the Scott-Knott test ($p>0.05$). CV = coefficient of variation.

Table 2
Electrical conductivity (µS.cm^{-1}. g^{-1}) of 12 canola seed lots under different imbibition periods and water volumes at 30 °C

	2 h			4 h			6 h			8 h			10 h		
Lot	25 mL	50 mL	75 mL	25 mL	50 mL	75 mL	25 mL	50 mL	75 mL	25 mL	50 mL	75 mL	25 mL	50 mL	75 mL
1	45.80 dA	32.88 cA	19.07 bA	68.23 eA	40.95 bB	26.35 bB	97.93 eA	49.82 dB	30.91 cB	85.90 eA	56.27 dB	42.37 cB	61.12 gA	54.95 fA	42.05 dA
2	79.09 cA	45.61 cB	30.20 bB	56.72 eA	38.58 bA	38.26 aA	59.03 fA	51.40 dA	40.92 cA	68.40 eA	83.20 cA	57.95 bA	183.32 cA	76.62 eB	57.69 cB
3	53.81 dA	25.39 cB	20.65 bB	55.93 eA	34.90 bB	25.33 bB	61.92 fA	34.29 eB	34.12 cB	86.34 eA	32.73 eB	45.05 cB	179.40 cA	95.09 dB	61.08 cC
4	112.47 bA	65.97 bB	42.25 bC	189.35 aA	84.68 aB	58.47 aC	194.26 bA	112.41 bB	66.69 bC	199.17 bA	104.82 bB	63.05 bC	256.39 bA	133.54 cB	75.55 cC
5	37.10 dA	24.23 cA	16.78 bA	68.80 eA	30.98 bB	25.67 bB	45.82 fA	31.67 eA	28.02 cA	84.21 eA	44.65 eB	36.75 cB	106.70 fA	66.95 eB	37.88 dC
6	43.89 dA	66.69 bA	42.22 aA	156.58 bA	85.00 aB	57.98 aC	174.17 cA	85.74 cB	60.23 bC	202.30 bA	106.07 bB	67.25 bC	273.07 aA	166.29 bB	82.75 bC
7	92.29 bA	54.33 bB	38.35 bB	82.81 dA	41.56 bB	36.71 aB	164.80 cA	68.53 dB	64.18 bB	108.94 dA	80.60 cB	72.25 bB	284.52 aA	157.98 bB	116.32 aC
8	249.74 aA	89.95 aB	55.73 aC	180.80 aA	93.37 aB	65.65 aC	245.33 aA	139.58 aB	87.27 aC	231.18 aA	128.85 aB	95.88 aC	153.87 dB	296.28 aA	102.66 aC
9	47.26 dA	26.31 cB	20.92 bB	63.83 eA	36.17 bB	27.44 bB	61.95 fA	41.19 eB	28.09 cB	80.93 eA	42.81 eB	28.18 cB	93.93 fA	45.93 fB	32.11 dB
10	69.57 cA	43.27 cB	32.20 bB	130.68 cA	77.39 aB	44.72 aC	175.19 cA	90.48 cB	63.25 bC	161.73 cA	87.74 cB	52.75 cC	106.14 dB	157.14 bA	75.24 bC
11	47.45 dA	26.53 cB	19.55 bB	57.80 eA	35.14 bB	26.45 bB	69.56 fA	39.61 eB	30.97 cB	71.77 eA	44.23 eB	30.15 cB	96.28 fA	51.29 fB	33.50 dB
12	70.57 cA	39.69 cB	25.01 bB	90.66 dA	44.46 bB	36.39 bB	125.09 dA	49.15 dB	31.05 cB	169.27 cA	69.44 dB	57.99 bB	131.40 eA	75.03 eB	49.36 cC
CV (%)								18.84							

Means followed by the same uppercase letter in the row in each period and lowercase letter in the column for each volume do not differ from each other according to the Scott-Knott (p>0.05). CV = coefficient of variation.

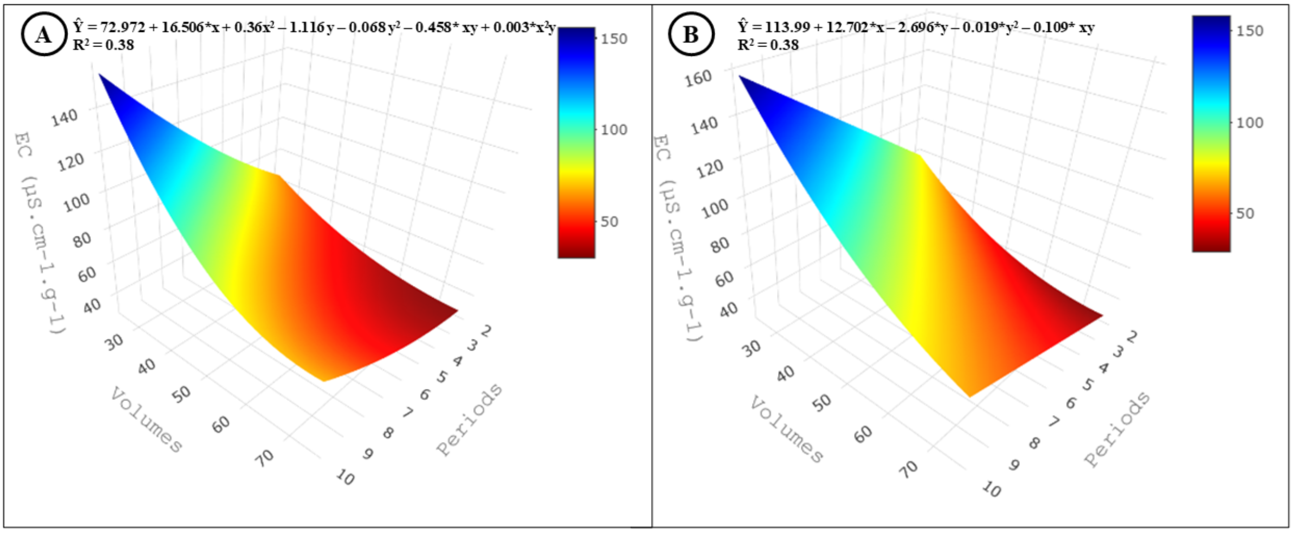

Figure 3. Response surface for the significant interaction between electrical conductivity (µS. cm-¹. g-¹), volumes (25, 50, and 75 mL), and periods (2, 4, 6, 8 and 10 h) at 25 °C (A) and 30 °C (B). Means of 12 canola seed lots.

The highest electrical conductivity values were recorded when seeds were soaked in smaller volumes, such as 25 mL, due to the higher concentration of leachates (ions) in the containers and reduced dilution, regardless of temperature (Figure 3). The 25 mL volume was sufficient to determine the electrical conductivity of the seeds, allowing for the stratification of vigor levels, as shown in Tables 1 and 2.

The 10-h imbibition period exhibited the highest conductivity values, confirming it as the optimal duration for solute leaching and electrical conductivity measurement. Similarly, the smaller imbibition volume consistently resulted in higher conductivity values compared to larger volumes, indicating a greater concentration of leachates. Similar findings were reported for ryegrass (Lopes & Franke, 2010) and zucchini (Dutra & Vieira, 2006) seeds, reinforcing the trend of greater leaching in smaller volumes. Response surface methodology is an optimization technique based on factorial designs, used to explore relationships between variables that may influence a response variable, such as process efficiency. This technique has been widely applied in process optimization and agricultural research (Faria & Pamplona, 2021; Myers et al., 2009).

Due to significant variation in Euclidean distance values and cophenetic correlation between seed lots across the analyzed variables, the original dataset was divided into five groups (Figure 4). These groups revealed significant differences in the similarity ranking of the lots. Groups I and II (black and red) comprised electrical conductivity test treatments, regardless of temperature, and were distinct from the other groups. Group III (blue) maintained the initial characterization of the lots, showing greater similarity to Group IV (green), which included electrical conductivity test treatments at 25 °C and 30 °C with a imbibition volume of 25 mL for 10 h.

Group V contained only electrical conductivity test treatments, independent of temperature, and showed dissimilarity from the others. According to Mingoti (2007), group separation is defined by the clusters that best explain the similarity results. The observed similarity between Groups IV and III supports the findings in Tables 1 and 2, demonstrating that the electrical conductivity test for canola seeds is effective using a 25 mL volume for a 10-h imbibition period.

Thus, the grouping and distribution of the seed lots formed distinct clusters, which were characterized by the physiological performance of the seeds, as determined by the initial characterization of the lots. The clustering analysis successfully distinguished and assessed the similarities among treatments in the electrical conductivity test.

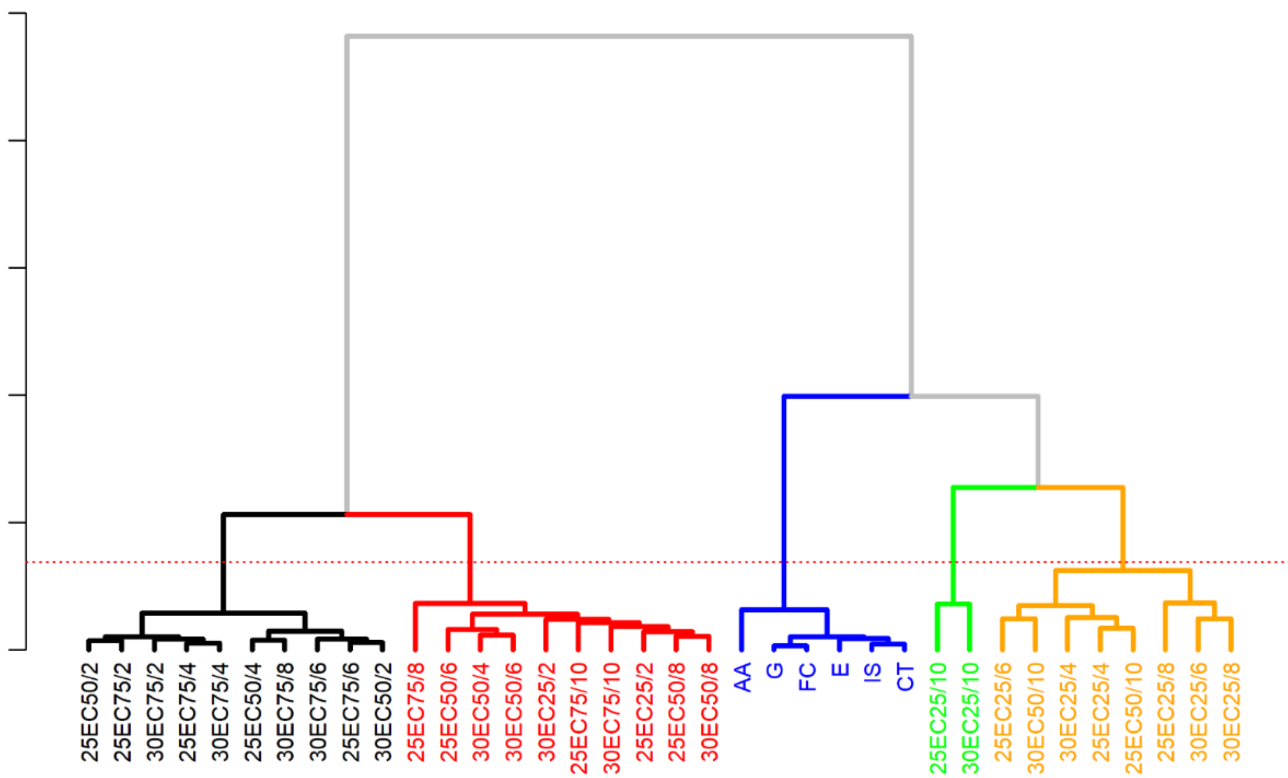

Figure 4. Dendrogram resulting from hierarchical cluster analysis with group formation according to the initial characterization of the lots and the electrical conductivity test in canola seeds. Each color represents a group formed.

Conclusion

The electrical conductivity test, performed with 50 seeds immersed in 25 mL of deionized water for 10 h at 25 °C or 30 °C, enables better stratification of vigor levels among the analyzed seed lots.

Acknowledgments

This study was supported by the Coordination for the Improvement of Higher Education Personnel - MAPA (CAPES) - Finance Code 001 and the National Council for Scientific and Technological Development (CNPq).

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