

# Canola seed vigor under controlled deterioration testing

## Vigor de sementes de canola pelo teste de deterioração controlada

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### Highlights

Canola is an economically important crop, with little assessment of its potential.  
Controlled deterioration testing can be used to assess the vigor of canola seeds.  
Seed lot characterization tests were combined with controlled deterioration testing.  
Controlled deterioration is practical and efficient at determining vigor.

### Abstract

Canola (*Brassica napus* L. var. *oleifera*) is an oilseed with considerable economic importance for human and animal consumption and biodiesel production. Vigor testing is essential in the search for greater advances in determining seed quality. In this respect, the present study aimed to adapt controlled deterioration testing to assess canola seed vigor. To that end, four seed lots of the Nuola 300 canola hybrid were initially characterized using the following tests: moisture content, thousand seed weight, germination first count, germination percentage, germination speed index, initial stand, seedling emergence percentage, emergence speed index, and cold testing. For controlled deterioration, the initial moisture content was adjusted to 15, 18, 20 and 22% and the samples were then placed in a water bath at 41 and 45 °C in a water bath for 24 hours, for germination and assessed five days after sowing. In canola seeds, controlled deterioration tests should be conducted at an 18% moisture content and temperature of 45 °C for 24 hours to evaluate physiological potential.

**Key words:** *Brassica napus* L. var. *oleifera*. Germination. Physiological potential. Physiological quality.

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## Resumo

A canola (*Brassica napus* L. var. *oleifera*) é uma espécie oleaginosa de grande importância econômica para alimentação humana e animal e para produção de biodiesel. Na busca por maiores avanços na obtenção de sementes de qualidade, o uso de teste de vigor é imprescindível. Nesse sentido, o objetivo do trabalho foi adequar a metodologia do teste de deterioração controlada para avaliação do vigor de sementes de canola. Para tanto, quatro lotes de sementes de canola do híbrido Nuola 300 foram avaliados quanto à caracterização inicial dos lotes, pelos testes: determinação do grau de umidade, peso de mil sementes, primeira contagem de germinação, germinação, índice de velocidade de germinação, estande inicial, emergência, índice de velocidade de emergência e teste de frio. Para o teste de deterioração controlada, o grau de umidade inicial foi ajustado para 15%, 18%, 20% e 22% e, posteriormente, as amostras foram expostas às temperaturas de 41 °C e 45 °C, em banho-maria, durante 24 horas, postas para germinarem e avaliadas ao quinto dia após a semeadura. O teste de deterioração controlada em sementes de canola deve ser conduzido com o grau de umidade a 18%, sob a temperatura de 45 °C, durante 24 horas, para avaliação do potencial fisiológico.

**Palavras-chave:** *Brassica napus* L. var. *oleifera*. Germinação. Potencial fisiológico. Qualidade fisiológica.

## Introduction

Considered the world's third largest oilseed crop, canola (*Brassica napus* L. var. *oleifera*), which belongs to the family *Brassicaceae*, was developed by breeding rapeseed (Guimarães et al., 2022). In Brazil, canola stands out for its high yield potential and plant characteristics, with considerable economic importance and potential for expansion given its possible use in crop rotation, interrupting pest and disease cycles and helping reduce soil compaction (Hryczyna et al., 2021).

Some studies have reported its potential in warmer climates, particularly for grain production in the Brazilian Cerrado, given its protein and oil content of approximately 24 to 27 and 38%, respectively (Rigon et al., 2017). However, research aimed at developing crop management technologies remains scarce, with a need for investment in breeding programs to ensure significant

yield gains and, consequently, expansion of the planted area (Santiago et al., 2022).

Seed quality is a key factor in crop yield because it determines the success or failure of production, since seeds represent the productive potential of plants (Bezerra et al., 2022). Thus, using seeds with good physiological quality is essential to adequate crop establishment (R. F. Araujo et al., 2011). Their physiological potential is generally determined via germination testing and complemented with vigor tests (Marcos, 2015).

One such vigor test is controlled deterioration, whereby seeds are aged under controlled temperature and humidity for a determinate period (Kruse, 1999). According to Powell and Matthews (1981), it is the most recommended test for small seeded vegetables, such as *Brassicas*, and can differentiate between seed lots at different stages of deterioration submitted to the same level of stress. The most vigorous lots

show better stress tolerance and superior germination (Krzyzanowski & Marcos, 2020a). Vegetable species tested included cauliflower (Kikuti & Marcos, 2008), cabbage (Bernardis et al., 2015) and sea kale (E. F. L. Araújo et al., 2017).

Given the lack of information on controlled deterioration testing in canola seeds, this study aimed to adapt the test methodology to assess its efficiency in identifying different levels of vigor in canola seed lots.

## Material and Methods

The experiments were conducted at the Seed Laboratory of the Agronomy Department of Universidade Federal do Vale do Jequitinhonha e Mucuri (UFVJM) in Diamantina, Minas Gerais state (MG), Brazil. Four seed lots of the Nuola 300 canola hybrid were used, provided by Empresa Celena Alimentos S/A and Universidade Federal de Lavras (UFLA), as well as seeds collected at the UFVJM experimental field (18° 9' S and 43° 21' W, altitude of 1,400 m) in the 2020/2021 growing seasons.

In initial characterization, the seed lots were assessed for physical and physiological quality based on the following:

### *Thousand seed weight*

Determined in accordance with the methodology of the Brazilian Ministry of Agriculture, Livestock and Supply Ministério da Agricultura, Pecuária e Abastecimento [MAPA] (2009), whereby eight repetitions of 100 seeds were weighed on an analytical

balance and the standard deviation and coefficient of variation calculated, with results expressed in grams.

### *Moisture content*

Obtained via the oven method at 105 °C for 24 hours (MAPA, 2009). Two repetitions were used for each lot, with a sample weight of 1 g of seeds per repetition.

### *Germination test*

Conducted according to the criteria established in Regulations for Seed Analysis (MAPA, 2009), with four repetitions of 50 seeds per lot, placed in plastic germination boxes (Gerbox®) (11 x 11 x 3.5 cm) on three sheets of germitest paper, moistened with distilled water at 2.5 times the weight of the dry paper. Next, the seeds were placed in a Biochemical Oxygen Demand (BOD) incubator under constant light and temperature (20 °C). Assessments were performed on day five (first count) and seven (last count) and the number of normal seedlings calculated. Counts were carried out daily to determine the germination speed index (GSI) as proposed by Maguire (1962), considering the number of seeds with a radicle at least 2 mm long, measured with a digital pachymeter.

### *Emergence test*

Four repetitions of 50 seeds per lot were performed. The seeds were sown in plastic boxes containing a mixture of sand and soil at a ratio of 2:1, moistened with

distilled water at a sandy soil field capacity of 60% (Krzyzanowski et al., 2020b). The boxes were placed in a growth chamber at 20 °C, under a constant photoperiod. After the onset of emergence, daily assessments were carried out and the initial stand was calculated on day five. The test ended when the emergence percentage remained stable for three days and the number of normal seedlings was then counted. For the emergence speed index (ESI), the number of emerged seedlings since emergence onset was counted daily, in line with Maguire (1962).

#### *Cold test*

Conducted based on the recommendations of Cicero and Vieira (2020), whereby four repetitions of 50 seeds per lot were seeded onto germination paper (Germitest®) moistened with water at the equivalent of 2.5 times the weight of the dry paper. Next, the papers were rolled up and grouped into fours (repetitions) with elastic bands, sealed in plastic bags and kept in BOD incubators at 10 °C for seven days. At the end of this period the rolls were removed from the bags, placed in a germination chamber at 25 °C and the percentage of normal seedlings calculated on the fifth day.

After analyses of physical and physiological quality for initial characterization of the seed lots, vigor testing was performed to adapt the controlled deterioration method:

#### *Controlled deterioration*

First, the moisture content of the seed lots was determined and then adjusted

to four different levels (15, 18, 20 and 22%) using the humid atmosphere method (Krzyzanowski & Marcos, 2020a). To that end, four repetitions of 50 seeds were distributed in a single layer onto a mesh screen suspended inside transparent plastic germination boxes (Gerbox®) containing 40 mL of distilled water. The boxes were then sealed and placed in a BOD incubator at 20 °C. The moisture content of the seeds was monitored via successive weighing until the final weight (P2) in grams was obtained. The formula proposed by Krzyzanowski and Marcos (2020a) was applied to achieve the desired moisture content (B), as follows:

$$P2 = [(100 - A) / (100 - B)] \times P1$$

where:

P2 = required weight at the desired moisture content (g).

A = initial seed moisture content (%).

B = desired seed moisture content (%).

P1 = initial seed weight (g).

Once the desired moisture content had been reached, the samples were placed in hermetically sealed aluminum bags in a BOD incubator at 10 °C for 24 hours to achieve equilibrium moisture content. The samples were then placed in a water bath at 41 and 45 °C for 24 hours at each temperature and left to cool at ambient temperature for a further 30 minutes. Next, the seed moisture content was determined by the oven method and germination test, as previously described, with the percentage of normal seedlings calculated on the fifth day after sowing (MAPA, 2009).

Tests to characterize the seed lots in terms of physical and physiological quality were performed using a completely randomized design, with four repetitions per lot. For controlled deterioration, the treatments were arranged in a triple factorial scheme, consisting of four seed lots for each of the four moisture levels (15, 18, 20 and 22%) and two temperatures (41 and 45 °C). Data on seed lot characterization and the controlled deterioration test were analyzed for normality and homogeneity of variances and then submitted to analysis of variance. The means were compared by Tukey's test ( $p < 0.05\%$ ). Next, a dendrogram of the initial characterization variables of the lots was constructed. Based on the clustering performed, Pearson's correlation coefficient ( $r$ ) was calculated, and principal component analysis (PCA) performed for all the combinations of vigor test results. The statistical analyses were performed using R. 4.1.2 software (R Core Team [R], 2022).

## Results and Discussion

The moisture content of the canola seed lots varied from 7.19 to 8.91% (Table 1). According to Marcos (2015), a uniform seed moisture content is important in order to standardize analyses and enable a reliable assessment of physiological potential. Coimbra et al. (2009) reported that similar moisture levels are vital in ensuring that tests are not affected by metabolic differences and the rate of seed water absorption and deterioration.

Canola seeds weigh less than 200 g and are therefore considered small (MAPA, 2009), with analyses to characterize the seed lots (Table 1) showing a variation of up to 5.05 g in thousand seed weight (TSW) between lots. There were no significant differences in seed quality between lots based on first count, germination percentage, germination speed index, emergence percentage and emergence speed index. The initial stand and cold tests indicated significant differences in vigor between lots, with lots 1 and 4 generally more vigorous and 2 and 3 less so.

**Table 1**

Moisture content = M (%), thousand seed weight = TSW (g), normal seedlings at first count = FC (%), germination percentage = G (%), germination speed index = GSI, initial stand = IS (%), seedling emergence percentage = E (%), emergence speed index = ESI and cold test = CT, obtained in initial characterization of canola seed lots

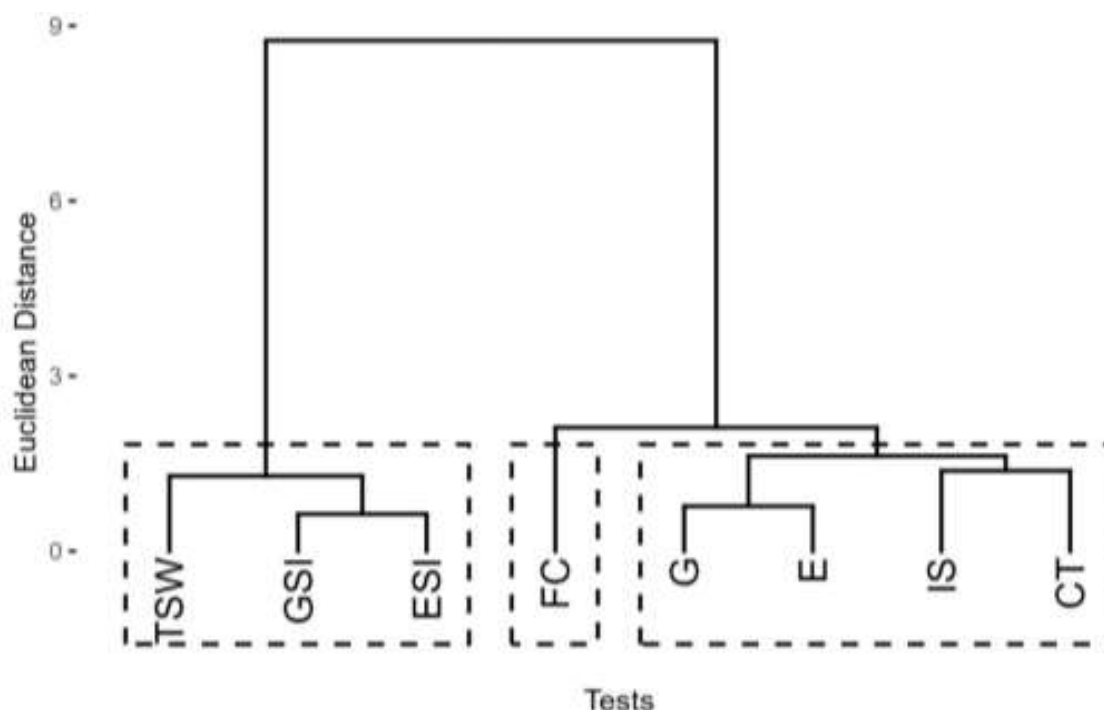
Lots	Tests								
	M (%)	TSW (g)	FC (%)	G (%)	GSI	IS (%)	E (%)	ESI	CT (%)
L1	7.19 b	5.05 a	96 a	99 a	19.34 a	92 a	97 a	11.72 a	98 a
L2	8.91 a	2.43 d	81 a	87 a	14.25 a	64 b	86 a	10.40 a	73 b
L3	7.67 b	3.22 c	73 a	89 a	14.82 a	91 a	95 a	10.22 a	71 b
L4	8.91 a	3.99 b	84 a	92 a	18.14 a	88 a	93 a	10.40 a	100 a
CV (%)	1.37	4.68	17.51	6.13	17.47	5.11	6.97	6.95	5.76

Means followed by the same lowercase letter in the column do not differ according to Tukey's test ( $p > 0.05$ ). CV (%) = coefficient of variation.

The cold test was used to assess the physiological quality of the canola seed lots, classifying them into two levels of vigor. Ávila et al. (2005) and Avila et al. (2008) used the cold test to evaluate the physiological potential of hybrid canola seeds and were able to differentiate between lots based on their vigor, corroborating the results of the present study. Cicero and Vieira (2020) reported that the cold test is one of the most widely used methods to determine seed vigor, whereby seeds are submitted to a combination of low temperature, high substrate water content and the possible action of pathogens, demonstrating the effectiveness of the test.

Cluster analysis made it possible to assess the similarity between analyses performed to characterized the lots (Figure

1), adopting a Euclidean distance of 3 (Hair et al., 2009). The initial characterization tests were separated into three groups: group I contained the variables TSW, germination speed index (GSI) and emergence speed index (ESI), which exhibited greater distance and dissimilarity in relation to the other groups; group II consisted of the first count (FC); with germination percentage (G), emergence percentage (E), initial stand (IS) and the cold test (CT) in group III, which differed from the others and is the largest group in terms of analyses (Figure 1). In this study, analyses to distinguish between groups were based on separating the groups that best explain the similarity results between tests, as highlighted by Mingoti (2007). As such, group III is suggested for future analyses under controlled deterioration.



**Figure 1.** Hierarchical clustering dendrogram with the formation of groups based on thousand seed weight (TSW), first count (FC), germination percentage (G), germination speed index (GSI), initial stand (IS), emergence percentage (E), emergence speed index (ESI) and the cold test (CT).



Seed moisture content was adjusted to 15, 18, 20 and 22%, with a relatively small variation between lots (Table 2). There were no significant moisture content variations, which is important for controlled deterioration

testing. Krzyzanowski and Marcos (2020a) underscored the importance of uniform seed moisture content for controlled deterioration in order to standardize assessments and obtain consistent results.

**Table 2**

**Average moisture content values after adjustment to 15, 18, 20 and 22% moisture at 41 and 45 °C for controlled deterioration testing in four canola seed lots**

Lots	Initial Moisture	Moisture content (%)							
		41 °C				45 °C			
		15%	18%	20%	22%	15%	18%	20%	22%
L1	7.19	15.35	18.47	20.84	22.23	15.44	18.56	20.33	22.63
L2	8.91	15.33	18.57	20.35	22.79	15.44	18.62	20.65	22.63
L3	7.67	15.64	18.27	20.42	22.49	15.34	18.45	20.47	22.28
L4	8.91	15.77	18.51	20.5	22.47	15.05	18.35	20.96	22.41

The results of controlled deterioration demonstrated significant interaction between the factors studied (Table 3). Seeds submitted to a temperature of 41 °C at 15, 20 and 22% moisture showed significant differences and the lots were therefore classified into two levels of vigor, whereas there was no significant difference between lots at 18%. At 45 °C, with moisture content

adjusted to 15, 20 and 22%, the lots were separated into two levels of vigor. Vigor stratification was better at 18% moisture, with lots 1 and 4 classified as high vigor, lot 3 intermediate and lot 2 low vigor and therefore poor physiological quality. In general, the ranking of seed lots in terms of vigor varied according to seed moisture content and exposure time.

**Table 3**

**Average germination rates (%) of four canola seed lots submitted to controlled deterioration using combinations of 15, 18, 20 and 22% moisture and temperatures of 41 and 45 °C**

Lots	Initial Moisture	Moisture content (%)							
		41 °C				45 °C			
		15%	18%	20%	22%	15%	18%	20%	22%
L1	7.19	96 a A	96 a A	71 b B	44 b C	94 a A	91 ab A	76 a B	51 b C
L2	8.91	80 b B	96 a A	91 a A	42 b C	78 b A	77 c A	76 a A	66 a B
L3	7.67	93 a A	89 a A	73 b B	69 a B	78 b A	84 bc A	76 a AB	69 a B
L4	8.91	72 b B	90 a A	69 b B	63 a B	84 b B	97 a A	64 b C	63 a C
CV (%)		6,6							

\*Means followed by the same uppercase letter in the rows and lower case in the columns do not differ according to Tukey's test ( $p > 0.05$ ). CV (%) = coefficient of variation.

The combination of adjusting moisture content to 18 % at a temperature of 45 °C in a water bath for 24 hours showed greater efficiency in differentiating between lots based on vigor via the controlled deterioration test (Table 3). The lot ranking at this moisture level was more consistent with the initial stand and cold tests results obtained in initial seed lot characterization (Table 1). Similar findings were reported by E. F. L. Araújo et al. (2017) in sea kale. Alves et al. (2011) obtained favorable results in controlled deterioration of arugula, with an adjusted moisture content of 18% at 45 °C.

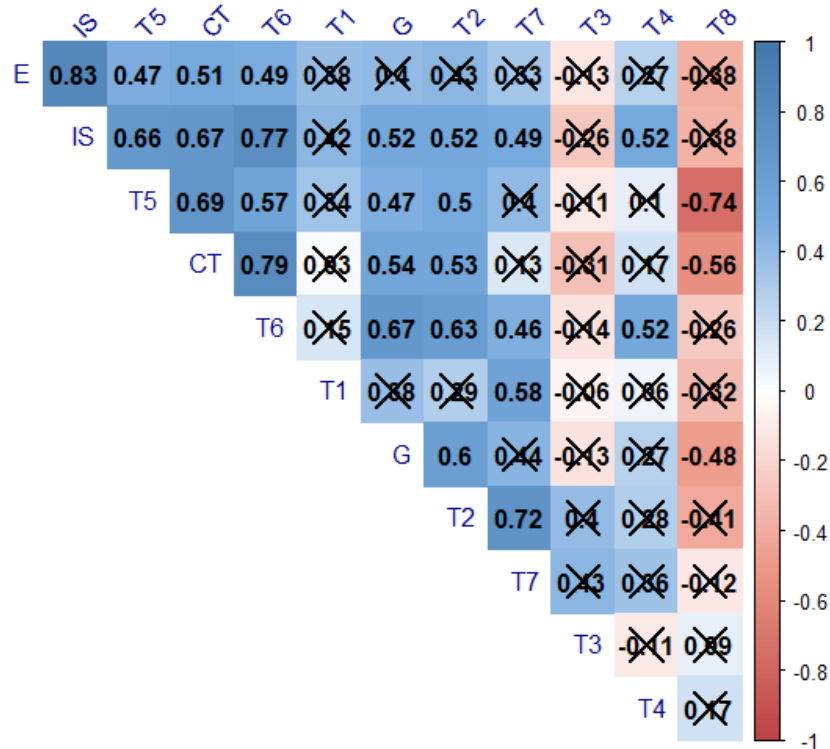
In regard to the performance of lots at higher moisture contents, particularly when adjusted to 22%, the germination percentage declined at both temperatures. Seeds adjusted to a high moisture content were more susceptible to unfavorable environmental conditions, so that the high moisture level at elevated temperatures was accompanied by less stress tolerance, which can affect seed performance due to intense metabolic activity.

Seeds with a high moisture content are more sensitive to adverse environmental conditions, raising stress levels and even causing death (Santos et al., 2003). Kikuti and Marcos (2008) reported unfavorable results in cauliflower seeds at an adjusted moisture content of 22%, with a decline in germination percentage. This reduced germination performance occurred because the seeds were exposed to high temperatures and relative humidity, causing changes that influence protein and nucleic acid synthesis as well as DNA metabolism (Vazquez et al., 1991).

Correlation analysis between the variables in group III and the controlled deterioration tests (Figure 2) showed that the treatment at 45 °C with 18% moisture (T6) obtained the highest correlation coefficients for both lots when compared to the values recorded in the initial characterization tests. Rodrigues et al. (2009) observed a significant correlation between emergence tests and controlled deterioration in sunflower seeds, since these tests help provide information that contributes to selecting seed lots.

According to PCA performed for each temperature (41 and 45 °C) in the four seed lots with different moisture contents (Figures 3 and 4), the first two components (PC 1 and PC2) combined explained most of the variance. At 41°C, PC1 and PC2 explained 84% of total variance (Figure 3), showing close correlations between the initial characterizations tests of the seed lots and the treatment with an adjusted moisture content of 15% (T1). For PCA at 45 °C, PC 1 and PC2 explained 86.4% of total variance (Figure 4), indicating a correlation between T6 (18% adjusted moisture content) and initial characterization testing, which explains the fact that most of the information provided by the variables is of greater relevance. As such, the eigenvectors are located close together and in the same directions, forming angles with each other in the dimensional plane. When the sum of PC1 and PC2 is greater than 80%, the PCA results can be considered effective at explaining total variance (Jolliffe & Cadima, 2016). According to Hongyu et al. (2016), the correlation coefficient assesses the discriminatory power of the variables in each principal component.

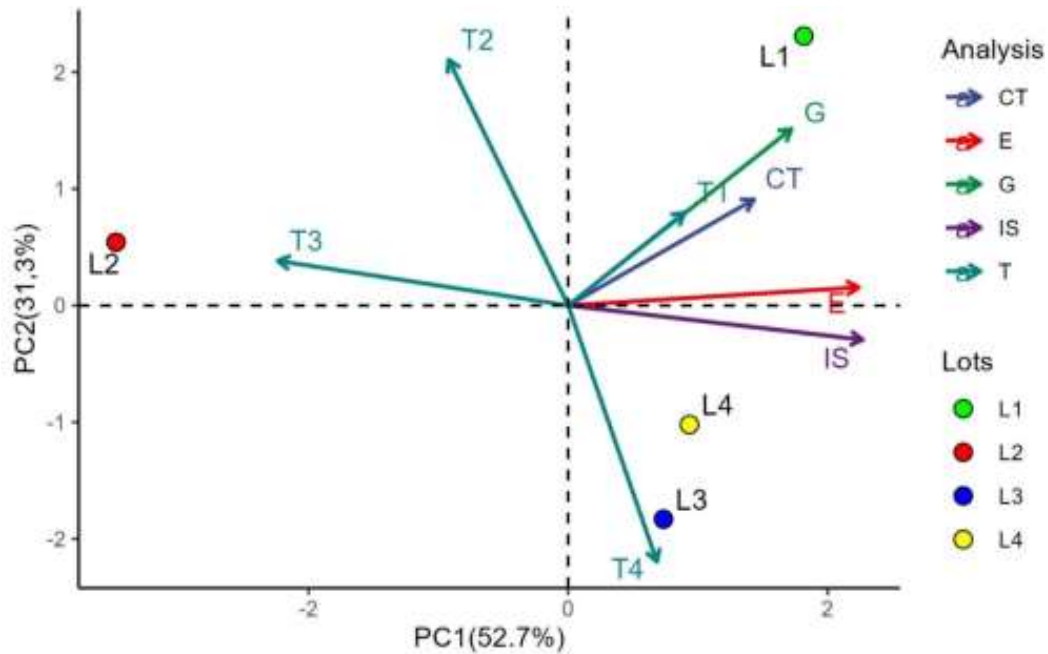




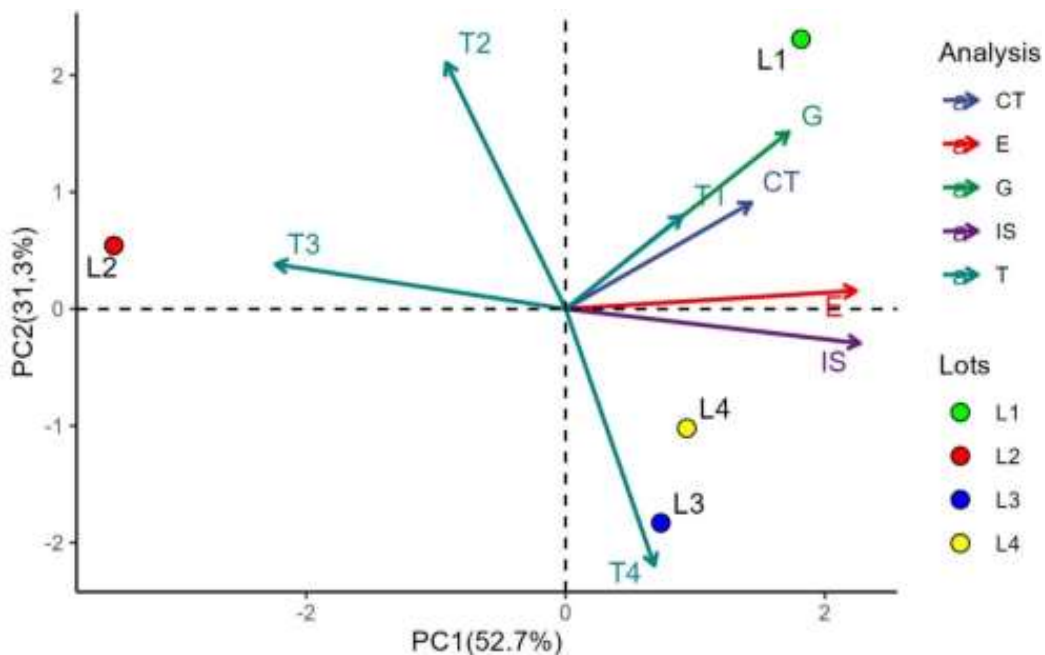
**Figure 2.** Estimated Pearson’s correlation (r) between the variables seedling emergence (E), initial stand (IS), germination percentage (G) and cold test (CT) and the controlled deterioration treatments in canola seeds: T1= 15%/41 °C, T2= 18%/41 °C, T3= 20%/41 °C, T4= 22%/41 °C, T5= 15%/45 °C, T6= 18%/45 °C, T7= 20%/45 °C and T8= 22%/45 °C. Values marked with an “X” exhibited no significant correlation at 5% probability according to the t test.

PC2 exhibited the lowest discriminatory power and cumulative variance at 41 and 45 °C (Figures 3 and 4). However, the angles between vectors for controlled deterioration in relation to the initial seed lot characterization tests represent possible seed deterioration. Thus, an increase in the desired moisture content reduced the percentage of normal seedlings after controlled deterioration (Table 3), especially at 22% moisture, characterizing an extreme adjustment rate. As such, the further the vector of the variable from the lot, the worse the performance of that seed lot in relation to the corresponding variable (J. de O. Araújo et al., 2021).

The proximity between vectors of physiological quality variables and vectors corresponding to T6 (18% moisture at 45 °C) (Figure 4) indicates a high correlation between this treatment and the results of germination tests, initial stand, emergence and the cold test in initial characterization (Table 1). Thus, based on simple correlation (Figure 2) and better discriminatory power, T6 can be confirmed as the best treatment in terms of differentiating between seed lots according to vigor by controlled deterioration (Table 3). The results obtained in the present study suggest that T6 was efficient at classifying canola seed lots according to physiological potential, since the findings were consistent with those recorded in traditional vigor tests.



**Figure 3.** Biplot of principal component analysis obtained via linear combination of the physiological variables seedling emergence (E), initial stand (IS), germination percentage (G) and cold test (CT) and controlled deterioration tests in canola seeds: T1= 15%/ 41 °C, T2= 18%/41 °C, T3= 20%/41 °C and T4= 22%/41 °C.



**Figure 4.** Biplot of principal component analysis obtained via linear combination of the physiological variables seedling emergence percentage (E), initial stand (IS), germination percentage (G) and cold test (CT) and controlled deterioration tests in canola seeds: T5= 15%/45 °C, T6= 18%/45 °C, T7= 20%/45 °C and T8= 22%/45 °C.

## Conclusion

Controlled deterioration of seeds with an 18% moisture content at 45 °C for 24 hours is efficient at evaluating the physiological potential of canola seeds.

Increasing seed moisture content to 22% reduced germination performance and vigor due to seed exposure to high temperatures and humidity.

Clustering the seed lot characterization variables made it possible to identify analyses important in stratifying lots via controlled deterioration.

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