eISSN 1981-1829

Ciência e Agrotecnologia
Science and Agrotechnology

Vigor of canola seeds through accelerated aging test and anatomical alterations

Vigor das sementes de canola pelo teste de envelhecimento acelerado e alterações anatômicas

Ítallo Jesus Silva[1*], Soryana Gonçalves Ferreira de Melo[1], Cássia Michele Cabral[2], Dayana Maria Teodoro Francino[2], Bruno Galvêas Laviola[3], Marcela Carlota Nery[1]

ABSTRACT

Canola is an oilseed crop of significant economic importance for food, feed production, and biodiesel, requiring high-quality seeds to ensure germination, uniformity, and productivity. This study evaluated the suitability of the accelerated aging test to assess seed vigor, as well as anatomical and histochemical alterations after artificial aging. Five seed lots of the Diamond cultivar were evaluated using germination, seedling emergence, and cold tests. The accelerated aging test was conducted using the traditional method and saturated solution with NaCl and KCl at 41 °C for 24 to 96 hours. Seed sections were stained for anatomical and histochemical analysis. The traditional method resulted in more significant seed deterioration, while the saturated solution with KCl for 24 hours preserved physiological quality, presenting cellular and staining patterns similar to non-aged seeds. After 96 hours in the traditional method, cellular disorganization and reduced protein bodies and lipid droplets were observed. The accelerated aging test using saturated solution with KCl at 41 °C for 24 hours proved effective in assessing seed vigor without compromising physiological integrity.

Index terms: Anatomy; *Brassica napus*; deterioration; germination; physiological potential.

RESUMO

A canola é uma oleaginosa de grande importância econômica para alimentação, a produção de rações e o biodiesel, demandando sementes de alta qualidade para assegurar germinação, uniformidade e produtividade. Este estudo avaliou o teste de envelhecimento acelerado para determinar o vigor das sementes, além de analisar alterações anatômicas e histoquímicas após envelhecimento artificial. Foram utilizados cinco lotes de sementes da cultivar Diamond, avaliados por testes de germinação, emergência de plântulas e frio. O teste de envelhecimento acelerado foi realizado pelo método tradicional e com solução saturada utilizando NaCl e KCl, a 41 °C, por 24 a 96 horas. Cortes de sementes foram corados para análise anatômica e histoquímica. O método tradicional resultou em maior deterioração das sementes, enquanto solução saturada com KCl por 24h preservou a qualidade fisiológica, apresentando padrões celulares e de coloração similares às sementes não envelhecidas. Após 96 horas no método tradicional, observou-se desorganização celular e redução de proteínas e lipídios. O envelhecimento acelerado com solução saturada de KCl a 41 °C por 24h mostrou-se eficaz na avaliação do vigor das sementes sem comprometer sua integridade fisiológica.

Termos para indexação: Anatomia; *Brassica napus*; deterioração; germinação; potencial fisiológico.

Introduction

Canola (*Brassica napus* L. var. *oleifera*) represents one of the most essential oilseeds globally, cultivated due to the high quality of its oil, utilized in both human nutrition and various industrial uses, including chemical, pharmaceutical, and biofuel production (Bocianowski & Liersch, 2022). In Brazil, it serves as an alternative crop in the cultivation system, particularly during intercropping periods, focusing on grain production containing approximately 24% to 27% protein and roughly 38% oil (Secchi et al., 2023).

The physiological quality of seeds plays a crucial role in stand establishment and initial plant development (Gularte, Macedo, & Panozzo, 2020). A challenge faced in research to assess the physiological quality of seeds lies in identifying vigor tests that detect deterioration events before the loss of seed germinability (Silva et al., 2019).

The requirements for an efficient vigor test include high sensitivity to differences in physiological potential not detected by the germination test and the ability to classify seed lots according to their performance when exposed to stress (Mathias & Coelho, 2021). The accelerated aging test detects

Agricultural Sciences

Ciênc. Agrotec., 49:e022124, 2025
http://dx.doi.org/10.1590/1413-7054202549022124

Editor: Renato Paiva

[1]Universidade Federal dos Vales do Jequitinhonha e Mucuri/UFVJM, Departamento de Agronomia, Diamantina, MG, Brasil
[2]Universidade Federal dos Vales do Jequitinhonha e Mucuri/UFVJM, Departamento de Ciências Biológicas, Diamantina, MG, Brasil
[3]Embrapa Agroenergia, Parque Estação Biológica/PqEB, Brasília, DF, Brasil

[*]Corresponding author: itallo.jesus@ufvjm.edu.br

Received in October 23, 2024 and approved in December 18, 2024

vigor differences among seed lots with similar germination and effectively evaluates the vigor of seeds from different species (Marcos-Filho, 2016). Some authors, such as Amaro et al. (2014), Lima et al. (2015), and Sales et al. (2022), found that the accelerated aging test in *Brassica* seeds provided satisfactory results for distinguishing the vigor of coriander, turnip, and kale.

Seed aging is a deterioration process associated with the final stage of development, leading to irreversible changes that affect their physiological potential. These changes can occur at the cellular level, involving anatomical and physiological modifications, especially under stress conditions, and are a natural part of the plant's life cycle until death (Popov et al., 2022). Anatomical and histochemical studies are essential for identifying cellular and tissue alterations associated with stress, as well as assisting in the mobilization of reserve compounds, providing insights into the loss of seed physiological quality (Cerri & Reale, 2020).

In light of the above, this study aimed to adapt the methodology of the accelerated aging test to assess the efficiency in identifying different levels of vigor among canola seed lots and evaluate, through anatomical and histochemical analyses, essential alterations in seed physiological quality after aging tests.

Material and Methods

The experiments were conducted at the Seed and Plant Anatomy Laboratories of the Department of Agronomy and Biological Sciences of Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), in Diamantina, Minas Gerais state (MG) (18°12'07" S, 43°34'24" W, altitude of 1.402 m), Brazil. Five seed lots of canola cultivar Diamond were used, provided by Empresa Celena Alimentos S/A, Universidade Federal de Lavras (UFLA) and EMBRAPA (Agroenergia), as well as seeds collected at the UFVJM experimental field (18° 12' 06" S and 43° 34'08" W, altitude of 1,386 m above sea level). In initial characterization, the seed lots were assessed for physical and physiological quality based on the following:

Moisture content - MC: obtained via the oven method at 105 °C for 24 h (Brasil, 2009). Two replications were used for each lot, with a sample weight of 1 g of seeds per replication.

Germination: conducted with four replicates of 50 seeds each per lot in plastic boxes (11.0 cm x 11.0 cm x 3.0 cm), moistened with water amount equivalent to 2.5 times the mass of dry paper and placed in a germinator at 20 °C under constant light (Brasil, 2009). Assessments were performed on the fifth day (germination first count - FC) and a final count (G) on the seventh day after planting. Ree expressed in percentage of normal seedlings. In addition, daily counts allowed the determination of the speed of germination index (GSI), as proposed by Maguire (1962).

Seedling emergence test: four replications of 50 seeds each per lot were evaluated. Seeds were distributed in plastic boxes, as above, 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%. The boxes were placed in a growth chamber at 20 °C under a constant photoperiod. After the onset of seedling emergence, daily counts were carried out, and the initial stand (IS) was calculated on day five after sowing. The test ended when the seedling emergence (E) counts remained stable for three consecutive days when the mean number of normal seedlings was registered (Krzyzanowski et al., 2020). For the speed of seedling emergence index (ESI), the number of emerged normal seedlings was counted daily, according to Maguire (1962).

Cold test (CT): conducted based on the recommendations of Cicero e Vieira (2020), whereby four replications of 50 seeds each per lot were distributed onto germination paper towel moistened with water equivalent to 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 a BOD incubator at 10 °C for seven days. At the end of this period, the rolls were removed from the bags and placed in a germination chamber at 25 °C, and the percentage of normal seedlings was determined on the fifth day.

Accelerated aging test procedures - Traditional Method (TM): four replications of 50 seeds each per lot were performed, placed in uniform layers on an aluminum screen placed inside accelerated aging plastic boxes containing 40 mL of water, providing a relative humidity (RH) of approximately 100%. These containers were then placed in a BOD at a temperature of 41 °C for 24h, 48h, 72h, and 96h. Before and after each period, the moisture content was determined, and the germination test was performed as described earlier; as described above, evaluation was performed on the fifth day after sowing. Saturated Salt Accelerated Aging (SS): conducted with four replications of 50 seeds each per lot, as described before (TM). However, 40 mL of saturated solutions of Sodium Chloride (SS-NaCl/76% RH), composed of 40 g of NaCl per 100 mL of water, of Potassium Chloride (SS-KCl/87% RH), composed of 32 g of KCl per 100 mL of water, were added to the accelerated aging plastic boxes containers (Marcos-Filho, 2020).

The anatomical and histochemical analyses were conducted after the accelerated aging tests, with samples of unaged seeds (US) - control, aged seeds by the accelerated aging test with KCl solutions for 24h (AS-KCl/24h) - considered efficient in the physiological evaluation of canola seeds, and aged seeds by treatment with the traditional method for 96 hours (AS-H_2O/96h) - considered a more drastic procedure.

Anatomical and histochemical characterization: The canola seeds US, AS-24h/KCl, and AS-96h/H_2O were first fixed in FAA70% (formaldehyde, acetic acid, and ethanol at 70%, in a ratio of 9:0.5:0.5) under vacuum for 48 hours and then stored in 70% ethanol (Kraus et al., 1998). Subsequently, the material was subjected to an increasing ethanol series for dehydration (80%, 90%, 95%, and 100% + resin 1:1) and embedded in methacrylate resin (Historesin - Leica), following the manufacturer's

recommendations. Cross-sections of 5 µm thickness were obtained using a manual rotary microtome (Model 829 Serial 71611) and stained with toluidine blue (O'Brien et al., 1964). The permanent slides were mounted in Clear 500® glass varnish. For histochemical analyses, freehand sections were obtained using a bench microtome (Euromex Mikrotom Grampeavel) and adhered to adhesive-moistened histological slides. These sections were treated with the reagents/dyes Xylidine Ponceau (XP) for total protein detection and Sudan IV for lipid detection (O'Brien & McCully, 1981). The slides were observed under a light microscope model Leica DM50, and photomicrographs were captured using a photomicroscope (Primo Star-Zeiss AxioCam ERc 5s). Subsequently, the photomicrographs were analyzed for structural changes, staining intensity, and any damages resulting from the treatments in the seed cells.

The physiological quality tests for lot characterization were conducted in an experiment with a completely randomized design, with four replications per lot. In the accelerated aging test, the experimental design was completely randomized in a triple factorial scheme, considering five lots in three different solutions (TM, SS-NaCl, and SS-KCl) and four aging periods (24h, 48h, 72h, and 96h). The lots characterization and accelerated aging test data were analyzed for normality and homogeneity of variances, followed by an analysis of variance; means were compared using Tukey's test (p<0.05%). A dendrogram of the variables was also constructed for the initial seed lots characterization. The Pearson's simple correlation coefficient (r) was calculated from this grouping, and multivariate principal component analysis (PCA) was performed for all combinations of vigor test results. All statistical analyses were conducted using the R statistical software, version 4.1.2 (R Core team, 2024).

Results and Discussion

The canola seed moisture content ranged from 7.2% to 9.2% among the lots evaluated (Table 1). The germination first count for lot 5 was observed to be higher than the others. Lots 1 and 5 showed higher vigor with respect to the speed of germination index, while lot 4 exhibited lower vigor. There were differences in germination percentage among the lots, with higher values from lots 1 and 5 and lower percentages in lot 4. Overall, the results obtained in initial stand, percentage, and speed of seedling emergence showed similarity in the classification of canola seed lots in terms of vigor, with lots 1 and 5 classified as superior performance and 4 as inferior in vigor. The cold test identified lot 4 as lower performance compared to the others. Seed quality is essential for successful crop establishment and high yield, highlighting the importance of uniform moisture content to standardize analyses related to seed physiological quality (Marcos-Filho, 2016). Avila et al. (2008) and Ávila et al. (2005), in their studies on the physiological quality of canola seeds, were able to classify and differentiate seed lots into different vigor levels using germination, cold, and seedling emergence tests, as performed in this study.

Through cluster analysis, it was possible to verify the similarity between the analyses performed in the initial characterization of the seed lots (Figure 1). The variables were separated into distinct clusters below the cutoff line by adopting the Euclidean distance as a reference. Therefore, the GSI and ESI tests are similar but exhibit dissimilarity with the other tests based on the distance. Thus, the variables G, FC, E, IS, and CT show similarity among themselves. In this study, the analysis for distinguishing the clusters was defined based on the separation of clusters that best explain the results of similarity between the tests. Thus, the clusters with the best similarity were used for future analyses with the accelerated aging test.

Regarding cluster grouping, Mingoti (2007) highlights that the distinction of clusters should be defined based on the cluster separation that best explains the similarity results among the tests. Each cluster represents a group of observations more similar to observations in other clusters (Hair et al., 2009).

Table 1: Mean values obtained in the tests of moisture content - MC, germination first count - FC, germination percentage - G, speed of germination index - GSI, initial stand - IS, percentage seedling emergence - E, speed of seedling emergence index - ESI and cold test - CT, obtained in initial characterization of canola seed lots.

Lot	Tests							
	MC (%)	FC (%)	G (%)	GSI	IS (%)	E (%)	ESI	CT (%)
L1	8.5 b	93 ab	98 a	19.9 a	96 ab	98 ab	20.3 ab	98 a
L2	7.3 c	84 b	90 bc	15.6 bc	75 c	89 b	14.5 c	76 b
L3	7.2 c	87 b	95 ab	17.8 ab	84 bc	94 ab	17.9 b	94 a
L4	7.8 c	73 c	88 c	12.9 c	73 c	76 c	12.6 c	68 c
L5	9.2 a	98 a	99 a	19.9 a	99 a	100 a	20.9 a	99 a
CV (%)	1.2	5.6	2.7	7.6	7.4	4.9	6.7	3.8

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.

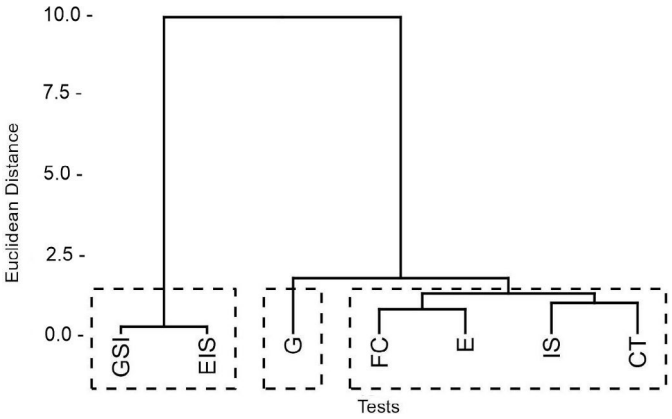

Figure 1: Hierarchical clustering dendrogram with the formation of groups based on germination first count (FC), germination percentage (G), speed of germination index (GSI), initial stand (IS), percentange of seedling emergence (E), speed of seedling emergence index (ESI) and the cold test (CT).

The moisture levels of the seed lots in each accelerated aging period did not show expressive variations after being subjected to different accelerated aging procedures in all treatments (Table 2). Thus, they remained within tolerable limits.

The variations in moisture content of the lots at each period remained within the recommended limit by Marcos-Filho (2016), which establishes tolerable variations of up to four percentage points. The decrease in seed vigor after TM aging and germination rate after aging exposure is likely due to the high moisture content during this period, as pointed out by Costa, Trzeciak and Villela (2008). Similar results were found by Lima et al. (2015) in cambre seeds.

According to the accelerated aging test results, there was a significant interaction of the studied factors (Table 3). The traditional, accelerated aging procedure led to a more drastic reduction in canola seed germination after aging, especially in the 96 h. On the other hand, the SS-NaCl resulted in less severe aging than the SS-KCl. However, results from SS-KCl for 24 h uniformly differentiated the lots, allowing for the distinction of lots closer to the germination test in the initial characterization. Thus, lots 1 and 5 showed higher vigor, and 4 was the lowest. Overall, the ranking of lots according to vigor levels varied depending on the exposure period to temperature and the use of saturated salt solution.

In the Pearson correlation analysis conducted with the variables obtained by the similarity of clusters with the treatments of the accelerated aging test (Figure 2), it is noteworthy that the use of SS-KCl for 24h showed the highest coefficients of simple correlation for both lots when compared with the results of the initial characterization tests of the lots. Conversely, Leeks et al. (2007), with *Brassica* seeds, also achieved greater efficacy in results when correlated with the SS-KCl for 24 h.

Table 2: Average percentage values of moisture content of canola seeds subjected to accelerated aging periods by traditional method (TM) and saturated solutions (SS) of NaCl and KCl.

Lot	TM			
	24h	48h	72h	96h
1	13.3	16.5	13.1	14.5
2	14.8	16.3	13.5	16.1
3	12.8	14.7	13.8	16.4
4	12.0	15.5	13.6	15.3
5	12.8	15.3	15.0	14.7
	SS-NaCl			
1	5.6	5.9	5.6	5.6
2	6.0	6.1	6.3	6.1
3	5.8	5.8	5.7	5.9
4	5.2	5.9	5.8	6.0
5	6.2	5.3	5.4	5.5
	SS-KCl			
1	8.7	7.4	7.1	7.0
2	8.6	8.3	7.2	8.0
3	8.3	7.5	8.9	7.0
4	7.9	8.0	7.1	7.8
5	8.9	7.4	7.4	6.1

The first two principal components (PC1 and PC2) explain the most accumulated variance, discriminating 82.3% of the total data variability (Figure 3). Thus, it was possible to observe close correlations between the initial lot characterization tests and treatments T2, T3, T5, and T11, as their vectors are in the same direction and form acute angles with each other in the dimension plane. However, it is essential to note that only treatment T3, with SS-KCl for 24 h, effectively stratified the lots into three levels of vigor (Table 3).

In PC2, a lower accumulated variance is observed, where treatments T4, T7, and T10, predominantly representative of the TM, show a departure from the vectors of the initial lot characterization tests, showing no correlation. This results in lower discriminative power, indicating a distancing from the other tests and treatments in the opposite direction and angle (Figure 3). This suggests a possible deterioration of the seeds, reflected by a low percentage of normal seedlings (Table 3), when compared to the other treatments, especially with an increase in the exposure period of the seeds to aging.

The proximity of the vectors of physiological quality variables to treatment T3 (Figure 3) indicates a correlation between the initial characterization variables and the mentioned treatment, highlighting a solid discriminatory

power for separating seed lots into different vigor levels by the accelerated aging test (Table 3). Thus, the results obtained in this study suggest that T3 was effective in classifying canola seed lots in terms of their physiological potential, maintaining consistency with the results obtained in traditional vigor tests. However, it becomes evident that treatment T10 results in a greater distancing of vectors in the opposite direction to the initial characterization tests. This indicates that the prolonged exposure period of the seeds to aging caused more pronounced deterioration with high humidity and temperature, resulting in drastic damage, as demonstrated by the low percentage of seed germination.

Table 3: Percentage of normal seedlings from the germination test after accelerated aging of the canola seed lots.

Lot	TM			
	24 h	48 h	72 h	96 h
1	74 aA	78 aA	81 aA	54 aB
2	77 aA	73 aAB	66 bB	3 bC
3	77 aA	18 cB	12 cB	1 bC
4	66 aA	48 bB	64 bA	49 aB
5	76 aA	74 aA	62 bB	8 bC
	SS-NaCl			
1	95 aA	98 aA	96 aA	91 abA
2	85 abA	93 aA	84 bA	83 abA
3	94 aA	95 aA	94 abA	93 aA
4	76 bA	80 bA	84 bA	81 bA
5	91 aA	98 aA	90 abA	92 aA
	SS-KCl			
1	89 aA	79 bA	81 aA	67 bcB
2	77 bcA	82 abA	79 aA	78 abA
3	88 abA	92 aA	77 aB	88 aA
4	67 cAB	65 cAB	76 aA	61 cB
5	92 aA	79 bB	70 aB	75 bB
CV (%)	7.97			

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

Combining all the characteristics of the variables through PCA can be considered effective in explaining the total variability of the observed data, as according to Jolliffe and Cadima (2016), data variability is considered acceptable and reliable when it reaches at least 80% of the total variance. As stated by Hongyu et al. (2016), the discriminatory power of the variables in each principal component is evaluated by the value of the correlation. Therefore, the farther the variable vector is from the batch, the lower the performance of that batch will be about the corresponding variable (Araújo et al., 2021). In this context, along with the Pearson correlation, the results obtained by PCA confirm that seed deterioration at high temperatures and RH contributed to the reduction in seed vigor. Marcos-Filho (2016) reports that higher temperatures and elevated RH increase seed viability loss.

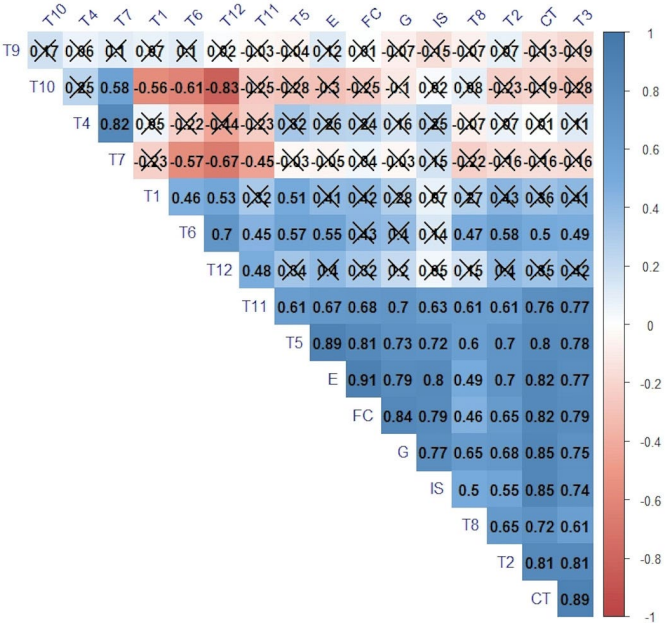

Figure 2: Estimated Pearson's correlation (r) between the variables seedling normal seedlings at emergence (E), initial stand (IS), first count (FC), germination percentage (G) and cold test (CT) and the treatments of the accelerated aging test conducted on canola seeds: T1 (TM/24h), T2 (SS-NaCl/24h), T3 (SS-KCl/24h), T4 (TM /48h), T5 (SS-NaCl/48h), T6 (SS-KCl/48h), T7 (TM /72h), T8 (SS-NaCl/72h), T9 (SS-KCl/72h), T10 (TM /96 h), T11 (SS-NaCl/96 h) e T12 (SS-KCl/96 h). Values marked with an "X" exhibited no significant correlation at 5% probability according to the t test.

The canola seed's anatomical analysis revealed several distinct structures, including seed coat, outer and inner cotyledons, and radicle (Figure 4. A, B, C, and D). The canola seed is exalbuminous, with two cotyledons that enclose the radicle, with the outer cotyledon being larger and enclosing the inner one. The canola seed has a thick seed coat and a uniseriate epidermis. The presence of palisade cells forming the aleurone layer is evident below the epidermis. The middle region of the canola seed cotyledons exhibits vascular tissues and palisade cells. This morphological characteristic of the cotyledons contributes to the identification and understanding of the structure and cellular composition of these seeds.

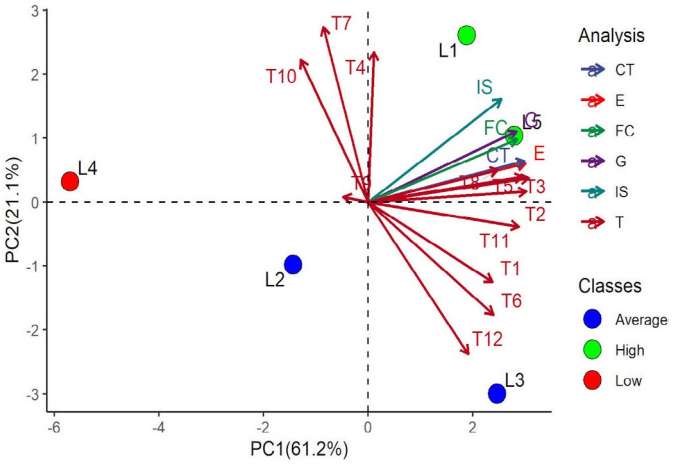

Figure 3: Biplot of principal component analysis obtained via linear combination of the physiological variables seedling emergence (E), initial stand (IS), germination percentage (G) and cold test (CT) and emergence (E), initial stand (IS), first count (FC), germination percentage (G) and cold test (CT) and the treatments of the accelerated aging test conducted on canola seeds: T1 (TM/24h), T2 (SS-NaCl/24h), T3 (SS-KCl/24h), T4 (TM /48h), T5 (SS-NaCl/48h), T6 (SS-KCl/48h), T7 (TM/72h), T8 (SS-NaCl/72h), T9 (SS-KCl/72h), T10 (TM/96 h), T11 (SS-NaCl/96 h) e T12 (SS-KCl/96 h).

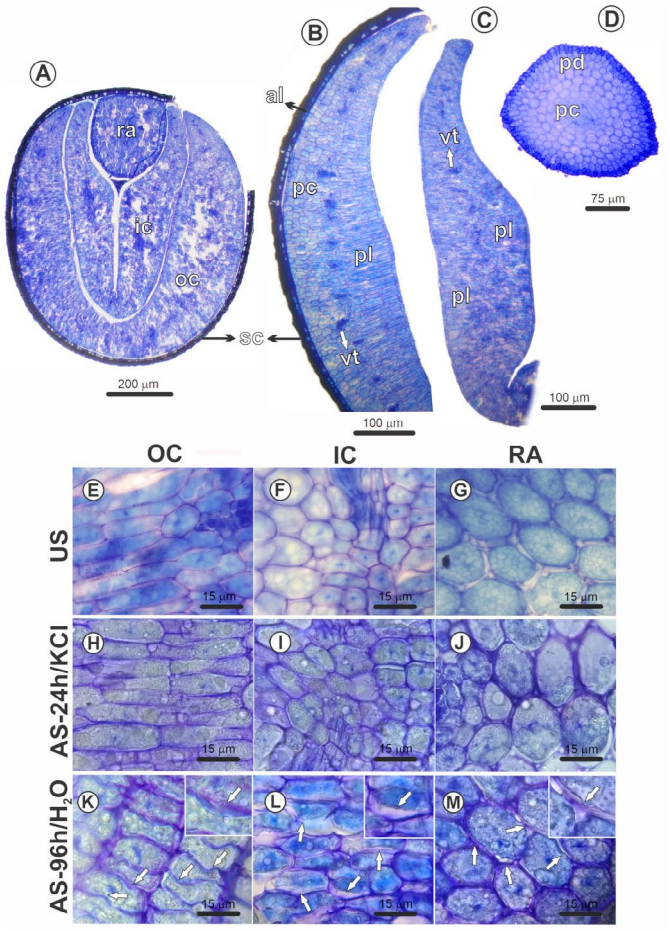

Figure 4: Transverse section of canola seed stained with toluidine blue. Whole seed (A). Outer cotyledon - OC (B). Inner cotyledon - IC (C). Radicle - Ra (D). Details of OC, IC, and Ra sectioned in unaged seeds - US and aged seeds - AS (E, F, G, H, I, J, K, L, M). ra: radicle, ic: inner cotyledon, oc: outer cotyledon, sc: seed coat, pl: palisade layer, al: aleurone layer, pd: protoderm, pc: procambium, and vt: vascular tissue. Black arrows indicate disarray and alteration in cell structure.

Canola seeds are classified as strictly exalbuminous (Groot & Caeseele, 2011). During maturation, rapeseed seeds, including canola, have degenerated endosperm while the tegument tightly encloses the embryo but not the endosperm (Hu et al., 2013). The canola seed's anatomical arrangement highlights the cotyledons' structural and functional complexity, confirming previous findings by Hu et al. (2013) and Zhang et al. (2022), Verboven et al. (2013) described that the cotyledons of canola seeds have spherical cells, with the hypocotyl/radicle centrally located, composed of small rounded cells. In *Brassica* seeds, lipids and proteins are often found as reserve sources, with lipids stored as lipid droplets and proteins as protein bodies (Robbelen & Thies, 1980). According to Borisjuk et al. (2013), the lipid content in the external cotyledon is higher than in the internal cotyledon in rapeseed seeds.

The anatomical observations of the canola seed constitution in the cotyledons revealed notable effects, significantly increasing intercellular spaces resulting from seed deterioration in the AS-H_2O/96h. This condition becomes more evident as the exposure period to the accelerated aging test by TM is prolonged. There was an increase in intercellular spaces, indicating disarrangement among the cells (Figure 4. K, L, and M). However, the US and AS-KCl/24h showed no changes, maintaining their cellular structures intact (Figure 4. E, F, G, H, I, and J). These findings indicate the structural deterioration of canola seed cells when subjected to prolonged stress conditions due to high temperature and RH during the aging test by TM, contrasting with the structural preservation observed in treatments with lower stress exposure.

The histochemical test results of canola seeds revealed the presence of reserves of protein compounds stained with XP reagent and lipid compounds stained with Sudan IV reagent in the parenchymal cells, both in the outer and inner cotyledons (Figure 5). The analyzed sections of the US and those subjected to aging by AS-KCl/24h exhibited a similar staining pattern (Figure 5. F, H, J, and L), indicating a uniform distribution of proteins. However, treatment with AS-H_2O/96h revealed slight changes in the shape and arrangement of cells, accompanied by a noticeable reduction in stained protein bodies, suggesting a decrease in protein production (Figure 5. N and P).

The outer and inner cotyledons were positively stained with Sudan IV, revealing the lipid droplets (Figure 5). No visual differences in staining were observed between the US treatments

and AS-24h/KCl (Figure 5. E, G, I, K), suggesting stability in lipid reserves even under stressful conditions. However, in the treatment with AS-H_2O/96h, there was a slight alteration in tissue structure and compaction, indicating an increase in intercellular spaces and a reduction in lipid droplets (Figure 5. M and O). This suggests a possible decrease in lipid production. Thus, the susceptibility of seeds to stress during aging may be directly related to their quality.

The anatomical findings of seeds subjected to accelerated aging TM indicate changes in cells, as Oliveira et al. (2011) observed in sorghum seeds, which exhibited irregular cell shapes ranging from elliptical to more rounded and a reduction in the number of protein bodies. According to Marcos-Filho (2016), exposure of seeds to high temperatures and RH tends to cause severe degenerative changes in seed metabolism, disrupting and losing membrane integrity and protein synthesis. Cellular damage can affect the accumulation of compounds in seeds, such as lipids and carbohydrates, which are generally consumed during germination and play a fundamental role in this process (Han et al., 2017).

Canola seeds exposed to high temperatures and relative humidity undergo degeneration due to the excessive production of reactive oxygen species (ROS), leading to cellular disorganization and degradation of reserves, which compromises their physiological quality, as evidenced by the loss of vigor (Figure 3). The AS-H_2O/96h accelerated aging test exacerbates this ROS production, directly impacting the seeds' physiological potential. The uncontrolled accumulation of ROS can cause cellular and structural damage, including changes in proteins and lipids, resulting in abnormal or inviable seedlings. Evaluating seed quality over time is essential to understanding the effects of aging on seed viability.

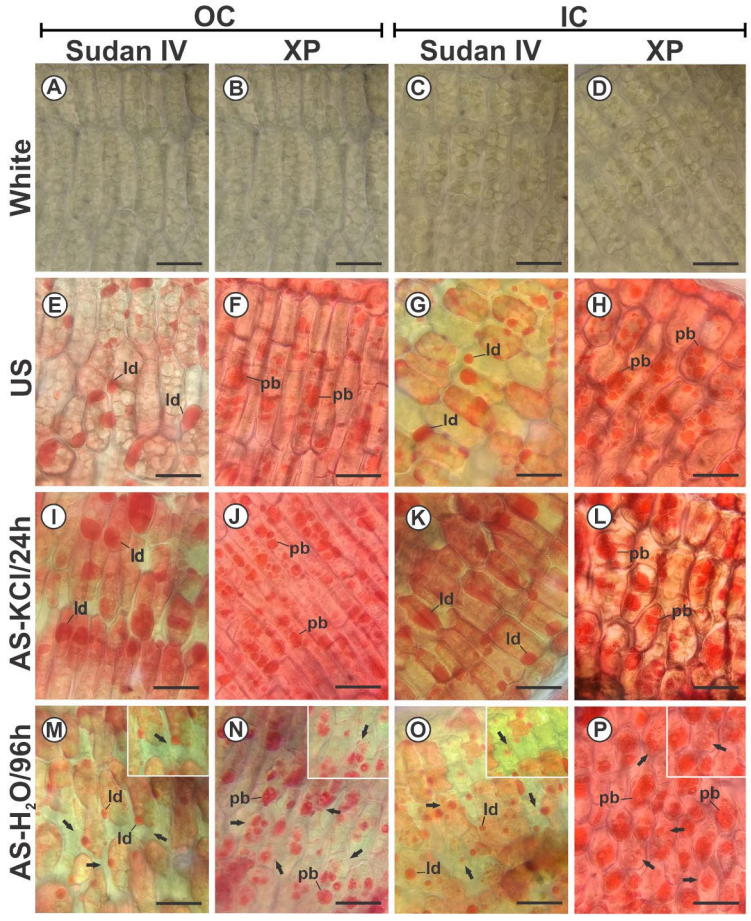

Figure 5: Transverse sections of outer cotyledon (OC) and inner cotyledon (IC) of canola seed stained with Sudan IV and Xylidine Ponceau (XP). Unstained sections - White (A, B, C, D). Unaged seeds (US): OC stained with Sudan IV (E), OC stained with XP (F), IC stained with Sudan IV (G), IC stained with XP (H). Aged seeds AS-KCl/24h: OC stained with Sudan IV (I), OC stained with XP (J), IC stained with Sudan IV (K), IC stained with XP (L). Aged seeds AS-H_2O/96h: OC stained with Sudan IV (M), OC stained with XP (N), IC stained with Sudan IV (O), IC stained with XP (P). Black arrows indicate alteration in cell structure and loss of reserves. ld: lipid droplet, pb: protein bodies. Scale bars: 15 µm.

The emergence of ROS is one of the mechanisms of cellular metabolic activity triggered by factors such as high temperature and humidity induced by changes in water content (Lah et al., 2023). Seed aging or deterioration is a natural process that triggers a series of cytological, physiological, biochemical, and physical changes, leading to reduced vigor and germination and, ultimately, to loss of viability (Choudhury & Bordolui, 2023). Zhang et al. (2021), during orthodox seeds' aging, cited changes such as membrane damage, organelle destruction, increased loss of reserve compounds, reduced respiratory rate, and overall cellular collapse.

In summary, through physical, physiological, anatomical, and histochemical analyses, all these observations reinforce the deleterious effects of deterioration related to high temperature and RH on canola seeds. Moreover, they are directly associated with the decline in vigor.

Conclusions

The accelerated aging test with a saturated solution of KCl at 41 °C for 24 hours effectively assesses the vigor of canola seeds without affecting the protein bodies and lipid droplets under stress conditions. High humidity and temperature in the traditional accelerated aging method disrupt cellular organization, increase intercellular spaces, and reduce protein bodies and lipid droplets, compromising seed vigor. The anatomical and histochemical analysis confirmed these effects and assisted in selecting the most suitable method for evaluating seed quality under stress conditions.

Author contributions

Conceptual idea: Silva, I. J.; Nery, MC; Methodology design: Silva, I. J.; Cabral, M. C.; Francino, D. M. T.; Nery, M. C.; Data collection: Silva, I. J.; Melo, S. G. F.; Data analysis and interpretation: Silva, I. J.; Francino, D. M. T.; Laviola, B. G.; Nery, M. C; Writing: Silva, I. J.; Editing: Silva, I. J.; Francino, D. M. T.; Nery M. C.

References

Amaro, H. T. R. et al. (2014). Accelerated aging test on crambe (*Crambe abyssinica Hochst*) seeds, FMS Brilhante cultivar. *Revista Ceres*, *61*(2):202-208.

Araújo, J. O. et al. (2021). Accelerated aging test and antioxidant enzyme activity to assess chickpea seed vigor. *Journal of Seed Science*, *43*:e202143038.

Avila, M. R. et al. (2008). Potassium fertilization in canola and its effect on yield and physiological and sanitary quality of seeds. *Acta Scientiarum Agronomy*, *26*(4):475-482.

Ávila, M. R. et al. (2005). Laboratory tests on canola seeds and correlation with seedling emergence in the field. *Journal of Seed Science*, *27*(1):62-70.

Bocianowski, J., & Liersch, A. (2022). Multidimensional analysis of diversity in genotypes of winter oilseed rape (*Brassica napus* L.). *Agronomy*, *12*(3):633.

Borisjuk, L. et al. (2013). Seed architecture shapes embryo metabolism in oilseed rape. *The Plant Cell*, *25*(5):1625-1640.

Brasil. (2009). Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Departamento Nacional de Produção Vegetal: Brasília, DF, Brasil, 399p.

Cerri, M, & Real, L. (2020). Anatomical characteristics of the main fruits: An overview. *Scientia Horticulturae*, *270*:109390.

Cicero, S. M., & Vieira, R. D. (2020). Testes de frio. In F. C. Krzyzanowski., R. D. Vieira., & J. B. França-Neto. *Vigor de sementes: Conceitos e testes*. Londrina: Abrates, (pp. 270-300).

Choudhury, A, & Bordolui, S. K. (2023). Concept of seed deterioration: Reason, factors, changes during deterioration and preventive measures to overcome seed degradation. *American International Journal of Agricultural Studies, 7*(1):41-56.

Costa, C. J., Trzeciak, M. B., & Villela, F. A. (2008). Physiological potential of brassica seeds with emphasis to the accelerated aging test. *Horticultura Brasileira*, *26*(2):114-148.

Groot, E. P., & Caeseele, L. A. V. (2011). The development of the aleurone layer in canola (*Brassica napus*). *Canadian Journal of Botany, 71*(9):1193-1201.

Gularte, J. A., Macedo, V. G. K., & Panozzo, L. E. (2020). Production and market of canola seeds in Brazil. *Brazilian Journal of Applied Technology for Agricultural Science*, *13*:1-9.

Hair, J. F. J. et al. (2009). *Multivariate data analysis*. London: Pearson, 907p.

Han, C. et al. (2017). Comparative metabolome analysis of wheat embryo and endosperm reveals the dynamic changes of metabolites during seed germination. *Plant Physiology and Biochemistry*, *115*:320-327.

Hongyu, K et al. (2016). Principal component analysis: Theoretical summary, application and interpretation. *Engineering and Science*, *5*(9):83-90.

Hu, Z. Y. et al. (2013). Seed structure characteristics to form ultrahigh oil content in rapeseed. *Plos One*, *8*(4):2-11.

Jolliffe, I. T., & Cadima, J. (2016). Principal component analysis: A review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *354*:2065.

Kraus, J. E. et al. (1998). Astra blue and basic fuchsin double staining of plant materials. *Biotechnic and Histochemistry, 73*(5):235-243.

Krzyzanowski, F. C. et al. (2020). Teste de vigor baseados em desempenhos de plântulas. In Krzyzanowski., R. D. Vieira., & J. B. França-Neto. *Vigor de sementes: Conceitos e testes.* Londrina: Abrates, (pp.79-123).

Lah, N. G. et al. (2023). An insight into the behaviour of recalcitrant seeds by understanding their molecular changes upon desiccation and low temperature. *Agronomy*, *13*(8):1-18.

Leeks, C. F. R. et al. (2007). Control of fungal contamination in the accelerated ageing test of *Brassica* spp. *Seed Science and Technology*, *35*(2):380-386.

Lima, J. J. P. et al. (2015). Accelerated aging and electrical conductivity tests in crambe seeds. *Ciência e Agrotecnologia*, *39*(1):7-14.

Maguire, J. D. (1962). Speed of germination: Aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, *2*(2):176-177.

Marcos-Filho, J. (2016). *Seed physiology of cultivated plants.* 2. ed. Londrina: Abrates, 660p

Marcos-Filho, J. (2020). Teste de envelhecimento acelerado. In F. C. Krzyzanowski., R. D. Vieira., & J. B. França-Neto. *Vigor de sementes: Conceitos e testes.* Londrina: Abrates, (pp.185-246).

Mathias, V., & Coelho, C. M. M. (2021). Correlation between vigor due to accelerated aging in pre-sowing and emergence of soybean seedlings in the field. *Semina: Ciências Agrárias*, *42*(2):455-470.

Mingoti, A. S. (2007). *Data analysis using multivariate statistics methods: An applied approach*. Belo Horizonte: Editora UFMG. 393p.

O'brien, T. P., & McColly, M. E. (1981). *The study of plant structure: Principles and selected methods*. Melbourne: Termarcarphi Pty. Ltd. 357p.

O'brien, T. P., Feder, N., & McCully, M. E. (1964). Polychromatic staining of plant cell walls by toluidine blue. *Protoplasma*, 59:368-373.

Oliveira, A. B. et al. (2011). Cytochemical evaluation during the germination of artificially aged and osmoprimed sorghum seeds under salinity. *Revista Ciência Agronômica*, *42*:223-231.

Popov, V. N. et al. (2022). Genetic mechanisms of aging in plants: What can we learn from them?. *Ageing Research Reviews*, *77*:101601.

R Core Team (2024). *R*: A language and environment for statistical computing. Vienna. R Foundation for Statistical Computing. Available in: https://www.R-project.org.

Robbelen, G., & Thies, W. (1980). Variation in rapeseed glucosinolates and breeding for improved meal quality. *Brassica crops and wild allies*, *89*:285-299.

Sales, T. S. et al. (2022). Accelerated ageing test and enzymatic expression in the evaluation of kale seed quality. *Semina: Ciências Agrárias*, *43*:1079-1094.

Secchi, M. A. et al. (2023). Effects of heat and drought on canola (*Brassica napus* L.) yield, oil, and protein: A meta-analysis. *Field Crops Research, 293*:108848.

Silva, G. Z. et al. (2019). Multivariate analysis and vigor tests to determine the quality of *Brachiaria decumbens* seeds. *Revista Ciência Agronômica*, *50*(2):291-299.

Verboven, P. et al. (2013). Void space inside the developing seed of *Brassica* napus and the modelling of its function. *New Phytologist*, *199*(4):936-947.

Zhang, Y et al. (2022). Multi-omics analysis dissects the genetic architecture of seed coat content in *Brassica napus*. *Genome Biology*, *23*(1):1-22.

Zhang, K. et al. (2021). Deterioration of orthodox seeds during ageing: Influencing factors, physiological alterations and the role of reactive oxygen species. *Plant Physiology and Biochemistry*, *158*:475-485.