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Accelerated ageing test and enzymatic expression in the evaluation of kale seed quality

Teste de envelhecimento acelerado e expressão enzimática na avaliação da qualidade de sementes de couve

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Highlights

The accelerated aging test allows evaluating the vigour of cabbage seeds.
Isoenzymes can be used as markers of the quality of aged kale seeds.
The accelerated ageing test methodology can be standardized for kale seeds.

Abstract

The demand for high-quality vegetable seeds and the production of vigorous seedlings has increased in recent years, as these characteristics are determining factors of production success. Vegetables are growing in national importance, and kale stands out as an important source of income for small farmers. The objective of this study was to adapt the traditional accelerated aging test methodology with a saturated NaCl solution of kale seeds and evaluate the enzymatic activity after the vigour test. Six batches of kale seeds were used, and the moisture content, weight of one thousand seeds, first germination count, germination, germination speed index, emergence, initial stand, and emergence velocity index were determined. In the accelerated ageing test, the seeds were submitted to the traditional accelerated ageing test method and the accelerated ageing test method with a saturated NaCl solution for ageing periods of 0, 24, 48, 72, and 96 hours. Electrophoretic analysis of superoxide dismutase (SOD), catalase (CAT), and alcohol dehydrogenase (ADH) isoenzymes was also performed. The accelerated ageing test, using the traditional method for 72 hours at 41 °C, is adequate for evaluating the physiological potential of kale seeds. The isoenzymatic analyses of SOD, CAT, and ADH demonstrate that the biochemical markers are efficient at differentiating kale seeds after accelerated ageing.

Key words: *Brassica oleracea* var. acephala. Isoenzymes. Saline solution. Vigour test.

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Resumo

A demanda por sementes de hortaliças com alta qualidade e a obtenção de mudas vigorosas tem aumentado nos últimos anos, pois estas características constituem fatores determinantes do êxito da produção. As hortaliças têm uma importância nacional crescente, dentre elas se destaca a couve, por ser importante fonte de renda para pequenos agricultores. Objetivou-se adequar a metodologia do teste de envelhecimento acelerado tradicional e com solução saturada de NaCl em sementes de couve e avaliar a atividade enzimática após o teste de vigor. Foram utilizados seis lotes de sementes de couve e determinou-se o grau de umidade, peso de mil sementes, primeira contagem de germinação, germinação, índice de velocidade de germinação, emergência, estande inicial e índice de velocidade de emergência. No teste de envelhecimento acelerado, as sementes foram submetidas ao método tradicional e com solução saturada de NaCl, por períodos de 0; 24; 48; 72 e 96 horas. Realizou-se também a análise eletroforética das isoenzimas superóxido dismutase (SOD), catalase (CAT) e álcool desidrogenase (ADH). O teste de envelhecimento acelerado, utilizando o método tradicional na combinação de 72 horas a 41 °C é adequado para avaliar o potencial fisiológico de sementes de couve. As análises isoenzimáticas superóxido dismutase (SOD), catalase (CAT) e álcool desidrogenase (ADH) demonstram que os marcadores bioquímicos são eficientes e promissores na diferenciação de sementes de couve após o envelhecimento acelerado.
Palavras-chave: *Brassica oleracea* var. *acephala.* Isoenzimas. Solução salina. Teste de vigor.

Introduction

Kale, Brassica oleracea var. acephala, is an annual shrubby vegetable that produces leaves that can be consumed raw in salads and juices, sautéed, or cooked in soups (Barbosa, Souza, Silva, Freire, & Orsi, 2017). When compared with other leafy vegetables, it stands out for its higher content of proteins, carbohydrates, fibre, calcium, iron, iodine, vitamins A and C and niacin, and it is commonly used in dietary programs (Fadigas et al., 2010; Trani et al., 2015).

In Brazil, kale is economically important, especially for small farmers, and is grown in all regions of the country. The 2017 Census of Agriculture estimated kale production at 343,127 tons and highlighted the southeastern region as having the highest production. The state of Rio de Janeiro represents 59% of the national production, and Minas Gerais corresponds to approximately 5% (Instituto Brasileiro de Geografia e Estatística [IBGE], 2017).

In Brazil, there is a growing demand for the production of better quality vegetable seeds as a result of improvements in commercial production systems (Lopes & Macedo, 2008). Despite these advances, much work needs to be conducted to not only achieve self-sufficiency in relation to production but also obtain an ideal plant stand. In this context, seeds with high vigour are a basic and fundamental element.

Vigour tests, unlike germination tests, are able to detect variations in the deterioration process. One of the most widely used tests is the accelerated ageing test (Panobianco & Marcos, 2001), which is based on the increased deterioration of seeds when exposed to the adverse conditions of high temperatures and humidity. Under these conditions, low-quality

seeds deteriorate more rapidly than more vigorous seeds (R. M. Barbosa, Costa, & Sá, 2011).

The traditional accelerated ageing test uses water inside a Gerbox to obtain a relative humidity of approximately 100%. Differences in water absorption by seeds when exposed to this humid atmosphere can result in marked variations in their moisture contents and, consequently, in their rates of deterioration (Jianhua & McDonald, 1997). To circumvent this problem, the authors proposed an alternative methodology, replacing water with a saturated saline solution, to obtain a relative humidity level lower than that used in the traditional ageing test method. Thus, water absorption by the seeds is slower, with the additional possibility of reducing the occurrence of microorganisms that may interfere with the test results. A saturated salt accelerated ageing solution was proposed for broccoli seeds (Tunes et al., 2012) at 44 °C for 24 hours based on the traditional method, and a method using an NaCl solution for chia seeds at 41 °C for 24 hours has also been conducted (Cardoso et al., 2019).

The main challenge facing research on vigour tests is the identification of parameters related to seed deterioration, which precede the loss of physiological potential. To address this issue, the protein electrophoresis technique stands out, as this technique enables the detection of the initial stages of deterioration through the activity of enzymes associated with the degradation and oxidation of reserve substances as well as the biosynthesis of new substances (Spinola, Cícero, & Melo, 2000).

These analyses are recognized for helping to identify the physiological quality of seeds and determine the factors that result in reduced vigour and viability (Andrade et al., 2018; Veiga et al., 2010).

Examples of these factors include enzymes with antioxidant functions, such as superoxide dismutase (SOD) and catalase (CAT), and enzymes that act on respiration, such as alcohol dehydrogenase (ADH), can be cited (Marcos, 2015).

The objective of this study was to adjust the methodology of traditional accelerated ageing tests with a saturated NaCl solution of kale seeds and to investigate the enzymatic activity in the seeds after the vigour test.

Materials and Methods

The experiments were conducted at the Seed Laboratory of the Department of Agronomy at the Federal University of Vales do Jequitinhonha and Mucuri (UFVJM) in Diamantina, MG, and at the Central Seed Laboratory of the Department of Agriculture at the Federal University of Lavras (Universidade Federal de Lavras - UFLA), in 2018.

The siliques were obtained from kale plants in an experimental set up in the Olericulture Sector of UFVJM, which is located at 1400 m altitude, 18°9'S, 43°21'O. The original parent plants of this study were those of the cabbage germplasm bank at UFVJM.

The seed harvest period varied from April 2016 to February 2017. The seeds were harvested in batches as the siliques changed from a light green to brown colour, with the seeds inside being a brown shade. Then, the seeds were manually extracted from the siliques. The seeds obtained from each plant and in each harvest season were combined

according to their batch and stored in paper bags under controlled conditions in a cold chamber at 10 °C and 50% relative humidity until the experiments were performed.

Six batches of half-sib progenies, where the "mother" plant was known but the pollen donor plant was unknown, were selected based on the highest number of seeds produced and were characterized in terms of physical and physiological quality as defined by the following tests:

The moisture content was determined by the oven method at 105 °C for 24 hours (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009), with two replicates of 0.3 g of seeds for each batch.

The weight of one thousand seeds (WOTS) was determined by the random count of eight replicates of 100 seeds, and then, the standard deviation, coefficient of variation and the values expressed in grams (g) were calculated (MAPA, 2009).

For the germination test, sowing was performed on Germitest paper substrate moistened with an amount of water equivalent to 2.5 times the dry weight of the paper in the Gerbox® boxes placed in a biological oxygen demand (BOD) germination chamber regulated at 20 °C with constant light (MAPA, 2009). Four replicates of 50 seeds were used, and the results were expressed as a percentage of normal seedlings on the fifth day (first count (FC)). The test ended on the tenth day (final count) (MAPA, 2009). The counts were performed daily to determine the germination speed index (GSI) and calculated according to the formula proposed by Maguire (1962), where the number of seeds with root protrusion with at least 1 mm of radicle is determined.

The seedling emergence (E) test was conducted with four replicates of 50 seeds per lot in box-type trays, with a sand and soil substrate at a ratio of 2:1. The substrate moisture was adjusted to 60% of the retention capacity (MAPA, 2009). After sowing, the trays were kept in BOD at 20 °C. Seedling E was calculated on the fifth day (initial stand) and the tenth day (final stand) after sowing based on the percentage of emerged seedlings. For the E speed index (ESI), the number of seedlings that emerged from the beginning of E was counted daily, and the calculation was performed according to Maguire (1962).

For the accelerated ageing test, four replicates of 50 seeds per lot were used. The seeds were arranged in a uniform layer on a fine mesh container placed on the surface of aluminium mesh coupled to a Gerbox® plastic box containing 40 mL of water. The Gerboxes were taken to BOD-type germination chambers at a temperature of 41 °C (Komba, Brunton, & Hampton, 2006) for periods of 0 (control), 24, 48, 72 and 96 hours. After each period, the degree of moisture was determined, and the germination test was performed as described above, evaluating the number of normal seedlings after the fifth day of sowing (MAPA, 2009; Marcos, 2015).

The accelerated ageing test using a saturated sodium chloride (NaCl) solution was performed using four replicates of 50 seeds per batch, similar to the traditional accelerated ageing method. However, 40 mL of saturated NaCl solution (40 g of NaCl in 100 mL of water) was added to the bottom of the plastic boxes, which provided a relative humidity of 76% (Jianhua & McDonald, 1997).

The enzymatic analysis was performed using the electrophoresis technique. For this

analysis, 4 g of seeds from each lot of kale was subjected to the traditional accelerated ageing test with the saturated NaCl solution at 0, 48 and 96 hours. The period was determined to include undamaged seeds (0 hours) and seeds at a medium stage of deterioration (48 hours) and at an advanced stage of deterioration (96 hours). They were manually macerated in the presence of liquid nitrogen and 1% (w/v) polyvinylpyrrolidone (PVPP) using a mortar and pestle and stored in a freezer at -86 °C.

The buffer used to extract the enzymes SOD, CAT, and ADH was Tris HCl 0.2 M at a pH of 8, and 0.1% β-mercaptoethanol was added at a ratio of 250 µL per 100 mg of seed sample. The material was vortexed and maintained overnight in a refrigerator, followed by centrifugation at 14,000 rpm for 60 minutes at 4 °C. The polyacrylamide gel was prepared at 7.5% (separator gel) and 4.5% (concentrator gel) for the electrophoretic run. The gel/electrode system used was Tris-glycine at a pH of 8.9. A total of 40 µL of supernatant from the samples was applied to the gel, and the electrophoretic run was performed at 150 V for 5 hours. The gels were developed for the enzymes SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and ADH (EC 1.1.1.1) according to the protocols of Alfenas (2006). For the qualitative analysis of the isoenzymatic systems, a visual interpretation of the electrophoresis gels was performed, taking into account the presence/absence and the intensity of each of the electrophoretic bands. For the quantitative analysis of the bands, the image analysis software ImageJ (Rasband, 1997) was used, and they were measured in pixels.

The experimental design used was completely randomized for all tests. For the accelerated ageing test, the data were analysed in a 6x5x2 factorial scheme (6 batches, 5 ageing periods, and 2 ageing methods). The data were subjected to analysis of variance, and the means were compared by Tukey's test at 5% probability for qualitative data. The statistical analyses were performed using the statistical software "R" (R Core Team [R], 2019).

Results and Discussion

Table 1 shows the data obtained that characterize the kale seed batches. The results of seed moisture content showed significant differences. However, the moisture content values ranged from 7.33% to 8.79% and were within the maximum tolerable limit, which should be 2.0%, so that the results expressed the effect of the treatments (Marcos, 2015). Variations in the initial moisture content between 6.9% and 7.6% in kale seed batches were also observed by Komba et al. (2006) and did not significantly affect the results of their accelerated ageing test.

The WOTS varied between 4.0 g and 3.2 g among batches (Table 1), indicating that they were small seeds because they weighed less than 200 g (MAPA, 2009). There was a difference between the batches regarding the weight of a thousand seeds, and the seeds of batch 2 weighed more than those in batches 1, 3, 4 and 5. This difference may have been due to the size of the seeds and not to the water content.

Table 1
Degree of moisture - M (%); weight of one thousand seeds - WOTS (g); normal seedlings in the first count - FC (%); germination - G (%); germination speed index - GSI; normal seedlings in the initial stand - IS (%); emergence - E (%) and emergence speed index - ESI- obtained from seeds of six kale batches

Batches	Tests							
	M (%)	WOTS (g)	FC (%)	G (%)	GSI	IS (%)	E (%)	ESI
1	8.76 a	3.4 bc	93 a	93 a	25.23 a	91 a	94 a	11.96 a
2	7.99 ab	4.0 a	86 a	88 a	23.76 a	87 a	91 a	11.95 a
3	7.68 b	3.3 c	94 a	95 a	25.75 a	84 a	89 a	11.50 a
4	7.33 b	3.2 c	90 a	91 a	26.02 a	89 a	92 a	12.76 a
5	8.79 a	3.3 c	89 a	95 a	24.92 a	86 a	92a	12.76 a
6	8.12 ab	3.8 ab	93 a	93 a	24.04 a	90 a	94 a	13.01 a
CV%	2.9	6.0	5.7	4.6	4.1	5.4	6.6	7.3

Means followed by the same letter in the column do not differ by Tukey's test at 5% significance.

For the percentage of normal seedlings obtained in the FC, germination (G), and GSI tests (Table 1), there was no significant difference between the batches. The germination values obtained were higher than the minimum standard for the commercialization of kale seeds established by Normative Instruction No. 42, which is 80% (MAPA, 2019).

For seedling E (Table 1), there was no significant difference between the batches for the normal seedlings in the initial stand (IS), at E, and in the ESI.

Table 2 shows the initial moisture content and that after the accelerated ageing tests by the traditional method and the saturated sodium chloride solution method.

The moisture content at the beginning of the tests (0 h) (Table 2), both in the traditional accelerated ageing method and the saturated NaCl solution method, varied from 1.84% to 1.79%. As in the initial characterization, this variation was less than 2% and was within the limit suggested by Marcos (2015) and did not compromise the results due to differences in the speed of moistening and seed deterioration during ageing.

Table 2
Moisture level (%) of the seeds of six kale batches subjected to periods of 0, 24, 48, 72, and 96 hours of accelerated ageing by the traditional accelerated ageing method (AA) and by the accelerated ageing method with the saturated NaCl solution (AASS)

Batches	Degree of moisture (%)				
	0 h	24 h	48 h	72 h	96 h
AA					
1	4.83	30.49	34.57	34.37	30.64
2	5.50	33.56	33.29	31.92	28.13
3	6.67	31.04	32.38	30.35	29.72
4	4.84	29.68	35.94	36.15	22.23
5	6.67	32.45	34.98	31.36	29.10
6	5.50	30.33	33.96	36.70	28.30
AASS					
1	5.17	9.62	8.70	9.21	9.74
2	5.23	10.61	10.03	10.70	10.23
3	6.33	10.70	10.02	9.96	10.18
4	4.94	8.42	8.97	9.66	8.32
5	6.73	10.07	9.19	9.31	10.15
6	5.34	8.84	9.62	9.94	8.35

However, after the traditional accelerated ageing test, the variations between the moisture degrees were greater than 2%, with the greatest range of variation observed in the 96-hour period, with a variation of 8.41%. These results have been observed to be more intense in smaller seeds, such as those of vegetables (Marcos, 2015), and this result was minimized in the accelerated ageing method with the saturated NaCl solution, where lower moisture levels were observed and were more uniform throughout ageing.

The seeds subjected to the traditional method reached higher moisture contents than those subjected to the method with the saturated NaCl solution. The same trend was observed in coriander (Radke, Reis, Gewehr, Tunes, & Villela, 2016), sesame (Nery et al., 2018), and amaranth (Martins et al., 2018) seeds and can be explained by moisture levels. The relative air humidity reached 100% for the seeds subjected to the traditional accelerated ageing test since typical seeds can vary based on relative humidity values.

Studies conducted in Brassica show that seeds tend to achieve hygroscopic equilibrium at higher water contents as the relative humidity increases (Costa, Trzeciak & Villela, 2008), which justifies the alternative use of saline solution in accelerated ageing. The substitution of water with a saturated salt solution allows a relative humidity of approximately 76% to be obtained. Once this relative humidity is achieved, water is absorbed more slowly by the seeds, and the reduced deterioration intensity results in less intense effects on the seeds and less variable results (Cardoso et al., 2019).

In the traditional accelerated ageing test and that with the saturated NaCl solution, there was a significant interaction between the batches, the periods, and the ageing methods (Table 3). Compared to the control ageing period (0 hours), the traditional accelerated ageing method resulted in a decrease in the percentage of normal seedlings after 48 hours for the seeds in batches 2, 3, and 5; after 72 hours, there was a decrease in the percentage of normal seedlings for the seeds in batches 1 and 4; and at 96 hours, there was a decrease in the percentage of normal seedlings for the seeds in batch 6 (Table 3). This same decrease was observed in parsley (Tunes et al., 2013) and coriander (Radke et al., 2016).

Table 3
Percentage of normal seedlings (%) obtained in the seed germination test of six kale batches subjected to periods of 0, 24, 48, 72, and 96 hours of accelerated ageing by the traditional accelerated ageing method (AA) and by the accelerated ageing method with the saturated NaCl solution (AASS)

Batches	Degree of moisture (%)				
	0 h	24 h	48 h	72 h	96 h
AA					
1	97 aAα	96 aAα	89 aAα	43 cBβ	20 bCβ
2	92 aAα	86 abABα	74 bBCα	64 bCβ	17 bDβ
3	96 aAα	96 aABα	84 abBβ	61 bCβ	36 aDβ
4	92 aAα	80 bAβ	83 abAβ	9 dBβ	21 bBβ
5	97 aAα	95 aABα	83 abBβ	70 bCβ	34 aDβ
6	94 aAα	94 aAα	91 aAα	87 aAβ	44 aBβ
AASS					
1	97 aAα	95 aAα	94 aAα	96 aAα	93 aAα
2	90 aAα	89 aAα	81 bAα	86 aAα	91 aAα
3	94 aAα	94 aAα	96 aAα	96 aAα	96 aAα
4	92 aAα	90 aAα	92 abAα	93 aAα	94 aAα
5	94 aAα	95 aAα	94 abAα	98 aAα	94 aAα
6	95 aAα	99 aAα	97 aAα	98 aAα	97 aAα
CV (%)			7.5		

Means followed by the same letter, uppercase in the row, lowercase in the column, and Greek between frames, do not differ by Tukey's test at 5% significance.

In the 72-hour period, it was possible to separate the batches into four quality levels, distinguishing batch 6 as being of superior quality; batches 1, 2, 3 and 5 being of intermediate quality; and batch 4 being of inferior quality.

The different periods of seed exposure to the accelerated ageing method with the saturated NaCl solution did not affect the seed germination of the batches, except at 48 hours (Table 3), where it was possible to distinguish the seeds from the batches at two vigour levels, with batches 1, 3 and 6 being the most vigorous and batch 2 being less vigorous than the others. However, this 42-hour period was less efficient in distinguishing germination, with seed germination in batches 4 and 5 being equal.

In comparing the ageing methods, the seeds subjected to the method with the saturated NaCl solution had a higher percentage of normal seedlings than those subjected to the traditional method, with more characteristic distinctions after 48 hours (Table 3). A possible explanation for this result is that the exposure of the seeds to a saturated salt solution during the accelerated ageing test reduced the relative humidity of the environment inside the Gerbox®. In the 24-hour period, only batch 4 differed from the other batches, with a lower percentage of normal seedlings identified during the traditional accelerated ageing than during the method with the saturated NaCl solution. In the 48-hour period, the same difference was observed for batch 4, in addition to batches 3 and 5. In the 72-hour and 96-hour periods, the effects on the percentage of normal seedlings were more intense.

These results contradict those obtained by Costa et al. (2008), who considered the accelerated ageing test following the traditional methodology for 96 hours and using diluted NaCl solution for 72 hours efficient in ranking kale seed batches. Komba et al. (2006) identified six kale seed batches at different vigour levels at 41 °C for 48 and 72 hours using the traditional accelerated ageing method.

The use of the saturated solution was efficient in obtaining normal seedlings, and although this method was advantageous for reducing fungal microflora, it did not differentiate the kale seed batches by quality levels, which is of great importance for vigour tests.

Regarding the analysis of enzymatic activity, the periods were chosen to evaluate the behaviour of the isoenzymes of seeds at different stages of deterioration, and period zero was considered undeteriorated kale seeds. Analysis of the enzymatic patterns of kale seeds revealed variations in the expression of the enzymes evaluated.

According to the zymogram of the SOD enzyme (Figure 1), in both ageing methods, there was SOD activity during all intervals and in the different batches. In the traditional accelerated ageing method, the highest SOD expression was observed for 96 hours in all batches, except for batch 3, indicating that this enzyme acts directly on the removal of O2-.

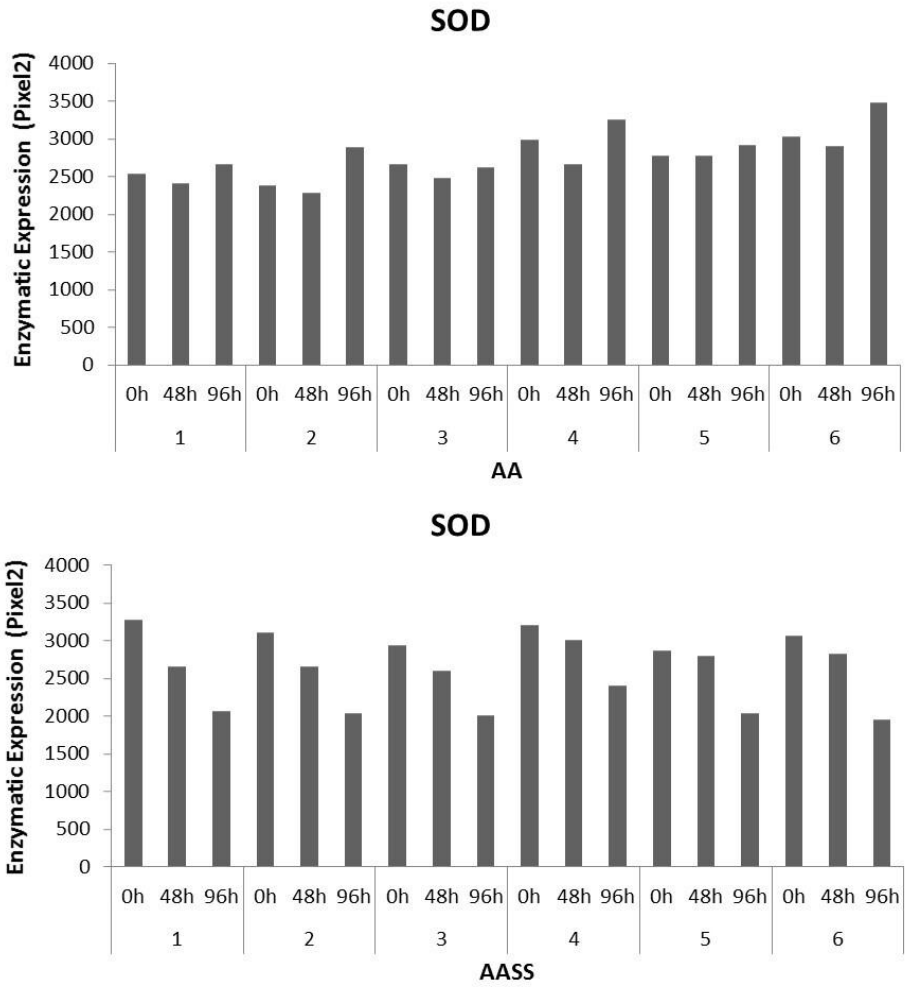

Figure 1. Expression of the enzyme superoxide dismutase (SOD) extracted from kale seeds from batches 1, 2, 3, 4, 5, and 6 subjected to accelerated ageing by the traditional accelerated ageing method (AA) and by the accelerated ageing method with the saturated NaCl solution (AASS) for different periods (0, 48, and 96 hours) and quantification of SOD enzyme activity using ImageJ.

In comparison to more vigorous seeds, less vigorous seeds have greater sensitivity to this SOD-related stress condition over a shorter time interval; however, this scenario was not observed in the present study since the seeds substantially reduced their physiological quality after only 96 hours, which was the same time interval over which the SOD enzyme showed higher activity. This same behaviour was observed by Nery et al. (2018) when studying the activity of the SOD enzyme in sesame seeds subjected to the traditional accelerated ageing test and saturated NaCl solution for different periods (0, 48 and 96 hours).

Conversely, in the accelerated ageing method with the saturated NaCl solution, the highest expression occurred in the 0-hour period, followed by that in the 48-hour and 96-hour periods, demonstrating lower SOD activity in the presence of salt.

In dry and nonaged seeds, the presence of reactive oxygen species (ROS) may originate from nonenzymatic reactions, such as lipid peroxidation, and the reactions of Amateur and Maillard, which are chemical reactions between the reducing agents amino acids and sugars since enzyme activity is likely reduced under these conditions (Murthy, Kumar, & Sun, 2003). Under conditions of accelerated ageing, seed imbibition occurs slowly, preceding germination, and consequently, the mobilization and degradation of reserves are intensified. It is evident that seed ageing is associated with lower activity of the antioxidant defence system but not with the complete loss of this capacity.

Figure 2 shows that in comparison to SOD, CAT behaved differently in terms of natural ageing in all of the studied kale seed batches; i.e., the CAT activity was high in the zero period (without ageing), with significant decreases in the periods of 48 hours and 96 hours.

In the deteriorated seeds, lower activity of this enzyme has been observed with lower efficiency of free radical scavenging systems and consequently less ability to prevent oxidative damage and accelerate the loss of viability (Marcos, 2015; Tabatabaei, 2015; Pires et al., 2017). According to Castro et al. (2017), when a seed is ageing, there is increased lipid peroxidation and reduced peroxide-removing enzyme activities such as that of catalase; i.e., the decrease in band intensity indicates that lipid peroxidation in the cell membrane is more frequently intense, causing increased permeability and, consequently, an advance in the deterioration process.

According to Mansouri-Far, Goodarzian-Ghahfarokhi, Saeidi and Abdoli (2015), this reduction in the activity of the antioxidant enzymes SOD and CAT is due to damage to RNA synthesis, which ultimately leads to decreased protein synthesis and inactivation of enzymes. Protein denaturation may result in decreased activity of antioxidant enzymes when subjected to artificial ageing or even a reduction in their concentration, decreasing their detoxification efficiency, as observed in the present study. When the permeability of the membrane becomes less selective, it diffuses through the normal membrane, acting in places other than the production site.

In the accelerated ageing method with the saturated NaCl solution, CAT expression showed variable behaviours among all batches. For batch 1, CAT activity was observed in all ageing periods; for batches 2, 3, 5 and 6, CAT activity occurred in the 0-hour and 48-hour periods; and for batch 4, it occurred in the 48-hour period (Figure 2).

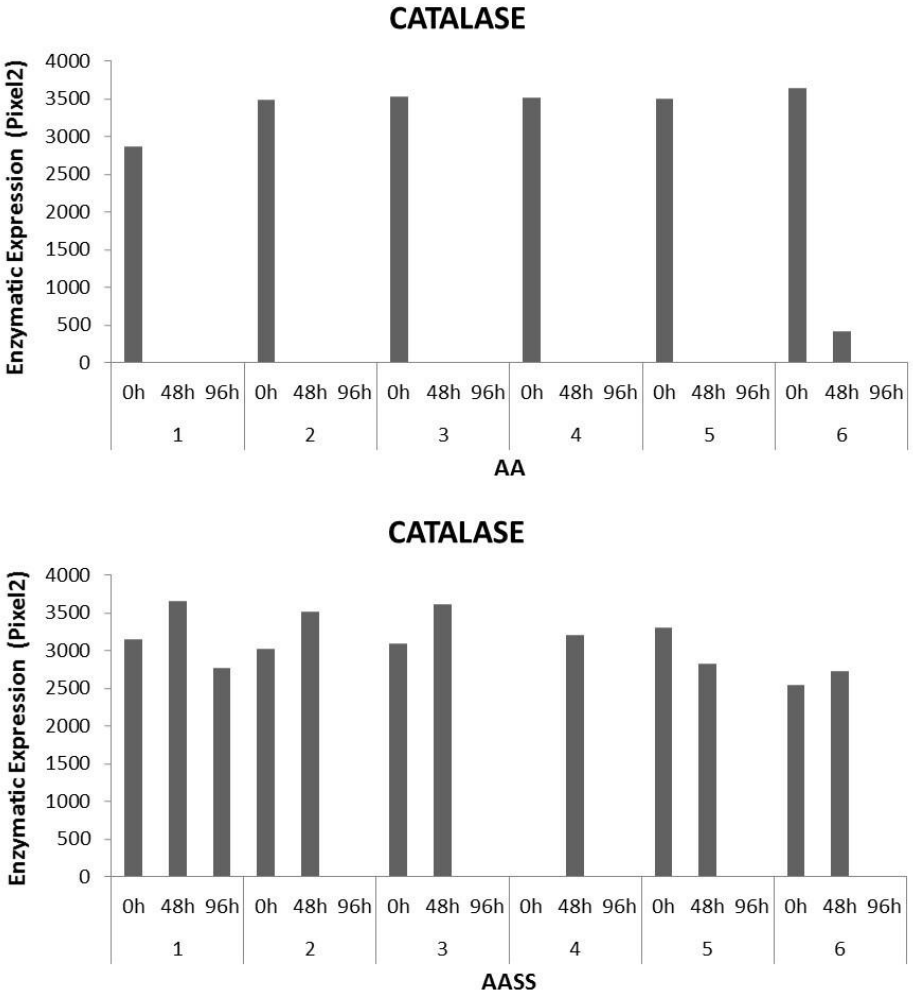

Figure 2. Catalase enzyme expression (CAT) extracted from kale seeds from batches 1, 2, 3, 4, 5, and 6 was subjected to accelerated ageing by the traditional accelerated ageing method (AA) and by the accelerated ageing method with the saturated NaCl solution (AASS) for different periods (0, 48, and 96 hours), and CAT enzyme activity was quantified using ImageJ.

With the absence of oxygen, the metabolism of fermentation by induction of ADH is favoured. According to Veiga et al. (2010), this enzyme is important because it converts acetaldehyde into ethanol, a less toxic compound, and slows the deterioration process. Thus, seeds are less sensitive to the deleterious effects of acetaldehyde with higher ADH activity (Carvalho, Mavaieie, Oliveira, Carvalho, & Vieira, 2014).

In this study, when subjected to ageing with a saline solution, the kale seed batches were more protected in more advanced periods of ageing because the expression of the ADH enzyme under these conditions (Figure 3) showed higher band expression. This result is in agreement with that observed in Table 3, where the seeds showed high germination values even after 48 hours and 96 hour under stress conditions, and with the

results of Carvalho et al. (2014), who found higher ADH expression in soybean cultivar seeds with better physiological quality.

Conversely, for the traditional accelerated ageing method, the expression of the ADH enzyme was observed only in seeds subjected to the 96-hour period in all batches (Figure 3).

The seeds subjected to conditions of accelerated ageing for 96 hours showed more advanced deterioration, as physiologically observed by the substantial reduction in germination in Table 3, which made them more susceptible to the action of acetaldehyde and thereby reduced their viability. Thus, the traditional accelerated ageing test method presented less favourable conditions for the seeds, and the possible increases in acetaldehyde production accelerated their deterioration, corroborating the results found for the percentage of normal seedlings (Table 3) and indicating the important role of this enzyme in response to environmental stresses. This scenario is also shown in Figure 3, where a greater accumulation of ADH occurs at 96 hours with the traditional accelerated ageing method.

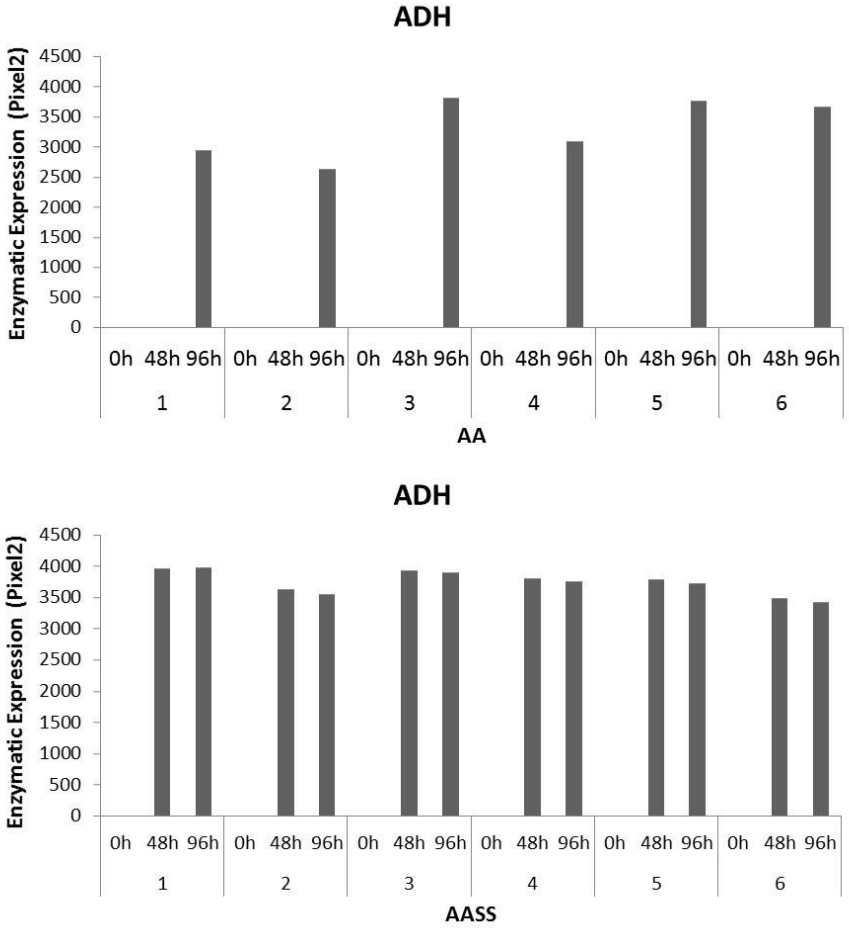

Figure 3. Expression of the alcohol dehydrogenase (ADH) enzyme extracted from kale seeds from batches 1, 2, 3, 4, 5 and 6 subjected to accelerated ageing by the traditional accelerated ageing method (AA) and by the accelerated ageing method with the saturated NaCl solution (AASS) for different periods (0, 48, and 96 hours) and quantification of ADH enzyme activity using ImageJ.

Conclusions

The traditional accelerated ageing test method for 72 hours at 41 °C is adequate for detecting the physiological potential of kale seeds.

The activity of the enzymes SOD, CAT, and ADH may be related to the physiological potential of kale seeds after accelerated ageing.

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