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BASIC AREAS
Article

Physiological potential and antioxidant metabolism during storage of soybean seeds contrasting with phenylpropanoid pathway compounds

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ABSTRACT: Differences in seed storage tolerance between soybean cultivars have been frequently observed. Thus, identifying compounds found in them that may be related to these differences is necessary to support the selection of cultivars with seeds with better storage potential. Therefore, this study aimed to evaluate changes in viability and vigor of soybean seeds during storage in two environments, relating them to antioxidant activity, production of reactive oxygen species, and metabolites of the phenylpropanoid pathway. The experimental design was completely randomized in a 4 × 5 factorial scheme (storage periods × cultivars). Cultivars with contrasting characteristics of lignin content, seed coat color, and isoflavone content in the seed were used, stored for six months in a cold and dry chamber and an uncontrolled environment. Every two months, viability and vigor, enzymatic activity (superoxide dismutase and catalase), and hydrogen peroxide content were evaluated. Storage in a cold and dry chamber maintains seed viability of cultivars A, C, and D; it is reduced in all cultivars in an uncontrolled environment. Seed vigor is reduced during storage. There is no association between the seeds' physiological quality and the superoxide dismutase and catalase activities. The increase in the hydrogen peroxide content in the seed coat is an indicator of the reduction in the seed physiological quality when stored in an uncontrolled environment. The difference in deterioration tolerance during storage is associated with the lignin content in the seed coat.

Key words: *Glycine max* (L.) Merrill, catalase, lignin, isoflavone, vigor.

INTRODUCTION

In the off-season, commercial soybean seeds are stored for a period ranging from six to eight months. During this period, deterioration is inevitable. However, the extent of physiological, physical, and biochemical changes in this process can be minimized, depending on environmental storage conditions and seed characteristics (Gebeyehu 2020).

The formation of free radicals occurs naturally with the seeds' aging. However, in stressful situations, such as storage under inadequate conditions, there is greater production of these compounds. In this context, reactive oxygen species (ROS) can be generated. The main ROS are the superoxide anion (O_2^-), the hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH), which are all harmful to cells (D'Autréaux and Toledano, 2007, Bhattacharjee 2012).

Seeds have antioxidant defense systems, which can be enzymatic or non-enzymatic to protect themselves against the ROS effects (Davar et al. 2013). The enzymes superoxide dismutase (SOD) and catalase (CAT) stand out among the enzymatic systems. SOD is present in all aerobic organisms and one of the most effective antioxidant enzymes.

This enzyme provides the first line of defense against the toxic effects of ROS, catalyzing the dismutation of two O_2^- radicals, in which one O_2^- is reduced to H_2O_2 and the other oxidized to O_2 (Gill and Tuteja 2010). On the other hand, CAT has an efficient mechanism to remove H_2O_2 formed in cells under stress conditions. It metabolizes H_2O_2 into H_2O and O_2 (Leung 2018).

Antioxidative enzymes play an essential role during seed storage (Hosamani et al. 2013). However, according to Copeland and McDonald (2001), these enzymes become less efficient to exert their catalytic activity with the evolution of the deterioration process. Thus, changes in enzymatic activity may indicate reduction in the seeds' physiological potential.

In addition to storage conditions, it has been reported that soybean cultivars have different responses regarding seed quality and longevity, which may be related to the greater or lesser activity of the defense enzyme systems and/or due to compounds present in the seeds, as metabolites from the phenylpropanoid pathway.

The phenylpropanoid route presents phenylalanine as the primary substrate. It includes a series of biochemical pathways, which, through several enzymes, branch out, generating several compounds, including lignin, anthocyanin, and isoflavones (Liu et al. 2015, Rock 2017).

The lignin present in soybean seed coats has been related to the physical and physiological quality of seeds, mainly due to its positive correlation with seed resistance to mechanical damage (Capeleti et al. 2005) and pre-harvest moisture deterioration (Huth et al. 2016). On the other hand, isoflavone and anthocyanin (the pigment responsible for the black color in soybean seed coats) can be related to seed quality mainly due to their antioxidant potential (Mujić et al. 2011, Bursać et al. 2017, Seo et al. 2017), which, together with the enzymatic defense system, can contribute to the greater tolerance of seeds to storage. However, studies of these metabolites related to conserving the seeds' physiological potential during storage are still scarce.

Thus, aiming to contribute to a better understanding of the factors that affect the seed deterioration process, the objective of this work was to evaluate changes in the viability and vigor of soybean cultivar seeds during storage in two environments, relating them to antioxidative activity, the production of ROS and metabolites of the phenylpropanoid pathway.

MATERIAL AND METHODS

The employed experimental design was completely randomized, in a 4 × 5 factorial scheme, with four replications. The factors consisted of four storage periods (zero, two, four, and six months) and five soybean cultivars, with contrasting characteristics of lignin content, color, and presence of anthocyanin in the seed coat and isoflavone content in the seeds (Table 1). The seeds used belonged to category C1 (first generation certified), with cultivar A produced in Uberaba, Minas Gerais state, Brazil, and the rest of the cultivars produced in Ponta Grossa, Paraná state, Brazil, all produced in the 2016/2017 crop season. The samples were collected from the seed lots and homogenized according to Brasil (2009). Approximately one month after the harvest, the trials began. The characterization of the seeds initial physiological quality used was carried out, and the cultivars A, B, C, D, and E presented 95, 91, 94, 92, and 94% of viability, respectively, and 88, 85, 91, 86, and 90% vigor, respectively, evaluated by the tetrazolium test (França-Neto and Krzyzanowski 2018).

The lignin content in the seed coat used to characterize the cultivars together with the seed coat color (presence of anthocyanin) and isoflavone content was determined using four replicates of 100 seeds for each treatment, which were initially immersed in water for 12 hours. After this procedure, the seed coats were removed and dried in an oven at 105°C for 24 hours. The mass of dry matter obtained was crushed and homogenized. Subsequently, 300 mg of seed coat was weighed, then subjected to centrifugation (3,300 rpm for 6 minutes) with different solutions (sodium and potassium phosphate; triton x-100; 1 M NaCl; deionized water and acetone) for obtaining the cell wall. After this process, the tubes with the samples were taken to a desiccator with vacuum and then taken to an oven at 60°C. After drying the samples, they were macerated, and the protein-free material was obtained. Afterwards, lignin was quantified using the acetyl bromide method (Moreira-Vilar et al. 2014). The results were expressed as a percentage.

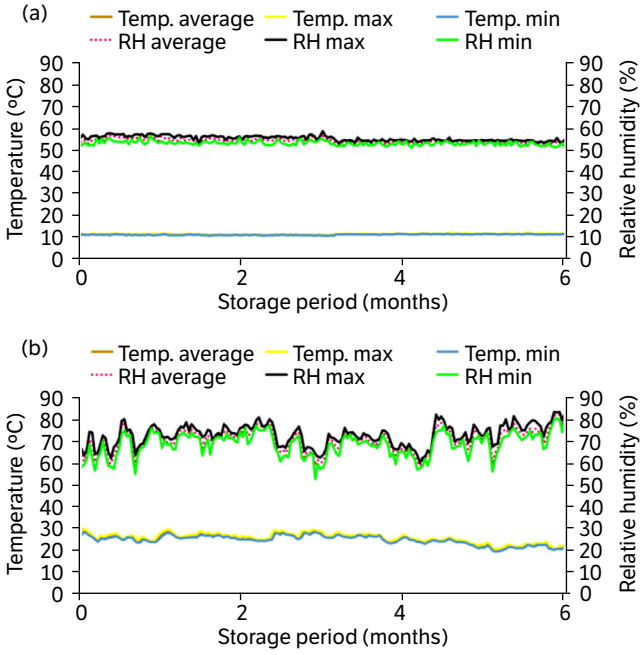
Table 1. Characterization of the soybean cultivars concerning the metabolites of the phenylpropanoid pathway (color, anthocyanin, and lignin content in the seed coat and isoflavone content in seeds).

ID	Cultivar	Seed coat			Seed
		Color	Anthocyanin	Lignin content (%)	Isoflavone content (mg·100 g^{-1} of flour)
A	BRSMG 715A	Black	+	14.38 a	332.78 a
B	DM 6563 IPRO	Yellow	-	4.43 b	301.37 b
C	BRS 413 RR	Yellow	-	4.19 c	233.74 c
D	BRS 1003 IPRO	Yellow	-	3.80 d	237.69 c
E	BMX Valente RR	Yellow	-	3.29 e	173.89 d

ID: acronym used to identify cultivars. Means followed by the same letter in the column do not differ by Tukey's test at 5% probability. Coefficient of variation – lignin content = 1.34% and isoflavone content = 3.79%.

The isoflavone content was determined according to the methodology of Carrão-Panizzi et al. (2002), in which the seeds were crushed and defatted with hexane, with constant agitation for 16 hours. Then, the samples were vacuum filtered. Afterwards, the material retained on the filter was kept at room temperature for 4 hours to evaporate the residual hexane. Next, 100 mg of each ground and defatted sample was transferred to 10-mL test tubes. Then, 4 mL of 70% ethanol solution containing 0.1% acetic acid was added. The tubes containing the samples and the extraction solution were homogenized every 15 minutes, for 1 hour, in a vortex-type shaker. After homogenization, the tubes containing the samples were sonicated for 30 minutes, and, subsequently, the supernatant (1.5 mL of extract) was transferred to Eppendorf tubes. They were centrifuged for 15 minutes, at 14,000 rpm, at 4°C. The supernatant was filtered through a membrane with pores of 0.45 µm, using 20 µL of the purified extract for injection in the ultra-pressure liquid chromatography (UPLC). According to modifications in the methodology recommended by Berhow (2002), the isoflavones quantification was performed in a liquid chromatograph equipped with a reverse-phase column of the ACQUITY UPLC BEH C18 type 1.7 µm, diameter 50 × 2.1 mm and auto-injector of samples. The results were expressed in mg·100 g^{-1} of flour.

The seeds were placed in paper packaging and stored for six months in two environments: cold and dry chamber (controlled temperature and relative humidity) and uncontrolled environment (under natural conditions). One kg of seeds of each cultivar was stored in each environment. During the experiment, the temperatures and relative humidity (RH) of the air in the two environments were monitored with a Data Logger model HT-500 equipment (Fig. 1).



Temp.: temperature; max: maximum; min: minimum.

Figure 1. Daily maximum, average, minimum temperature (°C) and daily maximum, average, and minimum relative humidity (RH – %) during the storage period of soybean seeds in a (a) cold and dry chamber and (b) uncontrolled environment.

During the storage period of the seeds (zero, two, four, and six months), the following evaluations were carried out:

- Tetrazolium test: conducted with 50 seeds per repetition, pre-conditioned in germitest paper moistened with distilled water for 16 hours, in a germinator at the constant temperature of 25°C. Afterwards, the seeds were entirely submerged in tetrazolium solution (2,3,5 triphenyl tetrazolium chloride), with the concentration of 0.075%, and kept at the temperature of 40°C for approximately 150 and 240 minutes for the yellow and black seed coat seeds, respectively. Subsequently, the seeds were classified as to viability and vigor according to the criteria proposed by França-Neto and Krzyzanowski (2018). The results were expressed as a percentage.
- The determination of enzymatic activity and hydrogen peroxide was performed separately in the seed coat and the embryo (cotyledon + embryonic axis) of soybean seeds, except for hydrogen peroxide in the seed coat of cultivar A, due to interference of seed color in the quantification. Tissues were macerated in a mortar in the presence of liquid nitrogen. Next, they were stored in an ultra-freezer, at -80°C, until the moment of analysis. The following sequence was performed to quantify the activity of antioxidative enzymes, SOD, and CAT:
  - Enzymatic extraction: about 100 mg of macerated tissue was transferred to Eppendorf-type tubes. Afterwards, 1.2 mL of extraction buffer (100 mM of potassium phosphate buffer – pH 7.5, 0.1 mM of ethylenediaminetetraacetic acid – EDTA, and 1% polyvinylpyrrolidone PVPP) was added. The homogenate was centrifuged at 15,000 g, at 4°C, for 20 minutes. Afterwards, the supernatant was stored in separate aliquots, at -80°C, for the determination of total proteins and antioxidant enzymes. The entire process was carried out on the ice and liquid nitrogen;
  - Determination of total proteins: by the method of Bradford (1976), using bovine serum albumin as a standard. It was used 0.05 mL of seed coat extract and 0.025 mL of the embryo. For quantification, 5 mL of Bradford reagent was added, and the samples were left to rest for 15 minutes; then, readings were taken in the spectrophotometer at 595 nm;
  - SOD activity: determined by the addition of several solutions in the following order: 1 mL of 50 mM potassium phosphate – pH 7.8, 0.4 mL of 14 mM methionine, 0.020 mL of 0.1 µM EDTA, 0.31 mL of Mili-Q water, 0.15 mL of nitrotetrazolium blue (NBT) 75 µM, 0.1 mL of enzymatic extract (sample) and finally 0.06 mL of 2 µM riboflavin, according to Krüger et al. (2017), with amendments. The reaction was carried out under the light of a 15-W fluorescent lamp kept inside a box. After 15 minutes of exposure to light, the lighting was interrupted, and the formazan, produced by the photoreduction of NBT, was measured in a spectrophotometer by reading the absorbance at 560 nm. White was obtained under the same conditions, but in the absence of light. The control consisted of all solutions except the sample and it was kept in the light. One unit of SOD (U SOD) was defined as the amount of enzyme needed to inhibit the photoreduction of NBT by 50%. Results were expressed as U SOD·mg protein^{-1};
  - CAT activity: determined as proposed by Azevedo et al. (1998), with minor modifications. Initially, a solution was prepared, in which 0.5 mL of 30% H_2O_2 was diluted in 200 mL of 100 mM potassium phosphate buffer pH 7.5. In each tube, 1 mL of this solution was added, and then the reaction was started by adding 0.1 mL of enzymatic extract (sample). This entire process was carried out with minimal lighting and with the tubes wrapped in aluminum foil. The activity was determined by monitoring the degradation of H_2O_2, recording the absorbance values at time 0, right after inserting the cuvette into the spectrophotometer, and after 1 minute. Readings were taken at 240 nm. Enzyme activity was calculated using the molar extinction coefficient of 36 $M^{-1}\cdot cm^{-1}$ (Anderson et al. 1995). Results were expressed in µmol of H_2O_2 $min^{-1}\cdot$mg protein^{-1};
- Hydrogen peroxide (H_2O_2): quantified using the method described by Alexieva et al. (2001). Approximately 100 mg of previously macerated tissue was transferred to Eppendorf tubes. Then, 1 mL of 0.1% trichloroacetic acid (TCA) was added. Then, the samples were centrifuged at 10,000 rpm, at 4°C, for 15 minutes. Subsequently, 0.2 mL of the supernatant was transferred to another tube and added with 0.2 mL of 100 mM potassium phosphate buffer (pH 7.5) and 0.8 mL of potassium iodide (KI) solution at 1 M. The blank consisted of the same solution. Still, the 0.2 mL of the sampled supernatant was replaced by 0.2 mL of TCA. The tubes, after brief homogenization, were placed on ice and kept in the dark for 1 hour. Finally, the reading was performed in a spectrophotometer at 390 nm. The H_2O_2 concentrations in the samples were calculated based on the calibration curve prepared with H_2O_2 standards. The results were expressed in µmol of $H_2O_2\cdot$g seed coat^{-1} and µmol of $H_2O_2\cdot$g embryo^{-1}.

Data were analyzed separately for each environment. Data were analyzed for normality and homogeneity of variances, using the Shapiro-Wilk and Hartley tests, respectively, which indicated no need for transformation. An analysis of variance was carried out in the viability and vigor data at 5% probability. Tukey's test compared cultivar means, and regression analysis was performed for storage periods. The analyses were performed using the computer program System for Analysis of Variance (SISVAR). The following were examined to verify the association between pairs of parameters measured in the experiment: the scatter diagram, the linear trend, and the Pearson correlation coefficient. This analysis was conducted in SAS/STAT® software, Version 9.4. Copyright© 2016 SAS Institute Inc.

RESULTS AND DISCUSSION

For physiological quality data, it is worth noticing that the seeds of the studied cultivars had similar viability and vigor in the period before storage (Table 2). This result is essential for works of this nature, since the initial quality of the seeds influences the intensity and the speed of deterioration and, consequently, the seed storage potential (Timóteo and Marcos-Filho 2013).

Table 2. Viability (TZ VIA) and vigor (TZ VIG), evaluated by the tetrazolium test, of the seeds of five soybean cultivars throughout the storage period in cold and dry chamber conditions and an uncontrolled environment*.

Cold and dry chamber						
Cultivar	TZ VIA (%)					
	Storage period (months)				Regression equation	R^2
	0	2	4	6		
A	95 a	94 a	95 a	95 a	ns	-
B	91 a	86 c	85 c	85 c	y = -0.95x + 89.6	0.73
C	94 a	93 ab	93 ab	92 ab	ns	-
D	92 a	89 bc	89 bc	89 bc	ns	-
E	94 a	93 ab	86 c	86 c	y = -1.55x + 94.4	0.85
Cultivar	TZ VIG (%)					
	0	2	4	6	Regression equation	R^2
A	88 a	89 a	85 a	84 a	y = -0.9375x + 89.125	0.79
B	85 a	78 b	72 c	72 c	y = -2.175x + 83.15	0.89
C	91 a	89 a	88 a	83 ab	y = -1.25x + 91.25	0.93
D	86 a	81 ab	81 ab	80 abc	y = -0.9625x + 84.825	0.80
E	90 a	89 a	76 bc	76 bc	y = -2.825x + 91.1	0.84
Uncontrolled environment						
Cultivar	TZ VIA (%)					
	0	2	4	6	Regression equation	R^2
A	95 a	92 a	91 a	91 a	y = -0.6125x + 94.15	0.74
B	91 a	88 ab	83 b	79 c	y = -2.125x + 91.5	0.99
C	94 a	92 a	86 b	86 b	y = -1.5x + 94	0.88
D	92 a	84 b	84 b	78 c	y = -2.1x + 90.8	0.89
E	94 a	87 b	85 b	81 c	y = -2.05x + 92.9	0.95
Cultivar	TZ VIG (%)					
	0	2	4	6	Regression equation	R^2
A	88 a	84 a	82 a	84 a	y = -0.7375x + 86.775	0.52
B	85 a	84 a	77 ab	66 bc	y = -3.2x + 87.6	0.89
C	91 a	84 a	80 ab	80 a	y = -1.8625x + 89.15	0.82
D	86 a	77 b	75 b	60 c	y = -4.025x + 86.45	0.93
E	90 a	85 a	78 ab	68 b	y = -3.65x + 91.2	0.98

*Means followed by the same letter in the column do not differ from each other (within each storage period), by Tukey's test, at 5% probability; ns: non-significant regression.

After two months of storage in the cold and dry chamber environment, it was already possible to differentiate the cultivars in terms of viability. At four and six months, cultivar A had higher values than cultivars B, D, and E (Table 2). Also, as shown in the regression equations in Table 2, there was a linear reduction in the percentage of viable seeds over the storage period in cultivars B and E. There was no significant difference in the viability throughout storage in the other cultivars (A, C, and D).

As for vigor, in a cold and dry chamber, the cultivars that showed the best results after two months of storage were cultivars A, C, and E, cultivars A, C, and D at four months, and cultivars A and C at six months, compared to cultivar B in the three periods (Table 2). Regarding the effect of the storage period, linear reductions in vigor were observed for all cultivars, but lower rates of decrease were verified in cultivars A, C, and D.

In the uncontrolled environment, after four months of storage, cultivar A, which has a black seed coat and higher levels of lignin and isoflavone, stood out compared to the others in terms of viability values (Table 2). Hosamani et al. (2013) also found greater viability in soybean seeds with black seed coat than yellow ones after storage under uncontrolled conditions.

For vigor, after six months of storage in an uncontrolled environment, the values found in cultivars B (66%), D (60%), and E (68%) stood out (Table 2). According to the classification proposed by França-Neto and Krzyzanowski (2018), these seeds have low vigor (≤ 74%) and should not be used as seeds. This recommendation is based on the fact that high vigor seeds provide faster and more uniform crop emergence, favor the growth of the aerial part and the root system, provide greater tolerance to environmental adversities, and positively contribute to the crop's productive potential (Bagateli et al. 2019).

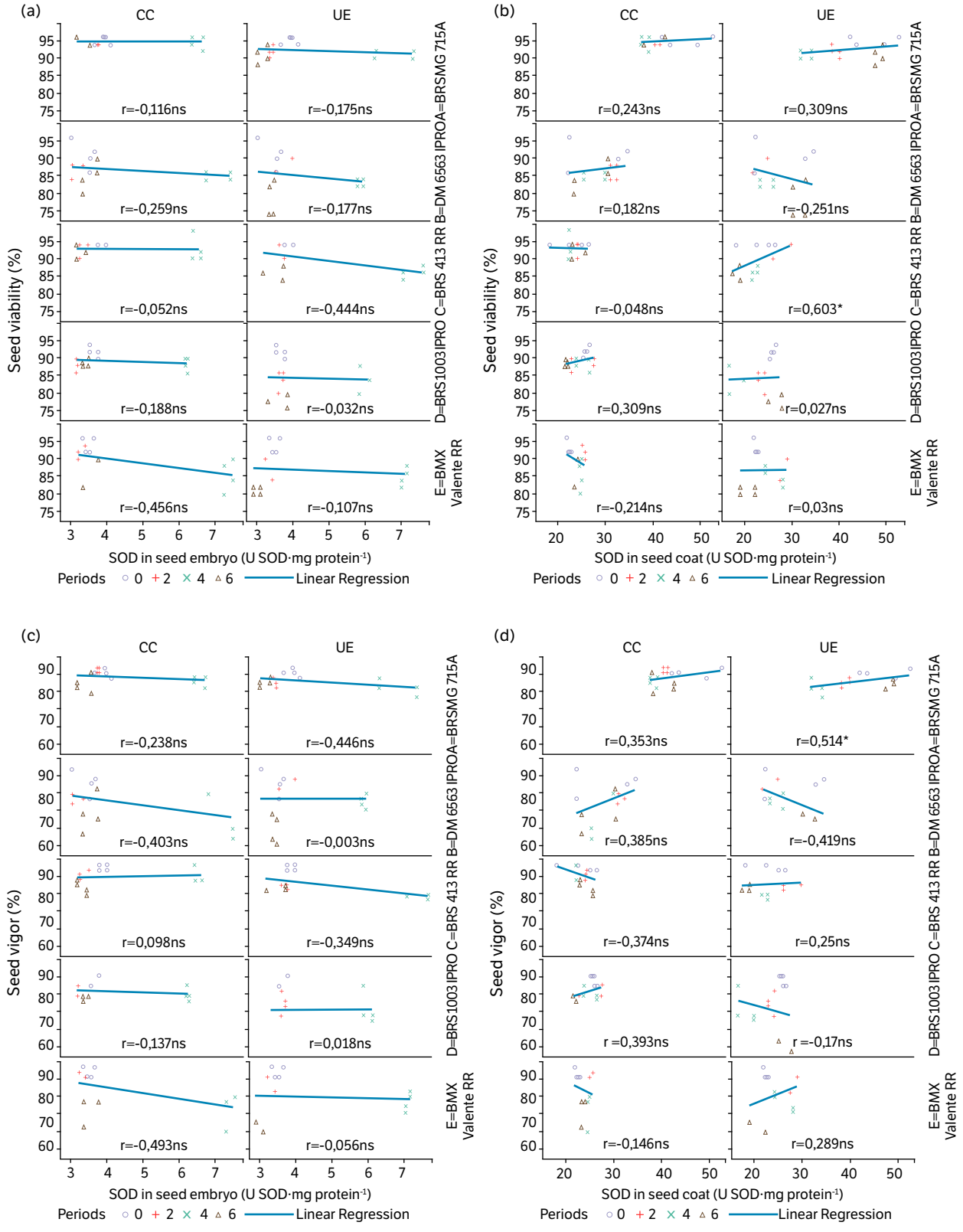
Over the storage period in the uncontrolled environment, linear reductions in viability and vigor were observed for all cultivars (Table 2). However, the rates of decrease between cultivars were distinct, being less pronounced in cultivars A and C, which presented reductions of 0.61 and 0.74 (cultivar A) and 1.50 and 1.86 (cultivar C) points percentage in the viability and vigor values, respectively, for each month of storage. Still, for vigor, the accentuated rates of decrease of cultivars B, D, and E stood out, which changed the classification of seeds from high and very high vigor (at the beginning of storage) to low vigor (after six months of storage), as mentioned before. Unlike what was observed in the cold and dry chamber environment, only cultivar B was classified as low vigor after the storage period.

The decreases observed in physiological quality during storage are due to the natural deterioration process of the seeds. In the present work, the cold and dry chamber environment provided the smallest reductions. It maintained the initial viability of seeds in three cultivars, while under uncontrolled conditions, none of the cultivars held their values. These results are related to the hygroscopic character of the seeds and to the temperature and RH values of the air present in the two environments, which, in a cold and dry chamber, were constant (11°C and 54% RH) and, in an uncontrolled environment, they showed fluctuations and higher values (variation from 19.2 to 30°C and 52 to 84% RH, with an average of 25°C and 71% RH) (Fig. 1), which intensified the deterioration process and reduced the storage potential of seeds stored in this environment.

The relationships of antioxidant enzyme activity, measured by SOD and CAT, and hydrogen peroxide with changes in the physiological quality of seeds can be observed in the panel data presented in Figs. 2, 3, and 4.

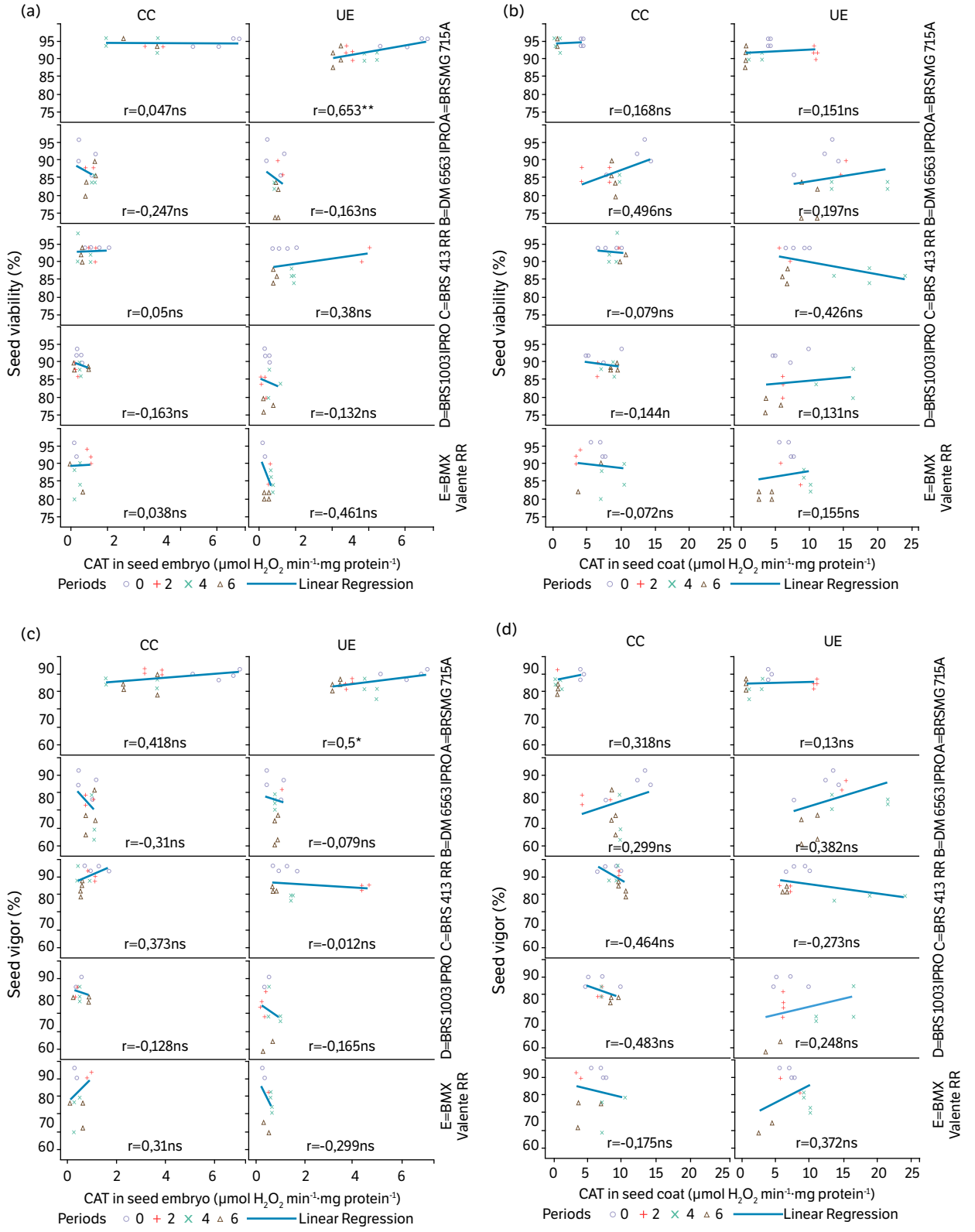
As for SOD, there was a significant correlation between the SOD seed coat and viability for cultivar C and vigor for cultivar A, both in the uncontrolled environment (Fig. 2). From these, it appears that the reductions in seed viability and vigor and the activity of this enzyme occurred together. However, the values of the correlation coefficients ($r = 0.60$ and 0.51) seen in these associations are highlighted.

For CAT, a positive correlation was found between the activity of this enzyme in the embryo and the values of viability ($r = 0.65$) and seed vigor ($r = 0.50$), specifically in cultivar A in the uncontrolled environment (Fig. 3). The reduction and/or loss of CAT may be associated with an increase in the accumulation of hydrogen peroxide. This enzyme catalyzes the decomposition of H_2O_2 into H_2O and O_2; however, it was not possible in this work to verify this fact. In the other cultivars and the seed coat, the loss of viability and vigor was not accompanied by an increase or decrease in CAT, regardless of the storage environment (Fig. 3).



r: Pearson's correlation coefficient; ns: correlation not significant; ** and * significant correlation at 1 and 5% probability, respectively.

Figure 2. Association between (a and b) seed viability and (c and d) vigor with the activity of the enzyme superoxide dismutase (SOD), evaluated in the (a and c) embryo and (b and c) seed coat, in (a, b, c, d, and e) five soybean cultivars throughout storage in a cold and dry chamber (CC) and an uncontrolled environment (UE).



r: Pearson's correlation coefficient; ns: correlation not significant; ** and * significant correlation at 1 and 5% probability, respectively.

Figure 3. Association between (a and b) seed viability and (c and d) vigor with catalase activity (CAT), evaluated in the (a and c) embryo and (b and d) seed coat, in (a, b, c, d, and e) five soybean cultivars throughout storage in a cold and dry chamber (CC) and an uncontrolled environment (UE).

Still, for SOD and CAT, it was found, in most cultivars, that the increase or decrease in the activity of these enzymes does not follow a pattern with the advance of the storage period in both evaluated tissues and environments (Figs. 2 and 3).

Unlike what was observed in this work, Balešević-Tubić et al. (2005, 2011) verified reductions in SOD activity during the storage of sunflower and soybean seeds, respectively. Furthermore, Hosamani et al. (2013) found that the activity of SOD and CAT increased in seeds of soybean cultivars with black seed coats and decreased in those with yellow seed coat at the end of the storage period under uncontrolled conditions of temperature and air RH. In addition, these authors mention the critical role of these enzymes in determining the behavior of cultivars compared to storage, given the smaller reduction in viability in seeds that showed superior antioxidant activity.

On the other hand, and corroborating the results of this work, Moriya et al. (2015) observed variations in the enzymatic activities of SOD and CAT in bean cultivars stored at 15±2°C and 50% RH. However, the enzymatic activity, evaluated in the embryonic axis and cotyledon of the seeds, was not significantly correlated with vigor tests, even in lots stored for up to three years, demonstrating that these enzymes had little influence on these seeds' deterioration process.

The results show the unlinking of the enzymes SOD and CAT activities with the deterioration process of the seeds of the soybean cultivars evaluated in this work. Thus, the lack SOD and CAT action in the cultivars' seeds that showed better maintenance of physiological quality throughout storage, in a cold and dry chamber, and especially in an uncontrolled environment, may be associated with other defense mechanisms present in the seeds. These defense mechanisms may be enzymatic or non-enzymatic antioxidant agents, such as ascorbic acid, tocopherol, flavonoids, glutathione reductase, ascorbate peroxidase (Gill and Tuteja 2010) or other compounds without antioxidant action.

For hydrogen peroxide (H_2O_2), evaluated in the seed embryo, positive and significant correlations were observed between this ROS and seed viability in cultivars C and D in an uncontrolled environment. In this tissue, positive correlations were also found between H_2O_2 content and seed vigor in cultivars C and D, in both storage environments, and negative correlation in seeds stored in a cold and dry chamber of cultivar B (Fig. 4).

As for the H_2O_2 evaluated in the seed coat, there was negative correlation between this variable and the viability and vigor of the seeds assessed in the two environments, indicating that the increase in the H_2O_2 content in the seed coat reduces the seeds' physiological quality, except in the seeds stored in a cold and dry chamber of cultivars C for viability and of cultivar D for viability and vigor (Fig. 4). Still, the strong relationship (> 0.70) between H_2O_2 in the seed coat and seed viability and vigor in most cultivars stands out.

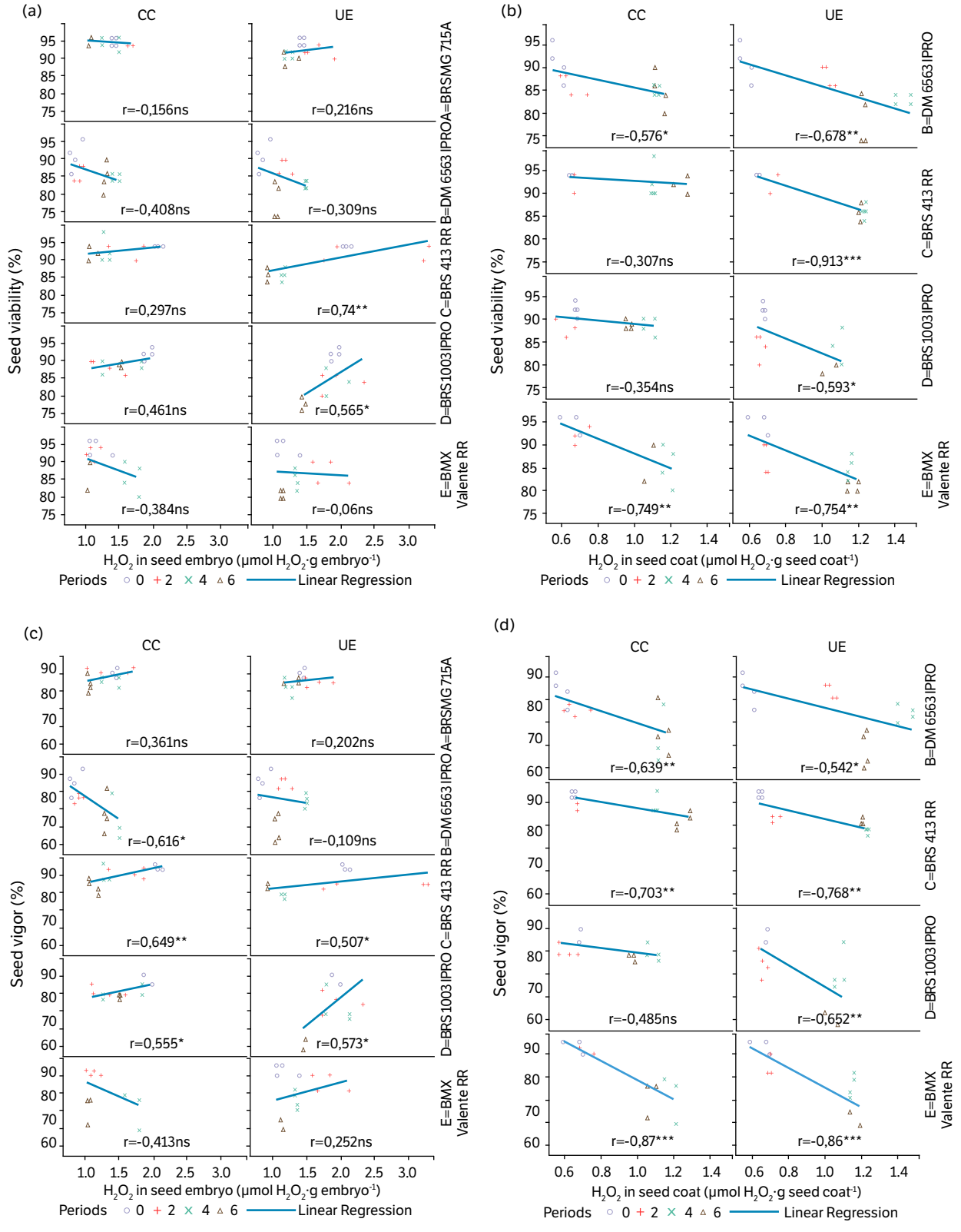
Similarly, however in another tissue, Tian et al. (2008) observed an increase in the content of H_2O_2 and superoxide radical in the embryonic axis of soybean seeds, and Lehner et al. (2008) associated the loss of viability in wheat seeds with an increase in H_2O_2, when the seeds were subjected to high temperature and RH, in both studies.

The correlations between H_2O_2 in the seed coat and physiological performance variables, in general, had the expected theoretical sign, since, according to Kumar et al. (2015), as the storage period increases, there is reduction in the physiological quality of the seeds, mainly due to the production and accumulation of ROS, especially in inadequate storage conditions.

The accumulation of ROS, such as H_2O_2, can be harmful to seeds due to the damage caused by these compounds to lipids, proteins, and DNA (Wojtyla et al. 2016). However, it is noticed that for cultivar C, in a cold and dry chamber, there was no correlation of H_2O_2 with viability, despite an increase in the content of H_2O_2 in the seed coat during storage (Fig. 4). This may be indicative that this cultivar is less vulnerable to increases in the concentration of H_2O_2, or even that its consumption (degradation) occurs quickly after its production. Furthermore, it is also noteworthy that this was the yellow seed coat cultivar that presented the smallest reductions in physiological quality after storage.

Despite the significant and negative correlation between H_2O_2 in the seed coat with viability and vigor, there was no progressive accumulation of H_2O_2 in the seed embryo, so there are other toxic products for cells contributing to the deterioration process, such as free radicals and aldehydes, produced from different reactions.

As for the metabolites of the phenylpropanoids route, there is no association between the levels of lignin and isoflavone and the initial physiological quality of the seeds (Table 3). This same behavior is observed between isoflavones with viability and vigor at six months of storage in both environments. Thus, it is inferred that the higher total isoflavone content did not contribute to the conservation of the initial physiological quality of the seeds. However, despite this result, the possible antioxidant action of isoflavones cannot be ruled out. For future work, it is suggested to quantify the profile of each type of isoflavone and verify their relationship with the seed storage potential.



r: Pearson's correlation coefficient; ns: correlation not significant; ***, ** and * significant correlation at <0.0001, 1 and 5% probability, respectively.

Figure 4. Association between (a and b) seed viability and (c and d) vigor with hydrogen peroxide (H_2O_2), evaluated in the (a and c) embryo and (b and d) seed coat, (a, b, c, d, and e) in five soybean cultivars throughout storage in a cold and dry chamber (CC) and an uncontrolled environment (UE).

Table 3. Pearson correlation coefficients (r) between lignin and isoflavone contents with physiological potential (viability and vigor) in seeds of five soybean cultivars, evaluated before storage (initial condition) and after six months of storage in a cold and dry chamber and an uncontrolled environment.

Variables	Initial condition		Cold and dry chamber		Uncontrolled environment	
	TZ VIA	TZ VIG	TZ VIA	TZ VIG	TZ VIA	TZ VIG
Lignin	0.339^{ns}	-0.012^{ns}	0.632**	0.404^{ns}	0.732**	0.660**
Isoflavone	-0.035^{ns}	-0.354^{ns}	0.358^{ns}	0.131^{ns}	0.370^{ns}	0.365^{ns}

^{ns}correlation not significant; ** and * significant at 1 and 5% probability, respectively; TZ VIA: viability assessed by the tetrazolium test; TZ VIG: vigor assessed by the tetrazolium test.

The lignin content was associated with maintaining the physiological quality of the seeds in both storage environments (Table 3). The relationship between these variables is probably because the lignin present in the seed coat of the seeds serves as a protection to the components of the seed coat itself and the embryonic axis and the seeds' reserve tissue during the storage period. This protection is possibly due to the properties of lignin, which confer lower permeability to seeds, leaving them less susceptible to deterioration (Krzyzanowski and França-Neto 2021), especially when stored in an uncontrolled environment. This environment, generally, presents temperature and RH of the air fluctuations, as well as these meteorological variables values superior to the storage under controlled conditions, as observed in the environments where this work was developed.

Therefore, based on the results, the importance of storing seeds under adequate temperature and air RH conditions is highlighted to guarantee the commercialization of quality seeds and avoid batches disposal and its use as grains, since expenses were made on seed production.

In addition, it was found that the high lignin content present in the seed coat provides better maintenance of their physiological quality. However, both this compound and the isoflavones did not interfere in the response of SOD and CAT enzymatic activity and the production of H_2O_2 throughout the storage period. Still, it was observed that the evaluation of the enzymes SOD and CAT, based on the separation of seed coat and embryo, was not efficient in evaluating the seed deterioration process. Nevertheless, other authors have observed contradictory results. These contradictions can be attributed to the lack of standard in the methodology used for enzymatic evaluation in seeds, the difference between species, and different chemical compositions and tissues evaluated within the same species. Thus, additional studies involving other tools are essential for a better understanding of the studied compounds that affected seed conservation during storage and identifying other compounds involved in this process.

CONCLUSION

Seed viability and vigor are reduced during storage, mainly in an uncontrolled environment. There is no association between the physiological quality of seeds with the activity of SOD and CAT. The increase in the hydrogen peroxide content in the seed coat is an indicator of the reduction in the seed physiological quality when stored in an uncontrolled environment. The difference in tolerance to deterioration during seed storage is not related to the total isoflavone content. It is associated with the lignin content in the seed coat, especially in an environment with air temperature and RH fluctuations.

AUTHORS' CONTRIBUTION

Conceptualization: Abati, J., Zucareli, C., Brzezinski, C. R., Mertz-Henning, L. M. and Henning, F. A.; **Methodology:** Abati, J., Zucareli, C., Moraes, L. A. C., Mertz-Henning, L. M., Krzyzanowski, F. C. and Henning, F. A.; **Investigation:** Abati, J. and Moraes, L. A. C.; **Formal Analysis:** Abati, J. and Lopes, I. O. N.; **Writing – Original Draft Preparation:** Abati, J.; **Writing – Review and Editing:** Abati, J., Zucareli, C., Brzezinski, C. R., Moraes, L. A. C., Lopes, I. O. N., Mertz-Henning, L. M., Krzyzanowski, F. C. and Henning, F. A.; **Resources:** Zucareli, C., Moraes, L. A. C., Mertz-Henning, L. M., Krzyzanowski, F. C. and Henning, F. A.; **Supervision:** Zucareli, C. and Henning, F. A.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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