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Physiological and enzymatic monitoring of treated seeds of cultivars soybean during storage

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ABSTRACT: The objective was to analyze the influence of industrial soybean seed treatments on physiological quality throughout the storage and to evaluate the worth of the alcohol dehydrogenase (ADH) enzyme as a marker for quality monitoring. The statistical design used was completely randomized in a 2 × 5 × 5 factorial schemes involving two cultivars, five phytosanitary treatments, and five evaluation periods during storage. Before treatment, the lignin content of the seed coat of the cultivars was analyzed. The physiological quality was evaluated by the first count of germination and the germination, and the ADH enzyme was also quantified. The deterioration of soybean seeds during storage was directly linked to the type of molecule and the viscosity of the spray solution used in the treatment of seeds. The treatment of soybean seeds with predominantly aqueous solution and the use of systemic insecticidal molecules, combined with fungicides and polymers, impaired the germination of the stored seeds, and the deleterious effects became greater over time. ADH has potential as a marker of the physiological quality of treated and stored soybean seeds.

Key words: alcohol dehydrogenase (ADH); chemical treatment of seeds; *Glycine max*; storability

Monitoramento fisiológico e enzimático de sementes tratadas de cultivares de soja durante o armazenamento

RESUMO: O objetivo foi analisar a influência de composições dos tratamentos industriais de sementes de soja sobre a qualidade fisiológica ao longo do armazenamento e avaliar o uso da enzima álcool desidrogenase (ADH) como marcador para o monitoramento da qualidade. O delineamento experimental foi inteiramente ao acaso em esquema fatorial 2 × 5 × 5, envolvendo duas cultivares, cinco tratamentos fitossanitários e cinco épocas de armazenamento. Previamente, realizou-se a análise do teor de lignina do tegumento das cultivares. A qualidade fisiológica foi avaliada por meio de primeira contagem de germinação e germinação, também foi quantificada a enzima ADH. A deterioração das sementes de soja ao longo do armazenamento está diretamente ligada ao tipo de molécula e à viscosidade da calda utilizada no tratamento de sementes. O tratamento de sementes de soja com calda predominantemente aquosa e a utilização de moléculas inseticidas sistêmicas, associado a fungicidas e polímero prejudicam a germinação das sementes armazenadas, sendo os efeitos deletérios maiores com o avanço do armazenamento. A enzima ADH tem potencial para possível marcador da qualidade fisiológica de sementes de soja tratadas e armazenadas.

Palavras-chave: álcool desidrogenase (ADH); tratamento químico de sementes; *Glycine max*; armazenabilidade

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Introduction

Soybean is among the most important crop in Brazil's agriculture industry and one of its main exports. In the 2020/2021 harvest, there was a record production, estimated at 136 million tons, 8.9% greater than that of the 2019/2020 harvest (Conab, 2021). One of the factors for the success of this crop is the adoption of new technologies by farmers, many of them relating to seeds, providing longevity to the seed, and achieving greater productivity in the field.

High quality soybean seeds have to high germination and vigor, sanitary quality, as well as a guarantee of physical and varietal purity. These factors are decisive in the production process and ensures uniform stands, increasing the chances of crop success (Krzyzanowski et al., 2018). In addition, the seed contains a technology and innovation package that was obtained through genetic improvement studies (Ferreira et al., 2016). In the seed production process, all steps are essential for obtaining a high-quality material. This fact reiterates the necessary precautions for storage because during this period, the effects of factors that lower the physiological quality of the seeds should be mitigated. Among the mitigating actions are the chemical treatments of seeds, in which compounds that can protect the seeds against the deleterious effects of pests and pathogens. Disease and pest control are performed in the initial period of crop establishment, favoring seedling emergence and development (Balardin et al., 2011). The treatment of seeds with fungicides favors the initial development of soybean seedlings, especially under water restriction in the soil (Carvalho et al., 2022).

Generally, seeds are chemically treated before sowing, both on the farmer's property and at the resale itself. However, with technological advancements in agriculture, seed production companies have begun to adopt techniques that seek to improve logistics and maximize crop yield, such as industrial seed treatment, which combines innovative equipment and techniques, including the use of new formulations containing fungicides, insecticides, and nematicides in the same treatment, as well as their carriers, which can maximize the efficiency of the products (Brzezinski et al., 2015). Despite their advantages, in some situations, some seed treatment molecules may have a phytotoxic effect on seeds and seedlings, which tends to grow with the seed storage time (Brzezinski et al., 2015; Ferreira et al., 2016). Depending on the anticipation period, the treatment before packaging can be harmful, but due to logistical issues in the flow of seeds during the planting window, treatment is performed in advance, especially in the case of industrial seed treatment (Carvalho et al., 2020).

Physiological quality must be maintained until sowing, so the period between seed field production and sowing, i.e., storage, is relevant. During storage, several factors are important, including environmental conditions (Smaniotto et al., 2014), genotype (Carvalho et al., 2014a), and the composition and volume of solution in the treatment (Santos et al., 2018), especially when insecticidal molecules that have a greater tendency for toxicity than fungicides are used (Rocha et al., 2020). Monitoring the time that the soybean seed can remain treated without impairing its physiological quality, the "seed safety", is extremely important (Carvalho et al., 2020). In addition to the commonly performed physiological tests, such as germination and vigor tests, other techniques can and should be used in monitoring throughout storage.

Lignin is a natural phenolic polymer present in the seed coat of soybeans, important for providing greater mechanical strength and defense in vascular plants (Dantas, 2012). França-Neto et al. (2016) state that the presence of lignin in soybean seeds confers, in addition to resistance to mechanical damage, greater tolerance to deterioration and maintains the integrity of the cell wall. Thus, the lignin content can influence the maintenance of the quality of the stored seeds, and its evaluation is important.

Another factor of great relevance in the evaluation of seed quality is enzymatic activity, considered as one of the main indicators of degenerative changes in seeds (Silva, 2019). The use of isoenzymatic markers can be used as a tool together with physiological tests to determine biochemical changes resulting from seed deterioration.

According to Veiga et al. (2010), the activity of the enzyme alcohol dehydrogenase (ADH) is extremely important, as it converts acetaldehyde into ethanol, which is a compound with less toxicity, and reduces the speed of the deterioration process. Thus, seeds with high expression of this enzyme are less subject to the harmful action of acetaldehyde. Carvalho et al. (2014a) when studying the physiological and isoenzymatic changes in stored soybean seeds, observed higher expressions of alcohol dehydrogenase in seeds that showed better physiological quality. Thus, this enzyme can be an ally for monitoring the quality of soybean seeds.

Thus, studies on the accurate monitoring of physiological quality and on technologies that try to predict physiological problems in treated and stored seeds, such as by measuring isoenzymes, are needed. The objective of this study was to analyze the influence of industrial soybean seed treatments on physiological quality throughout storage and to evaluate the use of the alcohol dehydrogenase (ADH) enzyme as a marker for quality monitoring.

Materials and Methods

Seeds from two soybean cultivars produced under the same edaphoclimatic conditions were selected after initial characterization of the lots. Cultivars with similar quality and vigor were used, called cultivar 1 and cultivar 2. Lignin in the seed coat was quantified, in order to detect differences between the materials regarding the amount of lignin present in the tegument. The analysis was performed before chemical treatment and storage, as this component is not variable after storage. The test was performed with two replications of 50 seeds at the Lignin Determination Laboratory of the Seeds and Grains Technological Center of Embrapa Soja, Londrina, Paraná, Brazil, according to Capeleti et al. (2005).

The moisture content of seeds before treatment oscillated in value close to 9%, with a difference between the lowest and the highest value of only 0.5% for the cultivars.

Seed treatment was performed in batches of 3 kg in a Momesso Arktos Laboratory L5K to simulate industrial treatment. The treatment solutions were a mix of fungicide and insecticide (Standak®Top: fipronil + pyraclostrobin + methyl thiophanate) plus polymer (treatment ST), the insecticide thiamethoxam (Cruiser®) + the fungicide fludioxonil + metalaxyl-M (Maxim XL) plus polymer (treatment CM), all in commercial doses, as well as the commercial polymer (Biochroma) (treatment P) or water alone (treatment W) (Table 1).

After the industrial treatment of the seeds, they remained in the shade at a temperature of approximately 20 °C for 20 min for drying. They were packed in multilayered kraft paper and stored in a cold chamber, with temperature kept at 11.5 °C and a relative humidity of 54%. The samples to be analyzed were separated at 0, 30, 60, 90, and 120 days of storage.

The physiological quality of the seeds was evaluated at the Laboratory of Seeds Analysis of the Federal University of Lavras (Universidade Federal de Lavras), through the following tests:

Germination: Four replicates of 50 seeds were uniformly distributed between two sheets of germitest paper, with a volume of distilled water for imbibition in the amount of 2.5 times the dry paper weight. Then, the rolls of paper were placed in a Mangelsdorf-type germinator at 25 °C. The evaluations were performed 8 days after sowing, and the results are expressed as percentages according to the criteria established in the Rules for Seed Analysis (Brasil, 2009).

The first germination count was performed in conjunction with the germination test. The number of normal seedlings was counted 5 days after sowing. The results are expressed as percentages (Brasil, 2009).

For the enzymatic determinations, after each storage period, seed samples were taken and stored at -86 °C so that the enzymatic analyses could be performed together at the end of the storage period. The biochemical analyses were performed using spectrophotometry at the Laboratory of Biotechnology Applied to Seeds of the Federal University of Lavras (Universidade Federal de Lavras), Lavras, Minas Gerais, Brazil. Enzymatic quantification by spectrophotometry was performed on 100 mg of extracted material per sample, which was weighed and homogenized in 800 µL of extraction buffer (100 mM HEPES, pH 7.5, 15% glycerol, 10 mM β-mercaptoethanol). ADH was incubated at 30 °C in reaction buffer containing 150 mM Tris-HCl, pH 8.0, NAD^+ 0.3 mg mL^{-1}, and 1% ethanol. The reduction of NAD^+ was monitored at 340 nm for 3 min with an Eon Biotek® microplate spectrophotometer. The enzymatic activity was calculated as the amount of NADH produced per minute of incubation (Yamanoshita et al., 2005). The results were expressed in $mmol \cdot min^{-1}$ $mgMF^{-1}$.

The statistical design was completely randomized in a 2 × 5 × 5 factorial scheme: two cultivars, five seed treatments, and five evaluation periods during storage. The data were subjected to analysis of variance with the aid of Sisvar® software (Ferreira, 2014) at 5% probability by the F test ($p < 0.05$). The means were compared using the Tukey test at 5%, or polynomial regression analyses were performed, choosing mathematical models at the 5% significance level that had the highest coefficient of determination.

Results and Discussion

Lignin is present in the seed coat and plays an important role in protecting seeds against mechanical injuries. Many authors report that the lignin content is related to the resistance of soybean seeds to mechanical damage, that is, when there are higher levels of this compound, the occurrence of damage is minimized and, consequently, better seed quality (Menezes et al., 2009; Gris et al., 2010; Feliceti, 2019).

However, the lignin contents in the seed coat were similar, with cultivar 1 quantified 3.02% and cultivar 2 quantified 3.15%, thus restricting any inference for this characteristic. All variables analyzed were significantly affected by the triple interaction between the cultivar, seed treatment, and storage factors, with satisfactory coefficients of variation (Table 2).

In the germination test, for the first count, similarities were observed between cultivars 1 and 2 at the beginning of storage, which confirms the similar initial qualities of the lots (Table 3). At this first evaluation, cultivar 2 seeds showed a difference between treatments, as the CM treatment provided fewer normal seedlings when compared to the C

Table 1. Composition and volumes of spray solution used in the industrial treatment of seeds of soybean cultivars.

Composition	mL·100 kg^{-1} of seeds					
	Standak®Top	Maxim XL®	Cruiser®	Polymer	Water	Volume of spray solution
No treatment (control) (C)	-	-	-	-	-	-
Fipronil + pyraclostrobin + thiophanate-methyl + polymer (ST)	200	-	-	250	0	450
Without chemical + polymer (P)	-	-	-	450	-	450
Without chemical + water (W)	-	-	-	-	450	450
Thiamethoxam + fludioxonil + metalaxyl-M + Polymer (CM)	-	100	250	200	0	550

Table 2. Analysis of variance of the first count of germination (1st G), germination percentage (G), and enzymatic expression of alcohol dehydrogenase (ADH) of treated and stored soybean seeds.

SV	GL	Mean square		
		1st G	G	ADH
Cultivar (C)	1	92.48	96.60	12.11
Treat. seed (TS)	4	619.10*	613.27*	14.96
Storage (S)	4	10572.58*	5733.20*	105.61*
C × TS	4	224.69*	543.95*	42.00
C × S	4	66.46	248.11*	17.04
TS × S	16	83.56	86.35*	45.32*
C × TS × S	16	111.78*	141.49*	39.82*
Residue	150	38.03	35.13	10.27
CV (%)		9.63	8.01	11.74

SV: source of variation. GL: degrees of freedom *Significant at 5% probability according to the F test ($p < 0.05$).

Table 3. Mean first germination count of soybean seeds of cultivars 1 and 2 at different storage times after treatment with phytosanitary products.

First germination count (%)			
Storage period	Composition	Cultivar	
		1	2
0 days	C	84 A a	88 A a
	ST	83 A a	79 A ab
	P	85 A a	85 A a
	W	76 A a	82 A ab
	CM	80 A a	71 A b
30 days	C	72 A a	80 A a
	ST	74 A a	67 B bc
	P	68 A ab	76 A ab
	W	59 A b	64 A c
	CM	65 A ab	58 A c
60 days	C	67 A a	73 A a
	ST	69 A a	69 A a
	P	65 A a	64 A a
	W	65 A a	68 A a
	CM	59 A a	66 A a
90 days	C	71 A a	73 A a
	ST	69 A a	70 A a
	P	65 A a	66 A a
	W	65 A a	68 A a
	CM	59 B b	70 A a
120 days	C	27 B b	45 A a
	ST	48 A a	40 A a
	P	52 A a	33 B ab
	W	29 A b	35 Aab
	CM	35 A b	27 B b

*Means followed by the same lowercase letter in a column in each storage period and by the same uppercase letter in a row do not differ from each other, at 5% probability by Tukey's test.

and P treatments, even before storage, probably related to the phytotoxic effect of the products in the germination test on paper. As seen by Rocha et al. (2020), substrates from tests with readily available water cause greater phytotoxicity, especially with some insecticidal molecules.

For cultivar 1, the seed treatments at 0 and 60 days of storage did not influence germination potential, which was also observed at 60 and 90 days for cultivar 2 (Table 3). Krzyzanowski et al. (2020) reported that the first germination count test can be used as a vigor test and that efficiency of this process is directly related to the level of seed deterioration. Thus, as the deterioration progresses, the germination speed slows. However, per Amaro et al. (2015), this test has low sensitivity, as it does not detect small differences in vigor between the lots.

At 30 days of storage, seeds of cultivar 1 treated only with W were fewer, whereas among seeds of cultivar 2, the W and CM groups had fewer seeds when compared to the C and P treatments. At 90 days, seeds of cultivar 1 treated with CM were also fewer (Table 3). Del Bem Junior et al. (2020) also observed lower physiological quality after storage in soybean seeds treated with the insecticide thiamethoxam. According to Rocha et al. (2020), insecticidal molecules hinder soybean germination and may cause greater seed phytotoxicity than fungicides.

Seed treatment can provide an advantage to seed longevity during the storage period (Mbofung et al., 2013), but the effects of the treatments depend on the molecule transmitted to the seeds and cannot be generalized. At 120 days of storage of seeds of cultivar 1, the groups treated with C, W, and CM showed lower germination on paper, while those treated with ST and P were higher. For cultivar 2, although it does not differ from P and W, CM was also assigned to the lower germination group on paper, and ST was assigned to the upper group, even after 120 days of storage (Table 3).

During storage, in the first germination count of seeds of cultivar 1, there was a more pronounced initial decreasing trend for C, W, and CM seeds, in which the values remained at this level up to 90 days and then abruptly decreased, that is, low values at the end of the storage period (Figure 1A). For ST and P, the decrease was linear, with milder effects on deterioration, and at the end of the storage period, those treatments showed higher values. These findings reiterate that deterioration during storage is directly linked to the type of molecule and the product used.

In the CM treatment, the insecticidal molecule present was thiamethoxam. Thiamethoxam is a systemic insecticide belonging to the neonicotinoid class, similar in action to clothianidin and imidacloprid. This class are among the most widespread insecticidal molecules in agricultural use. In the ST treatment, the insecticide molecule was fipronil, a pyrazole, which has a contact and ingestion mode of action (Martins et al., 2017).

In the seeds of cultivar 2, the initial deterioration trend (30 days) was greater in the ST, W, and CM treatments, while under C and P, this decrease occurred at 60 days. After this period, the values kept up similar trends, with a sharp decrease under all treatments at 120 days (Figure 1B). This was a different pattern from that of cultivar 1, indicating differences between the genotypes regarding tolerance to treatment and storage.

However, a seed coating of insecticides and fungicides protects the seeds; however, it may also be responsible for damage to the physiological quality of many seeds, with these damages occurring immediately after coating treatment

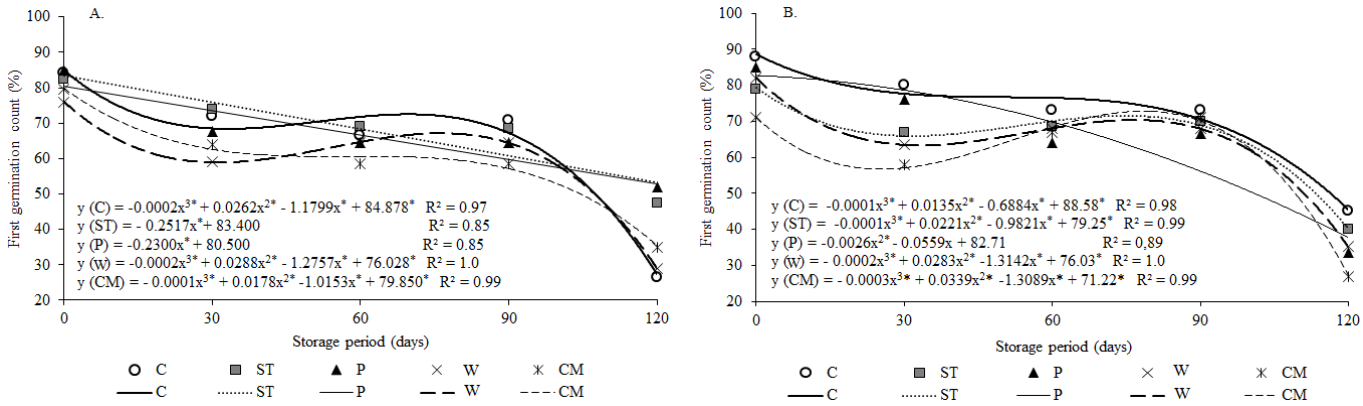

Figure 1. Percentage of normal seedlings from seeds of cultivar 1 (A) and cultivar 2 (B), obtained in the first germination count, on paper, during storage after treatment with phytosanitary products.

or after storage (Camilo et al., 2017; Rocha et al., 2020). In addition to the molecules involved in seed treatment, the composition and volume of the solution affect the safe storage of treated seeds (Santos et al., 2018).

The final germination count at time 0 was not different between the seed treatments regardless of the cultivar, thus highlighting the similarity in initial quality between the lots. At 30 days of storage, for treatments C and W, cultivar 1 showed lower values than cultivar 2. For cultivar 1, treatment W provided lower means in relation at C and ST treatments. It can be inferred that for cultivar 1, chemical treatment of seeds with aqueous predominance accelerates the process of seed deterioration when compared to cultivar 2. When analyzing the germination of seeds for cultivar 2, at 30 days, when they were subjected to treatments W and CM, they had lower germination than under C (Table 4).

At 60 days, for cultivar 1, seeds treated with the CM treatment resulted in lower germination, and those treated with ST had higher means. For seeds of cultivar 2, the CM treatment also yielded lower germination, while the highest was observed in the control treatment C (Table 4). At 60 days of storage, with the exception of group C of cultivar 2, none of the treatments with paper substrate reached the minimum germination percentage (80%) needed for commercialization (Brasil, 2013).

At 90 days of storage, seeds of cultivar 1 in the CM group had lower germination percentages than the others, the C and ST groups having the highest means (Table 4). This reiterates the effects of the higher phytotoxicity of some molecules on storage and germination when performed on paper substrate. Dan et al. (2013) concluded that treatment with thiamethoxam (Cruiser®350 FS) favored the physiological potential of soybean seeds for up to 30 days of storage.

At the end of the storage period (120 days), all treatments showed germination percentages below the minimum of 80% established for commercialization of soybean seeds in Brazil (Table 4) (Brasil, 2013). Seeds of cultivar 1 had the lowest germination under treatments C, W, and CM. For cultivar 2, the germination in paper with CM was lower than that in the control, which shows the phytotoxicity of these molecules during storage. Dan et al. (2013) found lower emergence of seedlings from soybean seeds treated with insecticide during storage. Rocha et al. (2020) found that insecticides have greater toxicity than fungicides, which may reduce the physiological quality during storage.

Table 4. Germination means for soybean seeds of cultivars 1 and 2 at five storage times after treatment with phytosanitary products.

Germination (%)			
Storage period	Treat. seed	Cultivar	
		1	2
0 days	C	87 A a	93 A a
	ST	90 A a	82 A a
	P	92 A a	91 A a
	W	81 A a	88 A a
	CM	88 A a	83 A a
30 days	C	80 B a	92 A a
	ST	86 A a	83 A ab
	P	78 A ab	86 A ab
	W	67 B b	78 A b
	CM	75 A ab	76 A b
60 days	C	71 B ab	83 A a
	ST	77A a	75 A ab
	P	72 A ab	75 A ab
	W	73 A ab	74 A ab
	CM	63 A b	71A b
90 days	C	77 A a	83 A a
	ST	80 A a	76 A a
	P	72 A ab	75 A a
	W	73 A ab	75 A a
	CM	63 B b	74 A a
120 days	C	42 B b	64 A a
	ST	73 A a	53 B abc
	P	75 A a	46 B bc
	W	51 A b	55 A ab
	CM	52 A b	42 B c

*Means followed by the same lowercase letter in a column and uppercase letter in a row do not differ at 5% probability by Tukey's test.

During storage, for cultivar 1, there was a marked decrease in initial germination under all treatments besides ST (Figure 2A), in which group the decrease in germination was linear, with a lower slope, which reflects the lower phytotoxicity of these molecules during storage. At 120 days of storage, seeds treated with this product and those treated only with P had higher means than the others.

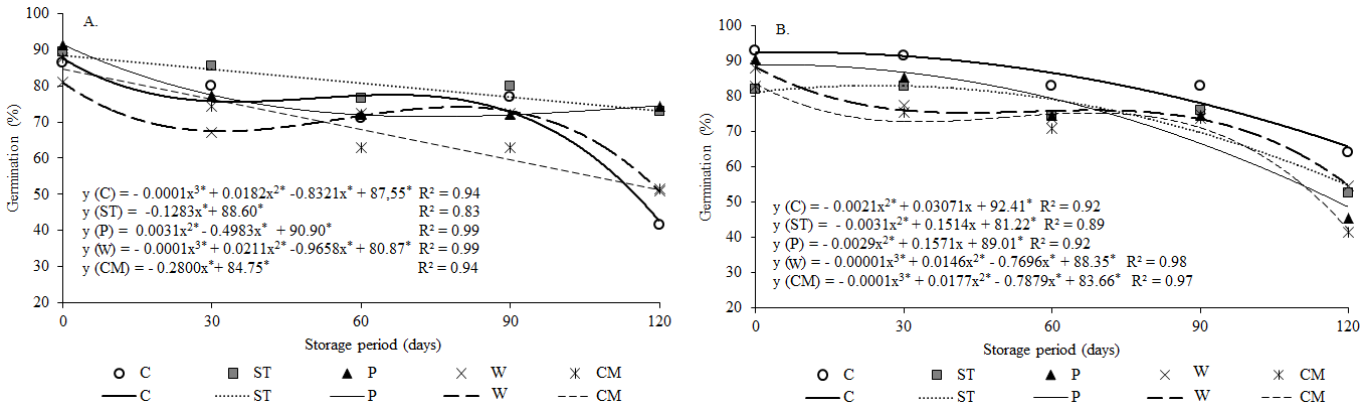

Figure 2. Percentage of normal seedlings from seeds of cultivar 1 (A) and cultivar 2 (B), obtained in the germination test, on paper, during storage after treatment with phytosanitary products.

Under the other treatments, after the initial drop in germination, the values remained steady until day 90. After this, germination drastically decreased, especially in groups C and W. In group CM, there was a linear trend of decreased germination with a high slope, resulting from phytotoxicity during germination, especially with the evaluation on paper substrate. One of the possible causes of the reduction in the number of normal seedlings in the germination test of soybean seeds treated with phytosanitary products may be linked to the substrate in which the seeds are sown, especially when the substrate has large amounts of readily available water and high concentrations of active ingredients per area.

Sene et al. (2020), when studying the effect of polymer combined or not with fungicides, found a beneficial effect of this type of association on stored rice seeds. Ferreira et al. (2016) reported that chemical treatment with mixtures containing the insecticide thiamethoxam did not affect the physiological quality of soybean seeds before storage or after two months of storage.

Seeds of cultivar 2, in the initial storage period (30 days), had a marked decrease in germination in the W and CM groups (Figure 2B). After this period, all treated seeds showed similar values at 90 days of storage - generally lower than that of group C. For all seeds with some type of treatment, ST, P, W, or CM, after 90 days, the decrease in germination on paper was accentuated, and at the end of the storage period, group C had a higher mean. This was not observed for cultivar 1 (Figure 2A). A negative effect of storage for 2 months on seeds treated with the insecticide fipronil + the fungicides pyraclostrobin and methyl thiophanate was reported by Ferreira et al. (2016).

During storage, there were no consistent differences in the expression of the enzyme ADH between the two cultivars when comparing each seed treatment applied (Table 5). There was a difference in the expression of the enzyme within each cultivar, such as at the beginning of storage, where the C and CM groups of cultivars 1 had lower values of enzyme expression. This was true up to 60 days, after which the product used did not affect the ADH expression for cultivar 1.

Conversely, for cultivar 2, differences in enzyme expression were observed as a function of the type of product used, except at 90 days, in which there was no difference between them.

Table 5. ADH in soybean seeds of cultivars 1 and 2 at five storage periods after treatment with phytosanitary products.

ADH (mol g ADH activity (mmol·min^{-1} mgMF^{-1})			
Storage period	Treat. seed	Cultivar	
		1	2
0 days	C	22.99 A bc	24.50 A b
	ST	29.87 A a	24.44 B b
	P	28.65 A ab	30.58 A a
	W	31.35A a	26.95 B ab
	CM	22.49 B c	30.03 A ab
30 days	C	26.25 A ab	23.76 A b
	ST	30.44 A a	26.38 A ab
	P	23.28 A b	24.98 A ab
	W	27.80 A ab	29.84 A a
	CM	29.04 A ab	29.23 A ab
60 days	C	25.11 B ab	29.55 A ab
	ST	26.40 A ab	24.57 A bc
	P	22.60 B b	33.01 A a
	W	21.16 A b	22.53 A c
	CM	30.30 A a	28.96 A ab
90 days	C	30.38 A a	34.52 A a
	ST	29.52 A a	31.69 A a
	P	30.28 A a	29.81 A a
	W	26.76 A a	29.09 A a
	CM	28.70 A a	29.83 A a
120 days	C	23.79 B a	27.67 A ab
	ST	25.17 A a	24.69 A ab
	P	25.33 A a	23.02 A b
	W	28.45 A a	26.79 A ab
	CM	26.82 A a	30.10 A a
Mean		26.91	27.86

*Means followed by the same lowercase letter in a column and uppercase letter in a row do not differ at 5% probability by Tukey's test.

At the end of the storage period, 120 days, seeds treated with CM showed the highest expression of the ADH enzyme (Table 5), and this treatment showed lower germination (Table 4). These findings might be related to a higher expression of the ADH substrate under exposure to CM - acetaldehyde, in the anaerobic pathway (Carvalho et al., 2014a).

During storage, the amount of ADH was in general higher in the seeds of cultivar 2 between 60 and 90 days, especially when they were treated with only P, compared to cultivar 1. No consistent difference was observed between the genotypes

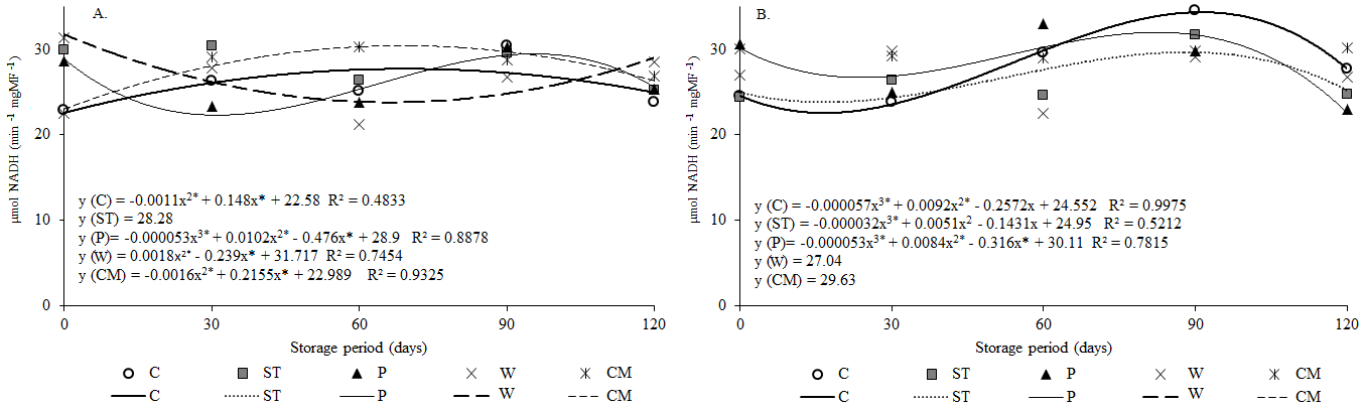

Figure 3. Quantification of ADH enzyme activity in treated and stored soybean seeds. (A) Cultivar 1. (B) Cultivar 2.

in the expression of the ADH enzyme, only variations as a function of the treatment and storage period (Figure 3).

ADH is important because it converts acetaldehyde into ethanol, a compound with less toxicity, so its presence in seeds during storage helps to reduce the deleterious effects of acetaldehyde (Marcos-Filho, 2015). Carvalho et al. (2014a) found that the activity of this enzyme contributed to the maintenance of seed quality during storage.

In seeds of soybean cultivars that showed better physiological quality, higher expression of ADH and isocitrate lyase and lower expression of malate dehydrogenase and peroxidase have been observed (Carvalho et al. 2014b). Similarly, Nery et al. (2018), studying the behavior of isoenzymes in sesame seeds subjected to accelerated ageing test, observed that cultivars with lower physiological quality showed the lowest expressions of the ADH enzyme.

Enzymes are products of gene expression and so are highly influenced by the environment because the genes that control their expression are expressed at certain stages of development and in specific organs and tissues or even under a certain stimulus (Malone et al., 2007). Our study suggests that storage combined with seed treatment activates a higher production of the ADH enzyme to protect the seeds from high acetaldehyde production, acting as a catalyst to reduce acetaldehyde to ethanol, thus reducing or trying to reduce the deleterious effects caused by lactate accumulation (Marcos-Filho, 2015). Therefore, the quantity of the ADH enzyme in stored soybean seeds may be a promising marker for their physiological quality. More studies will be needed at the transcriptomic and genomic levels to confirm it as a quality marker of stored soybean seeds.

Conclusions

The deterioration of soybean seeds during storage is directly linked to the type of chemical and the viscosity of the spray solution applied to seeds.

The treatment of soybean seeds with predominantly aqueous solution and the use of insecticidal molecules with systemic action, combined with fungicides and polymers, impair the germination in paper of the stored seeds. The deleterious effects are greater with longer storage time.

The enzyme alcohol dehydrogenase has potential as a marker of the physiological quality of treated and stored soybean seeds.

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