Revista Ciência Agronômica, v. 56, e202292842, 2025
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Storage potencial of soybeans cultivars under low temperature[1]

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ABSTRACT - Currently with a more demanding market, is necessary to produce better and high physiological quality cultivars of soybeans. In front of this new challenge, our objective was to evaluate the physiological quality of 11 soybean cultivars: CD201, SYN1263, SYN1279, BMX, UFLA1, CA115, CD215, CD202, Conquista, Savana, and BRS820 storage for 12 months. Evaluations were conducted through physiological like germination and vigor (accelerated aging and controlled deterioration) and isoenzymes analysis. The seeds were stored under controlled conditions at 10 °C and 10% relative humidity. It was assessed every four months (0, 4, 8, and 12). 200 seeds per treatment were used for each test, divided into 4 replications of 50 seeds. The number of normal plants was evaluated on the fifth and seventh days, expressed as a percentage value. Isoenzyme analysis of MDH, ADH, Esterase, and Catalase was made. The results were interpreted from the presence or absence of bands in the gel. An experimental design in randomized complete blocks, interpreting data using analysis of variance in a factorial scheme 11 x 4 (11 cultivars and 4 times of storage and averages compared by the test Scott-Knott 5% of probability and regression analysis. The statistical program used was Sisvar®. We found that cultivars Savana and Conquista showed low physiological quality, and the cultivars CD 215 and BMX showed high physiological quality during storage.

Key words: *Glycine max*. Isoenzymes. Soja. Tolerance. Crop development.

DOI: 10.5935/1806-6690.20250005
Editor-in-Chief: Prof. Alek Sandro Dutra - alekdutra@ufc.br
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Received for publication 09/06/2022; approved on 29/01/2024
[1]This paper is extracted from the dissertation of the lead author presented to the Postgraduate Program in Engenheria Agronomica, Universidad Federal de Lavras, Brasil. The authors wish to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)
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INTRODUCTION

Soybean is the most important oilseed in the world, with Brazil being the second largest producer of this seed, harvesting around 96.2 million tons per season. The quality control of soybean is crucial from its collection, chemical composition, nutritional quality, vigor, processing, and storage (BISHT *et al.*, 2015; MARTINS *et al.*, 2018).

The quality of seeds directly reflects the crop development, leading to plants with high vigor, population uniformity, and absence of seed-transmitted diseases (SILVA; LAZARINI; SÁ, 2010). Similarly, storage is a fundamental practice to maintain physiological quality and ensure the preservation of vigor and viability between collection and planting (AZEVEDO *et al.*, 2003), linked to the preservation of physiological and sanitary quality by reducing contamination, pest incidence, microorganisms, and minimizing deterioration. While the latter cannot be prevented, its speed can be minimized through appropriate procedures of production, harvesting, drying, processing, and transport (DELOUCHE, 2002; FRANÇA NETO *et al.*, 2010; GARCIA *et al.*, 2004; KRZYZANOWSKI, 2015; MAVAIEIE *et al.*, 2019).

High temperatures and the activity of microorganisms such as fungi and insects accelerate the respiratory processes of seeds, favoring the population growth of these organisms, thus increasing seed deterioration over time (MARCOS-FILHO, 2005). Likewise, enzymes involved in slowing down the damage caused by respiratory processes have been documented. For example, alcohol dehydrogenase (ADH) is an enzyme related to anaerobic respiration, promoting the reduction of acetaldehyde to ethanol. Acetaldehyde accelerates seed deterioration, therefore, increased ADH activity provides protection against the deleterious action of this compound (ZHANG *et al.*, 2008). Malate dehydrogenase (MDH) plays a significant role in the Krebs cycle, catalyzing the conversion of malate to oxaloacetate and producing NADH, which is a fundamental product in the production of ATP and essential intermediate compounds in cellular functioning (CARVALHO *et al.*, 2014; MAVAIEIE *et al.*, 2019). The intracellular enzyme catalase (CAT) found in plant peroxisomes has the ability to transform reactive oxygen species into harmless forms, such as the breakdown of hydrogen peroxide (BAILLY *et al.*, 2004). Esterase (EST) is an enzyme involved in lipid breakdown during the germination process, being relevant in the renewal of embryonic axis growth, especially in lipid-rich seeds like soybean (MAVAIEIE *et al.*, 2019).

The physiological quality of soybean seeds can vary depending on the genotypes, and this characteristic is important during the selection process in breeding programs (VERNETTI and VERNETTI, 1983). Therefore, there is a need to understand the genetic control for these traits (MEDEROS-RAMÍREZ; ORTIZ-PEREZ, 2021). Currently, there is a concern in selecting soybean genotypes with higher storage potential while preserving their physiological quality until planting time. However, there are not many studies that relate different storage tolerance levels among soybean cultivars (MAVAIEIE *et al.*, 2019). The objective of this research was to study the physiological quality of soybean seeds from 11 cultivars stored for 12 months in a cold chamber and obtain an isoenzyme expression profile during storage.

MATERIAL AND METHODS

Study Area

The work was conducted in two stages, one in the field and the other in the laboratory. The field phase was carried out at the experimental vegetable station of Hortiagro Semillas Ltda., in the municipality of Ijaci (Minas Gerais, Brazil), located 13 km northeast of the city of Lavras, at an elevation of 833 msnm, latitude: 21° 9’ 24’’ South, and longitude: 44° 55’ 34’’ West. Seed multiplication of 11 soybean cultivars (CD201, SYN1263, SYN1279, BMX potencia, UFLA1, CA115, MS8400, CD215, CD202, CONQUISTA, SAVANA) was carried out under controlled field conditions.

Subsequently, the seeds were collected at the R8 phenological stage (95% maturity and 18% moisture content), and then dried until reaching 12% moisture content. Circular size sieves ranging from 5.55 mm to 6.35 mm were used for seed processing. The seeds were weighed and stored in paper bags under controlled conditions in a cold chamber at the central seed laboratory of the Department of Agriculture of the Federal University of Lavras (UFLA), located in Lavras municipality, Minas Gerais, with a latitude of 21º 14” S, longitude of 45º 00”, and elevation of 918 msnm.

The experimental design employed was randomized complete block design with replications. The physiological tests evaluated were germination and vigor (controlled deterioration and accelerated aging). Each test was evaluated by counting normal plants, measurements taken on the fifth and eighth day, and results expressed as a percentage. In each test, 200 seeds per cultivar were used, divided into 4 replications of 50 seeds each, according to the Seed Analysis Standard (BRASIL, 2009).

Germination and Vigor Test (controlled deterioration and accelerated aging)

For the physiological tests evaluation, the seeds were treated with the insecticide Vitavax Thiram (200 mL per 100 kg of seeds) and water (300 mL per 100 kg of seeds). These tests were conducted at four different

times, 0, 4, 8, and 12 months. Subsequently, they were placed on moistened Germitest paper with water, equivalent to 2.5 times the weight of the dry substrate, and kept at a temperature of 25 °C in accordance with RAS (BRASIL, 2009) to ensure uniform humidity. Afterwards, each treatment with four rolls of Germitest paper containing 50 seeds for a total of 200 seeds/treatment were placed in a germinator regulated at 25 °C, where two evaluations were carried out by counting the number of normal plants on the fifth and eighth day after sowing.

Accelerated aging test

Mini cameras of the "Gerbox" type were used according to the RAS (BRASIL, 2009), where 42 g of seeds from each treatment were placed. The seeds were suspended on a grid, without coming into contact with the bottom of the box which previously contained 40 mL of distilled water. Subsequently, the mini cameras were placed in a germination chamber at 42 ºC for 82 h, ensuring maximum stress. After this time, they were removed and the germination test was carried out.

Controlled deterioration test

Aluminum envelopes coated with plastic and hermetically closed with 42 g of seeds from each treatment were used. These were kept in a water bath at 40 ºC for 48 h, following the protocol described by Marcos-Filho (2005). The seed moisture content was adjusted to 15% and placed in a cold chamber at 10 ºC/24 h. before being transferred to a germination chamber at a temperature of 42 ºC/48 h. On the fourth day, a germination test was performed according to the RAS guidelines (BRASIL, 2009).

Isoenzymatic analysis

After being harvested and processed, the seeds were stored at -80 ºC and evaluated at 0, 4, 8, and 12 months. For gene expression analysis, 50 ground seeds were used with liquid nitrogen and polyvinylpyrrolidone (PVP) antioxidant and divided into Eppendorf tubes with 100 mg each. Then, 300 uL of ethyl ether + 300 µL of water were added to remove the oil. Subsequently, they were centrifuged for 20 minutes at 14000 rpm at 4 ºC.

Enzyme extraction was performed with 250 µL of buffer (0.2 M Tris HCl pH 8 + 0.1% beta-mercaptoethanol), followed by vortex agitation and overnight incubation. Afterward, the samples were centrifuged at 4 ºC at 14000 rpm for 30 minutes. In the running gel, 50 µL of supernatant (separating gel - 7.5% polyacrylamide and concentrating gel - 4.5% polyacrylamide) were applied. The buffer gel/electrode system used was Tris glycine pH 8.9. Runs were performed at 150 volts for 6 hours. After electrophoresis, the enzyme was revealed following the protocol for each one (ALFENAS, 2006).

The statistical analysis of the obtained data was performed using the Sisvar® program (FERREIRA, 2011), through the analysis of variance of each test. The comparison of means was done using the Scott-Knott test, with a 5% probability. The design used was a completely randomized block design in a factorial design of 11x4 (11 cultivars and 4 evaluation periods: 0, 4, 8, 12 months).

RESULTS AND DISCUSSION

Physiological Tests

Significant differences ($F3; 30 = 15.826$; $P = 0.0001$, $p < 0.05$) were found in the analysis of variance for the physiological quality of soybean seeds in cultivars stored for different periods in germination and controlled deterioration tests on the fifth and eighth day, respectively. The cultivars Savana, Conquista, and BRS 820 were found to have the lowest evaluated physiological quality (Table 1). According to Baldoni (2013), these cultivars were also reported to have low physiological quality when evaluated through germination tests and accelerated aging, with no significant differences found ($p > 0.05$). This lack of differentiation may be due to the fact that the germination test does not provide information on the progress or potential for deterioration.

The evaluation period analyzed through regression showed significant differences for germination tests ($y = -4{,}1667x3 + 32x2 - 72{,}833x + 141$; $R^2 = 1$) (Figure 1a), controlled deterioration ($y = -9E\text{-}13x3 + 0{,}5x2 - 1{,}5x + 97$; $R^2 = 1$) (Figure 1b), and accelerated aging ($y = 1{,}8333x3 + 1{,}5x2 - 45{,}333x + 134$; $R^2 = 1$) (Figure 1c). A slight decrease in the physiological quality of the seeds at 4 months of storage can be considered, perhaps due to the presence of fungus in the seed at the time of collection. This decrease, along with the passage of time and exposure to low temperatures in a cold room, caused a decrease in its proliferation, allowing the potential vigor of the different soybean cultivars to be observed at 8 and 12 months. According to Mederos-Ramírez and Ortiz-Perez (2021), an increase in soybean seed germination has been found after 2 and 3 months of storage at room temperature, due to a reduction in the incidence of field fungi on the stored seeds.

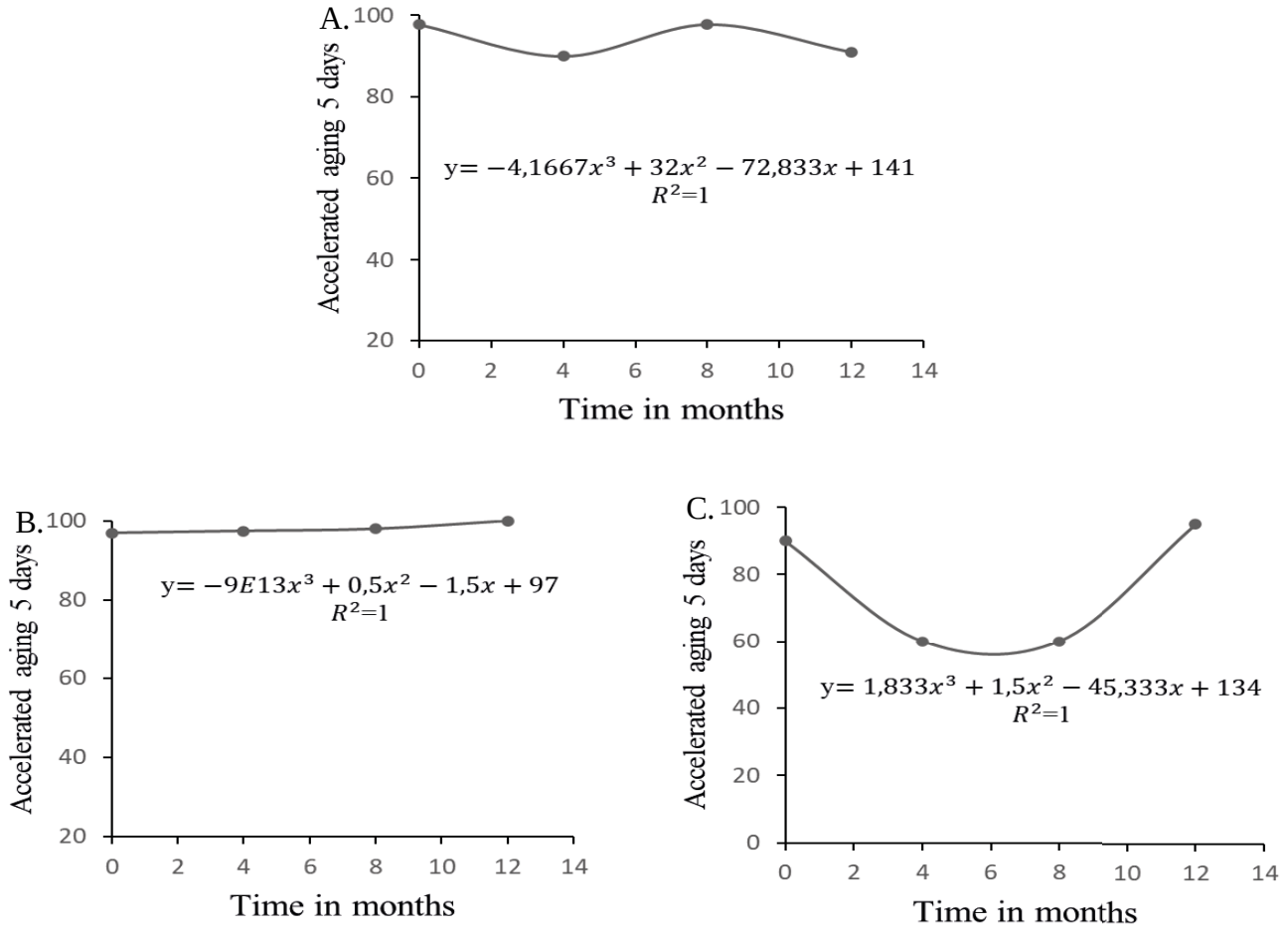
The results demonstrate efficiency in seed viability when stored in a cold chamber, after 6 months of storage, as also observed by Mavaieie *et al.* (2019) and Martins-Filho *et al.* (2001), after 8 months of storage in a cold chamber, the seeds of soybean cultivars were superior compared to those stored under non-controlled conditions). Analysis of variance showed interaction between evaluation periods and cultivars on day 7 of the controlled deterioration test. Germination test data and seed image analysis were used to perform an analysis of variance, and treatment means were compared using the Scott-Knott test, revealing

Table 1 - Mean germination (Ger), controlled deterioration (DC), and accelerated aging (EA) for the first and second count of seeds from 11 soybean cultivars

Cultivar	Ger. 5 Days	Ger. 8 Days	DC 5 Days	DC 8 Days	EA 5 Days	EA 8 Days
Savana	86 c	89 c	93 b	95 b	77 a	84 a
Conquista	90 b	93 b	95 b	96 b	75 a	80 a
BRS820	92 b	94 b	97 a	98 a	85 a	88 a
BMX	96 a	96 a	98 a	98 a	80 a	83 a
CD202	97 a	97 a	97 a	98 a	81 a	86 a
UFLA 1	97 a	98 a	98 a	98 a	86 a	91 a
CD 201	97 a	97 a	99 a	99 a	84 a	87 a
SYN 1263	97 a	98 a	98 a	98 a	77 a	82 a
CD 215	97 a	97 a	98 a	99 a	74 a	81 a
CA 115	97 a	98 a	97 a	99 a	63 a	69 a
SYN 1279	98 a	98 a	99 a	99 a	71 a	73 a
CV%	5.05	4.43	2.74	1.84	22.77	19.19

* Means followed by the same letter do not differ from each other according to the Scott-Knott test at a 5% probability

Figure 1 - Physiological quality of 11 soybean cultivars seeds during four storage periods: 0, 4, 8, and 12 months; A. Results corresponding to the fifth-day Germination tests. B. Eighth-day Germination tests. C. Controlled deterioration test on the fifth day



significant differences ($p \leq 0.0001$) (Table 2). An interaction was observed in the first three storage periods (months 0, 4, and 8) in the Savana and Conquista cultivars with poorer physiological quality. The accelerated aging test in different periods showed significant differences ($p < 0.05$) in treatments, with the CA115 cultivar being the most affected in its physiological quality after 8 months of storage, where a noticeable decline in the physiological quality of different cultivars can be observed (Table 3).

Isoenzyme Analysis

The selected cultivars for isoenzyme analysis, ranging from low to high quality, were Conquista, Savana, and the BMX and CD201 varieties, respectively. Their selection was based on previous research reports, highlighting their high physiological quality and commercial value in the market (BALDONI, 2013).

Seeds of the soybean cultivar BMX potency RR, have superior characteristics and maintain a minimum germination rate greater than 80% (JUVINO *et al.*, 2014). The CD201 cultivar is a conventional cultivar. It has been selected in different productivity and high physiological quality studies, and it has been reported to have a germination percentage greater than 92% during the first year of production according to Ludwing *et al.* (2011), and 88% in the second year with a productivity of 1,878 kg/ha. Thus, the results obtained can be seen in Figure 2, where the isoenzyme patterns of esterase, catalase, ADH, and MDH are shown for the four cultivars: CD201, BMX, Savana, and Conquista.

Table 2 - Mean values of the interaction of cultivars during the four storage periods

Cultivar	0 months	4 months	8 months
Savana	93 b	95 b	98 a
Conquista	98 a	94 b	91 b
BRS820	99 a	98 a	99 a
BMX	97 a	97 a	100 a
CD202	97 a	98 a	99 a
UFLA 1	99 a	97 a	99 a
CD 201	98 a	98 a	99 a
SYN 1263	99 a	97 a	99 a
CD 215	99 a	99 a	100 a
CA 115	98 a	97 a	100 a
SYN 1279	98 a	99 a	100 a

* Means followed by the same letter do not differ from each other according to the Scott-Knott test at a 5% probability

Table 3 - Mean interaction values at 8 months of storage for 11 soybean cultivars

Cultivar	Cultivar X Time (8 Months)
CA 115	17 b
SYN 1279	55 a
BRS 820	70 a
BMX	72 a
SAVANA	73 a
CD 201	73 a
CONQUISTA	77 a
CD 215	77 a
SYN 1363	82 a
CD 202	87 a
UFLA 1	88 a

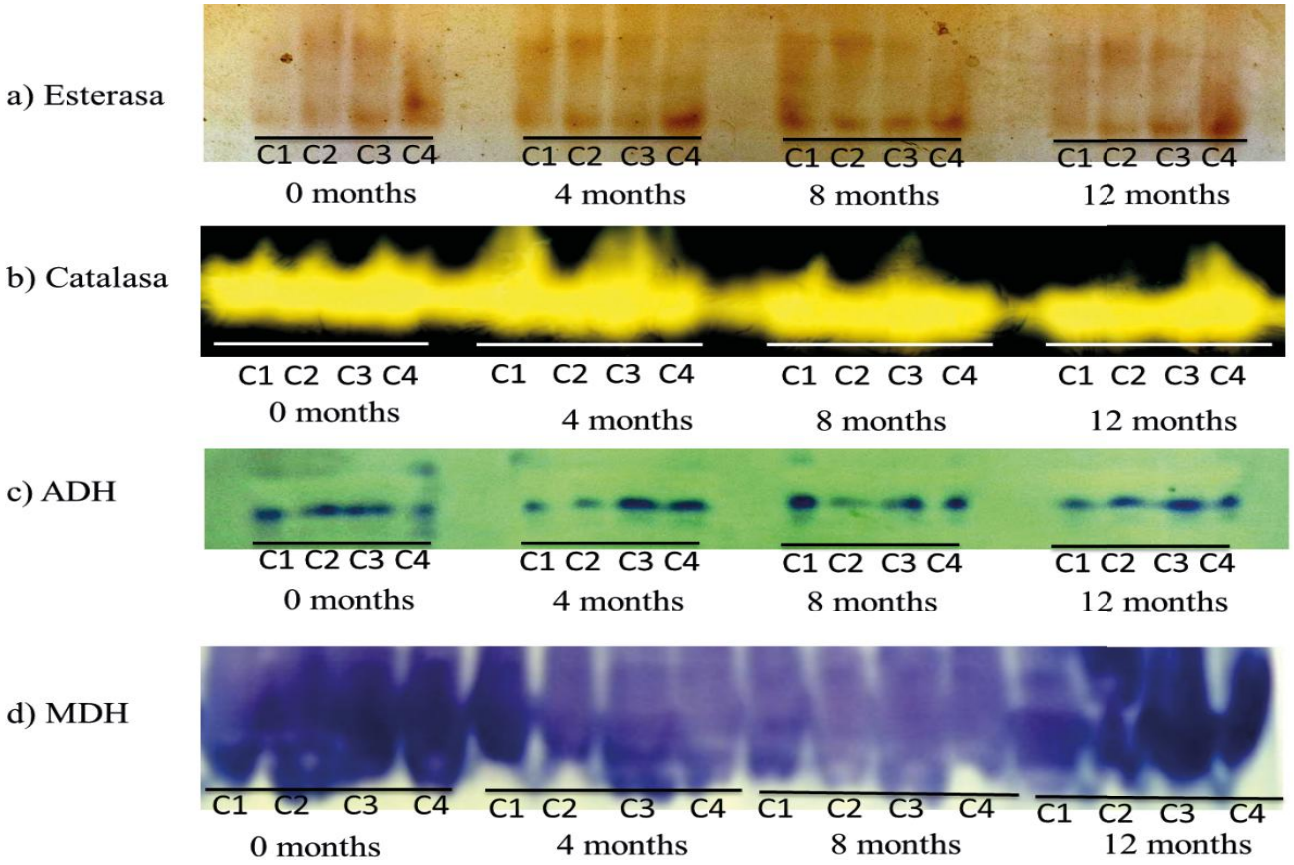
* Means followed by the same letter do not differ from each other according to the Scott-Knott test at a 5% probability level

The pattern found for the esterase enzyme shows that the Savana and Conquista cultivars have higher activity of this enzyme at 0 and 4 months, indicating deterioration. It also shows higher activity when there is more deterioration, as it degrades lipids during germination. At 8 months, activity increases for all four cultivars. At 12 months, there is a noticeable decrease for the high-quality cultivars, except for the Savana and Conquista cultivars. Mavaieie *et al.* (2019) found that, in cold storage, seeds have higher esterase activity compared to seeds stored in uncontrolled conditions throughout storage. Esterase plays a relevant role in the growth of the embryonic axis and the breakdown of lipids in the germination process, especially in oily seeds like soybeans. Esterase activity decreases after the sixth month, and according to Veiga *et al.* (2010), bands may disappear after nine or twelve months of storage. However, the Savana and Conquista cultivars maintained their enzymatic activity at 12 months, possibly due to their low quality. In contrast, the CD201 and BMX cultivars presented lower enzymatic activity, higher germination, and vigor (Table 1).

These differences were maintained throughout the storage period. The enzyme catalase (CAT), which involves the removal of hydrogen peroxide formed from enzyme activity and is considered the second line of defense in the antioxidant system after the enzyme SOD, showed high activity during the 0 and 4-month periods. Similarly, no differences in enzyme expression were observed between treatments within each storage period. However, in the 0 and 4-month periods, higher activity was found, contrary to what was found by Baldoni (2013), who found differences in enzyme activity between seeds collected at the R8 stage with higher expression and seeds collected 15 days after this stage with lower physiological quality.

In deteriorated seeds, lower enzyme activity (CAT) was also found, with lower efficiency of free radical scavenging systems. When the seed ages, lipid peroxidation increases and enzymatic activity of peroxide-scavenging enzymes decreases (BRACCINI *et al.*, 2000; MENEZES *et al.*, 2009). The expression of the alcohol dehydrogenase (ADH) enzyme showed activity during the 4 storage periods,

Figure 2 - Isoenzyme analysis of four cultivars (C1: CD201, C2: BMX, C3: Savana, C4: Conquista), representing two high-quality cultivars and two low-quality cultivars, respectively. Enzyme patterns for a) catalase, b) esterase, c) ADH, and d) MDH



with higher activity in high-quality cultivars in the 0, 8, and 12-month storage periods. The ADH enzyme is involved in the respiratory process and functions to remove acetaldehyde in seeds, as well as being relevant due to its conversion of acetaldehyde to ethanol, which is less toxic and reduces the rate of deterioration. Thus, seeds are less susceptible to the action of acetaldehyde when there is higher ADH enzyme activity (VEIGA *et al.*, 2010). According to Mavaieie *et al.* (2019), during storage, the expression of this enzyme is generally higher in seeds stored in cold chambers compared to seeds stored under uncontrolled conditions, especially at 6 and 8 months, contributing to the maintenance of quality during cold storage.

The expression of these enzymes in the 4 cultivars showed higher activity during the four storage periods, with seeds stored in cold chambers exhibiting higher activity of this enzyme compared to seeds stored under uncontrolled conditions. In general, seeds stored in cold chambers show elevated activity of malate dehydrogenases (MDH) until the end of storage, as also observed by Mavaieie *et al*. (2019). Similarly, Mavaieie (2019) emphasizes that the MDH enzyme has important physiological functions within the cell during the Krebs cycle, converting malate to oxaloacetate. Acting in respiration, it shows increased staining intensity or number of bands in seeds subjected to long storage periods, especially in seeds undergoing advanced deterioration.

CONCLUSIONS

Soybean seeds stored in cold storage maintain germination and vigor for indefinite periods of time, with different levels of tolerance to cold storage. Isoenzyme expressions are also maintained and can even increase when seeds are stored at low temperatures, affecting their expression according to the genotype and showing different behaviors during different storage periods.

ACKNOWLEDGMENTS

To the National Council for Scientific and Technological Development (CNPq) for the scholarship grant. To the Coordination for the Improvement of Higher Education Personnel (CAPES), Minas Gerais State Research Support Foundation (Fapemig) for the financial support, and to the University of Cauca (501100005682) for their assistance.

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