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Physiological and Biochemical Changes in Immature Seeds of *Tabebuia caraiba* During Storage

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HIGHLIGHTS

- The harvested *T. caraiba* seeds maintain high viability for 360 days of storage.
- Seeds harvested at 42 DAA have higher quality.
- The SOD and CAT activity is not related to the deterioration process of the seeds under storage.
- POX increases activity in response to the seed storage period.

Abstract: Studies about the viability and vigor of seeds during storage, especially on the characterization of enzymatic changes, may increase the harvesting efficiency of immature seeds and guarantee their viability for a prolonged period. Therefore, this study aimed to evaluate the behavior of immature seeds of *Tabebuia caraiba* during storage. For this, thirty selected mother plants at the anthesis stage located in São João do Cariri and Sumé, Paraíba, Brazil were selected. Flowers of each tree were marked, and the fruits were harvested at 35, 42, and 49 days after anthesis (DAA) and characterized according to their maturation stages. The seeds from each maturation stage and city were packed in paper bags and stored in a refrigerator (6 ± 2 °C) for 360 days. The seed quality, seedling vigor, and enzymatic activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) were evaluated at the beginning of the storage period and intervals of 90 days. The seeds of *T. caraiba* harvested at 35, 42, and 49 DAA maintained high viability for 360 days of storage. *T. caraiba* seeds stored for 360 days withstood with high vigor when harvested at 42 DAA. The CAT, SOD, and POX activity was correlated to the deterioration process of the seeds under storage conditions.

Keywords: Craibreira; Caatinga; Early harvest; Conservation; Antioxidant enzymes; Seed maturation and deterioration; Ipê amarelo.

INTRODUCTION

Popularly known as ipê - Amarelo or craibeira, the *Tabebuia caraiba* (Mart.) Bureau belongs to the Bignoniaceae family, with medium-sized trees that can reach up to 20 meters in height, and naturally occurs in the Caatinga, Cerrado, and Pantanal [1]. This species is mainly explored in the timber industry and the recovery of degraded areas [2]. This species is also explored in the chemical and pharmacological industry due to the presence of compounds with antinociceptive and antiedematogenic properties that help treat cancer and control mosquito larvae *Aedes aegypti* [3].

The fruits are dehiscent and have many winged seeds, which disperse naturally, exposing themselves to climatic conditions [4]. Therefore, monitoring the maturation process can help determine the ideal harvest point, avoid seed loss due to fruit dehiscence, and increase the number of high-quality seeds [5].

Seed maturity is characterized by a series of morphological, physical, and physiological changes that start with ovule fertilization and embryo formation and go until the seed is detached from the mother plant [6]. Early harvesting can be a viable strategy for obtaining high-quality seeds [7]. However, studies about the viability and vigor of seeds during storage are necessary to determine and adopt the best management strategies within this period.

Seeds of native species decrease their viability over time, which may last for weeks or months [8]. During storage, seeds lose quality due to deterioration, which occurs through physical, physiological, and biochemical factors. The intensity of these factors accelerates or delays the quality loss and death of the seeds [9].

Low germination is the first sign of deterioration due to storage, characterized by the loss of vigor and viability [10]. However, recent studies have also evaluated the cellular and molecular damage caused during seed deterioration caused by hydrolytic, oxidative, and peroxidative reactions [8,11,12]. Oxidative stress results from the imbalance between the production and elimination of reactive oxygen species (ROS), including superoxide anion (O_2^-), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) present in cells [13].

The seeds activate a primary defense antioxidant system, characterized by the activity of enzymes such as superoxide dismutase, catalase, and peroxidase to avoid cell damage [14]. High superoxide dismutase activities are related to increased production of H_2O_2, a key molecule for several metabolic processes within the cell, such as the production of free radicals and phenolic compounds [15]. Peroxidases eliminate H_2O_2 by oxidizing a co-substrate, and catalase converts H_2O_2 into H_2O and O_2 [13].

Storage conditions, which involve temperature, humidity, and appropriate packaging, are critical factors for efficient storage, reducing damage caused by it [8]. In tropical and subtropical areas, orthodox seeds should be stored in a dry state (6 to 8% moisture), below the environment relative humidity, with packaging materials appropriate for the environmental temperature [16].

Several studies have focused on determining the best storage conditions for several species, characterizing the events during storage. Thus, this study aimed to evaluate immature seeds of *Tabebuia caraiba* during storage to determine the proper maturation stage for storage.

MATERIAL AND METHODS

Research location

The field study was performed out in the municipalities of São João do Cariri (07º 23' 27" S, 36º 31' 58" W) and Sumé (7° 40′ 18″ S, 36° 52′ 54 ″ W), both located in the state of Paraíba, Brazil. According to the Köppen-Geiger classification, the climate of this region is classified as BSh- hot semi-arid, with summer rainfall and precipitation between 300 and 600 mm/year [17].

In each area, 30 *T. caraiba* mother tree were selected using as criteria their height (at least 10 m), stem diameter (greater than 40 cm), and phytosanitary appearance. At the anthesis, the flowers were marked with colored ribbons to monitor the development of fruits and seeds and harvested at 35, 42, and 49 days after anthesis (DAA), to characterize their maturation stages.

The harvested fruits were sent to the Seed Analysis Laboratory of the Universidade Federal da Paraíba, and visually classified based on the predominant epicarp color according to the Munsell's color chart (Figure 1) [18].

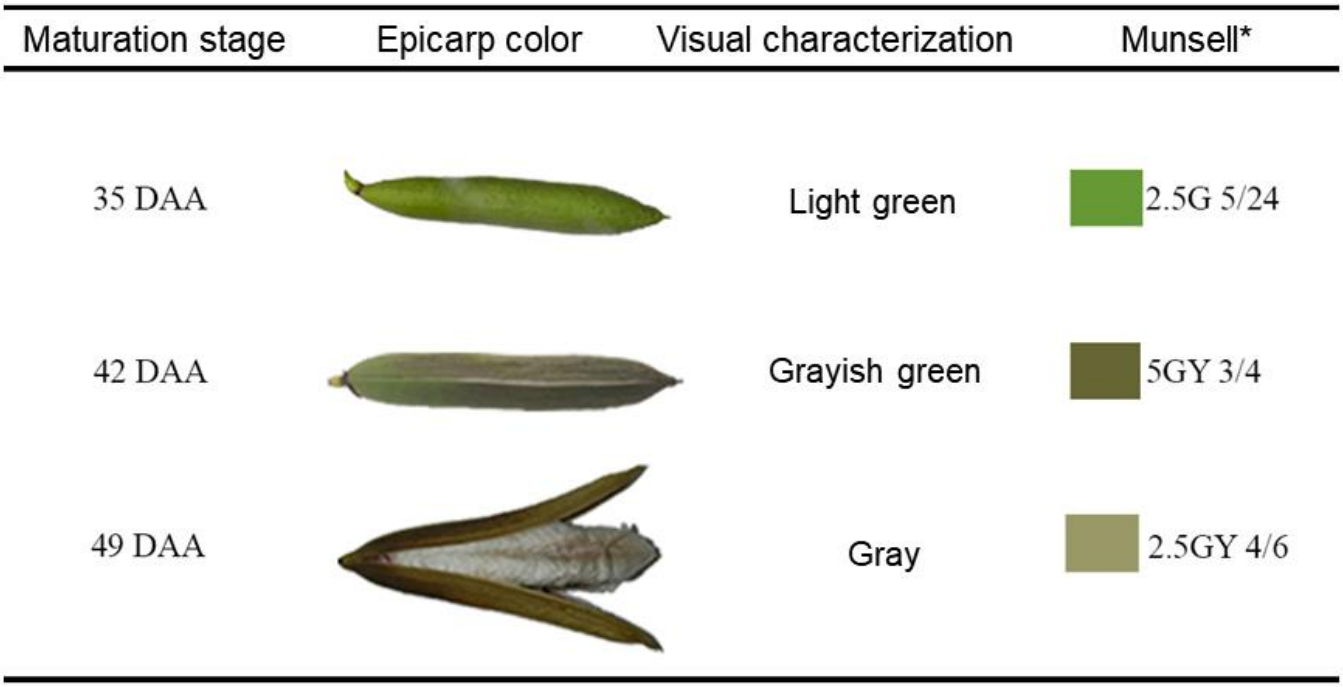

Figure 1. *Tabebuia caraiba* fruits color at different stages of maturation. * Munsell color charts for plant tissues.

The fruits remained on benches for five days under laboratory conditions (27 ± 3 ºC and 64 ± 20% RH) to facilitate the drying and release of the seeds. After the seed conditioning, the seed samples from each maturation stage and city were homogenized, packed in *Kraft* paper bags, and stored in a refrigerator (6 ± 2 °C) for 360 days (0, 90, 180, 270, and 360 days).

Physiological quality of the seeds

Seed samples with four replicates of 25 seeds were taken at the beginning of storage and in intervals of 90 days and evaluated for moisture content by the oven method at 105 ± 3 ºC for 24 hours [19]. The germination test was performed with four replicates of 25 seeds, which were placed to germinate on *germitest* paper moistened with distilled water in a quantity of 2.5 times the dry weight of the paper, and placed in a BOD (germination chamber (*Biological Oxygen Demand*) at 25 °C and 12 hours photoperiod, and the evaluations started after 21 days [20]. The first germination count started on the tenth day after sowing [20]. The germination speed index was obtained through daily counts of germinated seeds from the tenth to the twenty-first day, with subsequent calculation according to the equation proposed by [21].

The seed emergence test was performed with four replicates of 25 seeds, sown in plastic trays filled with previously sterilized washed sand, watered daily, and kept in a greenhouse (27 °C/72% RH). The evaluation started after 21 days by counting of emerged seedlings, and the result was expressed in percentage. The emergence speed index was obtained through daily counts of seedlings that emerged in the emergence test [21].

At the end of the germination test, the shoot and root length (cm) were measured with a ruler. Subsequently, the shoot and roots were separated and placed in *Kraft* paper bags to dry in a forced ventilation oven at 65 °C for 72 hours until they reached a constant weight, and then determined the shoot and root dry mass of the seedlings.

Antioxidant activity

A crude enzyme extract was prepared from the samples to determine the antioxidant activity of the enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX). For this, four replicates of 10 seeds were ground in a mortar using liquid nitrogen, and 300 mg of the processed seed samples were taken. Then, they were homogenized in 10 mL of 100 mM potassium phosphate buffer solution at pH 6.8, followed by centrifugation (4 °C at 12000 g for 30 minutes). The supernatant was collected and stored in an Eppendorf tube and frozen at -20 °C until the enzyme activity determination, according to the methodology proposed by [22].

SOD was determined by adding 50 µL of crude extract to a solution containing 13 mM methionine, 75 µL NBT, 100 nM EDTA (ethylenediamine tetraacetic acid) and 2 µM riboflavin in 3.0 mL phosphate buffer of 50 mM potassium, pH 7.8. After 5 minutes of incubation of the solution, performed by lighting in a fluorescent lamp chamber (15W at 25 °C), the end of the catalysis was determined by the interruption of light, followed by reading in a spectrophotometer at 560 nm [23]. The calculation of the specific activity of the enzyme considered the percentage of inhibition obtained between the sample volume and the protein concentration in it (µg μL^{-1}).

The CAT activity was measured in a spectrophotometer at 240 nm wavelength by monitoring the variation in the absorption of hydrogen peroxide at 80 seconds intervals. For this, 50µL of the crude enzymatic extract was added to a reaction medium with 950 µL of 50 mM potassium phosphate buffer, pH 7.0, supplemented with hydrogen peroxide at a final concentration of 12.5 mM using the molar extinction coefficient equal to 39.4 mM cm^{-1}, according to Peixoto [24]. The specific activity of CAT considered the concentration of soluble protein in the test.

POX activity was determined following the method described by Teisseire and Guy [25]. The reaction system consisted of 30 µL of diluted enzymatic extract, potassium phosphate buffer 50 mmol L^{-1}, pH 6.5, and pyrogallol (1,2,3-benzenetriol) 20 mmol L^{-1} and hydrogen peroxide (H_2O_2) 5 mmol L^{-1}, with a reaction time of 5 minutes at 25 °C. The specific activity of POX was expressed in µmol of purpurorogalin min^{-1} mg^{-1} of protein.

Experimental design and statistical analysis

The experiment was conducted using a complete randomized design with a 3 x 5 factorial scheme. Three maturation stages (35, 42, and 49 DAA) were combined with five storage periods (0, 90, 180, 270, and 360 days) for a total of 15 treatments. Each treatment was replicated four times for each city. Data was analyzed using ANOVA and Tukey's test ($p<0.05$), and a polynomial regression model was applied to examine the relationship between storage period and the variables. Correlation and PCA were used to assess the interrelationships between treatments and variables. All data analysis was performed using R software.

RESULTS

Seeds from São João do Cariri and Sumé were harvested at 35 and 42 DAA, the initial maturation stage, and their water content was measured at the beginning of storage (Figure 2). Results showed that seeds from São João do Cariri had 13.5% and 13.3% water content at 35 and 42 DAA, respectively, and decreased to 10.4% and 9.5% at 90 days of storage (Figure 2A). Similarly, seeds from Sumé (Figure 2B) had 13.0% and 14.2% water content at 35 and 42 DAA, respectively, and decreased to 10.2% and 9.5% at 90 days of storage. After 180 to 360 days of storage, water content of the seeds from both locations showed minimal variations, with mean values of 7.7% for seeds of São João do Cariri and 6.3% for Sumé.

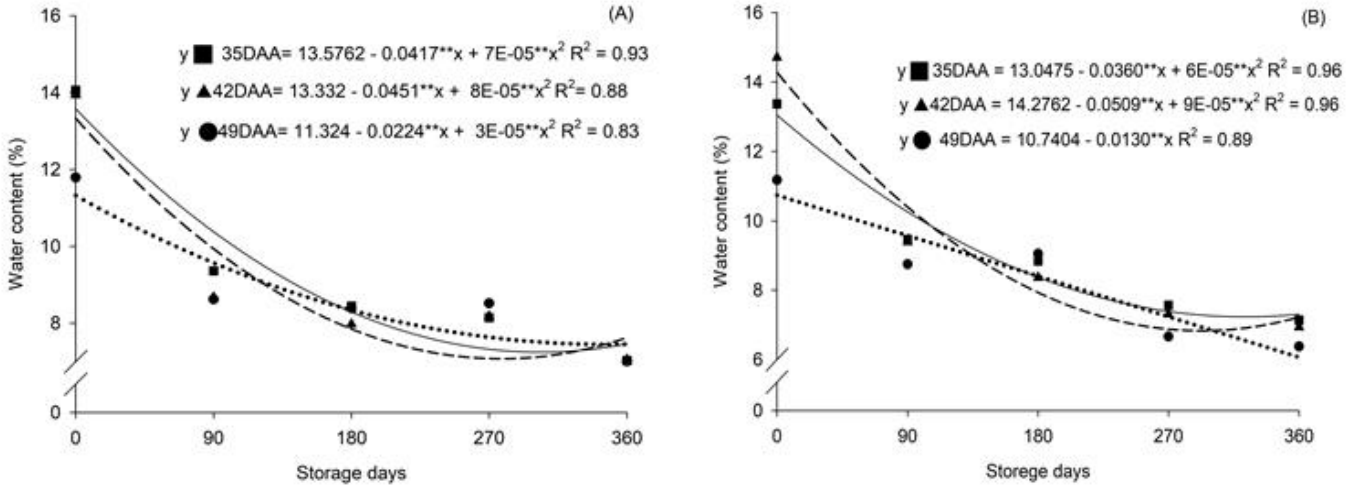

Figure 2. Water content of *Tabebuia caraiba* seeds harvested at different maturation stages in the municipalities of São João do Cariri (A) and Sumé (B) and stored for 360 days.

Seeds from São João do Cariri collected at 35 and 42 DAA showed a linear reduction in germination percentage (Figure 3A). At 360 days, 74.6% and 80.6% of seeds from each period germinated, respectively, representing a decrease of 21.1% and 14.8% compared to the beginning of storage. A quadratic polynomial model was used to analyze germination data in seeds and it best explained data of those harvested at 49

DAA, which showed the highest germination rate (97.7%) at 130 days of storage. Germination rate decreased as storage time increased, with 87% of seeds germinating at 360 days.

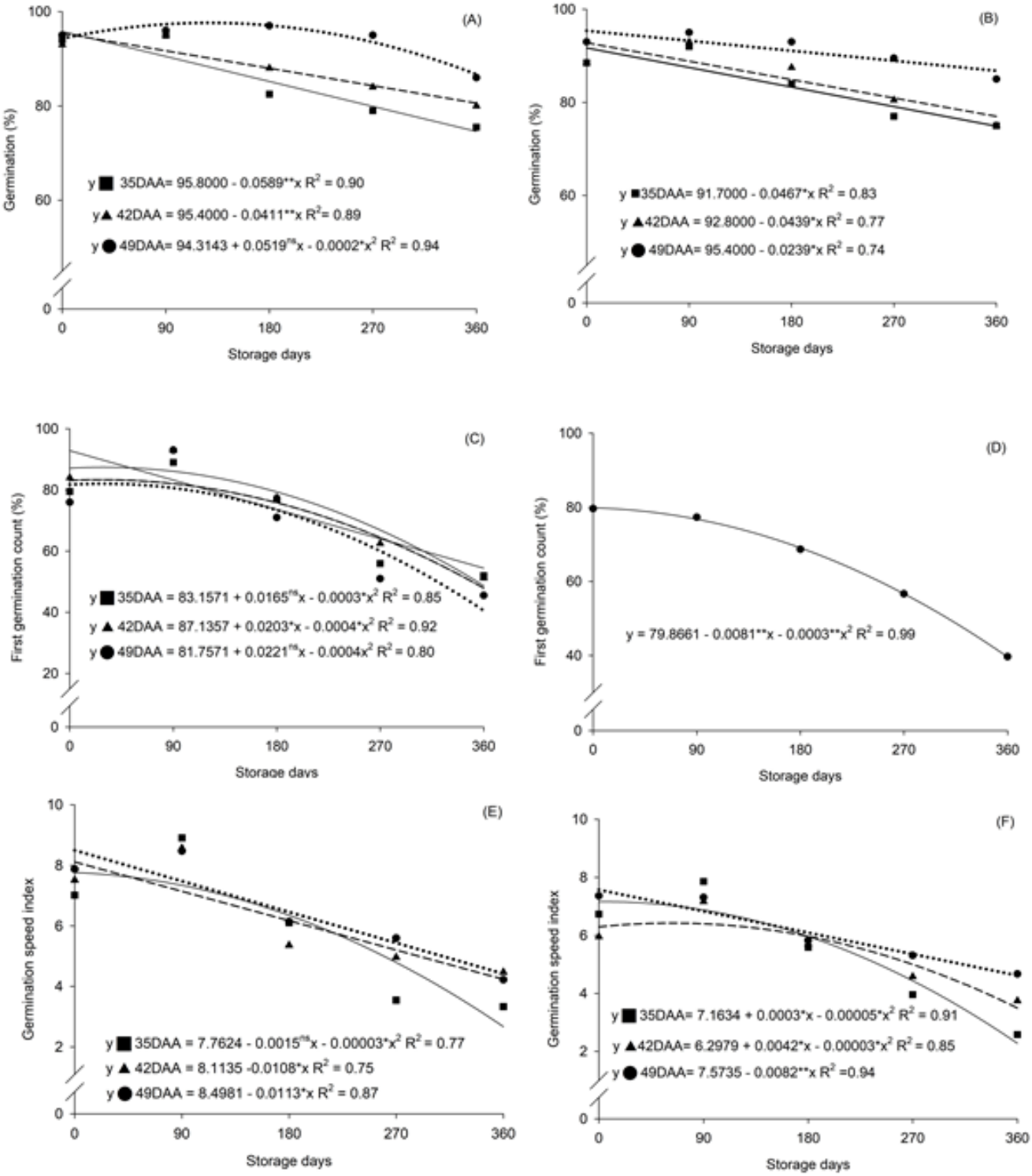

Figure 3. Germination, first count, and germination speed index of *Tabebuia caraiba* seeds harvested at different maturation stages from São João do Cariri (A, C, and E) and Sumé (B, D, and F), and stored for 360 days.

The germination decreased linearly in seeds from Sumé regardless of the maturation stages, with the highest values 91.6, 92.7, and 95.3% at 35; 42 and 49 DAA, respectively, obtained at the beginning of storage (Figure 3B). Notably, seeds harvested at 49 DAA had a lower reduction in the germination percentage, with 86.7% of seeds germinating at 360 days of storage. While those harvested at 35 and 42 DAA showed a reduction of 16.8 and 15.8%, respectively.

The seed vigor was assessed by the first germination count at different maturation stages (35, 42, and 49 DAA) and storage periods (0 to 360 days). For seeds collected in São João do Cariri (Figure 3C), the results showed that the highest seed vigor values were obtained for seeds harvested at 42 DAA, with 87.4% after 24 days of storage, which decreased thereafter. Seeds harvested at 35 and 49 DAA had the highest values of 83.3 and 80.1%, respectively, after 39 days of storage, which also decreased as storage time progressed. At the end of the storage period, the germination percentage in the first count was 50.2, 42.6, and 37.9% for the seeds harvested at 35, 42, and 49 DAA, respectively. For seeds harvested in Sumé, regardless of maturation stage, the highest germination in the first germination count (79.9%) was obtained at the beginning of storage, with a subsequent decrease, reaching 38% at 360 days of storage (Figure 3D).

For the germination speed index (GSI), the seeds collected in São João do Cariri showed the highest values at the beginning of storage, with the highest value (8.48) obtained at 49 DAA (Figure 3E). Similarly, seeds harvested at 35 and 42 DAA showed the highest values (7.76 and 8.10) at the beginning of storage, with a linear decrease as the storage period increased, reaching values of 3.33; 4.22 and 4.43 for seeds harvested at 35; 42 and 49 DAA, respectively, after 360 days of storage.

In seeds collected in Sumé, those harvested at 35 and 49 DAA showed a decrease in vigor as time progressed, with the highest values (7.16 and 7.56, respectively) observed at the beginning of storage (Figure 3F). It is noteworthy that this decrease in seeds harvested at 35 DAA after three days of storage was accentuated, resulting in the lowest value between the maturation stages, reaching values (0.79) close to zero at the end of the experiment. For those harvested at 42 DAA, the highest GSI (6.44) was observed 72 days after storage, followed by a decrease in vigor as the storage period increased.

The highest storage potential of seeds harvested at 42 DAA was also verified when vigor was evaluated through emergence and emergence speed index - GSI (Figure 4). The quadratic behavior adequately explained the seedling emergence data for all maturation stages of the seeds collected in the city of São João do Cariri, with the highest percentage of emergence (96.9%) obtained at the beginning of storage for seeds harvested at 49 DAA. Seeds harvested at 35 and 42 DAA showed the highest values (92.5 and 96.7%) at 27 and 108 days of storage, respectively. At 360 days of storage, seeds harvested at 42 DAA remained with the highest percentage of emergence (71.3%) (Figure 4A).

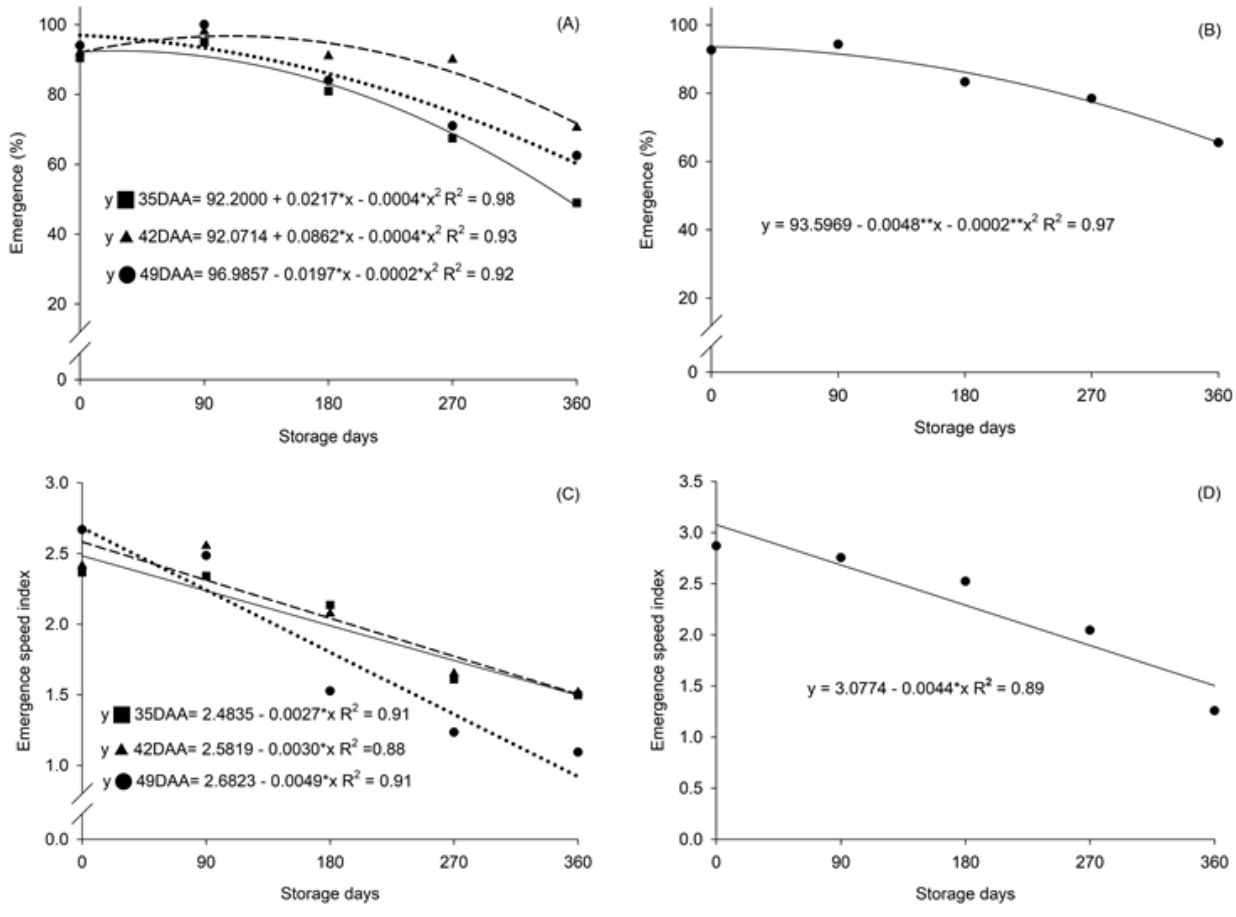

Figure 4. Emergence and emergence speed index of *Tabebuia caraiba* seedlings harvested at different

stages of maturation in the cities of São João do Cariri (A and C) and Sumé (B and D), and stored for 360 days.

In seeds from Sumé, the highest percentage of emergence (94%) was observed at the beginning of the storage period (3rd day), regardless of the maturation stage. At 360 days of storage, the emergence percentage was 67% (Figure 4B).

Seed vigor was evaluated using the GSI and the results showed that for seed samples harvested in São João do Cariri, there was a linear decrease in vigor for all maturation stages observed over the storage period. However, less mature seeds (harvested at 49 DAA) showed a more significant decline in vigor, with an index of 0.92 at 360 days of storage. In contrast, seeds harvested at 35 and 42 DAA had values of 1.51 and 1.50, respectively (Figure 4C).In seeds from Sumé, the GSI was not affected by the maturation stages, and its data were fitted to the decreasing linear model for the storage period factor, with an index of 1.49 after 360 days of storage (Figure 4D).

When seedling length was evaluated, it was possible to observe that the shoot length was more affected by the storage period. These data were fitted to the decreasing linear model, regardless of the harvest location (Figure 5A and 5B). The seeds harvested in São João do Cariri at 42 DAA originated seedlings of greater length (6.17 cm). However, at the end of the storage period (360 days), there was a decrease to 3.73 cm. The seeds harvested at 35 and 49 DAA had the highest shoot length (5.05 and 5.86 cm), which decreased to 3.83 and 3.50 cm, respectively (Figure 5A).

Seeds harvested in Sumé at 42 DAA originated seedlings of 6.03 cm, which decreased to 3.22 cm when stored for 360 days (Figure 5B). The seedlings from seeds harvested at 35 and 49 DAA showed the highest values for shoot length (5.0 and 5.71 cm) at the beginning of the storage period, followed by decreases up to 2.62 and 3.09 cm at 360 days, respectively.

Seeds from later maturation stages yielded the longest primary roots, as shown in Figure 5C and 5D. The primary root length of seeds harvested at 49 days after anthesis (DAA) in São João do Cariri reached a maximum of 21.56 cm after 62 days of storage (Figure 5C). However, seeds harvested at 42 DAA exhibited the longest primary root length at the end of the storage period, measuring 13.97 cm after 360 days. Seeds harvested at 35 DAA displayed the highest primary root length of 17.55 cm after 149 days of storage, which subsequently decreased to 13.0 cm by the end of the experiment. In Sumé, seeds harvested at 42 DAA exhibited the longest primary root length with a value of 16.93 cm after 83 days of storage. Nevertheless, seeds harvested at 35 and 49 DAA displayed the longest primary root lengths of 15.41 and 16.61 cm, respectively, after 126 and 77 days of storage. At the end of the storage period, the mean primary root length for seeds harvested at 35, 42, and 49 DAA was 12.03, 12.29, and 11.01 cm, respectively, as shown in Figure 5D.

Seedlings from seeds harvested at 49 DAA, in São João do Cariri, had the highest values for shoot and root dry mass of (1.68 and 0.44 g) at the beginning of storage (Figure 5E and 5G). However, at end of the storage period, seeds harvested at 42 DAA produced seedlings with the highest dry weight of shoot and root, measuring 1.04 and 0.22 grams, respectively. Seeds harvested at 35 DAA produced the seedlings with the lowest vigor, with maximum values of 1.22 and 0.31 g for shoot and roots, respectively.

Similarly, seeds harvested at 49 DAA in Sumé showed the highest values (1.48 and 0.47 g, respectively) for the shoot and root dry mass (Figure 5F and 5H). Seeds harvested at 42 DAA produced seedlings with a higher shoot and root dry mass at the end of the storage period, reaching up to 0.73 and 0.24 g, respectively. The shoot and root dry mass of seedlings from seeds harvested at 35 DAA had the lowest vigor, with maximum values of 1.26 and 0.38 g, respectively.

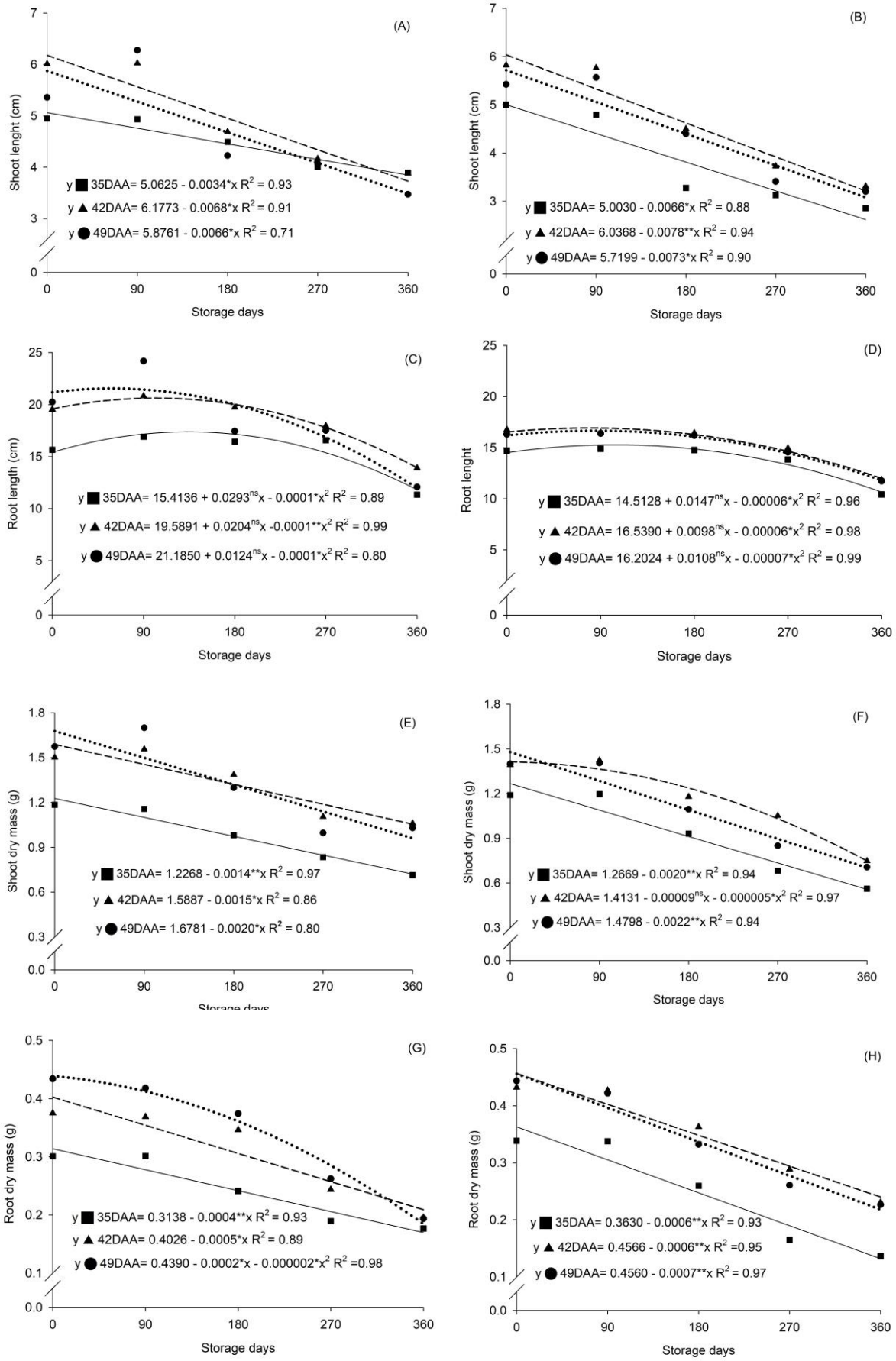

Figure 5. Shoot and root length, and shoot and root dry mass of *Tabebuia caraiba* seedlings harvested at different stages of maturation, in the cities of São João do Cariri (A, C, E, and G) and Sumé (B, D, F, and H), and stored for 360 days.

The metabolic reactions that occur during germination and storage of seeds lead to reactive oxygen species (ROS) production. These metabolic changes are observed through the activity of the enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) (Figure 6).

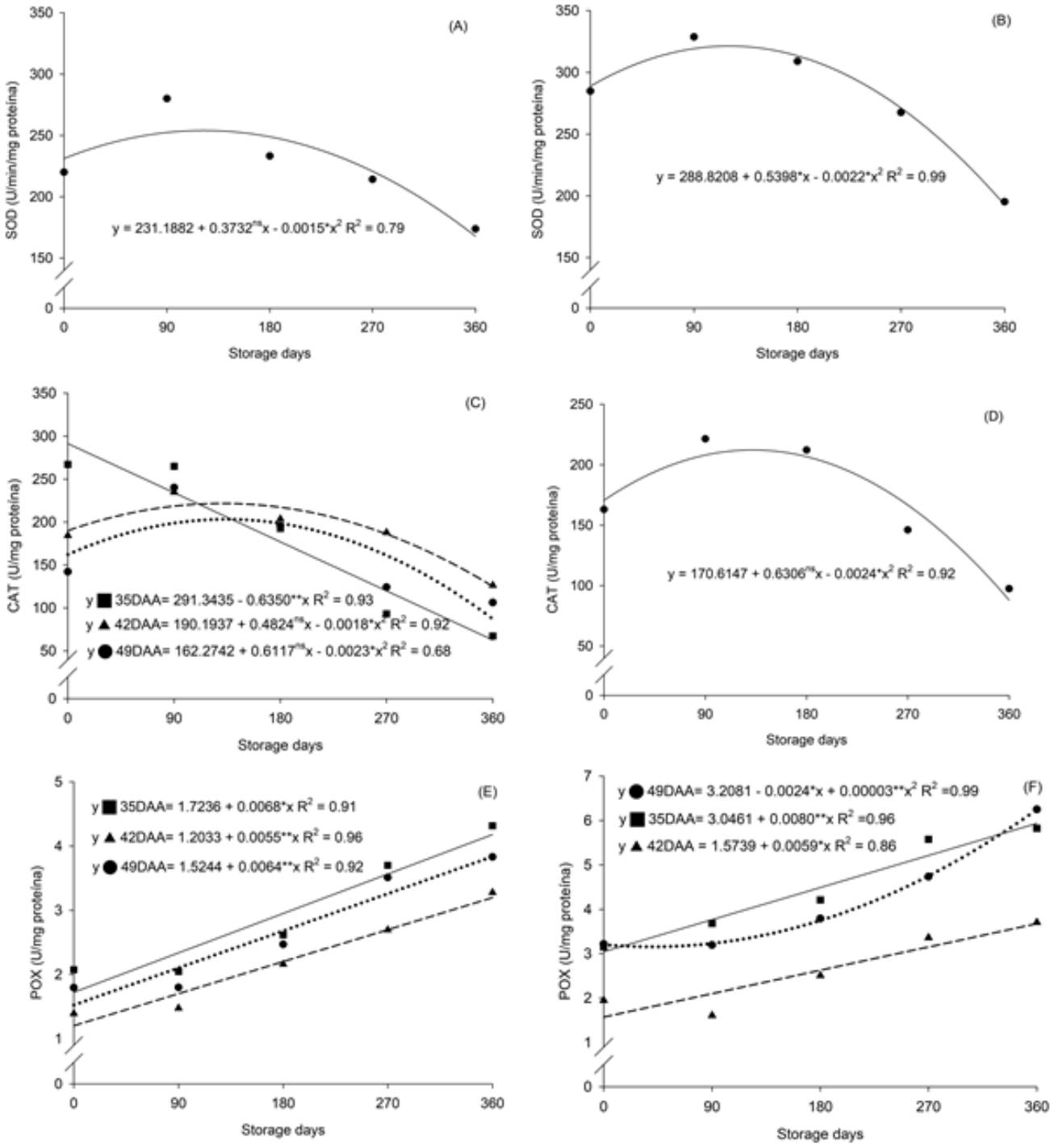

Figure 6. Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) enzyme activity in *Tabebuia caraiba* seeds harvested at different maturation stages, in the cities of São João do Cariri (A, C, and E) and Sumé (B, D, and F), and stored for up to 360 days.

The seed maturation stages had no significant effect on superoxide dismutase (SOD) activity, and the data was well-described by a quadratic model in samples collected from both cities, as observed in Figure 6A and 6B. In seeds harvested in São João do Cariri, the highest SOD values (254.4 units mg^{-1} of protein) were observed after 124 days of storage (Figure 6A), decreasing from that period onward. Similar behavior was observed in seeds from Sumé, with higher SOD activity (321.9 units mg^{-1} of protein) after 122 days of storage (Figure 6B).

The highest values for CAT activity (290.7 units mg^{-1} of protein) were obtained at 35 DAA at the beginning of storage in seeds harvested in São João do Cariri, followed by linear decreases over the storage period, reaching minimum values of 56.2 units mg^{-1} of protein at 360 days (Figure 6C). The highest CAT activity at the end of storage was observed in seeds harvested at 42 DAA, with values of 130.6 units mg^{-1} of protein. It is noteworthy that at the same stage of maturation, at 134 days of storage, the highest enzymatic activity of CAT was observed, with values of 222.5 units mg^{-1} of protein. Seeds harvested at 49 DAA had the highest CAT values at 133 days, followed by a decrease over the storage period, reaching 84.4 units mg^{-1} of protein.

The CAT activity was also not influenced by the maturation stages in seeds harvested in Sumé, only by the storage periods, reaching a maximum value of 212.03 units mg^{-1} of protein at 131 days, which decreased from that point onward (Figure 6D).

The POX activity increased as the storage period increased (Figure 6E and 6F). The lower POX activity was observed in seeds harvested at 42 DAA after 360 days of storage in seeds harvested in São João do Cariri, with 3.18 units mg^{-1} of protein, followed by those harvested at 49 and 35 DAA, with 3.82 and 4.17 mg $units^{-1}$ protein, respectively (Figure 6E). In seeds from Sumé, the highest activity (6.23 units mg^{-1} of protein) of POX occurred in seeds harvested at 49 DAA at the end of the storage period. The highest activity (5.93 units mg^{-1} of protein) was also recorded in seeds harvested at 42 DAA at the end of the storage period. Differently, seeds harvested at 35 DAA showed the lowest POX activity throughout the evaluated period, maintaining the activity of 3.69 units mg^{-1} of protein at the end of the storage period (Figure 6F).

Pearson's correlation analysis shows a strong association between the variables germination, initial growth, and enzymatic activity of *T. caraiba* seedlings from São João do Cariri (Figure 7A). It can be observed that the GER had a high correlation with the GSI (0.73). However, it is possible the highest positive correlations were obtained between the variables EME and ESI (0.87) and SDM and RDM (0.89). Regarding the negative correlations, it can be highlighted that the enzymatic activity of POX in relation to the EME and ESI variables, had the highest negative correlations with -0.86 and -0.81, respectively.

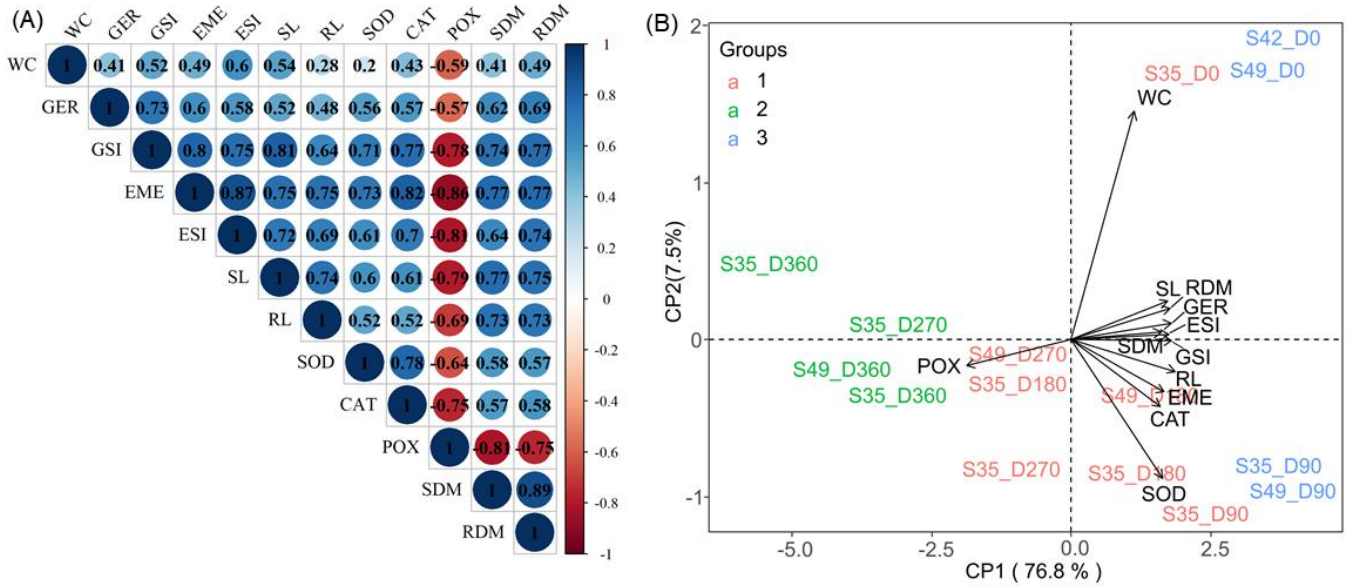

Figure 7. The Pearson correlation (A) and Principal Component Analysis (B) between water content (WC), germination (GER), first germination count (FGC), germination speed index (GSI), mean germination time (MGT), root length (RL), shoot length (SHL), seedling length (SEL), emergence (EME), emergence speed index (ESI), root dry mass (RDM), seedling dry mass (SDM), Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) in *Tabebuia caraiba* seeds harvested at different maturation stages, in São João do Cariri and stored for up to 360 days.

The principal component analysis for the interaction between treatments and variables for seeds harvested in São João do Cariri revealed a variability of 84.3%, with component 1 (CP1) showing variation of 76.5% , while CP2 had a variation of 7.5% (Figure 7B). It was possible to observe that there was the formation of three groups depending on the relationship between the treatments and analyzed variables, with group 1 consisting of seeds harvested at the 35 DAA stage in the storage periods of 0, 90, 180, 270 days and the of 49 DAA with 80 and 270 days of storage, correlating with the variables WC, GSI, RL, EME, POX, CAT and SOD. The second group was formed by the seeds of 35 DAA at 270 and 360 days and those harvested at 49 DAA and in the storage period and 360 days, which had a strong relationship with the enzymatic activity of POX. Group 3 was composed of seeds with 35DAA at 90 days of storage, those with 42 DAA at 0 days and 49DAA at 0 and 90 days of storage, especially associated with WC and CAT variables.

The Person's correlation indicates that GER had the highest positive relationship (0.76) with GSI in seeds harvested in Sumé. However, the greatest positive interactions occurred in the variables SDM and RDM (0.95), SL and SDM (0.91) and SL and RDM (0.88), indicating a high relationship in the initial growth of *T. caraiba* seedlings (Figure 8A). As for the negative correlation, the enzymatic activity of POX with the SDM and RDM variables were stood out with values of 0.81 and 0.79, respectively.

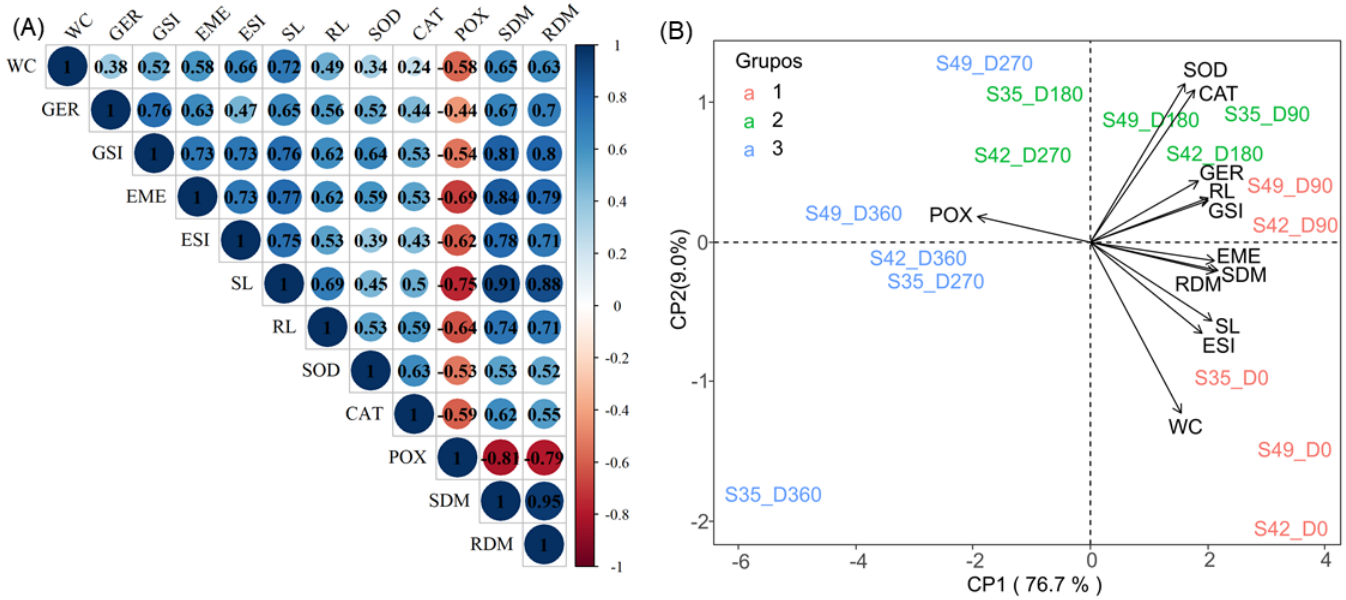

Figure 8. The Pearson correlation (A) and Principal Component Analysis (B) between germination (GER), first germination count (FGC), germination speed index (GSI), mean germination time (MGT), root length (RL), shoot length (SHL), seedling length (SEL), emergence (EME), emergence speed index (ESI), root dry mass (RDM), seedling dry mass (SDM), Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) in *Tabebuia caraiba* seeds harvested at different maturation stages, in Sumé and stored for up to 360 days.

The principal component analysis of the correlation between the treatments and the seed variables harvested in Sumé revealed a high degree of variability in the first two components, accounting for 85.7%. The first component, CP1, accounted for 76.7% of the variability, while the second component, CP2, accounted for 9.0% (Figure 8B). Additionally, the analysis revealed the formation of three distinct groups based on the correlation between the treatments and the analyzed variables. The first group was formed by seeds harvested at 35 DAA and 0 days of storage, those harvested at 42 and 49 DAA stored between 0 and 90 days, and associated with the variables WC, EME, ESI, SL, RDM and SDM.

The second group was composed of seeds harvested at 35 DAA and stored for 90 and 180 days, those harvested at 42 DAA and stored for 180 and 270 days, and those harvested at 49 DAA after 180 days, being mainly corelated to the GER and GSI and the enzymatic activity of CAT, POX and SOD (Figure 8B). Group 3, on the other hand, was formed by seeds harvested at 35 and 49 DAA and stored for 270 and 360 days and those harvested at 42 DAA and stored for 270 days, associated with the enzymatic activity of POX.

DISCUSSION

During the storage period, the moisture content of the seeds remained within acceptable limits for preserving seed quality. Despite a decrease in moisture over time, the seeds remained viable for germination under favorable conditions. Moisture management is crucial for seed conservation, as it impacts both the chemical composition and metabolic activity of the seeds [27].

It was possible to observe that the further away from the moment of fruit opening and seed dispersal, the higher was the seed moisture content. This behavior is due to their maturation process, given that high moisture content in the early stages of development are required for the metabolic processes to occur efficiently, with the proper formation of the different embryo tissues [6].

Although it may change between species, low moisture content aids in the efficient maintenance of seed storage of forest species [28]. Other species of *Tabebuia* were studied for storage, and moisture content of up to 15% were found to be adequate for storage at low temperatures [29].

Storage in *Kraft* paper bags promoted a decrease in the moisture content of the stored seeds, with a hygroscopic equilibrium obtained after 180 days of storage. This reduction was verified for all maturation

stages, even at different levels, considering that drier seeds lost less moisture at the beginning of storage. The hygroscopic balance depends on the environment and the seed genetic traits [30].

This behavior is essential, given that a lower metabolism is the primary strategy for conserving seeds during storage, which occurs by reducing moisture content and its maintenance at low temperatures [31]. The moisture removal allows the seed cytoplasm to reach a highly viscous state, in which active metabolism is practically impossible, being hampered by low molecular mobility [32].

The quality and viability maintenance of *T. caraiba* seeds withstood throughout storage and was linked to their maturation stage, given that at the beginning of storage, the germination percentage was above 90% in all maturation stages. The high viability of mature seeds during storage may reflect the drying process inside the fruit, occurring under natural and ideal conditions for the species [27].

Still, it is important to mention that seeds harvested at an immature stage (35 DAA) remained with germination above 80% until approximately 270 days of storage, which demonstrates that the storage conditions helped maintain the quality of the seeds. Seeds of *Jatropha curcas* at different stages of maturation only showed differences in their viability after 270 days of storage [33]. In contrast, seeds of *Tabebuia serratifolia* remained with high viability at 360 days of storage in refrigerator conditions [34]. *Tabebuia roseoalba* seeds also remained with high germination percentage after 360 days of storage, decreasing to 52% after 24 months [11].

The vigor assessed by the first count and germination speed index reduced at higher rates during storage, and this is associated with seed deterioration that occurs even under favorable storage conditions. Similar behavior was observed in the germination speed index of *T. serratifolia* seeds, with a reduction in vigor at 360 days, regardless of the storage conditions [34].

It is worth mentioning that seeds harvested at 35 DAA had lower GSI because they were not fully mature. The more complete the maturation process, the greater the seed vigor [33]. The maturation process must occur without interruption so that the seed can form all of its biochemical apparatus efficiently, considering its reserve tissues and its physiology [6].

As observed in germination, a reduction in seed vigor is observed through the percentage of emergence and GSI in response to the storage period. However, it is noteworthy that the maturation stage influences the maintenance of seed quality, and those harvested at 42 DAA showed greater vigor since they withstood with emergence above 70% after 360 days of storage.

Guedes [35] evaluated the quality of *T. caraiba* seeds stored in *Kraft* paper bags and a refrigerator and observed high reductions in the emergence, with values of 57% at 150 days, followed by a linear reduction in the GSI. These variables are of agricultural and ecological importance and are essential to identify seed properties that determine the potential for rapid and uniform emergence and the development of normal seedlings under a wide range of conditions [36].

Seedling growth indicates proximity between the different maturation stages at the end of storage, indicating that this behavior is more influenced by time, since seedling growth is reduced at the end of storage. Seeds of *J. curcas* harvested from dried fruits showed seedling length reduced during storage, attributed to enzymatic changes due to the high oil content of the seeds [27]. Hence, the high amount of oil present in seeds of species of the *Tabebuia* genus might be the cause of these changes. A linear decrease in the shoot length of seedlings of *T. roseoalba* stored seeds was also observed [11].

Seeds harvested in a more advanced maturation stage (42 and 49 DAA) tend to produce more vigorous seedlings. Thus, *T. caraiba* seedlings originated from seeds of these stages are more able to survive in the field and absorb a greater amount and diversity of nutrients from the soil [37].

Similarly, the shoot and root dry mass from seedlings of seeds harvested at 42 and 49 DAA were the most vigorous. The establishment of seedlings and a higher growth rate are related to a higher germination speed index, which favors the formation of the plant photosynthetic apparatus [38, 39]. Therefore, *T. caraiba* seeds harvested closer to the fruit dehiscence originate seedlings with greater chances of success in the field even after being stored for 360 days.

The enzyme antioxidant activity indicates the relationship between storage and seed deterioration. ROS act as signals for the antioxidant system during storage to prevent the accumulation of these substances at high levels within the cell, neutralizing them and preventing toxicity [13]. Therefore, the higher activity of SOD at 120 days of storage, on average, indicates higher quality in this period, given that the action of this enzyme is efficient in the oxidation and reduction of the superoxide anion to produce H_2O_2 and O_2 [37].

However, it is noteworthy that in this study, even with a reduction in the SOD and CAT activities, the seeds of *T. caraiba* remained viable. Seeds respond differently to ROS within an “oxidative frame,” which, depending on the concentration, may damage cells or even not be enough to activate the relevant metabolic pathways for the germination process [40].

In a study developed by Abbade and Takaki [11] with the storage of *T. roseoalba* seeds, the CAT enzyme

activity reduced as the storage period increased. Similar results were obtained in *Pterogyne nitens* seeds [41]. Borges [30] also observed reductions in SOD and CAT activity in *Melanoxylon brauna* Schott seeds as the storage period increased.

Compared to CAT, the POX enzyme is widely distributed in cell compartments, associated with cell walls and membranes, organelles, vacuoles, and cytosol, allowing greater mobility where its action is needed [42]. This enzyme acts as a defense mechanism and prevents quality loss, mainly due to ROS oxidation, such as phenols and some inorganic ions [33]. Using H_2O_2 as a receptor, POX plays a critical role in seed metabolism, enhancing defense mechanisms avoiding quality loss [43].

According to Pearson's correlation and the analysis of principal components, it is possible to highlight that there was a direct relationship between the enzymatic activity of POX and the process of deterioration of *T. caraiba* seeds, indicating that regardless of the stage of maturation of the seeds, storage contributes to the loss of seed quality, which can be represented by the increase in POX activity. During the deterioration process, a series of biochemical and physiological changes occur in the seeds, which can lead to an increase in the enzymatic activity of POX, and these changes can be associated with tests of germination and vigor of seeds and seedlings, used as markers of the process of seed deterioration [44].

CONCLUSION

Seed of *Tabebuia caraiba* obtained from light green (35 DAA), gray-green (42 DAA), and gray (49 DAA) color fruits remained viable after 360 days of storage when packaged in *Kraft* paper bags under low temperature and relative humidity conditions. The seeds harvested from gray-green fruits (42 DAA) exhibited high vigor after 360 days of storage. Additionally, the activity of superoxide dismutase (SOD) and catalase (CAT) were found to be correlated with the deterioration of *T. caraiba* seeds under storage conditions. The increase in peroxidase (POX) enzymatic activity can be used as an indicator of deterioration in *T. caraiba* seeds during storage.

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