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Oxidative metabolism and physiological quality of artificially aged *Arachis hypogaea* L. seeds[1]

Metabolismo oxidativo e qualidade fisiológica em sementes de *Arachis hypogaea* L., envelhecidas artificialmente

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ABSTRACT - Peanut (*Arachis hypogaea* L.) is an oleaginous plant that is well-adapted to the climatic conditions in the northeast of Brazil. However, there is little information on the existing cultivars in the region with regards to their behavior under stress conditions and the biochemical changes of the seeds. The objective of this study was to investigate the physiological and biochemical changes in aged peanut seeds. The experiments were conducted at the Seed Analysis Laboratories (LAS) and Biotechnology of CENLAG, Federal University of Agreste of Pernambuco (UFAPE). Seeds of two cultivars, IAC Tatu ST and Caiana, were artificially aged at 42 °C and 100% relative humidity for 96 h, and removed every 24 h for evaluation. Physiological potential was evaluated using germination, vigor test, and enzymatic modifications as the parameters. An increase in the accelerated aging period resulted in physiological and biochemical changes in the seeds of both cultivars. The activity of the enzymes in the aged peanut seeds was specific to the cultivar used, and the seeds of the cultivar Caiana had less vigor and decreased activity of the enzyme catalase.

Key words: Deterioration. Enzyme. Germinate. Oilseeds.

RESUMO - O amendoim (*Arachis hypogaea* L.) é uma planta oleaginosa que se destaca no Nordeste Brasileiro devido a sua adaptação as condições climáticas. No entanto, existem escassas informações sobre as características das cultivares existentes na região quanto ao seu comportamento em situações de estresses e as mudanças bioquímicas das sementes. O objetivo do trabalho foi estudar as alterações fisiológicas e bioquímicas em sementes envelhecidas de amendoim. Os experimentos foram conduzidos nos Laboratórios de Análise de Sementes (LAS) e Biotecnologia do CENLAG da Universidade Federal do Agreste de Pernambuco – (UFAPE). Foram utilizadas sementes de duas cultivares: IAC Tatu ST e Caiana, envelhecidas artificialmente a 42 °C e 100% de umidade relativa durante 96 horas, sendo retiradas amostras a cada 24 horas para avaliações fisiológicas e bioquímicas. A qualidade fisiológica e o metabolismo oxidativo foram avaliados por meio dos testes de germinação e vigor, e modificações enzimáticas (CAT e APX), respectivamente. O aumento do período de envelhecimento acelerado resultou em alterações fisiológicas e bioquímicas ocorridas nas sementes das duas cultivares, refletindo o efeito da deterioração causado pelo envelhecimento acelerado nas sementes de amendoim. A atividade das enzimas nas sementes envelhecidas de amendoim foi específica em função das cultivares, sendo que a atividade da enzima catalase foi reduzida nas sementes da cultivar Caiana, com menor vigor.

Palavras-chave: Deterioração. Enzima. Germinação. Oleaginosas.

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INTRODUCTION

Brazil is among the world's leading peanut producers (*Arachis hypogae* L.). This crop has attracted considerable economic interest, because its commercial exploitation presents a good opportunity for irrigated agriculture and has a great potential for expansion. The total production of peanuts in Brazil is estimated at more than 691 thousand tons, with an average productivity of 2.638 kg. ha^{-1} (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2021).

Research on the selection of more productive cultivars under the climatic conditions in the northeast of Brazil is of fundamental importance and has been the subject of several studies (BARBOSA *et al*., 2019; SILVA *et al*., 2018).

The Tatu cultivar is most commonly cultivated in São Paulo State and can grow successfully in several regions. It has an early cycle of approximately 90 to 100 days from planting to harvest. It belongs to the commercial group of Valencia and stands out in the market for occupying 10%–15% of the plantation area. In contrast, the cultivar Caiana has a cycle of less than 90 days and is adapted to the semi-arid northeast (SANTOS; GODOY; FÁVERO, 2013).

Peanuts, as well as other economically important crops, are propagated by seeds, and therefore, the physiological potential (germination and vigor) of seeds is an important consideration. Seed vigor provides precise information on differences in the physiological potential between seed lots, especially those with similar germination percentages. It enables the identification of seed lots with a higher probability of successful germination and growth after sowing and during storage (MARCOS FILHO, 2015).

The performance of seeds during storage can be monitored using vigor tests. The accelerated aging test evaluates the degree of tolerance in seeds exposed to adverse temperature conditions (between 40 °C and 45 °C) and relative air humidity (close to 100%), which increase the intensity and rate of deterioration (DUCATTI; SILVA; COIMBRA, 2016).

The effects of accelerated aging may be diverse, affecting viability and vigor due to the increase in metabolic activity. This leads to a higher consumption of reserves and results in lower seedling emergence speed. The susceptibility of seeds to stress during artificial aging can be correlated to the biochemical metabolism. Canola seeds, when subjected to accelerated aging, had lower levels of N, P and crude protein (SEYYEDI; AFSHARI; DANESHMANDI, 2018). As enzymes are metabolically active proteins, formed by N, a decline in their activity is expected when seeds are exposed to conditions of high temperature and relative humidity.

Enzymes are biocatalysts that are excellent indicators of seed deterioration because they accelerate the rate of biochemical reactions; however, they themselves do not change with the process. Enzymes are also known as life agents; this is an important term, since they determine almost all life processes in living organisms (TAIZ; ZEIGER, 2017).

Plants have an efficient antioxidant mechanism to reduce the effects of oxidative stress, and enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) are activated to remove free radicals from the cells (DAS; ROYCHOUDHURY, 2014).

During the germination of barley seeds previously exposed to the conditions of the accelerated aging test, there were increases in the activities of CAT and APX, characterizing a stress tolerance mechanism (MEI; SONG, 2010). The enzymatic system aims to prevent and reduce the irreversible damage caused by toxic products that are produced due to oxidative stress induced due to the actions of free radicals (CARNEIRO *et al*., 2011).

The objective of the present study was to investigate changes in the vigor of artificially aged seeds, as well as the relationship between enzymes (CAT and APX) and the accelerated aging of *A. hipogeae* L. seeds at different periods.

MATERIAL AND METHODS

The experiments were conducted at the Laboratory of Seed Analysis (LAS) and the Laboratory of Biotechnology of CENLAG, Federal University of Agreste of Pernambuco - UFAPE. Peanut seeds were harvested at Fazenda Sobradinho located at 09°45'09"S and 36°39'40"W at an average altitude of 264 m in the municipality of Arapiraca-AL in 2017. Two cultivars, Tatu and Caiana, were placed in nylon bags and brought back to the LAS for further experiments.

Previously selected peanut seed samples were distributed and incubated in gearbox-type boxes with aluminum screens in an accelerated aging chamber at 42 °C and 100% relative humidity for 24, 48, 72, and 96 h. The control group (0 h) contained seeds that were not incubated. Vigor tests were performed by collecting seed samples after 0, 24, 48, 72, and 96 h of aging, as previously described (DELOUCHE; BASKIN, 1973). Before and at each accelerated aging period, seed moisture content was determined using the greenhouse method à 105 ± 3 °C por 24 h (BRASIL, 2009).

Germination tests were performed with 4 replicates of 50 seeds per treatment. Seeds were germinated on the substrate between sand in plastic trays perforated at the bottom (dimensions: 29 × 22 × 05 cm) kept in a germinator at 25 °C for 10 days. These results were expressed as the percentage of germination, considering the number of seedlings that were classified as normal. The first germination count evaluations were conducted along with the germination test, including the registration of normal seedling percentages on the fifth day after the experiment was installed, as previously described (BRASIL, 2009).

The germination rate index was calculated as described by Maguire (1962) at the same time as the germination test, considering the daily counts of the normal seedlings from the fifth to the tenth day after sowing.

The length and dry mass of shoots and roots were measured using a millimeter ruler at the end of the germination test (50 seedlings per replicate). The results are expressed in centimeters and milligrams per second, respectively, as previously described (NAKAGAWA, 1999).

The activities of APX (EC 1.11.1.11) and CAT (EC 1.11.1.6) were also evaluated in addition to the growth characteristics. APX activity was determined according to the method described by Nakano and Asada (1981). This method involves the reduction of hydrogen peroxide in the medium of the crude extract by APX, with the reduction of the supplied ascorbic acid. The experiment was started at the time of H_2O_2 addition to the reaction medium, and the decreasing reading was assessed at 0–120 s intervals (measured every 30 s), by measuring the absorbance at 290 nm using a spectrophotometer. The activities of the total extract, expressed in UA/g MS/min, were determined by calculating the amount of extract that reduces the absorbance reading by 1 AU. CAT activity was determined in the control seed extracts by adding 100 μL of the enzyme extract to 2.9 mL of a solution containing 12.5 mL H_2O_2 and 50 mM potassium phosphate buffer (pH 7.0), and the absorbance at 240 nm was determined at 30 °C (HAVIR; MCHALE, 1987). Enzyme activity was calculated using a molar extinction coefficient of 36/M·cm.

The experimental design was completely randomized with four replicates. Analysis of variance (ANOVA) with subsequent polynomial regression was performed on the data associated with the measured variables.

RESULTS AND DISCUSSION

The initial water content of the seeds of the Tatu and Caiana cultivars varied from 5.99% a 6.49% (Table 1), the difference being less than 0.5% between cultivars, showing no statistical differences, suggesting that this parameter was in the range indicated for the test. Differences of 1 to 2% in water content between samples are not compromising. However, marked differences can cause changes in seed wetting speed during aging. After accelerated aging, the final water content varied by 0.2 percentage points, which according to Marcos Filho (1999) indicates a good uniformity of results, being essential for standardizing evaluations and obtaining consistent results.

Table 1 - Initial water content (%) of seeds of two peanut cultivars (*Arachis hypogaea* L.) and after five periods of accelerated aging à 42 °C

Initial water content and after accelerated aging		
Period (Hours)	TATU	CAIANA
0	5.99 a	6.49 a
24	12.40 a	14.61 a
48	12.87 a	16.32 a
72	12.58 a	16.25 a

* Means followed by the same lowercase letter on the line do not differ statistically from each other at 5% probability by the test Tukey

The initial germination percentage was 80% and 89% for the seeds of the two cultivars Caiana and Tatu, respectively, and this did not differ statistically (Figure 1A). The germination results are above 80%, therefore in accordance with the minimum standards for commercialization required by the Normative Instruction 45/2013, do Ministério da Agricultura Pecuária e Abastecimento (BRASIL, 2013). There was a linear decrease in germination and vigor (Figures 1A, B and C) after accelerated aging, which was expected, since the seeds were exposed to stress.

Germination was negatively affected, since the increase in temperature and prolonged exposure to thermal stress causes disorders seed metabolism disorders (SANTOS; ATAIDE; PIRES, 2019).

Seeds of the cultivar Tatu showed the greatest vigor when compared to those of the cultivar Caiana at 0, 24, and 48 h (Figure 1 B) during the first germination count, with a decrease of 32% and 86%, respectively, compared to the initial aging period. The vigor of the seeds of the two cultivars differed for up to 48 h of aging. The first germination count test is very useful for determining vigor, as it has a high correlation with seedling emergence in the field, making it possible to predict the performance of these seeds before seeding.

The germination rate index (Figure 1 C) indicated that the seeds of the cultivar Tatu had a high physiological potential (72.8) at 0 h (control), while those of the cultivar Caiana showed a lower germination rate (60.4). Seeds with a higher germination rate index have higher performance and greater resistance to abiotic and biotic stresses, which consequently contributes to the growth and development of seedlings (JUVINO *et al*., 2014).

The dry mass of roots, shoots, and cotyledons (Figure 2) were adjusted to the linear regression model, and a reduction in the dry mass of all parts of the seedlings was observed during the accelerated aging periods (24, 48, 72, and 96 h).

Figure 1 - Germination (A), first count (B), germination rate index (C) in the seeds of the two peanut cultivars as a function of periods of accelerated aging

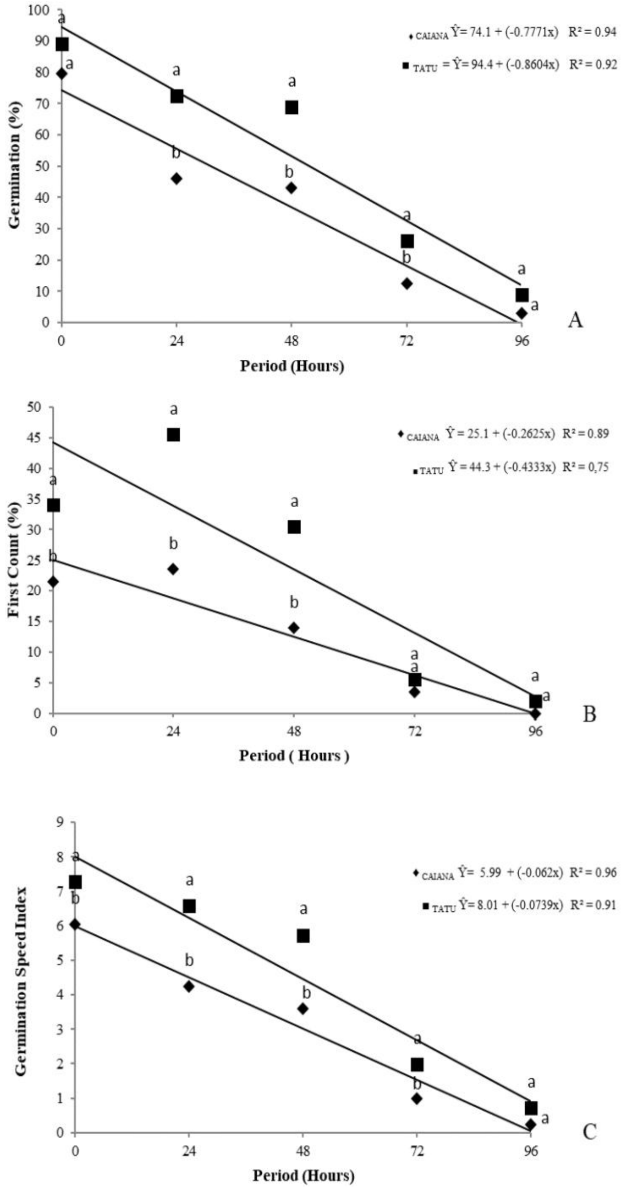
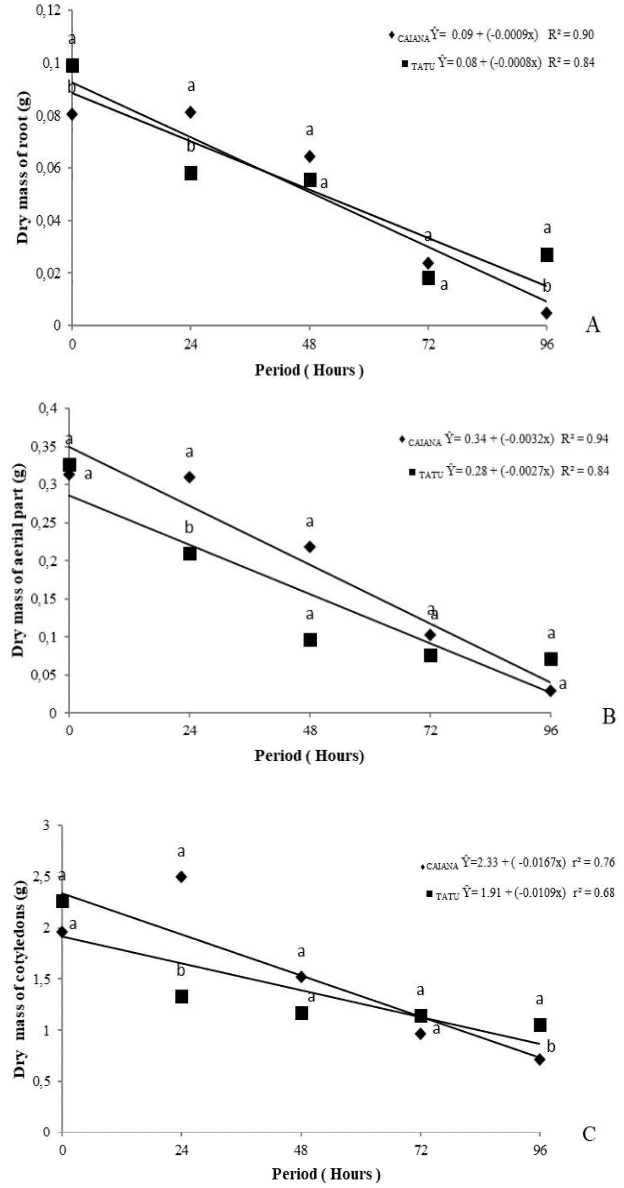


Figure 2 - Dry mass of root (A), aerial part (B) and cotyledons (C) of the seeds of the two peanut cultivars after periods of accelerated aging.)



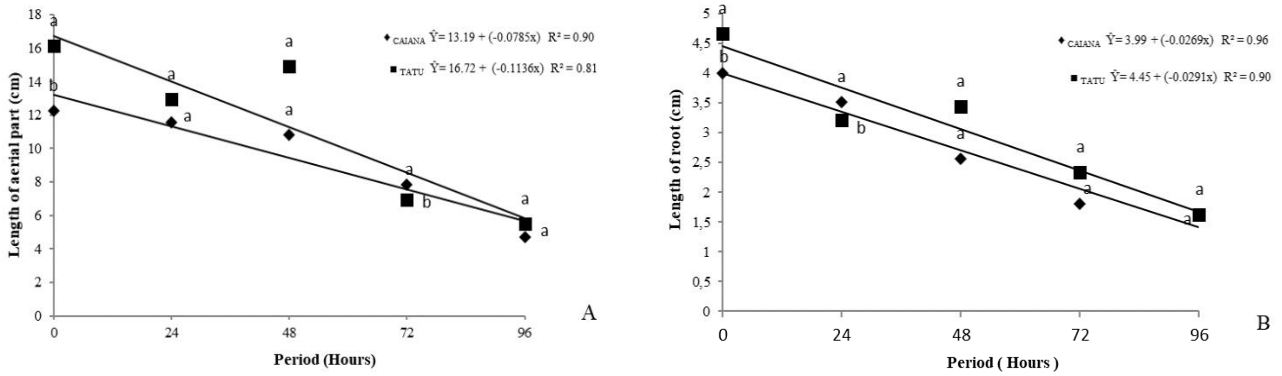
Seeds of the cultivar Tatu produced seedlings with higher dry mass values of the root; however, this did not differ from the seedlings derived from the cultivar Caiana seeds (Figure 2).

The evaluation of peanut seedling shoot and root lengths (Figure 3) revealed that the length of the artificial aging periods decreased during the increased exposure period in both cultivars. However, the cultivar Caiana showed higher sensitivity to stress, with a marked decrease in shoot and root length compared to that of the Tatu cultivar. This could be explained by the lower vigor of the Caiana cultivar.

The growth results, i.e., shoot and root lengths of the seedlings produced from these seed lots did not reveal significant differences, which is justified by the fact that batches of seeds with high germination are always of high vigor.

Vigor tests are efficient in identifying less advanced stages of seed deterioration, identifying reductions in plant growth and seedling establishment, and facilitating decision making in the choice of more vigorous seeds (WENDT *et al*., 2017).

Figure 3 - Length of aerial part (A) and root (B) of seedlings produced from the peanut seeds after periods of accelerated aging



However, physiological changes are observed at the time of aging, and the biochemical metabolism of the seed also undergoes modifications. CAT and APX play important roles in defense against reactive oxygen species (ROS) and help to protect the cell from oxidative stress. Thus, enzymatic activity is associated with defense against oxidative stress in tissues (LIMMONGKON *et al*., 2018).

Figure 4 shows an increase in CAT activity in the seeds of cultivar Caiana in the first 24 h of aging, gradually decreasing up to 96 h of aging due to seed deterioration. Water content increases respiratory activity, and therefore, more heat and water are released into the environment, leading to protein denaturation and lipid oxidation. It also causes variation in the degree of humidity during storage in environments without air humidity control, which impairs the conservation of their viability and vigor (MARCOS FILHO, 2015).

Silva *et al*. (2020) showed that seeds of *Dipteryx alata* Vog. showed decreased activity of catalase and superoxide dismutase due to accelerated aging, and therefore, such biochemical changes could be used as markers of seed deterioration. In *Dendrocalamus sikkimensis* Gamble seeds, the analyzes indicated decreased sugar, starch, protein content, decreased acid, alkaline and peroxidase phosphatase activity and increased amylase whit increasing seed aging, suggesting that the quick decline in the viability and germination it's related to biochemical changes (LAKSHMI; JIJEESH; SEETHALAKSHMI, 2021).

The metabolic changes due to stress were less prominent in the seeds of the cultivar Tatu. Reduced enzymatic activity was observed after 24 and 48 h of aging for the cultivars Caiana and Tatu, respectively, indicating that seeds of the cultivar Tatu can withstand greater periods of stress and have greater vigor. The increased exposure of seeds to accelerated aging leads to the accumulation of ROS, which cause lipid peroxidation, and therefore, lower membrane integrity and selectivity. This allows water to enter the cells faster, leading to reduced seed vigor (PENG *et al*., 2011).

Figure 4 - Activity of the enzyme Catalase (CAT) in the peanut seeds after periods of accelerated aging

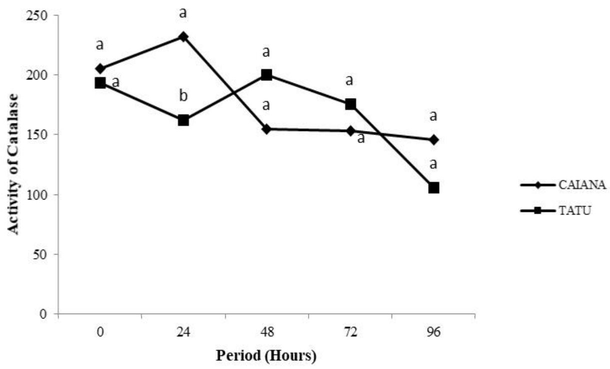
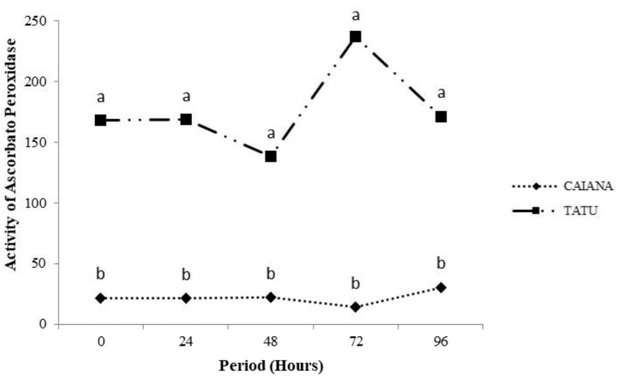


Figure 5 - Activity of the enzyme Ascorbate Peroxidase (APX) in the peanut seeds after periods of accelerated aging (UFAPE, 2018)



APX activity decreased after 72 h of accelerated aging, indicating the onset of seed deterioration (Figure 5). APX activity remained stable in the seeds of the cultivar Caiana, with a small increase observed after 72 h of aging. Therefore, it was assumed that the accelerated aging period of 96 h that was used to evaluate APX activity may not have been sufficient to cause changes in the enzyme.

Table 2 - Pearson (r) simple correlation coefficients for germination (GER), germination rate index (IVG), first germination count (PC), root dry mass MSPA), dry matter of cotyledons (MSC), root length (CR), shoot length (CPA), catalase (CAT) and ascorbate peroxidase (APX) in the peanut seeds subjected to accelerated aging (UFAPE, 2018)

	GER (%)	PC (%)	MSR (g)	MSPA (g)	MSCOT (g)	CR (cm)	CPA cm)	CAT	APX
IVG	0.99**	0.91**	0.45^{ns}	0.49^{ns}	0.51**	0.46^{ns}	0.75**	-0.18^{ns}	0.32^{ns}
GER		0.86**	0.44^{ns}	0.48^{ns}	0.52**	0.44^{ns}	0.74**	-0.22^{ns}	0.34^{ns}
PC			0.34^{ns}	0.35^{ns}	0.32^{ns}	0.34^{ns}	0.63**	-0.05^{ns}	0.27^{ns}
MSR				0.81**	0.56**	0.75**	0.46^{ns}	0.15^{ns}	0.10^{ns}
MSPA					0.67**	0.61**	0.53**	0.04^{ns}	0.05^{ns}
MSCOT						0.55**	0.68**	-0.16^{ns}	0.25^{ns}
CR							0.60**	0.08^{ns}	0.05^{ns}
CPA								-0.33^{ns}	0.26ns
CAT									0.18^{ns}

** r significante at 5% probability, ns r not significant by test t

APX is able to eliminate H_2O_2 more effectively during oxidative stress because it has a high affinity for this radical (HASANUZZAMAN *et al*., 2012). Therefore, the higher activity of this enzyme was represented by seeds at the beginning of the deterioration process, possibly due to the higher production of H_2O_2, which indicates the lower quality of the seeds from the cultivar Caiana (Figure 5). When aging seeds of *Jatropha curcas*, an oleaginous species, Suresh *et al*. (2019) observed that the reduced level of antioxidant enzyme activity could be negatively correlated with higher levels of malondialdehyde - MDA and H_2O_2. Furthermore, Ebone *et al*. (2020) highlighted in the embryonic axis of *Glycine max* seeds that the decline in APX and CAT activity due to aging, would be the responsible event for instability between antioxidant activity and ERO production.

Highly significant correlations were observed between the different combinations of germination, emergence, and vigor tests (Table 2). Pearson's correlation test revealed positive correlations between germination test and germination rate (r = 0.99 **), first count (r = 0.86 **), dry mass of the aerial part (r = 0.52 **), and shoot length of the aerial part (r = 0.74), indicating that the cultivar Tatu that showed the highest germination also showed the highest values for the vigor variables mentioned. This correlation of germination with several growth parameter tests that were performed provided evidence that the genetic potential of the studied cultivars was the common intrinsic factor affecting germination.

Among the tests based on seedling growth, the root dry weight was positively correlated with dry shoot mass (r = 0.81**), dry mass of cotyledons (r = 0.56**), and root length (r = 0.75**). The dry mass of the aerial part was highly correlated with the dry mass of cotyledons (r = 0.67**), root length (r = 0.61**), and shoot length (r = 0.53**). These results show that the seeds with the highest accumulation of dry mass correlated positively with the length of the seedlings, indicating that heavier seeds are able to produce more vigorous seedlings (Table 1).

CONCLUSION

1. The changes in the total activity of the catalase enzyme vary according to the peanut cultivar;
2. Seeds of the cultivar Tatu are more resistant to oxidative damage induced by accelerated aging than those of the cultivar Caiana;
3. Seeds of the cultivar Tatu are more resistant to accelerated aging and remain vigorous for a longer period.

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