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Watching the days go by: Aging during sunflower seed storage under distinct oxygen availability

Observando o passar dos dias: Envelhecimento de semente de girassol durante o armazenamento sob distinta disponibilidade de oxigênio

Rafael Agostinho Ferreira[1], Alisson Alves de Oliveira[1], Victor Navarro da Silva[1], Aline Aparecida Silva Pereira[1], Mateus Moreira Bernardes[1], Elisa Monteze Bicalho[1]*

[1]Universidade Federal de Lavras/UFLA, Lavras, MG, Brasil
*Corresponding author: elisa.bicalho@ufla.br
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ABSTRACT

The maintenance of seed viability is widely studied since preserving the physiological characteristics that will allow efficient germination and adequate field occupation is broadly pursued. However, even under optimal storage conditions, the aging process is inherent to the seed's life. In order to understand the effects of storage under low and normal oxygen conditions, this work sought to evaluate the physiological responses of two seed lots of two sunflower hybrids stored under different oxygen availability (normoxia and hypoxia) over a 360-day period. Aiming to investigate the effects of storage, the activities of the enzymatic antioxidant metabolism, hydrogen peroxide and MDA content, and the performance of viability, and vigor tests (tetrazolium test and electrolyte leakage) were performed with the stored seeds every 60 days. The hypoxia conditions were not able to keep seed viability over time, probably affecting negatively the embryonic axis. Throughout the evaluations, the viability tests demonstrated that the storage in the two experimental conditions was not able to contain the aging of the seeds. The increased content of H_2O_2 and MDA, associated with the enhanced electrical conductivity over time, indicate that there were losses by lipid peroxidation and that the aging process was not contained by storage under low oxygen availability.

Index terms: Oxidative damage; lipid peroxidation; ROS; *Helianthus annus*; hypoxia.

RESUMO

A manutenção da viabilidade das sementes é amplamente estudada uma vez que se busca preservar as características fisiológicas que permitirão uma germinação eficiente no campo. No entanto, mesmo em condições ideais de armazenamento, o processo de envelhecimento é inerente à vida da semente. Com o objetivo de compreender os efeitos do armazenamento em condições normais e de baixo oxigênio, este trabalho buscou avaliar as respostas fisiológicas de dois lotes de sementes de dois híbridos de girassol armazenados sob diferentes disponibilidades de oxigênio (normoxia e hipóxia) durante um período de 360 dias. Com o objetivo de investigar os efeitos do armazenamento, o metabolismo antioxidante enzimático, teor de peróxido de hidrogênio e MDA, bem como a viabilidade e o vigor (teste de tetrazólio e vazamento de eletrólito) foram realizados com as sementes armazenadas a cada 60 dias. As condições de hipóxia não foram capazes de manter a viabilidade das sementes ao longo do tempo, provavelmente afetando negativamente o eixo embrionário. Ao longo das avaliações, os testes de viabilidade demonstraram que o armazenamento nas duas condições experimentais não foi capaz de conter o envelhecimento das sementes. O aumento do conteúdo de H_2O_2 e MDA, associado ao aumento da condutividade elétrica ao longo do tempo, indicam que houve perdas por peroxidação lipídica e que o processo de envelhecimento não foi contido pelo armazenamento sob baixa disponibilidade de oxigênio.

Termos para indexação: Dano oxidativo; peroxidação lipídica; EROs; *Helianthus annus*; hipóxia.

INTRODUCTION

The loss of vigor in seeds during storage is being studied in recent years since it culminates in agronomiclosses impairing germplasm preservation due tothe aging (Kurek; Plitta-Michalak; Ratajczak, 2019; Ambarsi et al., 2021; Małecka et al., 2021). Although this process occurs in an irreversible way, the development of technologies that allow long-term storing seeds without the occurrence of losses is constant (Moncaleano-Escandon et al., 2013; De Castro Marais, et al., 2021).

The techniques that exploit changes in the storage conditions aim to control temperature, humidity and oxygen availability to provide suitable conditions for

seed metabolism since the variability in these conditions can lead to losses in vigor and viability (Ratajczak et al., 2019; De Vittis et al., 2020). Considering the storage conditions are controlled, seeds are less susceptible to external oscillations that can lead to increases in reactive oxygen species (ROS) (Biswas et al., 2020). An important highlight are the oxidative reactions, that result in electrons unpairing of the molecular oxygen, reacting with lipids from bilayer membrane and other cells constituents, inducing lipid peroxidation, protein carbonylation and DNA damage (El-Maarouf-Bouteau; Bailly, 2008; Zhang et al., 2021).

ROS accumulation above the scavenger capacity of antioxidant system can result in metabolic impairments, such as oxidative stress (Bailly, 2019; Sharma; Yadav; Sibi, 2020). Besides that, when storage conditions are controlled, seeds tend to reduce reserves mobilization for respiratory metabolism and maintain their vigor that, consequently, can prolong seed viability (Chhabra; Singh; Ansari, 2019; Zhang et al., 2021). Mostly, the damage that culminates in the loss of vigor by seeds is associated with hydrolytic, oxidative and peroxidative reactions (Mahjabin; Bilal; Abidi, 2015). Among these reactions are the enzymatic and non-enzymatic reactions that cause cellular and molecular damage leading to degradation of reserves (Hebelstrup; Moller 2015).

The oxidative reactions in dry or stored seeds, especially the Fenton and Maillard reactions, as well as mitochondrial activity, can lead to the formation of ROS, which in turn promote oxidative damage that causes seeds loss of vigor (Chhabra; Shabnam; Singh, 2019; Önder et al., 2020). During storage, ROS are originated mainly due to non-enzymatic reactions, such as the oxidation of polyunsaturated fatty acids (PUFA's) and by Maillard and Fenton reactions, as well as by the natural aging of seeds, thus oxidative stress plays a critical role in seed deterioration (Fotouo et al., 2020; Zhang et al., 2021). This is especially important in oilseed species, in which the lipid reserves are the main target of ROS attack (Whitehouse; Hay; Lusty, 2020).

Oxidative stress is the result of the imbalance between the production and elimination of ROS. In general, plant organisms have a network of agents responsible for the elimination of these ROS, including enzymatic and non-enzymatic antioxidant agents, composing a highly regulated line of defense against the action of these reactive molecules (Bailly, 2019). Among the main defense mechanisms, enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) and other peroxidases (POX) can be highlighted (Shaban, 2013).

As an inevitable consequence of oxidative damage in seeds, low germination rates are a major problem, since damage to seed reserves compromises the germination and establishment of seedlings, generating agronomic losses by seed aging processes (Tian et al., 2019). Thereby, storage techniques are focused in reduce the respiratory metabolism, reducing damages of oxidative stress (Wawrzyniak; Michalak; Chmielarz, 2020). This way, storing seeds under low oxygen conditions can promote benefits in reducing the ageing effects. Seeds are related to survive in conditions where can be deathly for plants (Zhang et al., 2021), so explore low oxygen availability can promote an increase in seed longevity (Pirredda et al., 2020).

In this work, we hypothesized that during storage under low oxygen availability, sunflower (*Helianthus annus*)seeds will keep their vigor by reducing the respiratory activity and consequently, avoiding ROS accumulation.We studied here one-year storage of two hybrids of sunflower (Helio 250 and Helio 251) seeds under two distinct oxygen availability. The seed reserves of this species are majority composed of lipids, which could accelerate aging, conducing the seed loss of vigor or even seed death. Therefore, we believe this work brings important contribution to seed storage techniques.

MATERIAL AND METHODS

Plant Material

Seeds from two sunflower hybrids were used in this experiment (Helio 250 and Helio 251 from HeliagroAgricultura e Pecuária©). Both materials were collected from two different periods. One lot, labeled 'STORED', consisted in seeds that were stored immediately after its harvesting in 2016 in a cold chamber until the set of the experiment. The other lot, labeled 'FRESH HARVESTED', comprised seeds harvested in 2019 which were readily subjected to the experimental conditions.

Experimental conditions

The experiment was carried out in the Laboratory of Plant Growth and Development (LCDP) at the Federal University of Lavras (Lavras-MG, Brazil). The experimental design was entirely randomized, in a 2x7 factorial scheme where two oxygen availability conditions (normoxia and hypoxia), around seven times of evaluation in each hybrid or year of production. The effects of the

different storage forms throughout the storage time were compared.The sunflower seeds were packaged in Kraft® paper bags depending on the way of storage (normoxia and hypoxia). The seeds from the hypoxic treatment were placed in a plastic bag and the air removed from the interior using a vacuum pump. The seeds from normoxia treatment were kept in paper bags in normal oxygen conditions, without any type of special storage.The seeds were assessed before of storage, and then every 60 days, for a period of 360 days of storage, totaling seven sampling over time. Each 60 days, the germination and biochemical parameters were carried out. The germination index, germination speed index, viability by tetrazolium test, electrolyte leakage and hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents, as well as enzymatic antioxidant system (SOD and CAT) were performed.

Germination assay

The seeds were placed into paper rolls using four repetitions of 50 sunflower seeds each; 25 seeds were used to measure the germination parameters and 25 seeds were collected for biochemical analysis. To conduct the germination test, plastic bags were used to wrap the sets of paper rolls with the seeds. The paper rolls were moistened with sterile water, in the proportion of 2.5 times the dry weight of the paper. Later the seeds were kept in the BOD, at a 25 ºC temperature with a 12/12 hours photoperiod, where the germination was counted daily over a period of 10 days. The germination assay was based on Seeds Analysis Rules (Brasil, 2009), and Germination speed index as described from Maguire (1962). Samples of 25 seeds used for biochemical analysis were collected after eight hours of imbibition, period that comprehend the phase II of germination, according to imbibition curve previously elaborated.

Electrical conductivity

The electrical conductivity test consisted of four replicates with 25 seeds each. Each replicate sample was placed to soak in a becker containing 75 mL of deionized water. Then, they were kept in a BOD chamber for 24 hours at a constant temperature of 25 °C, according to Vieira (1994). The electrical conductivity was measured with a conductivity meter, being the results expressed in $\mu S.cm^{-1}\ g^{-1}$ of seeds.

Seeds viability test

For this test, 25 seeds were pre-soaked in distilled water for 24 hours at 25º C. Afterwards, the seed coat was manually removed and a section was made through the tegument and between cotyledons to the center of the seed, then it was immersed in a 0.5% tetrazolium solution (2, 3, 5-triphenyltetrazolium chloride salt), pH 7, for a one-hourperiod at 25° C. The percentage of vigor and viability were calculated according to Silva et al. (2013).

Biochemical analysis

To perform the biochemical analyses, the seeds of each periodof storage were collected after eight hours of soaking on paper rolls. The seeds were frozen in liquid nitrogen and stored at -80 °C until analysis. To obtain an extract that could represent the totality of the seeds, a pool of ten seeds composed each replicate.

Hydrogen peroxide (H_2O_2)

The levels of H_2O_2 were quantified by the method of Velikova, Yordanov and Edreva, (2000). Four replicates of 100 mg of seeds each were grinded in liquid nitrogen and then homogenized with 1 mL of 0.1% Trichloroacetic Acid (TCA). Samples were centrifuged, and the reactions were carried out with 10 mM potassium phosphate buffer pH 7 and 1M potassium iodide (KI). The samples were read at spectrophotometer at 390 nm and the H_2O_2 amounts were measured by standard curve.

Lipid peroxidation

Lipid peroxidation estimation were performed by the quantification of Malondialdehyde (MDA) as described by Du and Bramlage (1992). 100 mg of seeds weregrinded in liquid nitrogen and then homogenized with 1 mL of 80% ethanol (three times, totaling 3 mL). The reactions were carried out with 1 mL of 20% TCA, 0.65% thiobarbituric acid (TBA) and 0.01% beta-hydroxytoluene (BHT). The estimation of lipid peroxidation was measured in spectrophotometer at 600, 440, and 532 nm.

Antioxidant enzymatic system assays

For the enzymatic extractions of catalase (CAT) and superoxide dismutase (SOD), about 100 mg of fresh material were grindedin liquid nitrogen and polyvinylpyrrolidone (PVPP) and homogenized with 1 mL of phosphate buffer (100 mM pH 7.8) with 100 mM EDTA and 1 mM ascorbic acid. The reaction medium for CAT comprised 67 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and an aliquot of the enzyme extract (Biemelt; Keetman; Albrecht, 1998). H_2O_2 consumption was measured through the absorbance at 240 nm as described by Anderson, Prassad and Stewart (1995).

SOD activity was measured using a reaction medium containing 50 mM phosphate buffer (pH 7.8), 13

mM L-methionine, 0.1 mM EDTA, 0.002 mM riboflavin and 0.075 mM NBT, as described by Giannopolitis and Ries (1977). The reaction occurred under fluorescent light (15 W) for 7 minutes. The blue formazan compound, derived from the reduction of NBT, was quantified at 575 nm. One unit of SOD was defined as the amount of enzyme required to inhibit NBT reduction by 50%.

Statistical analysis

The statistical analysis was performed with the software Rbio (Bhering, 2017). Data was subjected to two-way ANOVA and, in case of normal distribution, Tukey means test was selected at 5% significance. Data without a normal distribution were evaluated by a GLM analysis, assuming normality by observation of the qq-plot graphs and then applying the Tukey test at 5% of significance.

RESULTS AND DISCUSSION

The results showed herepointed out two relevant considerations: 1) the germination impairment in hypoxia condition is due to reductions in seed viability, mainly regarding to embryo axis; and 2) oxidative stress was marked over time of storage independent of the hybrid, oxygen conditions or the lots studied.

Germination percentage (G%) (Figure 1A) decreased in the hypoxia condition after 120 days of the storage, germination showed the lowest percentage of germinated seeds, even in fresh or previously stored seeds, for the two hybrids. These changes indicate that hypoxia was not efficient in promoting physiological conditions for the seeds to remain viable after removal from the storage condition. Seeds from both lots in the normoxia condition showed a germination percentage above 75%, reinforcing that the hypoxia condition was not able to keep viability over time. This is in agreement with observations of Morscher et al. (2015) and Bailly (2019).

Regarding the Germination Speed Index, stored seeds were more sensible over time than non-stored seeds from normoxia conditions. The highest GSI values that result in a higher germination (Figure 1B) could be observed for seeds from both lots under normoxia

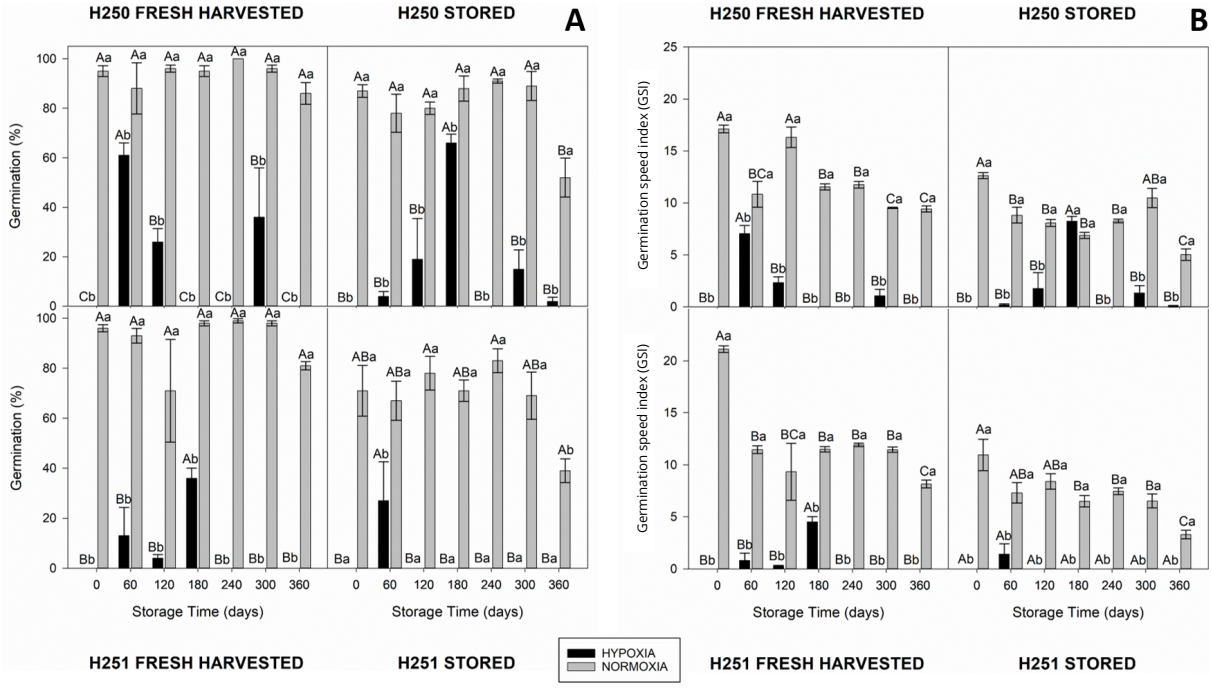

Figure 1: Germination percentage (A) and germination speed index (B) of *Helianthus annuus* hybrids seeds subjected to storage under normoxia (Grey bars) and hypoxia (black bars) during a 360-days period. Bars represent means ± standard error (n=4). Uppercase letters compare the storage period and lowercase letters compare the storage conditions (normoxia or hypoxia) within a certain storage period. Equal letters do not differ statistically at 5% significance level.

conditions. Under hypoxia conditions, only at 180 days a higher GSI could be observed for seeds from H250 hybrid, however, after that, this index decreased again showing compromises in germination, that results are similar to results obtained by Sahu et al. (2017) working with seeds under storage subjected to artificial aging process.

The viability of the seeds gradually decreased in storage conditions under low oxygen availability. At the initial characterization, all hybrids showed values of electrolyte leakage up to 9.03 µS/cm/g and all of the seeds have showed a vivid stained embryo, indicating that the seeds show high viability (Table 1). Although it is possible to observe staining in the cotyledons and in embryonic axis, the embryonic axis did not resist the storage under low oxygen availability (Figure 2). Moreover, the values of electrolyte leakage increased considerably throughout the experimental period; in hypoxic storage conditions the values observed are up to three times higher than those under normal oxygen conditions (Table 1). The increase in electrolyte leakage is associated with a higher incidence of membrane damage, and it is responsible for the low germination of the seeds since there is a compromise of the embryonic tissues, which can lead to embryo death (Liu et al., 2013).

Clearly, these changes were responsible for inducing redox homeostasis alterations that led to a loss of vigor in the seeds, increasing the mortality. The H_2O_2 content increased significantly after 120 days of evaluation (Figure 3A), although at low concentrations and under normal conditions tested. This molecule acts directly on signaling in seeds and plants. However, when in conditions that exceeds the "oxidative window" (Bailly; El-Maarouf-Bouteau; Corbineau, 2008), this molecule reacts with lipids, proteins and nucleic acids.

The content of MDA showed a prominent increase after 120 days of storage for all materials in both conditions (Figure 3B). These values indicated an intensification in lipid peroxidation regardless of the storage condition and seed hybrid, that could be associated with a greater increase of ROS, as showed for H_2O_2 levels. As can be seen, the H_2O_2 showed an enhancement in its endogenous concentration that accompanied the raise in MDA content, indicating an increased lipid peroxidation, an oxidative damage that contributes to the loss of vigor of seeds (Nagel et al., 2016).

These results indicated that lipid peroxidation might have occurred by the attack of ROS on both cell membranes and these seeds reserves, which is mostly lipid. Throughout the evaluation period, the highest values of MDA content could be observed in the seeds of hybrid H250 at 360 days of evaluation, which indicates that there was a process of aging of the seeds that was accentuated throughout the evaluations.

Figure 2: Images from the sunflower seeds at 360 days storage subjected to Tetrazolium test: A, B, C, D (hypoxia condition), seeds from hybrids H250 F+V, H251 F+V, H250 PS+V, H251 PS+V, and E, F, G, H (normoxia condition) from hybrids H250 F, H251 F, H250 PS, H251 PS – F+V: Fresh Harvested under hypoxia; PS+V: Pre-stored under hypoxia; F: Fresh harvested kept under normoxia; PS: Pre-stored kept under normoxia.

Table 1: Electrolyte leakage (µS/cm/g) and results of tetrazolium salt test (percentage of viable, inviable and dead seeds) of *Helianthus annuus* hybrids seeds subjected to storage under normoxia and hypoxia during a 360-days period. Uppercase letters compare the storage periodand lowercase letters compare thestorage conditions within a certain storage period. Equal letters do not differ statistically at 5% significance level. CV. cultivar; F. fresh seeds; PS. pre-harvested; V. hypoxia.

ELECTROLYTE LEAKAGE		TETRAZOLIUM TEST		
60 days				
CV. + STORAGE CONDITION	E. C (µS/cm/g)	VIABLE (%)	INVIABLE (%)	DEAD (%)
H250 F+V	8.52Ab	65Ba	34Ba	1Cc
H251 F+V	8.99Aab	38Cbc	50Aa	12Bc
H250 PS+V	8.86Aab	17Cc	45Aa	38Aa
H251 PS+V	8.97Aab	25Cbc	31Ba	44Aa
H250 F	8.74Aab	100Aa	0Cc	0Cc
H251 F	8.84Aab	92Aa	8Cb	0Cc
H250 PS	9.03Aa	92.8Aa	7.2Cb	0Cc
H251 PS	8.76Aab	100Aa	0Cc	0Cc
120 days				
CV. + STORAGE CONDITION	E. C (µS/cm/g)	VIABLE (%)	INVIABLE (%)	DEAD (%)
H250 F+V	10.07Aa	18Cb	72Aa	10Cc
H251 F+V	10.29Aa	3Dc	42Bab	55Bab
H250 PS+V	10.15Aa	23Cb	50Ba	27BCbc
H251 PS+V	10.27Aa	3Dc	7Cbc	90Aa
H250 F	10.04Aa	86Ba	12Cb	2Dc
H251 F	10.14Aa	98Aa	1Cc	1Dc
H250 PS	10.33Aa	96.8Aa	0.8Cc	2.4Dc
H251 PS	10.06Aa	88Ba	5.3Cc	6.6Dc
180 days				
CV. + STORAGE CONDITION	E. C (µS/cm/g)	VIABLE (%)	INVIABLE (%)	DEAD (%)
H250 F+V	61.03Ce	6Cd	0Cb	94Aa
H251 F+V	77.04Cde	74Bb	22Aab	0Cb
H250 PS+V	113.06ABbc	71Bb	23Aa	6Cb
H251 PS+V	174.54Aa	0Cd	0Cb	100Ca
H250 F	63.82Ce	89Aab	11Babc	0Ca
H251 F	61.92Ce	87Aab	13Bb	0Cb
H250 PS	97.68Be	98.4Aa	0Cb	1.6Cb
H251 PS	132.13ABb	93Aa	6.6Cbc	0Cb
240 days				
CV. + STORAGE CONDITION	E. C (µS/cm/g)	VIABLE (%)	INVIABLE (%)	DEAD (%)
H250 F+V	175.66ABb	9Dbc	0Aa	91Aa
H251 F+V	105.88Bc	2Dc	1Aa	97Aa
H250 PS+V	89.82Bc	6Dbc	13Aa	81Aa

Continue...

Table 1: Continuation.

H251 PS+V	311.45Aa	25Cbc	0Aa	74Ba
H250 F	97.37Bc	95Aa	3Aa	2Dc
H251 F	99.89Bc	96Aa	0Aa	4Db
H250 PS	166.69ABb	92.8Aa	3.2Aa	4Db
H251 PS	197.82ABb	66.6Bab	0Aa	33Cab
		300 days		
CV. + STORAGE CONDITION	E. C (µS/cm/g)	VIABLE (%)	INVIABLE (%)	DEAD (%)
H250 F+V	181.17BCbc	9Bb	25Bb	66Bab
H251 F+V	238.42Bab	0Cb	3Cc	97Aa
H250 PS+V	196.47BCbc	0Cb	50Aa	50Bab
H251 PS+V	333.17Aa	0Cb	0Cc	100Aa
H250 F	97.95Cc	100Aa	0Cc	0Cc
H251 F	93.62Cc	100Aa	0Cc	0Cc
H250 PS	167.42Cbc	99.2Aa	0Cc	0.8Cc
H251 PS	184BCbc	97.3Aa	0Cc	2.6Cc
		360 days		
CV. + STORAGE CONDITION	E. C (µS/cm/g)	VIABLE (%)	INVIABLE (%)	DEAD (%)
H250 F+V	170.26BCbc	0Db	4Cbc	96Aa
H251 F+V	89.09Cbcd	18Cb	45Aa	42Bbc
H250 PS+V	193.93Bab	9CDb	35Aa	56Bab
H251 PS+V	268.64Aa	0Db	0Cc	100Aa
H250 F	95.76Ccd	86Ba	11Bb	3Cc
H251 F	89.09Cd	99Aa	0Cc	1Cc
H250 PS	168.73BCbc	80Aa	0Cc	0Cc
H251 PS	190.49Bb	97.3Aa	1.3Cc	1.3Cc

Electrolyte Leakage (E. C (µS/cm/g)) and viability (obtained from tetrazolium-salt test) of Sunflower hybrid seeds during storage under normoxia and hypoxia.

The oxidative damage and, consequently electrolyte leakage, could be avoided by an efficient antioxidant system to maintain cellular homeostasis, however it was not observed here. During the evaluation period, it was observed that there were oscillations in the activity of SOD (Figure 4A). with a peak of its activity occurring in the first 120 days and then declining in all experimental conditions in all materials. The significant decrease in the activity of this enzyme that occurred after 120 days, is probably related to an imbalance in the capacity of extinction of the superoxide radical. These alterations could be observed in all conditions and for all materials. In order to eliminate the generated ROS, the enzymatic antioxidant metabolism acts by transforming ROS into molecules with less ability to interact with macromolecules and then reducing the possibility of oxidative damage occurrence (Diaz-Vivancos et al., 2013).

Trying to avoid the damages associated with ROS, catalase (Figure 4B) is one of the enzymes that act to clean up the H_2O_2 formed. This enzyme showed a similar activity for the different seed lots in both conditions of oxygen availability until the 120 days of the experiment. There was a decrease in the activity of this enzyme in the evaluations that comprised 180 and 240 days, with an increase in 300 and 360 days. This increase in activity might be seen as an attempt

to maintain the redox homeostasis of the seeds,which did not occur efficiently. The H250 hybrid seeds previously stored under low O_2 conditions showed higher activity, however it was not possible to observe the efficiency in guaranteeing the scavenging of ROS, which culminated in a low percentage of germination in the seeds under low O_2.

Although storing seeds under low oxygen availability is an option to promote the maintenance of desirable physiological characteristics, seeds under storage are inevitably subjected to aging processes that can culminate in the loss of germinability and viability. In order to control this process, it is essential to know the stored material and evaluate the best storage techniques to be adopted. In this work, it was possible to verify that low oxygen conditions were dangerous for embryonic axis of sunflower seeds, which was not able to keep viability over time. It could also be concluded that ageing reduced seed viability by increasing ROS and oxidative damage over time, independently of oxygen conditions. Therefore, this ageing during storage could be related to lipid losses of the cotyledon tissues, which are lipid-rich. Since seeds initiate the aging process after reaching physiological maturity and ROS formation is intrinsic to aerobic metabolism, we can suggest that some techniques such as seed priming or the adoption of molecules that can contain the formation of reactive oxygen species during storage may enable the maintenance of seed viability for long periods. Otherwise, the low oxygen conditions must be deeply investigated to stands out asa viable technique of storing seeds.

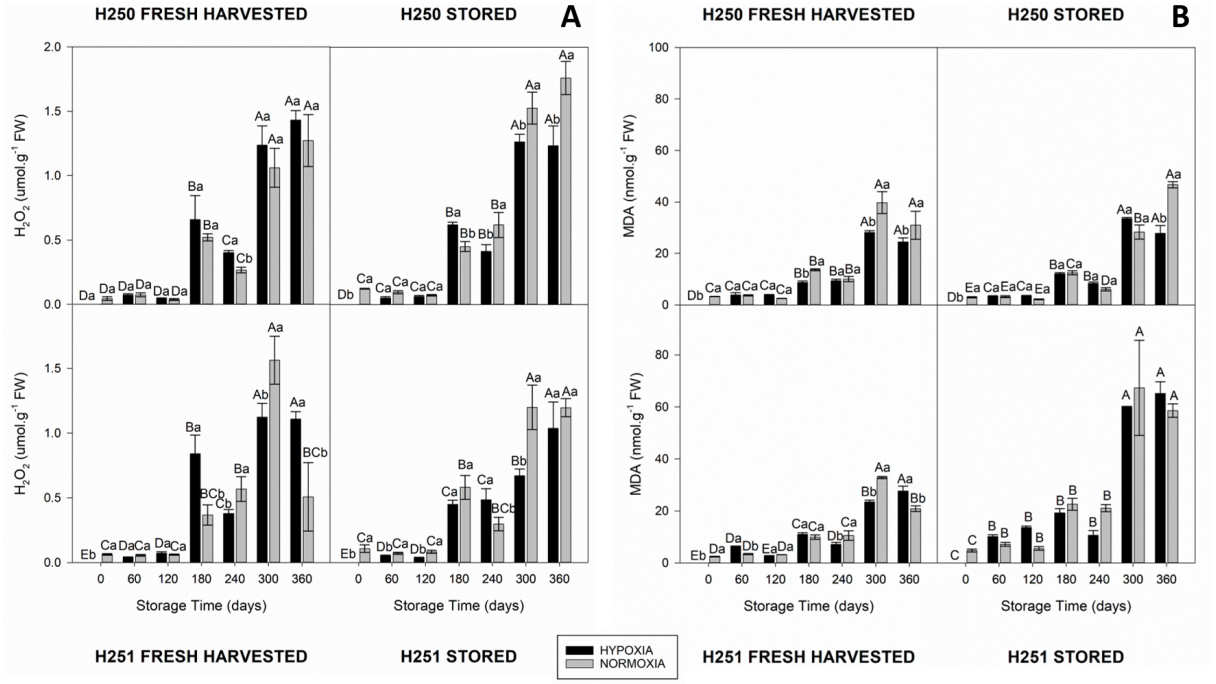

Figure 3: H_2O_2 (A) and MDA content (B) of *Helianthus annuus* hybrids seeds subjected to storage under normoxia (Grey bars) and hypoxia (black bars) during a 360-days period. Bars represent means ± standard error (n=4). Uppercase letters compare the storage period and lowercase letters compare the storage conditions (normoxia or hypoxia) within a certain storage period. Equal letters do not differ statistically at 5% significance level.

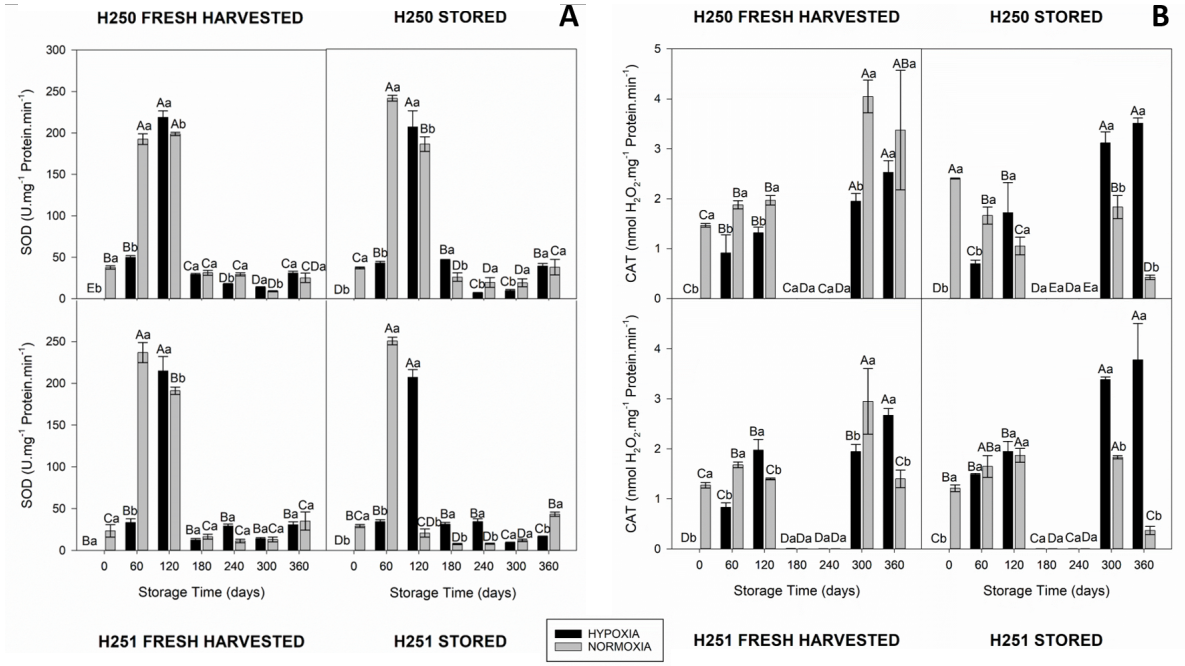

Figure 4: SOD content (A) CAT content (B) of *Helianthus annuus* hybrids seeds subjected to storage under normoxia (Grey bars) and hypoxia (black bars) during 360 days period. Bars are means ± standard error (n=4). Upper case letters compare storage period times and lower case letters compare storage conditions (normoxia or hypoxia) within a certain storage time. Equal letters do not differ statistically at 5% significance level.

CONCLUSIONS

Although storing seeds under low oxygen availability can promote the maintenance of desirable physiological characteristics, this condition can't stop aging processes that culminate in germinability and viability losses. Knowing the stored material is essential to adopt the best techniques aiming to control aging process. In this work, it was possible to verify that low oxygen conditions were dangerous for embryonic axis of sunflower seeds affecting the viability by increasing ROS and oxidative damage over time, independently of oxygen conditions.

AUTHOR CONTRIBUTION

Conceptual idea: Bicalho, E.M and Ferreira, R. A., Methodology design: Bicalho, E.M and Ferreira, R. A; Data collection: Ferreira, R. A.; Silva, V.N.; Alves, A. O.; Bernardes, M. M.; Pereira, A. A. P. Data analysis and interpretation: Bicalho, E.M; Ferreira, R. A.; Bernardes, M. M. Writing and editing: Ferreira, R. A.; Silva, V.N.; Alves, A. O.; Bernardes, M. M.; Pereira, A. A. P Bicalho, E.M.

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