Süleyman Demirel Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
Cilt 24, Sayı 3, 681-688, 2020

Süleyman Demirel University
Journal of Natural and Applied Sciences
Volume 24, Issue 3, 681-688, 2020

DOI: 10.19113/sdufenbed.793621

Determination of Hydrogen Peroxide Content and Antioxidant Enzyme Activities in Safflower (*Carthamus tinctorius* L.) Seeds After Accelerated Aging Test

Sercan ÖNDER*[1], Damla GÜVERCİN ÖNDER[2], Muhammet TONGUÇ[3]

[1,3]Isparta University of Applied Science, Faculty of Agricultural, Department of Agricultural Biotechnology, 32200, Isparta, Turkey
[2]Suleyman Demirel University, Faculty of Arts and Sciences, Department of Biology, 32260, Isparta, Turkey

(Alınış / Received: 11.09.2020, Kabul / Accepted: 30.10.2020, Online Yayınlanma / Published Online: 20.12.2020)

Keywords
Catalase,
Peroxidase,
Seed aging,
Superoxide dismutase

Abstract: Safflower is an important oleiferous crop species in the world. Aging tests are used to simulate cell damage occurring during the long term storage of seeds. In the present study, accelerated aging (AA) test was employed to investigate response of antioxidant enzymes in safflower. Four genotypes of safflower, previously classified as aging resistant (Bayer-6 and Bayer-12) and sensitive (Olas and Linas) based on AA test, were used as seed materials and AA treatments at 43 °C consisted of 5 different times (0, 48, 72, 96 and 120 h). Variance analysis were used and means were separated according to significance levels, and correlations were calculated between hydrogen peroxide (H_2O_2) content, superoxide dismutase (SOD) and catalase (CAT) activities. The H_2O_2 content, CAT, SOD and peroxidase (POD) activities were measured in control and AA treated seeds. Genotype, time and genotype x time interactions were all significant. While H_2O_2 content and SOD activity increased with AA time, CAT activity decreased in all genotypes throughout the experiment. POD did not show regular increase or decrease, its activity was specific to genotypes and time. Correlations between CAT activity and H_2O_2 content were significant negative for all genotypes, but between SOD activity and H_2O_2 content was positively correlated in AA treated seeds.

Hızlandırılmış Yaşlandırma Testi Sonrasında Aspir (*Carthamus tinctorius* L.) Tohumlarında Hidrojen Peroksit İçeriği ve Antioksidant Enzim Aktivitelerinin Belirlenmesi

Anahtar Kelimeler
Katalaz,
Peroksidaz,
Tohum yaşlanması,
Süperoksit dismutaz

Özet: Aspir dünyada önemli bir yağlı tohum bitkisidir. Yaşlandırma testleri, tohumların uzun süreli depolanması sırasında meydana gelen hücresel hasarın oluşturulmasını sağlayan testlerdir. Mevcut çalışmada hızlandırılmış yaşlandırma testi (AA) aspir tohumlarında antioksidant enzim aktivitelerinin incelenmesi için uygulanmıştır. Daha önceki çalışmada yaşlanmaya dirençli (Bayer-6 ve Bayer-12) yaşlanmaya hassas (Olas ve Linas) dört aspir genotipi tohum materyali olarak kullanılmıştır. AA, 43 °C 5 farklı zamanda (0, 48, 72, 96 ve 120 saat) gerçekleştirilmiştir. Sonuçlar varyans analizine tabi tutulmuş ve ortalamalar arasındaki önemlilik seviyeleri tespit edilmiştir. Ayrıca hidrojen peroksit (H_2O_2) içeriği ve katalaz (CAT) ve süperoksit dismutaz (SOD) aktiviteleri arasındaki korelasyon incelenmiştir. H_2O_2 içeriği, CAT, SOD ve peroksidaz (POD) aktiviteleri kontrol ve AA uygulanmış tohumlarda belirlenmiştir. Genotip, zaman ve genotip x zaman interaksiyonları önemli bulunmuştur. H_2O_2 içeriği ve SOD aktivitesi AA zamanına bağlı olarak artarken, CAT aktivitesi ise kullanılan tüm genotiplerde ise azalmıştır. POD aktivitesi düzenli bir şekilde artış veya azalış göstermemiş, aktivitedeki değişim genotip ve zamana göre farklılık göstermiştir. H_2O_2 içeriği ve CAT aktivitesi arasındaki korelasyon tüm genotiplerde önemli ve negatif olarak bulunurken; H_2O_2 içeriği ve SOD aktivitesi arasında AA uygulanmış tohumlarda pozitif korelasyon bulunmuştur.

* Corresponding author: sercanonder@isparta.edu.tr

1. Introduction

Safflower is an important oleiferous crop species in the world belonging to the Asteraceae family. Turkey ranked 8th in terms of the cultivated area (24.693 ha) and 5th in production (35.000 t) in 2018 [1]. As the arable land decreases as a result of the continuously growing world population, the only alternative to achieve high quality seeds is to increase agricultural productivity [2, 3]. Safflower is an alternative crop plant due to its tolerance to drought and salinity. Therefore, it is suitable to grow in arid and semi-arid areas where other oilseed crops cannot be grown economically [4, 5]. During the harvest and seed storage, seeds are exposed to different conditions leading to seed deterioration and reduced viability [6]. Storage of oilseeds is difficult than the other seeds because oilseeds are very susceptible to seed degradation [7]. In addition, safflower seed is categorized as weak storer since its viability and oil quality reduces after 1-1.5 years of storage under normal storage terms [8]. The two most important factors affecting seed quality and deterioration during aging are relative humidity and temperature [9]. A decrease of 1% in seed relative humidity or 5 °C in storage temperature increases seed life twice [10]. Seed aging thought to be caused by lipid peroxidation, changes in antioxidant enzyme activity, degradation in lipid and carbohydrates, changes in protein and enzyme structures, changes in energy metabolism and occurrence of reactive oxygen species (ROS) [11]. Plants possess antioxidant systems to protect against ROS. Seed aging could cause deterioration of antioxidant enzymes due to structural changes in macromolecular level [12-14]. The enzymatic antioxidant system has different enzymes scavenging ROS and maintaining physiological balance between ROS production and antioxidative defenses [7]. Superoxide dismutase (SOD) and catalase (CAT) are principal antioxidant enzymes in plants to eliminate ROS [13]. The production of ROS is one of the physiological results causing seed degradation [15, 16]. These free radicals may adversely affect the function of macromolecules and enzymatic activity by inducing enzymatic lipid peroxidation [13, 17, 18].

Although safflower is important for both the dyeing industry and oilseed crop; few studies have conducted to investigate the aging and vigor of seeds. Hence, examination of seed deterioration during the storage is important for seed storage and sustainable production. Accelerated aging (AA) is a commonly used test to evaluate seed vigor after exposing seeds to high humidity and high temperature [19]. There is a need to investigate the role of antioxidant enzymes in seed aging with AA testing and to examine the changes in the activity of antioxidant enzymes at different aging times. Oxidative lipid peroxidation was not the main reason of seed degradation during AA in safflower seeds [20]. Therefore, present study was carried out to investigate hydrogen peroxide (H_2O_2) accumulation and antioxidant enzyme activities (CAT, SOD and POD) between aging resistant and sensitive genotypes of safflower during the AA.

2. Material and Method

2.1. Seed material and accelerated aging test

The safflower genotypes (Bayer-6, Bayer-12, Linas and Olas) were used as seed materials. Adjustment of seed moisture content before and after surface sterilization, performing of AA tests, germination conditions were given in a previously published study [20]. Seeds of control treatment (0 h) were not exposed to aging conditions. These genotypes were differentiated based on their reaction to AA in a previous study and classify as aging resistant and sensitive [20]. Seeds were stored at 4 °C until the analyses.

2.2. Estimation of hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) extraction and measure from seeds of safflower were performed according to Velikove et al. [21]. Seeds were frozen with liquid nitrogen and grounded with 5 ml 0.1% (w/v) trichloroacetic acid. The mixture was centrifuged at 15.000 x g for 15 min at 4 °C. Supernatant (0.5 ml) was aliquoted and 1 ml 1 M KI and 0.5 ml 10 mM K_2HPO_4 buffer (pH 7.0) was added. Reaction mixture was subjected to dark conditions for 60 min. The absorbance values of the samples were read at 390 nm. H_2O_2 amount was calculated with a standard curve.

2.3. Antioxidant enzyme activity determination

For the determination of enzyme activities, 10 g seeds were grounded in 25 ml ice-cold extraction buffer and 0.5 g PVPP. For CAT analysis, 50 mM sodium phosphate (pH 7.0), for SOD and peroxidase (POD) analyses 100 mM sodium phosphate buffer (pH 6.4) was used as extraction buffer. Homogenates were centrifuged at 27.000 x g for 50 min at 4 °C and supernatants was used to measure enzyme activity [22].

The CAT activity was performed following Beers and Sizer [23] method. The CAT reaction mixture contained 0.5 ml H_2O_2 (40 mM), 2 ml sodium phosphate buffer (50 mM, pH 7.0) and 0.5 ml of the extracted enzyme. The decline of absorbance for H_2O_2 at 240 nm (ε_{240}= 43.6 mM^{-1} cm^{-1}) was recorded for 2 min. One unit of enzyme activity was estimated as the µmol of H_2O_2 decomposition per min. Specific activity of CAT was expressed as µmol of H_2O_2 degraded min^{-1} mg^{-1} protein.

SOD activity was performed following Constantine and Stanley [24] method. The SOD reaction mixture (3 ml) contained 13 mM methionine, 10 µM EDTA, 50

mM sodium phosphate buffer (pH 7.8), 2 µM riboflavin, 75 µM NBT and 0.1 ml of the enzyme extract. The reaction mixtures were lightened under light (60 µmol/m^2/s) for 10 min and the absorbance was monitored by recording at 560 nm using a spectrophotometer. Blank solutions were held in dark. Enzyme activity was estimated as the quantity of enzyme causing 50% reduction in absorbance. Activity of SOD was expressed as U mg^{-1} protein.

The POD activity was performed following Jiang et al. [25] method. The POD reaction mixture contained 2 ml buffer substrate (8 mM guaiacol, 100 mM sodium phosphate pH 6.4), 1 ml of 24 mM H_2O_2 and 0.5 ml of enzyme extract. Absorbance values were recorded twice at 30 s intervals at 460 nm. POD activity was expressed as ΔA_{460} min^{-1} mg^{-1} protein.

Protein amount was calculated using Bradford method [26]. Standard curve was prepared with BSA.

2.4. Statistical analysis

Analyses were performed using three replications. Data were analyzed with variance analysis (ANOVA) and differences between the means were separated by Duncan's multiple range test at $p \leq 0.05$ using the IBM SPSS Statistics 22.0 package.

3. Results

The H_2O_2 accumulation and antioxidant enzyme activities during the AA of safflower genotypes were investigated in the present study. Two aging resistant (Bayer-6 and Bayer-12) and aging sensitive (Olas and Linas) safflower genotypes were used as plant material. ANOVA results showed that genotypes, aging time and genotype x time interactions were significant (Table 1).

H_2O_2 content was estimated to verify free radicals accumulation and their effects during the AA in safflower seeds due to lipid peroxidation. Increased aging time caused an increase in the H_2O_2 content of all safflower genotypes (Table 1). Even 48 h AA was sufficient to increase H_2O_2 content of all genotypes compared to the control. After 120 h of aging in Bayer-12, Linas and Olas, H_2O_2 content increased by 25, 16 and 20%, respectively. However, Bayer-6 showed the highest H_2O_2 increase (12%) after 96 h of aging. There was a decrease in H_2O_2 content in Bayer-6, after 120 h of AA.

Increased AA time caused significant decrease in activity of the CAT enzyme in all four genotypes of safflower (Table 1). Among the studied genotypes, the highest decrease in CAT activity was exhibited

Table 1. Effect of AA on seed antioxidant enzyme activities and hydrogen peroxide (H_2O_2) content of safflower genotypes.

Genotype	Time (hour)	H_2O_2 (µmol g^{-1})	CAT (U mg^{-1} protein)	SOD (U mg^{-1} protein)	POD (U mg^{-1} protein)
Bayer-6	0	2.01^{d}	81.81^{a}	3.01^{c}	0.49^{b}
	48	2.19^{b}	68.66^{b}	3.17^{c}	0.49^{b}
	72	2.24^{ab}	64.86^{b}	3.25^{c}	0.49^{b}
	96	2.26^{a}	53.91^{c}	3.77^{b}	0.51^{ab}
	120	2.13^{c}	46.82^{c}	4.25^{a}	0.52^{a}
Bayer-12	0	2.92^{c}	91.70^{a}	2.17^{c}	0.58^{a}
	48	3.03^{bc}	80.34^{b}	2.23^{bc}	0.53^{b}
	72	3.19^{b}	74.21^{c}	2.46^{a}	0.58^{a}
	96	3.52^{a}	73.47^{c}	2.38^{ab}	0.49^{c}
	120	3.64^{a}	69.04^{c}	2.38^{ab}	0.48^{d}
Linas	0	2.62^{d}	102.35^{a}	2.30^{e}	0.85^{b}
	48	2.78^{c}	87.47^{b}	2.76^{d}	0.70^{d}
	72	2.88^{bc}	82.77^{bc}	2.90^{c}	0.44^{e}
	96	3.01^{ab}	76.65^{c}	3.25^{b}	0.91^{a}
	120	3.05^{a}	64.87^{d}	3.85^{a}	0.82^{c}
Olas	0	2.62^{b}	102.24^{a}	2.06^{c}	0.83^{b}
	48	2.98^{a}	88.39^{b}	3.11^{b}	0.53^{c}
	72	3.01^{a}	84.01^{b}	3.58^{a}	0.47^{e}
	96	3.08^{a}	75.92^{c}	3.60^{a}	0.86^{a}
	120	3.13^{a}	64.75^{d}	3.69^{a}	0.49^{d}
Genotype (G)		**	**	**	**
Time (T)		**	**	**	**
G x T		**	**	**	**
CV (%)		3.06	4.65	2.34	3.47

*Means followed by the same letters within columns are not significantly different at $p \leq 0.05$.
**Significant at $p \leq 0.01$

under 120 h aging conditions. After 120 h of aging in Bayer-6, Bayer-12, Linas and Olas, CAT activity decreased by 43, 25, 37 and 37%, respectively. The loss of CAT enzyme activity and accumulation of H_2O_2 were associated with loss of seed germination showing that CAT activity is necessary for the viability of the seeds. In this study, there was a significant negative correlation between CAT activity and H_2O_2 content after AA treatment in all genotypes (Fig. 1). However, the correlation was not as pronounced in aging resistant genotypes (Bayer-6 and Bayer-12) as compared to aging sensitive ones (Linas and Olas) that explains the excess ROS accumulation and loss of germination in these genotypes.

Superoxide dismutase is an enzyme catalyzing dismutation of superoxide radicals and plays

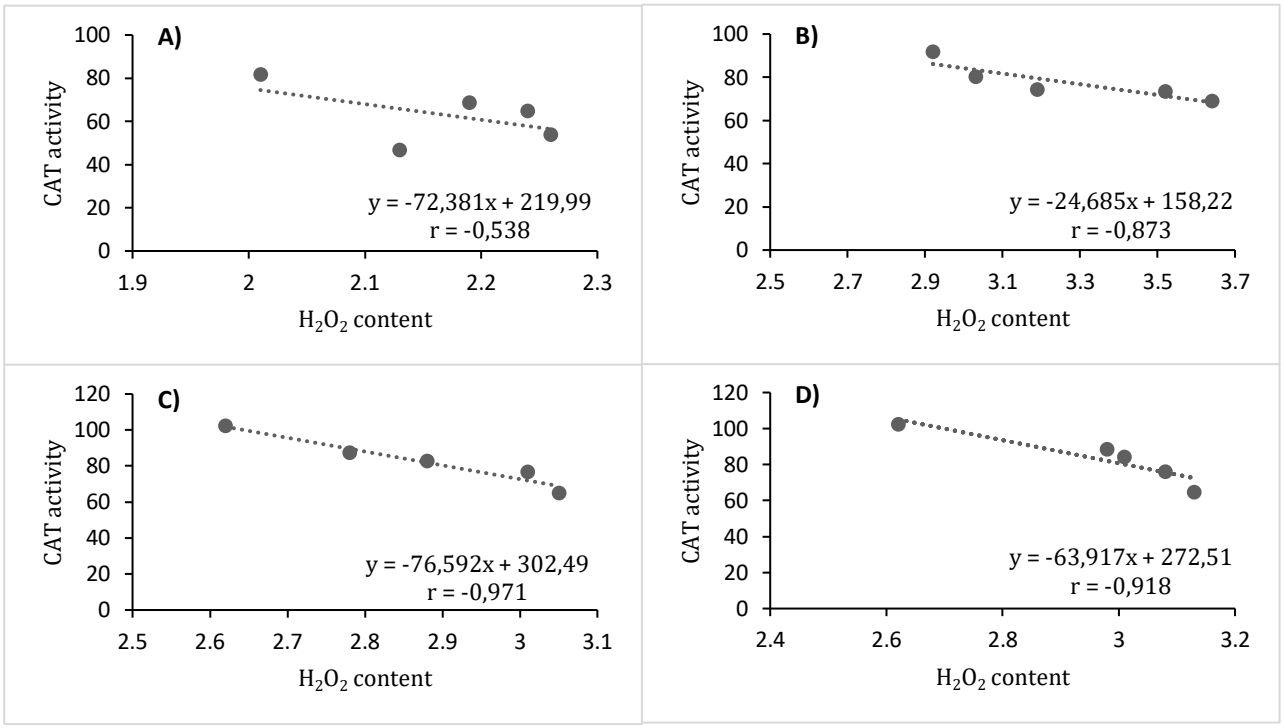

Fig. 1. Correlation analysis between H_2O_2 content and CAT activity in diferent safflower genotypes, A) Bayer-6, B) Bayer-12, C) Linas, D) Olas.

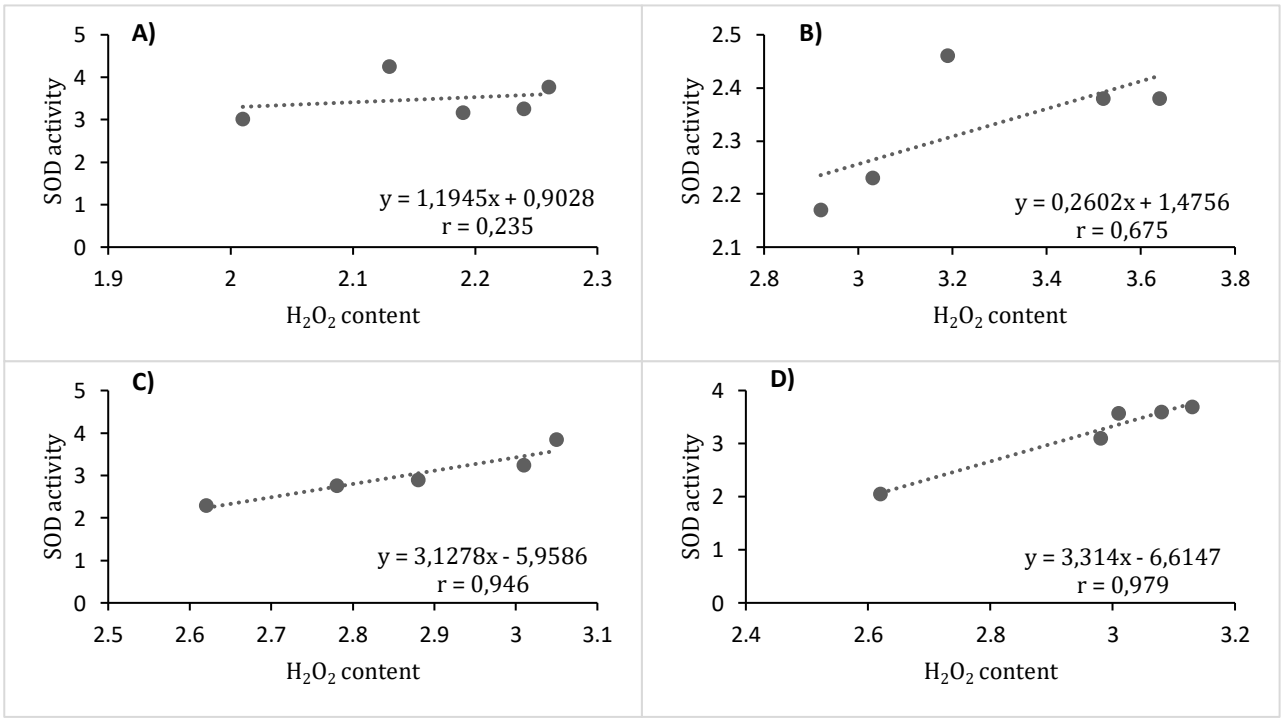

Fig. 2. Correlation analysis between H_2O_2 content and SOD activity in safflower genotypes, A) Bayer-6, B) Bayer-12, C) Linas, D) Olas.

important role in plant defense system. SOD activity increased with aging time in all genotypes, but increase in SOD activity in Bayer-12 was lower than the other genotypes and SOD activity increased till 72h aging and subsequently decreased at 96 h and did not change at 120 h of aging (Table 1). After 120 h of aging in Bayer-6, Linas and Olas, SOD activity increased by 41, 67 and 79%, respectively. However, Bayer-12 showed the highest SOD increase (13%) after 72 h of aging. The increase in SOD activity in aging sensitive (Linas and Olas) genotypes after 120 h of AA at 43 °C was significantly higher than in aging resistant (Bayer-6 and Bayer-12) genotypes. There was a positive correlation between SOD activities and H_2O_2 content after AA in safflower genotypes except for Bayer-6 (Bayer-6, r=0.235) (Fig. 2). However, correlation coefficient value is lower in Bayer-12 and there is a weak correlation (r=0.675).

POD activity was highly variable among the genotypes and aging times. POD activity in Bayer-6 started to increase at 96 h, whereas POD activity in Bayer-12 fluctuated between aging times and at the end decreased at 120 h. After 120 h of aging in Bayer-12, POD activity decreased by 17% but increased by 6% in Bayer-6**.** Among aging sensitive genotypes, Linas and Olas, POD activity has fluctuated during the aging times. At both genotypes, POD activity decreased regularly until 72 h of aging compared to control, but at 96 h of aging increased POD activity by 7% and 4% observed; respectively. After 120 h of aging in Linas and Olas, POD activity decreased by 48 and 43%, respectively.

4. Discussion and Conclusion

Seeds contain a complex defense system to protect themselves against damage during the aging and storage process. The enzymatic defense mechanism consists of many enzymatic mechanisms and these antioxidant enzymes catalyze regeneration and formation reactions to scavenge ROS. Seed deterioration and aging increase peroxide and ROS in seeds and cause cell damage due to oxidative stress [27, 28]. Changes in the activity of antioxidant enzymes are crucial biomarkers for observing biochemical changes during seed deterioration [29]. There are important changes in the activities of enzymes such as CAT, SOD and POD during the degradation and aging of seeds [30]. The AA test is a helpful tool for the study of different genotypes since the test simulates cell damage during prolonged storage and helpful to learn about the biochemical changes occurring in the seed [31].

The results of germination parameters such as; percent germination, mean germination time and normal seedling percentage after AA for safflower genotypes were published previously [20]. Results of germination tests indicated that AA test at 43 °C was favorable to assess the effects of aging in safflower since germination at specified time and temperature distinguished aging resistant and sensitive genotypes.

Seed deterioration is coincided with the accumulation of free radicals production as a result of metabolic events. During seed aging and storage, lipids react with oxygen to form hydrogen peroxides and free radicals. H_2O_2 accumulation during seed aging appears to have a strong negative correlation with seed germinability [7, 32]. The present study confirms the negative correlation between seed germination, increased H_2O_2 content and decreased seed viability after AA at 43 °C. In seed aging studies, ROS-like H_2O_2 plays a very important role in oxidation of all molecules found in cellular structures [7, 33]. Enzymatic lipid peroxidation, H_2O_2 accumulation and the formation of free radicals may be the main cause of seed degradation in oilseed plants such as safflower [20, 34].

Catalase is an enzyme that catalyzes the separation of H_2O_2 to H_2O and O_2 and does not need any reducing agent [35]. The CAT activities after AA observed in this study was similar to results of the previously reported studies for cotton [12], maize [36], onion [8] *Jatropha curcas* [7] and soybean [18] seeds. In okra seeds AA at 40 °C for 96 and 192 h of aging reduced CAT activity compared to control [28]. In onion seeds, there was a relationship between loss of viability and low catalase activity [37].

The significantly increased activity of SOD in safflower genotypes indicates that enzyme was active when the seeds were exposed to stress conditions at temperatures and moisture content. Studies for the effect of natural aging and AA on neem [38] and *J. curcas* [7] showed increased SOD activity to a certain aging period compared to control. Increased SOD activity is an effect on the arising of superoxide radicals, and these radicals are converted to less harmful H_2O_2 [7]. Therefore, high positive correlation in aging resistant genotypes (Linas and Olas) also explains higher seed viability observed in these genotypes. A study on the effect of AA at 45 °C on wheat seeds showed that two and four days of aging increased SOD activity compared to control [39]. Barreto and Garcia [40] reported that macaw palm seeds aged at 4, 8 and 12 days at 100% relative humidity at 45 °C hasn't affected SOD activity in for 4 days aged embryos, but it increased about 50% in embryos aged longer than 4 days.

POD activity increased aging resistant genotype Bayer-6 but decreased in Bayer-12 at 120 h of aging. POD activity fluctuated in aging sensitive genotypes but decrease at 10 h aging in safflower. Our results for POD activity were similar to POD activity determined in aged *J. curcas* seeds [7]. Decreased POD activity was observed during the aging of sunflower [41], radish [42] and onion [8] seeds as well. ROS causes damage to the molecules and can

play an important role in reducing antioxidant activity [43]. CAT, SOD, and POD activities were significantly affected by AA time, genotypes, and the interaction between aging time and genotypes. Changes in these enzyme activities eventually cause decreased protein synthesis, decreased sugar concentration in cells and inactivation of enzymes [44].

This study explores the antioxidant enzyme activity in aging sensitive (Linas and Olas) and aging resistant (Bayer-6 and Bayer-12) genotypes of safflower after AA test. Results showed that H_2O_2 content in aging genotypes is positively correlated with SOD activity and negatively correlated with CAT activity. There was high variability for antioxidant enzymatic activities among the studied genotypes. ROS like molecules accumulated during the AA and had detrimental effects on seeds exposed to AA. However, further studies are needed to better understand the underlying mechanisms of aging at the genotypic and cellular level.

Acknowledgment

We thank Dr. Sabri ERBAŞ for providing seed material used in this study.

References

[1] FAO,2020.http://www.fao.org/faostat/en/#data/QC (Erişim Tarihi: 10.08.2020)

[2] Verge, X. P. C., De Kimpe, C., Desjardins, R. L. 2007. Agricultural Production, Greenhouse Gas Emissions and Mitigation Potential. Agricultural and Forest Meteorology, 142(2-4), 255-269.

[3] Federico, G. 2010. Feeding the world: an economic history of agriculture, 1800-2000. Princeton University Press. Princeton, New Jersey, USA, 387p.

[4] Weiss, E. A. 2000. Oilseed crops. Blackwell Science. Oxford, 384p.

[5] Koç, H., Keleş, R., Ülker, R., Gümüşçü, G., Ercan, B., Göçmen Akçacık, A., Güneş, A., Özdemir, F., Özer, E., Uludağ, E. 2010. Bazı Aspir (*Carthamus tinctorius* L.) Hatlarının Verim, Verim Öğeleri ve Kalite Özellikleri İle Bu Özellikler Arasındaki İlişkilerin Belirlenmesi. Bitkisel Araştırma Dergisi, 2, 1-7.

[6] Delouche, J. C., Baskin, C. C. 2016. Accelerated Aging Techniques for Predicting the Relative Storability of Seed Lots. Seed Science and Technology, 1, 427-452.

[7] Suresh, A., Shah, N., Kotecha, M., Robin, P. 2019. Evaluation of Biochemical and Physiological Changes in Seeds of *Jatropha curcas* L. Under Natural Aging, Accelerated Aging and Saturated Salt Accelerated Aging. Scientia Horticulturae, 255, 21-29.

[8] Brar, N. S., Kaushik, P., Dudi, B. S. 2019. Assessment of Natural Ageing Related Physio-Biochemical Changes in Onion Seed. Agriculture, 9(8), 163.

[9] Probert, R., Adams, J., Coneybeer, J., Crawford, A., Hay, F. 2007. Seed Quality for Conservation is Critically Affected by Pre-Storage Factors. Australian Journal of Botany, 55(3), 326-335.

[10] Harrington, J. F. 1972. Seed storage and longevity (3nd). Academic Press, New York, 434p.

[11] Priestley, D. A. 1956. Seed aging. Cornell University Press, Ithaca, 304p.

[12] Goel, A., Goel, A. K., Sheoran, I. S. 2003. Changes in Oxidative Stress Enzymes During Artificial Ageing in Cotton (*Gossypium hirsutum* L.) Seeds. Journal of Plant Physiology, 160(9), 1093-1100.

[13] Bailly, C. 2004. Active Oxygen Species and Antioxidants in Seed Biology. Seed Science Research, 14(2), 93-107.

[14] Lehner, A., Mamadou, N., Poels, P., Come, D., Bailly, C., Corbineau, F. 2008. Changes in Soluble Carbohydrates, Lipid Peroxidation and Antioxidant Enzyme Activities in The Embryo During Ageing in Wheat Grains. Journal of Cereal Science, 47(3), 555-565.

[15] Parkhey, S., Naithani, S. C., Keshavkant, S. 2012. ROS Production and Lipid Catabolism in Desiccating *Shorea robusta* Seeds During Aging. Plant Physiology and Biochemistry, 57, 261-267.

[16] Sahu, B., Sahu, A. K., Thomas, V., Naithani, S. C. 2017. Reactive Oxygen Species, Lipid Peroxidation, Protein Oxidation and Antioxidative Enzymes in Dehydrating Karanj (*Pongamia pinnata*) Seeds During Storage. South African Journal of Botany, 112, 383-390.

[17] Bosco de Oliveira, A., Gomes-Filho, E., Enéas-Filho, J., Prisco, J. T., Alencar, N. L. M. 2012. Seed Priming Effects on Growth, Lipid Peroxidation, and Activity of ROS Scavenging Enzymes in NaCl-Stressed Sorghum Seedlings from Aged Seeds. Journal of Plant Interactions, 7(2), 151-159.

[18] Nazari, R., Parsa, S., Tavakkol Afshari, R., Mahmoodi, S., Seyyedi, S. M. 2020. Salicylic Acid Priming Before and After Accelerated Aging Process Increases Seedling Vigor in Aged Soybean Seed. Journal of Crop Improvement, 34(2), 218-237.

[19] Wang, F., Wang, R., Jing, W., Zhang, W. 2012. Quantitative Dissection of Lipid Degradation in Rice Seeds During Accelerated Aging. Plant Growth Regulation, 66(1), 49-58.

[20] Önder, S., Tonguç, M., Güvercin, D., Karakurt, Y. 2020. Biochemical Changes Stimulated by Accelerated Aging in Safflower Seeds (*Carthamus tinctorius* L.). Journal of Seed Science, 42, e202042015.

[21] Velikova, V., Yordanov, I., Edreva, A. 2000. Oxidative Stress and Some Antioxidant Systems in Acid Rain-Treated Bean Plants: Protective Role of Exogenous Polyamines. Plant Science, 151(1), 59-66.

[22] Wang, Y. S., Tian, S. P., Xu, Y., 2005. Effects of High Oxygen Concentration on Pro- and Anti-Oxidant Enzymes in Peach Fruits During Postharvest Periods. Food Chemistry, 91(1), 99-104.

[23] Beers, R. F., Sizer, I. W. 1952. A Spectrophotometric Method for Measuring The Breakdown of Hydrogen Peroxide by Catalase. The Journal Biological Chemistry, 195(1), 133-140.

[24] Constantine, N. G., Stanley, K. R. 1977. Superoxide Dismutases. Plant Physiology, 59(2), 315-318.

[25] Jiang, A. L., Tian, S. P., Xu, Y. 2002. Effects of Controlled Atmospheres with High-O_2 or High CO_2 Concentrations on Postharvest Physiology and Storability of ''Napoleon'' Sweet Cherry. Journal of Integrative Plant Biology, 44(8), 925-930.

[26] Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding. Analytical Biochemistry, 72(1-2), 248-254.

[27] Bewley, J. D., Bradford, K., Hilhorst, H. 2012. Seeds: Physiology of Development, Germination and Dormancy. Springer Science & Business Media, New York, 392p.

[28] Parmoon, G., Moosavi, S. A., Siadat, S. A. 2019. Performance of Iranian Okra Ecotypes Under Various Accelerated Aging Conditions. Horticultural Plant Journal, 5(1), 17-23.

[29] Silva, P. P., Sekita, M. C., Dias, D. C. F. S., Nascimento, W. M. 2016. Biochemical and Physiological Analysis in Carrot Seeds from Different Orders of Umbels. Revista Ciência Agronômica, 47(2), 407-413.

[30] Chhabra, R., Singh, S. T. 2019. Seed Aging, Storage and Deterioration: An Irresistible Physiological Phenomenon. Agricultural Reviews, 40(3), 234-238.

[31] Wu, X., Ning, F., Hu, X., Wang, W. 2017. Genetic Modification for İmproving Seed Vigor is Transitioning from Model Plants to Crop Plants. Frontiers in Plant Science, 8(8), 1-7.

[32] Ratajczak, E., Małecka, A., Bagniewska-Zadworna, A., Kalemba, E. M. 2015. The Production, Localization and Spreading of Reactive Oxygen Species Contributes to The Low Vitality of Long-term Stored Common Beech (*Fagus sylvatica* L.) Seeds. Journal of Plant Physiology, 174, 147-156.

[33] Kibinza, S., Vinel, D., Côme, D., Bailly, C., Corbineau, F. 2006. Sunflower Seed Deterioration as Related to Moisture Content During Ageing, Energy Metabolism and Active Oxygen Species Scavenging. Physiologia Plantarum, 128(3), 496-506.

[34] Mahjabin, S. B., Abidi, A. B. 2015. Physiological and Biochemical Changes During Seed Deterioration: A Review. International Journal of Recent Scientific Research, 6(4), 3416-3422.

[35] Anjum, N. A., Sharma, P., Gill, S. S., Hasanuzzaman, M., Khan, E. A., Kachhap, K., Mohamed, A. A., Thangavel, P., Devi, G. D., Vasudhevan, P., Sofo, A., Khan, N. A., Misra, A. N., Lukatkin, A. S., Singh, H. P., Pereira, E., Tuteja, N. 2016. Catalase and Ascorbate Peroxidase-Representative H_2O_2-Detoxifying Heme Enzymes in Plants. Environmental Science and Pollution Research, 23(19), 19002-19029.

[36] Vashisth, A., Nagarajan, S. 2009. Germination Characteristics of Seeds of Maize (*Zea mays* L.) Exposed to Magnetic Fields under Accelerated Ageing Condition. Journal of Agricultural Physics, 9, 50-58.

[37] Demirkaya, M., Dietz, K. J., Sıvrıtepe, H. O. 2010. Changes in Antioxidant Enzymes During Ageing of Onion Seeds. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38(1), 49-52.

[38] Sahu, A. K., Sahu, B., Soni, A., Naithani, S. C. 2017. Active Oxygen Species Metabolism in Neem (*Azadirachta indica*) Seeds Exposed to Natural Ageing and Controlled Deterioration. Acta Physiologiae Plantarum, 39(9), 197.

[39] Peng, Q. I. N., Zhiyou, K. O. N. G., Xiaohong, L. I. A. O., Yeju, L. I. U. 2011. Effects of Accelerated Aging on Physiological and Biochemical Characteristics of Waxy and Non-Waxy Wheat Seeds. Journal of Northeast Agricultural University, 18(2), 7-12.

[40] Barreto, L. C., Garcia, Q. S. 2017. Accelerated Ageing and Subsequent Imbibition Affect Seed Viability and The Efficiency of Antioxidant System in Macaw Palm Seeds. Acta Physiologiae Plantarum, 39(72), 1-8.

[41] Pallavi, M., Kumar, S. S., Dangi, K. S., Reddy, A. V. 2003. Effect of Seed Ageing on Physiological, Biochemical and Yield Attributes in Sunflower (*Helianthus annuus* L.) cv. Morden. Seed Research-New Delhı-, 31(2), 161-168.

[42] Scialabba, A., Bellani, L. M., Dell'Aquila, A. 2002. Effects of Ageing on Peroxidase Activity and Localization in Radish (*Raphanus sativus* L.) Seeds. European Journal of Histochemistry, 46(4), 351-358.

[43] Tabatabaei, S. A. 2015. The Changes of Germination Characteristics and Enzyme Activity of Barley Seeds Under Accelerated Aging. Cercetari Agronomice in Moldova, 48(2), 61-67.

[44] Zhu, F., Zhou, Y. K., Ji, Z. L., Chen, X. R. 2018. The Plant Ribosome-Inactivating Proteins Play Important Roles in Defense Against Pathogens and Insect Pest Attacks. Frontiers in Plant Science, 9(146), 1-14.