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EFFECTS OF MELATONIN AND TRYPTOPHAN APPLICATIONS ON VIABILITY AND GERMINATION PERFORMANCE OF TOMATO SEEDS DURING AND AFTER ARTIFICIAL AGING

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ABSTRACT

Although the diurnal fluctuations of melatonin (Mel) content in plants and its role in abiotic and biotic stress tolerance are well-documented, little is known about its changes within seeds and its potential effects on seed viability or the aging process. This study aimed to determine how artificial aging, induced by a controlled deterioration test, affects the Mel and tryptophan (Trp) content and seed viability. Furthermore, the study evaluated the effects of Mel and Trp applications on mitigating the impacts of aging in artificially aged seeds. Tomato seeds treated with 250 µM Mel and Trp were artificially aged for up to 8 days through controlled deterioration test after which Mel and Trp changes during ageing and the effect of treatments on seed viability and germination performance was determined. Seeds were also treated with Mel and Trp following artificial ageing in order to determine the effects of Mel and Trp on aged seeds. The positive effects of Mel and Trp applications on seed viability and vigor were particularly evident during and after artificial aging, compared to control seeds. It was observed that in control seeds subjected to controlled deterioration test, Mel and Trp contents exhibited an opposite trend. Applications of Mel and its precursor Trp, before and after artificial aging, significantly slowed down the aging process or alleviated the adverse effects of aging by protecting membrane structures against peroxidation and the accumulation of malondialdehyde (MDA) and H_2O_2. Moreover, indicators of seed deterioration such as electrical conductivity, MDA, and H_2O_2 contents were significantly reduced compared to untreated seeds, while the activities of antioxidant enzymes were boosted. In conclusion, the importance of Mel and Trp applications in preserving seed viability, minimizing storage losses, and slowing seed aging has been demonstrated, suggesting practical applications, particularly in preserving seeds of endangered species or valuable breeding materials.

Keywords: seed aging, controlled deterioration test, seed germination, seed viability, antioxidant enzymes

INTRODUCTION

The acquisition of high-quality seeds and healthy seedlings is a pre-request for successful crop production and cultivation [Wimalasekera 2015, Dwivedi et al. 2021]. The main factors determining seed quality include genetic and physical purity and physiological quality encompassing physical integrity, viability, and vigor. These factors influence the production, development, storage, and transportation of seeds [Hampton 2002, El-Maarouf-Bouteau 2022]. The conditions that seeds experience both before and after harvest are among the main factors affecting seed viability and quality [Dornbos 1995]. Adverse conditions such as relative humidity in storage, temperature, and the presence of oxygen can accelerate the aging process in

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seeds [De Vitis et al. 2020, El-Maarouf-Bouteau 2022]. However, as long as these factors are controlled, long-term storage with minimal loss of viability and vigor is possible [Shelar et al. 2008, Zinsmeister et al. 2020]. The controlled deterioration test has been successfully used by many researchers in recent years to predict field emergence performance and storage longevity, as well as to classify seed lots [Rahman et al. 2019, Zhou et al. 2020, Fatokun et al. 2022]. Today, it has become a recommended vigor test for small-seeded vegetable species by the ISTA Seed Vigor Committee [Powell 2022].

Melatonin (Mel, N-acetyl-5-methoxytryptamine) is an indoleamine whose existence was first identified in the bovine pineal gland by Lerner and colleagues [Lerner et al. 1958]. The discovery of Mel has generated significant interest in the scientific community and has added a new dimension to scientific research. Since its discovery, its presence has been demonstrated in evolutionarily diverse organisms such as unicellular organisms, fungi, algae, bacteria, animals, and plants [Liu et al. 2022]. Additionally, the existence of melatonin has been demonstrated in a wide variety of vegetables, fruits, seeds, grains, medicinal aromatic plants, ornamental, and wild plant species [Madebo et al. 2021, Wu et al. 2021, Altaf et al. 2023a, Muhammad et al. 2024]. In all living organisms, including plants, animals, algae, and bacteria, Mel is synthesized from the amino acid, tryptophan (Trp). Trp serves as the precursor not only for Mel but also for serotonin, a compound found in all plants and animals, and for another plant hormone called indole-3-acetic acid [Khattak et al. 2023, Tiwari et al. 2023]. In plants, Mel acts as a protective antioxidant similar to its role in animals, and it is involved in light/dark signaling. Moreover, Mel plays a significant role in improving tolerance to various environmental stress factors and in the growth and development of plants [Ramasamy et al. 2023, Sharma et al. 2024]. Research on stress tolerance of plants has accelerated with exogenous application of Mel and it has been reported that this molecule improves crop yield by mitigating the negative effects of abiotic stresses on plant growth [Ahmad et al. 2023]. Treatment of plants with Mel have been shown to play a protective role in various species under adverse environmental conditions such as drought [Imran et al. 2021, Altaf et al. 2022], low temperature [Korkmaz et al. 2017a, Korkmaz et al. 2022, Li et al. 2022, Zhang et al. 2023], high temperature [Kuppusamy et al. 2023, Yu et al. 2022], heavy metals [Ali et al. 2023, Altaf et al. 2023b], and salinity [Askari et al. 2023, Guo et al. 2023].

The effective role of Mel in conferring tolerance to various abiotic stress factors in plants has been demonstrated through studies conducted at the seedling or plant tissues. However, limited research exists on the potential effects of Mel on seed storability or seed aging. For instance, studies conducted with pepper, cucumber, and maize seeds have reported that during short-term storage (12 months), Mel content increased in winter months while decreasing in summer months [Kołodziejczyk et al. 2015, Köklü 2016]. On the other hand, Yakupoğlu et al. [2018, 2021] demonstrated that the endogenous Mel and Trp contents of lettuce seeds stored for 24 months changed in a circadian rhythm, and they observed that the Mel and Trp contents changed inversely with each other. That is, during the winter months when Mel content increased, Trp content was detected at its lowest levels. Additionally, similar changes in Mel content were observed in pepper seeds stored for two years, with Mel content increasing during the winter months and decreasing in the summer months. Similarly, in our previous study, we investigated the changes in Mel and its precursor-Trp content in tomato (*Lycopersicon lycopersicum* cv. Rio Grande) seeds stored for an extended period (28 months) under room temperature conditions, which caused significant viability losses [Karaca et al. 2023]. At the end of the research, changes similar to the above-mentioned results were observed in tomato seeds stored for 28 months, highlighting the importance of Mel and Trp applications in preserving viability, minimizing storage losses, and slowing aging in naturally ageing seeds. However, it is not known how the Mel and Trp contents change in artificially aged seeds and to what extent the treatments with these substances affect the deterioration caused by artificial aging. Therefore, in this study, we aim to elucidate the changes in Mel and Trp in artificially aged seeds through controlled deterioration tests and to determine the effects of Mel and Trp applications before and after artificial aging on seed viability. The results obtained from this research are expected to provide us with a better understanding of involvement of Mel and Trp in seed ageing and deterioration.

MATERIALS AND METHODS

Effects of Mel and Trp treatments on tomato seeds before artificial ageing

Plant material and seed treatments. Seeds of Rio Grande tomato (*Lycopersicon lycopersicum*) cultivar were purchased from Istanbul Seed Company, Turkey. Seed moisture content determination was carried out according to ISTA (2005) rules and found to be 8.45%. For Mel and Trp treatments, single layers of tomato seeds (in 100 g batches) were placed in trays between filter papers wetted with 250 µM Mel and Trp solutions and the trays were held at 25 °C in darkness for 24 hours. The concentration of Mel and Trp application (250 µM) was selected based on the results of Karaca et al. [2023]. Dry (untreated) seeds were accepted as control.

Controlled deterioration test. Treated and control seeds were placed between moist paper towels and allowed to imbibe to the weight necessary to reach 24% moisture content. Achievement of the desired weight was determined by periodic weighing. The seeds were then placed in air-tight glass bottles to allow moisture equilibration after which they were subjected to controlled deterioration test. Controlled deterioration test was performed with incubating the bottles at 47 °C in a water bath for varying durations of 0, 2, 3, 4, 6, and 8 days [Basak et al. 2006].

Chemicals and reagents. Mel and Trp along with other chemicals were purchased from Sigma-Aldrich Chemicals. Ten mg Mel and Trp were dissolved in ethanol (1 mL) and distilled water, respectively, then final volume was brought to 10 mL with adding double distilled water to make stock solution. Stock solutions were diluted with the mobile phase [see below] to obtain standard curves when calculating Mel and Trp contents of the seeds. All the measurements and tests were conducted in four replicates.

Measurements and analyzes. To determine the effects of Mel and Trp treatments on tomato seeds aged through controlled deterioration, seed samples were taken at the end of each ageing duration. At the end of each duration, following tests and analyses were conducted.

Extraction and analysis of Mel and Trp were performed according to the method of Korkmaz et al. [2014, 2017a,b] with slight modifications. In brief, 0.25 g of seed and 3 mL of ethyl acetate were shaked in darkness for 17 h at 4 °C in the test tubes. After 20 min of centrifugation at 6,000 *g* and 4 °C the supernatant was transferred to other tubes. The remaining residue was dissolved in methanol (0.5 mL), filtered (0.45 µm), and analyzed with HPLC. To determine Mel and Trp contents HPLC device (Prominance UFLC, Shimadzu) with fluorescence detector and Intersil ODS-2 (250 mm × 4.6 mm) column were used. An excitation and emission wavelength were 280 and 350 nm, respectively. The mobile phase consisted of methanol: 0.1 mM Na_2HPO_4/H_3PO_4 buffer (40 : 60, v/v, pH 4.5) and flow rate was 0.6 mL min^{-1}. The retention times of Trp and Mel were 6.6 and 15.6 min, respectively. Mel and Trp concentrations in each sample were calculated by comparison with the sample peak area with the calibration curves for Mel and Trp. The data obtained were expressed as ng g^{-1} fresh weight (FW).

Tomato seed were germinated in dark in temperature-controlled incubators at 14 ±1 °C (chilling conditions) or 25 ±1 °C (optimum conditions). Four replicates of 50 seeds were placed on filter paper moistened with 5 mL of distilled water in glass petri dishes (9 cm). The appearance of the radicle protrusion (2 mm) was considered sufficient for germination and the number of seeds germinated every day was determined and recorded until the numbers of germinated seeds have stabilized. Final germination percentage (FGP) and mean germination time (MGT) were calculated, from the total number of seed germinated, using Seed Germination v.1.0 software. Because no germination was observed at 14 °C and 25 °C for the seeds that were aged for 4 days and 6 days or more, respectively, FGP and MGT could not be calculated for those treatments.

Seed MDA and H_2O_2 contents were determined according to the method reported in Zhang et al. [2005] and Özden et al [2009], respectively. Additionally, electrical conductivity test was performed according to Vidigal et al. [2011]. For enzyme extractions the method described by Seckin et al. [2010] was followed and total soluble protein contents of the extracts were determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard. Catalase (CAT) and peroxidase (POX) activities were determined by the method of Güneş et al. [2007] and Herzog and Fahimi [1973], respectively.

Statistical analysis. The data were subjected to two factor (ageing duration and treatments) analysis of variance (ANOVA) using SAS statistical software program and least significant difference (LSD) test was used to determine the differences between treatments.

Effects of Mel and Trp applications on artificially aged tomato seeds. In the previous study conducted to investigate the effects of Mel and Trp applications on tomato seeds aged through controlled deterioration, the aging duration that reduced the germination percentage of tomato seeds to 60% at low temperature (14 °C), was determined as 3 days. Therefore, in order to examine the effects of Mel and Trp applications on the viability and germination performance of tomato seed, the seeds were subjected to controlled deterioration test for 3 days as explained previously. Following ageing, unaged seeds and seeds aged for 3 days were treated with 0 μM or 250 μM Mel and Trp after which they were dried back to original moisture content. After drying, seeds were germinated in dark in temperature-controlled incubators kept at 14 ±1 °C (chilling conditions) or 25 ±1 °C (optimum conditions). Also, the above tests and analyses were conducted to determine the effects of Mel and Trp applications on seeds after aging.

Statistical analysis. The data were subjected to two factor (treatments and aging) analysis of variance (ANOVA) using SAS statistical software program and least significant difference (LSD) test was used to determine the differences between treatments.

RESULTS

Effects of Mel and Trp applications on tomato seeds before artificial ageing

Seed Mel and Trp contents. The Mel and Trp contents of tomato seeds subjected to controlled deterioration for different durations after Mel and Trp applications are presented in Table 1. Regarding the effect on seed Mel levels, it was found that one of the two main factors in the study (the treatments) had a statistically significant effect, while the other factor, the aging duration, and the interactions between these factors were found to be non-significant. Upon examining the effect of treatments on seed Mel content, it was observed that Mel application resulted in significantly higher Mel levels compared to both control seeds and seeds treated with Trp. In the seeds treated with Mel, the Mel content was determined to be 5013.72 ng g^{-1} FW, whereas in the control seeds and seeds treated with Trp, it was 2.57 ng g^{-1} FW and 3.03 ng g^{-1} FW, respectively (Tab. 1). Additionally, no significant changes in Mel levels were observed with increasing aging duration; instead, small but insignificant decreases were observed compared to the beginning of aging. There was no significant interaction effect between the two main factors on the seed Mel content. Naturally, seeds treated with Mel exhibited a significantly higher Mel content compared to other treatments; however, statistically, this difference was not found to be significant. At the beginning of the experiment (day 0), the Mel content of seeds treated with Mel was 5295.75 ng g^{-1} FW and it increased slightly as aging progressed but ultimately decreased to 5103.19 ng g^{-1} FW by the end of the study on day 8. Similarly, considering the Mel content of control seeds, it was observed that in seeds aged for 2 days, the Mel content decreased, then increased again, reaching its highest value on day 4, and then decreased again with increasing aging duration (Tab. 1).

When examining the effect of controlled deterioration for different durations on the seed Trp content, it was observed that the Trp levels of Trp-treated seeds were similar to those of control seeds, but lower in seeds treated with Mel. The Trp content was 60.76 ng g^{-1} FW in seeds treated with Trp, 60.64 ng g^{-1} FW in control seeds, and decreased to 45.88 ng g^{-1} FW in seeds treated with Mel. Furthermore, when the Trp levels of the seeds were analyzed depending on different aging periods, it was found that the Trp content increased on the 2nd day of aging, then decreased slightly (on the 3rd and 4th days), but significantly increased with further aging (on the 6th and 8th days) – as in Table 1.

Seed germination. Germination test results conducted at 25 °C revealed that the mean germination time (MGT) of tomato seeds aged through controlled deterioration test for different durations (0, 2, 3, 4, 6 and 8 days) after Mel and Trp treatments increased with the progression of the aging period while FGP decreased (Tab. 2). As no seeds germinated after four days of aging, FGP and MGT could not be calculated for durations exceeding four days; therefore, the re-

Table 1. Mel and Trp contents of tomato seeds subjected to controlled deterioration for different durations after Mel and Trp applications. Values are means ±standard error (SE, n = 4)

Factors	Mel (ng g^{-1} FW)	Trp (ng g^{-1} FW)
Treatments		
Control	2.57 ±0.4 b	60.64 ±10.46 a
Mel	5013.72 ±92.3 a	45.88 ±8.54 b
Trp	3.03 ±0.3 b	60.76 ±11.71a
LSD $_{(0.05)}$	155.5	11.5
Ageing duration-AD (days)		
0	1767.49 ±752.6	15.47 ±2.04 d
2	1583.7 ±682.6	45.35 ±4.25 bc
3	1621.29 ±690.9	32.82 ±1.45c
4	1651.69 ±710.7	30.06 ±2.15 cd
6	1711.76 ±730.1	57.58 ±4.49 b
8	1702.7 ±727.5	153.29 ±13.54 a
LSD $_{(0.05)}$	–	16.2
Treatments*AD		
Control*0	1.48 ±0.05	10.58 ±0.64
Control*2	0.4 ±0.03	51.56 ±3.82
Control*3	1.9 ±0.31	36.56 ±2.41
Control*4	6.13 ±1.33	31.22 ±4.65
Control*6	3.26 ±0.47	75.58 ±4.77
Control*8	2.28 ±0.59	158.32 ±19.12
Mel*0	5295.79 ±75.9	11.11 ±1.59
Mel*2	4748.43 ±343	32.91 ±4.94
Mel*3	4859.43 ±87.7	27.66 ±0.95
Mel*4	4945.26 ±364.6	23.71 ±1.92
Mel*6	5130.21 ±143.8	54.61 ±3.53
Mel*8	5103.19 ±200.4	125.27 ±2.13
Trp*0	5.21 ±0.36	24.71 ±0.34
Trp*2	2.27 ±0.38	51.57 ±9.06
Trp*3	2.52 ±0.30	34.25 ±1.48
Trp*4	3.68 ±0.21	35.25 ±1.69
Trp*6	1.83 ±0.33	42.54 ±0.8
Trp*8	2.64 ±0.3	176.27 ±27.4
ANOVA		
Treatments	***	*
AD	NS	***
Treatments*AD	NS	NS

NS, *, **, ***, not significant, significant at $P < 0.05$, 0.01 or 0.001, respectively

sults of 6 and 8 days of aging were not included in the statistical analysis. Upon examining the effect of treatments on FGP, it was observed that the germination percentage was higher in seeds treated with Mel and Trp (69.6% and 71.3%, respectively) compared to control seeds (66.1%). Furthermore, when the effect of aging duration on the germination percentage of the seeds was considered, it was found that this effect was significant, with a notable decrease in FGP as the duration increased (Tab. 2). The germination percentage, which was 95.8% on day 0 (prior to controlled deterioration testing), decreased progressively with longer aging periods. It was 86.8% after 2 days of aging, 80.0% after 3 days, and 13.3% by the end of the 4th day. As shown in Table 2, the MGT for control seeds was 7.71 days, while it was 7.23 days for seeds treated with Mel and 7.20 days for those treated with Trp. The effect of different aging durations on MGT was significant, with MGT values increasing as the aging duration extended. Initially (day 0), the MGT was 3.67 days, and it increased to 13.22 days by the end of the 4th day. When examining the interaction of treatments with aging duration, it was observed that seeds treated with Mel and Trp generally had lower MGT values compared to control seeds as the aging duration progressed.

The effects of Mel and Trp applications on the germination performance of seeds aged for different durations using controlled deterioration testing at a low temperature (14 °C) are presented in Table 3. Because no germination was observed after more than 3 days of aging, FGP and MGT could not be calculated for these durations. Therefore, the results for 4, 6, and 8 days of aging were not included in the statistical analysis. FGP for control seeds was 75.2%, while it was significantly higher for seeds treated with Mel and Trp, 81.2% and 84.8%, respectively (Tab. 3). As the aging duration increased, FGP at 14 °C was negatively affected. Before the controlled deterioration (day 0), the germination percentage was 93.5%, which decreased to 82.3% after 2 days of aging and further to 65.3% by the end of the third day. The MGT for control seeds was 14.38 days, while for seeds treated with Mel and Trp, it was lower at 13.84 and 13.87 days, respectively (Tab. 3). Examining the effect of different aging durations on MGT at 14 °C revealed that germination speed decreased as the aging duration increased. Initially (day 0), the MGT was 9.76 days, which increased to the highest value of 16.53 days by the end of the second day and then decreased to 15.79 days by the end of the third day. The interaction of the two main factors in the study on FGP and MGT at 14 °C was also statistically significant. It was observed that FGP decreased with increasing the aging duration across all treatments (control, Mel, and Trp), while MGT increased (Tab. 3).

MDA and H_2O_2 contents and membrane permeability (EC). Mel and Trp significantly impacted the MDA and H_2O_2 contents, as well as the EC values, which indicate membrane integrity, during artificial aging of tomato seeds (Tab. 4). The MDA content of control seeds was 397 nmol g^{-1} FW, whereas the seeds treated with Mel and Trp had significantly lower MDA levels (378 nmol g^{-1} FW and 333 nmol g^{-1} FW, respectively). Initially (day 0), the MDA content of the seeds was 322 nmol g^{-1} FW, and significant increases in MDA content were observed as the aging duration progressed. These results indicated that Mel and Trp treatments significantly reduced the MDA contents of the seeds.

The treatments were found to significantly reduce the H_2O_2 content as well (Tab. 4). The H_2O_2 content in control seeds was 340 nmol g^{-1} FW, while seeds treated with Mel and Trp had lower levels (272 nmol g^{-1} FW and 220 nmol g^{-1} FW, respectively) compared to the control. It was observed that the H_2O_2 content of the seeds significantly increased with prolonged aging, reaching its highest level (346 nmol g^{-1} FW) by the 8th day of aging. When examining the effect of the interaction between the treatments and aging duration on H_2O_2 content, it was found that seeds treated with Mel and Trp generally had lower H_2O_2 content compared to the control seeds as aging progressed, and that H_2O_2 content increased with prolonged aging. At the start of the experiment (day 0), the H_2O_2 content of the control seeds was 273 nmol g^{-1} FW, which rapidly increased after 2, 3, and 4 days of aging, reaching the highest values of 381 nmol g^{-1} FW and 406 nmol g^{-1} FW at the end of 6 and 8 days, respectively. A similar pattern was observed in the H_2O_2 content of seeds treated with Mel and Trp; however, the H_2O_2 content of the treated seeds was lower compared to the control seeds (Tab. 4).

Mel and Trp applications resulted in significant decreases in seed EC values, with the lowest EC value obtained from seeds treated with Trp (Tab. 4).

Table 2. Final germination percentage (FGP) and mean germination time (MGT) at 25 °C of tomato seeds subjected to controlled deterioration for different durations after Mel and Trp applications. Values are means ±SE (n = 4)

Factors	FGP $_{25}$ (%)	MGT $_{25}$ (days)
Treatments		
Control	66.1 ±8.4 b	7.71 ±0.93 a
Mel	69.6 ±8.6 a	7.23 ±0.96 b
Trp	71.3 ±8.5 a	7.20 ±0.91 b
LSD $_{(0.05)}$	2.93	0.27
Ageing duration-AD (days)		
0	95.8 ±0.8 a	3.67 ±0.12 d
2	86.8 ±1.5 b	7.09 ±0.16 b
3	80.0 ±1.6 c	5.54 ±0.20 c
4	13.3 ±1.1 d	13.22 ±0.17 a
LSD $_{(0.05)}$	3.38	0.32
Treatments*AD		
Control*0	94.0 ±0.8	4.14 ±0.10 g
Control*2	84.5 ±3.4	6.64 ±0.20 de
Control*3	74.5 ±2.4	6.37 ±0.21 e
Control*4	11.5 ±1.7	13.69 ±0.37 a
Mel*0	97.0 ±1.7	3.55 ±0.1 h
Mel*2	86.5 ±1.0	7.12 ±0.32 cd
Mel*3	82.0 ±1.4	5.02 ±0.06 f
Mel*4	13.0 ±1.9	13.22 ±0.15 ab
Trp*0	96.5 ±1.5	3.30 ±0.08 h
Trp*2	89.5 ±2.9	7.50 ±0.10 c
Trp*3	83.5 ±2.1	5.23 ±0.15 f
Trp*4	15.5 ±2.2	12.75 ±0.17 b
ANOVA		
Treatments	**	***
AD	***	***
Treatments*AD	NS	***

NS, *, **, ***, not significant, significant at P < 0.05, 0.01 or 0.001, respectively

Table 3. Final germination percentage (FGP) and mean germination time (MGT) at 14 °C of tomato seeds subjected to controlled deterioration for different durations after Mel and Trp applications. Values are means ±SE (n = 4)

Factors	FGP $_{14}$ (%)	MGT $_{14}$ (days)
Treatments		
Control	75.2 ±4.8 b	14.38 ±0.83 a
Mel	81.2 ±3.8 a	13.84 ±0.93 b
Trp	84.8 ±2.7 a	13.87 ±1.01 b
LSD $_{(0.05)}$	4.23	0.36
Ageing duration-AD (days)		
0	93.5 ±1.0 a	9.76 ±0.19 c
2	82.3 ±1.7 b	16.53 ±0.15 a
3	65.3 ±2.9 c	15.79 ±0.15 b
LSD $_{[0.05]}$	4.29	0.32
Treatments*AD		
Control*0	92.0 ±1.8 ab	10.53 ±0.11 d
Control*2	78.5 ±3.4 de	16.38 ±0.34 ab
Control*3	55.0 ±1.3 g	16.25 ±0.16 ab
Mel*0	94.5 ±2.1 a	9.57 ±0.11 e
Mel*2	82.5 ±2.2 cd	16.61 ±0.26 a
Mel*3	66.5 ±4.2 f	15.32 ±0.20 c
Trp*0	94.0 ±1.2 a	9.19 ±0.22 e
Trp*2	86.0 ±2.4 bc	16.6 ±0.20 a
Trp*3	74.5 ±2.9 e	15.81 ±0.21 bc
ANOVA		
Treatments	***	**
AD	***	***
Treatments*AD	*	**

NS, *, **, ***, not significant, significant at $P < 0.05$, 0.01 or 0.001, respectively

Additionally, damage to cell membrane integrity increased with prolonged aging, as indicated by the rise in EC values. Initially (day 0), the EC value was 20.01 μS cm^{-1} g^{-1}, which increased with aging and reached the highest value of 48.64 μS cm^{-1} g^{-1} after 8 days of aging. Although the interaction effect between treatment and aging duration on EC values was found to be insignificant, it is evident that seeds treated with Mel and Trp generally had lower EC values compared to control seeds as aging progressed, and EC values increased with prolonged aging.

Catalase (CAT) and peroxidase (POX) enzyme activity. The effects of treatments applied to seeds before controlled deterioration aging on CAT and POX enzyme activities were presented in Table 5. The results indicated that treatments positively influenced the activities of both enzymes. The highest CAT and POX activities were observed in seeds treated with Mel and Trp, whereas the enzyme activities in control seeds were significantly lower. When examining the effects of different aging durations on CAT enzyme activity, there was a significant increase in enzyme

Table 4. MDA, H_2O_2 and EC contents of tomato seeds subjected to controlled deterioration for different durations after Mel and Trp applications. Values are means ±SE (n = 4)

Factors	MDA (nmol g^{-1} FW)	H_2O_2 (nmol g^{-1} FW)	EC (μS $cm^{-1}g^{-1}$)
Treatments			
Control	397 ±9 a	340 h10 a	39.94 ±2.43 a
Mel	378 ±7 b	272 ±8 b	28.82 ±2.24 b
Trp	333 ±9 c	220 ±16 c	25.95 ±2.1 c
LSD (0.05)	15.0	10.0	2.81
Ageing duration-AD (days)			
0	322 ±16 d	199 ±22 f	20.01 ±2.8 e
2	345 ±9 c	230 ±19 e	24.21 ±2.16 d
3	355 ±8 c	259 ±15 d	26.38 ±1.58 cd
4	376 ±13 b	302 ±12 c	30.03 ±2.22 c
6	399 ±8 a	327 ±12 b	40.15 ±2.64 b
8	417 ±10 a	346 ±14 a	48.64 ±2.05 a
LSD (0.05)	22.0	14.0	3.98
Treatments*AD			
Control*0	351 ±18	273 ±10 fg	30.91 ±5.09
Control*2	367 ±14	304 ±7 de	32.75 ±0.97
Control*3	380 ±8	320 ±13 d	33.25 ±0.96
Control*4	405 ±25	355 ±9 c	34.55 ±5.34
Control*6	424 ±14	381 ±2 b	50.9 ±2.38
Control*8	452 ±9	406 ±8 a	57.29 ±1.04
Mel*0	358 ±11	223 ±6 ij	15.04 ±0.4
Mel*2	353 ±14	233 ±7 hi	20.89 ±2.66
Mel*3	361 ±8	253 ±6 gh	24.14 ±0.85
Mel*4	380 ±21	292 ±4 ef	30.64 ±1.51
Mel*6	401 ±3	312 ±2 de	36.81 ±2.89
Mel*8	414 ±20	319 ±16 d	45.38 ±2.58
Trp*0	258 ±14	101 ±14 l	14.09 ±0.65
Trp*2	315 ±10	153 ±8 k	18.99 ±2.46
Trp*3	324 ±5	203 ±8 j	21.74 ±1.15
Trp*4	344 ±11	260 ±5 g	24.88 ±2.76
Trp*6	372 ±2	289 ±1ef	32.74 ±1.54
Trp*8	386 ±3	311 ±12 de	43.23 ±0.32
ANOVA			
Treatments	***	***	***
AD	***	***	***
Treatments*AD	NS	***	NS

NS, *, **, ***, not significant, significant at P < 0.05, 0.01 or 0.001, respectively

Table 5. CAT and POX enzyme activity of tomato seeds subjected to controlled deterioration for different durations after Mel and Trp applications. Values are means ±SE (n = 4)

Factors	CAT (U mg^{-1} protein)	POX (U mg^{-1} protein)
Treatments		
Control	0.56 ±0.06 b	0.0067 ±0.001 b
Mel	0.71 ±0.07 a	0.009 ±0.001 a
Trp	0.69 ±0.07 a	0.0082 ±0.001 a
LSD (0.05)	0.06	0.0015
Ageing duration-AD (days)		
0	0.77 ±0.07 b	0.0167 ±0.002 a
2	1.21 ±0.04 a	0.0069 ±0.001b c
3	0.73 ±0.04 b	0.007 ±0.001 bc
4	0.56 ±0.04 c	0.0081 ±0.001 b
6	0.38 ±0.02 d	0.0056 ±0 cd
8	0.29 ±0.01 e	0.0036 ±0 d
LSD (0.05)	0.08	0.0021
Treatments*AD		
Control*0	0.5 ±0.05 hi	0.0114 ±0.002 b
Control*2	1.13 ±0.01 bc	0.0073 ±0.002 c
Control*3	0.6 ±0.03 gh	0.0062 ±0 cde
Control*4	0.42 ±0.02 ijk	0.0057 ±0 cde
Control*6	0.39 ±0.02 i-l	0.0058 ±0 cde
Control*8	0.29 ±0.02 kl	0.0035 ±0.001 de
Mel*0	1.01 ±0.06 cd	0.0238 ±0.002 a
Mel*2	1.22 ±0.1 ab	0.0059 ±0.001 cde
Mel*3	0.71 ±0.03 fg	0.0072 ±0 c
Mel*4	0.6 ±0.08 gh	0.0069 ±0.001 cd
Mel*6	0.43 ±0.05 ij	0.0063 ±0 cde
Mel*8	0.32 ±0.02 jkl	0.0041 ±0 cde
Trp*0	0.78 ±0.06 ef	0.0148 ±0.002 b
Trp*2	1.27 ±0.08 a	0.0073 ±0.002 c
Trp*3	0.88 ±0.03 de	0.0076 ±0.002 c
Trp*4	0.65 ±0.05 fg	0.0115 ±0.002 b
Trp*6	0.31 ±0.02 jkl	0.0047 ±0 cde
Trp*8	0.27 ±0.02 l	0.0031 ±0 e
ANOVA		
Treatments	***	**
AD	***	***
Treatments*AD	***	***

NS, *, **, ***, not significant, significant at P < 0.05, 0.01 or 0.001, respectively

activity after 2 days of aging. However, as aging duration increased, substantial decreases in the activity of this enzyme were observed. For POX enzyme activity, a significant decrease was detected with increasing aging duration, likely due to increased stress effects. At the beginning of the experiment (day 0), POX activity was determined to be 0.0167 U mg^{-1} protein, which decreased to 0.0036 U mg^{-1} protein after 6 and 8 days of aging. When the interaction effects of treatments and aging duration on CAT activity of the seeds was examined, it was determined that the highest CAT enzyme activity was measured in seeds treated with Mel and Trp after the 2nd day of aging, while the lowest activity was observed in all treatments aged for 6 and 8 days. Similarly, POX activity was highest at the beginning of aging across nearly all treatments; however, as aging progressed, significant decreases in POX activity were observed.

Effects of Mel and Trp applications on artificially aged tomato seeds

Seed Mel and Trp contents. It has been determined that aging did not have a significant effect on the levels of Mel and Trp in seeds (Tab. 6). However, the treatments applied significantly affected the Mel and Trp contents of the seeds, Mel and Trp levels increasing with Mel and Trp treatments, respectively. There was no significant interaction observed between aging and the treatments on the Mel and Trp levels in seeds.

Seed germination. The statistical analysis revealed significant effects of the main factors (aging factor and treatments) on seed germination at 14 °C (Tab. 7).

Table 6. Mel and Trp contents of tomato seeds after Mel and Trp treatments following artificial aging. Values are means ±SE (n = 4)

Factors	Mel (ng g^{-1} FW)	Trp (ng g^{-1} FW)
Ageing		
– (Non-Aged)	1756.23 ±745.22	24.09 ±3.30
+ (Aged)	1743.17 ±738.40	28.61 ±3.53
LSD $_{(0.05)}$	89.2	7.51
Treatments		
Control	6.09 ±2.04 b	22.60 ±4.43 b
Mel	5226.76 ±59.64 a	20.25 ±2.32 b
Trp	16.24 ±1.41 b	36.21 ±3.35 a
LSD $_{(0.05)}$	109.2	9.20
Ageing*Treatments		
Non-Aged*control	0.88 ±0.26	26.95 ±7.67
Non-Aged*Mel	5250.77 ±53.96	14.95 ±1.77
Non-Aged*Trp	17.03 ±2.61	30.38 ±3.82
Aged*control	11.31 ±1.09	18.25 ±4.49
Aged*Mel	5202.76 ±115.33	25.54 ±1.80
Aged*Trp	15.45 ±1.43	42.04 ±3.88
ANOVA		
Ageing	N.S.	N.S.
Treatments	***	**
Ageing*Treatments	N.S.	N.S.

NS, *, **, ***, not significant, significant at $P < 0.05$, 0.01 or 0.001, respectively

Regarding the effect of aging factor, seeds aged for three days exhibited lower germination percentages compared to seeds without any aging treatment. While the germination percentage of non-aged tomato seeds was 90.8%, germination of seeds aged for three days was 73.0%. Furthermore, the germination percentage in control seeds was 75.3%, whereas seeds treated with Mel and Trp exhibited significantly higher germination rates compared to control seeds (86.5% and 84.0%, respectively). The interaction of two main factors on FGP at 14 °C was also found to be significant, and in all treatments (control, Mel, and Trp), seed germination decreased with the effect of aging. The germination percentage in control seeds without any aging treatment was 88.5%, whereas seeds treated with Mel and Trp showed similar percentages compared to control seeds (93.0% and 91.0%, respectively). However, in control seeds aged for three days, the germination percentage was 62.0%, whereas seeds treated with Mel and Trp had statistically significantly higher percentages compared to control seeds (80.0% and 77.0%, respectively). After the germination test conducted at low temperature (14 °C), the MGT of non-aged seeds was 9.19 days, which increased to 14.97 days when the seeds were aged for three days (Tab. 7). Additionally, the MGT was 12.72 days in control seeds, while in seeds treated with Mel and Trp, it was lower compared to control seeds (11.69 days and 11.83 days, respectively). However, the combined effect of aging and treatments on the MGT of seeds at 14 °C was found to be insignificant.

Table 7. Final germination percentage (FGP) and mean germination time (MGT) of tomato seeds at 14 °C and 25 °C after Mel and Trp treatments following artificial aging. Values are means ±SE (n = 4)

Factors	FGP_{25} (%)	MGT_{25} (days)	FGP_{14} (%)	MGT_{14} (days)
Ageing				
– (Non-Aged)	92.0 ±1.4 a	3.56 ±0.1 b	90.8 ±0.7 a	9.19 ±0.2 b
+ (Aged)	80.6 ±1.4 b	6.50 ±0.2 a	73.0 ±2.8 b	14.97 ±0.2 a
$LSD_{(0.05)}$	3.90	0.22	3.70	0.40
Treatments				
Control	83.3 ±2.5	5.49 ±0.6 a	75.3 ±5.1 b	12.72 ±1.1 a
Mel	88.3 ±2.5	4.64 ±0.5 c	86.5 ±2.8 a	11.69 ±1.1 b
Trp	87.5 ±2.8	4.98 ±0.6 b	84.0 ±3.1 a	11.83 ±1.1 b
$LSD_{(0.05)}$	4.79	0.27	4.53	0.49
Ageing*Treatments				
Non-Aged*control	89.5 ±1.3	3.95 ±0.2 d	88.5 ±0.9 a	9.72 ±0.1
Non-Aged*Mel	92.5 ±3.1	3.36 ±0.1 e	93.0 ±0.6 a	8.91 ±0.3
Non-Aged*Trp	94.0 ±2.6	3.37 ±0.1 e	91.0 ±1.3 a	8.93 ±0.2
Aged*control	77.0 ±1.3	7.02 ±0 a	62.0 ±2.2 c	15.72 ±0.3
Aged*Mel	84.0 ±2.9	5.91 ±0.1 c	80.0 ±2.9 b	14.48 ±0.2
Aged*Trp	81.0 ±1.7	6.58 ±0.2 b	77.0 ±3.4 b	14.73 ±0.2
ANOVA				
Ageing	***	***	***	***
Treatments	N.S.	***	***	**
Ageing*Treatments	N.S.	*	**	N.S.

NS, *, **, ***, not significant, significant at $P < 0.05$, 0.01 or 0.001, respectively

When the germination performance of seeds under optimum conditions (25 °C) was examined, it was observed that only the aging factor significantly affected the germination percentage, whereas the interaction with treatments did not have a significant effect (Tab. 7). The data revealed that seeds aged for three days exhibited a lower germination percentage (80.6%) compared to non-aged seeds (92.0%). Similarly, aging tomato seeds for 3 days significantly affected their germination rate under optimal conditions and resulted in slower germination compared to non-aged seeds. The MGT determined as 3.56 days for non-aged seeds increased significantly to 6.50 days for seeds aged for three days. The applications significantly influenced the MGT of the seeds, with the lowest MGT observed in seeds treated with Mel and the highest in control seeds (Tab. 7). Moreover, the interaction between the two main factors was also found to be significant, indicating that in all treatments (control, Mel, and Trp), the MGT of the seeds increased due to the effect of aging.

MDA, H_2O_2 and EC contents. It has been determined that the aging factor did not have a significant effect on the MDA content of the seeds, and both aged and non-aged seeds exhibited similar MDA contents (Tab. 8). However, the treatments applied significantly affected the MDA content, with Mel and Trp treated seeds having lower MDA contents compared to the control seeds (252 nmol g^{-1} FW and 286 nmol g^{-1} FW, respectively, compared to 390 nmol g^{-1} FW in control

Table 8. MDA H_2O_2 and EC contents of tomato seeds after Mel and Trp treatments following artificial aging. Values are means ±SE (n = 4)

Factors	MDA (nmol g^{-1} FW)	H_2O_2 (nmol g^{-1} FW)	EC (µS $cm^{-1}g^{-1)}$
Ageing			
– (Non-Aged)	293 ±33.9	286 ±48.5 b	30.31 ±4.77 b
+ (Aged)	326 ±20.9	475 ±76.6 a	39.67 ±5.84 a
LSD $_{(0.05)}$	72.0	121.0	6.84
Treatments			
Control	390 ±33.8 a	620 ±85.5 a	57.80 ±3.82 a
Mel	252 ±9.2 b	244 ±44.4 b	22.33 ±3.24 b
Trp	286 ±34 b	278 ±40.3 b	24.84 ±2.57 b
LSD $_{(0.05)}$	88.0	149.0	8.38
Ageing*Treatments			
Non-Aged*control	376 ±70.9	481 ±72.3	51.28 ±4.37
Non-Aged*Mel	230 ±6.9	198 ±39.8	20.54 ±2.63
Non-Aged*Trp	275 ±62.2	180 ±8.6	19.10 ±2.12
Aged*control	405 ±12.9	759 ±126.7	64.31 ±4.53
Aged*Mel	275 ±3.2	291 ±78.6	24.12 ±6.32
Aged*Trp	298 ±37.9	377 ±32.4	30.57 ±2.11
ANOVA			
Ageing	N.S.	***	*
Treatments	*	***	***
Ageing*Treatments	N.S.	N.S.	N.S.

NS, *, **, ***, not significant, significant at P < 0.05, 0.01 or 0.001, respectively

Table 9. CAT and POX enzyme activities of tomato seeds after Mel and Trp treatments following artificial aging. Values are means ±SE (n = 4)

Factors	CAT (U mg^{-1} protein)	POX (U mg^{-1} protein)
Ageing		
– (Non-Aged)	0.48 ±0.03 a	0.0086 ±0.001
+ (Aged)	0.36 ±0.02 b	0.0077 ±0.001
LSD $_{(0.05)}$	0.06	0.02
Treatments		
Control	0.39 ±0.04 b	0.0073 ±0.001
Mel	0.48 ±0.04 a	0.0091 ±0.001
Trp	0.39 ±0.02 b	0.008 ±0.001
LSD $_{(0.05)}$	0.1	0.03
Ageing*Treatments		
Non-Aged*control	0.49 ±0.03 ab	0.0077 ±0.001
Non-Aged*Mel	0.55 ±0.05 a	0.0097 ±0.001
Non-Aged*Trp	0.40 ±0.02 bcd	0.0084 ±0.002
Aged*control	0.30 ±0.03 d	0.0068 ±0.002
Aged*Mel	0.41 ±0.03 bc	0.0086 ±0.002
Aged*Trp	0.39 ±0.04 cd	0.0075 ±0.001
ANOVA		
Ageing	***	N.S.
Treatments	*	N.S.
Ageing*Treatments	*	N.S.

NS, *, **, ***, not significant, significant at $P < 0.05$, 0.01 or 0.001, respectively

seeds). The results presented in Table 8 indicated that the two main factors significantly affected the H_2O_2 content. In non-aged seeds, the measured H_2O_2 content (286 nmol g^{-1} FW) was significantly lower compared to those aged for three days (475 nmol g^{-1} FW). Additionally, it has been determined that the treatments significantly reduced the H_2O_2 content. In the control seeds, the H_2O_2 content was 620 nmol g^{-1} FW, whereas in seeds treated with Mel and Trp, the H_2O_2 content was lower (244 nmol g^{-1} FW and 278 nmol g^{-1} FW, respectively). Regarding the amount of membrane leakage, the EC value significantly increased from 30.31 μS $cm^{-1}g^{-1}$ in non-aged seeds to 39.67 μS $cm^{-1}g^{-1}$ in seeds aged for three days. Additionally, the EC value in control seeds was 57.80 μS $cm^{-1}g^{-1}$, while in Mel and Trp treated seeds, it was significantly lower (22.33 $cm^{-1}g^{-1}$ and 24.84 μS $cm^{-1}g^{-1}$, respectively). Furthermore, the interactions between the main factors (aging and treatments) were found to be insignificant for all three parameters.

Catalase (CAT) and peroxidase (POX) enzyme activity. It has been observed that aging for three days significantly affected the CAT enzyme activity of the seeds, reducing the activity from 0.48 U mg^{-1} protein in non-aged seeds to 0.36 U mg^{-1} protein with aging (Tab. 9). Furthermore, Mel and Trp treatments also significantly affected the CAT enzyme activity and the highest activity of 0.48 U mg^{-1} protein was observed in seeds treated with Mel. In contrast, seeds treated with Trp (0.39 U mg^{-1} protein) and control seeds (0.39 U mg^{-1} protein) exhibited similar enzyme activities. Additionally, significant interaction effects between the main factors affecting CAT activity were observed, except for Trp treatments, where aging

was found to reduce CAT enzyme activity. Regarding another stress enzyme, peroxidase (POX), it was determined that aging and treatments individually or together did not have a significant effect on its activity (Tab. 9).

DISCUSSION

In this study, the effects of changes in Mel and Trp contents in artificially aged tomato seeds through controlled deterioration tests, as well as the effects of Mel and Trp applications before and after aging on seed viability and germination performance, have been elucidated. Seeds treated with Mel and Trp prior to controlled deterioration exhibited higher Mel content compared to control seeds. Additionally, extending the aging period did not result in significant changes in Mel levels; rather, minor, and insignificant fluctuations were observed compared to pre-aging levels.

Mel plays various roles throughout all stages of plant development, including regulating the circadian rhythm [Korkmaz et al. 2017a,b, Yakupoğlu et al. 2021], promoting plant growth and development [Muhammad et al. 2024], and affecting processes from seed aging and germination to seedling growth and fruit ripening [Karaca et al. 2022, Sharma et al. 2024]. The content of Mel in plant tissues is significantly influenced by growing conditions, growth stages, environmental factors, and even the time of day when samples are taken [Arnao and Hernandez-Ruiz 2020, Korkmaz et al. 2022]. Studies on Mel in plants indicate that while this molecule is present continuously, its quantity fluctuates throughout the day, with synthesis rates increasing during darkness [Chang et al. 2021, Gao et al. 2023]. Moreover, the timing of sampling throughout the year is also crucial for determining Mel levels in plant tissues [Yakupoğlu 2016]. For instance, researchers have reported significant seasonal variations in Mel levels in eggplant plant throughout its life cycle [Korkmaz et al. 2017b]. Seasonal changes have also been observed during the 1-year storage of pepper [Korkmaz et al. 2018], corn, and cucumber seeds [Kołodziejczyk et al. 2015]. Additionally, similar results have been found in tomato seeds stored for 28 months [Karaca et al. 2023]. Researchers have suggested that increases in melatonin levels observed during winter months in all four species could indicate an evolutionary acquisition of a defense mechanism to protect the seeds against adverse environmental conditions. Mel is a potent free radical scavenger [Fathi et al. 2023, Muhammad et al. 2022], and high Mel levels serve as an antioxidant source in seeds freshly separated from the parent plant. In short, increased Mel levels under adverse environmental conditions act as a defense mechanism in seeds.

When examining the changes in Trp content, it was observed that seeds treated with Trp had significantly higher tryptophan content, reaching 176.27 ng g^{-1} FW on the 8th day compared to control seeds and other treatments. Additionally, seeds treated with Mel had lower tryptophan content (125.27 ng g^{-1} FW) compared to those treated with Trp on the 8th day. Additionally, an increase in Trp content was observed with aging. The variation in Trp content exhibited generally opposite changes compared to fluctuations in Mel content; that is, when Trp content generally increased in all treatments, Mel content tended to decrease. The results of this study show a similar relationship to the Mel content in tomato seeds stored for 28 months, where Mel levels peaked at the beginning of storage (0 months) and remained at low levels at 12 months (August 2018) and 24 months (August 2019), while Trp content showed increases parallel to the decreases in Mel levels [Karaca et al. 2023]. Similarly, Yakupoğlu et al. [2021] demonstrated that the endogenous Mel and Trp content in lettuce seeds stored for 24 months changed in a circadian rhythm, with Mel content and Trp content varying inversely with each other.

Mel is synthesized from the amino acid Trp through different pathways, with involvement of various enzymes during this synthesis process [Khattak et al. 2023]. Negri et al. [2021] have indicated that enzymes facilitating the transformation of molecules during Mel biosynthesis play a significant role in the changes observed in Mel and Trp levels. In particular, increased activity of certain enzymes along this pathway may enhance the biosynthesis of molecules, but excessive production of some molecules can lead to their accumulation in tissues, thereby negatively affecting the synthesis of other molecules. For instance, the conversion of Trp into serotonin occurs at much higher rates compared to serotonin's conversion into Mel, resulting in generally lower Mel levels observed in tissues. Additionally, the concentration of one mol-

ecule applied may suppress the other [Back 2021], or seeds may not require the biosynthesis of precursor molecules (e.g. lower Trp levels when Mel content is high), all of which could also vary by species or variety.

Treatments with Mel and Trp before controlled deterioration reduced the damage caused by aging and improved germination performance and MGT at both temperatures (14 °C and 25 °C). Moreover, in the continuation of this study, treating the tomato seeds artificially aged under controlled deterioration conditions (3 days at 47 °C with 24% humidity) with Mel and Trp also enhanced seed viability, FGP and MGT. Additionally, Mel and Trp treatments in both studies slowed down seed aging and improved germination performance compared to control seeds. It is well known that controlled deterioration test is a stress test developed to reveal vigor differences among seed lots of small-seeded vegetable species and it is successfully used in predicting field emergence performance, assessing storage life, and classifying the seed lots. For instance, Ermiş et al. [2015] subjected 12 tomato seed lots to controlled deterioration tests at 47 °C and 49 °C with 24% humidity for up to 120 hours and they suggested that early seedling emergence count could be used as an alternative and faster method to assess viability after controlled deterioration. Njie [2015] artificially aged seeds of various vegetable species (tomato, onion, cabbage, lettuce, and carrot) for 2 and 4 months under various temperature and humidity conditions and reported that seed viability decreased by 50% in almost all species after 2 months of storage at 27 °C and 60% relative humidity. It was also noted in the studies that as the aging period increased, the rate of deterioration in seeds also increased. Furthermore, in many studies, priming treatments have been suggested to act as a metabolic repair system that delays aging by organizing cellular structures and cell membranes [Tilden and West 1985].

Although detailed information about the role of Mel and Trp in seed aging is limited, recent studies have documented that Mel and Trp applications enhance germination performance of seeds under stressful conditions. For instance, Korkmaz et al. [2017a] indicated that exogenous Mel applications on pepper improved germination and emergence performance under chilling stress compared to control treatments. In a study investigating the effect of endogenous Mel content on seed germination under chilling stress, it was found that genotypes with high Mel content exhibited significantly better seed germination and seedling emergence under chilling stress conditions [Korkmaz et al. 2022]. García-Cánovas et al. [2024] studied the effects of Mel on germination and seedling growth in in aging sorghum seeds and reported that exogenous application of Mel in aging seeds had a biostimulator effect. Kolupaev et al. [2024] reported that exogenous Mel treatments increased the germination of 4-year-old triticale and rice seeds and this effect was linked to melatonin's regulation of the antioxidant system. Furthermore, it has been reported that Trp applications to pepper seeds under salt stress conditions positively affected germination and seedling emergence performance [Korkmaz et al. 2020]. The results of current research also clearly demonstrate the beneficial effects of Trp and Mel on the germination performance of artificially-aged seeds under stressful conditions.

Lipid structures in cell membranes undergo changes due to oxidation, resulting in alterations in membrane integrity, fluidity, permeability, and structure [Kravić et al. 2021, Li et al. 2022]. Lipids are oxidized by free radicals like H_2O_2, leading to the formation of detrimental products such as MDA. Therefore, determining the levels of H_2O_2 and MDA present in plant tissues and organs provides information about the integrity and functionality of membranes. Additionally, with aging, the permeability (conductivity) of cell membranes that facilitate the exchange of substances increases. Increased membrane permeability means that substances within the seed can leak uncontrollably into the external environment during water uptake, which is an undesired situation. The method used to test this phenomenon, electrical conductivity (EC) testing, is crucial for determining the relationship between aging, viability, and vigor in seeds [Özmen and Kenanoğlu 2024]. Our results have shown that EC, MDA, and H_2O_2 values increased parallel to the aging duration. Furthermore, seeds that did not undergo any aging process had lower EC, MDA, and H_2O_2 values compared to seeds aged for three days. Moreover, seeds treated with Trp and Mel both before and after aging generally exhibited lower EC, MDA, and H_2O_2 levels than control seeds. Similarly, it has been reported that lettuce seeds treated with Mel during a 2-year storage period had lower MDA and H_2O_2 contents compared to control seeds, and during this period, MDA and H_2O_2

levels in seeds were generally lowest when Mel levels were highest [Yakupoğlu et al. 2021]. Köklü [2016] reported that Mel significantly reduced EC, MDA, and H_2O_2 contents in stored seeds compared to untreated seeds. Karaca et al. [2023] also observed that Mel and Trp applications before storage significantly lowered the EC, MDA, and H_2O_2 contents of seeds.

Mel has been reported to directly play a role as an antioxidant in balancing membrane fluidity and lipid peroxidation in biological membranes such as mitochondria, chloroplasts, and plasma membranes, and it is effective in combating stress factors [Chrustek and Olszewska-Słonina 2021, Golding and Lee 2023]. Moreover, Mel regulates and enhances the activities of antioxidant enzymes such as POX, GR (glutathione reductase), SOD (superoxide dismutase), and CAT in plants under stress conditions [Colombage et al. 2023]. In this study, it was observed that pre-treatments before controlled deterioration increased CAT and POX enzyme activities. Additionally, applications of Mel and Trp to tomato seeds artificially aged through controlled deterioration tests resulted in positive improvements in CAT enzyme activity. Furthermore, higher CAT enzyme activity determined in seeds treated with Mel and Trp compared to control seeds is a strong indication that these treatments enhanced the activity of this enzyme. The results of this study are consistent with positive outcomes observed in various species studied, such as lettuce [Yakupoğlu et al. 2021], pepper [Korkmaz et al. 2017a], melon [Castanares and Bouzo 2019], and tomato [Karaca et al. 2023], where Mel applications have been shown to increase the activation of antioxidant enzymes and improve seed germination performance.

CONCLUSION

In this study, the changes in the viability of seeds artificially aged through controlled deterioration following Mel and Trp applications have been demonstrated for the first time. Furthermore, the effects of Mel and Trp applications on seeds aged through controlled deterioration have been identified to alleviate the impacts of aging. The positive effects of Mel and Trp applications on seed viability and vigor have been clearly observed, especially when compared to control seeds. Additionally, due to the stress effects in artificial aging, aging occurred more rapidly due to deformations in the seeds; however, the applications mitigated the severity of damage caused by aging. In controlled deterioration studies, it was found that control seeds without any treatment showed contrasting changes in Mel content compared to Trp content. Specifically, an increase in Mel content in artificially aged control seeds corresponded to a decrease in Trp content. Additionally, applications of Mel and Trp before and after artificial aging have significantly slowed down the aging process or alleviated the negative effects caused by aging by protecting membrane structures against peroxidation, electrolyte leakage, MDA, and H_2O_2 accumulation. Moreover, Mel and Trp applications have been observed to positively enhance the activities of antioxidant enzymes (CAT, POX). In conclusion, it is clear that pre-treatment with Mel and Trp, which serves as a precursor in Mel synthesis, can be a valuable tool to slow down seed aging. This is particularly important for the long-term storage of seeds of endangered species or valuable reproductive materials, highlighting the important practical applications.

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