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Melatonin and tryptophan effects on tomato seed deterioration during long-term storage

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ABSTRACT

Since the discovery of the melatonin, comprehensive research has been conducted to reveal the physiological role of melatonin and changes in melatonin contents in different plant species. Although variations in melatonin content of a plant within a day and roles of melatonin in tolerance to abiotic and biotic stressors were well-documented, there is not much information about fluctuations in seed melatonin and its pre-cursor-tryptophan contents and their potential effects on seed viability or ageing process. This study was conducted to unveil the changes in melatonin and tryptophan contents of tomato seeds stored for 28 months and to determine potential effects of pre-storage melatonin and tryptophan treatments on seed viability or ageing process. Tomato seeds were treated with melatonin and tryptophan (0 μM or 250 μM) for one day before storage and then stored at room temperature (25 °C) for up to 28 months. Effects of pre-storage melatonin and tryptophan treatments on seed quality and ageing were investigated through various tests and analyses. Seed melatonin contents changed significantly during the storage, reached the highest level at 12th and 24th months of storage, which corresponds to August 2018 and August 2019 while tryptophan levels exhibited an opposite trend during the months when melatonin peaked strongly. These results indicated that higher seed melatonin content in August is a strategy that tomato seeds have in order to ensure their life after leaving the mother plant and to establish their next generation. Pre-storage treatments reduced the ageing-induced damage significantly by lowering the seed MDA and H_2O_2 contents and improved germination performance especially at low temperature. Thus, melatonin and tryptophan treatments could be used as an effective tool to preserve seed viability, minimize storage losses and slow down the ageing process, which could have practical implications especially for long-term storage of seeds of endangered species or valuable breeding materials.

1. Introduction

Success in crop production and obtaining high yields largely depend on the use of quality seeds. Seed quality is defined as the sum of all the characteristics that determine the performance of seed lots. The main factors designating seed quality include genetic and physical purity and physiological quality, which includes the concepts of physical integrity, viability and vigor. These factors affect the production, development, storage and transportation of seeds (El-Maarouf-Bouteau, 2022; Domergue et al., 2019). Quality loss starts while the seed is still on the mother plant and covers the whole process from harvest to processing and from storage to planting in the field, but the highest quality losses occur during the storage (Ray and Bordolui, 2022; Bewley et al., 2013). When the seeds are stored under unsuitable conditions such as high humidity and temperature, aging occurs rapidly as a result of the oxidation of fatty acids and phospholipids by free radicals formed in the seed (Wang et al., 2022). Seed moisture and temperature are the two most important factors governing seed viability and storage life and changes in these factors determine the life of the seed (Domergue et al., 2019). For instance, according to Harington's rule, at moisture contents of between 5% and 14% and storage temperatures of between at 0 °C and 40 °C, every 1% decrease in seed moisture or 5.5 °C decrease in temperature the storage life of a seed is doubled (Harrington, 1973). Increasing the seed moisture content stimulates lipid peroxidation caused by reactive oxygen species. Temperature affects the rate of biochemical and physiological processes and high temperatures increase the rate of reactions that accelerate the deterioration of the seed (Wang et al., 2022). Therefore, low humidity and temperature are vital for a long storage life of the seeds.

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Melatonin (*N*-acetyl-5-methoxytryptamine), an indolamine, was first isolated from bovine adrenal gland more than 60 years ago (Lerner et al., 1958) and since its discovery, its existence has been proven in protozoa, fungi, algae, bacteria, animals and plants, which are organisms highly different from each other evolutionarily (Liu et al., 2022). Melatonin in plants was first found in 1995 as a result of studies conducted independently by two separate study groups (Dubbels et al., 1995; Hattori et al., 1995). The presence of highly variable amounts of melatonin has been detected in the seeds, fruits, leaves and roots of many plant species, including vegetables, fruits, seeds, cereals, medicinal and aromatic plants (Wu et al., 2021; Arnao and Hernández-Ruiz, 2020). The synthesis of melatonin in all living organisms starts from the amino acid tryptophan. Tryptophan is the precursor of not only melatonin, but also serotonin and a phytohormone found in all plants and animals, indole-3-acetic acid (IAA) (Rajora et al., 2022; Corpas et al., 2021). In addition to being a broad-spectrum antioxidant in all organisms, melatonin also acts as a photoperiodic regulator or a 24 h rhythm regulator. Moreover, melatonin plays an important role in increasing tolerance to various environmental stress factors and in the growth and development of plants (Fan et al., 2018; Rajora et al., 2022). The high content of melatonin found in plants living under adverse environmental conditions has accelerated the research on stress tolerance through the exogenous application of this molecule to plants and mitigated the negative effects of stress. Melatonin treatments played a protective role against various adverse environmental conditions such as high temperature (Martinez et al., 2018), low temperature (Korkmaz et al., 2017a), drought (Yang et al., 2019), heavy metals (Lei et al., 2021) and salinity (Bahcesular et al., 2020).

Studies revealing the effects of melatonin on seed storability or seed aging generally focused on the changes during short-term (1 year) storage. For instance, in studies conducted with pepper, cucumber and corn seeds, it was reported that melatonin content increased in winter months and decreased in summer months during one year of storage (Kołodziejczyk et al., 2015; Köklü, 2016). On the other hand, there is very limited number of studies on the changes in melatonin content of seeds stored for longer than one year. Yakupoğlu et al. (2021) documented that the endogenous melatonin and tryptophan contents of lettuce seeds stored for 24 months exhibited a circadian rhythm and that melatonin and tryptophan contents changed in opposite directions. In other words, seed melatonin content reached its peaks in winter months while the tryptophan level was at its lowest. In addition, similar changes in melatonin content were observed in pepper seeds stored for two years and seed melatonin content increased in winter months and decreased in summer months of both years (Yakupoğlu et al., 2018). Based on available data and observations, most of the information on melatonin being a broad spectrum antioxidant that enhances stress tolerance come from the investigations conducted at the whole plant level but very little data are available on seasonal fluctuations of seed melatonin and tryptophan contents and their involvement on seed viability or aging during long-term storage. Therefore, in this study, we hypothesized that changes in melatonin and its precursor tryptophan contents in tomato (*Lycopersicon lycopersicum cv.* Rio Grande) seeds during long time storage (28 months) under room temperature conditions would have an impact on seed viability and ageing. Additionally, the effects of exogenous melatonin and tryptophan treatments applied before the storage on tomato seed quality and deterioration were also investigated.

2. Materials and methods

2.1. Plant material and seed treatments

Seeds of "Rio Grande" tomato (*Lycopersicon lycopersicum)* cultivar produced in 2016 were purchased from Istanbul Seed Firm, Turkey. Seed moisture content determination was carried out according to ISTA (2005) rules and found to be 8.45%. For melatonin and tryptophan treatments, single layers of tomato seeds (in 100 g batches) were placed in trays between filter papers wetted with 250 μM melatonin or tryptophan solutions and the trays were held at 25 °C in darkness for twenty four hours. The concentration of melatonin and tryptophan application (250 μM) was selected based on the results of Yakupoğlu et al. (2018, 2021). Dry (untreated) seeds were accepted as control. Following treatment, seeds were washed for one minute under running tap water and dried back to their original moisture content under ventilated room conditions. After drying, the seeds were sealed in individually laminated plastic bags (5 g) covered with aluminum foil and placed at 25 °C (incubator) for 28 months from the beginning of August 2017 to December of 2019. Control seeds (untreated seeds) having the same moisture content were also packaged and stored under the same conditions as mentioned above.

2.2. Chemicals and reagents

Melatonin and tryptophan along with other chemicals were purchased from Sigma-Aldrich Chemicals. One mg melatonin were dissolved in ethanol (1 mL), then final volume was completed double distilled water to 10 mL to make stock solution. Ten mg of tryptophan were dissolved in distilled water and the total volume was completed to 10 mL as mentioned above. Stock solutions were diluted with the mobile phase (see below) to obtain standard curves when calculating melatonin and tryptophan contents of the seeds. All the measurements and tests were conducted in four replicates.

2.3. Measurements and analyzes

Seeds samples were taken every two months (every 4 months for CAT enzyme activity and seed moisture) starting from the beginning of storage to determine seasonal variations in melatonin and tryptophan contents and the impacts of melatonin and tryptophan treatments on seed aging during storage. At the end of each storage time, following testes and analyses were conducted.

2.3.1. Melatonin and tryptophan analysis

Extraction and analysis of melatonin and tryptophan were performed according to the method suggested by Korkmaz et al. (2014, 2017b). In brief, 0.25 g of seed and ethyl acetate (3 mL) were placed in the test tubes the tubes were shaked in darkness for 17 h at 4 °C. The tubes were centrifuged at 6000 g and 4 °C for 20 min after which the supernatant was transferred to another tube. The remaining sample was washed again by adding ethyl acetate (0.5 mL) and the combined washing solutions were evaporated using vacuum concentrator. The remaining residue was dissolved in methanol (0.5 mL), filtered (0.45 μm), and analyzed with HPLC.

Shimadzu brand (Prominance UFLC model) HPLC device with fluorescence detector and Intersil ODS-2 (250 mm x 4.6 mm) column were used for determine melatonin and tryptophan content. 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 tryptophan and melatonin were 6.6 and 15.6 min, respectively. Melatonin and tryptophan concentrations in each sample were calculated by comparison with the sample peak area with the calibration curves for melatonin and tryptophan. The data obtained were expressed as ng g^{-1} fresh weight (FW) of tissues.

2.3.2. Seed germination

Tomato seed were germinated in dark in temperature-controlled incubators kept at 14±1 °C (chilling stress conditions) or 25±1 °C (optimum conditions). Fifty seeds in four replicates were placed on filter paper moistened with distilled water (5 mL) 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. From the total number of seed germinated, final germination percentage (FGP) and mean germination time (MGT) were calculated using Seed germination v.1.0 software.

2.3.3. MDA (malondialdehyde) content

MDA content was determined according to the method reported in Zhang et al. (2005). Seeds (0.25 g) were homogenized in 6 mL 10% trichloroacetic acid and centrifuged at 10,000 g for 15 min. One mL of the supernatant was mixed with 4 mL of 0.6% thiobarbituric acid (TBA). The mixture was boiled at 100 °C for 20 min, cooled at optimum conditions and its absorbance was measured at 532, 600, and 450 nm. The MDA content was calculated using following equation.

$$\text{MDA}\ (\text{nmol g}^{-1}\ \text{FW}) = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

2.3.4. H_2O_2 content

H_2O_2 content was determined using the methods of Özden et al (2009). Seeds (0.25 g) were homogenized in 1% trichloroacetic acid (3 mL) and centrifuged at 10,000 g and 4 °C for 10 min. Subsequently, 0.75 mL of the extract was mixed with 0.75 mL of K-P buffer (pH 7.0) and 1.5 mL of KI after which its absorbance was measured at 390 nm. The content of H_2O_2 was calculated from a standard curve plotted in the range from 1 to 100 nmol mL^{-1} and H_2O_2 concentration was expressed as nmol g^{-1} FW.

2.3.5. Electrical conductivity test

Electrical conductivity test was performed as reported in Vidigal et al. (2011). Fifty seeds in four replicates were placed flasks containing 50 mL of distilled water and the flasks were kept at 25 °C for 24 h in darkness. Subsequently, the seeds were filtered away and the electrical conductivity (EC) of the solution was measured with a Hanna-215 model conductivity meter and the results were expressed as mean μS cm^{-1} g^{-1} FW.

2.3.6. Seed moisture content and catalase enzyme activity

Seed moisture content and catalase (CAT) enzyme activity was determined every four months with the beginning of storage. Moisture determination was made according to ISTA rules (ISTA, 2005) in 4 replications.

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) activity was determined by the method of Güneş et al. (2007). For this purpose, reaction liquid was obtained by adding 50 mM sodium phosphate buffer solution (pH 7.0) containing 0.1 mM EDTA. Afterwards, 70 μL crude enzyme extract, 920 μL reaction liquid with 0.1 mM EDTA and 10 μL 3% H_2O_2 were added into the spectrophotometer cuvette, respectively and the change in the absorbance at 240 nm was recorded. For blank sample, distilled water was used instead of enzyme extract. Enzyme activity was expressed as unit mg^{-1} protein.

2.3.7. Statistical analysis

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

3. Results

3.1. Seed melatonin and tryptophan contents

Melatonin and tryptophan levels of tomato seeds stored for 28 months after melatonin and tryptophan treatments were significantly affected and seasonal fluctuations were observed in the melatonin and tryptophan content of seeds (Table 1). During the storage, time-dependent fluctuations were observed in the melatonin levels of the seeds. The melatonin content of seeds, which was quite high (447.2 ng g^{-1} FW) at the beginning of storage, decreased during the first 6 months, but then increased and reached the highest level of 2018 in the 12th month (August-339.7 ng g^{-1} FW). The seed melatonin content, which initially decreased in the second year of storage, increased again and reached to its highest level (116.7 ng g^{-1} FW) of 2019 in the 24th month of storage (August), but at the end of the study, it slightly decreased to 85.3 ng g^{-1} FW. With melatonin treatments, seed melatonin levels (403.04 ng g^{-1} FW) were found to be significantly higher than the control seeds and seeds treated with tryptophan (1.38 ng g^{-1} FW and 4.59 ng g^{-1} FW, respectively) (Table 1).

As it was in melatonin contents, time-dependent variations were also observed in the tryptophan levels of the seeds during the storage. Seed tryptophan content was quite low at the beginning of storage (32.55 ng g^{-1} FW) but increased significantly especially in the 4th (December 2017, 90.33 ng g^{-1} FW), 8th (April 2018, 105.24 ng g^{-1} FW) and 14th months (October 2018, 61.77 ng g^{-1} FW). In addition, the tryptophan content of the seeds increased again at the end of the 24th month of storage (August 2019, 73.44 ng g^{-1} FW), but decreased at the 28th month (December 2019) and reached to initial levels measured at the beginning of the study. The highest seed tryptophan content was measured at 8th month during the entire storage period. It was observed that pre-storage treatments had significant effects on the tryptophan contents of the seeds and the highest tryptophan content was found in the tryptophan-treated

Table 1
Effect of melatonin and tryptophan treatments during 28 month-long storage on melatonin and tryptophan contents of tomato seeds. Values are means ± SE (n = 4).

Factors	Melatonin (ng g^{-1} FW)	Tryptophan (ng g^{-1} FW)
Storage Time-ST (Months)		
0	447.2±187.8 **a**	32.55±5.62 **gh**
2	136.9±56.1 **c**	84.83±13.68 **b**
4	89.2±37.6 **de**	90.33±18.31 **b**
6	78.0±32.9 **ef**	68.59±5.59 **cd**
8	109.9±47.4 **cde**	105.24±3.54 **a**
10	114.1±48.5 **cde**	75.44±8.42 **c**
12	339.7±143.7 **b**	29.11±6.55 **h**
14	104.5±43.8 **cde**	61.77±4.08 **d**
16	85.2±36.3 **def**	42.98±2.90 **f**
18	51.9±22.0 **f**	52.93±3.29 **e**
20	84.3±36.8 **def**	37.24±7.19 **fg**
22	106.7±45.7 **cde**	65.38±8.58 **d**
24	116.7±49.7 **cd**	73.44±14.9 **c**
26	94.8±39.6 **de**	33.15±1.33 **gh**
28	85.3±36.3**def**	30.77±0.74 **gh**
LSD $_{(0.05)}$	**37.04**	**7.99**
Treatments		
Control	1.38±0.2 **b**	33.52±3.04 **c**
Melatonin	403.04±41.0 **a**	66.44±4.20 **b**
Tryptophan	4.59±0.8 **b**	76.79±4.96 **a**
LSD $_{(0.05)}$	**16.56**	**3.57**
ANOVA		
ST	***	***
Treatments	***	***
ST*Treatments	***	***

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

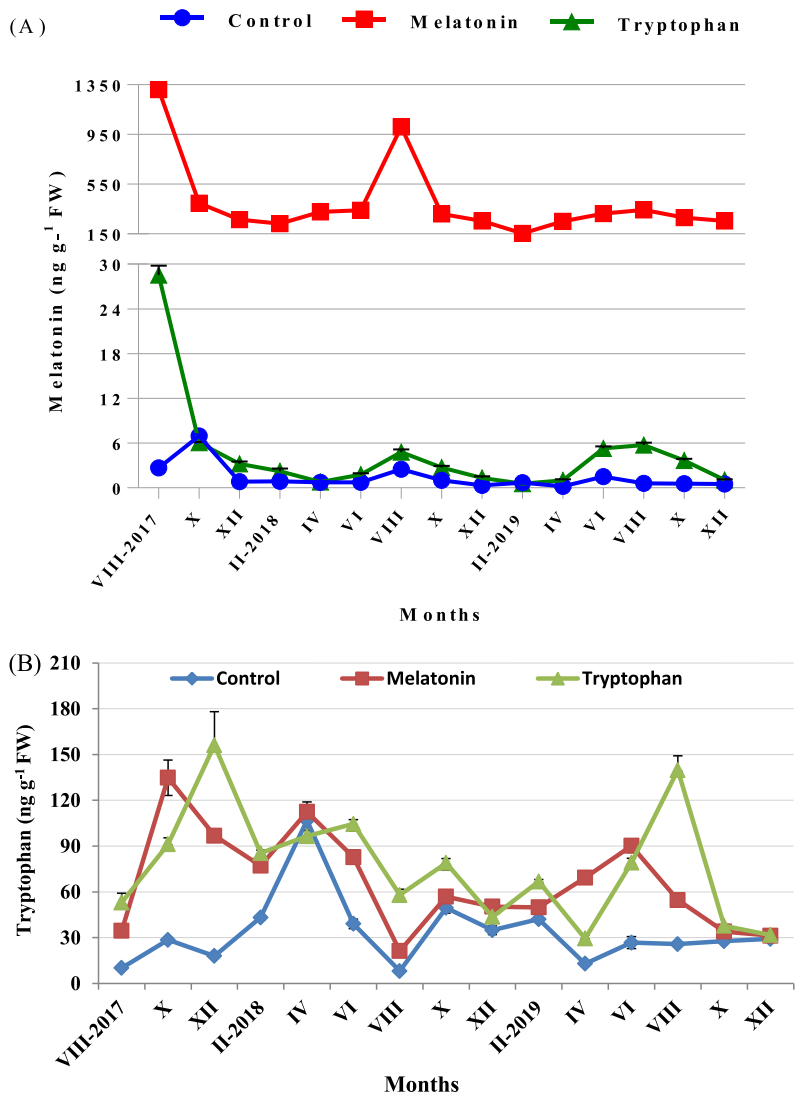

Fig. 1. Changes in melatonin (A) and tryptophan (B) levels of tomato seeds during 28-month storage. The results are expressed as mean values of four measurements ± standard error.

seeds, while the lowest tryptophan content was found in the control seeds (Table 1).

When the fluctuations in melatonin content of seeds in individual treatments were examined, it was seen that seed melatonin contents reached to the highest level at the end of 12th and 24th months of storage, which corresponds to August 2018 and August 2019 (Fig. 1A). In addition, melatonin content of melatonin-treated seeds was found to be much higher than the control seeds and tryptophan-treated seeds. Melatonin content of 1310.5 ng g^{-1} FW (August 2017) of melatonin-treated seeds at the beginning of storage changed throughout the year and decreased to 254.5 ng g^{-1} FW (December 2019) at the end of the 28th month. The melatonin content of control seeds and tryptophan-treated seeds exhibited similar changes. Especially in tryptophan-treated seeds, seed melatonin content, which was found to be quite high at the beginning of storage, decreased with the progress of storage and reached to the level of control seeds, but increased again at the 12th and 24th months of storage and exceeded the melatonin content of the control seeds.

When the changes in the tryptophan content of the seeds stored for 28 months were examined (Fig. 1B), it was observed that the tryptophan content of the control seeds, which was low at the beginning, reached the levels of the seeds treated with tryptophan and melatonin by the 8th month of storage. Afterwards, the tryptophan content, which decreased in all applications, decreased to the lowest level of the year in the 12th month (August 2018), but tryptophan-treated seeds had higher tryptophan content than the other seeds. Then, the seed tryptophan content, which was similar in all treatments until the 18th month of storage, showed different changes depending on treatments and decreased to similar levels at the 28th month, the end of the study.

3.2. Seed germination

Melatonin and tryptophan applications significantly affected the final germination percentage (FGP) and mean germination time (MGT) of tomato seeds at 14 °C and 25°C during 28 months of storage (Table 2). MGT at 14 °C and 25 °C during the storage period up to 28 months increased with increasing storage time, while the FGP decreased. Under chilling stress (14 °C) conditions, FGP, which was 93.0% before storage, decreased to 76.3% after 28 months of storage. In addition, pre-storage melatonin and tryptophan treatments significantly improved FGP of seeds as compared to the control seeds (80.9%) and increased it to 87.3% and 86.2%, respectively. MGT, which was 9.08 days at the beginning of storage, increased to 15.37 days at the end of 28 month-long storage. Additionally, MGT decreased significantly from 14.33 days in control seeds to 11.84 days and

Table 2
Effect of melatonin and tryptophan treatments during 28 month-long storage on final germination percentage (FGP) and mean germination time (MGT) of tomato seeds at 14 °C and 25 °C. Values are means ± SE (n = 4).

Factors	14°C FGP (%)	25°C FGP (%)	14°C MGT (days)	25°C MGT (days)
Storage Time-ST (Months)				
0	93.0±1.0 **a**	95.8±1.3 **a**	9.08±0.9 **g**	2.98±0.14 i
2	91.2±0.8 **a**	94.3±1.1**ab**	11.12±0.2 **f**	3.06±0.15 **h**i
4	89.0±0.8 **b**	93.7±0.8 **b**	12.10±0.2 **de**	3.18±0.17**gh**
6	88.3±1.4 **bc**	93.3±0.7 **b**	11.66±0.4 **ef**	3.30±0.20 **fg**
8	88.5±1.0 **b**	92.3±0.9 **bc**	11.71±0.5 **ef**	3.35±0.16 **f**
10	88.0±0.9 **bc**	92.3±0.6 **bc**	11.61±0.4 **ef**	3.38±0.16 **f**
12	87.3±0.8 **bc**	90.7±1.0 **cd**	11.99±0.5 **de**	3.55±0.16 **e**
14	86.3±0.9 **cd**	89.2±0.7**de**	12.23±0.4 **de**	3.63±0.16 **de**
16	84.3±0.8 **de**	88.0±0.8 **ef**	12.41±0.4 **d**	3.69±0.17**cd**
18	83.0±0.9 **ef**	87.7±0.8 **efg**	13.08±0.3 **c**	3.73±0.18 **cd**
20	81.7±1.2 **f**	86.7±0.9 **fg**	13.57±0.4 **c**	3.81±0.19 **bc**
22	79.3±1.4 **g**	85.8±0.9 **gh**	14.56±0.4 **b**	3.93±0.20 **b**
24	78.0±1.4 **gh**	84.5±1.3 **h**	14.86±0.4 **ab**	4.12±0.25 **a**
26	77.3±1.4**gh**	84.0±1.2 **h**i	15.04±0.4 **ab**	4.12±0.25 **a**
28	76.3±1.6 **g**	82.3±1.3 i	15.37±0.4 **a**	4.13±0.25 **a**
LSD $_{(0.05)}$	**2.02**	**2.04**	**0.64**	**0.14**
Treatments				
Control	80.9±0.9 **c**	86.2±0.7 **b**	14.33±0.2 **a**	4.44±0.07 **a**
Melatonin	87.3±0.6 **a**	91.1±0.5 **a**	11.84±0.3 **b**	3.22±0.04 **b**
Tryptophan	86.2±0.6 **b**	90.8±0.6 **a**	11.90±0.2 **b**	3.13±0.05 **c**
LSD $_{(0.05)}$	**0.90**	**0.91**	**0.28**	**0.06**
ANOVA				
ST	***	***	***	***
Treatments	***	***	***	***
ST*Treatments	NS	NS	**	***

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

11.90 days, respectively, in the seeds treated with melatonin and tryptophan. However, under optimum (25 °C) conditions, the germination percentage, which was 95.8% before the storage, decreased to 82.3% at the end of 28 month-long storage. Pre-storage melatonin and tryptophan treatments significantly improved FGP as compared to the control seeds (86.2%) and were found to be 91.1% and 90.8%, respectively. Moreover, the MGT, which was 2.98 days before the storage, reached to 4.13 days after up to 28 months of storage and it was determined as 4.44 days in the control seeds, 3.22 days and 3.13 days in the seeds treated with melatonin and tryptophan, respectively.

Changes in germination percentages of seeds stored after treatments for 28 months at 14 °C and 25 °C are presented in Fig. 2A and B. Under chilling stress (14 °C) conditions, FGP of control seeds decreased to 70% while pre-storage melatonin and tryptophan treatments significantly improved the FGP of seeds (80% and 79%, respectively) (Fig. 2A). In germination tests performed under optimum (25 °C) conditions at the end of storage, the FGP of the seeds treated with melatonin and tryptophan were found to be 85% and 84%, respectively, while control seeds had an FGP of 78% (Fig. 2B).

Similarly, as the storage period progressed, MGT values of all treatments increased steadily and pre-storage melatonin and tryptophan treatments resulted in significantly lower MGT values compared to the control seeds (Fig. 2C and D). Under chilling stress, MGT was 17.1 days in the control seeds and 14.3 days and 14.7 days in melatonin and tryptophan-treated seeds, respectively at the end of storage period (Fig. 2C). On the other hand, under optimum conditions, MGT was found to be 5.3 days in the control seeds and 3.5 days and 3.5 days in melatonin and tryptophan-treated seeds, respectively (Fig. 2D). These results indicated that pre-storage melatonin and tryptophan treatments had similar effects on FGP and MGT at 14 °C and 25 °C temperatures and pre-storage melatonin and its precursor-tryptophan treatments improved germination performance especially at low temperatures.

3.3. MDA, H_2O_2 and EC contents

Pre-storage melatonin and tryptophan treatments significantly affected the MDA and H_2O_2 contents of tomato seeds and the EC value, which is an indicator of membrane integrity, during 28 months of storage (Table 3). In particular, the MDA content, which was determined as 221.7 nmol g^{-1} FW at the beginning of storage, increased to the highest level with 448.6 nmol g^{-1} FW in the 24th month of storage and slightly decreased at the end of the storage (28th month) to 373.6 nmol g^{-1} FW. When the effect of pre-storage applications on MDA content was examined, the MDA content of control seeds (441.4 nmol g^{-1} FW) was found to be significantly higher than the seeds treated with melatonin and tryptophan (246.5 nmol g^{-1} FW and 243.7 nmol g^{-1} FW, respectively) (Table 3). The H_2O_2 content, which was determined as 294.1 nmol g^{-1} TA at the beginning of storage (0th month-August 2017), reached the highest values starting from the 24th month of storage. The seed H_2O_2 content of control seeds (652.1 nmol g^{-1} FW) was also significantly higher than melatonin and tryptophan-treated seeds (262.4 nmol g^{-1} FW and 252.4 nmol g^{-1} FW, respectively) (Table 3). No difference was observed between the effects of pre-storage melatonin and tryptophan treatments on the MDA and H_2O_2 contents of the seeds at the end of storage. Additionally, the EC value, which was 68.99 μS $cm^{-1}g^{-1}$ at the beginning of storage, reached its highest level with 87.53 μS $cm^{-1}g^{-1}$ in the 22nd month of storage. It was observed that pre-storage treatments reduced EC values, thus slowing down the progression of membrane deterioration. The EC value was 82.05 μS $cm^{-1}g^{-1}$ in the control seeds, 53.51 μS $cm^{-1}g^{-1}$ in melatonin-treated seeds and 50.19 μS $cm^{-1}g^{-1}$ in tryptophan-treated seeds (Table 3).

Seed MDA (Fig. 3A) and H_2O_2 (Fig. 3B) contents followed a variable course during 28 months of storage. However, control seeds always had significantly higher MDA and H_2O_2 contents than the treated seeds. In addition, time-dependent fluctuations were

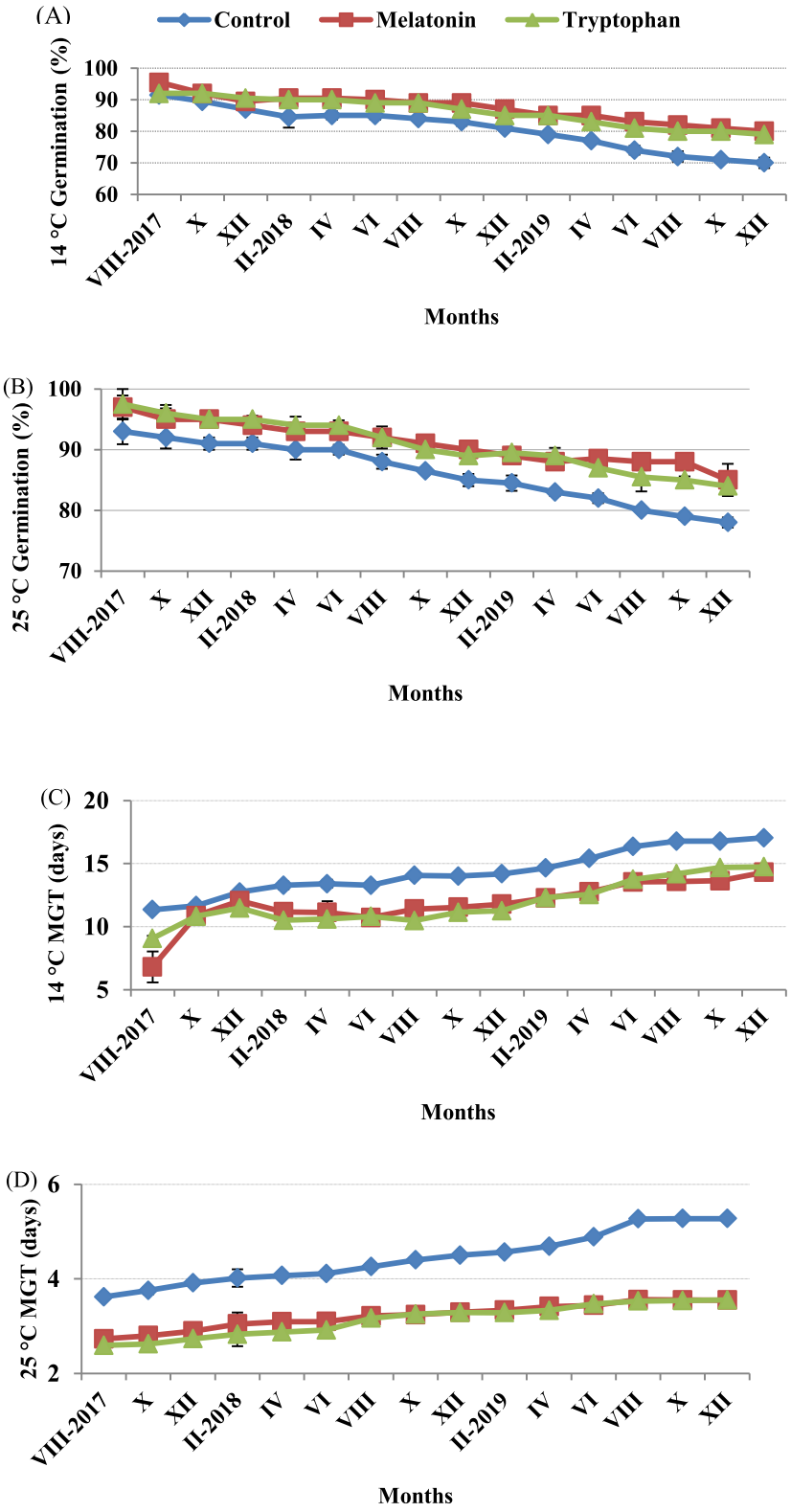

Fig. 2. Changes in final germination percentage (FGP) (A and B) and mean germination time (MGT) (C and D) of tomato seeds at 14 °C and 25 °C during 28-month storage. The results are expressed as mean values of four measurements ± standard error.

Table 3
Effect of melatonin and tryptophan treatments during 28 month-long storage on MDA, H_2O_2 contents and EC values of tomato seeds. Values are means ± SE (n = 4).

Factors	MDA (nmol g^{-1} FW)	H_2O_2 (nmol g^{-1} FW)	EC (μS $cm^{-1}g^{-1}$)
Storage Time-ST (Months)			
0	221.7±14.6 **g**	294.1±35.5 **gh**	68.99±2.77 **cd**
2	268.3±28.6 **fe**	393.1±75.7 **cd**	79.31±3.17 **b**
4	242.8±18.8 **fg**	351.5±77.2 **def**	54.58±6.11**e**
6	363.3±32.7 **bc**	458.6±70.4 **b**	42.75±4.99 **fg**
8	287.2±34.6 **de**	355.8±55.8 **de**	22.69±2.79 **h**
10	271.1±28.8 **ef**	436.2±81.4 **bc**	63.28±4.74 **d**
12	327.2±32.5 **cd**	322.5±68.7**efg**	72.9±4.71 **c**
14	233.9±26.4 **fg**	248.2±39 **h**	67.37±5.79 **cd**
16	331.7±37.9 **bc**	423.5±67 **bc**	68.07±5.69 **cd**
18	345.6±57.6 **bc**	299.5±40.2 **g**	39.74±3.53 **g**
20	325.0±28.1 **cd**	302.6±52.9 **fg**	48.57±5.09 **ef**
22	272.2±32.8 **ef**	306.5±52.1**efg**	87.53±6.35 **a**
24	448.6±54.9 **a**	522.6±57.0 **a**	68.18±6.69 **cd**
26	345.7±30.4 **bc**	552±56.5 **a**	71.48±5.68 **c**
28	373.6±28.4 **b**	567.9±56.7**a**	73.32±4.84 **b**
LSD $_{(0.05)}$	**43.7**	**50.1**	**6.23**
Treatments			
Control	441.4±14.9 **a**	652.1±20.7 **a**	82.05±2.76 **a**
Melatonin	246.5±9.6 **b**	262.4±13.9 **b**	53.51±2.35 **b**
Tryptophan	243.7±9.2 **b**	252.4±12.5 **b**	50.19±2.10 **c**
LSD $_{(0.05)}$	**19.5**	**22.4**	**2.78**
ANOVA			
ST	***	***	***
Treatments	***	***	***
ST*Treatments	***	***	***

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

observed in the MDA and H_2O_2 contents of the seeds throughout the storage, but an increase was detected in all treatments starting from 24th month of storage (Fig. 3B). Membrane leakage values (Fig. 3C) exhibited a similar change with the other parameters during the storage period and they increased in the 2nd month of storage (October 2017) in all treatments but decreased to the lowest level in the 8th month (April 2018). As the storage process progresses, EC values again decreased to the lowest levels of the year in the 18–20th months (February–April 2019) and then increased in the 22nd months (June 2019). Similar to MDA and H_2O_2 values, EC values of the control seeds were almost always higher than the other treated seeds.

3.4. Catalase (CAT) enzyme activity and seed moisture content

When the effect of storage time up to 28 months on CAT enzyme activity was examined, it was observed that the enzyme activity followed a time-dependent course with the progress of storage time (Table 4). The CAT activity of the seeds determined before the storage (0.41 U mg^{-1} protein) followed a fluctuating course during the storage and reached its highest level at the end of the 24th month (0.75 U mg^{-1} protein) but decreased to the pre-storage level at the end of storage. CAT enzyme activity of the control seeds (0.36 U mg^{-1} protein) was found to be lower than the seeds treated with melatonin and tryptophan (0.48 U mg^{-1} protein and 0.49 U mg^{-1} protein, respectively) (Table 4). The moisture content of the seeds increased significantly during the storage over two years, even though they were stored in sealed plastic packages after drying back to their initial moisture content (Table 4). The seed moisture content, which was around 8.45% at the beginning of storage, was found to be 8.81% in the 12th month of storage. Increases in seed moisture content were observed with the progress of storage and it was determined as 9.31% at the end of 28 months of storage. In addition, seed moisture of control seeds (9.02%) was significantly lower in seeds treated with melatonin and tryptophan (both having moisture content of 8.78%) (Table 4).

The CAT activity of tomato seeds stored for 28 months followed a fluctuating course during the storage (Fig. 4A). Generally lower enzyme activity of the control seeds as compared to the other treated seeds indicated that these treatments enhanced the activities of this antioxidant enzyme. CAT activity, which exhibited a different course depending on the treatments in the first 16 months of storage, increased in all treatments in the 24th month, but decreased to the pre-storage levels in the 28th month, the end of the study (Fig. 4A). Seed moisture rose sharply at the end of the first year of storage and continued to increase steadily until the end of the second year (Fig. 4B). In particular, control seeds were found to have greater moisture contents as compared to the seeds treated with melatonin and tryptophan. Moisture content at the end of storage, which was 9.56% in control seeds, was 9.24% in melatonin-treated seeds and 9.14% in tryptophan-treated seeds (Fig. 4B).

4. Discussion

There is not much detailed information in the literature regarding the changes in melatonin and tryptophan content of seeds stored for long term. In this study, changes in the concentrations of these molecules in tomato seeds stored for 28 months were revealed for the first time. Melatonin contents of control seeds and seeds pre-treated before storage displayed similar and significant seasonal changes and reached the highest level in 2018 August (12th month) and 2019 August (24th month) (Fig. 1A). Melatonin contents of seeds treated with melatonin and tryptophan were also found to be higher than the control seeds.

It was reported that melatonin was permanently present in plants acting as a circadian rhythm regulator (Ahn et al., 2021), but its concentration in the tissues varied during the day reaching its peaks in the dark (Chang et al., 2021). In addition, the growing conditions, growth stages and environmental factors and even the sampling time during the day have a significant effect on the melatonin content of plant tissues (Wu et al., 2021; Arnao and Hernandez-Ruiz, 2021). For instance, similar changes were reported in *Chenopodium rubrum* L. (Wolf et al., 2001), cherry fruits (Zhao et al., 2012), Malbec grape variety (Boccalandro et al., 2011), lupine (*Lupinus albus*) seedlings (Arnao and Hernández-Ruiz, 2015), *Arabidopsis* plant (Li et al., 2020; Hernández et al., 2015) and eggplant seedlings (Korkmaz et al., 2017b). Present findings revealed that seed melatonin contents also showed significant seasonal changes throughout the year. Similar seasonal changes were observed in pepper (Korkmaz et al., 2018) and maize and cucumber (Kołodziejczyk et al., 2015) seeds stored for 1 year. The researchers stated that elevated melatonin levels in winter months during the storage might be an indicator of a defense mechanism to protect the seeds against adverse environmental conditions and that increased melatonin levels under adverse environmental conditions functioned as a defense mechanism in the seed. Melatonin is a potent free radical scavenger (Fan et al., 2018; Muhammad et al., 2022; Reiter et al., 2017) and therefore high melatonin levels act as a source of antioxidants in seeds just separated from the mother plant. Since the seed is no longer connected to the mother plant after shedding, the successful formation of the next generation can occur in seeds separated from the mother with high melatonin content, providing significant protection against aging and/or harsh environmental conditions. It could be deduced from the results of the current study that melatonin, which is found in high amounts in the seeds in August, is a strategy that tomato seeds have in order to ensure their life after leaving the mother plant and to establish their next generation.

The change in tryptophan content, the precursor of melatonin, generally exhibited an opposite trend to fluctuations in melatonin content (Fig. 1B); that is, in all treatments, the amount of melatonin decreased when the amount of tryptophan increased. Tryptophan content remained at very low levels, especially at the beginning of storage (0th month), 12th month (August 2018) and 26th months

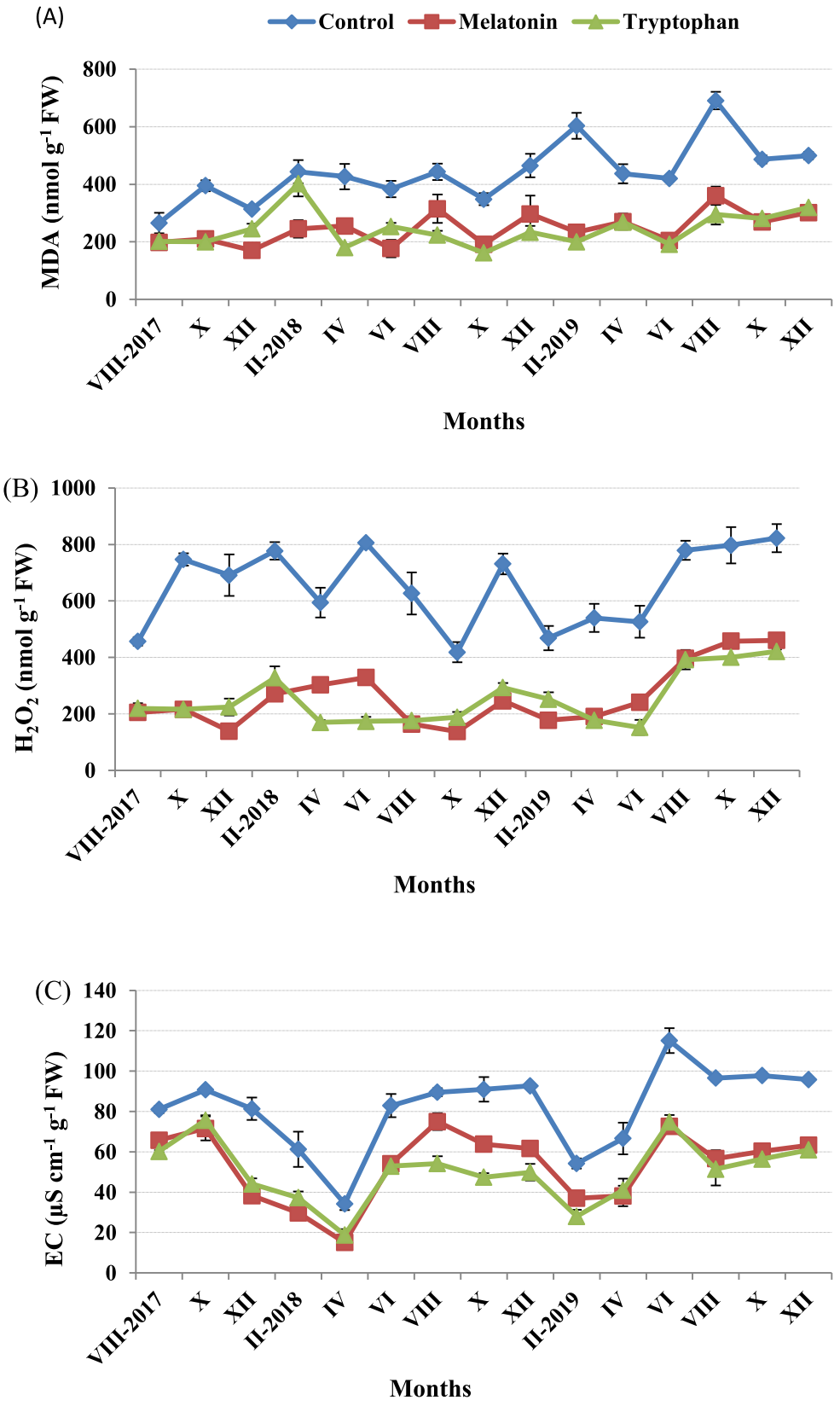

Fig. 3. Changes in MDA (A), H_2O_2 (B) contents and EC values (C) of tomato seeds during 28-month storage. The results are expressed as mean values of four measurements ± standard error.

Table 4
Effect of melatonin and tryptophan treatments during 28 month-long storage on CAT activity and seed moisture content of tomato seeds. Values are means ± SE (n = 4).

Factors	CAT activity (U mg^{-1} protein)	Seed Moisture Content (%)
Storage Time-ST (Months)		
0	0.41±0.04 **cd**	8.45±0.06 **e**
4	0.32±0.06 **ef**	8.52±0.06 **e**
8	0.30±0.05 **f**	8.77±0.06 **d**
12	0.40±0.02 **cd**	8.81±0.05 **cd**
16	0.37±0.02 **de**	8.88±0.1**cd**
20	0.56±0.02 **b**	9.01±0.09 **bc**
24	0.75±0.04 **a**	9.12±0.12 **ab**
28	0.43±0.01 **c**	9.31±0.06 **a**
LSD $_{(0.05)}$	**0.05**	**0.22**
Treatments		
Control	0.36±0.03 **b**	9.02±0.07 **a**
Melatonin	0.48±0.03 **a**	8.78±0.06 **b**
Tryptophan	0.49±0.03 **a**	8.78±0.07 **b**
LSD $_{(0.05)}$	**0.03**	**0.13**
ANOVA		
ST	***	***
Treatments	***	**
ST*Treatments	***	NS

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

(October 2019), when the melatonin contents peaked and elevated tryptophan levels were observed in parallel with decreases in melatonin levels. Korkmaz et al. (2017a) observed a similar relationship between tryptophan and melatonin content in the roots and leaves of the eggplant plants. The researchers monitored melatonin and tryptophan changes in the seeds during the day and in the leaves and roots throughout the growing season and reported that melatonin and tryptophan contents changed in an opposite way, that is, if the concentration of one in the tissues increased, the concentration of the other compound decreased. Moreover, Yakupoğlu et al. (2021) revealed that the endogenous melatonin and tryptophan contents of lettuce seeds stored for 24 months changed in a circadian manner and that the melatonin and tryptophan contents varied in opposition to each other. In other words, during the winter months when the melatonin content peaked strongly, tryptophan content was determined at the lowest levels.

Melatonin is synthesized from the precursor of tryptophan, an aromatic amino acid, by a 4-stage biosynthesis pathway in which different enzymes play a role in each stage, and this pathway is briefly outlined as tryptophan → tryptamine/5-hydroxytryptophan → serotonin → 5-methoxytryptamine/n-acetyl serotonin → melatonin (Mannino et al., 2021). Therefore, it was stated that the enzymes that enable the conversion of molecules during melatonin biosynthesis had an important effect on the changes in melatonin and tryptophan levels (Negri et al., 2021). In particular, the

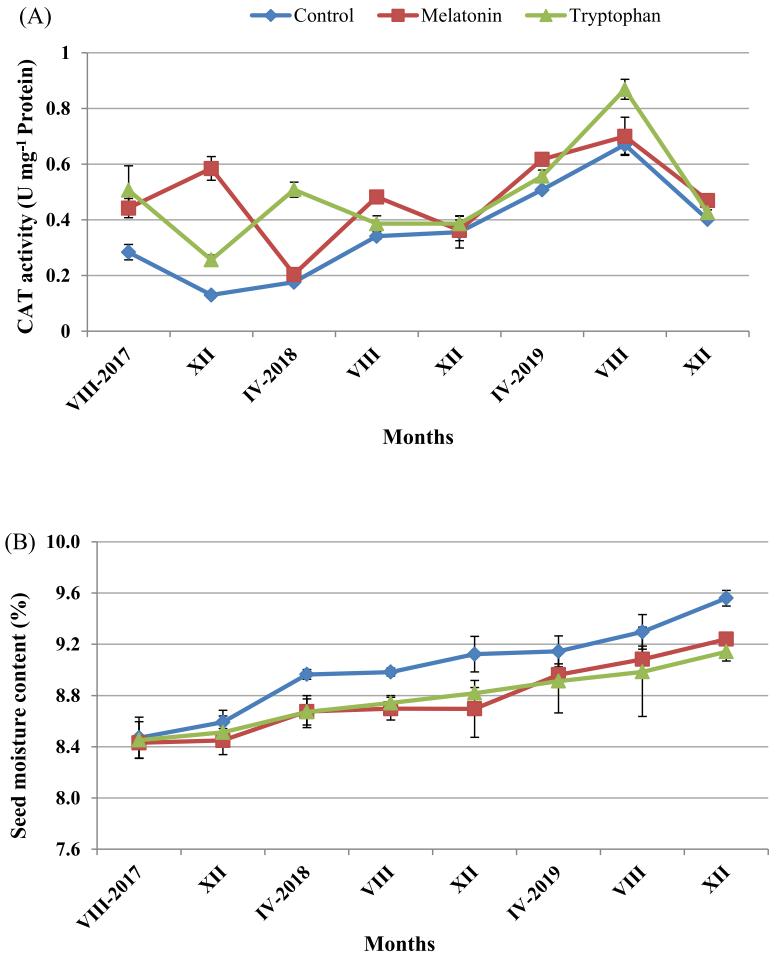

Fig. 4. Changes in the activity of CAT (A) and moisture content (B) of tomato seeds during 28-month storage at room temperature (25 °C). The results are expressed as mean values of four measurements ± standard error.

increase in the activities of some enzymes along this pathway may increase the biosynthesis of molecules, while the accumulation and/or excessive production of some molecules may adversely affect melatonin biosynthesis. For instance, low melatonin levels are generally observed in tissues because the conversion of tryptophan into serotonin occurs at much higher rates than the conversion of serotonin to melatonin. It is also known that the promotion of serotonin production, especially with the effect of environmental conditions such as stress and time of day and year (circadian rhythm), negatively affects melatonin production (Back et al., 2016).

Moisture content of seeds in the study increased significantly during the storage over 28 months, even though they were stored in sealed plastic packages after drying to initial moisture content (Fig. 4B). While seed moisture content varied slightly in the first year, which was 8.45% at the beginning, it increased sharply at the end of the second year of storage and reached to 9.56%. In addition, as a result of the treatments applied to the seeds, significant changes occurred in the moisture content of the seeds. Storing the seeds with low moisture content under low temperature conditions prolongs the survival of the seed. It was reported that high ambient moisture and temperature conditions adversely affected lettuce seed longevity in long term storage (Demir et al., 2016). It was reported that storing lettuce seeds with initial moisture content of 7% in sealed plastic begs for 24 months at ambient conditions resulted in elevated seed moisture levels which adversely affected the seed viability (Yakupoğlu et al., 2021). The authors stated that lower seed moisture content was essential to maintain seed viability during relatively long term storage. In this study, seed moisture content increased progressively over the course of storage time in all treatments, especially the control seeds and due to relatively high storage temperature (25 °C), viability and vigor losses became increasingly evident over time. However, seed treatments with melatonin and tryptophan were effective to some extent in preventing these losses.

Pre-storage melatonin and tryptophan treatments alleviated the effect of ageing-induced damage and improved germination performance and mean germination time at both temperatures (14 °C and 25 °C). Although there is not much detailed information about the role of melatonin and tryptophan in seed aging, recent studies have documented that the application of melatonin and tryptophan improved the germination performance of seeds under stress conditions. For instance, Korkmaz et al. (2017a) stated that the exogenous melatonin treatments had positive effects on germination and emergence performance of pepper seeds under chilling stress. Also, melatonin treatments increased the germination performance of *Arabidopsis thaliana* seeds under heat stress by 60% as compared to control treatments (Hernández et al., 2015). A study aimed to develop new stress tolerant cultivars investigated the effect of seed melatonin content of 28 pepper genotypes and reported that genotypes with high endogenous melatonin content had higher germination and emergence percentages under chilling stress conditions. (Korkmaz et al., 2022). The researchers stated that the melatonin content of the seeds of the genotypes varied between 0.64 and 5.03 ng g^{-1} and the genotypes with less than 2 ng g^{-1} seed melatonin content had germination and seedling emergence values of 50% or below. It was also reported that tryptophan applications to pepper seeds under salt stress conditions positively affected germination and seedling emergence performance (Korkmaz et al., 2020). Present findings clearly revealed the effects of tryptophan and melatonin treatments on germination performance of long term-stored seeds under stress conditions.

Changes in membrane integrity, fluidity, permeability and structure are observed after the deterioration of the lipids in cell membranes as a result of oxidation (Kravić et al., 2021). MDA could be given as an example of the products formed due to lipid peroxidation caused by the actions of free radicals such as H_2O_2. Therefore, determination of H_2O_2 and MDA contents in plant tissues and organs is important in terms of providing information about the integrity and functionality of the membranes as a result of deteriorations caused by lipid oxidation. Seed MDA (Fig. 3A) and H_2O_2 (Fig. 3B) contents increased significantly during the storage period and followed a course that varied from month to month during the year and these changes were generally dependent on melatonin content. For instance, MDA content decreased after 12th (August-2018) and 24th (August-2019) months in storage, when the seed melatonin content peaked strongly. Also, seeds treated with tryptophan and melatonin almost always had lower levels of MDA and H_2O_2 contents than the control seeds. All these revealed that the MDA content of the seeds showed a change closely related to the course in the content of melatonin and that the treated seeds had lower MDA and H_2O_2 content as compared to the control seeds. Similar to present findings, it was reported that lettuce seeds treated with melatonin had lower MDA and H_2O_2 contents than the control seeds during 2 years of storage, and that MDA and H_2O_2 levels were generally at their lowest levels in the months when the melatonin levels were the highest (Yakupoğlu et al., 2021).

Loss of membrane integrity due to the damaged phospholipids in cell membranes causes increased membrane permeability and the leakage of electrolytes and the other substances from the cells (Kurek et al., 2019). Another reason for increased electrolyte leakage is the peroxidation of lipids present in cell membranes (Li et al., 2022). The electrical conductivity of tomato seeds during long-term storage (28 months) showed a similar change in all treatments and decreased in the months following the months when the melatonin content peaked (Fig. 3C). In addition, it was determined that control seeds had higher EC values during the entire storage period as compared seeds treated with melatonin and tryptophan. Similarly, the effectiveness of seed treatments with melatonin were investigated in various species including maize (Jiang et al., 2016; Simlat et al., 2018), cucumber (Zhang et al., 2014), soybean (Wei et al., 2015), pepper (Korkmaz et al., 2017a), cotton (Chen et al., 2020) and *Limonium bicolor* (Li et al., 2019) and it was reported that melatonin treatment reduced stress-induced lipid peroxidation and membrane leakage while increasing antioxidant activities. Such findings indicated that damage caused by free radical-induced membrane oxidations could be mitigated by exogenous seed treatments.

It was reported that melatonin played an essential role as antioxidant directly in stabilization of lipid peroxidation and membrane fluidity of biological membranes such as mitochondria, chloroplasts and plasma and combating against stress factors (Wu et al., 2021; Arnao and Hernández-Ruiz, 2020). Melatonin also regulates and promotes the activities of antioxidant enzymes including CAT in plants under stress (Arnao and Hernández-Ruiz, 2021). In this study, it was observed that pre-storage treatments increased the CAT enzyme activity which was also parallel to the change melatonin content. For instance, CAT enzyme activity increased at 12th and 24th months, when the melatonin content peaked strongly (Fig. 4A). In addition, enzyme activity of the seeds treated with melatonin and tryptophan was higher than the control seeds. Melatonin treatments increased antioxidant enzyme activity of lettuce (Yakupoğlu et al. 2021), pepper (Köklü, 2016), maize (Deng et al., .2017) and soybean (Wei et al., 2015) seeds and improved germination performance (Rajora et al. 2022) under various stress conditions. Additionally, it was reported that melatonin treatments significantly protected the membrane structures of pepper (Korkmaz et al. 2017a) and maize (Cao et al., 2019) seeds germinated under chilling stress against peroxidation and MDA accumulation.

5. Conclusion

In this study, how the melatonin content of tomato seeds treated with melatonin and its precursor tryptophan changed during 28 months of storage was demonstrated for the first time. In addition,

the effects of pre-storage melatonin and tryptophan treatments on aging of seeds during the storage were also determined. Seasonal fluctuations were observed in seed melatonin and tryptophan contents during 28 month long storage. The melatonin contents of the seeds peaked at the end of 12th and 24th months of storage, which corresponds to August 2018 and August 2019. On the other hand, tryptophan levels showed an opposite trend during the months when melatonin peaked strongly. The low MDA and H_2O_2 levels detected in the months following the months when the melatonin content in the seeds reached the highest levels showed that melatonin slowed down the aging process and also played a direct role in the reduction of lipid peroxidation in seeds. Although seasonal fluctuations were detected in melatonin contents during long-term storage, it is still not fully clear what triggers or manages these changes in seed tissues. Therefore, further research is required to determine the factors governing melatonin biosynthesis, particularly the effects of the presence and/or amount of precursor molecules in seed tissues at different times of the year, and long-term seasonal fluctuations in seeds of different species. In this way, physiological roles of seasonal changes in the melatonin content of stored seeds could be better understood. Pre-storage treatments, on the other hand, significantly slowed down the aging process by protecting the membrane structures against peroxidation, MDA and H_2O_2 accumulation and increasing the activities of antioxidant enzymes. The fact that pre-storage treatment with melatonin and its pre-cursor-tryptophan, could be used as a highly valuable tool to slow down seed ageing may have important practical applications, especially for long-term storage of seeds of endangered species or valuable breeding materials.

CRediT authorship contribution statement

Aygül Karaca: Investigation, Conceptualization, Data curation, Funding acquisition, Formal analysis, Writing – review & editing. **Şebnem Köklü Ardıç:** Data curation, Formal analysis. **Abdullah Havan:** Data curation, Formal analysis. **Muhammet Ömür Aslan:** Data curation, Formal analysis. **Gökçen Yakupoğlu:** Investigation, Conceptualization. **Ahmet Korkmaz:** Investigation, Conceptualization, Data curation, Funding acquisition, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ahn, H.R., Kim, Y.J., Lim, Y.J., Duan, S., Eom, S.H., Jung, K.H., 2021. Key genes in the melatonin biosynthesis pathway with circadian rhythm are associated with various abiotic stresses. Plants 10, 129. https://doi.org/10.3390/plants10010129.

Arnao, M.B., Hernández-Ruiz, J., 2015. Functions of melatonin in plants: a review. J. Pineal Res. 59, 133–150. https://doi.org/10.1111/jpi.12253.

Arnao, M.B., Hernández-Ruiz, J., 2020. Is phytomelatonin a new plant hormone? Agronomy 10, 95. https://doi.org/10.3390/agronomy10010095.

Arnao, M.B., Hernández-Ruiz, J., 2021. Melatonin as a regulatory hub of plant hormone levels and action in stress situations. Plant Biol. https://doi.org/10.1111/plb.13202.

Back, K., Tan, D.X., Reiter, R.J., 2016. Melatonin biosynthesis in plants: multiple pathways catalyze tryptophan to melatonin in the cytoplasm or chloroplasts. J. Pineal Res. 61, 426–437. https://doi.org/10.1111/jpi.12364.

Bahcesular, B., Yildirim, E.D., Karaçocuk, M., Kulak, M., Karaman, S., 2020. Seed priming with melatonin effects on growth, essential oil compounds and antioxidant activity of basil (*Ocimum basilicum* L.) under salinity stress. Ind. Crops Prod. 146, 112165. https://doi.org/10.1016/j.indcrop.2020.112165.

Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H., 2013. Seeds: Physiology of Development, Germination and Dormancy, 3rd ed. Springer, New York.

Boccalandro, H.E., Gonzalez, C.V., Wunderlin, D.A., Silva, M.F., 2011. Melatonin levels, determined by LC-ESI-MS/MS, fluctuate during the day/night cycle in *Vitis vinifera* cv Malbec: evidence of its antioxidant role in fruits. J. Pineal Res. 51, 226–232. https://doi.org/10.1111/j.1600-079X.2011.00884.x.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Cao, Q., Li, G., Cui, Z., Yang, F., Jiang, X., Diallo, L., Kong, F., 2019. Seed priming with melatonin improves the seed germination of waxy maize under chilling stress *via* promoting the antioxidant system and starch metabolism. Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-51122-y.

Corpas, F.J., Gupta, D.K., Palma, J.M., 2021. Tryptophan: a precursor of signaling molecules in higher plants. Hormones and Plant Response. Springer, Cham, pp. 273–289.

Chang, T., Zhao, Y., He, H., Xi, Q., Fu, J., Zhao, Y., 2021. Exogenous melatonin improves growth in hulless barley seedlings under cold stress by influencing the expression rhythms of circadian clock genes. PeerJ 9, e10740. https://doi.org/10.7717/peerj.10740.

Chen, L., Liu, L., Lu, B., Ma, T., Jiang, D., Li, J., Zhang, K., Sun, H., Zhang, Y., Bai, Z., Li, C., 2020. Exogenous melatonin promotes seed germination and osmotic regulation under salt stress in cotton (*Gossypium hirsutum* L.). PLOS One 15, e0228241. https://doi.org/10.1371/journal.pone.0228241.

Demir, I., Ozden, E., Kara, F., Hassanzadeh, M., Mavi, K., 2016. Effects of ambient storage temperature and seed moisture content on seed longevity of lettuce (*Lactuca sativa*). J. Exp. Agric. Int. 12, 1–5. https://doi.org/10.9734/ajea/2016/25526.

Deng, B., Yang, K., Zhang, Y., Li, Z., 2017. Can antioxidant's reactive oxygen species (ROS) scavenging capacity contribute to aged seed recovery? Contrasting effect of melatonin, ascorbate and glutathione on germination ability of aged maize seeds. Free Radic. Res. 51, 765–771. https://doi.org/10.1080/10715762.2017.1375099.

Dubbels, R., Reiter, R.J., Klenke, E., Goebel, A., Schnakenberg, E., Ehlers, C., Schiwara, H.W., Schloot, W., 1995. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. J. Pineal Res. 18, 28–31. https://doi.org/10.1111/j.1600-079X.1995.tb00136.x.

Domergue, J.B., Abadie, C., Limami, A., Way, D., Tcherkez, G., 2019. Seed quality and carbon primary metabolism. Plant Cell Environ. 42, 2776–2788. https://doi.org/10.1111/pce.13618.

El-Maarouf-Bouteau, H., 2022. The seed and the metabolism regulation. Biology 11, 168. https://doi.org/10.3390/biology11020168.

Fan, J., Xie, Y., Zhang, Z., Chen, L., 2018. Melatonin-A multifunctional factor in plants. Int. J. Mol. Sci. 19, 1528. https://doi.org/10.3390/ijms19051528.

Günes, A., Inal, A., Baggi, E.G., Coban, S., Pilbean, D.J., 2007. Silicon mediates changes to some physiological and enzymatic parameters symptomatic for oxidative stress in spinach (*Spinacia oleracea* L.) grown under B toxicity. Sci. Horti. 113, 113–119. https://doi.org/10.1016/j.scienta.2007.03.009.

Harrington, J.F., 1973. Biochemical basis of seed longevity. Seed Sci. Technol. 1, 453–461.

Hattori, A., Migitaka, H., Iigo, M., Itoh, M., Yamamoto, K., Ohtani-Kaneko, R., Hara, M., Suzuki, T., Reiter, R.J., 1995. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochem. Mol. Biol. Int. 35, 627–634.

Hernández, I.G., Gomez, F.J.V., Cerutti, S., Arana, M.V., Silva, M.F., 2015. Melatonin in *Arabidopsis thaliana* acts as plant growth regulator at low concentrations and preserves seed viability at high concentrations. Plant Physiol. Biochem. 94, 191–196. https://doi.org/10.1016/j.plaphy.2015.06.011.

ISTA, 2005. International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.

Jiang, C., Cui, Q., Feng, K., Xu, D., Li, C., Zheng, Q., 2016. Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. Acta Physiol. Plant. 38, 82. https://doi.org/10.1007/s11738-016-2101-2.

Kołodziejczyk, I., Balabusta, M., Szewczyk, R., Posmyk, M.M., 2015. The levels of melatonin and its metabolites in conditioned corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) seeds during storage. Acta Physiol. Plant. 37, 105. https://doi.org/10.1007/s11738-015-1850-7.

Korkmaz, A., Değer, Ö., Cuci, Y., 2014. Profiling the melatonin content in organs of the pepper plant during different growth stages. Sci. Horti. 172, 242–247. https://doi.org/10.1016/j.scienta.2014.04.018.

Korkmaz, A., Karaca, A., Kocacinar, F., Cuci, Y., 2017. The effects of seed treatment with melatonin on germination and emergence performance of pepper seeds under chilling stress. J. Agric. Sci. 23, 167–176.
Korkmaz, A., Yakupoğlu, G., Köklü, Ş., Cuci, Y., Kocaçınar, F., 2017. Determining diurnal and seasonal changes in tryptophan and melatonin content of eggplant (*Solanum melongena* L.). Turk J. Bot. 41, 356–366. https://doi.org/10.3906/bot-1611-48.
Korkmaz, A., Köklü, Ş., Yakupoğlu, G., 2018. Investigating the effects of melatonin application on the ageing process of pepper seeds. Acta Horti. 1204, 9–16. https://doi.org/10.17660/ActaHortic.2018.1204.2.
Korkmaz, A., Gerekli, A., Yakupoğlu, G., Karaca, A., Köklü, Ş., 2020. Seed treatment with tryptophan improves germination and emergence of pepper under salinity stress. Acta Horti. 1273, 441–448. https://doi.org/10.17660/ActaHortic.2020.1273.57.
Korkmaz, A., Düver, E., Szafrańska, K., Karaca, A., Ardıç, Ş.K., Yakupoğlu, G., 2022. Feasibility of using melatonin content in pepper (*Capsicum annuum*) seeds as a physiological marker of chilling stress tolerance. Funct. Plant Biol. 49, 832–843. https://doi.org/10.1071/FP22005.
Köklü, Ş., 2016. Investigating the Effects of Melatonin on the Ageing Process of Pepper SEEDS. Kahramanmaraş Sütçü İmam University, Institute for Graduate Studies in Science and Technology. (Turkish with English abstract M.Sc. Thesis).
Kurek, K., Plitta-Michalak, B., Ratajczak, E., 2019. Reactive oxygen species as potential drivers of the seed aging process. Plants 8, 174. https://doi.org/10.3390/plants8060174.
Kravić, N., Dragičević, V., Milivojević, M., Babić, V., Žilić, S., 2021. Antioxidants from maize seeds and accelerated ageing. Selekcija I Semenarstvo 27, 47–57. https://doi.org/10.5937/SelSem2102047K.
Lerner, A.B., Case, J.D., Takahashi, Y., Lee, T.H., Mori, W., 1958. Isolation of melatonin, the pineal factor that lightness melanocytes. J. Am. Chem. Soc. 80, 2587–2592. https://doi.org/10.1021/ja01543a060.
Lei, K., Sun, S., Zhong, K., Li, S., Hu, H., Sun, C., Zheng, Q., Tian, Z., Dai, T., Sun, J., 2021. Seed soaking with melatonin promotes seed germination under chromium stress *via* enhancing reserve mobilization and antioxidant metabolism in wheat. Ecotoxicol. Environ. Saf. 220, 112241. https://doi.org/10.1016/j.ecoenv.2021.112241.
Li, J., Zhao, C., Zhang, M., Yuan, F., Chen, M., 2019. Exogenous melatonin improves seed germination in *Limonium bicolor* under salt stress. Plant Sign. Behav. 14, 1659705. https://doi.org/10.1080/15592324.2019.1659705.
Li, D., Wei, J., Peng, Z., Ma, W., Yang, Q., Song, Z., Sun, W., Yang, W., Yuan, L., Xu, X., Chang, W., Rengel, Z., Shen, J., Reiter, R.J., Cui, X., Yu, D., Chen, Q., 2020. Daily rhythms of phytomelatonin signaling modulate diurnal stomatal closure *via* regulating reactive oxygen species dynamics in Arabidopsis. J. Pineal Res. 68, e12640. https://doi.org/10.1111/jpi.12640.
Li, W., Niu, Y., Zheng, Y., Wang, Z., 2022. Advances in the understanding of reactive oxygen species-dependent regulation on seed dormancy, germination, and deterioration in crops. Front. Plant Sci. 13, 826809. https://doi.org/10.3389/fpls.2022.826809.
Liu, K., Jing, T., Wang, Y., Ai, X., Bi, H., 2022. Melatonin delays leaf senescence and improves cucumber yield by modulating chlorophyll degradation and photoinhibition of PSII and PSI. Environ. Exp. Bot. 200, 104915. https://doi.org/10.1016/j.envexpbot.2022.104915.
Mannino, G., Pernici, C., Serio, G., Gentile, C., Bertea, C.M., 2021. Melatonin and phytomelatonin: Chemistry, biosynthesis, metabolism, distribution and bioactivity in plants and animals-an overview. Int. J. Mol. Sci. 22, 9996. https://doi.org/10.3390/ijms22189996.
Martinez, V., Nieves-Cordones, M., Lopez-Delacalle, M., Rodenas, R., Mestre, T.C., Garcia-Sanchez, F., Rubio, F., Nortes, P.A., Mittler, R., Rivero, R.M., 2018. Tolerance to stress combination in tomato plants: new insights in the protective role of melatonin. Molecules 23, 535. https://doi.org/10.3390/molecules23030535.
Muhammad, I., Yang, L., Ahmad, S., Mosaad, I.S., Al-Ghamdi, A.A., Abbasi, A.M., Zhou, X.B., 2022. Melatonin application alleviates stress-induced photosynthetic inhibition and oxidative damage by regulating antioxidant defense system of maize: a meta-analysis. Antioxidants 11, 512. https://doi.org/10.3390/antiox11030512.
Negri, S., Commisso, M., Avesani, L., Guzzo, F., 2021. The case of tryptamine and serotonin in plants: a mysterious precursor for an illustrious metabolite. J. Exp. Bot. 72, 5336–5355. https://doi.org/10.1093/jxb/erab220.
Özden, M., Demirel, U., Kahraman, A., 2009. Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. Sci. Horti. 119, 163–168. https://doi.org/10.1016/j.scienta.2008.07.031.
Rajora, N., Vats, S., Raturi, G., Thakral, V., Kaur, S., Rachappanavar, V., Kumar, M., Kesarwani, K.A., Sonah, H., Sharma, R.T., Deshmukh, R., 2022. Seed priming with melatonin: a promising approach to combat abiotic stress in plants. Plant Stress 4, 100071. https://doi.org/10.1016/j.stress.2022.100071.
Ray, J., Bordolui, S.K., 2022. Seed quality deterioration of tomato during storage: effect of storing containers and condition. Biol. Forum 14, 327–334.
Reiter, R.J., Rosales-Corral, S., Tan, D.X., Jou, M.J., Galano, A., Xu, B., 2017. Melatonin as a mitochondria-targeted antioxidant: one of evolution's best ideas. Cell Mol. Life Sci. 74, 3863–3881. https://doi.org/10.1007/s00018-017-2609-7.
Seckin, B., Turkan, I., Sekmen, A.H., Ozfidan, C., 2010. The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (sea barleygrass) and *Hordeum vulgare* L. (cultivated barley). Environ. Exp. Bot. 69, 76–85. https://doi.org/10.1016/j.envexpbot.2010.02.013.
Simlat, M., Ptak, A., Warchol, M., Moranska, E., Piórkowska, E., 2018. Melatonin significantly influences seed germination and seedling growth of *Stevia rebaudiana* Bertoni. PeerJ 6, e5009. https://doi.org/10.7717/peerj.5009.
Vidigal, D.S., Dias, D.C.F.S., Dias, L.A.S., Finger, F.L., 2011. Changes in seed quality during fruit maturation of sweet pepper. Sci. Agric. 68, 535–539. https://doi.org/10.1590/S0103-90162011000500004.
Wang, B., Yang, R., Ji, Z., Zhang, H., Zheng, W., Zhang, H., Feng, F., 2022. Evaluation of biochemical and physiological changes in sweet corn seeds under natural aging and artificial accelerated aging. Agronomy 12, 1028. https://doi.org/10.3390/agronomy12051028.
Wei, W., Li, Q.T., Chu, Y.N., Reiter, R.J., Yu, X.M., Zhu, D.H., Zhang, W.K., Ma, B., Lin, Q., Zhang, J.S., Chen, S.Y., 2015. Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. J. Exp. Bot. 66, 695–707. https://doi.org/10.1093/jxb/eru392.
Wolf, K., Kolár, J., Witters, E., van Dongen, W, van Onckelen, H., Macháčková, I., 2001. Daily profile of melatonin levels in *Chenopodium rubrum* L. depends on photoperiod. J. Plant Physiol. 158, 1491–1493. https://doi.org/10.1078/0176-1617-00561.
Wu, X., Ren, J., Huang, X., Zheng, X., Tian, Y., Shi, L., Dong, P., Li, Z., 2021. Melatonin: biosynthesis, content, and function in horticultural plants and potential application. Sci. Horti. 288, 110392. https://doi.org/10.1016/j.scienta.2021.110392.
Yakupoğlu, G., Köklü, Ş., Karaca, A., Düver, E., Klicic, A., Korkmaz, A., 2018. Changes in melatonin content of pepper seeds during storage. Acta Horti. 1273, 425–432. https://doi.org/10.17660/ActaHortic.2020.1273.55.
Yakupoğlu, G., Köklü, Ş., Karaca, A., Düver, E., Reiter, R.J., Korkmaz, A., 2021. Fluctuations in melatonin content and its effects on the ageing process of lettuce seeds during storage. Acta Sci. Pol. Hortorum Cultus. 20, 77–88. https://doi.org/10.24326/asphc.2021.3.10.
Yang, W.J., Du, Y.T., Zhou, Y.B., Chen, J., Xu, Z.S., Ma, Y.Z., Chen, M., Min, D.H., 2019. Overexpression of TaCOMT improves melatonin production and enhances drought tolerance in transgenic *Arabidopsis*. Int. J. Mol. Sci. 20, 652. https://doi.org/10.3390/ijms20030652.
Zhang, J.H., Huang, W.D., Liu, Y.P., Pan, Q.H., 2005. Effects of temperature acclimation pretreatment on the ultrastructure of mesophyll cells in young grape plants (*Vitis vinifera* L. cv. Jingxiu) under cross-temperature stresses. J. Integ. Plant Biol. 47, 959–970. https://doi.org/10.1111/j.1744-7909.2005.00109.x.
Zhang, H.J., Zhang, N., Yang, R.C., Wang, L., Sun, Q.Q., Li, D.B., Cao, Y.Y., Weeda, S., Zhao, B., Ren, S., Guo, Y.D., 2014. Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA_4 interaction in cucumber (*Cucumis sativus* L.). J. Pineal Res. 57, 269–279. https://doi.org/10.1111/jpi.12167.
Zhao, Y., Tan, D.X., Lei, Q., Chen, H., Wang, L., Li, Q.T., Gao, Y., Kong, J., 2012. Melatonin and its potential biological functions in the fruits of sweet cherry. J. Pineal Res. 55, 79–88. https://doi.org/10.1111/jpi.12044.