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Postharvest melatonin treatment reduces chilling injury and enhances antioxidant capacity of tomato fruit during cold storage

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Key words: enzyme activity, lycopene, melatonin, proline, tomato fruit.

Abstract: In this study, tomato fruit was treated with 50, 100 or 200 µM melatonin and then stored at 5°C for 28 days to investigate the effect of melatonin treatment on chilling injury, nutritional quality and changes in the antioxidant system. Tomato fruit developed chilling injury, manifested as surface pitting and irregular red color development during storage. These chilling injury symptoms, ion leakage and malondialdehyde content were significantly reduced, and proline and carotenoids contents were significantly increased by melatonin treatment. Meanwhile, melatonin substantially reduced O_2^- production rate and H_2O_2 content, which result from significantly higher activities of superoxide dismutase, catalase, and peroxidase than control during the storage. These results suggest that melatonin treatment can effectively enhance chilling tolerance and reduce chilling injury. The reduction in chilling injury by melatonin may be associated with enhanced enzymatic and non-enzymatic antioxidants, in favor of membrane integrity and thus low cellular and tissue damage.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Cold storage is one of the most effective postharvest technologies to preserve the quality of fresh produces from the time of harvest until final preparation for human consumption in food chain (Bourne, 2006). However, cold storage imposes great risk on postharvest commodities sensitive to chilling injury (CI). Tomato (*Lycopersicon esculentum*), as one of the most important tropical crops, is typically cold sensitive (Hong and Gross, 2006). The most common visual injury symptoms of CI depicted for tomato fruit include irregular ripening and red color development as well as surface pitting on the fruit. Furthermore, as the chilled tissues are weakened, they become prone to decay and microbial spoilage. This phenomenon limits postharvest life and leads to significant degradation of

produce quality (Wang, 1993).

Melatonin, first discovered in tomato in 1995, accumulates in the fruits as they mature (Dubbels *et al*., 1995; Hattori *et al*., 1995). Melatonin content, as an endogenous signaling molecule, increases in response to abiotic and biotic stress, such as drought, salinity, chilling, and pathogens to protect against damage caused by them (Zhang *et al*., 2014; Arnao and Hernández-Ruiz, 2015; Liu *et al*., 2016). Accumulation of higher levels of melatonin in horticultural crops is beneficial not only for human health, but also for prolonging storability (Tan *et al*., 2012). Melatonin, a naturally occurring indoleamine, acts as endogenous elicitor and signaling molecule for plants growth and development, decreasing of biotic and abiotic stress, as well as a potent hydroxyl radical scavenger and antioxidant (Zhang *et al*., 2014; Zhang and Zhang, 2014; Manchester *et al*., 2015; Zhang *et al*., 2015). Melatonin contribution has been evidenced in a semilunar rhythm in macroalgae guarding this plant against high temperature stress (Tal *et al*., 2011). Melatonin treatment decreased apoptosis chilling-induced in carrot suspension cells. Moreover, melatonin treatment alleviated chilling-induced shrinkage and disruption of carrot cell plasma membranes (Lei *et al*., 2004). It has been reported that melatonin treatment reduced chilling injury in peach fruits by enhancement of chilling tolerance and provoking of defense response during cold storage (Cao *et al*., 2016). Soleimani Aghdam and Rezapour Fard (2017) reported that melatonin treatment at 100 µM decreased strawberry fungal decay resulting from higher superoxide dismutase (SOD) activity, associated with lower catalase (CAT) and ascorbate peroxidase (APX) activities as well as higher phenylalanine ammonia lyase (PAL) enzyme activity leading to higher total phenols and anthocyanins accumulation along with higher DPPH scavenging capacity. Likewise, marssonina apple blotch caused by fungus *Diplocarpon mali* decreased by melatonin treatment at 0.1 mM which is caused by higher H_2O_2 accumulation leading to enhancing pathogenesis related (PR) proteins accumulation such as peroxidase, chitinase and b-1,3-glucanase, and triggering phenylpropanoid pathway by enhancing phenylalanine ammonia lyase (PAL) enzyme activity (Yin *et al*., 2013). It has been reported that the attenuating of postharvest physiological deterioration in cassava roots by melatonin treatment, obtained by lower H_2O_2 accumulation as a result of increasing antioxidant enzymes; SOD, CAT and GR activities causing higher membrane integrity indicated by lower malondialdehyde (MDA) accumulation (Ma *et al*., 2016). Gao *et al.* (2016) reported that lower O^-_2 and H_2O_2 accumulation in melatonin treated peach fruits resulted from higher antioxidant enzymes SOD, CAT, APX activities, concurrent with lower lipoxygenase (LOX) enzyme activity leading to higher membrane integrity indicated by lower MDA accumulation.

In addition to antifungal and antioxidant activities, melatonin is useful in increasing postharvest sensory and nutritional quality of fresh produces (Meng *et al*., 2015; Cao *et al*., 2016; Gao *et al*., 2016; Liu *et al*., 2016; Ma *et al*., 2016). It has been reported that preveraison melatonin-treated grape berries showed higher endogenous melatonin accumulation, which not only enhances berry size and weight, indicated by higher sugars accumulation and higher endogenous hormones GA/ABA ratio, but also enhances synchronicity of berry ripening (Meng *et al*., 2015). It has been observed that preharvest melatonin-treated tomato fruits showed higher fruits weight caused by higher sugars accumulation, as well as higher organic acids accumulation results in tomato fruits with favorable flavor. Moreover, higher lycopene and ascorbic acid contents were observed in preharvest melatonin-treated tomato fruits (Liu *et al*., 2016).

Since there is a lack of knowledge about the influence of melatonin on chilling tolerance of tomato fruit during low temperature storage, the present work was initiated to determine the efficacy of postharvest melatonin treatment on chilling demonstrations and enhanced fruits visual and nutritional qualities through augmenting antioxidant capacity of tomato fruits frequently encountered under cold storage.

2. Materials and Methods

Fruit and treatment

Tomato fruit (*Lycopersicon esculentum* Mill. cv Banemi) were harvested at the mature green stage (i.e., liquefying locular tissue, seeds not cut with a knife) (Saltveit, 1991) from a local producer in Mohammad Shahr, Karaj (Iran) and then immediately transported to Karaj Agricultural Engineering and Engineering Research Institute Laboratory. The green stage of maturity with homogeneous size and randomly allotted into three groups (100 fruits per grop) for treatment in triplicate by dipping of fruits at 0 (control), 50, 100 and 200 µM melatonin solution for

5 min at 20°C. The selected concentrations were based on published effects of these compounds on peach, strawberries and cherry tomatoes (Sun *et al*., 2015; Cao *et al*., 2016; Soleimani Aghdam and Rezapour Fard, 2017). Following immersion, the fruits were dried for 1 h at room temperature. The tomato fruits were then put in plastic baskets, covered with a perforated plastic bag to retard weight loss and stored at 5°C with 80-85% relative humidity for 4 weeks. The seven-day intervals during storage at 5°C followed by shelf life at 20°C for 1 and 3 days, the development of chilling injury and ripening characteristics as well as enhanced fruits nutritional quality through augmenting antioxidant capacity were measured, respectively (Ding *et al*., 2002).

Measurements of chilling injury and ripening characteristics

Chilling injury (CI) of fruits was evaluated at 20°C for 1 day after the 7-, 14- 21 or 28-day cold-storage periods. Symptoms of tomato fruit chilling injury were manifested as surface pitting and large green patches or blotchy yellow areas resulting from loss of full red color development ability (Wang, 1993). The severity of the symptoms was assessed visually according to the following four-stage scale: 0= no pitting; 1= pitting covering <25% of the fruit surface; 2= pitting covering <50%, but >25% of surface; 3= pitting covering <75%, but >50% of surface and 4= pitting covering >75% of surface. The average extent of chilling-injury damage was expressed as a chilling-injury (CI) index, which was calculated using the following formula:

$$\text{CI index (\%)} = \{[(\text{CI level}) \times (\text{number of fruit at the CI level})]/(\text{total number of fruits}) \times 4\} \times 100.$$

For determining the effect of different treatments on ripening, fruits following 28 days storage, were incubated in diffused light at 20°C for 3 days to full red color development (Ding *et al*., 2002). Measurement of full red color development in terms of carotenoids accumulation was conducted. Lycopene and β-carotene were determined by the method described by Nagata and Yamashita (1992). The amount of 0.1 g of fruit tissue was mixed with 20 mL of hexane:acetone solution (3:2). An aliquot was taken from the supernatant and measured at 453, 505, 645, and 663 nm in a spectrophotometer. The content of lycopene and β-carotene was estimated using the following equations:

$$\text{Lycopene} = -0.0458\ A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453}$$
$$\beta\text{-carotene} = -0.216\ A_{663} - 1.220\ A_{645} + 0.304\ A_{505} + 0.452\ A_{453}$$

The results were expressed in milligrams per 100 g fresh weight (mg 100 g^{-1} FW).

Measurements of ion leakage and malondialdehyde content

Ion leakage was measured at 20°C for 3 days after the 7-, 14-, 21- or 28- day cold-storage period according to the method of Zhao *et al.* (2009). 3 mm thick of mesocarp tissues were excised from equator part of 5 fruits. Disks were put into aqueous 0.1 M mannitol and shaken at 100 cycles/min for 2 h. The conductivity of the solution (L1) was measured with a conductivity meter. Solutions were boiled for 10 min and then cooled to 20°C. The conductivity of tissues (L2) was measured. Ion leakage was calculated as the ratio of L1 to L2. Malondialdehyde (MDA) content was measured at 20°C for 3 days after the 7-, 14-, 21- or 28- day cold-storage period using the thiobarbituric acid method described by Zhao *et al.* (2009) with modification. Absorbance at 532 nm was recorded and corrected for nonspecific absorbance at 600 nm. The amount of MDA expressed as µmol MDA per gram of pulp.

Measurement of proline content

Proline content was measured at 20°C for 3 days after the 7-, 14-, 21- or 28- day cold-storage period using the acid ninhydrin method described by Shan *et al*. (2007). Proline in tissues was extracted with 30 mL L^{-1} sulfosalicylic acid at 100°C for 10 min with shaking. The extract was mixed with an equal volume of glacial acetic acid and acid ninhydrin reagent and boiled for 30 min. After cooling, the reaction mix was partitioned against toluene and the absorbance of the organic phase was recorded at 520 nm. The resulting values were compared with a standard curve constructed using known amounts of proline and expressed as µg proline g^{-1} fresh weight (FW).

Measurements of O_2^- production rate and H_2O_2 content

The O_2^- production rate and H_2O_2 content were measured at 20°C for 3 days after the 7-, 14-, 21- or 28- day cold-storage period. O_2^- production was measured using the method of Elstner (1976) with modification. 4 g of fruit tissue was homogenized with 5 ml of 50 mM phosphate buffer (pH 7.8) and then centrifuged at 8000×g for 20 min at 4°C. The supernatant was used for the determination of O_2^- production and expressed as nmol g^{-1} FW min^{-1}.

For H_2O_2 measurement, 2 g of fruit tissue was homogenized with 5 ml of cold acetone and then centrifuged for 15 min at 8000×g at 4°C, the supernatant was collected immediately for H_2O_2 analysis according to the method of Patterson *et al.* (1984).

H_2O_2 content was expressed as nmol g^{-1} FW.

Enzyme extraction and analysis

Enzyme activities were measured at 20°C for 3 days after the 7-, 14-, 21- or 28- day cold-storage period. 5 g of fruits tissue were homogenized with 50 mmol/L phosphate buffer (pH 7) containing 0.2 mmol/L EDTA and 2% PVP. The homogenate was centrifuged at 12,000×g for 20 min at 4°C and the supernatant was used. SOD (EC 1.15.1.1) activity was determined according to Giannopolitis and Ries (1977) with modification. One unit of SOD activity was defined as enzyme that caused 50% inhibition of nitro blue tetrazolium reduction by recording the absorbance at 560 nm. According to Zhang *et al*. (2013) with modification, 1 unit of CAT (EC 1.11.1.6) activity was defined as 0.01 decrease in absorbance at 240 nm per min. POD (EC 1.11.1.7) activity was determined according to Maehly and Chance (1954) with modification. One unit of POD was defined as 0.01 increase of absorbance at 470 nm as a result of guaiacol oxidation.

Statistical analysis

Experiments were performed using a completely randomized design. All statistical analyses were performed with SAS 9.2 software package. Data were analyzed by one-way analysis of variance (ANOVA). Mean comparisons were performed using HSD in Tukey's test for comparing treatment group at level of 1% (P<0.01) on three biological replicates.

3. Results

Chilling injury and ripening characteristics

The chilling injury (CI) symptoms were expressed on control group as surface pitting and large green patches or blotchy yellow areas resulting from loss of full red color development ability (Wang, 1993), only after 7-day of cold storage and following shelf life at 20°C for 1 and 3 days, respectively (Fig. 1). No significant difference in CI was observed between the control and 50 µM melatonin-treated fruit. Whereas, melatonin-treated groups with 100 or 200 µM underwent normal ripening at 20°C and only few visual chilling-injury symptoms were observed after 14 days storage at 5°C (Fig. 1). Fruits treated with 100 or 200 µM melatonin maintained the same quality as before chilling-temperature storage except for developing a slight yellow pigmentation, and the effect of the used formulas increased with increasing their concentrations (Fig. 2A). The results indicate that a 14-day storage was the maximum that could be tolerated by untreated mature green fruit. In this experiment, treatments with higher concentrations (100 or 200 µM) of melatonin were more effective in protecting against chilling injury than lower concentration (50 µM).

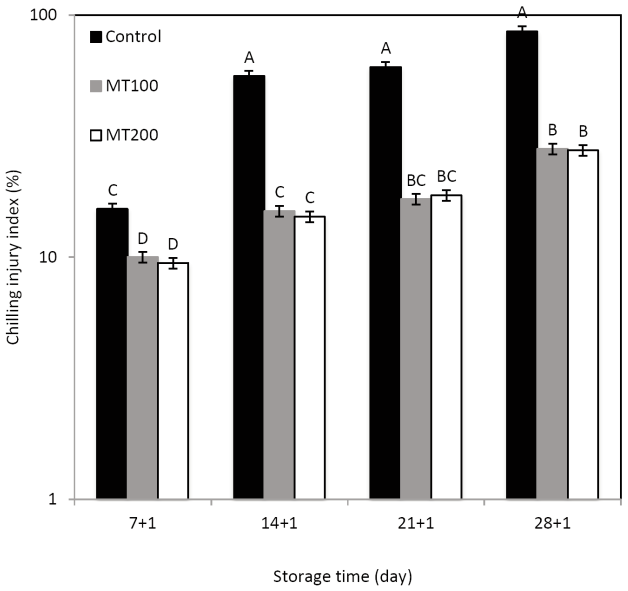

Fig. 1 - Chilling injury (CI) index (%) of tomato fruits treated with 100 and 200 µM during storage at 5°C and after 1 day of shelf life. All data are presented as a mean of three biological replicates, and vertical bars indicated the standard errors. Different letters indicate significant differences at P<0.01.

For examining the effect of melatonin treatment on color development of fruit after cold storage, mature green tomatoes were transferred to 20°C for 3 days for ripening. Treatment with 100 or 200 µM melatonin, prior to 5°C storage, was effective at alleviating chilling injury; this treatment category resulted in normally fruit ripening and uniform red color development caused by significantly (P<0.01) more lycopene and β-carotene accumulations, and the effect of the used formulas on fruit ripening and eventually visual quality increased with increasing their concentrations (Fig. 2). However, control and melatonin-treated group with 50 µM failed to develop the normal red color, with lower lycopene and β-carotene values, demonstrated by large green patches or blotchy yellow areas. Interaction effects and time of storage had no meaningful influence on these traits.

Ion leakage, malondialdehyde content

Ion leakage and MDA, as a consequence of membrane damage, are credible parameters for CI development and degree for postharvest tomato fruit

(Zhao *et al*., 2009). In this experiment, significantly the highest ion leakage was detected in control group (P<0.01) (Fig. 3A). However, no significant differences were statistically found in the ion leakage incidence between melatonin-treated groups with 100 or 200 µM. As shown in figure 2B, MDA content showed a similar pattern of change during storage.

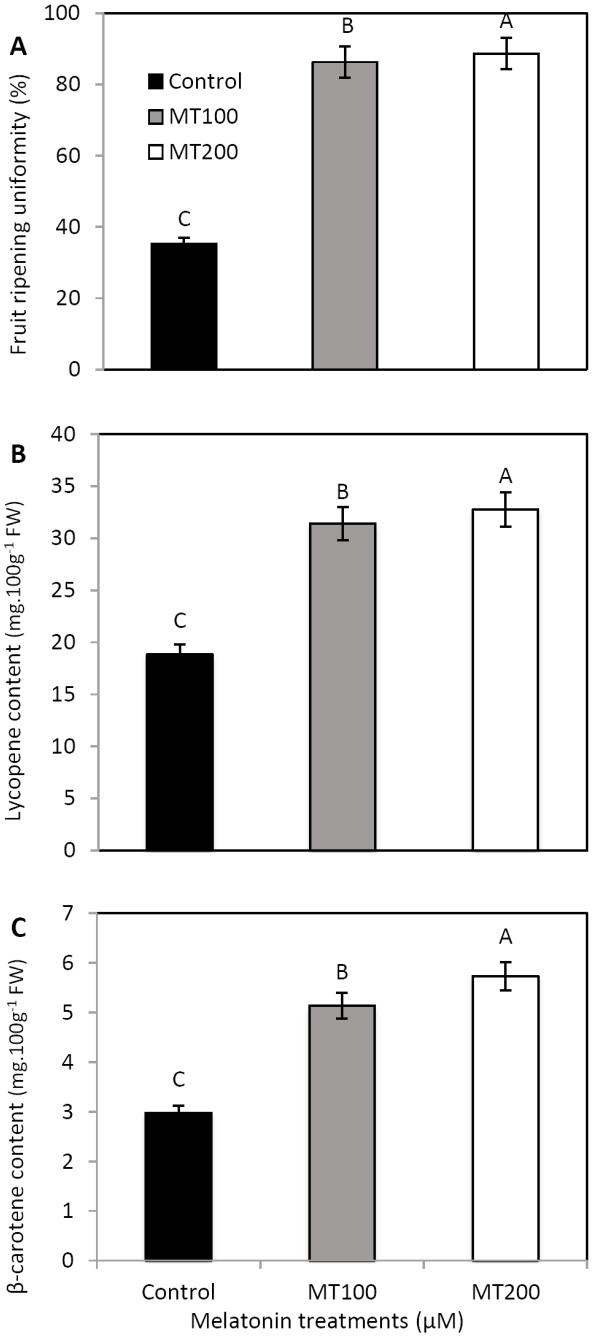

Fig. 2 - Fruit ripening uniformity (%), lycopene (A) and β-carotene (B) contents of tomato fruits treated with 100 and 200 µM during storage at 5°C and after 3 days of shelf life. All data are presented as a mean of three biological replicates, and vertical bars indicated the standard errors. Different letters indicate significant differences at P<0.01.

MDA content was significantly (P<0.01) lower in melatonin-treated groups with 100 or 200 µM compared with control at the same time of cold storage, and the highest level observed about 14 to 21 days (Fig. 3B).

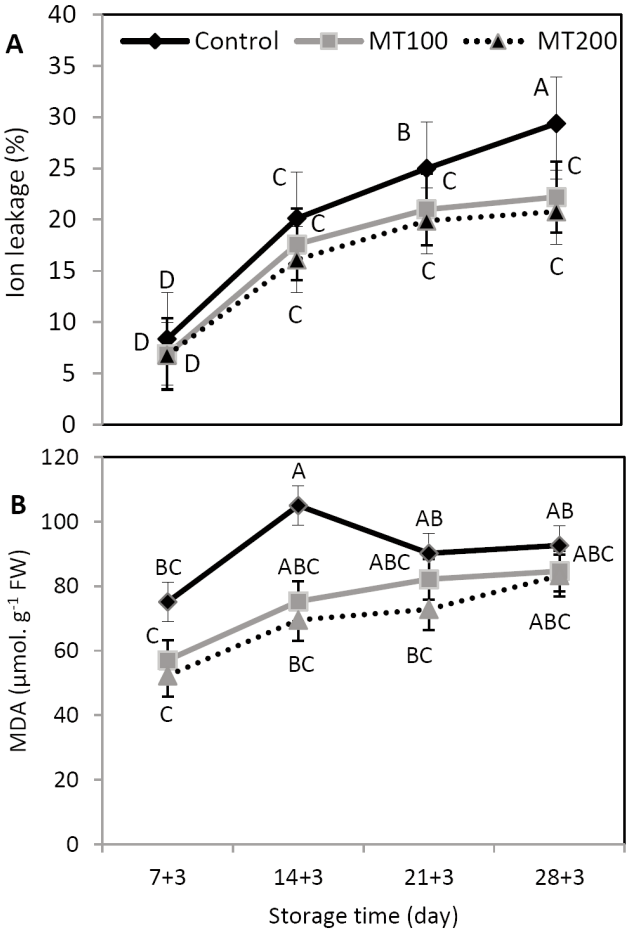

Fig. 3 - Ion leakage (%) (A) and MDA (B) content of tomato fruits treated with 100 and 200 µM melatonin during storage at 5°C and after 3 days of shelf life. All data are presented as a mean of three biological replicates, and vertical bars indicated the standard errors. Different letters indicate significant differences at P<0.01.

Proline content

There was a peak of proline content appearing in 14-day in all groups, which suggested that low temperature induced the proline synthesis mechanism in fruits (Zhao *et al*., 2009). However, proline accumulation was about 2 times high in the melatonin-treated groups with 100 and 200 µM compared to control from 14-day to the end of storage period, and the effect of the used formulas on proline content increased with increasing their concentrations (Fig. 4). No significant difference in proline content was observed between the control and 50 µM melatonin-treated fruit.

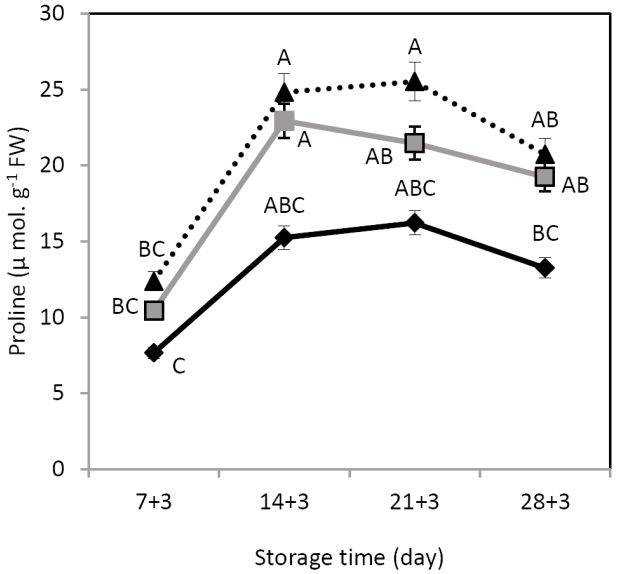

Fig. 4 - Prolin content of tomato fruits treated with 100 and 200 µM melatonin during storage at 5°C and after 3 days of shelf life. All data are presented as a mean of three biological replicates, and vertical bars indicated the standard errors. Different letters indicate significant differences at P<0.01.

O_2^- production and H_2O_2 content

In figure 5, the measured levels of O_2^- and H_2O_2 were shown as an influence of low temperature to ROS generation in fruits exposure to chilling stress. Contents of O_2^- and H_2O_2 remained relatively unchanged in control and melatonin-treated groups within the first 14 days of cold storage. Thereafter, both O_2^- and H_2O_2 contents increased rapidly, treatment with 100 and 200 µM melatonin significantly (P<0.01) restrained the enhancement of O_2^- and H_2O_2 contents, and again the effect of the used formulas on O_2^- production rate and H_2O_2 content decreased with increasing their concentrations (Fig. 4). No significant difference in O_2^- and H_2O_2 contents was observed between the control and 50 µM melatonin-treated fruit.

SOD, CAT, POD activities

As depicted in figure 6A, the SOD activity in both control and melatonin-treated groups steadily increased during storage, nonetheless significantly the highest SOD activity was observed in melatonin treated groups with 200 and 100 µM throughout the storage, respectively (P<0.01). The changes of CAT and POD activities in tomato fruit showed a similar pattern during the cold storage. The activities of both enzymes in control and melatonin-treated groups oscillatory increased with storage time. Melatonin treatment significantly promoted the increases in activities of CAT and POD, the activities of both enzymes were significantly higher (P<0.01) in these groups than those in control group during the whole storage (Fig. 6).

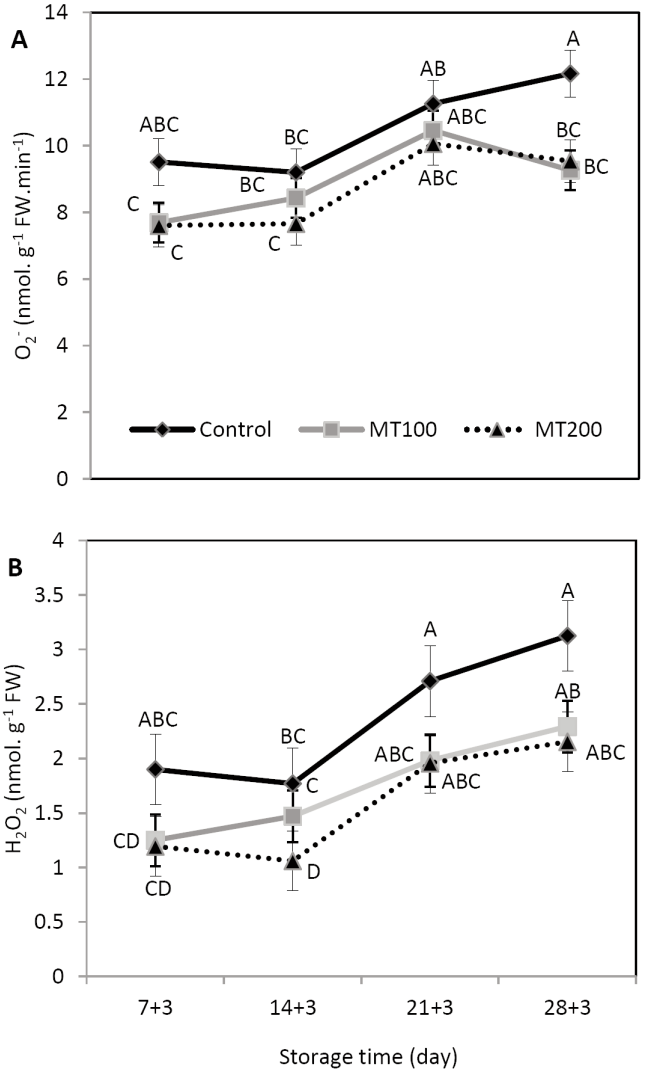

Fig. 5 - O_2^- Production (A) and H_2O_2 (B) content of tomato fruits treated with 100 and 200 µM during storage at 5°C and after 3 days of shelf life. All data are presented as a mean of three biological replicates, and vertical bars indicated the standard errors. Different letters indicate significant differences at P<0.01.

4. Discussion and Conclusions

Little information is available on melatonin treatment of horticultural commodities, even though there are many reports suggesting that melatonin is an endogenous signaling molecule for the activation of certain plant defense responses and the onset of the tolerance has often been correlated with the accumulation of defense-related enzymes and compounds (Zhang *et al.*, 2014; Arnao and Hernández-

Ruiz, 2015; Liu *et al.*, 2016). Exogenous melatonin application has been shown to result in an improved chilling tolerance and reduced incidence of chilling injury in peach and strawberry fruits (Cao *et al*., 2016; Soleimani Aghdam and Rezapour Fard, 2017). In this experiment, we found that melatonin treatment could effectively not only reduce development of surface pitting on the fruit and irregular ripening and full red color development (large green patches or blotchy yellow areas), the typical chilling injury

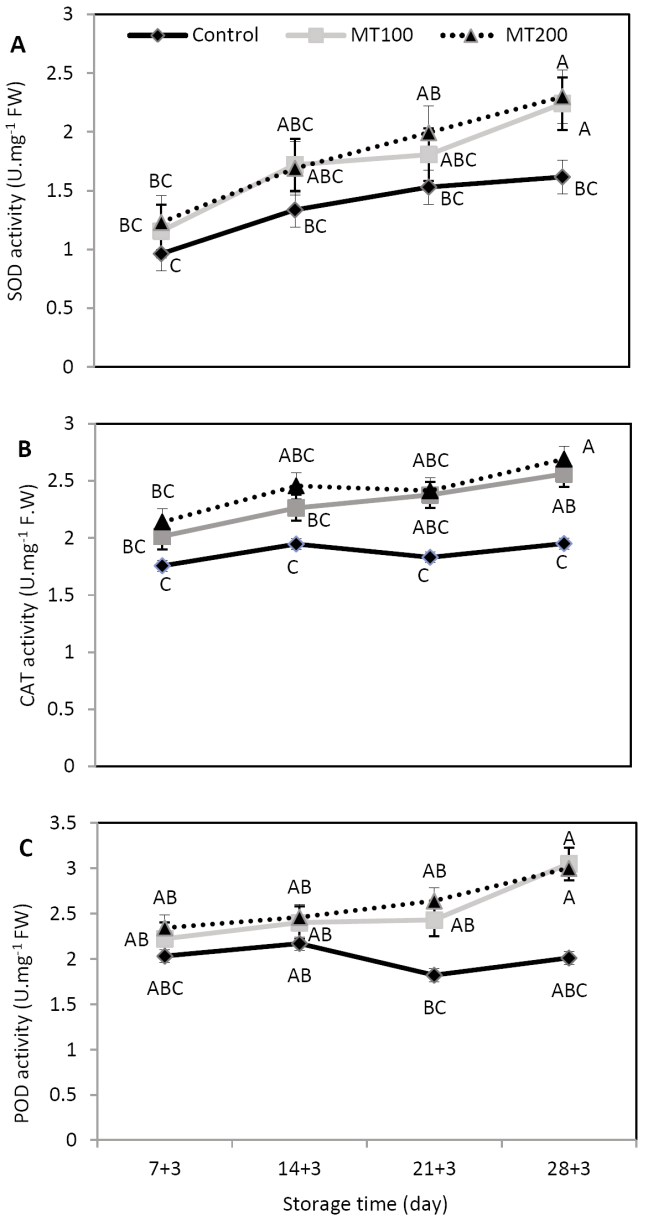

Fig. 6 - Superoxide dismutase (A), Catalase (B) and peroxidase activities (C) of tomato fruits treated with 100 and 200 µM during storage at 5°C and after 3 days of shelf life. All data are presented as a mean of three biological replicates, and vertical bars indicated the standard errors. Different letters indicate significant differences at P<0.01.

symptoms in tomato fruit, but also enhance fruits nutritional quality. This indicates that postharvest treatment with melatonin increased chilling tolerance in tomato fruit. Since melatonin treatment is easy to set up, inexpensive and safe, even if higher amounts are accumulated in the plant (Tan *et al*., 2012), it could be a functional method to decrease chilling injury, maintain quality and prolong shelf life of tomato fruit.

Carotenoids, highly characteristic phytochemicals, known to be potent ROS scavengers and antioxidants, act as a cell proliferation inhibitor and hindering of cancer cell growing (Tijskens and Evelo, 1994; Levi *et al*., 2001; Giovannucci *et al*., 2002; Stahl and Sies, 2005). During maturation/ripening, the green pigment chlorophyll degrades and carotenoids are synthesized. Carotenoids, particularly lycopene and β-carotene, represent the primary components of ripe fruit pigmentation in tomato pericarp and are responsible for the characteristic color of ripe tomatoes, conferring deep red and orange colors, respectively. These carotenoids largely influence flavor and nutritional qualities as well as commercial value and enhances consumer acceptance of fresh tomato fruit (Tijskens and Evelo, 1994). In this study, higher accumulation of lycopene and β-carotene in melatonin treated groups with 100 and 200 µM not only contributed to alleviate chilling injury to fruit, but also lead to normally fruit ripening with uniform red color development (panels B and C of Fig. 2). It has been reported that in tomatoes, the contents of lycopene and β-carotene increase from the green to the fully ripe stage (Fraser *et al*., 1994). Melatonin may affect directly or indirectly other carotenoid genes and/or enzymes in tomato fruit. This could be the case for example of lycopene cyclases, which is responsible for the formation of β-carotene from lycopene, which its accumulation is a ripening-related event in tomato (Giovannoni, 2001). The higher levels of these compounds in melatonin-treated red ripe fruits may be associated with a general acceleration in ripening and with some of the associated transcriptional events, leading to the color change of tomato fruit (Guo, 2015; Sun *et al*., 2015). Therefore, the improved capability of full red color development in chilling-faced melatonin-treated group is one of the most important outcome of this study for the quality of tomato.

Proline, an important amino acid, has been considered as a cellular osmotic regulator, protein stabilizer, free-radical scavenger, and lipid peroxidation inhibitor in plant (Sun *et al*., 2015). The elevated level

of proline found to be associated with improved cold tolerance in chilling-sensitive plants (Zhao *et al*., 2009; Shang *et al*., 2011; Zhang *et al*., 2013; Cao *et al*., 2016). Our findings are in agreement with the above reports (Zhang *et al*., 2010), because a significantly ($P<0.01$) higher content of proline was observed in melatonin-treated tomato fruits with 100 or 200 µM during the whole storage period along with the reduced CI incidence (Fig. 4). Cao *et al*. (2016) reported that higher transcripts of *PpP5CS* and *PpOA* were observed in melatonin-treated peach fruits which provokes proline accumulation. Zhao *et al*. (2009) claimed that proline levels in a tissue may be an effective indicator for CI analysis in postharvest tomato fruits.

The reduction of cell energy and/or induction of alterations in membrane integrity are occurred in chilling-sensitive horticultural commodities at low temperatures. Reducing scavenging potency through such factors as chilling-related inactivation of antioxidants and/or obstructed antioxidant turnover may result in the enhanced ROS generation. Chilling temperatures destroy the balance between ROS formation and defense mechanisms which cause oxidatively chilling injury and consequent cellular damage (Hodges *et al*., 2004). It is figured that antioxidant enzymes, SOD, CAT, and POD are the primary enzymatic scavenging mechanism of ROS that contribute to attenuate chilling injury to fruit (Sala and Lafuente, 2000; Mondal *et al*., 2004; Ding *et al*., 2007; Imahori *et al*., 2008). Thus, this balance between the generation and scavenging of ROS is crucial to cell survival during cold storage and is thought to be a major mechanism of resistance to chilling stress. It has been reported that in harvested commodities enhanced enzymatic antioxidant activities result in the improved chilling tolerance. A higher antioxidant enzyme activity was indicated in the chilling-tolerant mandarins compared with the chilling sensitive cultivars (Sala, 1998). In many other studies, enhancement of antioxidant enzyme activity through a number of postharvest treatments (e.g. heat shock, low temperature conditioning and superatmospheric oxygen treatment) provoked chilling tolerance and alleviated chilling injury to fruit (Wang, 1995; Sala and Lafuente, 2000; Zheng *et al*., 2008). Neutralizing of the O_2^- by SOD is the initial step of cell defense against free radicals (Bowler *et al*., 1992). CAT is one of the enzymes that protect cells against ROS because it catalyzes the decomposition of H_2O_2 to form O_2^- and H_2O_2 (Imahori *et al*., 2008). POD catalyzes H_2O_2 dependent oxidation of substrate (Fu and Huang, 2001). In the present work, the higher increases in activities of SOD, CAT, and POD concurrent with reduced O_2^- and H_2O_2 content in melatonin-treated groups than those in control group were indicated (Fig. 6). While, the levels of H_2O_2 and O_2^- significantly increased during the development of irreversible chilling injury symptoms surface pitting and irregular ripening and full color development in control group. Treatment with melatonin significantly alleviated these chilling-induced damages and increased the activities of SOD, CAT, and POD under cold stress. The increased SOD activity could enhance the ability of the fruit to dismutate superoxide radicals, while the increases in CAT and POD activities would contribute to the stronger omitting of hydrogen peroxide (Lukatkin, 2002), which may give an explanation for the lower levels of O_2^- and H_2O_2 observed in melatonin-treated groups. These results suggest that effect of melatonin in reducing the incidence of chilling injury was correlated to enhanced enzymatic scavenging mechanism of ROS. In melatonin-treated tomato fruits the continues functions of SOD, CAT, and POD may be associated with higher stress resistance and eventually extended shelf life.

Membrane lipid peroxidation may be one of the first events in the manifestation of CI, in which phase MDA as a final product of polyunsaturated fatty acid oxidation was produced and damaged to cell membrane, resulted in ion leakage (Lukatkin, 2002; Imahori *et al*., 2008). As depicted in panels A and B of figure 3, the increase in ion leakage and MDA from 14-day of storage period indicates that cold storage caused a distinct deterioration of membrane integrity and activation of lipid peroxidation in the non-treated control group, which could be attributed to the decreases in SOD and CAT activities as well as in antioxidant compounds including lycopene, β-carotene and proline. These reductions induced by chilling stress favor accumulation of O_2^- and H_2O_2, which can result in lipid peroxidation. Ion leakage and MDA content may be a reflection of CI development and fruit cold tolerance (Zhao *et al*., 2009). Furthermore, Posmyk *et al*. (2005) reported that ion leakage intensity and MDA content in a tissue can be a reliable indicators of the structural integrity of the membranes of plants exposed to low temperature. Given to these results, prevention of MDA accumulation and subsequent ion leakage by melatonin treatment could be related to a low degree of lipid peroxidation, which could result from the maintenance of high enzymatic and non-enzymatic antioxidants. It has been reported that melatonin efficiently con-

tributes to membrane integrity maintenance, and in turn, alleviates symptoms and severity of CI (Cao *et al*., 2016; Soleimani Aghdam and Rezapour Fard, 2017). Treatment with melatonin attenuated chilling induced shrinkage and disruption of carrot cell plasma (Lei *et al*., 2004).

As a whole, the results of this study show that melatonin treatment can effectively enhance chilling tolerance and reduce chilling injury of tomato fruit. The reduction in chilling injury by melatonin may be associated with enhanced enzymatic and non-enzymatic antioxidants, in favor of not only membrane integrity as well as low cellular and tissue damage, but also fruits visual and nutritional qualities. Practically, considering the economic aspect and nutritional risks of melatonin treatment, this compound may be used as an efficient bio-molecule for protecting tomato fruits encountered with chilling.

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References

ARNAO M.B., HERNÁNDEZ-RUIZ J., 2015 - *Functions of melatonin in plants: a review*.- J. Pineal Res., 59: 133–150.

BOURNE M.C., 2006 - *Selection and use of postharvest technologies as a component of the food chain*. - J. Food Sci., 69: 43-46.

BOWLER C., VAN MONTAGU M., INZ D., 1992 - *Superoxide dismutase and stress tolerance*. - Annu. Rev. Plant Physiol., 43: 83-116.

CAO S., SONG C., SHAO J., BIAN K., CHEN W., YANG Z., 2016 - *Exogenous melatonin treatment increases chilling tolerance and induces defense response in harvested peach fruit during cold storage*. - J. Agri. Food Chem., 64(25): 5215-5222.

DING C.K., WANG C.Y., GROSS K.C., SMITH D.L., 2002 - *Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit.* - Planta, 214(6): 895-901.

DING Z.S., TIAN S.P. , ZHENG X.L., ZHOU Z.W, XU Y., 2007 - *Responses of reactive oxygen metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress*. - Physiol Plantarum., 130(1): 112-121.

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.

ELSTNER E.F., 1976 - *Inhibition of nitrite formation hydroxylam-mouium-chloride: A simple assay for superoxide dismutase*. - Anal. Biochem., 70: 616-620.

FRASER P.D., TRUESDALE M.R., BIRD C.R., SCHUCH W., BRAMLEY P.M., 1994 - *Carotenoid biosynthesis during tomato fruit development*. - Plant Physiol., 105: 405-413.

FU J., HUANG B., 2001 - *Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress*. - Environ. Exp. Bot., 45: 105-114.

GAO H., ZHANG Z.K., CHAI H.K., CHENG N., YANG Y., WANG D.N., CAO W., 2016 - *Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit*. - Postharvest Biol. Technol., 118: 103-110.

GIANNOPOLITIS C.N., RIES S.K., 1977 - *Superoxide dismutases: I occurrence in higher plants*. - Plant Physiol., 59(2): 309-314.

GIOVANNONI J.J., 2001 - *Molecular regulation of fruit ripening*. - Annu. Rev. Plant Physiol. Plant Mol. Biol., 52: 725-749.

GIOVANNUCCI E., RIMM E.B., LIU Y., STAMPFER M.J., WILLETT W.C., 2002 - *A prospective study of tomato products, lycopene, and prostate cancer risk*. - J. Natl. Cancer Inst., 94: 391-398.

GUO Y.D., 2015 - *Melatonin promotes ripening and improves quality of tomato fruit during harvested life*. - J. Exp. Bot., 66: 657-668.

HATTORI A., MIGITAKA H., IIGO M., ITOH M., YAMAMOTO K., OHTANIKANEKO 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.

HODGES D.M., LESTER G.E., MUNRO K.D., TOIVONEN A., 2004 - *Oxidative stress: importance for postharvest quality*. - HortSci., 39: 924-929.

HONG J.H., GROSS K.C., 2006 - *Maintaining quality of fresh-cut tomato slices through modified atmosphere packaging and low temperature storage.* - J. Food Sci., 66(7): 960-965.

IMAHORI Y., TAKEMURA M., BAI J., 2008 - *Chilling-induced oxidative stress and antioxidant responses in mume (*Prunus mume*) fruit during low temperature storage*. - Postharvest Biol. Technol., 49(1): 54-60.

LEI X.Y., ZHU R.Y., ZHANG G.Y., DAI Y.R., 2004 - *Attenuation of cold-induced apoptosis by exogenous melatonin in carrot suspension cells: The possible involvement of polyamines*. - J. Pineal Res., 36: 126–131.

LEVI F., PASCHE C., LUCCHINI F., LA VECCHIA C., 2001 - *Dietary intake of selected micronutrients and breast-*

cancer risk. - Int. J. Cancer., 91: 260-263.

LIU J., ZHANG R., SUN Y., LIU Z., JIN W., SUN Y., 2016 - *The beneficial effects of exogenous melatonin on tomato fruit properties*. - Sci. Hortic., 207: 14-20.

LUKATKIN A.S., 2002 - *Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants: The activity of antioxidant enzymes during plant chilling*. - Russian J. Plant Physiol., 49: 782-788.

MA Q., ZHANG T., ZHANG P., WANG Z.Y., 2016 - *Melatonin attenuates postharvest physiological deterioration of cassava storage roots*. - J. Pineal Res., 60(4): 424-434.

MAEHLY A.C., CHANCE B., 1954 - *The assay of catalase and peroxidase*. - Methods Biochem. Anal., 1: 357-424.

MANCHESTER L.C., COTO-MONTES A., BOGA J.A., ANDERSEN L.P., ZHOU Z., GALANO A., VRIEND J., TAN D.X., REITER R.J., 2015 - *Melatonin: An ancient molecule that makes oxygen metabolically tolerable*. - J. Pineal Res., 59: 403-419.

MENG J.F., XU T.F., SONG C.Z., YU Y., HU F., ZHANG L., XI Z.M., 2015 - *Melatonin treatment of pre-veraison grape berries to increase size and synchronicity of berries and modify wine aroma components*. - Food Chem., 185: 127-134.

MONDAL K., SHARMA N.S., MALHOTRA S.P., DHAWAN K., SINGH R., 2004 - *Antioxidant systems in ripening tomato fruit*. - Biol. Plant., 48: 49-53.

NAGATA M., YAMASHITA I., 1992 - *Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit.* - J. Jpn. Soc. Food Sci. Technol., 39: 925-928.

PATTERSON B.D., MACKAE E.A., FERGUSON I.B., 1984 - *Estimation of hydrogen peroxide in plant extracts using titanium*. - Anal. Biochem., 139: 487-492.

POSMYK M.M., BAILLY C., SZAFRÁNSKA K., JANA K.M., CORBINEAU F., 2005 - *Antioxidant enzymes and isoflavonoids in chilled soybean (*Glycinemax *(L.) Merr.) seedling*. - J. Plant Physiol., 162(4): 403-412.

SALA J.M., 1998 - *Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits*. - Postharvest Biol. Technol., 13: 255-261.

SALA J.M., LAFUENTE M.T., 2000 - *Catalase enzyme activity is related to tolerance of mandarin fruits to chilling*. - Postharvest Biol. Technol., 20(1): 81-89.

SALTVEIT M.E., 1991 - *Determining tomato fruit maturity with nondestructive* in vivo *nuclear magnetic resonance imaging*. - Postharvest Biol. Technol., 1: 153-159.

SHAN D.P., HUANG J.G., YANG Y.T., GUO Y.H., WU C.A., YANG G.D., GAO Z.G., ZHENG C.C., 2007 - *Cotton GhDREB1 increases plant tolerance to low temperature and is negatively regulated by gibberellic acid*. - New Phytol., 176(1): 70-81.

SHANG H.T., CAO S.F., YANG Z.F., CAI Y.T., ZHENG Y.H., 2011 - *Effect of exogenous γ-aminobutyric acid treatment on proline accumulation and chilling injury in peach fruit after long-term cold storage*. - J. Agric. Food Chem., 59: 1264-1268.

SOLEIMANI AGHDAM M., REZAPOUR FARD J., 2017 - *Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (*Fragaria anannasa *cv. Selva) by enhancing GABA shunt activity*. - Food Chem., 221: 1650-1657.

STAHL W., SIES H., 2005 - *Bioactivity and protective effects of natural carotenoids*. - Biochim. Biophys. Acta, 1740(2): 101-107.

SUN Q.Q., ZHANG N., WANG J.F., ZHANG H.J., LI D.B., SHI J., LI R., WEEDA S., ZHAO B., REN S.X., GUO Y.D., 2015 - *Melatonin promotes ripening and improves quality of tomato fruit during postharvest life*. - J. Exp. Bot., 66(3): 657-668.

TAL 0., HAIM A., HAREL O., GERCHMAN Y., 2011 - *Melatonin as an antioxidant and its semi-lunar rhythm in green macroalga* Ulva *sp*. - J. Exp. Bot., 62(6): 1903-1910.

TAN D.X., HARDELAND R., MANCHESTER L.C., KORKMAZ A., MA S., ROSALES-CORRAL S., REITER R.J., 2012 - *Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science.* - J. Exp. Bot., 63(2): 577-597.

TIJSKENS L.M., EVELO R.W., 1994 - *Modelling colour of tomatoes during postharvest storage*. - Postharvest Biol. Technol., 4(1-2): 85-89.

WANG C.Y., 1993 - *Approaches to reduce chilling injury of fruits and vegetables*. - Hort. Rev., 15: 83-95.

WANG C.Y., 1995 - *Effect of temperature preconditioning on catalase, peroxidase, and superoxide dismutase in chilled zucchini squash*. - Postharvest Biol. Technol., 5: 67-76.

YIN L., WANG P., LI M., KE X., LI C., LIANG D., MA F., 2013 - *Exogenous melatonin improves Malus resistance to Marssonina apple blotch*. - J. Pineal Res., 54(4): 426-434.

ZHANG H.M., ZHANG Y., 2014 - *Melatonin: A well-documented antioxidant with conditional pro-oxidant actions*. - J. Pineal Res., 57: 131–146.

ZHANG N., SUN Q.Q., ZHANG H.J., CAO Y.Y., WEEDA S., REN S.X., GUO Y.D., 2015 - *Roles of melatonin in abiotic stress resistance in plants*. - J. Exp. Bot., 66(3): 647-656.

ZHANG N., ZHANG H.J., ZHAO B., SUN Q.Q., CAO Y.Y., LI R., WIU X.X., WEEDA S., LI L., REN S., REITER R.J., GUO Y.D., 2014 - *The RNA-seq approach to discriminate gene expression profiles in response to melatonin on cucumber lateral root formation*. - J. Pineal Res., 56(1): 39-50.

ZHANG X., SHEN L., LI F., ZHANG Y., MENGA D., SHENG J., 2010 - *Up-regulating arginase contributes to amelioration of chilling stress and the antioxidant system in cherry tomato fruits*. - J. Sci. Food Agric., 90: 2195-2202.

ZHANG Z., HUBER D.J., RAO J., 2013 - *Antioxidant systems of ripening avocado (*Persea americana *Mill.) fruit following treatment at the preclimacteric stage with aqueous 1-methylcyclopropene*. - Postharvest Biol. Technol., 76: 58-64.

ZHAO D.Y., SHEN L., FAN B., LIU K.L., YU M.M., ZHENG Y., DING Y., SHENG J.P., 2009 - *Physiological and genetic properties of tomato fruits from 2 cultivars differing in chilling tolerance at cold storage*.- Food Chem., 74: 348-352.

ZHENG Y.H., RAYMOND W.M.F., WANG S.Y., WANG C.Y., 2008 - *Transcript levels of antioxidative genes and oxygen radical scavenging enzyme activities in chilled zucchini squash in response to superatmospheric oxygen*. - Postharvest Biol Technol., 47: 151-158.