Scientia Horticulturae 254 (2019) 222–227

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Melatonin treatment promotes endogenous melatonin accumulation and triggers GABA shunt pathway activity in tomato fruits during cold storage

Yavar Sharafi[f], Morteza Soleimani Aghdam[a,*], Zisheng Luo[b,*], Abbasali Jannatizadeh[a], Farhang Razavi[c], Javad Rezapour Fard[d], Boukaga Farmani[e]

[a] Department of Horticultural Science, Imam Khomeini International University, Qazvin, Iran
[b] Zhejiang University, College of Biosystems Engineering and Food Science, Key Laboratory of Agro-Products Postharvest Handling Ministry of Agriculture, Zhejiang Key Laboratory for Agro-Food Processing, Hangzhou, 310058, People's Republic of China
[c] Department of Horticulture, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
[d] Department of Horticultural Sciences, Faculty of Agricultural Sciences, Urmia University, Urmia, Iran
[e] Department of Food Science and Technology, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Tabriz, Iran
[f] Department of Horticulture, Faculty of Agriculture, Shahed University, Tehran, Iran

ARTICLE INFO

Keywords:
DPPH scavenging capacity
Low temperature storage
Phenols accumulation
Phenylpropanoid pathway activity
ROS scavenging capacity
Signaling H_2O_2 accumulation

ABSTRACT

Higher H_2O_2 accumulation in tomato fruits during storage at 4 °C for 7 days by melatonin treatment at 100 μM may serve as signaling molecule for promoting endogenous melatonin accumulation by trigerring melatonin biosynthesis *TDC*, *T5H*, *SNAT,* and *ASMT* genes expression during storage at 4 °C for 28 days. Also, higher GABA shunt pathway activity demonstrating by higher GAD, GABA-T and SSADH enzymes activity in tomato fruits treated with melatonin may be responsible for promoting phenylpropanoid pathway activity demonstrating by higher *PAL* gene expression and enzyme activity giving rise to higher phenols accumulation and higher DPPH scavenging capacity during storage at 4 °C for 28 days. Hence, exogenous melatonin treatment may serve as beneficial procedure for response to chilling stress in tomato fruits by signaling H_2O_2 accumulation responsible for promoting endogenous melatonin accumulation accompanying by stimulating GABA shunt pathway activity.

1. Introduction

Cold storage has been employed for extending postharvest life accompanying by keeping sensory and nutritional quality of tomato fruits. But, tomato fruits suffer from chilling injury during cold storage. Due to the high socio-economic value of tomato fruits, promising procedures have been employed for ameliorating chilling injury in tomato fruits during cold storage (Aghdam et al., 2019c; Aghdam et al., 2019d).

In plants, melatonin (*N*-acetyl-5-methoxytryptamine) exerts as a universal biostimulator and signaling biomolecule (Galano and Reiter, 2018). Owing to melatonin superior reactive oxygen species (ROS) scavenging cascade, exogenous melatonin application as well as endogenous melatonin accumulation may be pivotal for ameliorating oxidative stress in horticultural crops during cold storage (Aghdam et al., 2019a; Cao et al., 2018b; Gao et al., 2018; Jannatizadeh, 2019; Liu et al., 2018). In addition to ROS scavenging cascade, melatonin serves as signaling molecule via perception by G protein-coupled receptor (PMTR1) at plasma membrane giving rise to cytosolic Ca^{2+} raising and signaling H_2O_2 accumulation via NADPH oxidase enzyme activity (Wei et al., 2018). So, exogenous melatonin application, as well as endogenous melatonin accumulation, may be beneficial for ameliorating stresses, delaying senescence and keeping sensory and nutritional quality of horticultural crops during cold storage. In recent years, exogenous melatonin application has been employed for ameliorating stresses, delaying senescence and keeping sensory and nutritional quality of horticultural crops during cold storage (Aghdam and Fard, 2017; Aghdam et al., 2019a,c; Cao et al., 2018a; Cao 2016; Gao et al., 2018; Gao et al., 2016, Hu 2018; Jannatizadeh, 2019; Jannatizadeh et al., 2019, Liu 2019; Zhang et al., 2018). Cao et al. (2018b) stated that the ameliorating chilling injury in peach fruits by melatonin treatment (100 μM) may attribute to signaling H_2O_2 accumulation during 7 days of cold storage which may be responsible for provoking ROS scavenging system activity giving rise to lower O_2^- and H_2O_2 accumulation during 28 days of cold storage. Liu et al. (2018) stated that the delaying strawberry fruits senescence by melatonin treatment (100 μM) may attribute to promoting endogenous melatonin accumulation arising from higher melatonin biosynthesis *tryptophan decarboxylase (TDC)*, *tryptamine 5-hydroxylase (T5H)*, *serotonin N-acetyltransferase (SNAT)*

* Corresponding authors.
E-mail addresses: soleimaniaghdam@eng.ikiu.ac.ir (M.S. Aghdam), luozisheng@zju.edu.cn (Z. Luo).

https://doi.org/10.1016/j.scienta.2019.04.056
Received 28 February 2019; Received in revised form 17 April 2019; Accepted 18 April 2019
Available online 04 May 2019
0304-4238/

and *N-acetylserotonin methyltransferase (ASMT)* genes expression accompanying by higher phenols and flavonoids accumulation giving rise to higher DPPH and ABTS scavenging capacity, which both are pivotal for ameliorating H_2O_2 accumulation. Beneficial effects of exogenous melatonin application for ameliorating chilling injury in tomato fruits during cold storage may ascribe to keeping membrane integrity demonstrating by lower electrolyte leakage and malondialdehyde (MDA) accumulation attributing to supplying sufficient intracellular energy arising from promoting higher H^+-ATPase, Ca^{2+}-ATPase, cytochrome c oxidase (CCO), and succinate dehydrogenase (SDH) enzymes activity, preserving membrane unsaturated/saturated fatty acids (unSFA/SFA) ratio owing to higher linoleic and linolenic acids accumulation concomitant with lower palmitic, stearic and oleic acids accumulation arising from higher *fatty acid desaturase 3* (*FAD3*) and *7* (*FAD7)* genes expression accompanying by lower *phospholipase D (PLD)* and *lipoxygenase (LOX)* genes expression and enzymes activity and eliciting endogenous polyamines, proline, and nitric oxide accumulation by triggering *ZAT2/6/12* genes expression giving rise to prompting *CBF1* signaling pathway (Aghdam et al., 2019c; Jannatizadeh et al., 2019).

During postharvest life, functioning γ-aminobutyric acid (GABA) shunt pathway activity is fundamental for ameliorating stresses, delaying senescence and keeping sensory and nutritional quality of horticultural crops during cold storage ((Aghdam et al., 2019b; Han 2018; Palma et al., 2019). During senescence and stresses, GABA shunt pathway is initiated by glutamate decarboxylase (GAD), which is responsible for GABA biosynthesis. Then, GABA transaminase (GABA-T) is responsible for the production of succinic semialdehyde (SSA) in mitochondria, which enter the tricarboxylic acid cycle by succinic semialdehyde dehydrogenase (SSADH) as succinate, accompanying with NADPH production. The GABA shunt pathway activity under oxidative stress operates as an electron donor to mitochondrial electron transport chain in addition to NADH production (Shelp et al., 2017). Insufficient energy and skeleton carbons during chilling stress may ascribe to succinyl-CoA ligase and oxoglutarate dehydrogenase enzymes vulnerability to damaging ROS accumulation (Sweetlove et al., 2002). Since ameliorating chilling stress require higher intracellular ATP and carbon skeletons, functioning GABA shunt pathway is fundamental for furnishing NADH and succinate for mitochondrial tricarboxylic acid cycle and electron transport chain, which is pivotal for providing sufficient intracellular ATP and carbon skeletons accompanying by ameliorating H_2O_2 accumulation (Michaeli and Fromm, 2015; Palma et al., 2019).

The goal of our study was to evaluating the roles of endogenous melatonin accumulation and GABA shunt pathway activity in tomato fruits treated with melatonin in response to chilling stress during cold storage.

2. Materials and methods

2.1. Fruits and melatonin treatment

The tomato fruits cv. Izmir were harvested at mature green stage and then treated with 100 μM according the previous study by Aghdam et al. (2019c). For melatonin treatment, fruits were immersed in 10 L of fresh melatonin solution for 5 min and in distilled water as a control. Fruit were allowed to completely dry at room temperature before storage at 4 ± 0.5 °C and 85–90% RH for 28 days. For biochemical and gene expression analysis, 15 fruit per replicate of each treatment were sampled after 7, 14, 21 or 28 days' cold storage.

2.2. Endogenous H_2O_2 and melatonin accumulation

The titanium (IV) procedure at 410 nm was employed for analyzing H_2O_2 accumulation according to Patterson et al. (1984). The H_2O_2 accumulation was expressed as μmol g^{-1} fresh weight. The melatonin accumulation was analyzed by HPLC as stated by Sun et al. (2015). The melatonin accumulation was expressed as ng g^{-1} fresh weight.

2.3. GABA shunt pathway activity

Pseudomonas fluorescens GABase (Sigma) at 340 nm was employed for analyzing of GABA accumulation enzymatically as suggested by Deewatthanawong et al. (2010). GABA accumulation was expressed as μmol g^{-1} fresh weight. GAD enzyme activity was analyzed based on GABA production, as stated by Bartyzel et al. (2003). GAD enzyme activity was expressed as μmol GABA mg^{-1} protein s^{-1}. GABA-T enzyme activity was analyzed based on alanine production according to Ansari et al. (2005). GABA-T enzyme activity expressed as μmol alanine mg^{-1} protein s^{-1}. SSADH enzyme activity was analyzed based on succinate production as stated by Thorburn et al. (1993). SSADH enzyme activity was expressed as μmol succinate mg^{-1} protein s^{-1}.

2.4. Phenols accumulation and DPPH scavenging capacity

Phenylalanine ammonia lyase (PAL) enzyme activity was analyzed as stated by Chen et al. (2008). PAL enzyme activity was expressed by U mg^{-1} protein. Phenols accumulation was analyzed by the Folin–Ciocalteu procedure (Singleton and Rossi, 1965). Phenols accumulation was expressed as mg gallic acid equivalent (GAE) g^{-1} fresh weight. DPPH scavenging capacity (%) was analyzed as stated by Nakajima et al. (2004).

2.5. Genes expression assay by RT-qPCR

Total RNA extraction and cDNA synthesis was performed as stated

Table 1
The primers used for qRT-PCR.

Gene name	Accession numbers	Functional annotations	Primer sequences (5′-3′)
TDC	Solyc09g064430	Melatonin biosynthesis	F: TGCTCTAGACTCCGTCACCACAAAGC R: CGCGGATCCCCCAGAAATCCAGCCAC
T5H	Solyc09g014900	Melatonin biosynthesis	F: TGCTCTAGATGATTTCGTCTCGTTCT R: CGCGGATCCCTCTTCTTCATCCCACT
SNAT	Solyc10g074910	Melatonin biosynthesis	F: TGCTCTAGATCTTCCCTACCCACTAT R: CGCGGATCCACATCAACATCTCCTCC
ASMT	Solyc03g080180	Melatonin biosynthesis	F: TGCTCTAGAGCTTTCTTGTTCGCTAT R: CGCGGATCCGTCCATCAGGGAGTGTC
PAL	Solyc03g042560	Phenols biosynthesis	F: ATTGGGAAATGGCTGCTGATT R: TCAACATTTGCAATGGATGCA
Actin	Solyc03g078400	Marker gene	F: TGGTCGGAATGGGACAGAAG; R: CTCAGTCAGGAGAACAGGGT

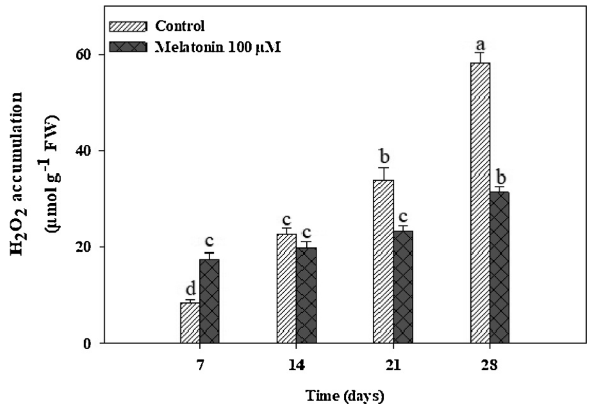

Fig. 1. Endogenous H_2O_2 accumulation in tomato fruits treated with melatonin during cold storage. Data shown as mean ± SE (n = 3). Significantly values were shown according to Tukey's test at $P < 0.05$.

by Zhao et al. (2011). *TDC*, *T5H*, *SNAT*, *ASMT* and *PAL* genes expression (Table 1) was assayed according to Aghdam et al. (2019c).

2.6. Statistical analysis

The investigate was conducted using a factorial design with melatonin treatment (0 and 100 µM) and storage time (7, 14, 21 and 28 days) as the two factors (n = 3, 120 fruits per each replicate, 30 fruits per each sampling time). All analyses were performed with SPSS software version 20 (SPSS Inc., Chicago, IL, USA). Means were compared by Tukey's test and differences at $p < 0.05$ were considered significant.

3. Results

3.1. Endogenous H_2O_2 accumulation

As shown in Fig. 1, tomato fruits treated with melatonin at 100 µM exhibited significantly higher signaling H_2O_2 accumulation during 7 days of cold storage. After 7 days of cold storage, tomato fruits treated with melatonin exhibited significantly lower damaging H_2O_2 accumulation (Fig. 1; $P < 0.01$) demonstrates ameliorating oxidative stress in tomato fruits which may arise from endogenous melatonin accumulation exhibiting superior ROS scavenging attributes (Galano and Reiter, 2018).

3.2. Endogenous melatonin accumulation

As seen in Fig. 2, tomato fruits treated with melatonin at 100 µM exhibited significantly higher melatonin accumulation ($P < 0.01$) during cold storage. Higher melatonin accumulation in tomato fruits treated with melatonin at 100 µM may ascribe to higher melatonin biosynthesis *TDC, T5H, SNAT,* and *ASMT* genes expression during cold storage (Fig. 2; $P < 0.01$).

3.3. GABA shunt pathway activity

As seen in Table 2, tomato fruits treated with melatonin at 100 µM exhibited significantly higher GABA shunt pathway activity demonstrating by higher GAD, GABA-T and SSADH enzymes activity ($P < 0.01$) during cold storage.

3.4. Phenols accumulation and DPPH scavenging capacity

Tomato fruits treated with melatonin at 100 µM exhibited significantly higher *PAL* gene expression (Fig. 3; $P < 0.01$) and enzyme activity (Table 3; $P < 0.01$) giving rise to higher phenols accumulation (Table 3; $P < 0.01$) and higher DPPH scavenging capacity (Table 3; $P < 0.01$) during cold storage.

4. Discussion

Higher H_2O_2 accumulation in tomato fruits during storage at 4 °C for 7 days by melatonin treatment may serve as signaling molecule for response to chilling stress. Signaling H_2O_2 accumulation in tomato fruits treated with melatonin during 7 days of cold storage may arise from perception of melatonin on plasma membrane by PMTR1 receptor giving rise to cytosolic Ca^{2+} raising and signaling H_2O_2 accumulation via NADPH oxidase enzyme activity. Signaling H_2O_2 accumulation in tomato fruits treated with melatonin during 7 days of cold storage may serve as defense response to chilling stress in tomato fruits by promoting endogenous melatonin accumulation (Lee and Back, 2017; Yuan et al., 2016). Also, lower damaging H_2O_2 accumulation after 7 days of cold storage in tomato fruits treated with melatonin demonstrates ameliorating oxidative stress in tomato fruits which may arise from endogenous melatonin accumulation exhibiting superior ROS scavenging attributes (Galano and Reiter, 2018). Melatonin exhibits ROS scavenging cascade, so one melatonin molecule can scavenge up to 10 ROS, compared to ascorbic acid, tocopherols, and glutathione antioxidants, which can only scavenge one ROS per one molecule (Galano and Reiter, 2018). Also, owing to the amphiphilic nature, melatonin exhibits higher intracellular distribution, causing bidirectional recycling of ascorbic acid, tocopherols, and glutathione antioxidants, giving rise to heightening cellular ROS scavenging capacity. Melatonin is fundamental for the formation of the cellular antioxidant network, which operations as a bridge for joining cellular hydrophilic and lipophilic antioxidants, which are pivotal for bidirectional recycling of antioxidants, giving rise to heightening cellular ROS scavenging capacity (Galano and Reiter, 2018; Tan et al., 2013). As well as direct ROS scavenging cascade, melatonin is efficient for heightening ROS scavenging systems activity (Galano and Reiter, 2018; Tan et al., 2013). Accordingly, after 7 days of cold storage, lower damaging H_2O_2 accumulation in tomato fruits treated with melatonin may attribute to melatonin operation in heightening cellular ROS scavenging capacity.

Higher endogenous melatonin accumulation in tomato fruits treated with melatonin may ascribe to signaling H_2O_2 accumulation during 7 days of storage at 4 °C (Lee and Back, 2017; Wei et al., 2016). Higher melatonin biosynthesis *TDC, T5H, SNAT,* and *ASMT* genes expression in tomato fruits treated with melatonin may ascribe to signaling H_2O_2 accumulation giving rise to higher melatonin accumulation during cold storage. In plants, tryptophan is a precursor in melatonin biosynthesis (Back et al., 2016). TDC is responsible for the decarboxylation of tryptophan to tryptamine or 5-hydroxytryptophan to serotonin in the cytoplasm. T5H is responsible for the hydroxylation of tryptamine to serotonin in the endoplasmic reticulum. SNAT is responsible for the acetylation of serotonin to *N*-acetylserotonin or 5-methoxytryptamine to melatonin in chloroplasts. ASMT is responsible for *O*-methylation of *N*-acetylserotonin to melatonin or serotonin to 5-methoxytryptamine in cytoplasms. Accordingly, SNAT is responsible for melatonin biosynthesis in cytoplasm and ASMT is responsible for melatonin biosynthesis in chloroplast, so cytoplasm and chloroplast are prevailing cellular site for melatonin biosynthesis in plants (Back et al., 2016). In addition to cytoplasm and chloroplast, plants mitochondria have melatonin biosynthesis capacity by SNAT enzyme activity (Wang et al., 2017). During higher ROS accumulation in response to drought stress in apple, higher mitochondrial melatonin accumulation operation as a powerful ROS scavenging strategy for ameliorating drought stress (Wang et al., 2017). Also, transgenic *Arabidopsis* ectopically expressing apple *SNAT* gene exhibited higher melatonin accumulation in mitochondria, ameliorating drought stress by acting as a powerful ROS scavenger (Wang et al., 2017). Since electron transport chain in mitochondria during postharvest respiration is prevailing ROS producing sites by electron leakage, having melatonin biosynthesis capacity in mitochondria is

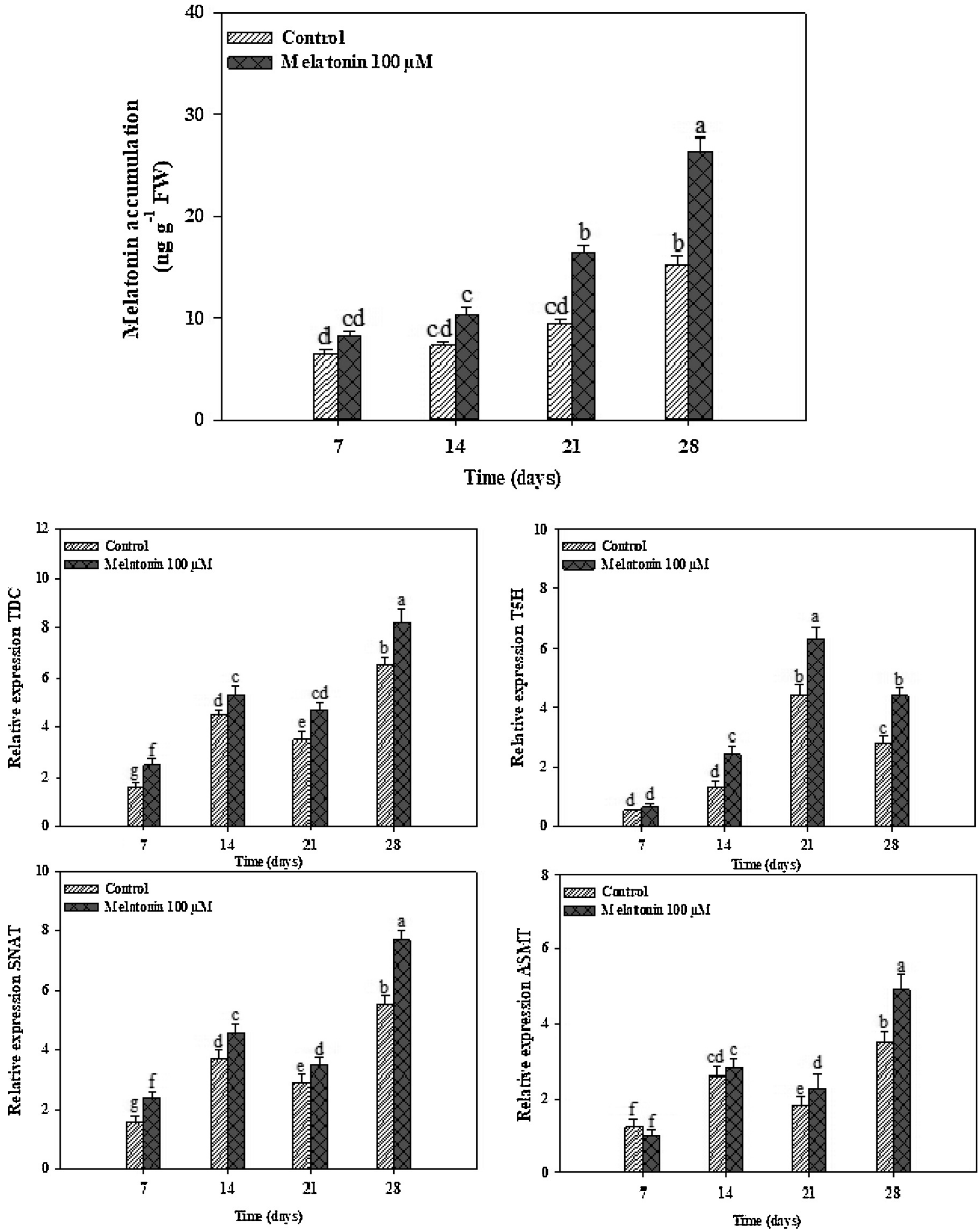

Fig. 2. Endogenous melatonin accumulation accompanying by *TDC*, *T5H*, *SNAT* and *ASMT* genes expression in tomato fruits treated with melatonin during cold storage. Data shown as mean ± SE (n = 3). Significantly values were shown according to Tukey's test at $P < 0.05$.

pivotal for ameliorating oxidative stress by detoxifying mitochondrial ROS in horticultural crops during postharvest life (Wang et al., 2017). Accordingly, higher endogenous melatonin accumulation by trigerring melatonin biosynthesis *TDC, T5H, SNAT,* and *ASMT* genes expression in tomato fruits treated with melatonin may be beneficial for ameliorating oxidative stress during cold storage. So, keeping membrane integrity as shown by lower electrolyte leakage and MDA accumulation in tomato fruits treated with melatonin as stated by Aghdam et al. (2019c) may arise from higher endogenous melatonin accumulation exhibiting ROS scavenging capacity.

Due to C-terminal calmodulin (CaM)-binding domain, higher Ca^{2+}/CaM may be pivotal for stimulating GABA shunt pathway activity by enhancing GAD enzyme activity (Igamberdiev and Hill, 2018; Shelp et al., 2017). Higher GABA shunt pathway activity demonstrating by higher GAD, GABA-T and SSADH enzymes activity in tomato fruits treated with melatonin may ascribe to cytosolic Ca^{2+} raising by melatonin perception on plasma membrane by PMTR1 receptor. Higher GABA shunt pathway activity is responsible for furnishing NADH and succinate for mitochondrial tricarboxylic acid cycle and electron transport chain, which is pivotal for providing sufficient intracellular

Table 2
GAD, GABA-T and SSADH enzymes activity in tomato fruits treated with melatonin during cold storage.

Time (day)	Treatment	GABA shunt pathway activity			
	Melatonin (μM)	GAD activity (μ mol mg^{-1} protein s^{-1})	GABA-T activity (μ mol mg^{-1} protein s^{-1})	SSADH activity (μ mol mg^{-1} protein s^{-1})	GABA accumulation (μ mol g^{-1} FW)
7	0	10.27 ± 0.46 f	24.21 ± 1.42 d	32.32 ± 1.65 f	0.55 ± 0.028 e
	100	12.48 ± 0.44 ef	28.85 ± 1.25 d	36.58 ± 1.07 f	0.53 ± 0.036 e
14	0	15.32 ± 0.66 e	30.25 ± 1.19 d	46.24 ± 1.38 e	0.87 ± 0.055 de
	100	18.58 ± 0.62 d	38.53 ± 1.48 c	50.25 ± 0.62 de	0.95 ± 0.055 d
21	0	20.58 ± 0.98 d	37.04 ± 1.18 c	55.49 ± 1.58 cd	1.42 ± 0.096 c
	100	29.52 ± 0.83c	42.54 ± 1.43 c	65.84 ± 1.60 b	1.12 ± 0.067 cd
28	0	38.31 ± 1.1 b	52.24 ± 1.99 b	62.57 ± 1.20 bc	2.86 ± 0.172 a
	100	48.67 ± 0.93 a	84.19 ± 2.79 a	76.57 ± 1.42 a	2.12 ± 0.135 b

Mean values ± SE (n = 3). Different letters indicate significant differences among samples determined by Tukey's test ($P < 0.05$).

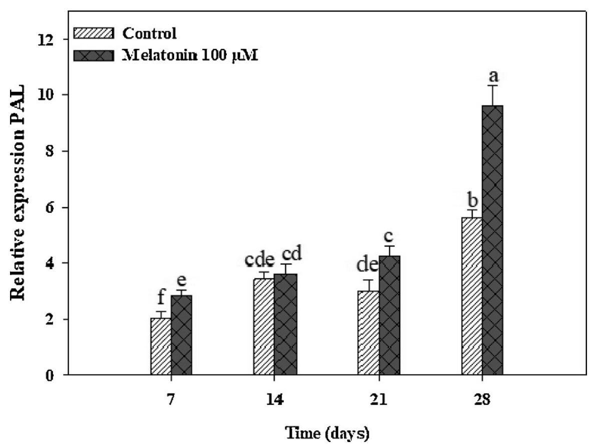

Fig. 3. PAL gene expression in tomato fruits treated with melatonin during cold storage. Data shown as mean ± SE (n = 3). Significantly values were shown according to Tukey's test at $P < 0.05$.

Table 3
Phenols accumulation and DPPH scavenging capacity in tomato fruits treated with melatonin during cold storage.

Time (day)	Treatment	Phenolic Metabolism		
	Melatonin (mM)	Phenols accumulation (mg GAE g^{-1} FW)	DPPH scavenging capacity (%)	PAL activity (U mg $^{-1}$ protein)
7	0	1.49 ± 0.092 c	36.79 ± 2.83 d	17.87 ± 2.14 d
	100	1.85 ± 0.038 c	42.07 ± 2.51 cd	24.87 ± 1.53 d
14	0	1.68 ± 0.062 c	38.58 ± 2.16 d	22.62 ± 1.46 d
	100	2.03 ± 0.083 c	48.67 ± 0.91 bc	34.47 ± 1.64 c
21	0	1.9 ± 0.044 c	42.53 ± 1.42 cd	36.13 ± 1.38 c
	100	2.96 ± 0.127 ab	55.78 ± 1.47 b	45.73 ± 2.24 b
28	0	2.54 ± 0.116 b	49.2 ± 1.69 bc	40.7 ± 0.95 bc
	100	3.44 ± 0.26 a	70.57 ± 3.58 a	58.45 ± 2.56 a

Mean values ± SE (n = 3). Different letters indicate significant differences among samples determined by Tukey's test ($P < 0.05$).

ATP and carbon skeletons accompanying by ameliorating H_2O_2 accumulation during chilling stress (Aghdam and Fard, 2017; Palma et al., 2016, 2019; Palma et al., 2015). Accordingly, higher GABA shunt pathway activity in tomato fruits treated with melatonin may be a defense response to chilling stress in tomato fruits during cold storage by contribution to ameliorating H_2O_2 accumulation and providing intracellular ATP and carbon skeletons for phenylpropanoid pathway activity. So, keeping membrane integrity as shown by lower electrolyte leakage and MDA accumulation in tomato fruits treated with melatonin as stated by Aghdam et al. (2019c) may arise from higher GABA shunt pathway activity beneficial for ameliorating H_2O_2 accumulation and providing intracellular ATP. Sufficient NH_4^+ supplying by PAL enzyme activity is key for supplying glutamate for GABA shunt pathway activity via GS/GOGAT cycle (Aghdam and Fard, 2017). Therefore, higher GABA shunt pathway activity in tomato fruits treated with melatonin may attribute to higher PAL enzyme activity serving as glutamate supplier by GS/GOGAT cycle activity. Also, Aghdam and Fard (2017) stated that the ameliorating fungal decay in strawberry fruits by melatonin applying attributed to higher GABA shunt pathway activity serving as sufficient intracellular ATP and skeleton carbons supplier for PAL enzyme activity leading to higher phenols and anthocyanins accumulation and higher DPPH scavenging capacity. Accordingly, higher PAL enzyme activity in tomato fruits treated with melatonin may arise from higher GABA shunt pathway activity serves as sufficient intracellular ATP and skeleton carbons supplier for phenylpropanoid pathway activity. Higher phenols accumulation in tomato fruits treated with melatonin not only are vital for response to chilling stress but also are favorable for human health. According to Wei et al. (2018), perception of melatonin on plasma membrane by PMTR1 receptor giving rise to cytosolic Ca^{2+} raising and signaling H_2O_2 accumulation via NADPH oxidase enzyme activity. Hence, in addition to higher GABA shunt pathway activity, higher PAL enzyme activity in tomato fruits treated with melatonin may ascribe to signaling H_2O_2 accumulation (Wang et al., 2015).

5. Conclusion

Our results demonstrated the valuable effects of melatonin treatment at 100 μM on triggering defense response to chilling stress in tomato fruits during cold storage. So, stimulating GABA shunt pathway activity in tomato fruits treated with melatonin may serves as sufficient intracellular ATP and skeleton carbons supplier for phenylpropanoid pathway activity and ameliorating damaging H_2O_2 accumulation during cold storage. Also, promoting endogenous melatonin accumulation by trigerring melatonin biosynthesis *TDC*, *T5H*, *SNAT* and *ASMT* genes expression in tomato fruits treated with melatonin may serves as powerful ROS scavenging strategy during cold storage. Signaling operation of melatonin by perception on plasma membrane by PMTR1 receptor giving rise to cytosolic Ca^{2+} raising and signaling H_2O_2 accumulation via NADPH oxidase enzyme activity. Signaling H_2O_2 accumulation may be responsible for promoting endogenous melatonin accumulation and stimulating GABA shunt pathway activity in tomato fruits during cold storage. Therefore, exogenous melatonin treatment may serve as beneficial procedure for response to chilling stress in tomato fruits during cold storage by signaling H_2O_2 accumulation responsible for promoting endogenous melatonin accumulation accompanying by stimulating GABA shunt pathway activity.

References

Aghdam, M.S., Fard, J.R., 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.

Aghdam, M.S., Jannatizadeh, A., Nojadeh, M.S., Ebrahimzadeh, A., 2019a. Exogenous melatonin ameliorates chilling injury in cut anthurium flowers during low temperature storage. Postharvest Biol. Technol. 148, 184–191.

Aghdam, M.S., Kakavand, F., Rabiei, V., Zaare-Nahandi, F., Razavi, F., 2019b. γ-Aminobutyric acid and nitric oxide treatments preserve sensory and nutritional quality of cornelian cherry fruits during postharvest cold storage by delaying softening and enhancing phenols accumulation. Sci. Hortic. 246, 812–817.

Aghdam, M.S., Luo, Z., Jannatizadeh, A., Sheikh-Assadi, M., Sharafi, Y., Farmani, B., Fard, J.R., Razavi, F., 2019c. Employing exogenous melatonin applying confers chilling tolerance in tomato fruits by upregulating ZAT2/6/12 giving rise to promoting endogenous polyamines, proline, and nitric oxide accumulation by triggering arginine pathway activity. Food Chem. 275, 549–556.

Aghdam, M.S., Moradi, M., Razavi, F., Rabiei, V., 2019d. Exogenous phenylalanine application promotes chilling tolerance in tomato fruits during cold storage by ensuring supply of NADPH for activation of ROS scavenging systems. Sci. Hortic. 246, 818–825.

Ansari, M.I., Lee, R.H., Chen, S.C.G., 2005. A novel senescence-associated gene encoding γ-aminobutyric acid (GABA): pyruvate transaminase is upregulated during rice leaf senescence. Physiol. Plant. 123, 1–8.

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.

Bartyzel, I., Pelczar, K., Paszkowski, A., 2003. Functioning of the γ-aminobutyrate pathway in wheat seedlings affected by osmotic stress. Biol. Plant. 47, 221–225.

Cao, S., Song, C., Shao, J., Bian, K., Chen, W., Yang, Z., 2016. Exogenous melatonin treatment increases chilling tolerance and induces defence response in harvested peach fruit during cold storage. J. Agric. Food Chem.

Cao, S., Bian, K., Shi, L., Chung, H.H., Chen, W., Yang, Z., 2018a. Role of melatonin in cell-wall disassembly and chilling tolerance in cold-stored peach fruit. J. Agric. Food Chem. 66, 5663–5670.

Cao, S., Shao, J., Shi, L., Xu, L., Shen, Z., Chen, W., Yang, Z., 2018b. Melatonin increases chilling tolerance in postharvest peach fruit by alleviating oxidative injury. Sci. Rep. 8, 806.

Chen, Jy., He, Lh., Jiang, Ym., Wang, Y., Joyce, D.C., Ji, Zl., Lu, Wj., 2008. Role of phenylalanine ammonia-lyase in heat pretreatment-induced chilling tolerance in banana fruit. Physiol. Plant. 132, 318–328.

Deewatthanawong, R., Rowell, P., Watkins, C.B., 2010. γ-Aminobutyric acid (GABA) metabolism in CO2 treated tomatoes. Postharvest Biol. Technol. 57, 97–105.

Galano, A., Reiter, R.J., 2018. Melatonin and its metabolites versus oxidative stress: from individual actions to collective protection. J. Pineal Res., e12514.

Gao, H., Zhang, Z.K., Chai, H.K., Cheng, N., Yang, Y., Wang, D.N., Yang, T., Cao, W., 2016. Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. Postharvest Biol. Technol. 118, 103–110.

Gao, H., Lu, Z., Yang, Y., Wang, D., Yang, T., Cao, M., Cao, W., 2018. Melatonin treatment reduces chilling injury in peach fruit through its regulation of membrane fatty acid contents and phenolic metabolism. Food Chem. 245, 659–666.

Han, S., Nan, Y., Qu, W., He, Y., Ban, Q., Lv, Y., Rao, J., 2018. Exogenous gamma-aminobutyric acid (GABA) treatment contributes to regulation of malate metabolism and ethylene synthesis in apple fruit during storage. J. Agric. Food Chem.

Hu, W., Tie, W., Ou, W., Yan, Y., Kong, H., Zuo, J., Ding, X., Ding, Z., Liu, Y., Wu, C., Guo, Y., Shi, H., Li, K., Guo, A., 2018. Crosstalk between calcium and melatonin affects postharvest physiological deterioration and quality loss in cassava. Postharvest Biol. Technol. 140, 42–49.

Igamberdiev, A.U., Hill, R.D., 2018. Elevation of cytosolic Ca2+ in response to energy deficiency in plants: the general mechanism of adaptation to low oxygen stress. Biochem. J. 475, 1411–1425.

Jannatizadeh, A., 2019. Exogenous melatonin applying confers chilling tolerance in pomegranate fruit during cold storage. Sci. Hortic. 246, 544–549.

Jannatizadeh, A., Aghdam, M.S., Luo, Z., Razavi, F., 2019. Impact of exogenous melatonin application on chilling injury in tomato fruits during cold storage. Food Bioprocess Technol.

Lee, H.Y., Back, K., 2017. Melatonin is required for H2 O2 - and NO-mediated defense signaling through MAPKKK3 and OXI1 in Arabidopsis thaliana. J. Pineal Res. 62.

Liu, C., Zheng, H., Sheng, K., Liu, W., Zheng, L., 2018. Effects of melatonin treatment on the postharvest quality of strawberry fruit. Postharvest Biol. Technol. 139, 47–55.

Liu, J., Yang, J., Zhang, H., Cong, L., Zhai, R., Yang, C., Wang, Z., Ma, F., Xu, L., 2019. Melatonin inhibits ethylene synthesis via nitric oxide regulation to delay postharvest senescence in pears. J. Agric. Food Chem. 67, 2279–2288.

Michaeli, S., Fromm, H., 2015. Closing the loop on the GABA shunt in plants: are GABA metabolism and signaling entwined? Front. Plant Sci. 6, 419.

Nakajima, J.-i., Tanaka, I., Seo, S., Yamazaki, M., Saito, K., 2004. LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries. BioMed Res. Int. 2004, 241–247.

Palma, F., Carvajal, F., Ramos, J.M., Jamilena, M., Garrido, D., 2015. Effect of putrescine application on maintenance of zucchini fruit quality during cold storage: contribution of GABA shunt and other related nitrogen metabolites. Postharvest Biol. Technol. 99, 131–140.

Palma, F., Carvajal, F., Jamilena, M., Garrido, D., 2016. Putrescine treatment increases the antioxidant response and carbohydrate content in zucchini fruit stored at low temperature. Postharvest Biol. Technol. 118, 68–70.

Palma, F., Carvajal, F., Jimenez-Munoz, R., Pulido, A., Jamilena, M., Garrido, D., 2019. Exogenous gamma-aminobutyric acid treatment improves the cold tolerance of zucchini fruit during postharvest storage. Plant Physiol. Biochem.: PPB 136, 188–195.

Patterson, B.D., MacRae, E.A., Ferguson, I.B., 1984. Estimation of hydrogen peroxide in plant extracts using titanium (IV). Anal. Biochem. 139, 487–492.

Shelp, B.J., Bown, A.W., Zarei, A., 2017. 4-Aminobutyrate (GABA): a metabolite and signal with practical significance. Botany 95, 1015–1032.

Singleton, V., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Sun, Q., Zhang, N., Wang, J., Zhang, H., Li, D., Shi, J., Li, R., Weeda, S., Zhao, B., Ren, S., Guo, Y.-D., 2015. Melatonin promotes ripening and improves quality of tomato fruit during postharvest life. J. Exp. Bot. 66, 657–668.

Sweetlove, L., Heazlewood, J., Herald, V., Holtzapffel, R., Day, D., Leaver, C., Millar, A., 2002. The impact of oxidative stress on Arabidopsis mitochondria. Plant J. 32, 891–904.

Tan, D.-X., Manchester, L.C., Liu, X., Rosales-Corral, S.A., Acuna-Castroviejo, D., Reiter, R.J., 2013. Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. J. Pineal Res. 54, 127–138.

Thorburn, D.R., Thompson, G.N., Howells, D.W., 1993. A fluorimetric assay for succinic semialdehyde dehydrogenase activity suitable for prenatal diagnosis of the enzyme deficiency. J. Inherit. Metab. Dis. 16, 942–949.

Wang, Z., Ma, L., Zhang, X., Xu, L., Cao, J., Jiang, W., 2015. The effect of exogenous salicylic acid on antioxidant activity, bioactive compounds and antioxidant system in apricot fruit. Sci. Hortic. 181, 113–120.

Wang, L., Feng, C., Zheng, X., Guo, Y., Zhou, F., Shan, D., Liu, X., Kong, J., 2017. Plant mitochondria synthesize melatonin and enhance the tolerance of plants to drought stress. J. Pineal Res.

Wei, Y., Hu, W., Wang, Q., Liu, W., Wu, C., Zeng, H., Yan, Y., Li, X., He, C., Shi, H., 2016. Comprehensive transcriptional and functional analyses of melatonin synthesis genes in cassava reveal their novel role in hypersensitive-like cell death. Sci. Rep. 6, 35029.

Wei, J., Li, D.X., Zhang, J.R., Shan, C., Rengel, Z., Song, Z.B., Chen, Q., 2018. Phytomelatonin receptor PMTR1-mediated signaling regulates stomatal closure in Arabidopsis thaliana. J. Pineal Res., e12500.

Yuan, S., Guan, C., Liu, S., Huang, Y., Tian, D., Cui, X., Zhang, Y., Yang, F., 2016. Comparative transcriptomic analyses of differentially expressed genes in transgenic melatonin biosynthesis ovine HIOMT gene in switchgrass. Front. Plant Sci. 7, 1613.

Zhang, Y., Huber, D.J., Hu, M., Jiang, G., Gao, Z., Xu, X., Jiang, Y., Zhang, Z., 2018. Delay of Postharvest Browning in Litchi fruit by melatonin via the enhancing of anti-oxidative processes and oxidation repair. J. Agric. Food Chem. 66, 7475–7484.

Zhao, R., Sheng, J., Lv, S., Zheng, Y., Zhang, J., Yu, M., Shen, L., 2011. Nitric oxide participates in the regulation of LeCBF1 gene expression and improves cold tolerance in harvested tomato fruit. Postharvest Biol. Technol. 62, 121–126.