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Exogenous Melatonin Delays Methyl Jasmonate-Triggered Senescence in Tomato Leaves

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Abstract: Leaf senescence represents the last stage of leaf development and is highly regulated by plant hormones and environmental factors. Leaf senescence limits growth and yields in crops, leading to a significant portion of agricultural loss. It is thus crucial to develop strategies to delay this physiological process. Melatonin, an extensively studied molecule, has been demonstrated to play a role in the regulation of leaf senescence in plants. Here, we report the role of exogenous melatonin in the alleviation of methyl jasmonate (MeJA)-induced senescence in tomato (*Solanum lycopersicum*) leaves. The application of melatonin led to slower degradation of chlorophyll, reduced electrolyte leakage, decreased malondialdehyde (MDA) content, and reduced reactive oxygen species (ROS) levels in tomato leaves incubated with MeJA. In addition, melatonin repressed the upregulation of senescence-related genes (*SAG* and *SEN*) and chlorophyll degradation genes (*SGR1* and *PAO*) in tomato leaves exposed to MeJA. Furthermore, melatonin stimulated the activity of a Calvin-Benson Cycle enzyme sedoheptulose-1,7-bisphosphatase (SBPase) and alleviated the inhibition of *SlSBPASE* (tomato SBPase gene) expression and in MeJA-treated tomato leaves, suggesting an action of melatonin on the capacity for carbon fixation during senescence. Collectively, these results support a role for melatonin in the alleviation of MeJA-induced senescence in tomato leaves. This work also presents a case study that melatonin may be a useful agent in the delay of crop senescence in agricultural practice.

Keywords: melatonin; senescence; chlorophyll; reactive oxygen species; photosynthesis; tomato

1. Introduction

Senescence is the final stage of leaf development and is highly regulated by internal factors, such as developmental age and hormone level, and environmental factors, including drought, heat, dark, nutrient deficiency, and UV-B irradiation [1]. Senescent leaves undergo dramatic changes in gene expression, metabolism, and cell structures. The most noticeable feature of leaf senescence is the loss of chlorophyll as a consequence of accelerated chlorophyll degradation [2]. In addition, during senescence, the expression of senescence-associated genes is largely boosted, while the expression of photosynthesis-related genes is dramatically down regulated [2–5]. Another important characteristic of leaf senescence is the excessive accumulation of reactive oxygen species (ROS) caused by an imbalance between production and scavenging of ROS [6,7]. Although it is an evolutionarily developmental process important for optimal production of offspring and survival of plants under some unfavorable environmental conditions, leaf senescence limits growth and yields in crops, leading to a significant portion of agricultural loss. It is thus crucial to understand this physiological process so as to develop strategies to control and delay this process.

Melatonin (*N*-acetyl-5-methoxytryptamine) has gained widespread attention among biologists since it was first discovered in the bovine pineal gland [8]. The physiological functions of this molecule in animals have been extensively investigated. It has been demonstrated that melatonin plays diverse

roles in the regulation of circadian rhythms, mood, sleep, body temperature, retina physiology, sexual behavior, seasonal reproduction, and the immune system in humans and animals [9,10]. In 1995, two groups identified the existence of melatonin in plants [11,12], and since then, melatonin has been found present in a variety of plant species. A great many studies have documented the diverse functions of melatonin in plants. The well-established role of melatonin is to act as an antioxidant in plants. Melatonin enhances antioxidative potential by stimulating activities of antioxidant enzymes and increasing levels of non-enzymatic antioxidants, thereby reducing lipid peroxidation and relieving oxidative stress [13,14]. As an indoleamine, melatonin also functions as an auxin-like hormone in the regulation of the growth of roots and shoots in different species, including tomato, cucumber, maize, wheat, and soybean [15]. Melatonin has been further found to play critical roles in the responses to environmental stresses in plants, such as drought [16,17], cold [18–20], heat [21], salinity [22,23], heavy metal toxicity [24], and methyl viologen-induced oxidative stress [25,26]. Additionally, melatonin acts as a signaling molecule that plays a role in the defense against pathogens in plants [27,28].

Melatonin also slows down the process of stress-induced senescence in plants. The role of melatonin in the delay of senescence has been observed in a number of species, such as Arabidopsis, apple, grape, cucumber, rice, peach, ryegrass, cassava, and kiwifruit [22,29–37]. It has been shown that melatonin delays dark-induced senescence and reduces chlorophyll degradation in barley leaves [38]. The observation that melatonin suppresses the upregulation of senescence-associated genes in leaves of drought-induced senescence in apple trees further supports the role of melatonin in the regulation of senescence [29]. In a recent study, it has been shown that melatonin delays senescence of kiwifruit leaves by improving antioxidant capacity and enhancing flavonoid biosynthesis [36]. Melatonin has also been implicated in the prevention of chlorophyll degradation by downregulating chlorophyll degradation enzymes, such as chlorophyllase and pheophorbide a oxygenase [39]. Importantly, the mutation of a rice gene *OsMTS1*, which codes for a methyltransferase in the melatonin biosynthetic pathway, reduces melatonin production and triggers premature leaf senescence in rice leaves, providing direct evidence for the role of melatonin in the leaf senescence [40].

Jasmonates (JAs) are a group of plant hormones, including jasmonate acid and its derivatives, such as methyl jasmonate (MeJA) [41,42]. JAs have been documented to mediate multiple plant developmental processes and stress responses in plants [43–47]. A number of studies have established a role for JAs in the induction of leaf senescence in both model species and crop species, including Arabidopsis, maize, and rice [48–50]. Recently, JA has been shown to induce leaf senescence in tomato plants [51]. However, the interplay between JA and melatonin remains largely unclear.

The objective of this study was to investigate whether melatonin influences the process of MeJA-triggered leaf senescence in tomato plants. In this work, we found that exogenous melatonin slowed down chlorophyll degradation, reduced electrolyte leakage, and decreased the inhibition of photosynthetic capacity in MeJA-treated tomato leaves. In addition, we observed that melatonin suppressed the upregulation of senescence-associated genes and chlorophyll degradation genes in MeJA-treated tomato leaves. Interestingly, melatonin alleviated the JA-induced inhibition of *SlSBPASE* expression and sedoheptulose-1,7-bisphosphatase (SBPase) activity in tomato leaves. These results support a role for melatonin in the alleviation of MeJA-triggered senescence in tomato leaves.

2. Materials and Methods

2.1. Plant Materials and Treatment

Tomato (*Solanum lycopersicum* cv. Micro-Tom) seeds were germinated and grown in growth substrate (peat: vermiculite 3:1 *v*/*v*) in 12 cm × 12 cm × 10 cm plastic pots and the growth conditions were as follows: CO_2, 400 μmol mol^{-1}; light, 300 μmol m^{-2} s^{-1}; day/night temperature, 25/20 °C; relative humidity, 60–65%; photoperiod, 16 h.

Fully expanded leaves were detached from tomato plants at the 8-leaf stage and were divided into four types of groups: (1) control: detached leaves were incubated in water in the dark for 12 h and

were then incubated in water under 16 h light/8 h dark cycles for 5 days (d); (2) control + MT: detached leaves were incubated in 50 μM melatonin in the dark for 12 h and were then incubated in water under 16 h light/8 h dark cycles for 5 d; (3) MeJA: detached leaves were incubated in water in the dark for 12 h and were then incubated in 100 μM MeJA under 16 h light/8 h dark cycles for 5 d; (4) MeJA + MT: detached leaves were incubated in 50 μM melatonin in the dark for 12 h and were then incubated in 100 μM MeJA under 16 h light/8 h dark cycles for 5 d. Each group contained 30 leaves, and the process was repeated three times. All leaves were incubated at a constant temperature of 25 °C and relative humidity of 60%. Leaf samples were collected at the end of treatment for further analysis.

2.2. Measurement of Chlorophyll Content

For measurement of chlorophyll content, 4 detached leaves were collected from one repeat following different treatments. Leaf discs were punched with a cork borer from 4 leaves and were pool incubated in 80% acetone (*v*/*v*) in the dark until leaf discs appeared colorless. The same procedure was taken for another two repeats. Absorbance at 647 and 664 nm was measured and used for the calculation of chlorophyll content according to the formula 20.3 × A647 − 8.04 × A664. The results were presented as the average of three repeats.

2.3. Measurement of Reactive Oxygen Species (ROS) Accumulation

Leaf samples were collected following treatment to determine the accumulation of H_2O_2 and O_2^-. The H_2O_2 was extracted with 5% (*w*/*v*) trichloroacetic acid and measured by monitoring the absorbance of the titanium-peroxide complex at 410 nm according to a previous study [52]. O_2^- was detected using nitroblue tetrazolium (NBT), as previously reported [53].

2.4. Measurement of Malonaldehyde (MDA) Content

Leaf samples were collected following treatment to determine malonaldehyde (MDA) content, as described previously [54,55]. MDA was extracted with trichloroacetic acid and assessed using thiobarbituric acid. Absorbance of the supernatant at 450, 532, and 600 nm was measured for the quantification of MDA.

2.5. Measurement of Electrolyte Leakage

Electrolyte leakage was measured according to the method previously described [56]. Leaf samples were collected following treatment and incubated in deionized water overnight. The conductivity of the incubated solution was measured as E1. Then, leaves were boiled, and the conductivity of the solution was measured as E2. The relative electrolyte leakage was calculated as E1/E2.

2.6. Measurement of Antioxidant Enzyme Activities

Leaf samples were collected following treatment for the measurement of SOD and CAT. Leaf samples (0.1 g) were extracted with 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (*w*/*v*). The extraction was centrifuged, and the supernatant was used for measurement of activities of SOD and CAT, as described previously [57,58].

2.7. Determination of SBPase Activity

Leaf samples were collected following treatment for measurement of SBPase activity. SBPase activity was measured, as described previously [59,60]. Leaf samples (0.1 g) were extracted in extraction buffer containing 50 mM Hepes (pH 8.2), 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 2 mM benzamidine, 2 mM aminocaproic acid, 0.5 mM phenylmethylsulfonyluoride (PMSF), and 10 mM dithiothreitol (DTT). For SBPase activity assay, 20 μL of protein samples were added to 80 μL of assay buffer containing 50 mM Tris, 15 mM $MgCl_2$, 1.5 mM EDTA, 10 mM DTT, 2 mM SBP and incubated at 25 °C for 5 min. The reaction was terminated by adding 50 μL 1 M perchloric acid. The samples

were centrifuged, and the supernatant was assayed for phosphate. Samples of 50 µL and phosphate standards (0–0.5 mM NaH_2PO_4) were incubated with an 850 µL molybdate solution (0.3% ammonium molybdate in 0.55 M H_2SO_4) for 10 min. A total of 150 µL malachite green (0.035% malachite green and 0.35% polyvinyl alcohol) was added and incubated for a further 45 min at room temperature. Absorbance at 620 nm was measured for the calculation of SBPase activity.

2.8. Measurement of Photosynthesis and Maximum Photochemical Efficiency

Leaves from different treatments were dark-adapted for 30 min, and minimal fluorescence from a dark-adapted leaf (Fo) was measured with a portable fluorometer (PAM-2000, Walz, Germany), and following a saturating pulse, maximal fluorescence from a dark-adapted leaf (Fm) was obtained, which allowed us to calculate maximum photochemical efficiency (Fv/Fm). Carbon assimilation rate was measured with a portable photosynthesis system (LI 6400, LI-COR Biosciences, Lincoln, NE, USA).

2.9. Measurement of Transcript Abundance by Quantitative Real-Time PCR

Relative transcript abundance was measured using quantitative real-time PCR, as previously described [61]. RNA was extracted from leaves following different treatments and was used as a template for cDNA synthesis. Quantitative real-time PCR was performed using SYBR® Premix Ex Taq™ according to manufacturer's instructions (TaKaRa, Dalian, China). The tomato actin gene (GenBank Accession No. AB695290) was used as a reference gene. Each real-time PCR reaction was performed in a 25 µL final volume on an iQ5 Multicolor Real-Time PCR Detection System (BIO-RAD, Hercules, CA, USA) with the following program: 1 cycle of 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C, 30 s at 60 °C. The primers used in this study are listed in Table 1.

Table 1. Primers used in RT-PCR.

Primer Name	Primer Sequence (5′-3′)
SAG-F	TGCAGTAGCAGCTATGGAAGG
SAG-R	ACACCATCTGCTGCCTGGTAT
SEN-F	AGGGTAGTGGAAATCTTGGAG
SEN-R	GTTCCTTCAGCAATTGCTTTA
PAO-F	TCATCTCCTCTTAGAGTAGCTGC
PAO-R	ACCCACTGAGATCCAGATTTATC
SlSBPASE-F	CGTGACATCTCCAACAGCTAAGG
SlSBPASE-R	CATCGCTGCTGTAACCTCCAG
SGR1-F	CTCAAGGCTTTTGTTCATGGAG
SGR1-R	AGCCAAGGCATAACACTCACTG
Actin-F	ATGTATGTTGCTATTCAGGCTGTG
Actin-R	TAACCCTCGTAGATAGGGACAG

2.10. Statistical Analysis

The values presented in this study are the means ± SDs. Student's t-test test was performed to compare the difference between treatments. Asterisks indicate significant difference at ** $p < 0.01$ or at * $p < 0.05$.

3. Results

3.1. Melatonin Alleviates MeJA-Induced Senescence in Tomato Leaves

MeJA has been demonstrated to induce leaf senescence in plants. To understand the role of melatonin in the MeJA-induced senescence in tomato leaves, detached tomato leaves were pretreated with 50 µM melatonin for 12 h and were subsequently incubated in 100 µM MeJA for 5 d. We found that tomato leaves exposed to MeJA displayed a yellowing phenotype compared with tomato leaves in the control, while melatonin pretreatment led to the alleviation of MeJA-induced leaf chlorosis

(Figure 1A). Chlorophyll content is an important physiological marker of leaf senescence [5]. We thus quantified the chlorophyll content of tomato leaves that had been subjected to different treatments. It was shown that chlorophyll content was markedly decreased in tomato leaves incubated with MeJA, whereas melatonin pretreatment reduced MeJA-induced chlorophyll loss (Figure 1B), suggestive of the effect of melatonin on the alleviation of MeJA-induced senescence in tomato leaves.

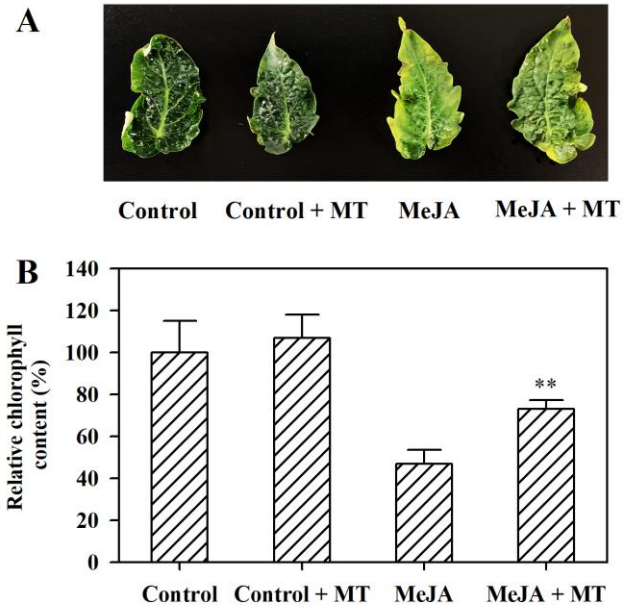

Figure 1. Melatonin alleviated MeJA-induced senescence in detached tomato leaves. (**A**) Phenotypes of detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. (**B**) Chlorophyll contents of detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. The MeJA treatment was performed by incubating detached leaves in 100 μM MeJA for 5 d under 16 h light/8 h dark cycles. The chlorophyll content in the control leaves was set to 100%, and the relative chlorophyll contents in the leaves of the other three treatments were calculated accordingly. The values presented are means ± SDs (n = 3). Asterisks indicate significant difference at ** $p < 0.01$ between MeJA treatment and MeJA + MT treatment.

3.2. Melatonin Represses the Upregulation of Senescence-Related Genes and Chlorophyll Degradation Genes in MeJA-Treated Tomato Leaves

To validate the involvement of melatonin in the regulation of MeJA-induced senescence in tomato leaves, we examined the expression of senescence-related genes, including *SAG* (senescence-associated gene, Solyc02g076910.2) and *SEN* (senescence, Solyc12g008460.1), chlorophyll degradation genes, including *SGR1* (senescence-inducible chloroplast stay-green protein 1, Solyc08g080090.2) and *PAO* (pheide a oxygenase, Solyc11g066440.1). *SAG*, *SEN*, *SGR1,* and *PAO* were dramatically upregulated by MeJA treatment, confirming that senescence was induced by MeJA in tomato leaves. However, melatonin pretreatment significantly suppressed the upregulation of *SAG*, *SEN*, *SGR1,* and *PAO* in tomato leaves incubated with MeJA. No significant changes in the expression of these genes were found in the control leaves and leaves pretreated with melatonin but without subsequent MeJA treatment (Figure 2). These results suggest that melatonin influencing MeJA-induced senescence by inhibiting the expression of senescence-related genes and chlorophyll degradation genes.

3.3. Melatonin Reduces Electrolyte Leakage and MDA Content in MeJA-Induced Tomato Leaves

Electrolyte leakage is generally associated with leaf senescence [5]. We found that electrolyte leakage was increased significantly in tomato leaves incubated with MeJA compared with control leaves. However, melatonin pretreatment reduced electrolyte leakage in MeJA-treated tomato leaves

(Figure 3A). We further assessed lipid peroxidation by measuring malondialdehyde (MDA) content. The results showed that MeJA treatment led to a dramatic increase in MDA content in tomato leaves, while pretreatment with melatonin reduced the accumulation of MDA in MeJA-treated tomato leaves (Figure 3B). These results suggest that MeJA induces membrane damage, while melatonin is able to relieve MeJA-induced lipid damage in tomato leaves.

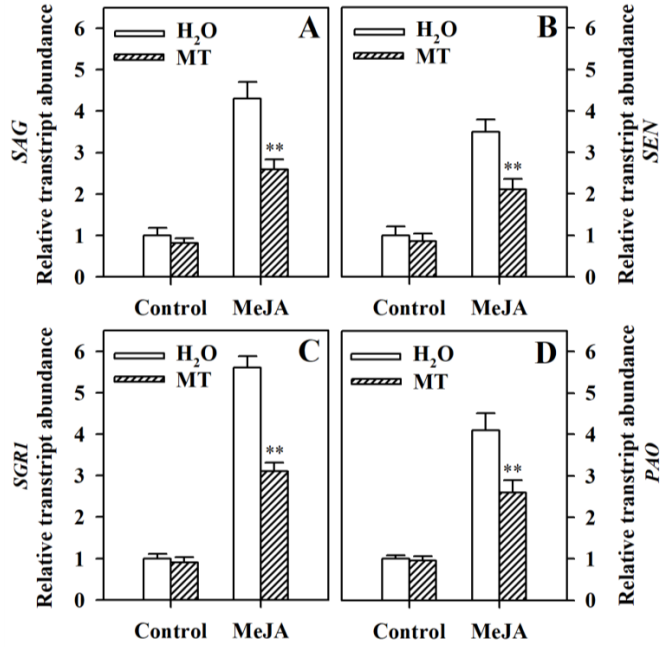

Figure 2. Melatonin suppressed the upregulation of senescence-related genes and chlorophyll degradation genes in detached tomato leaves treated with MeJA. The MeJA treatment was performed by incubating detached leaves in 100 μM MeJA for 5 d under 16 h light/8 h dark cycles. (**A**) Relative transcript abundance of *SAG*. (**B**) Relative transcript abundance of *SEN*. (**C**) Relative transcript abundance of *SGR1*. (**D**) Relative transcript abundance of *PAO*. The expression level in the control leaves was set to 1, and the relative expression levels in the leaves of the other three treatments were calculated accordingly. The values presented are means ± SDs (n = 6). Asterisks indicate significant difference at ** $p < 0.01$ between treatments.

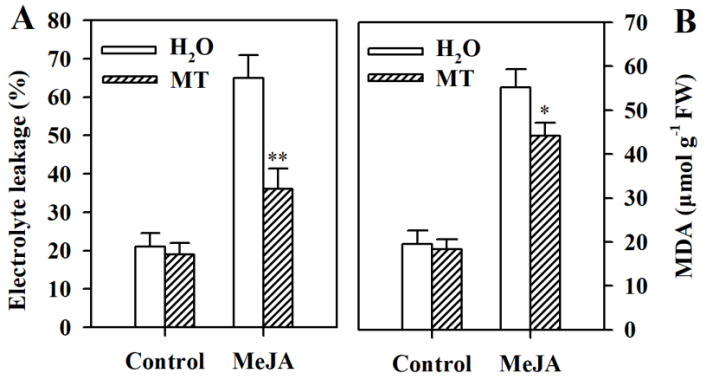

Figure 3. Melatonin decreased electrolyte leakage and malondialdehyde (MDA) content in MeJA-treated tomato leaves. (**A**) relative electrolyte leakage in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. (**B**) MDA content in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. The MeJA treatment was performed by incubating detached leaves in 100 μM MeJA for 5 d under 16 h light/8 h dark cycles. The values presented are means ± SDs (n = 3). Asterisks indicate significant difference at * $p < 0.05$ or ** $p < 0.01$ between treatments.

3.4. Melatonin Decreases Levels of Reactive Oxygen Species (ROS) in MeJA-Treated Tomato Leaves

It has been reported that ROS is involved in the trigger of leaf senescence [22]. To evaluate whether the role of melatonin in the alleviation of MeJA-induced leaf senescence was associated with the alteration of ROS content, we measured the levels of H_2O_2 and O_2^- in tomato leaves pretreated with or without melatonin that had been subsequently subjected to MeJA treatment. The results showed that exposure to MeJA substantially enhanced accumulation of H_2O_2 and O_2^-, while exogenous application of melatonin suppressed the MeJA-induced enhancement of H_2O_2 and O_2^- levels in detached tomato leaves (Figure 4). The levels of H_2O_2 and O_2^- did not show significant difference between control leaves and those pretreated with melatonin but without subsequent MeJA treatment. These results suggest that melatonin mitigates MeJA-induced senescence, at least partly, by reducing ROS content.

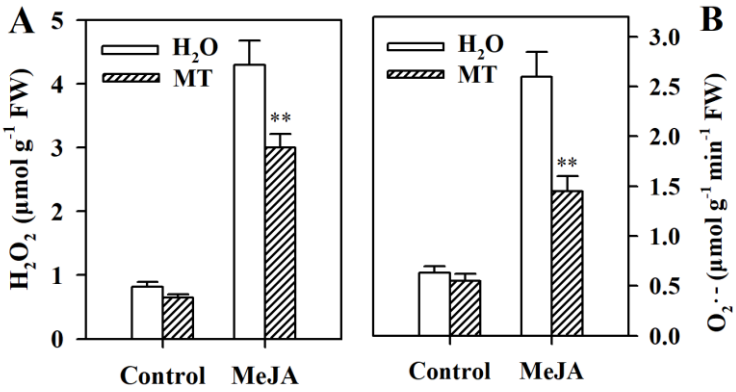

Figure 4. Melatonin reduced the level of reactive oxygen species (ROS) in MeJA-treated tomato leaves. (**A**) H_2O_2 content in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. (**B**) O_2^- content in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. The MeJA treatment was performed by incubating detached leaves in 100 μM MeJA for 5 d under 16 h light/8 h dark cycles. The values presented are means ± SDs (n = 3). Asterisks indicate significant difference at ** $p < 0.01$ between treatments.

3.5. Melatonin Stimulates Activities of Antioxidant Enzymes in MeJA-Treated Tomato Leaves

The capacity for the removal of excess ROS is largely dependent on the activities of antioxidant enzymes under unfavorable growth conditions. It is recognized that melatonin is a well-known antioxidant, playing an important role in the scavenging of ROS in plants. To explain the observed decrease in lipid peroxidation and ROS content in melatonin-treated tomato leaves exposed to MeJA, we measured activities of representative antioxidant enzymes, including SOD and CAT. It was found that in response to MeJA, activities of both SOD and CAT were increased, and exogenously applied melatonin further promoted activities of these two enzymes. However, melatonin did not have a pronounced effect on the activities of SOD and CAT in tomato leaves without MeJA treatment (Figure 5).

3.6. Melatonin Mitigates the Inhibition of Photosynthetic Capacity in MeJA-Treated Tomato Leaves

One of the primary consequences of senescence is the reduced photosynthetic capacity. In the present study, following exposure to MeJA for 5 d, tomato leaves without melatonin pretreatment exhibited a substantial reduction in photosynthetic capacity as demonstrated by a decreased carbon assimilation rate and maximum photochemical efficiency while leaves pretreated with melatonin showed relatively high photosynthetic capacity (Figure 6). These results imply that melatonin protects tomato leaves against MeJA-induced damage to the photosynthetic system.

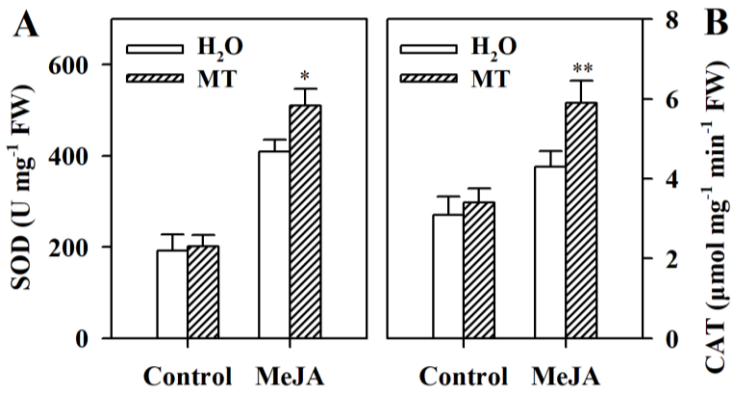

Figure 5. Melatonin stimulated activities of antioxidant enzymes in MeJA-treated tomato leaves. (**A**) SOD activity in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. (**B**) CAT activity in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. The MeJA treatment was performed by incubating detached leaves in 100 μM MeJA for 5 d under 16 h light/8 h dark cycles. The values presented are means ± SDs (n = 3). Asterisks indicate significant difference at * $p < 0.05$ or ** $p < 0.01$ between treatments.

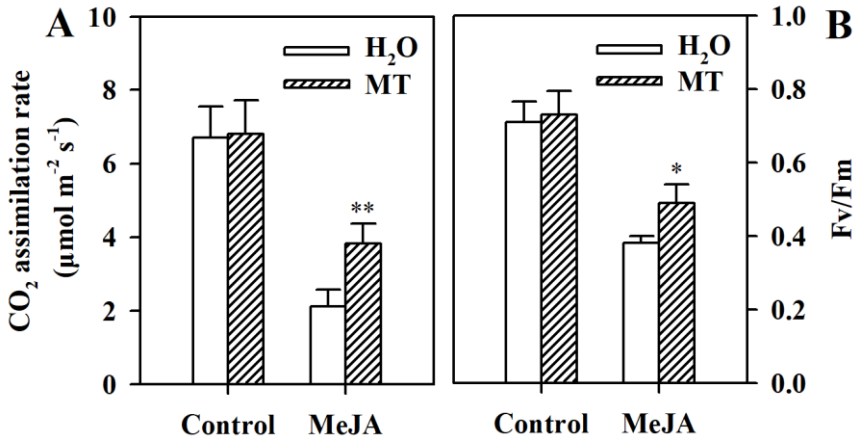

Figure 6. Melatonin improved photosynthetic capacity in MeJA-treated tomato leaves. (**A**) Carbon assimilation rates in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. (**B**) Photochemical efficiency (Fv/Fm) in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. The MeJA treatment was performed by incubating detached leaves in 100 μM MeJA for 5 d under 16 h light/8 h dark cycles. The values presented are means ± SDs (n = 3). Asterisks indicate significant difference at * $p < 0.05$ or ** $p < 0.01$ between treatments.

3.7. Melatonin Increases the Expression of SlSBPASE and Promotes SBPase Activity in MeJA-Treated Tomato Leaves

Sedoheptulose-1,7-bisphosphatase (SBPase) is an enzyme in the Calvin-Benson cycle and has been demonstrated to be crucial for photosynthetic carbon assimilation, growth, and stress tolerance in tomato plants [20,62]. A previous study has revealed that MeJA suppresses the expression of *SlSBPASE* (Solyc05g052600.2) that encodes SBPase in tomato. In this study, we found that the expression of *SlSBPASE* was severely suppressed in tomato leaves incubated with MeJA for 5 d, while melatonin pretreatment significantly relieved the suppression (Figure 7A). Consistent with *SlSBPASE* expression, SBPase activity was dramatically decreased by MeJA, while it was promoted by melatonin pretreatment

in MeJA-treated tomato leaves (Figure 7B). These results suggest that melatonin may be involved in the regulation of MeJA-induced leaf senescence by acting on the photosynthetic carbon fixation process.

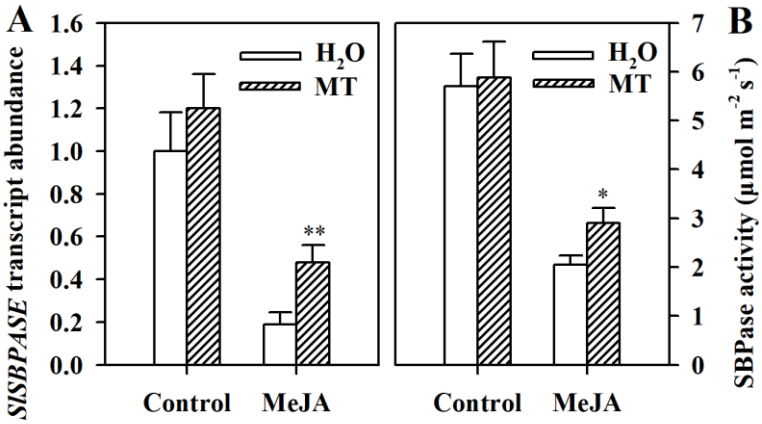

Figure 7. Melatonin promoted expression of *SlSBPASE* and increased SBPase activity in MeJA-treated tomato leaves. (**A**) Relative transcript abundance of *SlSBPASE* in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. (**B**) SBPase activity in detached tomato leaves pretreated with or without melatonin and subsequently treated with or without MeJA. The MeJA treatment was performed by incubating detached leaves in 100 μM MeJA for 5 d under 16 h light/8 h dark cycles. The expression level in the control leaves was set to 1, and the relative expression levels in the leaves of the other three treatments were calculated accordingly. The values presented are means ± SDs (n = 3). Asterisks indicate significant difference at * $p < 0.05$ or ** $p < 0.01$ between treatments.

4. Discussion

Leaf senescence represents the last stage of leaf development and is a critical phase of the plant life cycle. Leaf senescence is important to sustain plant fitness by remobilizing nutrients and energy from aging leaves to younger developing tissues. The limited nutrients are further utilized for growth, development, reproduction, and defense to various types of stresses. Under optimal growth conditions, leaf senescence occurs naturally dependent on developmental age. However, under environmental stresses, leaf senescence is accelerated, leading to precocious senescence. In the latter case, leaf senescence is a deleterious process in agriculture, causing nutrient loss and restricting yields in crops. As a ubiquitous and extensively studied molecule in plants, melatonin has been demonstrated to play a wide range of roles in growth, development, and responses to abiotic and biotic stresses. Melatonin protects plants against diverse environmental factors, including drought, salinity, extreme temperature, sodic alkaline, and heavy metal toxicity [16–18,20,24,26,33,63–65]. Importantly, melatonin has been found to play a role in the delay of leaf senescence in fruit tree species, vegetable crops, and grasses [16,22,29–31,66,67]. However, the role of melatonin in the alleviation of JA-induced leaf senescence in plants is still largely unknown. In the present study, we investigated the effect of exogenous melatonin on the alleviation of MeJA-induced senescence in tomato leaves. We conclude that melatonin is involved in the alleviation of MeJA-induced senescence in detached tomato leaves and the evidence includes (1) melatonin decelerated MeJA-induced degradation of chlorophyll; (2) melatonin improved photosynthetic capacity in MeJA-treated tomato leaves; (3) melatonin repressed MeJA-induced expression of senescence-related genes and chlorophyll degradation genes; (4) melatonin decreased accumulation of ROS by enhancing activities of antioxidant enzymes in tomato leaves exposed to MeJA; (5) melatonin stimulated SBPase activity in MeJA-treated tomato leaves.

The decline in chlorophyll content is a typical feature of leaf senescence. In senescent leaves, chlorophyll undergoes accelerated degradation, leading to leaf yellowing. It has been reported that chlorophyll degradation is associated with the upregulation of chlorophyll degradation genes, such as

SGR1 and *PAO* [35,50,68–70]. In this study, we found that exposure to MeJA led to a marked decline in chlorophyll content in detached tomato leaves. Consistently, gene expression analysis showed that the expression of two chlorophyll degradation genes *SGR1* and *PAO* was substantially boosted. However, melatonin pretreatment repressed the upregulation of *SGR1* and *PAO* in MeJA-treated tomato leaves, suggesting a role for melatonin in the prevention of MeJA-induced chlorophyll degradation. Further analysis revealed that MeJA treatment significantly promoted the expression of the senescence marker genes in this study. The expression of *SAG* and *SEN* was dramatically increased in tomato leaves exposed to MeJA compared with melatonin-pretreated leaves exposed to MeJA, confirming that melatonin was involved in the mitigation of MeJA-induced senescence in tomato leaves. These observations indicate that melatonin may act as a negative regulator of chlorophyll degradation and MeJA-induced senescence.

It has been established that excessive accumulation of ROS triggers leaf senescence, thus reducing intracellular ROS assists in the extension of leaf longevity [71]. Overproduction of ROS during leaf senescence leads to the disruption of plasma membrane integrity [1]. It is, therefore, recognized that increased lipid peroxidation and electrolyte leakage are key characteristics associated with leaf senescence [71,72]. In this study, we found that the production of ROS was significantly enhanced in detached tomato leaves treated by MeJA. Along with the increase in ROS level was the elevation of electrolyte leakage and MDA content, suggestive of membrane damage caused by MeJA-induced accumulation of ROS. However, the application of melatonin obviously reduced the endogenous level of ROS, with the electrolyte leakage and MDA content being decreased accordingly. These results suggest that melatonin may relieve senescence-related membrane damage by decreasing the accumulation of ROS. Increasing evidence suggests a role for melatonin as an antioxidant molecule. It is thus likely that melatonin aids in the increment of antioxidant potential in senescent tomato leaves, which results in declined content of ROS. This explanation is supported by our observation that exogenous melatonin stimulated activities of antioxidant enzymes, including SOD and CAT, in tomato leaves exposed to MeJA for 5 d. Similar results were also observed in melatonin-treated ryegrass in the dark [35]. Therefore, the improved antioxidant potential by melatonin may contribute to the alleviation of MeJA-induced senescence in tomato leaves.

Leaf senescence is also featured by a reduction in photosynthetic capacity, which can be assessed by measuring carbon assimilation rate and photochemical efficiency (Fv/Fm) [1,13,20,73]. Melatonin has proved effective in the promotion of photosynthetic capacity under stressed conditions, such as cold, drought, salinity, and acid rain [13,62,63,73,74]. In the present work, the carbon assimilation rate and Fv/Fm were substantially repressed in tomato leaves incubated with MeJA for 5 d, whereas melatonin-pretreated leaves with subsequent MeJA treatment exhibited relatively higher carbon assimilation rates and Fv/Fm. These results indicate that melatonin is associated with the alleviation of photosynthesis suppression in MeJA-treated tomato leaves. Sedoheptulose-1,7-bisphosphatase (SBPase) is involved in the carbon fixation in the Calvin-Benson cycle. Previous studies have established that maintaining SBPase activity is crucial for photosynthetic performance under abiotic stresses [20,62,75]. To examine the potential impact of melatonin on SBPase during MeJA-induced senescence in tomato leaves, we measured *SlSBPASE* transcript abundance and SBPase activity and found that exposure to MeJA for 5 d led to a severe reduction in *SlSBPASE* transcript abundance and SBPase activity in non-melatonin-pretreated leaves compared with that in melatonin-pretreated leaves, demonstrating that melatonin action on SBPase at the transcript and protein levels may contribute to upregulation of photosynthetic performance of MeJA-treated tomato leaves.

In summary, our study demonstrates that melatonin relieves MeJA-induced senescence in tomato leaves. Melatonin reduces the degradation of chlorophyll by downregulating the expression of chlorophyll degradation genes in MeJA-induced senescent leaves. Moreover, melatonin controls the levels of ROS and reduces membrane damage by promoting antioxidant capacity in MeJA-induced senescence in tomato leaves. Finally, melatonin alleviates the MeJA-induced repression of

photosynthetic capacity by partly acting on SBPase in tomato leaves. This study represents a case that melatonin could be exogenously applied for the delay of crop senescence.

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