Postharvest Biology and Technology 222 (2025) 113419

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Melatonin improves the postharvest life of cut ruscus foliage after a long storage condition

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ARTICLE INFO

Keywords:
Danae racemosa
Melatonin treatment
Leaf senescence
Yellowing
Hormonal balance
Thidiazuron

ABSTRACT

This study aimed to investigate the effects of various pulse treatments on the postharvest longevity and physiological quality of cut *Danae racemosa* branches. The cut foliage was treated with deionised water (CTRL), 10 μM thidiazuron (TDZ), 100 μM melatonin (MEL), 300 μM 5-aminolevulinic acid (AA), 300 μM ascorbic acid (VIT C), and 300 μM tocopherol (VIT E) for 24 h. The foliage was then subjected to simulated refrigerated transport (four weeks, at 6–8 °C in the dark) and retail conditions for up to 30 d (photoperiod: 12 h light/dark, light intensity: 50 μmol m^{-2} s^{-1}, temperature: 20–24 °C). Measurements included the water uptake rate, weight change, vase life, chlorophyll *a* fluorescence, colour index, chlorophyll content, ethylene production, and hormone balance. The results showed that TDZ or MEL treatments improved the water balance, delayed senescence, and enhanced photosynthetic efficiency, with higher chlorophyll concentration (1.3–1.6 g kg^{-1} FW) and avoided ethylene increase (0.2–0.3 pL kg^{-1} h^{-1}). Hormonal analysis revealed a decrease in ABA and an increase in cytokinin content in MEL-treated branches, indicating the role of MEL in modulating hormonal balance to extend vase life. These findings indicate that MEL is an effective postharvest treatment for preserving the visual and physiological quality of cut foliage, comparable to the well-established benefits of the TDZ.

1. Introduction

The ornamental sector, driven by high quality, faces significant challenges due to the sensitivity of fresh products, which are vulnerable to losses during post-harvest stages. International trade and globalisation have expanded production areas, leading to the need for careful handling of ornamental products transported over long distances to maintain their quality and longevity (Ferrante et al., 2015). Additionally, the emerging consumer preference for quality and sustainability further emphasizes the need for improved standards in the industry (Gabellini and Scaramuzzi, 2022).

Cut foliage represents a significant economic contributor to the floriculture industry, with an international worth of approximately US $ 1.4 billion (Faust and Dole, 2021). Cut foliage are branches with leaves that are used to add colour, texture, and depth to floral arrangements and bouquets, serving as a crucial greenery or filler in ornamental design, and is especially important for preparing arrangements of flowers that are naturally without leaves (Faust and Dole, 2021). Among 100 plant species used as cut foliage, *Danae racemosa* (L.) *Moench,* commonly called ruscus, Alexandrian laurel, or poets' laurel, is highly appreciated by the consumer and flower arrangement industry for its elegant, arching stems adorned with attractive, elliptical, glossy, sturdy, waxy, vivid green, and evergreen leaves (Shen et al., 2013).

Cut foliage generally outlasts cut flowers but may lose quality during prolonged storage (Pacifici et al., 2013). Long-distance transport systems to reach higher remunerative markets in a sustainable manner could make it challenging to maintain the high quality of these products. The major post-harvest issues that compromise the marketability/commercial value of this species are generally represented by leaf yellowing, leaf desiccation, leaf abscission, and weight variation (weight loss), as this foliage are sold by weight (Pacifici et al., 2007; Bulgari et al., 2015). Leaf senescence represents the last stage of leaf development and commonly appears in an age-dependent manner, as well as when plants face environmental stressors, including extreme temperatures, water deficiency, or prolonged low-light conditions/darkness (Lim et al., 2007). Leaf yellowing is commonly the

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https://doi.org/10.1016/j.postharvbio.2025.113419
Received 21 November 2024; Received in revised form 10 January 2025; Accepted 22 January 2025

initial phenomenon observed, resulting from the degradation of chlorophyll (Chl) and Chl-protein complexes (Chen et al., 2021). The plant hormone ethylene plays a pivotal role in leaf aging by exerting both direct and indirect influences on metabolic regulation (Kader, 1985; Iqbal et al., 2017). Leaf yellowing is prevalent in numerous cut flowers, including Alstroemeria, Chrysanthemum, lilies, and tulips, as well as in some cut foliage, such as cut *D. racemosa* (Van Doorn et al., 1992; Ferrante et al., 2001, 2002, 2003, 2005). During the postharvest distribution chain, another important alteration affecting cut foliage is the weight loss because it is separated from the roots, leading to detachment from the mother plant, followed by transfer to storage facilities under environmental conditions that are different from those in the cultivation environment. Weight loss in cut foliage is associated with water potential imbalances and respiratory metabolism caused by improper water conductance, which may be caused by xylem vessel occlusion, air embolism, or increased leaf transpiration (van Doorn, 1997). Considering that the most cut foliage is sold by weight, a decrease in weight of these products during the distribution chain is directly translated into economic losses.

Ensuring foliage health is crucial to enhancing longevity and quality, countering post-harvest aging. Modern agriculture relies on chemicals to boost yield and quality, but their environmental and health impacts are concerning. Many chemicals that inhibit the ethylene biosynthesis such as aminooxyacetic acid, (AOA) or its action (silver thiosulfate, STS), employed to extend the vase life of cut flowers and foliage, may soon be banned because of their environmental impact (Scariot et al., 2014). Postharvest treatments with cytokinins, such as phenylurea and thidiazuron (N-phenyl- N'-1,2,3-thiadiazol-5-yl urea, TDZ), with cytokinin-like activity, effectively delay leaf yellowing in many cut flowers and foliage, even at low concentrations (Ferrante et al., 2002; Bulgari et al., 2015). TDZ effectively delayed leaf yellowing is several cut flowers or potted plants (Ferrante et al., 2002, 2003; Mutui et al., 2005; Jiang et al., 2009). Recent focus on sustainable production highlights the use of exogenous natural compounds with antioxidant properties as eco-friendly alternatives to chemicals. These compounds protect cellular membranes, support photosynthesis, prevent chlorophyll degradation, and extend the vase life of floricultural products under post-harvest stress (Scariot et al., 2014; Ferrante et al., 2015). Ascorbic acid (vitamin C) enhanced water uptake and prolonged the vase life of cut flowers such as lisianthus (*Eustoma grandiflora*), snapdragons, gerberas, and gladioli (Abdulrahman et al., 2012; Mehdikhah et al., 2016; Ravanbakhsh et al., 2016; Budiarto et al., 2022). It plays a crucial role in photosynthesis and plant growth acting as an enzyme cofactor, antioxidant, and electron transporter in the plasma membrane and chloroplasts (Szarka et al., 2013). High levels of ascorbic acid boosted the antioxidant system and protected plants from oxidative damage (Conklin, 2001). Tocopherols, also known as vitamin E, played a crucial role in delaying plant senescence. Tocopherol deficiency in transgenic tobacco (*Nicotiana tabacum* L.) plants showed an enhanced senescence phenotype (Abbasi et al., 2009). By stabilising cellular membranes, scavenging free radicals, maintaining chlorophyll levels, and regulating ethylene, tocopherols helped in the mitigation of the detrimental effects of oxidative stress and senescence in plants (Simancas, Munné-Bosch, 2015). The application of chlorophyll biosynthesis precursors, such as 5-aminolevulinic acid (AA), could potentially extend the post-production longevity of cut foliage because it enhanced leaf photosynthesis under stressful environmental conditions (Hotta et al., 1997). AA serves as a biosynthetic precursor to all biological tetrapyrroles, including chlorophyll and heme, which are crucial components of plant photosynthesis and growth. N-acetyl-5-methoxytryptamine, commonly known as melatonin, is a naturally occurring indoleamine produced through decarboxylation of tryptophan, a primary amino acid in metabolism. It has well-known roles in animals and humans, such as sleep physiology, circadian rhythms, and sexual behaviours (Reiter et al., 2014). Melatonin exhibits a wide range of functions in plants such as germination, photosynthesis, and growth, acting as an antioxidant that directly eliminates reactive oxygen species (ROS), activated the antioxidant system, and influenced gene expression (Arnao and Hernández-Ruiz, 2020). Furthermore, melatonin has been reported to interact with other plant hormones such as salicylic acid, abscisic acid, and ethylene to activate defence mechanisms under stress conditions (Arnao and Hernández-Ruiz, 2018, 2019). In addition, melatonin has been shown to play a significant role in delaying leaf senescence during abiotic stresses, such as heat, drought, darkness, salinity, and cold (Liang et al., 2018; Xu et al., 2019; Zhang et al., 2016; Wang et al., 2012).

Melatonin has been used as preservative in cut flowers to extend their vase life. Both free and nanochitosan-encapsulated forms of melatonin have been found to significantly enhance the longevity and quality of cut flowers (Zulfiqar et al., 2023; Seyed Hajizadeh et al., 2024). For instance, in *Gerbera jamesonii* treated with melatonin and nanoencapsulated melatonin promoted cell membrane stability, maintained higher levels of carbohydrates and flavonoids, and increased antioxidant enzyme activity, ultimately extending vase life up to 12 d when treated with 0.1 mM nanoencapsulated melatonin (Seyed Hajizadeh et al., 2024). In tuberose flowers, melatonin applications mitigated arsenic-induced stress, enhancing growth traits, chlorophyll content, and vase life (Zulfiqar et al., 2023). Notably, preharvest melatonin applications have also shown promising results for postharvest cut flower performance. In tuberose, preharvest melatonin treatment increased vase life by up to 4 d, boosted total soluble proteins, soluble sugars, and catalase activity, and reduced oxidative stress markers (Zulfiqar et al., 2023). In a similar study, the melatonin applied in vase solution at the concentrations of 0.1 or 0.5 mM improved the vase life of cut peony (Chen et al., 2024). These findings suggest that melatonin's protective effects can be activated before harvest, potentially providing a novel approach for enhancing cut flower quality.

This study focuses on optimizing postharvest handling techniques for cut ruscus foliage to extend its vase life and preserve quality during transportation to distant markets. By applying innovative, eco-friendly pulse treatments (ascorbic acid, tocopherol, melatonin, and aminolevulinic acid) before 30 d of refrigerated transport, the display quality of cut ruscus foliage was evaluated during the recovery phase, with particular attention to delaying leaf senescence and comparing melatonin's effects with thidiazuron's well-established impact on leaf yellowing. The novelty of this research lies in proposing melatonin as a highly effective treatment for prolonging the longevity of cut foliage. While melatonin's role in delaying senescence has been documented in various horticultural species (Liu et al., 2024), no prior study has evaluated its effects under conditions simulating the full postharvest stages of cut green foliage, including refrigerated shipping and retail storage. The findings presented here could offer innovative protocols for using melatonin to manage postharvest phases, preserving the quality of cut foliage throughout the commercialization process.

2. Material and methods

2.1. Plant materials and simulation of transport and permanence to retail shelves

Cut branches of *Danae racemosa* (L.) Moench were harvested in the morning and selected based on current commercial quality standards (absence of visual defects on the leaves) by a commercial company (Floratoscana, Pescia, PT, Italy) The cut foliage were immediately transported to the postharvest laboratory at Scuola Superiore Sant'Anna (Pisa, Italy), and trimmed to a length of 60 cm to ensure homogeneous samples for the experiments. Chemical treatments were prepared in deionised water to final concentrations of (i) deionised water (CTRL), (ii) 10 μM thidiazuron (TDZ, Sigma-Aldrich, Italy), (iii) 100 μM melatonin (MEL, Sigma-Aldrich, Italy), (iv) 300 μM 5-aminolevulinic acid (AA, Sigma-Aldrich, Italy), (v) 300 μM ascorbic acid (VIT C, Sigma-Aldrich, Italy), and (vi) 300 μM tocopherol (VIT E, Sigma-Aldrich, Merk Italy). For the pulse treatments, cut ruscus branches were placed in distilled water (control – untreated), in aqueous solutions containing 10 μM thidiazuron (TDZ), 100 μM melatonin (MEL), 300 μM 5-aminolevulinic acid (AA), 300 μM tocopherol (vitamin E, VIT E), and 300 μM ascorbic acid (vitamin C, VIT C). Cool white light in the growth chamber was provided by L 36 W/76 fluorescent tubes (Osram, Munich, Germany), and the pulse treatments were carried out for 24 h under a 12:12 h dark:light (50 μmol^{-1} m^{-2} s^{-1}) cycle at room temperature (22–24°C). Afterward, the cut stems were transferred to distilled water and placed in a dark storage cell for four weeks at a temperature of 6–8 °C and a relative humidity of 80 % to simulate container transportation. After the transport simulation, the plants were transferred to a room for the simulation of the retailed environment (SRE). The SRE conditions during the 30 d of permanence were as follows: photoperiod of 12 h light/dark (50 μmol^{-1} m^{-2} s^{-1} light intensity), 20–24 °C, and 40–70 % RH. A total of 60–70 ruscus branches were used for each treatment, and the experiments were repeated twice from October to December 2021 and 2022. An overview of the experimental design and the effects of pulse treatments is presented in Fig. 1 (A and B).

2.2. Water uptake rate

The water uptake rate was determined using the gravimetric method, according to the methodology described by van Doorn et al. (2002) and Vieira et al. (2011). After storage, the stems were placed in individual tubes, initially weighed, and then deionised water was added. The tubes were weighed periodically for 30 d under SRE environmental conditions, with and without stems. To negate the effects of evaporation, the upper extremities of the tubes were coated with four layers of polyvinyl chloride (PVC) film. The water uptake rate of each solution was estimated as the volume of solution absorbed and expressed as a daily rate for a cut ruscus branches (H_2O g d^{-1} $stem^{-1}$).

2.3. Weight loss (%)

The initial fresh weight was assigned the value of 100 %. The fresh weight change was estimated as a percentage of the initial weight of the stems, according to the following formula:

$$FWC = (FWST \times 100)/IWST$$

FWC: fresh weight change (%)

FWST: final fresh weight of stem

IWST: initial fresh weight of the stem

The sampling points were 0, 4, 9, 16, 24, and 30 d during the SRE conditions.

2.4. Vase life evaluation

Vase life was visually evaluated by monitoring leaf yellowing of cut branches placed under SRE conditions. The initial symptoms of senescence were determined by daily observations of basal, medial, and apical leaf yellowing. Vase life was defined as the number of days until the branches showed more than five leaves with 50 % of the yellowing area.

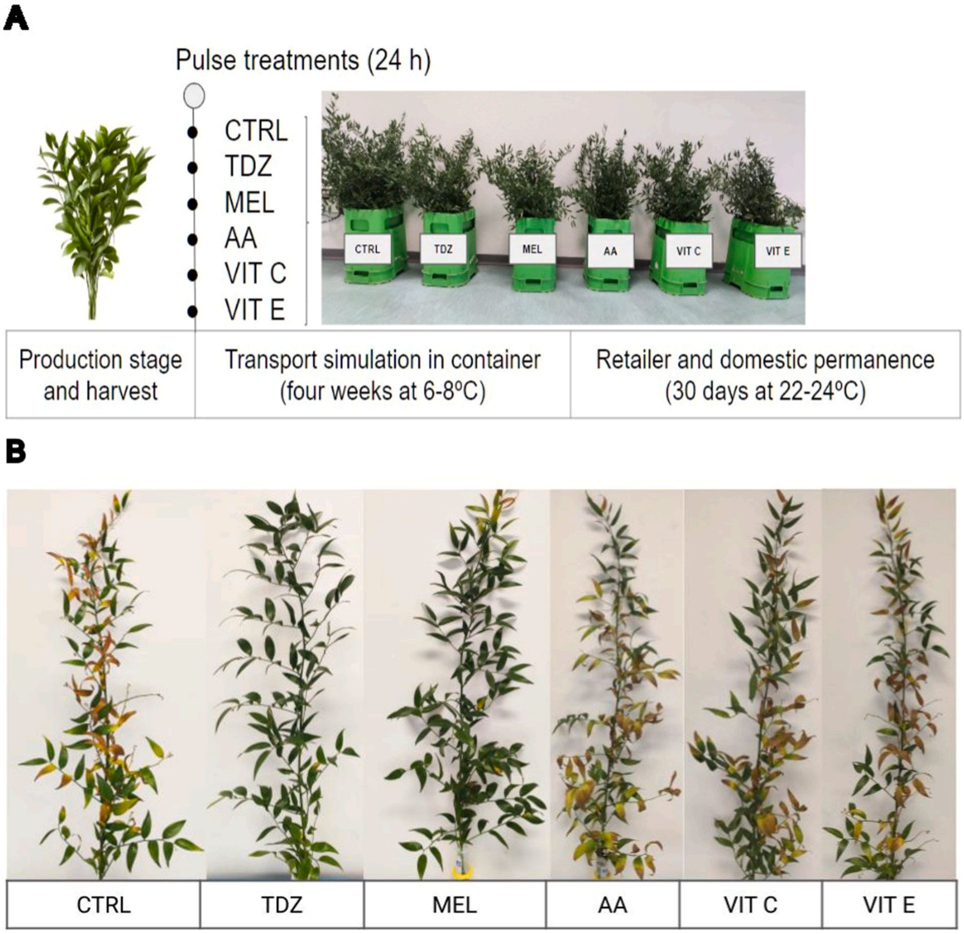

Fig. 1. Postharvest cut ruscus foliage. (A) Experimental design and (B) effects of pulse treatments at the end of the trial.

2.5. Chlorophyll *a* fluorescence

The chlorophyll *a* fluorescence measurements were performed using a portable chlorophyll fluorometer (Handy Pea, Hansatech Instruments Ltd., UK) (Strasser et al., 2004). Ruscus leaves were dark-adapted using leaf clips (4 mm diameter) for 30 min and then exposed to an excitation light intensity (ultra-bright red LEDs with a peak at 650 nm) of 3000 μmol m^{-2} s^{-1} provided by three high-intensity light-emitting diodes. Chlorophyll *a* fluorescence was assessed throughout vase life under SRE conditions. Acronyms, equations, and explanations of the JIP test parameters employed in the present investigation can be found in the user manual of the Handy PEA Chlorophyll Fluorimeter.

2.6. Chromatic variation of cut ruscus branches

To monitor the progression of leaf yellowing during SRE, non-destructive *in vivo* analysis and destructive analysis were performed using a colorimeter and chlorophyll content quantification, respectively.

Yellowing index: ten leaves from different branches in each treatment were used to measure leaf colour. The colour of the leaves was measured at three different time points, the average value was recorded using a Minolta CR 400 Chroma portable colorimeter (Minolta Co., Osaka, Japan), and the CIELAB colour space coordinates L* , a* , and b* were determined. The colorimeter was calibrated using a standard white calibration plate at the beginning of the measurement. The yellowing index (YI) was calculated using the following formula: YI = 142.86 b/L (Hirschler, 2012).

Chlorophyll determination: a pure methanol solution was used for extraction. The extraction protocol reported by Maggini et al. (2013) was used with minor modifications. Frozen fresh tissue samples (0.03 g) were soaked in 1.5 mL of extraction solvent in 3 mL tubes. The tubes were sonicated twice for 30 min in an ice bath and stored overnight at −20 °C. After separating the supernatant, the extraction was repeated on the pellet with 1.5 mL of fresh extraction solvent. The two aliquots of the supernatant were pooled and used for chlorophyll quantification. Absorbance was measured at 665.2 and 652.4 nm. Total chlorophyll content was calculated using Lichtenthaler (1987) and expressed on a fresh weight (g kg^{-1} FW) basis.

2.7. Ethylene determination

Ethylene production was measured by enclosing 10–15 cm long apical portions of branches in airtight containers (100 mL). Two mL gas samples were taken from the headspace of the containers after 1 h of incubation at room temperature. The ethylene concentration was measured by a gas chromatograph (HP 8890, Hewlett-Packard, Menlo Park, CA) using a flame ionisation detector (FID), a stainless-steel column (150 ×0,4 cm ø packed with Hysep T), column and detector temperatures of 70 °C and 350 °C, respectively, and helium carrier gas at a flow rate of 30 mL min^{-1}. Quantification was performed against an external standard, and the results were expressed on a fresh weight basis (pL h^{-1} kg^{-1} FW). Ethylene evolution was assessed throughout the vase life during throughout 24 d of SRE conditions (1, 9, 16 and 24 d).

2.8. Hormonal balance in MEL-treated cut branches

Tissue processing and quantitative analysis of ABA and ABA catabolites, cytokinins, auxins, and gibberellins in these samples was performed using a modified procedure described by Lulsdorf et al. (2013) and carried out at Creative Proteomics (NY, USA). The analysis was performed on a UPLC/ESI-MS/MS utilising a Waters ACQUITY UPLC system, which was equipped with a binary solvent delivery manager and a sample manager coupled to a Waters Micromass Quattro Premier XE quadrupole tandem mass spectrometer via a Z-spray interface. MassLynx and QuanLynx (Micromass, Manchester, UK) were used for data acquisition and analysis. Quantitative analysis was performed using the Multiple Reaction Monitoring (MRM) function of the MassLynx v4.1 (Waters Inc.) control software. The resulting chromatographic traces for each analyte (endogenous phytohormone) and their respective deuterium-labelled internal standards were quantified offline using QuanLynx v4.1 software (Waters Inc), wherein each trace was integrated, and the resulting ratio of signals (analyte/internal standard) was compared with a previously constructed calibration curve to yield the amount of analyte present (ng per sample). Calibration curves were generated from the MRM signals obtained from the standard solutions based on the ratio of the chromatographic peak area for each analyte to that of the corresponding internal standard. QC samples, internal standard blanks, and solvent blanks were also prepared and analysed along with each batch of tissue samples. Results of the analysis of these samples were expressed in mg kg^{-1} of sample.

2.9. Statistical analysis

Data were subjected to statistical analysis using GraphPad Prism9 software (GraphPad Software, San Diego, CA, USA). To stabilise the variance and normalise the percentage data, an arcsine transformation was used. Statistical analyses were conducted using the software, and the significance of differences was tested using two-way ANOVA ($p < 0.05$). Differences among means were determined using Tukey's post-hoc test. Student's *t*-test was used to compute pairwise comparisons between group means. Each experiment was repeated at least twice. Different letters within the same time point denote significant differences among the different pulse treatments at $p < 0.05$

3. Results

3.1. Water uptake rate in cut ruscus branches

In the cut ruscus branches treated with AA, the water uptake was significantly higher after 4 d of vase life compared to the other treatments(MEL, TDZ, VIT E, and VIT C) or the untreated control (CTRL), and then gradually decreased significantly after 9 d (Fig. 2A). No significant change in water uptake was observed between the CTRL branches and those treated with VIT E, VIT C, MEL, and TDZ for up to 16 d of vase life (Fig. 2A). After 24 and 30 d of storage, the foliage treated with TDZ and MEL showed a better water uptake rate than the control. The best water balance over time was observed in foliage treated with TDZ and MEL (Fig. 2A). Weight change during the vase life of treated and untreated cut branches (Fresh weight change, FWC) was expressed as percentage (Fig. 2B). In cut branches treated with AA the FWC was significantly higher after four days of vase life compared to the other treatments (MEL, TDZ, VIT E, and VIT C) and the untreated control (CTRL). Subsequently, there were no statistically significant differences compared with the control. The cut foliage treated with TDZ, MEL, VIT C, and VIT E did not show significantly different values of FWC compared to the CTRL up to 16 d of vase life (Fig. 2B). Subsequently, the values were higher in the MEL and TDZ treatments at 24 and 30 d of vase life and in cut foliage treated with VIT C after 30 d, compared to the CTRL (Fig. 2B).

3.2. Photosynthetic performance in cut ruscus branches

The post-harvest performance of treated *D. racemosa* branches was evaluated by measuring chlorophyll *a* fluorescence during the 30 d of SRE conditions (Fig. 3). The maximum quantum efficiency of photosystem II (Fv/Fm ratio) showed a gradual decline during the SRE period in all treatments, except for the cut foliage treated with MEL and TDZ (Fig. 3A). The Fv/Fm ratio in cut foliage treated with MEL or TDZ was significantly higher than the control at 16, 24, or 30 d of SRE. In contrast, cut branches treated with AA showed a sharp drop in Fv/Fm compared to the control after 16 d of vase life. In the cut foliage treated with VIT C, Fv/Fm significantly increased compared to the control only after 24 d of SRE, whereas no significant changes were observed at other

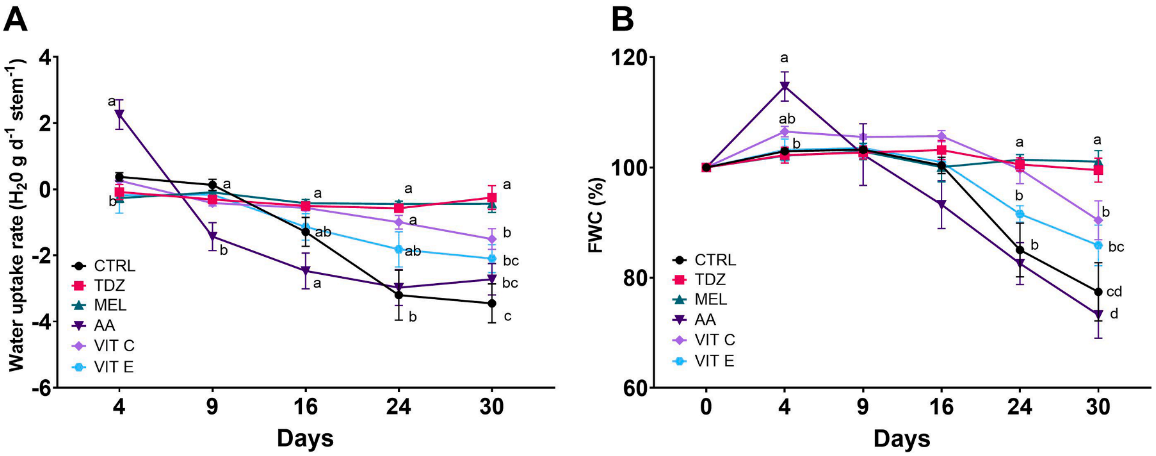

Fig. 2. Water uptake rate (A) and fresh weight change (FWC) (%) (B) of previously pulse-treated cut ruscus branches using 10 μM thidiazuron (TDZ), 100 μM melatonin (MEL), 300 μM 5-aminolevulinic acid (AA), 300 μM tocopherol (VIT E), and 300 μM ascorbic acid (VIT C) or untreated (CTRL). Values are presented as means (n = 10) and standard error bars. The measurements were carried out at 0, 4, 9, 16, 24, and 30 d during the SRE condition after four weeks of container transport simulation at 6–8 °C.

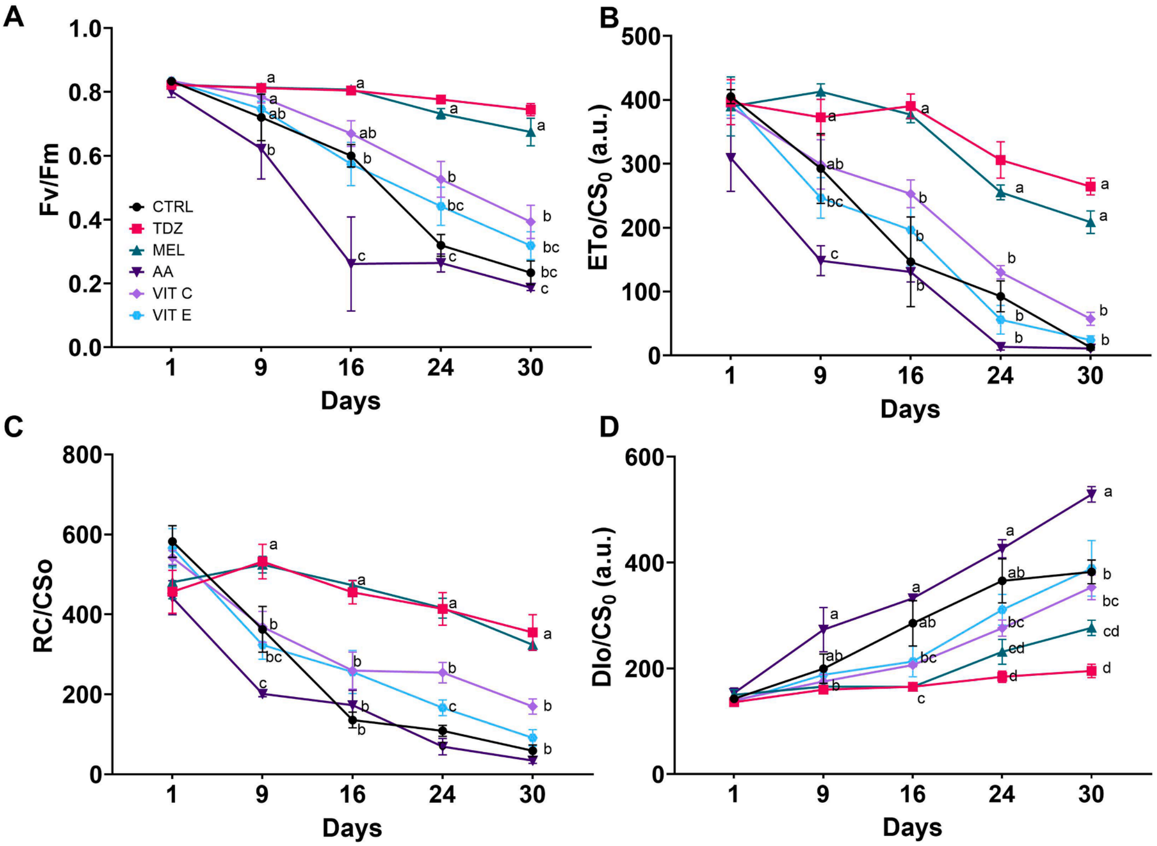

Fig. 3. Chlorophyll *a* fluorescence parameters were measured in the leaves of cut ruscus foliage exposed to pulse treatments containing 10 μM thidiazuron (TDZ), 100 μM melatonin (MEL), 300 μM 5-aminolevulinic acid (AA), 300 μM tocopherol (VIT E), and 300 μM ascorbic acid (VIT C) or not exposed (CTRL). (A) Maximum quantum efficiency of photosystem II (Fv/Fm); (B) Electron transport flux per cross-section (CS), ETo/CS; (C) Density of reaction centre per CS, RC/CS: (D) dissipation energy per CS, DIo/CS. Values are presented as means (n = 5) and standard error bars. The measurements were carried out at 1, 9, 16, 24, and 30 d during the SRE condition and after four weeks of container transport simulation at 6–8 °C.

sampling times. Fluorescence data were monitored for 30 d in the leaves of cut ruscus branches subjected to pulse treatments. The JIP test provides information on energy flow and potential photosynthetic activity. As the number of days of SRE increased in all treated and untreated cut foliage, a gradual reduction in electron transport flow per cross-section (ETo/CSo) was observed (Fig. 3B). However, cut ruscus branches treated with TDZ or MEL significantly showed higher ETo/CSo values than the CTRL and those treated with VIT C, VIT E, or AA after 16, 24, and 30 d of SRE. Similarly to ETo/CSo parameter, the density of reaction centers measured at Fo (RC/CSo) showed a gradual decrease during SRE condition at room temperature (Fig. 3C). However, in leaves of cut foliage treated with MEL and TDZ, the RC/CSo responsible for absorption and electron transfer maintained significantly higher values compared to the CTRL and other treated cut ruscus branches after 9 d and until the end of SRE period (30 d). Finally, the energy dissipated per cross section (DIo/CSo) slightly increased during the vase life of the cut foliage (Fig. 3D). The most rapid increase over time was observed in the cut foliage treated with AA after 9 d, whereas cut branches treated with MEL and TDZ showed significantly lower values compared to the control after 16, 24, or 30 d of SRE.

3.3. Changes in total chlorophyll concentration and yellowness index

The chlorophyll concentration did not change during the initial SRE period (until the 9th d) in all treatments (Fig. 4A). Total chlorophyll content decreased after 16 d in cut ruscus branches treated with AA and after 24 d in treatments with VIT C, VIT E, and CTRL. After 30 d of vase life, the cut foliage treated with MEL or TDZ maintained a significantly higher chlorophyll concentration compared to all other treatments (Fig. 4A). The colour of the leaf surface was also monitored non-destructively using the yellowness index determined with a colorimeter (Fig. 4B). As observed for the total chlorophyll, the yellowness index remained stable during the initial SRE period (9 d) in all treatments. Subsequently, an increase in its values was observed in the cut foliage treated with AA after 16 d, in the CTRL cut ruscus branches, and in those treated with VIT C or VIT E after 24 d. Treatments with TDZ or MEL slowed leaf yellowing, as shown by the significantly lower yellowness index values compared to other treatments after 30 d of SRE.

3.4. Ethylene evolution in cut ruscus foliage

To evaluate the effectiveness of exogenous pulse treatments in prolonging the longevity of cut foliage, ethylene production, a hormone involved in the aging process of plant tissues, was determined as a physiological marker of leaf senescence progression. Endogenous ethylene production was measured in leaves of treated branches after 30 d of SRE (Fig. 5). In the cut foliage treated with AA, a significant increase in ethylene production was observed compared to the control and other treated branches after 9 d of SRE. Ethylene biosynthesis in the control cut foliage significantly increased after 16 d compared to the cut ruscus branches treated with TDZ, MEL, VIT C, or VIT E. After 24 and 30 d of SRE, leaves of branches treated with TDZ or MEL did not show any significant increase in ethylene production compared to initial levels. In these two treatments the ethylene production was significantly lower if compared with the cut foliage treated with VIT C, VIT E, AA, or the control.

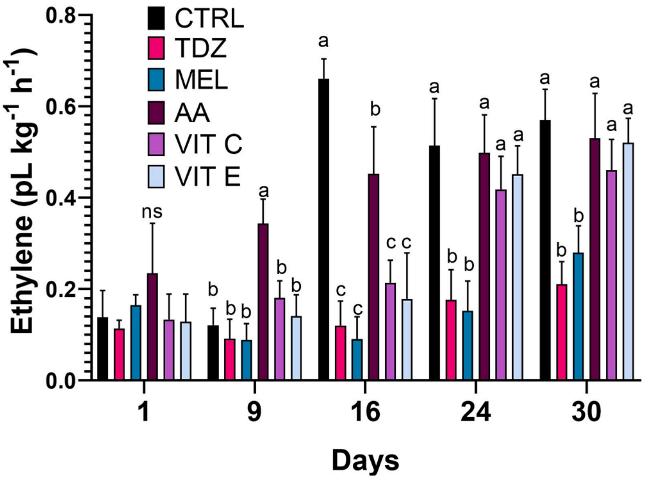

Fig. 5. Ethylene evolution in leaves of cut ruscus foliage exposed to pulse treatments containing 10 μM thidiazuron (TDZ), 100 μM melatonin (MEL), 300 μM 5-aminolevulinic acid (AA), 300 μM tocopherol (VIT E), and 300 μM ascorbic acid (VIT C) or not exposed (CTRL). Values are presented as means (n = 5) and standard error bars. The measurements were carried out at 1, 9, 16, 24, and 30 d during SRE condition and after four weeks of container transport simulation at 6–8 °C.

3.5. Hormone profiles in cut ruscus foliage melatonin treated

The metabolomic investigation assessed the quantification of a comprehensive panel of plant hormones in cut ruscus leaves after 16 d (at the initial stage of the senescence process in untreated cut foliage) clearly showed that melatonin acted as a regulatory hub of plant hormone metabolism, mediating the increase or decrease of their endogenous levels. Results reported in Table 1 show the presence of biologically active ABA and some of its metabolites in cut ruscus foliage.

Values are presented as means (n = 6) and standard error values. Pair-wise statistical comparisons were calculated with a two-tailed Student's *t*-test and different letters for each hormone detected (within the row) between MEL-treated branches and non-treated (CTRL) ones, denote significant differences at $p < 0.05$.

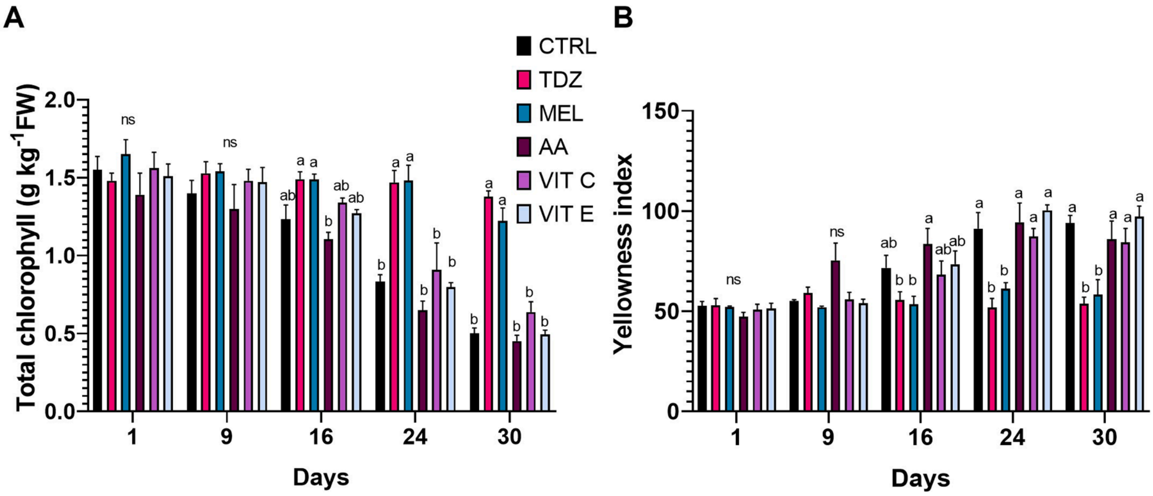

Fig. 4. Changes in total chlorophyll content (A) and yellowness index (B) in leaves of cut ruscus foliage exposed to pulse treatments containing 10 μM thidiazuron (TDZ), 100 μM melatonin (MEL), 300 μM 5-aminolevulinic acid (AA), 300 μM tocopherol (VIT E), and 300 μM ascorbic acid (VIT C) or not exposed (CTRL). Values are presented as means (n = 5) and standard error bars. The measurements were carried out at 1, 9, 16, 24, and 30 d during SRE condition and after four weeks of container transport simulation at 6–8 °C.

Table 1
Plant hormone profiles in ruscus leaves pulse-treated with melatonin after 16 d of SRE condition. The cut foliage was stored at 6–8 °C to simulate container transportation. Results are expressed on fresh weight (FW).

Plant hormone (mg kg^{-1} FW)		Non-treated branches (CTRL)	Melatonin treated branches (MEL)
ABA and ABA metabolites	ABA	112.0 ± 6.24 a	66.0 ± 8.54 b
	DPA	1159.0 ± 75.11a	614.3 ± 31.6 b
	ABAGE	101.3 ± 14.90	75.7 ± 11.91
	PA	60.3 ± 8.95 a	28.7 ± 2.73 b
	7'OH-ABA	7.3 ± 1.20	5.5 ± 0.87
	t-ABA	13.0 ± 1.53	11.0 ± 0.60
Cytokinins	t-ZOG	18.0 ± 4.04 b	42.3 ± 2.73 a
	c-ZOG	5.0 ± 0.78	5.3 ± 0.33
	c-ZR	1.3 ± 0.33	1.6 ± 0.29
	iPR	1.0 ± 0.58	0.7 ± 0.32
Auxins	IAA	45.7 ± 3.33	54.9 ± 2.40

The concentration of cis-ABA and its catabolites, dihydrophaseic acid (DPA), and phaseic acid (PA) were significantly higher CTRL cut ruscus foliage than in the MEL-treated ones. On the other hand, ABA catabolites obtained through 7'-hydroxylation and glucosilation (resulting in 7'OH-ABA and ABAGE, respectively) did not show significant differences between the treated and untreated cut ruscus branches. Regarding cytokinin-related compounds, the Z catabolism product zeatin-O-glucoside (ZOG), both cis- and trans-isomers, was detected, and the (*trans*) zeatin-O-glucoside (*t*-ZOG) was significantly higher in the MEL-treated cut foliage (Table 1). The biosynthetic precursors zeatin riboside (cis-isomer) and isopentenyladenosine (iPR) were not significantly different between treated and untreated cut ruscus branches. The auxin, indole-3-acetic acid, showed a slight increase, although differences were not statistically significant, between the control and treated cut ruscus branches. Overall, data reported indicated that melatonin treatment resulted in a decrease in ABA levels, as the overall ABA content in control branches was greater than that in melatonin-treated cut foliage (Fig. 6A), and an increase in cytokinin levels, as the overall cytokinin content in melatonin-treated branches was greater than that in control leaves (Fig. 6B).

4. Discussion

The visual appearance of the cut foliage is the most important quality parameter for determining the value of these ornamental crops. Considering the nature of the floriculture market and the ongoing globalisation process, postharvest handling of the product through preconditioning treatments using low environmental impact compounds to improve quality performance during the postharvest phases (packaging, transportation, storage, etc.) is crucial from the perspective of current sustainability-oriented consumption patterns (Ferrante et al., 2015; Gabellini and Scaramuzzi, 2022). The present study investigated the effects of different pre-conditioning treatments/agents on the physiology of cut ruscus foliage during long-term storage and subsequently vase life as a new strategy to enhance postharvest handling practices. Physiological and biochemical responses were monitored to understand the beneficial effects of treatments on quality retain of cut foliage. These treatments were performed to slow down the senescence process and leaf yellowing to ensure high quality during long-distance transportation or extended storage periods.

Several studies have shown that the highly regulated natural process of leaf senescence can be triggered by extremely low light conditions and darkness, which foliage inherently experience during transportation and storage (Pacifici et al., 2013; Bulgari et al., 2015; Franzoni et al., 2024).

In this study, pulse applications of TDZ, AA, VIT C, VIT E, or MEL before 30 d of refrigerated transportation were studied (to preserve the quality and prolong the vase life of cut ruscus foliage) by identifying key metabolic processes which play a role in the quality and lifespan of cut ruscus foliage. The vase life of cut ruscus can be determined either by fresh weight change (%), as cut foliage is typically sold by weight, or by the change in leaf colour from green to yellow, which is the most visible sign of leaf senescence and quality deterioration (Bulgari et al., 2015). Weight loss is primarily due to water loss through transpiration, reduced water uptake, and other processes associated with postharvest senescence (Franzoni et al. 2024). Our results suggest that only the TDZ or MEL treatments maintained a better water uptake rate during the simulated time spent at the retailer, which, in turn, resulted in less change in fresh weight percentage. However, the AA, VIT C, or VIT E treatments did not have any effect on preserving the water uptake rate and consequently preventing weight loss, showing results similar to those of the control foliage at the end of the experiment. The effectiveness of TDZ or MEL compared to other treatments in preserving vase life and quality has been confirmed by chlorophyll *a* fluorescence parameters, the delay in leaf yellowing measured quantitatively through the determination of chlorophyll content and by the yellowing index, and the regulation of hormonal phytohormones, particularly ethylene which is widely acknowledged as a senescence-promoting hormone through autocatalytic induction (Iqbal et al., 2017). Cytokinins, particularly TDZ, have long been recognised for their ability to delay leaf yellowing and extend the vase life of many ornamental species (Ferrante et al., 2009; Jiang et al., 2009; Trivellini et al., 2015). Cytokinins are biosynthesised in roots; therefore, when cut flowers and foliage are

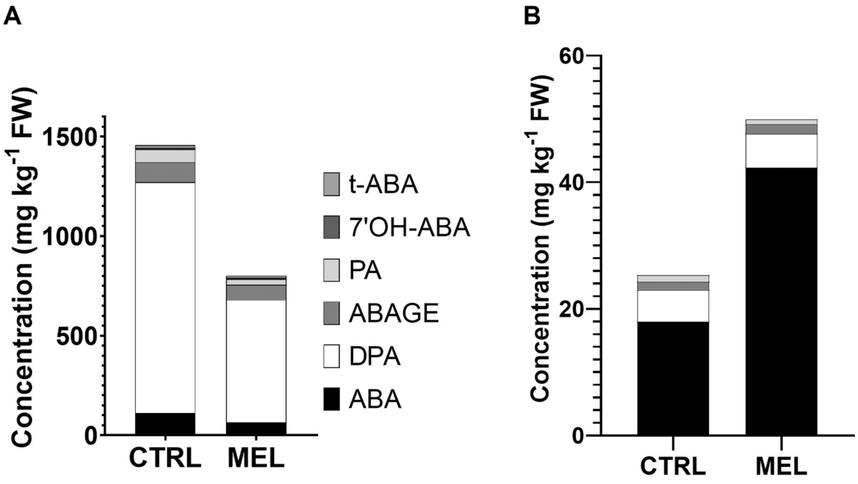

Fig. 6. (A) ABA and ABA catabolites and (B) cytokinin content in cut ruscus ABA leaves pulse-treated with melatonin after 16 d of SRE. The cut foliage was previously stored at 6–8 °C to simulate container transportation. Values are presented as global means (n = 6).

harvested, the lack of endogenous supply of these essential plant hormones occurs (Kakimoto, 2003; Janowska and Andrzejak, 2022). The reduction in endogenous cytokinins, combined with the dark conditions, accelerates the onset of leaf senescence (Eason, 2006; Guo et al., 2021). Since TDZ is not metabolised by plants, its activity lasts longer than that of other cytokinins (Genkov and Ivanova, 1995) and promotes the conversion of cytokinin ribonucleotides to more biologically active ribonucleosides (Capelle et al., 1983). The exogenous application of this compound effectively delayed chlorophyll degradation, leaf yellowing, and senescence (Nisler, 2018). In addition, the beneficial effect on the performance of the photosynthetic system could suggest that TDZ-treated plants respond more favourably to longer storage durations. Multiple studies cited by Gururani et al. (2015) have shown that the external application of cytokinins affects photosynthesis under abiotic stress, emphasising the vital role of these phytohormones in enhancing photosynthetic efficiency in challenging environments. Furthermore, the application of TDZ in our study demonstrated the ability to reduce stress-induced ethylene levels after harvest and during dark storage, thereby mitigating its harmful effects while simultaneously delaying the onset of senescence, as previously reported by Mutui et al. (2015) in Pelargonium cuttings.

Similar to the well-known and already established effect of TDZ, MEL treatments exerted senescence inhibition on *D. racemosa* L., thereby increasing the vase life of cut foliage. This finding was associated with the evolution of fresh weight (FW), as MEL treatments helped maintain water relations for a longer duration during postharvest vase life. Achieving a proper balance between organ dehydration and water uptake is crucial for preserving cell turgor in cut ornamental plants (Reid and Jiang, 2012). As previously reported for cut peony flowers (Wang et al., 2024), MEL treatment helped to maintain the water uptake rate, presenting a promising strategy to improve the aesthetic appeal and longevity of cut ornamental commodities. The MEL treatment appeared to preserve the health status of cut ruscus foliage, as demonstrated by the analysis of chlorophyll *a* fluorescence parameters, higher chlorophyll content, and a reduced yellowness index, as shown by both *in vivo* and destructive analyses. These results suggest that the photosynthetic apparatus performed better in the MEL treatment than in the VIT C, VIT E, ALA, and control foliage treatments. Exogenous application of MEL in cucumber has been reported to delay natural leaf senescence by decreasing the expression of the senescence-related gene *SAG12* and the cell death-related gene *PDCD*, while increasing chlorophyll content by downregulating chlorophyll degradation genes and maintaining a normal chloroplast structure (Liu et al., 2022). This leads to improved activity of PSI and PSII reaction centres, enhanced electron transfer efficiency, and higher levels of photosynthesis-associated proteins compared to those in H_2O-treated seedlings (Liu et al., 2022). Furthermore, dark-induced leaf senescence in cut gardenia was significantly suppressed by exogenous MEL treatment, as evidenced by increased chlorophyll content and fluorescence parameters (Fv/Fm, Fv/F0, and Y (II)), reduced leaf programmed cell death in darkness, elevated endogenous melatonin levels, enhanced antioxidant enzyme activities, and preserved anatomical integrity (Zhao et al., 2017). The role of MEL as in ROS scavenger has been observed in leaves and fruit, increasing the activity of detoxification enzymes (Cortés-Montaña et al., 2025; Yu et al., 2025). The MEL seems to have an effect in reducing cellular damages and preserving leaf functionality or integrity. Similar results were observed in this study, which demonstrated that exogenous MEL significantly inhibited chlorophyll degradation and decreased the potential efficiency of photosystem II in ruscus leaves exposed to darkness. This was achieved by mitigating PSII damage, as indicated by reduced energy dissipation, the maintenance of active reaction centres, and electron transport, and ultimately preserving the overall function of ruscus leaves after prolonged simulated dark exposure. The positive effect of MEL on chlorophyll and chlorophyll *a* fluorescence parameters have been reported in crops under drought conditions (Du et al., 2024).

Given the anti-senescent effects observed in MEL-treated foliage—evidenced by reduced leaf yellowing, slower chlorophyll degradation, improved water retention, and sustained photosynthetic activity—combined with the established role of melatonin in regulating other plant hormones (Arnao and Hernández-Ruiz, 2007; Sun et al., 2015; Gao et al., 2018; Arnao and Hernández-Ruiz, 2020), a metabolomic analysis of plant hormones was carried out to explore these effects in greater depth. Metabolomic analysis, which assessed the quantification of a comprehensive panel of plant hormones in ruscus leaves after 16 d (at the early stage of the senescence process in untreated foliage), clearly showed that melatonin acts as a regulatory hub for plant hormone metabolism by mediating the increase or decrease in their endogenous levels. Specifically, there is an observed increase in hormones known to be negative regulators of senescence, such as cytokinins, and a decrease in hormones considered inducers of senescence, such as ethylene, ABA, and its metabolites (Iqbal et al., 2017).

Melatonin treatments on endogenous hormone levels and the physiological consequences involve many plant growth processes, ranging from enhanced germination to delayed senescence (Arnao and Hernández-Ruiz, 2018). During tomato ripening, melatonin increased ethylene production by upregulating the expression of 1-*amino-cyclopropane-carboxylic acid synthase 4 (ACS4)* gene, (Sun et al., 2015). On the contrary, melatonin reduced ethylene production in pears, which resulted in a lower loss of firmness (Zhai et al., 2018) and etiolated lupin hypocotyls (Arnao and Hernández-Ruiz, 2007). Ethylene production did not increase in cut ruscus foliage treated with MEL. These results, along with previous findings on other plant organs, suggest that MEL may have the potential to delay senescence, extend vase life, and prevent physiological alterations, including chlorophyll degradation and water imbalance.

Numerous studies have shown that MEL can slow leaf senescence in various plant species when exposed to challenging conditions, such as drought, darkness, and ABA-induced stress (Ma et al., 2010; Tan et al., 2019; Wang et al., 2012). ABA, widely known as a, is a key inducer of leaf senescence, with its concentrations increasing at the onset of senescence under both optimal and stressful conditions, such as drought, salinity, extreme temperatures, or darkness (Lim et al., 2007; Wang et al., 2016). Moreover, under salt stress and drought condition, exogenous melatonin treatment led to a rapid reduction in ABA levels by increasing the expression of ABA catabolism genes and decreasing the expression of 9-cis-epoxycarotenoid dioxygenase (NCED), resulting in an improved response to these adverse conditions (Zhang et al., 2014: Li et al., 2015). In this study, MEL treatment significantly affected ABA content during the postharvest life of cut ruscus foliage stored in prolonged darkness, acting as an antagonist to the endogenous increase in ABA and thereby regulating leaf senescence.

Cytokinins can delay leaf senescence and promote chlorophyll retention, as shown by Richmond and Lang (1957). This effect was further demonstrated by the autoregulation of cytokinin biosynthesis during senescence using IPT under the control of the SAG12 promoter (Gan and Amasino, 1995). Moreover, the total cytokinin content rapidly declined with aging (Hönig et al., 2018). These findings highlight the significance of this study, where MEL's role in enhancing or maintaining high cytokinin levels reflects its impact on the overall quality and longevity of cut ruscus foliage. Notably, the (trans) Zeatin-O-glucoside (t-ZOG), an inactive but convertible storage form (Brzobohaty et al., 1993), was significantly higher in MEL-treated cut foliage. This suggests that MEL treatment may activate cytokinin glucosyl transferases, which form O-glucosides, key regulators of cytokinin homeostasis with the potential to delay leaf senescence.

5. Conclusion

In conclusion, this study highlights the importance of visual quality in cut foliage, a key factor in their commercial value. With the increasing emphasis on sustainability and the globalized nature of floriculture, optimizing postharvest treatments to preserve foliage quality is essential. The findings demonstrate that preconditioning treatments, specifically using TDZ or MEL, effectively maintain water balance, delay leaf yellowing, and enhance photosynthetic activity in cut ruscus. These treatments proved more effective than VIT C, VIT E, and AA in preserving the physiological and aesthetic properties during extended storage. The efficacy of MEL was linked to their influence on hormone regulation—specifically reducing ethylene and ABA levels while increasing cytokinins, antioxidant activity, and maintaining photosynthetic performance. MEL treatment may act as an antagonist to ABA, a key senescence-promoting hormone, potentially mitigating its effects on leaf aging. While the observed decrease in ABA levels and increase in cytokinin content suggest a role in delaying senescence, further verification through pharmacological experiments is required to confirm this mechanism. Integrating these treatments into postharvest protocols offers a sustainable and impactful approach to improving the marketability and vase life of cut ornamental foliage, aligning with evolving market demands.

Funding

This work was supported by the PSR 2014–2020 (Rural Development Programme), Measure 16.2. (PIF 27/2017). Project name: “LECOSFLO - Logistica e conservazione eco-sostenibile per il Florovivaismo Toscano”.

CRediT authorship contribution statement

Franzoni Giulia: Methodology, Data curation, Writing – review & editing. **Ferrante Antonio:** Writing – original draft, Funding acquisition, Methodology, Writing – review & editing. **Trivellini Alice:** Writing – original draft, Methodology, Data curation, Conceptualization, Formal analysis, Writing – review & editing. **Mensuali Anna:** Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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