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RESEARCH ARTICLE

Investigating the effect of methyl jasmonate and melatonin on resistance of *Malus crabapple* ‘Hong Jiu’ to ozone stress

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Abstract

Ozone (O_3) is an adverse environmental factor posing damage to ornamental plants. Thus, it is important to seek an effective way of enhancing plant tolerance to O_3-induced damage. Methyl jasmonate (MJ) and melatonin (MT) are plant growth regulators (PGRs) involved in plant abiotic stress responses. In this study, compared with the control group of plants without ozone, the influence of exogenous MJ (0, 10, 50, 100, and 150 μM) and MT (0, 0.1, 0.5, 2.5, and 12.5 μM) on the resistance of *Malus crabapple* ‘Hong Jiu’ was evaluated under O_3 stress (100 ± 10 nL/L for 3 h). Our data revealed that levels of MDA were significantly enhanced following O_3 treatment compared with plants without O_3. O_3 induced the activities of antioxidant enzymes and the accumulation of non-enzymatic antioxidants. While lower malondialdehyde (MDA) content, greater activities of antioxidant enzymes, and higher levels of soluble protein and non-enzymatic antioxidants were observed in PGRs-pretreated plants than in non-PGRs-pretreated plants under O_3 stress. Based on the above results and air pollution tolerance index (APTI), an exogenous supply of MJ and MT to *Malus crabapple* ‘Hong Jiu’ seedlings was protective for O_3-induced toxicity. The present study provides new insights into the mechanisms of MJ and MT amelioration of O_3-induced oxidative stress damages in *Malus crabapple* ‘Hong Jiu.’

Keywords Ozone stress · Methyl jasmonate · Melatonin

Abbreviations

ANOVA	Analysis of variance
APTI	Air pollution tolerance index
AsA	Ascorbic acid
CAT	Catalase
GSH	Glutathione
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
MJ	Methyl jasmonate
MT	Melatonin
NBT	Nitroblue tetrazolium
O_2	Oxygen
O_2^-	Superoxide anion
O_3	Ozone
OTC	Open-top chamber
PGRs	Plant growth regulators
POD	Peroxidase
ROS	Reactive oxygen species
RWC	Relative water content
SD	Standard deviation
SDAU	Shandong Agricultural University
SE	Standard error
SOD	Superoxide dismutase
TBA	2-Thiobarbituric acid
TCH	Total chlorophyll

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Introduction

Ozone (O_3) is the major constituent of photochemical smog (a combination of smoke and fog). Since it was first investigated, it has become evident that O_3 is by far the most important air pollutant toxic to plants worldwide (Krupa et al. 2007). In recent years, near-surface ozone pollution has become common in many cities of Europe, North America, Japan, and China, with the increase of automobile exhaust emissions and heavy industrial energy consumption. Current models predict that ozone will recover from the effects of man-made ozone-depleting gases (Secretariat 2011). Nearly one-quarter of the earth's surface is currently at risk from tropospheric ozone in excess of 60 nL/L (Morgan et al. 2006; Zhang et al. 2011). Unlike all other climatically important trace gases, ozone is toxic, and increases in its concentration will result in serious environmental damage (Fishman 1991). Generally, exposure to O_3 concentrations in excess of 60 nL/L for several hours causes injury to ozone-sensitive plants (Smith et al. 2012), which is typically expressed as tiny purple-red, yellow, or black spots (described as stipple) or sometimes as a general even discoloration, reddening, or bronzing (Wang and Chen 1985; Lie et al. 2014a; Wan et al. 2014). Therefore, studying the impact of future concentrations of near-surface O_3 on plants is a focus of scholars in China and abroad (Gaucher et al. 2006; Wang et al. 2011, 2016a).

The leaf is the main medium for ozone to enter plant tissue and is therefore the first sensor of plant ozone stress (Lie et al. 2014b). As a strong oxidant, O_3 enters the leaf tissue through stomata and is converted to reactive oxygen species (ROS) (Xie et al. 2009). The production of ROS is considered the main cause of the serious effect of O_3 on the normal growth and development of plants. Supplementation of O_3-exposed plants with exogenous substances can alleviate oxidative stress–induced damage through reduced lipid peroxidation and enhancement of antioxidant defense systems (Zheng et al. 2006; Xie et al. 2009). For instance, melatonin (MT) is a crucial molecule involved in plant abiotic stress responses, and previous work supports a role for MT as a free radical scavenger (Reiter et al. 2000; Galano et al. 2011), directly scavenging ROS in cellular compartments and thereby mitigating oxidative stress in plants (Reiter et al. 2015; Ding et al. 2017). Methyl jasmonate (MJ) is another phytohormone identified as a vital cellular regulator that mediates diverse developmental processes and defense responses against biotic and abiotic stresses (Cheong and Choi 2003). Studies have shown the effects of MJ on oxygen-scavenging enzyme activities and membrane lipid composition (Wang 1999; Ali et al. 2006). Moreover, MJ and MT protect plants against a variety of environmental stresses, such as cold, heat, salinity, and drought (Yang et al. 2011; Zou et al. 2011; Liu et al. 2016; Gao et al. 2017b; Yang et al. 2017).

Malus crabapple 'Hong Jiu' is an ornamental plant of the *Malus* genus (Rosaceae), which was independently bred by Professor Shen Xiang from Shandong Agricultural University. It is horticulturally important and planted for the brilliant autumn and winter colors of its fruits. Thus, exploring MJ- and MT-mediated O_3 tolerance in *Malus crabapple* 'Hong Jiu' is of practical significance. The objectives of the present work were to determine the potential effects of MJ and MT on O_3 damage to *Malus crabapple* 'Hong Jiu' and to investigate the possible physiological mechanisms of MJ- and MT-mediated responses to O_3 stress in *Malus crabapple* 'Hong Jiu.'

Material and methods

Plant material, stress exposure, and chamber description

The *Malus crabapple* 'Hong Jiu' from Shandong Agricultural University (SDAU) in Tai'an was chosen as the experimental material. The seeds of *Malus crabapple* 'Hong Jiu' with the same germination were selected to be sown in plastic pots filled with matrix and vermiculite (2/1 v/v) and cultured in the greenhouse of Shandong Agricultural University until plants were 6 months old with 10–12 expanded leaves.

We have done two separate experiments on plants with good growth and relatively consistent growth characters from May to June 2017. The highest monthly average temperature was recorded in May (29 °C) and June (31 °C), while the lowest monthly average temperature was recorded in May (16 °C) and June (20 °C). The average monthly relative humidity during the study period was found to be 52.6% in May and 59.8% in June (provided by the office of Tai'an meteorological department).

The one with various concentrations of MJ (0, 10, 50, 100, and 150 μM) were applied to seedling leaves, and the other one with MT concentrations (0, 0.1, 0.5, 2.5, and 12.5 μM) were applied, with 0 μM distilled water between them. After 15 days, all specimens were exposed to O_3 of 100 ± 10 nL/L for 3 h (9:00–12:00 a.m) in an open-top chamber (OTC) at the Forestry College's experimental station at SDAU (Gong et al. 2017). *Malus crabapple* 'Hong Jiu' seedlings grown without MJ or MT treatment under no ozone stress were used as controls. There were nine pots (6 plants per pot) with similar growth conditions per treatment arranged in a complete randomized design and the experiment was conducted three times.

An open-top chamber should provide more natural conditions than a closed chamber (Heagle et al. 1973). Here, the chamber (Fig. 1) consisted of an outer steel frame covered with polyethylene plastic film and an inner aeration pipe and was open to the atmosphere at the top. Oxygen (O_2) in the oxygen tank entered the ozone generator (SK-CFG-10P, Sankang, China), which produced O_3 to be output to the pipe. The O_3 was blown into the chamber by a fan (SF2-2, Shenli, China). An ozone detector (DR70C-O_3, Wosaite, China) in the chamber monitored the O_3 concentration in real time and transmitted data to a computer for observation and storage through the universal USB/RS-485/422 converter UT-890. A rotor flow meter in the ozone generator was used to adjust the O_2 flow, and thereby control the concentration of O_3 in the chamber.

Malondialdehyde content analysis

Lipid peroxidation was determined by calculating the rate of malondialdehyde (MDA), according to Heath and Packer (1968) who used the 2-thiobarbituric acid (TBA) as substrate. The absorbance was measured at 532 nm using a UV/VIS spectrophotometer (UV-2450, Shimadzu, Japan; the same as below). The value for nonspecific absorbance at 600 nm was subtracted.

Soluble protein content analysis

A total of 0.5 g of frozen leaves was used to determine protein content according to the Bradford method (Kruger 1994). The Bradford assay relies on the binding of the dye Coomassie Blue G250 to protein.

Antioxidant enzyme activity assays

Catalase (CAT) activity was spectrophotometrically measured by analyzing the decomposition of hydrogen peroxide (H_2O_2) at 240 nm for at least 3 min (Aeobi 1974). Assays of peroxidase (POD) activity were carried out using guaiacol as the hydrogen donor (Putter 1978). Superoxide dismutase (SOD) activity was determined by measuring nitroblue tetrazolium (NBT) reduction by the superoxide anion (O_2^-) according to Beauchamp and Fridovich (1971).

Determination of glutathione and ascorbic acid content

Glutathione (GSH) content was measured by an enzymatic cycling assay method as described by Griffith (1980). A simple and sensitive procedure for the spectrophotometric determination of AsA was used as described by Besada (1987), which involved the formation of ferroin.

Air pollution tolerance index

APTI is an index used to quantify the tolerance of plants to air pollutants (Pandey et al. 2016), (Singh and Rao 1983). It is mainly based on four parameters, namely AsA, leaf extract pH, total chlorophyll (TCH), and relative water content (RWC). The AsA content of leaf samples was determined using the above method. TCH content was determined following the spectrophotometric method of Arnon (1949), and leaf extract pH was recorded with a glass electrode pH meter (SX-620, Sanxin, China) according to Pandey et al. (2015a, b). The RWC percentage was calculated using the fresh weight, turgid weight, and dry weight of leaf samples according to Sen and Bhandari (1978). Finally, APTI was calculated by the following mathematical expression (Singh and Rao 1983):

$$\mathrm{APTI} = [A(T+P)+R]/10$$

where A is the AsA content in mg/g, T is the TCH in mg/g, P is the leaf extract pH, and R is the RWC in percentage.

Gradation and classification of APTI

Plant species can be divided into four different tolerance groups (Liu et al. 1983; Zhang et al. 2016) by comparing the APTI value of each target species with the mean APTI value of all studied species together with its standard deviation (SD). Accordingly, a species can be classified as tolerant (T) if its APTI is higher than the mean APTI plus SD, as moderately tolerant (MT) if its APTI value is between the mean APTI and mean APTI plus SD, as intermediate (I) if its APTI value is between the mean APTI minus SD and mean APTI, and as sensitive (S) if its APTI value is lower than the mean APTI minus SD.

Data analysis

Each experiment was repeated at least three times. Values are expressed as means ± standard error (SE). The data were statistically analyzed using SPSS v. 22.0 and comparisons were performed using a one-way analysis of variance (ANOVA) together with Duncan's test for independent samples. In all cases, the confidence coefficient was set at $P < 0.05$.

Results

MJ and MT effects on MDA content in *Malus crabapple* 'Hong Jiu' under O_3 stress

One of the detrimental effects of ozone stress on plants is O_3-induced oxidative damage to cell membranes. MDA levels were quantified in the leaf tissue of *Malus crabapple* 'Hong Jiu' to investigate the level of lipid peroxidation and oxidative damage in response to O_3 stress and whether MJ and MT maintained cell membrane stability under O_3 stress. MDA were substantially increased in O_3-treated plants compared with those in control plants, while MJ and MT pretreatment significantly reduced the content of MDA in *Malus crabapple* 'Hong Jiu' under O_3 stress (Fig. 2). As shown in Fig. 2a, O_3 significantly increased MDA content in leaves by 133.89% for *Malus crabapple* 'Hong Jiu,' compared with the control. Addition of 100 or 150 μM MJ resulted in lower MDA content than 0 μM MJ, with decreases of 55.03% and 48.49%, respectively. The lowest MDA content was observed under 2.5 μM MT, with a decrease of 38.23% (Fig. 2b) compared with 0 μM MT. These results suggest that MJ and MT reduces oxidative stress and maintains cell membrane integrity.

MJ and MT effects on soluble protein content in *Malus crabapple* 'Hong Jiu under O_3 stress

Overall, the accumulation of soluble protein was concomitant with increased MJ and MT concentrations in stressed *Malus crabapple* 'Hong Jiu' plants compared with control plants (Fig. 3). Specifically, when the concentrations of MJ and MT were increased to 150 μM and 2.5 μM, respectively, the soluble protein content was significantly enhanced ($P < 0.05$) and was 97.83% and 35.63% higher, respectively, than in 0 μM, indicating that tolerance to O_3 stress was increased.

MJ and MT effects on antioxidant enzyme activities in *Malus crabapple* 'Hong Jiu' under O_3 stress

To further study the roles of MJ and MT in the possible alleviation of O_3-induced oxidative stress, we examined the activities of three key antioxidant enzymes in *Malus crabapple* 'Hong Jiu' leaves. Under O_3 stress, the activities of antioxidant enzymes were significantly induced compared with non-stressed plants. Application of exogenous MJ and MT further enhanced enzyme activities accumulation in *Malus crabapple* 'Hong Jiu' with O_3 treatment. Compared with CK, the activities of CAT, POD, and SOD under 0 μM MJ with O_3 treatment were significantly increased by 85.00%, 50.00%, and 50.60%, respectively. Compared with 0 μM MJ, the activities of CAT, POD, and SOD under 150 μM were significantly increased by 51.35%, 70.83%, and 38.88%, respectively ($P < 0.05$; Fig. 4a–c). Among the MT treatments, the activities of CAT, POD, and SOD were significantly increased by 90.43%, 95.65%, and 28.87% under 2.5 μM MT compared with 0 μM ($P < 0.05$; Fig. 4d–f). This result indicated that MJ and MT alleviated O_3 toxicity by increasing antioxidant enzyme activities in plant tissues.

MJ and MT effects on GSH and AsA contents in *Malus crabapple* 'Hong Jiu' under O_3 stress

Figure 5 illustrates a significant stimulation of GSH and AsA contents following O_3 treatment in MJ- and MT-pretreated plants. O_3 significantly increased GSH content in leaves by 38.60% for *Malus crabapple* 'Hong Jiu' compared with the control, and the GSH content was highest under 150 μM MJ and was significantly increased by 94.12% compared with 0 μM, while the AsA content under 10–150 μM MJ was significantly increased by 16.54–35.20% compared with 0 μM (Fig. 5a, b; $P < 0.05$). The maximum increase in GSH content was observed under 2.5 μM MT (64.89% higher than 0 μM). Similar results were also observed for AsA content, with an increase of 12.10% (Fig. 5c, d; $P < 0.05$).

APTI of stressed *Malus crabapple* 'Hong Jiu' plants under different MJ and MT concentrations

The measured values of the four biochemical parameters and the calculated APTIs for non-stressed and stressed *Malus crabapple* 'Hong Jiu' plants with different MJ and MT concentrations are shown in Table 1. Among the four parameters of APTI, AsA content was found to be highest under 150 μM MJ (2.19 ± 0.03) and 2.5 μM MT (1.78 ± 0.02). Furthermore, the activity of AsA is pH-controlled and thus the AsA content of plants is generally greater at higher pH and smaller at lower pH (Pandey et al. 2015a, b). Therefore, the highest pH levels were recorded in the same MJ and MT treatments. O_3 significantly decreased chlorophyll contents and RWC in leaves for *Malus crabapple* 'Hong Jiu,' compared with plants without the O_3. In stressed plants with PGRs treatments, the highest chlorophyll contents were found under 150 μM MJ (1.50 ± 0.07) and 2.5 μM MT (1.52 ± 0.06). RWC (in percentage) was also highest under 150 μM MJ (82.79 ± 3.03) and 2.5 μM MT (85.76 ± 1.42). Considering these measurements, for the MJ treatments, the APTI values ranged from 7.88 ± 0.41 to 10.06 ± 0.30 (Table 1A). The highest APTI value occurred under 150 μM MJ, while 10 μM MJ showed the lowest value. The MJ treatments with APTI values above the mean (9.23) were (listed from high to low) 150, CK, 100, 0and 50 μM. An overall mean APTI value of 9.40 was obtained for the MT treatments containing CK, with the highest value of 10.01 ± 0.14 at 2.5 μM MT and the lowest value of 8.89 ± 0.08 at 0 μM MT (Table 1B). Two MT treatments showed values above the mean, CK, 2.5 and 12.5 μM, while the other treatments showed lower values than the mean.

Gradation of APTI and tolerance assessment

Table 1 shows that the MJ and MT treatments induced varying degrees of tolerance to O_3 stress. The mean and SD of APTI for the MJ treatments were 9.23 and 0.73, while those for the MT treatments were 9.40 and 0.41. Therefore, plants under 10 μM MJ and 0 μM MT were classified as sensitive (S), while those under 0.1 and 0.5 μM MT were classified as intermediate (I). The control plants and those under 0, 50, and 100 μM MJ were classified as moderately tolerant (MT), as were plants under a MT concentration of 12.5 μM. Plants under 150 μM MJ and 2.5 μM MT were classified as tolerant (T). Usually, when plants are classified as T, they can be considered tolerant to O_3 stress.

Discussion

Among the various pollutants present in nature, O_3 is one of the major causative factors in free radical formation in plants, limiting plant growth (Krishnaveni 2013; Gao et al. 2017a). At present, the ground-level O_3 concentration exceeds the damage threshold (40 nL/L) of sensitive plants in many parts of the world and causes visible damage to them (Feng et al. 2014; Jia 2016). O_3 can enter plant tissues through the stomata and induce oxidative stress damage by causing the formation of ROS. ROS can react with lipids and proteins, causing membrane damage and enzyme inactivation, and eventually decrease plant growth and biomass (Mittler 2002).

Thus, it is important to seek an effective way of enhancing plant tolerance to O_3-induced oxidative damage. MJ has been shown to alter plant metabolism (Sun et al. 2017; Hou et al. 2017). Moreover, MJ has been recently demonstrated to protect plants against diverse abiotic stresses, such as cold, heat, drought, salt, and heavy metal toxicity (Ji et al. 2009; Meng et al. 2009; Yang et al. 2015). In addition, many studies have shown that MT can not only regulate plant growth, but also improve the resistance of plants to drought, salt damage, heavy metals, UV radiation, high temperature, chilling injury, and other stress (Wang et al. 2016a, b; Wu and Jia 2017; Gong and Shi 2017). In the present study, we investigated the physiological and metabolic effects of MJ and MT on *Malus crabapple* 'Hong Jiu' under O_3 stress.

Cell membranes are susceptible to O_3-induced oxidative stress. MDA content is generally considered a reliable indicator of cellular damage (Zhou et al. 2017). Dramatic increases in MDA content was found in *Malus crabapple* 'Hong Jiu' under O_3 stress, suggesting severe lipid peroxidation and plasma membrane injury. Treatment with MJ and MT at appropriate concentrations, such as 150 μM MJ and 2.5 μM MT, could partially reverse the deleterious effects brought about by O_3 stress, as shown by lower MDA and higher protein contents (Figs. 2 and 3). Yang et al. (2011) also observed that MJ application decreased the MDA content in *Phalaenopsis* seedlings under high temperature stress. MT treatment was observed to have the same effect on MDA content by Ding et al. (2017). Accumulation of ROS affects proteins. The functionality of proteins can be affected by ROS through oxidation of amino acid side chains or by secondary reactions with aldehydic products of lipid peroxidation. Both primary and secondary reactions can introduce carbonyl groups into proteins, and the appearance of such groups is taken as evidence of oxidative stress (Gonçalves et al. 2007; Ramakrishna and Rao 2012). In our study, supplementation with MJ and MT completely reversed the damage trend and increased the protein content in O_3-stressed seedlings, implying that cellular proteins were protected from ROS-mediated oxidative damage. Consistent with this, Zhao and Dai (2012) reported that the protein content can reflect the resistance of a plant. The increase in protein content we observed might indicate greater tolerance to stress as proposed by Meng et al. (2016) and Zhu et al. (2017) in plant leaves. Therefore, these are important indicators to assess O_3 stress damage. The lowest MDA content and the highest soluble protein content in *Malus crabapple* 'Hong Jiu were observed in plants treated with 150 μM MJ and 2.5 μM MT, indicating that these are the optimal concentrations for treatment with MJ and MT in our experiments.

To scavenge ROS and counter oxidative stress, plants have evolved an efficient antioxidant defense system. O_3 (100 ± 10 nL/L for 3 h) induced the activities of antioxidant enzymes and the accumulation of non-enzymatic antioxidants (Figs. 4 and 5). The results correspond with Alscher et al. (2002) and Wu et al. (2011). However, Zheng et al. (2005) reported that long-term or high-intensity ozone stress destroys plant antioxidant defense systems. Previous studies have established that MJ and MT promote the activities of antioxidant enzymes and the accumulation of non-enzymatic antioxidants to counteract the harmful effects caused by various environmental stresses (Wang 1999; Fan et al. 2015; Ding et al. 2017). SOD constitutes the first line of defense against ROS in plants, catalyzing the detoxification of O_2^- to H_2O_2 and O_2 (Hu et al. 2016). In the present study, a significant increase in SOD activity was observed in O_3-stressed seedlings with foliar application of MJ and MT, suggesting SOD has an important role in removing O_2^- induced by oxidative stress (Fig. 4c, f). CAT and POD further break down H_2O_2 to H_2O and O_2. Consistent with this, the MJ and MT treatments dramatically stimulated the activities of CAT and POD (Fig. 4a, b, d, e), implying MJ and MT have a protective effect through efficient scavenging of ROS. These results agree well with Ye (2015), who reported that MJ increased the CAT, POD, and SOD activities under high temperature stress in *Actinidia deliciosa*. Similarly, Jiang et al. (2016) reported that MT enhanced the levels of antioxidant enzymes under stress in plant seedlings. Additionally, non-enzymatic antioxidants, GSH and AsA, were substantially increased in O_3-stressed *Malus crabapple* 'Hong Jiu' with application of MJ and MT (Fig. 5). Increased contents of GSH and AsA have been reported to increase tolerance to stress (Nagalakshmi and Prasad 2001; Mahalingam et al. 2006). Our results further support roles for MJ and MT in improving the antioxidant capacity of plants under stress conditions.

To evaluate the susceptibility of *Malus crabapple* 'Hong Jiu' plants under MJ and MT treatments to O_3 stress, four parameters, namely AsA, TCH, RWC, and pH, were measured and used to calculate the APTI of each treatment. Plants with a high index value are tolerant to air pollutants and vice versa (Singh et al. 1991). The present study showed that plants under the 150 μM MJ and 2.5 μM MT treatments were tolerant to O_3 stress, with the highest APTI values (Table 1). The tolerance of plants to air pollutants varies with these parameters (Ogunkunle et al. 2015; Pandey et al. 2015a, b). AsA is an antioxidant involved in the defense against ROS and thus affects the resistance to air pollution in plants (Pathak et al. 2011). An increased level of AsA in leaves has been reported to increase air pollution tolerance in plants (Mittler 2002; Suganthi. et al. 2013). Furthermore, higher leaf extract pH levels indicate greater tolerance to air pollution (Singh and Verma 2007; Pandey et al. 2015a, b). Similarly, higher TCH content might increase tolerance to air pollutants in plants (Rai and Panda 2014; Fan et al. 2015). The RWC of a leaf is associated with protoplasmic permeability; thus, plants with higher RWC values are probably more tolerant to air pollutants (Singh et al. 1991). High RWC within the plant leaf helps to maintain physiological balance under stress conditions such as exposure to air pollution when transpiration rates usually remain high.

On the basis of APTI, the tolerance levels of plants under different MJ and MT treatments were classified into four groups including tolerant (T), moderate tolerant (MT), intermediate (I), and sensitive (S), using the formula described by Liu et al. (1983). Our data show that plants under 150 μM MJ and 2.5 μM MT were classified as T, suggesting they were tolerant to O_3 stress (Table 1). These results are consistent with previous indexes and APTI values. The susceptibility levels of *Malus crabapple* 'Hong Jiu' plants under MJ and MT treatments to O_3 stress, as indicated by their index values, compared well with the physiological responses. Thus, APTI determination provides a reliable method for screening sensitive or tolerant plants under stress.

Conclusion

In conclusion, O_3 (100 ± 10 nL/L for 3 h) induced oxidative stress damage in *Malus crabapple* 'Hong Jiu' causing membrane damage, inducing the activities of antioxidant enzymes and the accumulation of non-enzymatic antioxidants. Treatment with MJ and MT at appropriate concentrations can improve the tolerance of *Malus crabapple* 'Hong Jiu' to O_3 stress. The optimal concentrations were 150 μM for MJ treatment and 2.5 μM for MT treatment. Exogenous MJ and MT had a protective effect on lipid peroxidation, protein oxidation, membrane integrity, and the antioxidant defense system in *Malus crabapple* 'Hong Jiu' and thus significantly alleviated O_3-induced oxidative stress damage. Moreover, 150 μM MJ had a greater effect than 2.5 μM MT on tolerance to O_3 stress in terms of soluble protein content, SOD activity, accumulation of non-enzymatic antioxidants, and APTI value. The focus of future research should be to elucidate the mechanism by which MJ and MT confer tolerance to O_3 stress at the cellular and molecular levels.

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