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Mitigation of Chilling Stress Effects on Eggplant Seedlings by Exogenous Application of Melatonin

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ABSTRACT

The purpose of this study was to explore the possibilities of improving chilling stress tolerance of eggplant seedlings through exogenous melatonin (MEL) application. Eggplant (Hadrian F_1) seedlings were treated with various concentrations (0, 1, 5 or 25 µM) of MEL via soil drench after which they were subjected to chilling stress at 5°C/10°C (night/day) for 3 days. Following stress imposition, the efficacy of MEL applications on enhancing chilling stress tolerance was determined by several physical and physiological measurements and biochemical analyses. The results demonstrated that exogenous application of MEL alleviated the adverse effects of chilling stress in eggplant seedlings. Among the MEL concentrations tested, 5 µM was determined as the most effective concentration since antioxidant enzyme (CAT, POX and APOX) and photosynthetic activities increased while visual and membrane damage decreased in 5 µM MEL-treated seedlings. Also, these results are the first experimental evidence that exogenous application of MEL could improve chilling stress tolerance in eggplant, but further detailed studies are necessary to better understand the mechanism in acquiring chilling tolerance.

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Introduction

Melatonin (MEL, N-acetyl-5-methoxytryptamine), an indolamine that has been identified to be ubiquitously present in almost all living organisms, was first isolated in 1958 from the bovine pineal gland (Lerner et al., 1958). Even though some preliminary results had been documented previously (Van Tassel et al., 1993), two different groups of researchers (Dubbels et al., 1995; Hattori et al., 1995) reported the first solid evidence that MEL was present in higher plants. Following its discovery in plants, MEL has been found in varying amounts in seeds, fruits, leaves and roots of many plant species such as medicinal herbs, vegetables, fruits and cereals (Debnath et al., 2019). Although it was documented that tissue MEL content varies not only from species to species but also among the varieties within a species (Paredes et al., 2009), the generative organs and seeds generally have the highest MEL content among the plant organs (Arnao, 2014; Nawaz et al., 2016).

MEL in plants is synthesized from the aromatic amino acid-tryptophan in four successive steps each one of which is catalyzed by different enzymes (Back et al., 2016). The same pre-cursor also produces one of the most common auxins in plants, indole-3-acetic acid, via a slightly different pathway and therefore MEL exhibits auxin-like functions (Fan et al., 2018). MEL is known to function as a circadian and photoperiodic rhythm regulator in animals as well as in plants (Kolár and Macháčková, 2005; Reiter et al., 2015) and plays a vital role in the perception of time during the day and within the year (Cardinali and Pevet, 1998). In addition to being a daily and seasonal rhythm regulator in plants, the role of MEL as a potent antioxidant in plants has been proved by several studies (Debnath et al., 2019; Korkmaz et al., 2017). MEL as a strong antioxidant in plants can regulate and promote the activities of antioxidant enzymes such as peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) (Yakupoğlu et al., 2018; Arnao and Hernández-Ruiz 2019a). It also plays as a direct antioxidant role in balancing biological membranes (mitochondria,

chloroplast and plasma), scavenging reactive oxygen species (ROS) and lipid peroxides (Catala 2007; Garcia et al., 2014) and reducing the electron leakage from the inner mitochondrial membrane by reducing the formation of ROS (Reiter et al., 2001).

Plants with higher endogenous MEL content are reported to better adapt to unfavorable environmental conditions and exposure to stressful conditions causes significant increase in MEL content of plant tissues (Fan et al., 2018; Sharif et al., 2018). In recent years, number of studies involving exogenous MEL application to enhance plant's tolerance to abiotic stress conditions have increased dramatically. For example, MEL application was found to promote the germination performance of cucumber seeds at low temperature (15°C) (Posmyk et al., 2009). Germination of pepper seeds treated with various concentrations of MEL was significantly increased under chilling stress (Korkmaz et al., 2017). Similarly, protective roles of MEL as an antioxidant in plants exposed to such abiotic stresses as high salinity (Li et al., 2012), heavy metals (Posmyk et al., 2008), drought (Cui et al., 2017), and high temperature (Shi et al., 2015) were also reported.

Eggplant is the most sensitive vegetable species to low temperatures in *Solonacea* family and for optimal plant growth, average temperatures of 24-29°C during the day and 18 to 24°C at night are preferred (Peirce, 1987). The chilling temperatures (those below 15°C but above 0°C) that frequently occur in unheated greenhouse during fall and winter production can substantially hinder the eggplant growth and reduce its yield (Adamczewska-Sowińska et al., 2016). Based on available data and observations, it is evident that exogenous MEL applications have potential to enhance the stress tolerance of plants in agricultural production. However, there is no information available on the involvement of MEL in boosting the tolerance of eggplant seedlings under chilling stress. Therefore, this research was carried out to determine the effect exogenously applied MEL in various concentrations on tolerance of eggplant seedlings exposed to chilling stress conditions.

Materials and Methods

Plant Material, Chemicals and MEL Treatments

The seeds of Hadrian F_1 eggplant cultivar, provided from Antalya Tarım Seed Company (Antalya, Turkey), was used as material in this research. This cultivar which is used in single crop cultivation in greenhouse has 25-27 cm long fruits weighing approximately 220-250 grams. The seeds were planted into flat cells (75 cm^3) containing a mixture of perlite and peat in the ratio of 1:3. The flats were placed in a growth chamber at 25/20°C (day/night) under cool fluorescent lamps providing approximately 225 µmol $m^{-2}s^{-1}$ light for 16 h day^{-1}. When seedlings reached 4 fully developed leaf stages, MEL at various concentrations (0, 1, 5 and 25 µM) was applied as soil drench. These melatonin concentrations were selected after conducting preliminary experiments using a wider range of melatonin concentrations. One day after the MEL application, half of the plants were exposed to chilling stress (5/10°C, night/day, 12/12 h, night/day, %65-75 RH) for 3 days while the other half continued to grow under optimum conditions (25/20°C, night/day, 12/12 h, night/day, 65-75% RH) in growth chamber. MEL and all other chemicals used in this study were products of Sigma-Aldrich Chemicals (St. Louis, MO, USA).

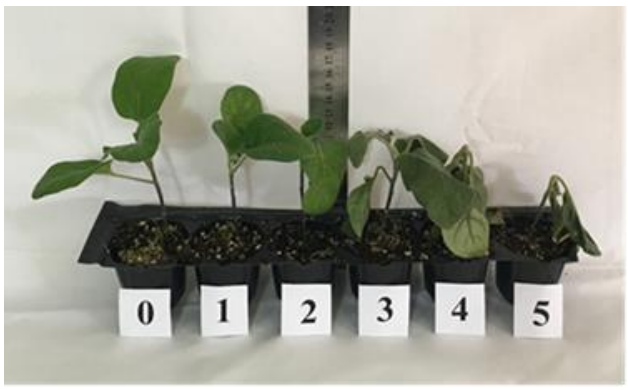

Figure 1. Visual assessment of the effects of chilling stress.

Measurements and Data Collection

At the end of stress period, the extent of chilling stress injury was determined by visually examing the plants and using the slightly modified scale reported by Korkmaz (2002) as shown in Figure 1: 0: No necrotic damage, 1: Slight damage, 2: Moderate damage, 3: Severe damage, 4: extensive damage and 5: Plant dead.

A partly modified version of the method reported by Korkmaz et al. (2014) was used to determine MEL content in plant tissues. Samples were analyzed using Prominance UFLC HPLC system (Shimadzu, Kyoto, Japan) equipped with RF-20A fluorescence detector utilizing an Inertsil ODS-2 column (GL Sciences150 × 4.6 mm, 5 µm). All seedlings were cut at the growth medium level and their shoot and root fresh weights were determined. Leaf areas of the plants were determined with the aid of the LiCor LI-3000 series leaf area meter. Photosynthetic parameters (net photosynthetic rate-A, stomatal conductance-*gs*, and transpiration-E) were measured with portable photosynthesis meter (Walz, GFS3000). During the measurements, the light intensity was 225 µmol m^{-2} s^{-1} and leaf temperature was maintained at 25°C, and chamber CO_2 concentration of 380 ppm was supplied by a CO_2 injector. Tissue malondialdehyde (MDA) content, indicative of the oxidative damage caused by chilling stress, was determined by following the method of Zhang et al. (2005).

Electrolyte leakage (EC_1/EC_2), a measure of membrane permeability, was determined according to Campos et al. (2003) with slight modifications. Leaf chlorophyll content (expressed as chlorophyll a + chlorophyll b) and relative water content (RWC) were determined by using the methods reported in Lichtenthaler (1987) and Korkmaz et al. (2010), respectively. H_2O_2 content in plant tissues was determined according to the method described in Özden et al. (2009). Extraction for antioxidant enzymes was performed as described in Seçkin et al. (2010). The activities of CAT (E.C.1.11.1.6), POX (E.C. 1.11.1.7) and APOX (EC 1.11.1.11) were measured according to the methods described in Güneş et al. (2007), Dolatabadian et al. (2008) and Nakano and Asada (1981), respectively.

Statistical Analysis

The experimental design was randomized complete block design with four replications each having 12 plants. Entire experiment was repeated twice and the data obtained in both repetitions were combined for statistical analysis (n=8). Analysis of variance (ANOVA) was performed using the SAS v.8.2 statistical software program (2001) and LSD (minimum significant difference) test was used to determine the differences among the treatments.

Results

The MEL levels determined in leaves and roots of the seedlings are presented in Table 1. MEL levels in both leaves and roots increased significantly with exogenous MEL treatment applied as soil drench and the highest MEL levels (5.5-7.0 ng g^{-1} FW in leaves and 7.5-11.7 ng g^{-1} FW in roots) were determined in seedlings treated with 5 µM and 25 µM MEL while the untreated seedlings (0 µM MEL) had the lowest levels of MEL (3.2-3.5 ng g^{-1} FW in leaves and 4.9-5.6 ng g^{-1} FW in roots).

Visual damage index and leaf area of plants subjected to chilling stress and optimum conditions are presented in Table 2. Obviously, no visible visual damage was found in the seedlings grown under optimum conditions. However, chilling stress caused substantial damage on eggplant seedlings and untreated plants (0 µM MEL) developed characteristic chilling injury symptoms in moderate to severe levels while those treated with 5 µM and 25 µM had only slight injury symptoms. MEL application enhanced the leaf growth of the seedlings under both optimum and chilling stress conditions; however, the increase caused by MEL applications was not statistically significant.

Chilling stress significantly affected plant growth as indicated by reduced seedling shoot and root fresh weight (Table 3). Even though MEL application had an insignificant effect on shoot fresh weight under optimum conditions, shoot fresh weight under stress conditions was increased 30% by 25 µM MEL application compared to non-MEL-treated plants. Additionally, MEL application enhanced significantly the chlorophyll content of seedlings grown under optimum conditions and seedlings treated with 5 µM MEL had the highest chlorophyll content. Under chilling stress conditions, however, the increase in chlorophyll content caused by MEL application was not statistically significant.

The effects of MEL applications on membrane permeability (EC_1/EC_2) and RWC values of seedlings grown under optimum and chilling stress conditions are given in Table 4. The highest membrane damage indicated by higher permeability values (42.6-48.4%) were obtained from seedlings not treated with MEL and seedlings treated with 5 µM MEL under chilling conditions and 1 µM and 25 µM MEL applications (37.1-39.0%) significantly reduced membrane damage. Similarly, MEL pre-treatment of seedlings also improved the RWC of seedlings especially under chilling stress conditions and seedlings treated with 5 µM and 25 µM MEL exhibited significantly higher RWC values (89.2-89.4%) compared to those not treated with MEL (77.2%).

Table 1. Melatonin (MEL) content in the leaves and roots of eggplant seedlings

MEL (µM)	Chilling stress	Leaf MEL (ng g^{-1} FW)	Root MEL (ng g^{-1} FW)
0	-	3.2 ± 0.3^{c}	5.6 ± 0.4^{cd}
1	-	4.6 ± 0.4^{bc}	6.6 ± 1^{bcd}
5	-	6.3 ± 0.6^{a}	$7.5\pm1.5^{a-d}$
25	-	7.0 ± 1^{a}	$9.4\pm0.7^{a-d}$
0	+	3.5 ± 0.4^{c}	4.9 ± 0.7^{d}
1	+	4.16 ± 0.3^{bc}	$9.8\pm2.9^{a-c}$
5	+	5.5 ± 0.7^{ab}	11.7 ± 2.8^{a}
25	+	6.8 ± 0.4^{a}	11.0 ± 1.8^{ab}
$LSD_{0.05}$		1.6	4.9

Table 2. The effects of melatonin (MEL) application on visual damage index and seedling leaf area

MEL (µM)	Chilling stress	Visual damage index (0-5)	Leaf area (cm^2)
0	-	0 ± 0^{d}	$63.6\pm11.3^{a-c}$
1	-	0 ± 0^{d}	78.2 ± 7.9^{a}
5	-	0 ± 0^{d}	81.3 ± 6.7^{a}
25	-	0 ± 0^{d}	72.6 ± 11.9^{ab}
0	+	2.99 ± 0.24^{a}	42.6 ± 8.3^{c}
1	+	2.17 ± 0.08^{b}	48.8 ± 7.1^{bc}
5	+	1.33 ± 0.17^{c}	$55.7\pm10.3^{a-c}$
25	+	1.50 ± 0.25^{c}	$56.7\pm7.7^{a-c}$
$LSD_{0.05}$		0.39	25.86

Table 3. The effects of melatonin (MEL) application seedling shoot and root fresh weight and chlorophyll content

MEL (µM)	Chilling stress	Shoot fresh weight (mg $plant^{-1}$)	Root fresh weight (mg $plant^{-1}$)	Chlorophyll content (mg g^{-1} FW)
0	-	2120 ± 210^{ab}	322 ± 21^{a}	41.7 ± 3.4^{b}
1	-	2320 ± 150^{ab}	323 ± 61^{a}	45.4 ± 1.3^{ab}
5	-	2550 ± 180^{a}	331 ± 23^{a}	49.0 ± 1.0^{a}
25	-	2220 ± 250^{ab}	300 ± 20^{a}	45.2 ± 3.2^{ab}
0	+	1570 ± 190^{d}	223 ± 31^{b}	44.3 ± 2.6^{ab}
1	+	1810 ± 120^{bcd}	262 ± 34^{ab}	45.6 ± 1.7^{ab}
5	+	1960 ± 170^{bcd}	291 ± 23^{ab}	47.7 ± 2.5^{ab}
25	+	2050 ± 210^{bc}	290 ± 21^{ab}	48.6 ± 1.1^{a}
$LSD_{0.05}$		408	7	6.5

Table 4. The effects of melatonin (MEL) application on electrolyte leakage (EC_1/EC_2) and relative water content (RWC) of eggplant seedlings

MEL (µM)	Chilling stress	EC_1/EC_2 (%)	RWC (%)
0	-	38.0±3.0^{b}	87.2±3.3^{ab}
1	-	35.1±3.5^{b}	91.4±2.3^{a}
5	-	38.3±4.4^{b}	91.3±1.4^{a}
25	-	34.4±3.3^{b}	90.8±1.8^{a}
0	+	48.4±4.0^{a}	77.2±2.6^{c}
1	+	37.1±3.5^{b}	82.5±2.0^{bc}
5	+	42.6±3.7^{ab}	89.4±2.1^{a}
25	+	39.0±3.6^{b}	89.2±2.5^{a}
$LSD_{0.05}$		9.1	6.6

Table 5. Effects of melatonin (MEL) applications leaf MDA, proline and H_2O_2 contents

MEL (µM)	Chilling stress	MDA (nmol g^{-1} FW)	Proline (nmol g^{-1} FW)	H_2O_2 (nmol g^{-1} FW)
0	-	210±52	293±04^{d}	370±7
1	-	180±33	296±05^{d}	322±11
5	-	150±35	300±05^{cd}	381±6
25	-	150±40	297±5^{cd}	410±10
0	+	220±41	304±5^{b-d}	523±10
1	+	230±33	317±10^{a-c}	390±8
5	+	230±32	331±11^{a}	334±7
25	+	240±40	324±8^{ab}	421±12
$LSD_{0.05}$		-	22	-

Table 6. The effects of melatonin (MEL) applications on the net photosynthetic rate (A), transpiration (E) and stomatal conductivity (*gs*) of eggplant seedlings

MEL (µM)	Chilling stress	A (µmol m^{-2} s^{-1})	E (mmol $m^{-2}s^{-1}$)	(gs) mmol m^{-2} s^{-1}
0	-	5.69±1.09^{a}	0.87±0.23^{ab}	53.3±13.6^{ab}
1	-	5.31±0.93^{ab}	0.85±0.18^{ab}	51.1±11.5^{ab}
5	-	3.88±0.46^{bc}	0.89±0.22^{ab}	54.6±14.0^{a}
25	-	4.01±0.30^{bc}	0.95±0.22^{a}	58.1±13.7^{a}
0	+	2.40±0.37^{cd}	0.42±0.10^{bc}	25.2±5.8^{bc}
1	+	2.86±0.34^{b-d}	0.50±0.10^{a-c}	30.0±6.0^{a-c}
5	+	3.82±0.94^{bc}	0.54±0.13^{a-c}	32.8±7.6^{a-c}
25	+	2.21±0.23^{d}	0.34±0.06^{c}	20.0±3.7^{c}
$LSD_{0.05}$		1.59	0.47	29.20

When the effect of MEL applications on MDA and H_2O_2 contents of seedlings were examined, it was seen that MEL application had no significant effect on these variables (Table 5). Proline content, however, was increased significantly by MEL treatments under chilling stress conditions while, on the other hand, did not change under non-stress conditions. In the seedlings exposed to the chilling stress, the lowest proline content was measured in 0 µM MEL-treated plants (304 nmol g^{-1} FW) while 5 µM MEL-treated seedlings had significantly higher proline content (331 nmol g^{-1} FW).

Chilling stress suppressed all gas exchange parameters, resulting in significant reductions in A, *gs* and E (Table 6). Soil application of MEL decreased A considerably while slightly increasing E and *gs* of plants grown under optimum conditions. Under chilling stress conditions, highest A (3.82 µmol^{-2} s^{-1}), E (0.54 mmol m^{-2} s^{-1}), and gs (32.8 mmol m^{-2} s^{-1}) were measured in seedlings treated with 5 µM MEL. However, even though 5 µM MEL treatment increased A, E and *gs* of eggplant seedlings under chilling stress by 59%, 29% and 30%, respectively, compared to non-MEL-treated plants, these enhancements were not statistically significant.

The effects of MEL applications on antioxidant enzyme activities of seedlings grown in optimum and chilling stress conditions were also investigated and it was observed that chilling stress caused significant increases in the activities of these enzymes (Table 7). MEL applications boosted the activities of all enzymes in seedlings grown under optimum conditions and the highest CAT (696.5 U mg^{-1} protein), POX (39.5 U mg^{-1} protein) and APOX (14.7 U mg^{-1} protein) activities were measured in the seedlings treated with 5 µM MEL. However, increasing MEL concentration to 25 µM resulted in significant decreases in CAT and APOX activities. A similar effect was observed in the seedlings exposed to chilling stress and the highest CAT and POX activities were determined in seedlings treated with 5 µM MEL while 1 µM and 5 µM MEL applications resulted in the highest APOX activity.

Table 7. The effect of melatonin (MEL) applications on the activities of CAT, POX and APOX enzymes

MEL (µM)	Chilling stress	CAT (U mg^{-1} protein)	POX (U mg^{-1} protein)	APOX (U mg^{-1} protein)
0	-	181.1±9.8^{f}	31.7±1.7^{cd}	7.6±0.8^{e}
1	-	340.2±11.5^{e}	31.9±1.4^{cd}	10.0±0.7^{cd}
5	-	696.5±17.8^{b}	39.5±2.7^{ab}	14.7±0.7^{a}
25	-	344.0±8.0^{e}	35.9±2.7^{a-c}	9.3±0.1^{de}
0	+	291.8±13.7^{e}	31.3±1.8^{cd}	8.6±0.4^{de}
1	+	579.4±28.8^{c}	34.4±2.8^{bc}	12.7±1.0^{b}
5	+	844.2±53.5^{a}	42.0±4.3^{a}	11.7±0.4^{bc}
25	+	454.3±15.0^{d}	27.0±2.1^{d}	9.0±0.1^{de}
$LSD_{0.05}$		69.0	7.3	1.7

Discussion

MEL levels in eggplant leaves and roots increased significantly with exogenous MEL applied as soil drench and the highest MEL levels were determined in seedlings treated with 25 µM MEL while the untreated seedlings (0 µM MEL) had the lowest levels of MEL. These results show that MEL applied to the roots is taken up by the plants and transported within the plant. MEL levels in water hyacinth leaves increased significantly with exogenous application of 5 µM MEL compared to untreated leaves (Tan et al., 2007a). Additionally, exogenous MEL applied to barley leaves increased the amount of MEL in leaves depending on the concentrations (Arnao and Hernandez-Ruiz, 2008) while tissue MEL content of rice seedlings grown under cold stress increased significantly by MEL application via three different methods (seed soaking, foliar sprey and root immersing) (Han et al., 2017). No visible visual damage was found in the seedlings grown under optimum conditions. However, chilling stress caused damage at moderate to severe level on eggplant seedlings not treated with MEL (0 µM) while those treated with 5 µM and 25 µM were only slightly damaged. Generally, MEL application increased the tolerance of eggplant seedlings to chilling stress and plants showed no signs of visual damage except small necrotic areas and slight turgor loss at the leaf tips indicating that plants treated with MEL suffered less damage under chilling stress conditions. Tan et al. (2007b) reported that the survival rate of pea plants grown in soils polluted with heavy metals increased with MEL application. It has been reported that the application of exogenous MEL against salt stress in alfalfa (Cen et al., 2020) and cucumber (Zhang et al., 2020) promoted plant growth and increased tolerance to salt by increasing antioxidant enzyme activities.

Even though MEL application reduced the chilling-induced sypmtoms under chilling stress conditions, its effect on plant growth was not substantial. It was reported that soybean plants raised from the seeds treated with 50 and 100 µM MEL had higher leaf area and plant height compared to plants obtained from seeds not treated with MEL (Wei et al., 2015). *Arabidopsis thaliana* seedlings treated with 20 µM MEL exhibited 50% survival rate under high temperature stress (45°C for 120 min) while all control plants were dead (Shi et al., 2015). In another study where *Arabidopsis* plants were exposed to low temperature stress (4°C) for varying durations (72 to 120 hours), MEL application at different concentrations (10-30 µM) increased seedling fresh weight, root length and stem height (Bajwa et al., 2014).

MEL applications slightly increased the total chlorophyll content of seedlings under chilling stress and the highest chlorophyll content was measured in seedlings treated with 25 µM MEL. Chilling stress caused significant reductions in all photosynthetic parameters but MEL applications at lower concentrations slightly increased the net photosynthetic rate, stomatal conductivity and transpiration rate. MEL is known to protect seedlings against chlorophyll degradation and it delays senescence and boosts the photosynthetic capacity of plants grown under various stress conditions (Tan et al., 2012). For example, MEL applied as soil drench at 100 µM concentration has been reported to increase the photosynthetic activity in addition to delaying chlorophyll degradation and protecting against leaf aging in apple seedlings exposed to drought stress (Wang et al., 2013). In another study examining the effects of exogenously applied MEL in salt-stressed corn plants, it was found that photosynthesis increased by 19% by MEL application in plants exposed to stress (Jiang et al., 2016).

Osmoprotectants such as glycinebetaine, proline and sugars have significant roles in plant's tolerance to stressful conditions and they act in osmotic adjustment, stabilization of membranes and scavenging ROS in tissues (Dar et al., 2016). Proline content increased in response to chilling stress and it was further boosted by MEL applications reaching its highest level in seedlings treated with 5 µM MEL. These findings were consistent with those of Zhang et al. (2017), who reported that MEL pre-treatment of melon seedlings exposed to cold stress (12/6°C) for 7 days significantly enhanced tissue proline content compared to control plants. Moreover, measuring electrolyte leakage is widely used to estimate membrane permeability while determining the level of MDA which is by-product of lipid peroxidation is considered as a well-known indicator of oxidative stress caused by ROS such as O_2 and H_2O_2 (Zhang et al., 2014). In the current study, chilling stress resulted in the accumulation of MDA in parallel with the change of H_2O_2 level, indicative of oxidative damage caused by lipid peroxidation in eggplant seedlings. Treating the plants with MEL significantly reduced tissue electrolyte leakage and, though not statistically significant, lowered MDA and H_2O_2 accumulation in seedlings exposed to chilling stress. Similarly, it was reported that exogenous MEL application reduced the ROS burst, decreased electrolyte leakage and MDA accumulation in maize plants exposed to drought stress (Ahmad et al., 2019) and melon seedlings under cold stress (Zhang et al., 2017).

Plants have evolved effective protective mechanisms to cope with the damage due to lipid peroxidation in biological membranes. Such mechanisms might involve augmenting the activities of free radical and peroxide-scavenging antioxidant enzymes such as CAT, POX and SOD (Dey et al., 2007). As a result of MEL applications CAT, POX and APOX enzyme activities increased markedly in seedlings grown under both optimum and stress conditions. The highest enzyme activities were measured in seedlings treated with 1 µM and 5 µM MEL and increasing the MEL concentration further to 25 µM resulted in significant reductions in the activities of these enzymes. Besides being a hormone, MEL is known as endogenous free radical scavenger and potent broad-spectrum antioxidant via stimulating antioxidant enzymes thereby alleviating oxidative stress (Sharif et al., 2018). Numerous studies reported that exogenous application of MEL in various crops enhanced their stress tolerance by boosting their antioxidant capacity. For example, Korkmaz et al., (2017) reported that seed application of MEL in the range of 0-25 µM improved pepper seed germination and seedling emergence performance under chilling conditions by enhancing the activities of SOD, CAT and POX enzymes. Similarly, Ding et al. (2017) found that foliar application of MEL mitigated the adverse effects of cold stress in tomato plants by dramatically stimulating the activities of SOD, CAT, POX and APOX enzymes in addition to non-enzymatic antioxidants such as ascorbate and glutathione.

In summary, our results demonstrated that exogenous MEL alleviated the adverse effects of chilling stress in eggplant seedlings. Among the MEL concentrations tested, 5 µM was determined as the better concentration since antioxidant enzyme (CAT, POX and APOX) and photosynthetic activities increased while visual and membrane damage decreased in 5 µM MEL-treated seedlings through root appliaction. The optimum MEL concentration (5 µM) that boosted chilling stress tolerance seem to be lower than those reported by other studies. Numerous studies reported that concentrations above 25 µM even 50 µM or higher were effective in conferring stress tolerance (Arnao and Hernández-Ruiz, 2019b). However, it should be noted that most studies employed foliar application method which may affect or reduce the uptake of MEL by plant tissues. MEL has a protective role in the eggplant seedlings grown under chilling stress conditions and its optimum concentration may be helpful in elevating chilling stress tolerance in several crops. Also, our results offer the first experimental evidence that exogenous application of MEL could improve chilling stress tolerance in eggplant; however, the mechanism in acquiring chilling tolerance needs further investigating.

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