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Effects of melatonin applications on certain biochemical characteristics of strawberry seedlings in lime stress conditions

Gülden BALCI*

Department of Horticulture, Faculty of Agriculture, Yozgat Bozok University, Yozgat, Turkey

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Abstract: The aim of this study is to determine the effects of different doses of melatonin (MEL) applications (0, 5, 10 µM) on certain biochemical characteristics of strawberry seedlings grown in calcareous stress conditions. The experiment was conducted in the pots filled with lime-added mixture at the rate of 1% and peat perlite at the rate of 1:1 with frigo seedlings belonging to "Albion" strawberry cultivar. In order to examine the effects of MEL application against lime stress in different development stages; analyses of proline, total phenolic content, and lipid peroxidation were performed on leaf samples taken in 3 different periods. MEL applications in strawberry seedlings grown in a controlled and calcareous condition increased the proline amount while decreasing lipid peroxidation (MDA). As a result, it was detected that MEL applications relieve the effects of lime stress and the dose of 10 µM generally offers better results.

Key words: Lime, lipid peroxidation, MDA, melatonin, proline, strawberry, total phenolic

1. Introduction

Being a species in the family *Rosaceae* and the genus *Fragaria* and having an important place among the berry fruits, strawberry (*Fragaria* X *ananassa Duch.*) is grown in many parts of the world (Hancock, 1999). Strawberry, which is perennial, herbaceous, and evergreen, is among the fruits preferred by millions of people in the world with its flavor, vitamin, and mineral content. According to the data of 2019, the amount of strawberry grown in Turkey is 440,968 t and 8,337,099 t in the world[1] and it is very difficult to grow it in calcareous soil. The optimum soil pH level for strawberries is between 6.0 and 6.5 (Kepenek, 2002; Çakaryıldırım, 2004). When strawberry is grown in calcareous soils, certain problems such as yield losses, slowing in the growth of the plant, and chlorosis can be observed (Hancock, 1999).

Melatonin (MEL) is an indoleamine isolated first in 1958 from bovine pineal tissue (Lerner et al., 1958). MEL, which is secreted more in dark environments, functions in the perception of time in animals and humans in day and year. MEL hormone has gained importance in human nutrition in recent years with its antioxidant and anticarcinogen effects as well as organizing daily rhythm (Aguilera et al., 2015; Yakupoğlu et al., 2018;). It was revealed that MEL, believed to be found only in vertebrates for a long time, was also found in plants with two different studies conducted in 1995 (Dubbles et al., 1995; Hattori et al., 1995). It has been reported that MEL content in plants is higher in seeds and fruits. It is thought to result from MEL's function in the antioxidant defense mechanism (Korkmaz et al., 2018).

Maintaining plant growth and productivity is essential in the cultivation made under environmental stress conditions (Chernyshuk et al., 2020; Kaya et al., 2020). Therefore, some plant growth regulators are used for increasing the tolerance against stress conditions (Ercisli et al., 2003). Many studies reveal that plants increase MEL production under negative environmental conditions (Arnao and Hernandez-Ruiz, 2009; Tal et al., 2011; Arnao and Hernandez-Ruiz, 2013; Byeon and Back, 2014). There are many studies putting forward that MEL applied to plants externally relieves membrane fluidity and lipid peroxidation as an antioxidant (Catala, 2007; Garcia et al., 2014) and increases proline and total phenolic amount (Korkmaz et al., 2018; Arnao and Hernandez-Ruiz, 2015). As a result of the literature review, a study focusing on the effects of MEL applications on the storage duration in strawberry was found (Liu et al., 2018); however, no study was discovered regarding the MEL applications in strawberry growing.

[1] Food and Agriculture Organization of the United Nations (2020). FAOSTAT [online]. Website http://www.fao.org/faostat/en/#data/QC [Accessed 09 April 2020].

* Correspondence: gulden.balci@bozok.edu.tr

In light of these data, our experiment aims to determine the effects of different doses of MEL applications on some biochemical characteristics of strawberry seedlings grown under lime stress.

2. Material and methods

2.1. Materials

This study was carried out in the nonheated greenhouse on the field belonging to Yozgat Bozok University. In our experiment, "Albion" cultivar, one of the day-neutral strawberries, was used. Albion, which is very productive and high-quality, is successfully grown in areas with high altitude (Balcı et al., 2017).

Our experiment was carried out by filling the peat perlite mixture at the rate of 1:1 in 2-L pots (165 × 155 × 140 mm) (Sahin et al., 2002). Lime addition was not made to the pots in the %0 lime group, and %1 lime was added to the group to which lime stress was applied in terms of weight. In Table 1, the environment pH values belonging to the beginning and end of the experiment related to the groups are given. The pH values of the environment were determined according to Kacar (2012).

The frigo seedlings belonging to Albion strawberry cultivar were planted in the pots on 28.03.2018. The strawberry seedlings were once fertilized with "Nutritect 18-18-18 TE" commercial fertilizer (15.05.2018).

2.2. Method

2.2.1. Melatonin application

In our experiment, 3 different doses of MEL (0, 5, and 10 μM) were evaluated. The prepared solution was stored in a light-proof way and applied to the plant root as 150 mL per seedling. MEL was applied to the plants in the experiment for 2 times. The first application was given in the form of root zone immediately after planting (28.03.2018) and the second application was given on 22.06.2018.

2.2.2. Taking leaf samples

Leaf sampling was performed in three different periods with an aim to determine the MEL effect on lime stress in strawberry seedlings in different development stages. The first leaf sample was taken in the period when the strawberry seedlings had 4 leaves (25.04.2018), the second sample was taken in the blooming period 2 months after planting (25.06.2018), and the last sample was taken 3 months after planting in the fruit stage (26.07.2018). On these harvesting dates, the leaves reaching their maximum sizes were picked and placed in ice immediately and stored at –20 °C until the analyses were conducted.

2.2.3. Evaluated criteria

The amount of proline was calculated according to the method by Bates et al. (1973) and the results were given as μmol proline/g (fresh weight). The total phenolic amount was determined according to Singleton and Rossi (1965) by using the Folin–Ciocalteu colorimetric method and the results were given in gallic acid equivalent (mg/g). The lipid peroxidation was calculated according to Zhang et al., (2007) and the calculated results were given as μmol/g in fresh weight.

Table 1. The pH values of the environment belonging to the experimental groups.

	Initial pH values	Final pH values		
		0 MEL	5 MEL	10 MEL
%0 lime	7.74	7.68	7.77	7.78
%1 lime	8.41	7.95	8.01	7.90

2.2.4. Evaluation of the data

The experiment was set up as with three repeats (10 plants in each repeat), two applications (%0 and %1 lime application) and 3 doses (0, 5, 10 μM) according to the factorial experimental design in the randomized parcels. For calculating the averages of all data obtained during the research, "Microsoft Office XP EXCEL" was used and the statistical analyses were evaluated in SPSS 20.0 package program. As a result of the statistical analysis, Duncan's multiple range test (Duncan's multiple comparison test) was applied by using the same package program to determine the difference between the environments showing difference. The significance level between the differences in the statistical evaluation of the results was determined as 0.05.

3. Results and discussion

3.1. Total phenolic content

Throughout the experiment, it was observed that MEL applications had important effects on the total phenolic substance (Table 2). In the first sampling period, the highest total phenolic substance was obtained from 10 μM MEL application (11.19 mg GAE/g) in the %0 lime group while the lowest amount was obtained from 0 μM MEL application in the %0 lime group (0.68 mg/g). In the second sampling period, the highest total phenolic substance content was determined in the plants to which 10 μM was applied in the %0 lime group with 13.78 mg/g while the lowest amount was found in the plants to which 5 μM MEL was applied in the %0 lime group with 9.08 mg/g. In the last sampling, the highest content was found in the plants (17.51 mg GAE/g) to which 10 μM was applied in the lime group while the lowest content was detected in the plants (7.67 mg/g) to which 10 μM was applied in the lime group.

Plants promote phenylpropanoid biosynthesis through the PAL enzyme under biotic and abiotic stress conditions; thus, many secondary metabolites are synthesized.

Table 2. The effects of MEL application on the total phenolic substance amount of strawberry plants under lime stress conditions.

Applications	%0 lime	%1 lime
Beginning of growth		
0 MEL	0.677e	0.677e
5 MEL	0.949d	0.949d
10MEL	11.193a	11.193a
Flowering		
0 MEL	11.765b	11.765b
5 MEL	9.082d	9.082d
10MEL	13.784a	13.784a
Harvesting		
0 MEL	8.618cd	8.618cd
5 MEL	12.412bc	12.412bc
10MEL	14.116ab	14.116ab

Mean followed by different letters within columns differ significantly ($p < 0.05$).

Table 3. The effects of MEL application on the proline amount of strawberry plants under lime stress conditions.

Applications	%0 lime	%1 lime
Beginning of growth		
0 MEL	0.032ns	0.031
5 MEL	0.040	0.034
10MEL	0.039	0.034
Flowering		
0 MEL	0.039bc	0.041bc
5 MEL	0.041bc	0.048b
10MEL	0.023c	0.095a
Harvesting		
0 MEL	0.039b	0.041b
5 MEL	0.041ab	0.048ab
10MEL	0.023ab	0.095a

Mean followed by different letters within columns differ significantly ($p < 0.05$).

Phenolic compounds are one of them and many studies suggest that phenolic compounds increase in a stressful environment (Pejakovic et al., 2016; Çetin and Daler, 2017). In our experiment, it was observed that 10 µM MEL applications increased the total phenolic contents of the strawberry plants. In their study, Shi et al. (2015) put forward that MEL applications increased the amount of organic acids, sugars, and amino acids in the tissues in the bermuda grass. It was determined that a higher amount of total phenolic substance is accumulated when MEL is externally applied to the wheats under cold stress (Turk et al., 2014).

3.2. Proline

It was observed in our experiment that MEL applications had important effects on the proline amount (Table 3).

In the first sampling, it was observed that MEL application statistically did not have any effect on the proline contents in strawberry plants under lime conditions, but MEL applications increased the proline contents numerically. In the second sampling, the highest proline content was obtained in 10 µM application under lime condition with 0.095 µmol proline/g and the lowest proline was detected in 10 µM application in the %0 lime group with 0.023 µmol proline/g. In the last sampling, the highest proline content was obtained in 10 µM application under lime stress as 0.114 µmol proline/g and the lowest proline was detected in 0 µM MEL application in the %0 lime group and under lime stress as 0.058 and 0.055 µmol proline/g, respectively.

Stress conditions cause changes in secondary metabolites such as amino acids (proline, etc.), amines, and sugar. These compounds are not active in normal conditions but accumulate intensively in the cytoplasm under stress conditions (Chen and Murata, 2002; Vardharajula et al., 2011; Çetin and Daler, 2017). When the plants in the %0 lime group and under lime stress are analyzed among themselves, proline contents increased with MEL applications in general. It was observed that the amount of proline was higher in the plants under the lime stress and MEL application increased the amount of proline. In the studies conducted, it was revealed that the exogenous MEL applications increased the amounts of amino acids in bermuda grass (*Cynadon dactylon*) plants exposed to stress factors such as low temperature, drought, and salinity. It was determined that MEL application increased the amount of proline in grape seedlings under the drought stress and wheats grown at low temperatures (Meng et al., 2014; Turk et al., 2014; Shi et al., 2015).

3.3. Lipid peroxidation

In our experiment, the effects of MEL on lipid peroxidation (MDA) are shown in Table 4. In the first sampling, the highest MDA value was obtained from the plants whose MEL application was not carried out under the lime stress and those whose 5 µM application was performed in the %0 lime group (2.554 and 2.55 µmol/g, respectively). The lowest MDA value was determined in the plants to which 10 µM MEL was applied (1.616 µmol/g) under lime stress conditions. In the second sampling, the highest MDA

Table 4. The effects of MEL application on the MDA amount of strawberry plants under lime stress conditions.

Applications	%0 lime	%1 lime
Beginning of growth		
0 MEL	1.876ab	2.554a
5 MEL	2.550a	2.012ab
10MEL	1.763ab	1.616ab
Flowering		
0 MEL	3.642ab	3.731a
5 MEL	2.531c	2.005c
10MEL	2.431c	2.805bc
Harvesting		
0 MEL	2.631a	2.529a
5 MEL	1.863b	1.804bc
10MEL	1.515c	1.992b

Means followed by different letters within columns differ significantly ($p < 0.05$).

value was found (3.731 μmol/g) in the plants whose MEL application was not carried out under lime stress, and the lowest values were observed in the plants to which 5 μM MEL was applied under lime stress conditions and to which 10 μM MEL was applied in the %0 lime group (2.005 and 2.431 μmol/g, respectively). In the last sampling, the highest MDA value was obtained in the plants on which MEL application was not conducted under the %0 and %1 lime stress (2.631 and 2.529 μmol/g, respectively), and the lowest value was determined in the %0 lime plants with 10 μM MEL application (1.515 μmol/g).

It is known that MEL plays a direct role as an antioxidant in the protection of the biological membranes and it is effective in the struggle against the membrane fluidity and lipid peroxidation (Catala, 2007; Garcia et al., 2014). In our experiment, it is seen that the MEL applications decreased the MDA values. It was revealed that the lipid peroxidation decreased substantially in the seedlings under the heavy metal (copper) stress, which were obtained from the red cabbage seeds that had MEL application before October (Posmyk et al., 2008). It was reported for the strawberries that the MDA amount decreased significantly during the storage in the fruits that had MEL application after the harvest (Liu et al., 2018).

4. Conclusion

Strawberry plants, produced and consumed across the world, have almost no resistance to lime soil conditions. High lime conditions lead to severe chlorosis in strawberries and reduce the plant growth and the yield substantially. It was found out in our study, where we examined the effect of the melatonin applications on some of the biochemical properties in the strawberry plants grown under the lime stress conditions, that the MEL applications eased the lime stress conditions. In all of the criteria studied, 10 μM MEL application was observed to generally set forth better results.

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