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Effects of melatonin on salt stressed garlic plants: Time-dependent monitoring of leaf morphology in early plant phase

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ABSTRACT

In this study, the effects of melatonin to garlic were investigated under salinity stress in the early plant growth phase. The experiment was carried out under semi-controlled greenhouse conditions in the spring season. Garlic cloves were soaked in solutions of different concentrations of melatonin (0, 1, 2, 4, and 8 mM) during a 12-hour period at room temperature and dark conditions. Salt stress was generated by adding NaCl (0, 100, 200, and 400 mM) to irrigation water. The complete randomized design was used with three replications. At the end of the study, it was found that the 2 mM Melatonin treatment had the highest effect on the leaf length under 400 mM salinity. In the same stress condition, the maximum leaf amount was obtained in 4 mM MEL treated plants. When the time-dependent change in the leaf length was examined, it was observed that there was a separation between MEL applications from the first week in high salinity environment. Leaf waxiness, leaf erect, and leaf color intensity values were not affected by the Melatonin applications. It was concluded that application of 2 mM and 4 mM melatonin significantly increased salt tolerance of garlic plants in the early plant growth phase.

Key words: *Allium sativum*, NaCl, tolerance, variation.

INTRODUCTION

Worldwide garlic production was 28.164.055 tons in 2017 from 1.577.779 hectares according to the latest data of the United Nations Food and Agriculture Organization. According to the same data, Turkey produces 148.133 tons of garlic annually, which is 0.53% of world production, and it ranks as the 13th largest garlic producer (Anonymous, 3). Garlic can be grown in almost every region of Turkey due to its high adaptability. However, commercial production is carried out in agricultural areas in the transitional zone. Production declines have started to become an increasing problem due to using saline irrigation water in these areas.

World arable areas are critical for the salinity problem. Out of the total area of the cultivable area on Earth (14 million km^2), approximately 20% is heavily influenced by salinity and half of that faces various concentrations of salinity (Shahbaz and Ashraf, 12). Since the pineal tissue is only present in the nervous system of vertebrates, and the functions of melatonin are hormonal, this compound was initially determined as a neuronal hormone (a neurohormone) (Gundy *et al.*, 8). The several factors such us advancement in photosynthetic capacity, prevention of chlorophyll degradation, increase of growth parameters and activities of antioxidant enzymes, and improving of root morphology related characteristics may be assumed to affect alleviating effects of melatonin (Altaf, 1).

Synthesizing of melatonin in plants plays a role as an antioxidant or a modulator of growth and development (Fleta, 7). In addition, melatonin has been found to eliminate free radicals in humans and animals and has therefore been recognized as a broad-spectrum antioxidant. The adoption of this hypothesis proves the idea that melatonin can undertake similar activities in plants. Observable increases in the amount of melatonin in plants grown under stress conditions are directed to protecting plants from harmful effects caused by oxidative stress (Venegas *et al,.* 13). There are some questions that need clarification regarding the mechanism by which plants take up melatonin. Although the biosynthetic pathway has been explained, the molecular signals that regulate the formation of melatonin have not yet been precisely defined. In addition, there are uncertainties about the genes involved in melatonin biosynthesis in many species. The most acceptable hypothesis is that melatonin accumulates in protein-independent lipid stores and gains functionality (Hardeland, 10).

Some attempts have been made to improve garlic salt tolerance, including the use of exogenous substances, such as salicylic acid (Shama *et al.,* 11) foliar nutrition (El-Fadel and Mohamed, 6) and selenium (Se) (Astaneh *et al.,* 4). Despite these attempts, there is still a need for data concerning garlic growth under saline conditions, and inhibitory effects of melatonin. Our objective was to determine the detailed leaf features of garlic grown under salt stress and exogenous melatonin application.

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MATERIALS AND METHODS

The study was conducted between March and May 2019 in the semi-controlled greenhouse of Erciyes University. A local garlic genotype obtained from Malatya province was used as plant material. The experiment was conducted with three replications by a completely randomized design.

Garlic bulbs obtained from a local producer are divided into cloves. Same size cloves are chosen for the next steps. These cloves are soaked in different concentrations of melatonin solutions (1, 2, 4, and 8 mM) for 12 hours at 21˚C temperature under dark condition before the sowing. The cloves in the control group were kept in distilled water under the same conditions. After the application of melatonin cloves were planted in 2-liter pots on 28 February 2019. The pots were filled with 1.8-liter peat + perlite mixture before planting. Salt stress treatments were carried out with four treatments of NaCl (0 mM, 100 mM, 200 mM, 400 mM). The application of saline irrigation began two days after the cloves were planted. In this way, irrigation was repeated 19 times between 06 March and 14 May with two or three days periods. All agronomic applications were made as required for about three months. During this period, the minimum temperature was 10 °C and the maximum temperature was 23°C. Data on five traits were recorded on an individual basis from six plants randomly chosen in each treatment in the early plant growth phase. In order to monitor the periodical development of leaf length, a total of 10 measurements were performed in weekly periods. A scale of 0-5 was used in order to express the visible signs of leaf waxiness, leaf erect, leaf color intensity by salt stress on garlic. Statistical analysis was conducted using the JMP software. The data from the experiment were subjected to a general analysis of variance (ANOVA).

RESULTS AND DISCUSSION

According to the results of variance analysis, the effect of irrigation water salinity is statistically significant for all parameters measured. Melatonin application had a statistically significant effect only on leaf length and leaf amount values ($P < 0.01$). In addition, the interaction between melatonin applications and salinity had no statistically significant effect on any of the parameters.

The length of leaves varied between 12.77 and 40.40 cm (Table 1). Even though these values decreased with respect to the degree of the salinity, the doses of MEL affected the direction and severity of this decreasing. In the control group (not irrigated with salt water), the highest length of leaves was obtained in 2 mM Melatonin application (40.40 cm). In the maximum salt stress condition (400 mM), the treatment of 2 mM Melatonin also resulted in an increase in the length of leaves compared to the control (24.43 and 19.83 cm respectively). However, under this stress condition, the most severe reduction was observed in the highest dose of melatonin (8 mM).

Table 1. Leaf lengths of 70-day old garlic plants (cm).

Melatonin (mM)	Salinity (mM)				
	0	100	200	400	Average
0	35.50	30.00	25.60	19.83	27.73 b
1	34.33	31.33	28.50	19.90	28.52 ab
2	40.40	30.17	27.83	24.43	30.71 a
4	31.67	33.83	30.20	20.27	28.99 ab
8	32.87	27.00	25.17	12.77	24.45 c
Average	34.95 A	30.47 B	27.46 C	19.44 D	

*Means within a column that have a different small letter are significantly different from each other. Means within a row that have a different capital letter are significantly different from each other. P < 0.01.

When the time-dependent change in the leaf length was examined, it was observed that there was no separation between MEL applications until 28 March in a salt-free environment. Until this date, all plants have leaves about 22-24 cm long. As can be seen from the same graph, during this time, the greenhouse's night temperature is 10-12˚C and the daytime temperature is around 16-18˚C. However, as the temperature values started to rise, leaf growth was accelerated in the plants treated with 2 mM MEL, while other plants showed a slower development. Especially when the daytime temperature reaches 21˚C, this difference has gradually increased. The most obvious result obtained from this graph is that the application of 2 mM MEL promotes leaf growth for a long time in garlic plants which are not under salt stress conditions.

When the periodic changes of leaf elongation were analyzed, the results of 2 mM MEL application under the highest salt stress conditions were different from the others (Fig. I). Although this differentiation occurred in the salt-free group about one month after planting cloves, it became apparent from the first week under high-stress conditions. These results are parallel with the results of Amorim *et al.* (2). In that study, five levels of water salinity varying from 0.6 to 3.0 dS m-1 and two modes of water application were tested under greenhouse conditions in garlic plants. During the study, the development of the plant was measured in one month periods. At the end of the study, it was observed that garlic plants are relatively tolerant of salinity at the bulb formation stage and initial growth up to 30 days. The salinity

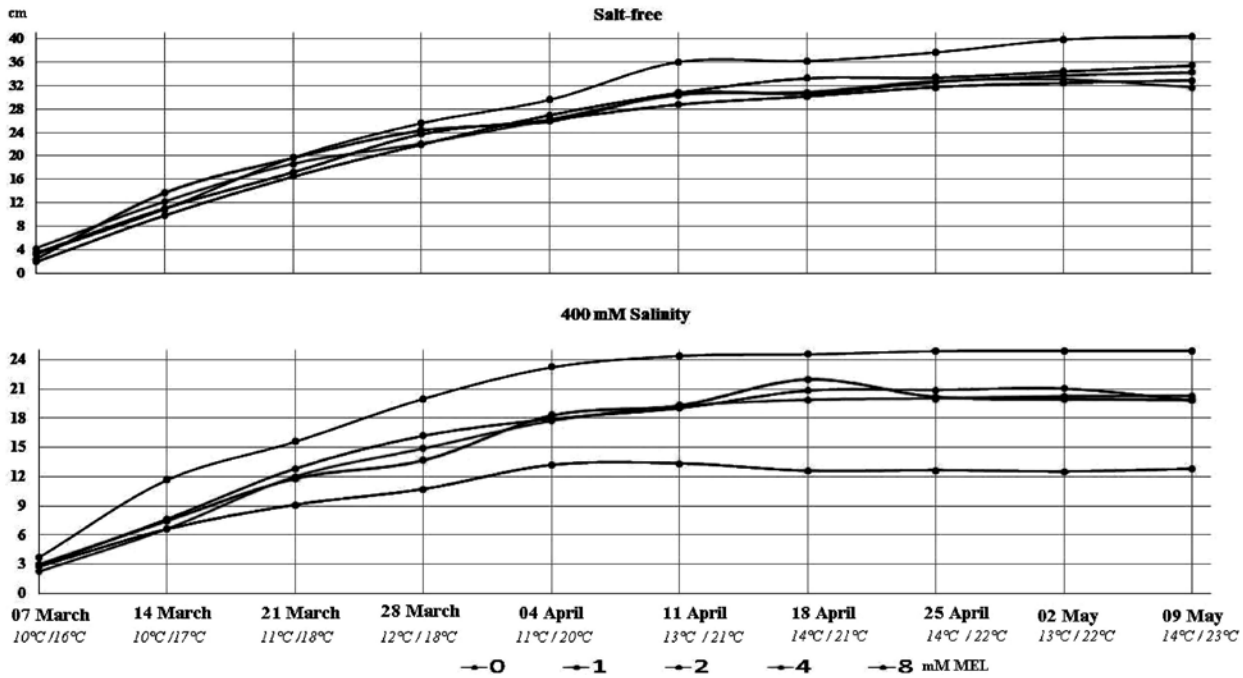

Fig. 1. Weekly changes of leaf length.

levels caused affecting aerial parts during the period of 30-60 days while the bulb was affected only between 60-90 days. Wang *et al.,* (14) examined the influences of exogenous melatonin on growth and antioxidant system in cucumber plants under 200 mM NaCl stress conditions. The results revealed that the melatonin-applied plants significantly increased growth mass and antioxidant protection. Under stress, it was observed that melatonin and the resistance of plants displayed a concentration effect.

The results of leaf numbers were not similar to leaf length results (Table 2). Irrigation applications containing 100 mM and 200 mM NaCl did not lead to a decrease in the number of leaves. Only 400 mM NaCl dose caused a decrease in the number of leaves. The effect of MEL applications on this character is parallel to the leaf length. Although the highest number of leaves was obtained from 2 mM MEL application, no statistically significant difference was found between 1 mM, 2 mM, and 4 mM MEL applications. The highest dose of MEL yielded the same result as control (10.33). In plants, irrigated with non-saline water, and non-treated with MEL, the increase of the number of leaves continued until April 25th. However, this value increased for one more week in all MEL treated plants.

In plants irrigated with the highest NaCl dose (400 mM), the increase in the number of leaves of all plants was very close to each other until March 28, after which some concentrations were separated

Table 2. Leaf numbers of 70-day old garlic plants.

Melatonin (mM)	Salinity (mM)				
	0	100	200	400	Average
0	10.00	11.33	11.33	8.67	10.33 b
1	13.00	15.00	13.00	10.00	12.75 a
2	13.33	13.67	11.67	10.00	11.67 a
4	13.00	13.00	13.00	10.67	12.42 a
8	13.00	9.67	11.67	7.00	10.33 b
Average	12.47 A	12.53 A	12.13 A	9.27 B	

*Means within a column that have a different small letter are significantly different from each other. Means within a row that have a different capital letter are significantly different from each other. $P < 0.01$.

(Fig. 2). Especially plants treated with 4 mM MEL showed more active growth when the in-greenhouse daytime temperature rose above 20°C (04th April). The growth rate in plants treated with 1 mM and 2 mM MEL increased after April 25, when the temperature increased above 22°C (25th April).

The decrease in vegetative characters under salt stress is a commonly reported phenomenon and it was used as a sensitive indicator in several plants. In the study of El-Fadel and Mohamed (6), the increasing salinity of irrigation water reduced leaf growth. Also, N and P uptake were gradually and significantly

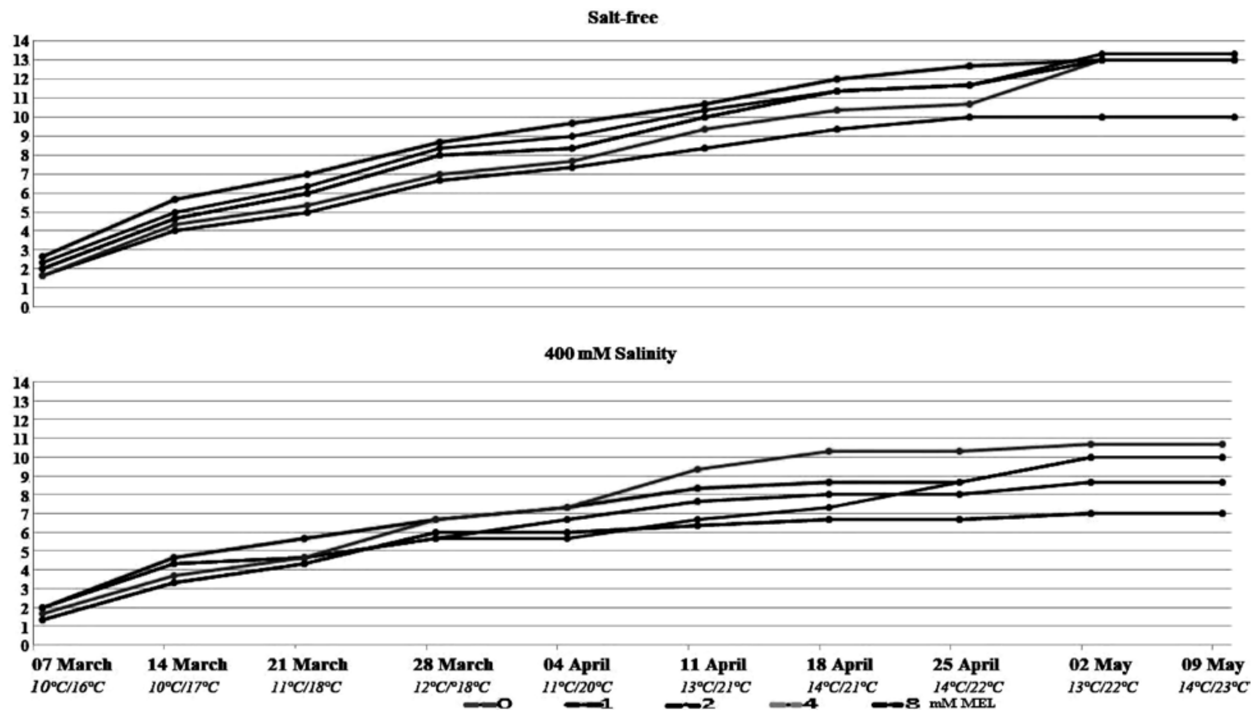

Fig. 2. Weekly changes of leaf number.

reduced by increasing irrigation water salinity up to its highest level. Vegetative growth, chemical content and green yield, significantly improved with nutrients applied through the foliar spray. Also in the study of Wu *et al*. (15), it was recognized that salt stress significantly repressed growth in melon seedlings, while exogenous treatment of melatonin and Ca^{2+} relieved the inhibition. Additional investigation revealed that exogenous application of melatonin or Ca^{2+} increased the photosynthetic rate and water use efficiency in salt-stressed melon seedlings.

Data on leaf waxiness, leaf erectness, and leaf color intensity are shown in Table 3. All these values decreased due to increasing salt concentrations. The effect of MEL applications on these characters was not found to be statistically significant. In the study of Hanci and Cebeci (9), five different onion varieties were exposed to salt stress at different doses. As a result of this study, waxiness in the leaves of all cultivars increased due to increasing salt doses. This result is contrary to the results obtained from our study.

When the studies that included garlic and melatonin were examined in the literature review, a limited number of studies using these two components were reached. In the study of Badria (5) surveyed the melatonin contents of some edible plants in Egypt. At the end of the study, 58.4 µg Melatonin was found in 100 g garlic samples. No studies have investigated the effect of exogenous melatonin application on abiotic stress conditions in garlic plants. From this point, this is the first study in this field. When all the results were evaluated together, it was found that increased irrigation water salinity caused decreases in measured values. In particular, a 2mM MEL application has been shown to inhibit these decreases. When the time-dependent leaf length change was examined, it was found that the positive effect of 2 mM MEL application at high salinity conditions has been observed from the second week. In the same conditions, the positive contribution to the number of leaves was mostly observed in the 4 mM MEL application. This effect appeared later than leaf length. (6^{th} week). As mentioned earlier, this is the first study to investigate the effect of melatonin on garlic plants. Therefore, in order to reach a more accurate conclusion, detailed studies are needed. In particular, increasing the number of melatonin doses used; evaluation of different genotypes; and the creation of fully controlled environmental conditions will serve this purpose better.

DECLARATION

The authors declare no conflict of interest.

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Table 3. Some leaf parameters of garlic plants at different salinity conditions.

Salinity (mM)	Melatonin (mM)	Waxiness	Erect	Color intensity of leaf
0	0	4.33	3.67	4.00
	1	5.00	3.67	4.00
	2	4.00	4.00	3.67
	4	4.00	3.67	3.67
	8	3.33	3.33	3.33
	Average	4.13 a	3.67 a	3.73 a
100	0	3.33	3.00	3.00
	1	2.67	2.67	3.00
	2	2.67	3.33	2.33
	4	3.33	3.33	3.00
	8	2.67	3.67	3.00
	Average	2.93 b	3.20 b	2.87 b
200	0	2.33	2.33	2.33
	1	2.33	3.00	3.00
	2	2.33	2.33	2.33
	4	2.33	3.00	2.67
	8	2.00	2.00	1.67
	Average	2.27 c	2.53 c	2.40 c
400	0	2.00	1.67	1.67
	1	1.33	1.33	1.00
	2	1.67	1.67	1.67
	4	1.33	2.00	2.00
	8	1.33	1.33	1.00
	Average	1.53 d	1.60 d	1.47 d

*Means within a column that have a different small letter are significantly different from each other $P < 0.01$.

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