Effect of melatonin on vegetative and chemical traits of tomato shoots growing under salt stress conditions in vitro

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Abstract

This study was conducted in the Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering at the College of Agriculture, Al-Qasim Green University during the year 2023-2024. To study the effect of melatonin on vegetative and chemical indicators of tomato plants, Serin cultivar, under salt stress in vitro, the experiment included two factors, melatonin at three concentrations (0, 0.2, 0.4) mg.L-1 and sodium chloride(NaCl) at three concentrations (0, 50, 100) mmol.L-1. The results showed that the interaction treatment of sodium chloride (0 mmol.L-1+ 0 mg.L -1melatonin) was superior in the percentage of dry weight (21.94%), and the interaction treatment of sodium chloride (0 mmol.L-1 + 0.2 mg.L-1 melatonin) was excelled in the number of leaves, shoots length, chlorophyll content, percentage of nitrogen, protein and potassium (3.467 leaves/shoot, 12.49 cm, 33.33 mg. 100 g-1 fresh weight, 1.387%, 8.67%, 2.92%) respectively .

Keywords: tissue culture, tomato, salt stress, melatonin,

introduction

Tomato, scientific name Solanum Lycopersicon, belongs to the Solanaceae family and is considered one of the most important and widely consumed vegetables in the world. It has beneficial effects on human health because it contains high levels of potassium, antioxidants, and many vitamins, and is a good source of carbohydrates and many fatty and amino acids and minerals (9). Tomatoes occupy an advanced position among the most consumed crops in Iraq, and the cultivated area in Iraq for the year 2021 is about 130,999 dunums, with a production of 744,166 tons (5.(

The problem of salinity in soil or water is considered one of the most prominent obstacles facing agriculture in various regions of the world, especially arid and semi-arid regions, causing damage or death to plants at high concentrations (11). Salt stress is considered one of the types of environmental stresses that have a clear negative impact, as it affects the plant from several aspects, including osmotic stress, ion toxicity, ion imbalance, stress resulting from hormonal imbalance, and oxidative stress, as it hinders enzyme activity and the efficiency of photosynthesis, increases osmotic stress, ion toxicity, and the generation of oxygen free radicals. (ROS) in plant cells (1(

Many mechanisms have been used to increase the resistance of plants to salinity, including growth regulators and stimulants such as hormones, vitamins, and some organic substances. Melatonin is considered a hormone that plays a role in regulating

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biochemical processes in plants, such as the development of roots and shoots and seed germination It has an important role in confronting abiotic stresses such as salinity, drought, and temperature. It regulates It balances oxidation and reduction reactions, as it works to remove free radicals (ROS), where it acts as a line of defense against oxidative stress and protects plant cells (10). The use of in vitro culture techniques in studying the mechanics and physiology of tolerance to salinity, as it provides a promising solution to what this provides. Techniques from a controlled environment in terms of salt content and environmental conditions, as such studies may be difficult under field conditions due to their interference with various other stresses related to the soil and climate, and the interference of such factors with salinity leads to the difficulty of conducting the process of testing, evaluating, and understanding the mechanics of salt tolerance (8) Therefore, this study aimed to study the effect of melatonin in alleviating the effect of salt stress on tomato in vitro.

Materials and methods

The experiment was conducted in the Plant Tissue Culture Laboratory, College of Agriculture, Al-Qasim Green University, 2023-2024, to study the effect of melatonin on the biochemical traits of tomato seedlings of Serin cultivar growing under salt stress in vitro. The study was connected according to a (CRD)completely randomized design with two factors and ten replicates. The first factor included three concentrations of(M) melatonin (0, 0.2, 0.4) mg.L-1, and the second factor included three concentrations of(S) NaCl (0, 50, 100) mmol.L-1. In this experiment, it used tomato seeds of Serin cultivar, which were obtained from a local agricultural company. The tomato seeds were washed with running water, then transferred to a Laminar Flow Cabinet and sterilized with 70% alcohol for 30 seconds, after which they were sterilized with a solution of(NaOCL2) sodium hypochlorate (1%) with add 3 (1-2DROPS) drops of Tween 20. After completing the sterilization process, the seeds were washed with sterile distilled water three times to remove the effect of the sterilizing material inside the planting booth. In this experiment, the prepared(MS) Murashige and Skoog medium was used with the addition of (15%) sucrose and (7) g/L agar. The medium was then placed on a thermal magnetic mixing device to dissolve the agar and homogenize the nutrient medium. Then the medium was distributed directly into the culture bottles at a rate of (50) ml. It was sterilized in an Autoclave at a temperature of 121°C and a pressure of 1.04 kg/cm2 for 20 minutes. The seeds were grown on the nutrient medium, 5 seeds per bottle, inside the Laminar Air Flow Cabinet, and incubated in the growth room for 21 days. After that, the growing top was cut and planted on the same nutrient medium, with the addition of(BA)Benzyl adenine 2 mg.L-1, with the addition of the experimental parameters: sodium chloride concentrations. And melatonin. The crops were incubated in a growth room at a temperature of 25 ± 1 and 16 hours of light with 8 hours of darkness for a period of four weeks to study the effect of melatonin and sodium chloride on the traits of number of leaves, shoot length, chlorophyll content, percentage of dry weight, percentage of nitrogen, percentage of protein and percentage of potassium.

Results and discussion-:

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Number of leaves (leaf. Shoot-1(

The results shown in Table (1) showed no significant differences between the concentrations of sodium chloride salt in the number of leaves on tomato shoots growing under salt stress in vitro, where the treatment without addition excelled by giving it the highest number of 2.933 compared to the rest of the treatments. It is noted from the same table that there are significant differences in the addition of melatonin, where the treatment adding melatonin 0.4 mg.L-1 recorded the highest number of leaves of 2.933 compared to the treatment without addition, which recorded the lowest number of 2.211, while the interaction treatment 0 mmol.L-1 sodium chloride + 0.2 mg.L-1 melatonin recorded the highest number of 3.467 .

Table (1) Effect of melatonin on the number of leaves in tomato seedlings growing under salt stress conditions in vitro (leaf. Shoot-1(

Average NaCl	**Melatonin mg.L^{-1}**			**NaCl mmol.L^{-1}**
	0.4	**0.2**	**0.0**	
2.933	3.133	3.467	2.200	0 : **NaCl**
2.456	2.733	2.400	2.233	50 **: NaCl**
2.611	2.933	2.700	2.200	100 : **NaCl**
	2.933	2.856	2.211	**Average Melatonin**
S:0.5079	**M: 0.5079**	**S*M :0.8797**		**L.S. D**

Length of shoots (cm(

The results shown in Table (2) showed significant differences between the concentrations of sodium chloride salt in the length of tomato shoots growing under salt stress in vitro, where the treatment without addition was superior by giving it the highest length of 11.07 compared to the rest of the treatments. It is noted from the same table that there are significant differences in the addition of melatonin, as the treatment with the addition of melatonin 0.2 mg.L-1 recorded the highest shoot length of 11.13 compared to the treatment without addition, which recorded the lowest length of 8.3, while the interaction treatment 0 mmol.L-1 sodium chloride + 0.2 mg.L -1melatonin recorded the highest length of 12.49 .

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Table (2) Effect of melatonin on shoot length in tomato plants grown under salt stress in vitro (cm(

Average NaCl	Melatonin mg.L-1			NaCl mmol.L-1
	0.4	0.2	0.0	
11.07	12.22	12.49	8.51	0 : NaCl
9.52	9.36	11.13	8.08	50 : NaCl
9.18	9.44	9.79	8.31	100 : NaCl
	10.34	11.13	8.3	Average Melatonin
S:1.116	M: 1.116	S*M :1.933		L.S. D

Percentage of dry weight

The results shown in Table (3) showed significant differences between the concentrations of sodium chloride salt in the percentage of dry matter in tomato shoots grown under salt stress in vitro, as the treatment without addition was excelled by giving the highest percentage of 21.23 compared to the rest of the treatments. It is noted from the same table that there are significant differences in the addition of melatonin, as the treatment with the addition of melatonin 0.4 mg.L-1 recorded the highest percentage of 19.85 compared to the treatment without addition, which recorded the lowest percentage of 17.85, while the interaction treatment 0 mmol.L-1 sodium chloride + 0.0 mg.L-1 melatonin recorded the highest percentage of 21.94.

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Table (3) Effect of melatonin on dry weight percentage in tomato shoots grown under salt stress conditions in vitro

Average NaCl	Melatonin mg.L-1			NaCl mmol.L-1
	0.4	**0.2**	**0.0**	
21.23	20.11	21.64	21.94	0 : **NaCl**
19.33	20.63	19.15	18.21	50 **:** **NaCl**
16.63	18.79	17.72	13.39	100 : **NaCl**
	19.85	19.5	17.85	**Average Melatonin**
S : 2.822	**M: 2.822**		**S*M: 4.888**	**L.S. D**

Chlorophyll content in shoots (mg. 100 g-1 fresh weight)

The results shown in Table (4) showed significant differences between the concentrations of sodium chloride salt in the chlorophyll content in tomato shoots grown under salt stress in vitro, as the treatment without addition excelled by giving the highest content of 27.45 compared to the rest of the treatments. It is noted from the same table that there are significant differences in the addition of melatonin, as the treatment with the addition of 0.4 mg.L-1 melatonin recorded the highest content of 26.27 compared to the treatment without addition, which recorded the lowest content of 18.34, while the interaction treatment 0 mmol.L-1 sodium chloride + 0.2 mg.L-1 melatonin recorded the highest content of 33.33 .

Table (4) Effect of melatonin on the percentage of chlorophyll in tomato plants grown under salt stress conditions in vitro(%)

Average NaCl	Melatonin mg.L-1			NaCl mmol.L-1
	0.4	**0.2**	**0.0**	
27.45	29.04	33.33	19.97	0 : **NaCl**
21.76	25.29	22.07	17.92	50 **:** **NaCl**
21.46	24.48	22.78	17.12	100 : **NaCl**
	26.27	26.06	18.34	**Average Melatonin**
S: 4.414	**M: 4.414**	**S*M :7.645**		**L.S. D**

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Nitrogen%

The results in Table (5) showed significant differences between the concentrations of sodium chloride salt in the percentage of nitrogen in tomato shoots grown in vitro, as the concentration without addition was superior by giving the highest percentage of 1.243% compared to the concentration of 100 mmol.L-1 which recorded the lowest percentage of 0.900%. It is noted from the same table that there is a significant effect when adding melatonin in the percentage of nitrogen, as the concentration of 0.4 mg.L-1 was superior by giving the highest percentage of 1.180% compared to the comparison treatment which recorded the lowest percentage of 0.911%. While the interaction treatment 0 sodium chloride + 0.2 mg.L-1 melatonin recorded the highest percentage of 1.387%.

Table (5) Effect of melatonin on the percentage of nitrogen in tomato plants grown under salt stress conditions in vitro(%)

Average NaCl	Melatonin mg.L^{-1}			NaCl mmol.L^{-1}
	0.4	0.2	0.0	
1.243	1.268	1.387	1.073	0 : NaCl
1.078	1.180	1.024	1.031	50 : NaCl
0.900	1.091	0.981	0.628	100 : NaCl
	1.180	1.131	0.911	Average Melatonin
S: 0.1254	M: 0.1254	S*M :0.2173		L.S. D

-Protein%

The results in Table (6) showed significant differences between the concentrations of sodium chloride salt in the percentage of protein in tomato shoots grown in vitro, as the concentration without addition was superior by giving the highest percentage of 7.77% compared to the concentration of 100 mmol.L-1 which recorded the lowest percentage of 5.63%. It is noted from the same table that there is a significant effect when adding melatonin in the percentage of protein, as the concentration exceeded 0.4 mg.L-1 and recorded the highest percentage of 7.37% compared to the comparison treatment which recorded the lowest percentage of 5.69%. While the intervention treatment 0 sodium chloride + 0.2 mg.L-1 melatonin recorded the highest percentage of 8.67%.

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Table (6) Effect of melatonin on the percentage of protein in tomato shoots grown under salt stress conditions in vitro(%)

Average NaCl	Melatonin mg.L^{-1}			NaCl mmol.L^{-1}
	0.4	0.2	0.0	
7.77	7.93	8.67	6.71	0 : NaCl
6.74	7.37	6.4	6.45	50 : NaCl
5.63	6.82	6.13	3.93	100 : NaCl
	7.37	7.07	5.69	Average Melatonin
S : 0.784	M: 0.784	S*M : 1.358		L.S. D

Potassium percentage

The results in Table (7) showed significant differences between the concentrations of sodium chloride salt in the percentage of potassium in tomato shoots grown in vitro, as the concentration without addition was superior by giving the highest percentage of 2.38% compared to the concentration of 100 mmol.L-1, which recorded the lowest percentage of 1.82%. It is noted from the same table that there is a significant effect when adding melatonin on the percentage of calcium, as the concentration exceeded 0.4 mg.L-1 and recorded the highest percentage of 2.37% compared to the comparison treatment, which recorded the lowest percentage of 1.50%. While the interaction treatment 0 sodium chloride + 0.2 mg.L-1 melatonin recorded the highest percentage of 2.92% .

Table (7) Effect of melatonin on the percentage of potassium in tomato plants grown under salt stress conditions in vitro(%)

Average NaCl	Melatonin mg.L^{-1}			NaCl mmol.L^{-1}
	0.4	0.2	0.0	
2.38	2.72	2.92	1.50	0 : NaCl
1.91	2.23	1.84	1.67	50 : NaCl
1.82	2.16	1.97	1.33	100 : NaCl
	2.37	2.25	1.50	Average Melatonin
S :0.555	M :0.555	S*M: 0.960		L.S. D

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Discussion

The results of the tables showed a negative effect of salt stress on the studied traits of tomato plants, which may be attributed to the decrease in vital activities inside the cell such as photosynthesis and respiration as a result of the osmotic effect of sodium chloride salt in the nutrient medium, which leads to a decrease in the absorption of water and nutrients by the plant (7). Other negative effects of salinity include the accumulation of toxic ions of sodium and chloride, causing a decrease in the activity of meristematic tissues, inhibition of cell division and elongation, and thus a decrease in growth (3). On the other hand, the results of the tables showed a significant role for melatonin in the studied traits under salt stress conditions in vitro. Melatonin works to increase the efficiency of carbon metabolism while inhibiting the activity of free radicals ROS under salt stress conditions (4). Melatonin showed significant differences in increasing plant height, number of leaves and dry weight, which may be due to its role in IAA metabolism, as melatonin is similar to IAA, both of which are from the same amino acid (2). Melatonin is also a regulator of many vital processes within the plant, as it works at low concentrations to stimulate plant growth and improve the efficiency of the photosynthesis process, as it works to protect the structure of chloroplasts from oxidation (6.(

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