Effect of melatonin on Hormonal Balance and Antioxidant Enzyme Activity in Potato Shoots Under salt stress in vitro

Emad J.Ch. Al-Naylle(1) Mohammed J. H.Al-Shareefi(2) Duraid K.A. Al-Taey(3(

1,3Department of Horticulture and landscape, College of Agriculture Al-Qasim Green University, Babylon, Iraq.

E-mail:Emad.jasim@agre.uoqasim.edu.iq

Abstract

This study was conducted in the Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering, College of Agriculture, Al-Qasim Green University, during the year 2023-2024. To investigate the effect of the growth regulator melatonin on the content of growth regulators in potato cultures (Cultivar Burin) under in vitro salt stress, the experiment included two factors: the first factor was sodium chloride at four concentrations (0, 50, 75, and 100) mmol L-1, and the second factor was melatonin at three concentrations (0, 0.4, and 0.6) mg L-1. The results showed that sodium chloride had a negative effect on the studied traits, as the concentration (100 mmol.L-1) led to a decrease in the shoots' content of internal growth regulators (auxin, cytokinin and gibberellins) and recorded the lowest rate (151.50, 49.39, 92.12 µg.kg-1 fresh weight) respectively, while the same concentration showed an increase in the activity of the Peroxidase enzyme and recorded a rate of 25.71 absorption units. min.g-1, while the treatment (75 mmol.L-1) of NaCl salt was excelled and gave the highest rate in the activity of the Catalase enzyme, which amounted to 14.55 absorption units. min.g-1. The results also showed that adding melatonin to the nutrient medium had a positive effect on the studied traits. The melatonin treatment (0.6 mg.L-1) achieved the highest levels of endogenous growth regulators (auxin, cytokinin, and gibberellins) in the shoots, giving them 173.96, 69.79, and 117.22 µg.kg-1 fresh weight, respectively. The same treatment also recorded the lowest catalase activity, with an average of 12.12 MU/min g-1. while, the melatonin treatment (0.4 mg.L-1) recorded the lowest peroxidase activity, with an average of 18.44 MU/min g-1.

Keywords: Catalase, Peroxidase, Tissue Culture, Potato, Salt Stress, Melatonin

Introduction

Potato (Solanum tuberosum L.) is a vital and strategic crop in the world, alongside wheat, rice, and corn, in global human nutrition. This is due to its richness in energy and carbohydrates, low in fat, and high in fiber, minerals, vitamins, and antioxidants. Potato cultivation faces many challenges, foremost among which is the problem of salinity, which limits plant growth and productivity in many regions of the world, particularly arid and semi-arid regions. Salinity causes a significant decline in the growth and yield of plants growing in these soils due to hormonal, nutritional, and enzyme imbalances, as well as the toxic effects of ions [2]; [7]. Salinity is also one of the environmental stresses with a clear negative impact, as it affects plants in several ways, including osmotic stress, ionic

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toxicity, ionic imbalance, stress resulting from hormonal imbalance, and oxidative stress. This hinders enzyme activity and photosynthetic efficiency, and increases osmotic pressure, ionic toxicity, and the generation of free oxygen radicals (ROS) in plant cells. [13];[3], and since potatoes are one of the crops that contribute to providing food and nutritional security in light of the increasing population growth in developing countries, therefore, one of the most important challenges facing decision-makers is finding and developing different strategies to deal with such conditions and variables to ensure the sustainability of food and nutritional security for future generations [4];[15], therefore, researchers have turned to using many techniques to study the mechanics and physiology of plant tolerance to salinity, and one of these techniques is the in vitro cultivation technique, as this technique provides a homogeneous growth medium in terms of salt content and environmental conditions, as such studies may be difficult under field conditions due to their interaction with various other stresses related to soil and climate, and the interaction of such factors with salinity leads to difficulty in conducting the testing and evaluation process and understanding the mechanism of tolerance [6]. Due to the widespread occurrence of soil and irrigation water salinity, which limits the expansion of potato cultivation in Iraq, it has become necessary to use methods to reduce the severity of the harmful effects. Recent research has turned to the use of chemical or mineral substances that help increase the growth rate and production in such conditions. In the past few years, the clear and effective effect of melatonin has drawn the attention of researchers, as it is considered one of the hormones that play a major role in regulating biochemical processes in plants, such as the development of roots and buds and seed germination. It also has an important role in confronting abiotic stresses such as salinity, drought and temperature, as it regulates and balances oxidation-reduction reactions, as it works to remove free oxygen radicals (ROS), as it works as a line of defense against oxidative stress and protects plant cells [18]; [19]. Therefore, this research aimed to study the effect of melatonin in mitigating the harmful effects of salt stress on potato 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 vegetative and chemical traits of potato cultivar Borin grown under salt stress in vitro. The study was implemented according to a two-factor CRD design with ten replications. The first factor included four concentrations of NaCl (0, 50, 75, 100) mmol L-1, and the second factor included three concentrations of melatonin (0, 0.4, 0.6) mg L-1. In this experiment, Buren potato seeds of Elite class, which is widely cultivated in Iraq and was obtained from Nahar Al-Awrad Potato Trading Company, were used. Infected and mechanically damaged tubers were sorted and excluded. Then, medium-sized tubers were taken and washed with running water to remove dirt and dust, then left to dry. After that, they were incubated at a temperature of 20-25°C in the dark for two weeks to break the dormancy phase and stimulate the growth of vegetative buds. After germination, the vegetative growths were removed from the potato tubers and washed with running water for 30 minutes, then washed with distilled water. Then, the ends of the buds were dipped in paraffin wax to prevent the penetration of the material used for sterilization into the bud

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tissues. After that, the vegetative growths were transferred to a laminar air flow cabinet, and the buds were immersed in ethyl alcohol for (10 seconds). Then, the buds were placed for 15 minutes in a 1% sodium hypochlorite solution (Naocl). Use a commercial bleach containing 5% sodium hypochlorite and add 3 drops of Tween-20 to reduce surface tension and increase the efficiency of surface sterilization. After that, the buds were washed with sterile distilled water three times to remove the effect of the sterilizing substance. After completing the sterilization process, the sterilized plant parts were transferred and placed in sterilized Petri dishes inside the laminar air flow cabinet. The sterilized vegetative buds were planted on the (MS) culture medium for the emergence of cultures after removing the part surrounded by paraffin wax, using sterile tissue culture tools. In this experiment, the ready-made MS culture medium was used, with sucrose added to it at a rate of (15%) and agar at a rate of (7) g/L. The medium was then placed on the thermal magnetic mixing device to dissolve the agar and homogenize the culture medium. Then, the medium was distributed directly into the culture bottles at a rate of (10) ml and sterilized in an Autoclave device at a temperature of 121°C and a pressure of 1.04 kg/cm2 for 20 minutes. One bud was planted for each elephant with a metal plug (9 cm long and 3 cm in diameter). Then the cultures were incubated at 23-25°C and light intensity of 1000 lux for 16 hours of light and 8 hours of darkness. After 3-4 weeks, the vegetative buds grew and became ready for the excision of the growing tip. The growing tip was separated using special sterile tools and blades to ensure the non-transmission of pathogens. In this experiment, bottles containing 20 ml of the prepared culture medium weighing 4.34 g. L-1 with the addition of sucrose 30 g. L-1 and myo-inositol 100 mg. L-1 with the addition of 2 mg. L-1 BA + 0.5 mg. 1 L-1 GA3 was grown directly in the nutrient medium (MS) at a rate of 2 plant parts per glass bottle. After that, the cultures were transferred to the growth chamber and incubated at a temperature of 25 ± 1 and 16 hours of light with 8 hours of darkness for six weeks. The process was repeated several times until the required number of cultures was reached to study the effect of sodium chloride and the growth regulator melatonin on the content of the shoots of growth regulators auxin, cytokinin and gibberellins.

The study included the study of two factors:

First factor: sodium chloride (NaCl) at four concentrations:

- First concentration: 0
- Second concentration: 50 mmol L-1
- Third concentration: 75 mmol L-1
- Fourth concentration: 100 mmol L-1

Second factor: melatonin at three concentrations:

- First concentration: 0
- Second concentration: 0.4 mg L-1
- Third concentration: 0.6 mg L-1 L-1

Results:

Auxin content of shoots (IAA) (µg.kg-1 fresh weight(

The results shown in Table (1) showed significant differences between sodium chloride concentrations. The content of the endogenous hormone auxin in potato shoots growing under in vitro salt stress decreased with increasing sodium chloride concentration. The control treatment (without sodium

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chloride addition) significantly excelled, producing the highest auxin content of 169.65 µg.kg-1 fresh weight, compared to the 100 mmo.L-1 sodium chloride treatment, which produced the lowest auxin content of 151.50 µg.kg-1 fresh weight. The same table also shows a significant effect of adding melatonin, with the 0.6 mg.L-1 melatonin treatment producing the highest auxin content of 173.96 µg.kg-1. kg-1 fresh weight, compared to the control treatment (without adding), which produced the lowest auxin content of 147.4 µg kg-1 fresh weight. While, the interaction treatment (NaCl 0 mmol L-1 + Melatonin 0.6 mg L-1) recorded the highest level of 187.15 µg kg-1 fresh weight.

Table (1) Effect of melatonin on auxin content in Borin potato cultures grown under salt stress conditions in vitro (µg kg-1 fresh weight.(

sodium chloride)Nacl(Mmol.L^{-1} (S)	**Growth regulator melatonin (M) mg.L^{-1}**			**Effect sodium chloride**
	0	**0.4**	**0.6**	
Nacl: 0	**146.85**	**164.94**	**187.15**	**169.65**
Nacl: 50	**151.13**	**166.94**	**181.25**	**166.44**
Nacl: 75	**146.26**	**161.84**	**171.12**	**159.74**
Nacl: 100	**145.34**	**152.58**	**156.31**	**151.50**
Effect Melatonin	**147.4**	**164.14**	**173.96**	
L.S.D	**S : 1.344 M : 1.164 S*M : 2.328**			

Cytokinin content of potato shoots

)µg.kg-1 fresh weight(

The results shown in Table (2) showed significant differences between sodium chloride concentrations. The endogenous hormone cytokinin content in potato shoots growing under in vitro salt stress decreased with increasing sodium chloride concentration. The control treatment (without sodium chloride addition) significantly excelled, producing the highest content of 65.20 µg.kg-1 fresh weight, compared to the 100 mmo.L-1 sodium chloride treatment, which produced the lowest cytokinin content of 49.39 µg.kg-1 fresh weight. The same table also shows a significant effect of adding melatonin, with the 0.6 mg.L-1 melatonin treatment producing the highest cytokinin content of 69.79 µg.kg-1. fresh weight, compared to the control treatment (without adding), which produced the lowest cytokinin content, reaching 43.98 µg kg-1 fresh weight. While, the interaction treatment (NaCl 0 mmol L-1 + Melatonin 0.6 mg L-1) recorded the highest level, reaching 81.53 µg kg-1 fresh weight

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Table (2) Effect of melatonin on cytokinin content in Borin potato cultures grown under salt stress conditions in vitro (µg kg-1 fresh weight.(

sodium chloride)Nacl(Mmol.L^{-1} (S)	Growth regulator melatonin (M) mg.L^{-1}			Effect sodium chloride
	0	0.4	0.6	
Nacl: 0	44.03	70.03	81.53	65.20
Nacl: 50	48.19	60.69	77.25	62.05
Nacl: 75	42.73	58.41	65.10	55.41
Nacl: 100	40.96	51.94	55.28	49.39
Effect Melatonin	43.98	60.27	69.79	
L.S.D	S : 0.719 M : 0.623 S*M : 1.245			

Gibberellin content of potato shoots (µg.kg-1 fresh weight(

The results shown in Table (3) showed significant differences between sodium chloride concentrations. The content of the endogenous hormone gibberellin in potato shoots growing under in vitro salt stress decreased with increasing sodium chloride concentration. The control treatment (without sodium chloride addition) significantly excelled the control treatment, producing the highest content of 112.80 µg.kg-1 fresh weight, compared to the treatment (100 mmo.L-1) of sodium chloride, which produced the lowest gibberellin content of 92.12 µg.kg-1 fresh weight. The same table also shows a significant effect of adding melatonin, as the 0.6 mg.L-1 melatonin treatment produced the highest gibberellin content of 117.22 µg.kg-1. fresh weight, compared to the control treatment (without adding), which yielded the lowest gibberellin content, reaching 86.71 µg kg-1 fresh weight. While, the interaction treatment (NaCl 0 mmol L-1 + Melatonin 0.6 mg L-1) recorded the highest level, reaching 132.52 µg kg-1 fresh weight.

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Table (3) Effect of melatonin on gibberellin content in Borin potato cultures grown under salt stress conditions in vitro (µg kg-1 fresh weight.(

sodium chloride)Nacl(Mmol.L^{-1} (S)	Growth regulator melatonin (M) mg.L^{-1}			Effect sodium chloride
	0	0.4	0.6	
Nacl: 0	86.68	119.20	132.52	112.80
Nacl: 50	91.08	108.02	124.40	107.83
Nacl: 75	84.97	102.28	113.58	100.28
Nacl: 100	84.12	93.86	98.38	92.12
Effect Melatonin	86.71	105.84	117.22	
L.S.D	S : 1.071 M : 0.927 S*M : 1.854			

Peroxidase activity in shoots (absorption

unit.min.g-1(

The results shown in Table (4) showed significant differences in the effect of sodium chloride on the activity of the Peroxidase enzyme in potatoes grown under salt stress in vitro. The treatment (100 mmol L-1) of sodium chloride was excelled and produced the highest rate of Peroxidase activity, reaching 25.71 absorption unit.min.g-1. compared to the control treatment (without adding) of sodium chloride, which produced the lowest rate of Peroxidase activity, reaching 14.28 absorption unit.min.g-1. The same table shows a significant effect of the growth regulator melatonin on the activity of the Peroxidase enzyme, as The control treatment (without melatonin addition) excelled and produced the highest Peroxidase activity rate of 22.09 MU/min g-1, compared to the melatonin treatment (0.4 mg.L-1), which produced the lowest Peroxidase activity rate of 18.44 MU/min g-1. While, the interaction treatment (NaCl 100 mmol L-1 + melatonin 0 mg L-1) recorded the highest Peroxidase activity rate of 28.70 MU/min g-1.

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Table (4) Effect of melatonin on the Peroxidase activity of potato cultivar Borin grown under salt stress conditions in vitro (MU/min g-1.(

sodium chloride)Nacl(Mmol.L^{-1} (S)	Growth regulator melatonin (M) mg.L^{-1}			Effect sodium chloride
	0	0.4	0.6	
Nacl: 0	16.72	13.20	12.93	**14.28**
Nacl: 50	18.84	15.17	16.63	**16.88**
Nacl: 75	24.11	20.23	22.69	**22.34**
Nacl: 100	28.70	25.16	23.27	**25.71**
Effect Melatonin	**22.09**	**18.44**	**18.88**	
L.S.D	**S : 1.304**	**M : 1.129**	**S*M : 2.259**	

Catalase activity in shoots (absorption

units.min.g-1(

The results shown in Table (5) showed significant differences in the effect of sodium chloride on the activity of the catalase enzyme in potatoes grown under salt stress in vitro. The 75 mmol L-1 treatment of sodium chloride was excelled and produced the highest rate of catalase activity, reaching 14.55 absorption units.min.g-1. This did not differ significantly from the 100 mmol L-1 treatment of sodium chloride, which recorded a rate of 14.40 absorption units.min.g-1. However, it differed significantly from the control treatment (without adding) of sodium chloride, which produced the lowest rate of catalase activity, reaching 10.59 absorption units.min.g-1. The same table shows a significant effect of the growth regulator melatonin on the activity of the catalase enzyme. The control treatment (without melatonin addition) excelled the control treatment, producing the highest rate of catalase activity, reaching 14.76 MU/min g-1, compared to the melatonin treatment (0.6 mg.L-1), which produced the lowest rate of catalase activity, reaching 12.12 MU/min g-1. While, the interaction treatment (NaCl 100 mmo.L-1 + melatonin 0 mg.L-1) recorded the highest rate, reaching 17.59 MU/min g-1.

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Table (5) Effect of melatonin on the activity of the catalase enzyme in potato plants (Borin variety) grown under salt stress conditions in vitro (MU/min g-1.(

sodium chloride)Nacl(Mmol.L^{-1} (S)	Growth regulator melatonin (M) mg.L^{-1}			Effect sodium chloride
	0	0.4	0.6	
Nacl: 0	12.02	9.10	10.65	10.59
Nacl: 50	13.07	12.09	12.48	12.55
Nacl: 75	16.35	14.43	12.87	14.55
Nacl: 100	17.59	13.15	12.47	14.40
Effect Melatonin	14.76	12.20	12.12	
L.S.D	S : 0.746 M : 0.646 S*M : 1.291			

Discussion

The results of the tables showed a negative effect of salt stress on the studied traits of potato cultures. This may be attributed to a decrease in intracellular vital activities, such as photosynthesis and respiration, due to the osmotic effect of sodium chloride in the culture medium, which leads to reduced water and nutrient absorption by the plant. [12]; [17] Other negative effects of salinity include the accumulation of toxic sodium and chloride ions, causing decreased activity in meristematic tissues, inhibition of cell division and elongation, and consequently, reduced growth. [14]; [15] The increased activity of peroxidase and catalase enzymes may be due to the mechanism followed by the plant to increase its tolerance to stress by increasing the activity of oxidative enzymes. The plant's production of active oxygen radicals increases under stress conditions, disrupting cellular metabolism and impeding physiological processes. Therefore, the plant works to increase the activity of some enzymes, such as CAT and POD, due to their ability to remove oxygen radicals and to avoid the negative effects resulting from salinity. [1]; [5]; [16] On the other hand, the results showed a significant role of melatonin in the studied traits under in vitro salt stress conditions. Melatonin increases the efficiency of carbon metabolism while inhibiting the activity of free radicals (ROS) under salt stress conditions [16]; [10]. This may be due to its role in IAA metabolism, as melatonin is similar to IAA, as both are from the same amino acid. Melatonin also acts as a regulator of a wide range of essential plant processes. It has the ability to enhance the efficiency of photosynthesis and promote plant growth when present in low amounts. More specifically, it works to prevent oxidation in the structure of chloroplasts [8]; [11.[

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